BIOLOGICAL REGULATION

THE BLOOD ANTICOAGULANT PROTEIN S DRIVES ORAL SQUAMOUS CELL CARCINOMA TUMORIGENESIS

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Introduction: Tyro3, Axl and MerTK (TAM) are proto-oncogenes implicated in various cancers, including in Oral Squamous Cell Carcinoma (OSCC). The blood anticoagulant Protein S (PROS1) was recently shown to acts as a TAM agonist, however its role in cancer was not explored. Objectives: To investigate the role of PROS1 in OSCC tumorigenesis.

Methods: PROS1 expression levels were determined in OSCC cell lines by Real-time quantitative PCR (RT-qPCR) and western blot (WB) analysis. Lenit-viral mediated Sh-RNA knockdown (kd) of PROS1 was performed in SCC-1 and SCC-25 - two human OSCC cell lines. The effect of PROS1 kd was verified and tested using various functional assays. Proliferation and viability were measured by BrdU incorporation and XTT assay. Migration, invasion and ability to form colonies in soft agar were assayed. The effect of PROS1-kd in-vivo was tested using the xenograft transplantation model. Molecularly, activation of oncogenic signaling pathways, and expression of key effector genes was tested following kd by WB, RT-PCR and Immunostaining.

Results: High PROS1 expression is detected among OSCC lines, and in tissues from OSCC patients. Effective PROS1 kd in two OSCC lines revealed a reduced proliferation rate, and reduced migratory and invasive potentials compared to their control-treated counterparts. PROS1 kd cells also significantly reduced the tumor burden and growth in xenografts, compared to control-treated cells. Altogether these results suggest PROS1 maintains cancer characteristics in OSCC. Interestingly, the receptor tyrosine kinase Axl and it downstream effector Akt, are down regulated following PROS1 kd in OSCC, indicating this signaling pathway mediates OSCC tumorigenic features. Conclusion: We provide novel evidence indicating that PROS1 is involved in OSCC tumorigenesis, affecting proliferation, migration, invasiveness and growth in soft agar. Mechanistically, PROS1 maintains high Axl expression levels, allowing Axl-mediated activation of the downstream Akt signaling pathway, known to drive proliferation in cancer cells.

SOX9 REGULATES CEACAM1 AND IMMUNE RESISTANCE IN MELANOMA CELLS

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Malignant melanoma is a highly aggressive and fatal form of skin cancer. Treatment options have increased in the past years, however, melanoma therapy is still far from reaching its full potential. As melanoma cells are immunogenic, they instigate an adaptive immune response and production of anti-tumor T-cells. A central factor in this interaction is CEACAM1, a transmembrane glycoprotein previously shown in our lab to protect melanoma cells from lymphocyte-mediated killing. SOX9 [SRY (sex determining region Y)-box 9], is a transcription factor with an essential role in embryonic development and adult tissues. It was found to have a major role in normal melanocytes during maturation from melanoblasts and in the cell response to UVB exposure. Bioinformatic analysis revealed several binding sites for SOX9 in the CEACAM1 promoter. Therefore, we aim to study the role of SOX9 in regulation of CEACAM1 expression and immune resistance in melanoma cells. SOX9 knockdown by siRNA in melanoma cell lines demonstrated an up-regulation of CEACAM1 expression (protein and RNA) in comparison to control cells. Furthermore, SOX9-knockdown melanoma cells were more resistant to Tumor Infiltrating Lymphocytes (TILs) mediated killing. SOX9 overexpression showed a decrease in CEACAM1 promoter activity in luciferase reporter assays. However, deletion of 8 putative SOX9 binding sites did not eliminate this effect, suggesting SOX9 regulates CEACAM1 indirectly. Our results show that SOX9 regulates the expression of CEACAM1 in and immune-resistance in melanoma cells. Regulation of CEACAM1 by SOX9 is at a transcriptional level, but probably occurs indirectly. Therefore, we hypothesize that SOX9 regulates immune resistance in a CEACAM1 dependent manner. Further understanding in the regulation of CEACAM1 in melanoma can provide new understating into melanoma biology and immune crosstalk, and help develop new approaches to melanoma therapy.

INDUCTION OF CELLULAR SENESCENCE AND HAIR FOLLICLE STEM CELL DYSFUNCTION UPON P16INK4A EXPRESSION IN THE SKIN

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Cellular senescence is a physiologic stress response program, in which cells cease to proliferate and undergo dramatic alterations in morphology, metabolic activity and protein secretion. Despite its importance in aging, tumor suppression and development, many fundamental aspects of cellular senescence remain poorly elucidated. Senescence is executed most often by the p14ARF/p53 and p16INK4a/Rb pathways, which are interconnected, yet their distinct contributions to the senescence program, and the consequence of their activation on the surrounding tissue are not fully understood. We have developed mice that allow inducible activation of p16INK4a in the epidermis of the skin. Using this model, we set out to study the effects of its activation on senescence induction, on the skin structure and on hair follicle stem cell function. We found that p16INK4a activation induced the formation of senescent cells in the epidermis, however with efficiency lower than that induced by p14ARF in a similar model. p16INK4a activation in the skin also induced a pronounced epidermal thickening, which was caused by enlargement of keratinocytes. Interestingly, upon long-term induction, p16INK4a altered the normal epidermal differentiation pattern, causing an accumulation of differentiated epidermal cell layers, changes that were distinct from those observed during normal aging. This was accompanied with a complete hair loss (alopecia) due to the disruption of hair follicle stem cell function and the hair follicle destruction. In the long-term induced mice, multiple changes in the dermal structure were also observed. Our findings indicate that long term p16INK4a activation exerts complex effects on skin physiology and structure, in both cell autonomous and non-autonomous manners.

MITOCHONDRIAL RESPIRATION CAPACITY AND SENSITIVITY TO GLYCOLYSIS BLOCKADE IN LUNG CANCER CELL LINES

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Introduction: One of the metabolic perturbations in cancer cells is the Warburg effect; glycolysis is preferred over oxidative phosphorylation (OXPHOS), even in the presence of oxygen. The precise mitochondrial alterations that underlie the increased dependence of cancer cells on aerobic glycolysis for energy generation may serve as an escape mechanism from apoptosis. Here, we aimed to profile the mitochondrial activity in different lung cancer cell lines in reference to their glycolytic activity and to their sensitivity to metabolic modifications.

Methods: The metabolic profile of A549 and H358 cell lines were tested before and after glycolysis blockade (glucose starvation, 2DG) and mitochondrial induction (FCCP). Glycolysis inhibition and mitochondrial activity were assessed by western-blot quantification of key enzymes involved in the glycolysis pathway (e.g. Hexokinase I/II, glyceraldehyde-3-phosphate dehydrogenase, pyruvate kinase 2) and of mitochondrial coded proteins (e.g. ND1, ATP6 synthase). The oxygen consumption rates (OCR) and extra cellular acidification rate (ECAR) were measured by XFe24 extracellular flux analyzer. Further, mitochondrial index was compared to the cells' sensitivity to glycolysis inhibition.

Results: A549 cells were highly affected by glucose inhibition/starvation accompanied by ineffective mitochondrial compensation. On the other hand, H358 cells recovered completely from glucose starvation through mitochondrial hyper-activation (Fig 1); At the basal level (when no material was applied), A549 cells that were starved had a decrease of 68% in the ECAR, as compared to non-treated cells. Their recovery was limited after glucose injection (23 vs.41 mpH/min). In comparison, H358 cells had a 43% decrease in their glycolysis rate with a full recovery after glucose injection (44-46 mpH/min; pre & post respectively). Mitochondrial respiration was very low for A549 cells under starvation, while significantly increased in H358 cells (223 vs.143 pmol/min, *Pv<0.0001). Respectively, the expression level of mitochondrial coded proteins was higher in the cells that demonstrated higher mitochondrial capacity (Fig 2). **Conclusion:** Cells with high mitochondrial capacity may tolerate glucose starvation/ blockade, while a limited mitochondrial reserve exposes the cells to higher sensitivity to glycolysis stress. This might suggest a potential therapeutic avenue with a companion predictive test.

DEPLETION OF LATS KINASES ALTERS P53 TO PROMOTE CELL MIGRATION

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Proper cellular response to internal and environmental challenges requires profound interactions between components of different signaling pathways. Specifically, deregulation of the wiring between different tumor suppressor pathways may lead to neoplastic transformation or enable tumor progression and metastasis. The tumor suppressor p53 interacts with numerous signaling pathways to orchestrate a broad range of cell fates, most notably induction of cell cycle arrest and apoptosis. Additionally, pro-survival effects of p53 are being increasingly appreciated. Previous studies from our lab demonstrated multiple interactions between the Hippo kinase Lats2 and p53 in response to different types of stress as well as in stem cell differentiation. We now provide evidence that attenuation of Lats1 and Lats2 (Lats1/2) may modulate the physical and functional properties of p53 in unstressed cells. Using mass spectrometry combined with global transcriptome analysis, we were able to characterize different aspects of p53 activity following depletion of Lats1/2. Interestingly, depletion of Lats1/2 causes p53 to regulate a set of "non-canonical" transcriptional targets, some of which have been associated previously with cancer-associated mutant p53 isoforms. These changes in p53 may account for our surprising finding that, although wild type p53 is commonly believed to inhibit cell migration as part of its tumor suppressive features, it actually contributes to the enhanced migration of Lats1/2-depleted cells. Overall, we suggest a novel mechanism in which Hippo pathway kinases maintain p53 in its proper tumor suppressor state. Attenuated expression and activity of those kinases, as occurs in various cancers, might allow p53 to exert non-canonical activities that eventually benefit the cancer cells.

THE ROLE OF CELLULAR SENESCENCE, A STRESS RESPONSE MECHANISM, IN BREAST CANCER DEVELOPMENT

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Breast cancer is one of the most common human malignancies and a leading cause of death among women worldwide. Therefore, understanding the mechanism driving this disease is of great importance. Cellular senescence is considered central mechanism of tumor suppression, as it induces irreversible cell cycle arrest in response to strong oncogenic signals. However, studies have shown that senescent cells secrete a host of cytokines, which can influence various cells in their environment and thereby exert either tumor-promoting or tumor-suppressing effects. This suggests more complex, non-cell-autonomous functions of the senescence program may take place during tumorigenesis. The fate and function of senescent cells in tumor lesions is thus unclear. It is not known whether these cells are retained in the tissue or are removed from it, and how this affects tumor progression. Here, I aim to uncover the impact of senescent cells on their environment and on surrounding cells within breast cancer lesions. To do this, I generated transgenic mice that allow the induction of p14ARF or p16Ink4A, the main activators of the senescence program, within developing breast tumors. This experimental system enables me to induce these factors in different stages of tumor progression, and also within mammary stroma components. Upon short term activation of p16Ink4A, large numbers of p16-positive cells are detected in the lesions, whereas at longer activation few transgene expressing cells are detected, suggesting either active removal or rapid dilution of p16-expressing cells. To test the effects of senescence in tumor stroma on tumor growth and characteristics, I induced p16Ink4A in blood vessels of developing tumors using an endothelium-specific driver. Prolonged activation of the transgene at an early stage of tumor growth resulted in a significant decrease in blood vessel numbers indicating p16Ink4a block of tumor vessel development. In contrast, p16Ink4A induction in advanced tumors did not affect vessel numbers, yet led to decreased number of proliferating cells in the lesions, suggesting decreased endothelial function. These findings lay the ground for detailed analysis of the fate and effects of senescent breast cancer cells, and of senescent tumor endothelial cells, on tumor growth and progression.

ADAR1 REGULATES THE RESISTANCE OF MELANOMA CELLS TO T CELLS VIA MIR-222 AND ICAM1

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The blossom of immunotherapy in melanoma highlights the need to delineate mechanisms of immune resistance. Recently, we have demonstrated that the RNA editing protein, adenosine deaminase acting on RNA-1 (ADAR1) is down-regulated during metastatic transition of melanoma, which enhances melanoma cell proliferation and tumorigenicity. Here we investigate the role of ADAR1 in melanoma immune resistance. Importantly, knockdown of ADAR1 in human melanoma cells induces resistance to tumor infiltrating lymphocytes in a cell contact-dependent mechanism. We show that ADAR1, in an editing-independent manner, regulates the biogenesis of miR-222 at the transcription level and thereby Intercellular Adhesion Molecule 1 (ICAM1) expression, which consequently affects melanoma immune resistance. ADAR1 thus has a novel, pivotal, role in cancer immune resistance. Corroborating with these results, the expression of miR-222 in melanoma tissue specimens was significantly higher in patients who had no clinical benefit from treatment with ipilimumab as compared to patients that responded clinically, suggesting that miR-222 could function as a biomarker for the prediction of response to ipilimumab. These results provide not only novel insights on melanoma immune resistance, but also pave the way to the development of innovative personalized tools to enable optimal drug selection and treatment.

THE LATS2-P53 TUMOR SUPPRESSOR AXIS

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The Lats2 tumor suppressor is primarily recognized as a central kinase of the Hippo pathway, however evidence is accumulating for the involvement of Hippo pathway components in non-Yap/Taz effector outcomes. Our research has focused on crosstalk between Lats2 and the p53 pathway. Previously, we showed that the Lats2-p53 axis is critical for the maintenance of chromosome number integrity, activation of p53 in response to oncogenic stress and apoptosis of transformed cells. Cells surviving sustained oncogenic activity suppress Lats2 expression, diminish p53 induction and emerge with features of transformation, such as genomic instability. We are currently exploring other "non-canonical" functions of Lats2, in stem cell differentiation and in metabolic homeostasis. Together, our data suggest that restraining the activity of Lats2 may have multipronged implications in cell transformation and tumor progression.

HIGH THROUGHPUT SCREENING OF PRIMARY VERSUS METASTATIC ISOGENIC CELL LINES REVEALS A NOVEL ROLE FOR SNX18 IN MIGRATION OF MELANOMA CELLS

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Melanoma is a high-grade malignant tumor of pigment producing cells. Melanoma aggressiveness is partially measured through its proliferative, invasive, migratory and immune evasive qualities. These qualities vary even among genotypically identical melanoma, thus discriminate primary from metastatic lesions in terms of differential aggressive phenotype. MicroRNAs (miRNAs) are small non-coding RNAs that post-transcriptionally regulate mRNA molecules. Their deregulation has been shown to play an essential role in cancer development and progression. In order to reveal new players involved in the regulation of aggressive features we employed 3 high-throughput screens for the analysis of 2 isogenic melanoma cell lines which originated from a primary (WM-115) and metastatic (WM-266-4) lesions of the same melanoma patient. We identified 89 proteins (Welsh test, FDR threshold 0.05), 146 miRNAs and 1492 mRNAs that were differentially expressed (FC≥2) between these 2 cell lines. miRNAs potential targets were predicted (TargetScan & miRDB) and intersected with the differentially expressed proteins and mRNAs followed by literature screen and prioritization. Sorting Nexin18 (SNX18) protein level was ~6 fold down-regulated in the metastatic cell-line. Remarkably, no change in mRNA levels was observed. Moreover, 49 up-regulated miRNA were predicted to target SNX18. Over-expression of SNX18 in WM-266-4 cell line had no effect on their proliferation rate (XTT assay) but showed 2 fold decrease in migration kinetics as measured by Real-Time Cell Analysis assay (p-value=1.87E-25). Preliminary results show similar trend in A375 melanoma cell line. These results suggest a possible role for SNX18 in migration of melanoma cell lines. To the best of our knowledge this is the first report of SNX18 in context of cancer-related features. Finding novel molecular players and deciphering their mechanism of action might pave the way towards improved tools for future therapy and better understanding of the malignant transformation process.

EZH2 ACTS VIA NOTCH TO DRIVE INTRATUMORAL HETEROGENEITY IN BREAST TUMORS

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Human breast cancer is a heterogeneous disease; composed of moderate well-differentiated subtypes as well as aggressive undifferentiated subtypes each with distinct clinical behavior, and unique marker expression. Tumors of the “basal-like” subtype exhibit poor differentiation and aggressive clinical behavior, yet it is currently unclear what factors drive this differentiation state and no targeted therapy currently exists to treat these malignancies. The central aim of our study is to uncover regulatory genes that determine the subtype identity of breast cancer cells, and thereby their aggressive traits. We have identified the existence of a subpopulation of cells with progenitor-like features, found in basal-like cell lines and patient tumor samples, and marked by co-expression of markers of both lineages of the normal breast – thus termed “bi-lineage” cells. We found that this population is maintained by EZH2 both in vitro and in vivo, via the regulation of the Notch pathway and cell-replication decitions. We are currently working to further characterize the presence and function of the bi-lineage cells in tumor initiation and progression and are working to dissect the regulatory networks that govern the basal-like differentiation state. To do so we are conducting a broad-scale shRNA screen, based on the tools we have developed thus far, to detect additional functional regulators of the basal-like state.

REGULATION OF COMPLEMENT-DEPENDENT CYTOTOXICITY BY MICRORNAS

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Insertion of the C5b-9 membrane attack complex (MAC) into the plasma membrane of target cells can cause rapid cell death or, at sublytic doses, trigger diverse cellular signals. For protection, cells eliminate the MAC from their surface by endocytosis, through caveolae, and by ectocytosis, in a process regulated by the mitogen-activated protein kinase ERK and by mortalin, the mitochondrial heat shock protein 70. In addition, cells regulate MAC activation and assembly by using the membrane complement regulatory proteins CD46, CD55 and CD59. As we have shown, modulation of mortalin expression level had a major impact on cell sensitivity to complement-dependent cytotoxicity (CDC); overexpression was protective and knockdown enhanced sensitivity. Recently, we have examined 3 microRNAs (miRs), miR-200b, miR-200c and miR-217, that were predicted to regulate mortalin protein expression and examined their contribution to complement resistance. Enhanced expression of miR-200c or miR-217 in K562 cells lowered the expression level of mortalin mRNA. In contrast, miR inhibitors specific for miR-200b, miR-200c or miR-217 enhanced mortalin mRNA level. Surprisingly, these miR modulators had no significant effect on total mortalin protein levels. Metabolic labeling analysis demonstrated that the cells increased mortalin protein synthesis in response to reduction in mortalin mRNA level. Cells overexpressing miR-200c or miR-217 showed reduced sensitivity to CDC while inhibition of miR-200c and miR-217 enhanced cell death. miR-200c overexpression reduced MAC deposition and enhanced C9 removal from the cells. Overexpression of miR-200c or miR-217 also enhanced expression of CD46 and CD55 but not of CD59. Furthermore, MAC-induced mortalin translocation to the plasma membrane and release from the cells was observed in cells over-expressing miR-200c. This study has identified miR-200b, miR-200c and miR-217 as regulators of mortalin, CD46 and CD55 expression and thus as regulators of cell resistance to complement-dependent cytotoxicity. Cell exposure to a sublytic dose of complement was shown to increase expression of miR-200b and miR-200c, thus suggesting that complement MAC exerts a feedback regulatory effect on these miRs level.

GSK-3 REGULATION OF AUTOPHAGY/LYSOSOME PATHWAY PROMOTES PRO-CANCER ACTIVITY

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The role of Glycogen synthase kinase-3 (GSK-3) in regulating cancer cell growth is not completely understood, and some studies reached opposite conclusions regarding the role of GSK-3 in promoting neoplasia. In this study we examined possible relationships between GSK-3 with cancer cell growth and metabolic regulation. We examined the regulation of the mammalian target of rapamycin complex 1 (mTORC1), and autophagy/lysosome pathway by GSK-3. We used MCF-7 human breast cancer cells as our model system. We found that GSK-3 is a positive regulator of mTORC1. Subsequently, GSK-3 suppressed autophagy as indicated by increased levels of p62/SQSTM1 and LC3II that reflected increased amount of autophagosomes. In addition, GSK-3 suppressed autophagic flux manifested by reduced lysosomal acidification. We further found that lysosomal acidification is reduced in MCF-7 cells: the cells expressed high levels of p62/SQSTM1 and increased amount of autophagosomes as pronounced by increased LC3II levels as compared to non-cancer MCF-10 epithelial cells. Subsequently, treatment with various GSK-3 inhibitors restored lysosomal acidification, enhanced autophagic flux and inhibited mTORC1. GSK-3 inhibitors inhibited MCF-7 cell proliferation.
Altogether, we show that GSK-3 suppresses autophagic activity via mTORC1 and regulation of lysosomal activity. In respect to metabolic effect, Rotenone that inhibits mitochondria function, reduced lysosome acidification which was rescued by GSK-3 inhibitors indicating mechanisms dependent on ATP supply. Taken together, our study places GSK-3 as a key regulator coordinating cellular homeostasis. GSK-3 inhibitors may be useful in targeting mTORC1 and lysosomal acidification for cancer therapy.

REGULATION OF CEACAM1 PROTEIN EXPRESSION IN BRAF-MUTANT HUMAN METASTATIC MELANOMA CELLS

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Malignant melanoma is among the most aggressive types of human cancers and is responsible for 80% of skin cancer–related deaths worldwide. BRAF is a key component of MAPK pathway, which is involved in the control of cell growth, proliferation and migration. BRAF is mutated in 50%-70% of melanoma cases, with V600E responsible for more than 90% of the cases. Vemurafenib, FDA approved BRAF inhibitor, achieves high and rapid response rate. However, secondary resistance develops in all cases within varying periods of time. CEACAM1 is a transmembrane glycoprotein that protects melanoma cells from immune attack by NK and T cells. CEACAM1 expression in primary melanoma lesions strongly predicts the development of metastasis and poor survival. Our results show strong correlation between BRAF mutation status and CEACAM1 expression in melanoma cells derived from patients. Cells treated with MAPK inhibitors (MAPKi) showed down regulation in CEACAM1 expression, only among the BRAFV600E melanoma lines in a dose- and exposure time- dependent manner. Furthermore mRNA amount of CEACAM1 decreased following MAPKi treatment, suggesting a transcriptional regulation. In parallel, Vemurafenib-resistant cell system showed an increase of membrane CEACAM1 expression and mRNA amount, compared to control. Investigation of CEACAM1 transcription regulation show significant reduction in CEACAM1 promoter (pCEACAM1) activity following treatment with MAPKi. ETS1, a downstream effector of the MAPK pathway, was identified as a regulator of the promoter of CEACAM1. Our results indicate a reduction of pCEACAM1 activity following ETS1 binding site deletion from pCEACAM1. ETS1 over expression enhanced the activity of CEACAM1 promoter and mutant ETS1T38A showed dominant negative effect. Thus we hypothesize that CEACAM1 is regulated at the transcriptional level through MAPK pathway activated by BRAFV600E mutation via ETS1. Finally, CEACAM1 down-regulation by MAPKi rendered the cells more sensitive to immune attack. These results provide a new view on a potential immunological mechanism of action of MAPKi in melanoma, as well as on the aggressive phenotype observed in drug-resistant cells.

THE UBIQUITIN LIGASE E6AP UBIQUITYLATES β-CATENIN AND REGULATES B-CATENIN STABILITY AND TRANSCRIPTIONAL ACTIVITY

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The E6AP ubiquitin ligase catalyzes the ubiquitylation and proteasomal degradation of several cellular proteins. Recently, we have shown that E6AP can stabilize β-catenin and that this activity is enhanced by the human papillomavirus (HPV) E6 oncoprotein. In the present study we show that E6AP interacts with β-catenin and ubiquitylates it in a nonproteolytic manner, through atypical ubiquitin linkage that involves K63, K29 and K11, but not K48. The Ubiquitylation of β-catenin by E6AP is specific, it requires the ubiquitin ligase activity of E6AP and is independent of the phosphorylation of β catenin by GSK3β and activity of the "β-catenin destruction complex". E6 is not involved in the E6AP mediated ubiquitylation of β-catenin. Interestingly, the E6AP-induced stabilization and activation of β-catenin/TCF transcription, are absolutely dependent on the activity GSK3β, the susceptibility of β-catenin to GSK3β phosphorylation and the ubiquitin ligase activity of E6AP. Collectively, our studies uncover a role for E6AP in regulation of β-catenin ubiquitylation, stability and transcriptional activity, raising the possibility that the nonproteolytic ubiquitylation of β-catenin by E6AP may be a necessary but not sufficient for stabilizing β-catenin and stimulating its transcriptional activity.

SIMILARITIES BETWEEN C5B-9-INDUCED NECROSIS AND TNF-INDUCED NECROPTOSIS: THE RIP1 AND RIP3 KINASES

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Upon insertion of complement C5b-9 complexes into cell membranes, cells undergo a rapid process of programmed necrosis. Only few of the intracellular molecular events activated by C5b-9 and regulate life and death decisions have been identified. The receptor-interacting protein kinases RIP1 and RIP3 are essential players in necrotic cell death, named also necroptosis, activated upon binding of TNF-α to TNF-receptor 1 and of LPS to toll-like receptor. We have investigated the involvement of RIP1 and RIP3 in C5b-9-induced programmed necrosis. The effects of necrostatin-1 (Nec-1), an inhibitor of RIP1 kinase activity, and necrosulfonamide (NSA), a RIP3 inhibitor, on cell death were tested. Nec-1 and NSA markedly inhibited complement-dependent cytotoxicity (CDC) of K562 erythroleukemia cells, BT474 breast cancer cells and HT-29 colon carcinoma cells. Mouse fibroblasts lacking RIP3 were less sensitive to CDC than wildtype fibroblasts. In addition, Nec-1 reduced CDC of wildtype but not of RIP3-knockout fibroblasts. Knocking down RIP3 expression in K562 cells with shRNA also desensitized them to CDC. In contrast, overexpression of RIP3 by transfection of a RIP3 plasmid, but not of a kinase dead mutant of RIP3, enhanced CDC. Fluorescence Resonance Energy Transfer (FRET) and co-immunoprecipitation analyses demonstrated binding of RIP1 to RIP3 in K562 cells treated with a sublytic dose of C5b-9. Analysis under a confocal microscope showed co-localization of C5b-9 with RIP3 at the cell membrane and with RIP1 in the cytoplasm. Direct binding of RIP1 or RIP3 from K562 cell extracts to purified human C9 were demonstrated by a cell-free binding assay. Our results demonstrate that complement C5b-9 induces a RIP1/RIP3-dependent necrotic cell death in human and murine cells. They provide a deeper insight into the process of complement-dependent cytotoxicity and will promote identification of novel modifiers of CDC for intervention in autoimmunity and cancer immunotherapy.

ADAR1-MEDIATED REGULATON OF MELANOMA INVASION

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The main RNA-editing enzyme, Adenosine Deaminase Acting on RNA-1 (ADAR1), is silenced in many metastatic tumors, including melanoma. We have recently shown that ADAR1 suppresses several cancer features, as its downregulation alters cell morphology, facilitates cell-cycle, proliferation, and dramatically enhances the tumorigenicity in-vivo. We further demonstrated that ADAR1 controls the expression of >100 microRNAs, which regulate hundreds of genes that account for the observed phenotype. Cutaneous melanoma is a highly metastasizing neoplastic disease, and its malignant potential has been previously associated with integrin beta-3 (ITGB3) expression, a known oncogene, strongly linked to the acquisition of invasive properties of many tumors. However, only little is known about the regulation of ITGB3 expression in cancer cells. ITGB3 is upregulated during the transition from dysplastic nevi to tumorigenic melanomas, inversely to the substantial reduction in ADAR1 expression. We show in several cell lines that silencing of ADAR1 directly enhances melanoma cell invasiveness and ITGB3 expression. The enhanced invasion is corrected when ITGB3 is blocked with monoclonal antibodies. Experiments with a series of melanoma cell lines transfected with wild type or catalytically inactive ADAR1 mutants show that this phenomenon is independent of RNA-editing. Mechanistically, we found that ADAR1 controls ITGB3 expression both at the post transcriptional and transcriptional level, via miR-30a and miR-22 and PAX6 transcription factor, respectively, which are described here as direct regulators of ITGB3. The novel ADAR1-dependent and RNA-editing-independent regulation of invasion presented here, mediated by ITGB3, as well as the ADAR1-controlled regulation of ITGB3 expression, strongly points on a central involvement of ADAR1 in cancer progression and metastasis. These findings provide novel insights on the process of cancer development with potential implications for future translational medicine.

ASPARTATE METABOILSM LINKS THE UREA CYCLE WITH NUCLEIC ACID SYNTHESIS TO PROMOTE CANCEROUS PROLIFERATION

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Argininosuccinate synthase (ASS1) is a urea cycle cytosolic enzyme that conjugates aspartate transported across the mitochondria and citrulline. In the liver, this is a critical step in conversion of nitrogenous waste to urea, whereas in most other tissues, it is the penultimate step in arginine synthesis. Citrullinemia is a urea cycle disorder caused by germline mutations that lead to decreased flux through ASS1. Citrullinemia type I (CTLN I) is caused by ASS1 deficiency and citrullinemia type II (CTLN II) is caused by deficiency in mitochondrial aspartate transporter citrin. In contrast to the established role of ASS1 in ureagenesis, it was found to be somatically silenced in multiple cancers for which the purpose is unknown. Whereas ASS1 silencing renders the tumors auxotrophic for arginine, we hypothesized that down-regulation of ASS1 has an arginine-independent survival effect by redirecting of aspartate towards pyrimidine synthesis. Supported by computational modeling and using multiple methodologies including studies of fibroblasts from patients with CTLN I and CTLN II, cancer cells, clinical data, robust informatics analysis of multiple tumors, we show that ASS1 is a key regulator of the mitochondria-derived aspartate flux. Silencing of ASS1, leads to preferential diversion of aspartate away from the synthesis of arginine and urea to pyrimidine synthesis. Decreasing aspartate flux to pyrimidine synthesis by either expressing ASS1 in cancer cells that have endogenous silencing, or by blocking the transport of aspartate through the mitochondrial membrane by inhibiting Citrin, decreases cell proliferation due to decreased nucleic acid synthesis. Our results demonstrate that ASS1 silencing is a novel mechanism to support nucleic acid synthesis in cancers and provides the first metabolic link between the urea cycle enzymes and pyrimidine synthesis.

NK-MEDIATED IMMUNE SURVEILLANCE OF SENESCENT CELLS

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NK-cell-mediated immune surveillance of senescent cells is one component of the coordinated process whereby cellular senescence limits the extent of liver fibrosis and facilitates wound repair. Recent studies also suggest that senescent cell recognition and clearance by immune cells promotes tumor regression in established tumors. Our results demonstrate that senescent cells are preferentially recognized and killed by NK cells. We show that DNA damage-induced senescent fibroblasts specifically upregulate the NKG2D immune ligands MICA, ULBP1 and ULBP2. Furthermore, we demonstrate that ERK signaling contributes to this immune ligand upregulation, independent of p53 and NFκB. Importantly, the upregulation of MICA and ULBP2 on the cell surface of senescent cells is pivotal for the NK-mediated recognition, since interference with the receptor-ligands interactions inhibits the NK-mediated clearance both in vitro and in vivo to limit liver fibrosis and facilitate tissue repair. Lastly, we demonstrate that the granule exocytosis pathway, but not the death receptor pathway, is necessary for the specific killing of senescent fibroblasts and stellate cells by NK cells and participates in the clearance of senescent activated HSCs to limit liver fibrosis. We suggest that this pathway bias is mediated by the upregulation of Dcr2, a decoy receptor for the death ligand TRAIL, by senescent cells. Therefore, NK-cell-mediated recognition of senescent cells via NKG2D ligands and killing through NKG2D signaling and the granule exocytosis pathway contributes to immune surveillance of senescent cells in vitro and in vivo.

DEFERASIROX INDUCES APOPTOSIS OF CD34+/CD38− LEUKEMIC STEM CELLS

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Acute myeloid leukemia (AML) mortality rate is high due to poor treatment response and disease relapse. Leukemia stem cells (LSCs) are believed to be responsible for this since they are insensitive to current therapeutic agents. There is an urgent need for new therapeutic strategies aimed at eradicating AML stem cells. HIF1α is essential for LSC maintenance and targeting HIF1α selectively eliminates LSC. Deferasirox is an iron chelator used to reduce chronic iron overload. In this study, we aim to elucidate the ability of deferasirox to induce apoptosis and to target HIF1α in AML LSCs. CD34+CD38- LSC-like cells isolated from the leukemic CD34+ KG1a cell-line exhibited increased sensitivity to deferasirox with an IC50 of 1.3μM compared to 8.9μM for the more mature CD34+CD38+ cells. These CD34+CD38- LSC-like cells were less sensitive to ARA-C compared to the CD34+/CD38+ cells. Deferasirox was >2-fold more efficient in inducing apoptosis in the CD34+CD38- cells, compared to the CD34+CD38+ cells (74+7.2% 32+6.2%, respectively). Deferasirox demonstrated a synergistic effect with ARA-C on both CD34+CD38− and CD34+CD38+ KG1a fractions. Similar results were observed with CD34+CD38-CD123+ LSCs and CD34+CD38+CD123+ progenitor cells isolated from AML patients bone marrow samples. AML patient LSCs were 2-fold more sensitive to deferasirox treatment showing 62+15% apoptosis compared to only 34+9% in leukemic progenitor cells. The increased cell death was accompanied by an increment of reactive oxygen species (ROS) levels which was more prominent in the LSCs. Furthermore, deferasirox enhanced the cytotoxic effects of ARA-C on both the LSCs and the leukemic progenitor cells. These data indicate that deferasirox is cytotoxic to AML cells; however, it is more specific to AML stem cells. Since deferasirox is an NFkB inhibitor which regulates HIF1α, and since HIF1α is essential for AML stem cell maintenance, we studied the effect of deferasirox on HIF1α expression. HIF1α expression was downregulated between 40-60% in CD34+CD38- KG1a LSC-like cells and in CD34+CD38- CD123+ cells isolated from an AML patient while it was upregulated in CD34+CD38+ KG1a cells following deferasirox treatment. These data hint that deferasirox selectively targets AML-LSCs. We describe a novel anti-LSC property of deferasirox, originally developed as an iron chelator. Clinically relevant concentrations of deferasirox were cytotoxic in vitro to AML cells but even more potent against LSCs. We believe that deferasirox exerts its cytotoxic effect, at least partially, by downregulating HIF1α levels in LSC. Pending further characterization, deferasirox can be considered a potential therapeutic agent for eradicating LSCs.

MIR-451 INHIBITS CELL GROWTH BY TARGETING NAMPT IN ACUTE LYMPHOBLASTIC LEUKEMIA

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We have previously shown that microRNA-451 (miR-451) was significantly decreased in pediatric precursor B-cell acute lymphoblastic leukemia (ALL) patient samples and this downregulation was associated with a high relapse rate. miR-451 was already reported as a tumor suppressor gene also in other types of cancer, however, its clinical significance and molecular mechanism in ALL is still not understood. In this study we explored the contribution of miR-451 to ALL progression using a mice xenograft ALL model and revealed its direct functional target in ALL. Tumor growth was significantly reduced in mice injected with cells over-expressing miR-451, supporting the role of miR-451 as a tumor suppressor gene in ALL. Based on bioinformatic analysis we found that nicotinamide phosphoribosyltransferase (NAMPT) is a potential target of miR-451. NAMPT is the rate-limiting enzyme in the NAD+ biosynthetic pathway. As NAD metabolism is usually high in disease conditions, it has been proven that NAMPT is over expressed in various cancers and inflammatory disorders. We demonstrated the inverse association between miR-451 expression levels and NAMPT protein levels in ALL cell line. Using luciferase reporter analysis we proved that this association is a result of a direct binding of miR- 451 to NAMPT. Then, we used FK866, a potent NAMPT inhibitor, which causes the depletion of intracellular NAD+ levels and ultimately induces apoptosis. We identified that cells expressing low levels of miR-451 resulting in increased NAMPT expression were significantly more sensitive to treatment. These observations demonstrate that miR-451 may play an important role in ALL progression via NAMPT regulation. Thus, miR-451 expression levels, could be used as a biomarker for the identification of patients that could benefit from treatment with NAMPT inhibitory agents.

HEPARANASE ENHANCES TUMOR GROWTH AND CHEMO-RESISTANCE BY AUGMENTING AUTOPHAGY

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The endo-β-glucuronidase heparanase is the only enzyme in mammals capable of cleaving heparan sulfate, activity that is highly implicated in tumor metastasis, tumor angiogenesis and inflammatory disorders. Heparanase is secreted as a latent protein that gets internalized and subjected to proteolytic processing and activation in lysosomes. The role of heparanase under normal conditions has not been revealed yet. Here, we provide evidence that heparanase resides within autophagosomes. Moreover, autophagy extent was decreased in heparanase knockout mice and cells, and was augmented significantly by heparanase over expression in transgenic mice and cancer-derived cells, evident by immunofluorescent staining, immunoblotting and electron microscopy. Furthermore, we show that the pro-tumorigenic properties of heparanase are mediated, at least in part, by its pro-autophagic function. This is demonstrated by decreased cell survival, colony number and size, and tumor xenografts growth by inhibitors of the lysosome (chloroquine) and heparanase (PG545) alone and in combination. We also show that heparanase over expressing cells are more resistant to stress and chemotherapy, resistance that also results from increased autophagy and was reversed by chloroquine. Collectively, the results indicate, for the first time, a role for endogenous heparanase in modulating autophagy that endows cancer cells with growth advantage and chemo resistance.

SENSITIZING B- AND T- CELL LYMPHOMA CELLS TO PACLITAXEL/ABRAXANE-INDUCED DEATH BY AS101 VIA INHIBITION OF THE VLA-4-IL10-SURVIVIN AXIS

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Cancer cell resistance to chemotherapy is a major concern in clinical oncology, resulting in increased tumor growth and decreased patient survival. Manipulation of apoptosis has emerged as a new therapeutic strategy to eliminate cancer cells. The focus of this study resides within a novel approach to target survivin, an integrator of both cell death and mitosis. This protein plays a pivotal role in the resistance of tumors to chemotherapy, especially to paclitaxel. The data herein demonstrate an indirect repression of survivin in both B- and T-cell lymphoma and human NHL by the nontoxic tellurium compound, AS101 [ammonium trichloro(dioxoethylene-o,o')tellurate], via inhibition of tumor autocrine IL10-STAT3-Survivin signaling. As a result of survivin abrogation, sensitization of lymphomas to paclitaxel or to Abraxane, the new albumin-stabilized nanoparticle formulation of paclitaxel, occurs both in vitro and in vivo. Importantly, inhibition of lymphoma cell IL10 secretion is mediated by inactivation of the VLA-4 integrin, recently shown to be an important target of AS101. This activity is followed by inhibition of the PI3K-AKT axis that mediates IL10 suppression. Because a wide variety of lymphomas and other tumor types express VLA-4 and secrete IL10 in an autocrine manner, inhibition of survivin with a small nontoxic agent has vast clinical significance in modulating chemosensitivity in many tumor types. Mol Cancer Res. 2015 Mar;13(3):411-22

P53 MEDIATED EPIGENETIC REGULATION FOLLOWING GLUCOSE STARVATION

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DNA methylation is an epigenetic mechanism that affects gene expression. Disruption of this modification leads to a variety of pathologies, including cancer. In recent years, there are several works showing the crosstalk between the tumor suppressor p53 and enzymes that are involved in catalyzing or modifying DNA methylation. However, the concept of epigenetic repression by p53 is rather understudied. Glucose is one of the most important metabolites, used as the major source for cellular energy. Also it is well established that cancer cells are using higher amounts of glucose, both for their increased energy demands and for anabolic needs associated with the high demand of newly synthesized macromolecules for cell proliferation. Therefore, we focused on glucose starvation as an activating stress signal for p53. We show that in response to glucose starvation p53 is activated to arrest cellular proliferation and a binding between p53 and a DNA methyltransferase enzyme is observed. Also, we have indications that p53 repression of cellular proliferation is partially due to DNA methylation. Finally, our data demonstrates the p53 can utilize the DNA methylation machinery to affect the temporal glucose stress response.

DECONSTRUCTING THE INTRACELLULAR EVENTS LEADING TO ENHANCED MELANOMA CELL PROLIFERATION BY THE CEACAM1 PROTEIN

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Expression of the CEACAM1 protein in primary cutaneous melanoma lesions predicts the development of metastasis and poor survival. We have previously identified CEACAM1 as a new immune checkpoint and generated a novel blocking antibody as a potential immunotherapeutic agent. Recently, we found that CEACAM1 facilitates cell cycle and net proliferation of melanoma cells, as well as in vivo tumorigenicity in a xenograft animal model. These results indicate on a dual role of CEACAM1 in melanoma. CEACAM1 acts as immune modulator by transferring inhibitory signals to lymphocytes as a result of trans-homophilic interactions. In order to understand if the same interactions lead to melanoma enhanced proliferation, the interactions were abrogated in different ways: blocking antibody and point mutation of amino acids involved in the interaction (arginine 43 and glutamine 44 were changed to serine and leucine). Proliferation assay showed that this specific interaction doesn't involve in CEACAM1 effect on proliferation. CEACAM1 is a transmembrane glycoprotein contains extracellular, transmembrane and intracellular domain. Previously we showed that the entire protein is crucial for proliferation. In order to deconstruct the intracellular events that lead to enhanced proliferation, we created a series of point mutations in CEACAM1. The constructs were stably transfected into CEACAM1-negative melanoma cells and tested for proliferation. Remarkably, mutation analysis identifies the ITIM residues (tyrosine 493 and tyrosine 520) as well as serine 508 to hold a critical role in CEACAM1-mediated proliferation. Indeed, triple mutation entirely abrogates the proliferative effect of CEACAM1 on melanoma cells both in vitro and in a xenograft animal model. Accordingly, the role of SHP phosphatases was studied. Co-IP assays showed more interactions of SHP-1with CEACAM1 in melanoma cells transfected with wild type compare to triple CEACAM1 mutant, unlike SHP-2 that showed no difference. The results of the Co-IP assays were supported by knockdown assays with siRNA that showed only the effect of SHP-1 on proliferation but not SHP-2. This implies that the effect is mediated by SHP-1. Finally, we found that CEACAM1 affect two important cell cycle effectors: mir-34 and p21. Cells transfected with CEACAM1 showed lower levels of p21 (RNA and protein) and mir-34 (RNA) compare to cells with mock transfection. In conclusion, we propose a new pathway that facilitating melanoma cell proliferation, mediated by recruitment of SHP-1 to key residues in CEACAM1 protein and then, down regulation of cell cycle effectors like mir-34 and p21.

DORMANCY, ANGIOGENESIS, METASTASIS

HIGHLY EFFECTIVE HEPARANASE-BASED THERAPY FOR MESOTHELIOMA

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Malignant mesothelioma is a highly aggressive form of cancer that develops from cells of the mesothelium - the protective lining that covers many internal organs of the body. It has a poor prognosis because of the lack of markers for early diagnosis, and resistance to conventional therapies, underscoring the need for novel treatments. Mesothelioma tumors express high levels of heparanase, the sole mammalian endoglycosidase degrading heparan sulfate (HS) side chains of HS-proteoglycans in the extracellular matrix (ECM) and cell surface. Heparanase activity facilitates cell invasion and releases growth- and angiogenesis-promoting factors that are stored as a complex with HS in the ECM and tumor microenvironment. We demonstrate that heparanase is expressed and enzymatically active in several pleural mesothelioma cell lines. Heparanase gene silencing attenuates cell invasion in vitro and tumor xenografts growth in-vivo. Likewise, heparanase inhibitor (PG545) attenuated cell invasion and anchorage independent growth of mesothelioma cell lines. Moreover, PG545 reduced mesothelioma tumor xenografts growth by inhibiting angiogenesis and Akt signaling. Strikingly, PG545 significantly increased the survival of mice implanted orthotopically with mesothelioma cells. In conclusion, heparanase play an important role in mesothelioma tumor progression, thus encouraging further development of heparanase inhibitors (e.g. PG545) for this rare and deadly malignancy.

LOXL2: A NOVEL REGULATOR OF TUMOR DORMANCY AND CELLULAR PLASTICITY

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Breast cancer that recurs, as metastatic disease many years after primary tumor resection and adjuvant therapy appears to arise from tumor cells that disseminated early in the course of the disease but did not develop into clinically apparent lesions. These long-term surviving, disseminated tumor cells maintain a state of dormancy and are resistant to conventional therapies that target actively dividing cells. The mechanisms responsible for maintaining the survival and outgrowth of dormant tumor cells remain largely unknown. We present here for the first time the role of LOXL2 in the switch of disseminated dormant tumor cells to proliferative growth. Dormant MCF-7 tumor cells ectopically expressing LOXL2 (MCF-LOXL2) transitioned from tumor dormancy to metastatic growth in a 3D model system that models tumor dormancy. Expression of LOXL2 in the dormant MCF-7 cells promoted epithelial mesenchymal transition (EMT) and cancer stem cell (CSC)-like phenotype of the cells. The flow sorted CSC-like cells mediated the transition from tumor dormancy to metastatic growth in the 3D BME system. Whereas, the non-cancer stem cells expressing LOXL2 remained dormant. Intriguingly, the outgrowth of the dormant tumor cells was followed by loss of luminal markers such as GATA3 and estrogen receptor alpha (ERa). Furthermore, nuclear expression of LOXL2 was required for EMT, acquisition of CSC-like phenotype and their outgrowth. Similarly, conditional silencing of LOXL2 expression in MCF-7 cells inhibited their EMT and significantly reduced their CSC like phenotype. Last, we demonstrate that conditional expression of endogenous LOXL2 in MCF-7 cells by signals from the tumor microenvironment such as hypoxia promoted EMT thus acquiring CSC -like phenotype, and Knockdown of LOXL2 inhibited this acquisition. Hence, these findings may have profound clinical importance given that breast cancer recurrence if often associated with resistance to anti-estrogen therapy and is common in luminal breast cancer patients. Therefore, the results of our overarching research may in the future identify LOXL2 as a potential therapeutic target to prevent and treated breast cancer recurrence.

BRAIN METASTASIS: THE IMPACT OF SURGICAL STRESS, IMMUNE STIMULATION, AND NK CELLS

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Brain metastasis (BrM) have poor prognosis, and prophylactic approaches are scarce. Surgical stress responses have been shown to promote metastasis in peripheral organs through their immune-suppressive impacts and through direct effects on the malignant tissue and host physiology. However, BrM have not been studied in these respects, and the unique brain immune milieu, blood supply, and BBB, may react differently to such neuroendocrine challenges. Thus, we studied the effect of laparotomy, the role of NK cells, and CpG-C immune-stimulation – a TLR-9 agonist having minimal adverse effects in humans – in early stages of brain and lung metastasis. Two syngeneic animal models were used: 3LL/D122 in C57BL/J6 mice, and MADB106 mammary adenocarcinoma in F344 rats. Tumor cells were injected either through the tail vein or employing a novel internal carotid injection approach we have developed, which generates BrM with minimal injection-related interferences to brain blood flow, yet ensuring that all injected cells reach brain circulation. Employing both models and inoculation approaches in naïve and NK-depleted animals, our results indicate that NK cells surprisingly have no impact on BrM, while profoundly controlling lung metastases. On the other hand, laparotomy significantly enhanced brain and lungs tumor infiltration, and CpG-C reduced it, overcoming the effects of laparotomy. Thus, surgery is a significant risk factor for BrM through yet unknown mechanisms, and CpG-C treatment may be used prophylactically in cancer patients.

αvβ3 INTEGRIN-TARGETED PGA-PACLITAXEL NANOCUNJUGATE AS NEOADJUVANT TREATMENT PREVENTING METASTASIS DEVELOPMENT OF BREAST CANCER

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Prevention of metastasis growth presents an unmet clinical need. Anti-angiogenic therapy might provide an alternative way to manipulate cancer, yet it did not materialize into clinical practice. Therefore, combination of anti-angiogenic therapy with cytotoxic therapy directed to the metastatic cancer cells, offers a promising therapeutic approach. Paclitaxel (PTX) is a widely-used potent cytotoxic drug, however, its use is limited by severe side effects, caused by the hydrophobic drug and its solubilizing agents. We designed and synthesized a novel polyglutamic acid (PGA)-PTX-E-[c(RGDfK)2] nano-sized conjugate. Polymer conjugation converted PTX to a water-soluble macromolecule, which passively targeted the tumor tissue exploiting the enhanced permeability and retention (EPR) effect, while extravasating via the leaky tumor neovasculature. PGA is enzymatically-degradable by cathepsin B, leading to PTX release. The E-[c(RGDfK)2] serves as an additional active targeting to αvβ3 integrin. Integrins play a key role in cell matrix interactions. The highly restricted integrin αvβ3 is overexpressed on tumor endothelial and some epithelial cells, during tumor growth, invasion, and metastasis. PGA-PTX-E-[c(RGDfK)2] displayed a potent anti-angiogenic therapy. Mice bearing orthotopic mammary tumors demonstrated preferential tumor accumulation of the RGD-bearing conjugate, leading to enhanced antitumor efficacy and a marked decrease in toxicity compared with free PTX[1]. We developed a mouse model that mimics the clinical setting, of mammary cancer metastases following resection of the primary tumor. Integrin αvβ3 expression was detected on mCherry-labeled MDA-MB-231 mammary adenocarcinoma cells while circulating in the bloodstream of mice. Using this model, PGA-PTX-E-[c(RGDfK)2] conjugate prevented breast cancer metastases formation following surgical removal of the primary tumor. Taken together, our conjugate alters the pharmacokinetics of free PTX. Inclusion of an active targeting moiety to integrin expressing-cells, have the potential to prevent breast cancer metastasis development as an anti-angiogenic and anticancer adjuvant therapy. 1. Eldar-Boock et al. Biomaterials. 2011;32(15):3862-74

MAGNETIC RESONANCE DETECTION OF LYMPHATIC BREAST CANCER METASTASIS IN A XENOGRAFT MODEL BY HYPERPOLARIZED 13C-PYRUVATE

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Breast cancer is the most commonly diagnosed cancer among women. Besides significant progress in therapeutic strategies, the prognosis of metastatic breast cancer (MBC) is poor with a five years survival rate around 25%. An early event of MBC is the dissemination of tumor cells in the lymphatic system to form metastasis in lymph nodes (LN). Thus, a key challenge is the ability to non-invasively detect and characterize the metastatic LN in order to improve patient management. The emergence of dissolution dynamic nuclear polarization (DNP) technique in combination with magnetic resonance imaging or spectroscopy (MRI/MRS) has opened new avenues for real-time imaging of the metabolism. This cutting-edge method enables the detection of a hyperpolarized metabolite and its metabolic products and therefore has the sensitivity to identify tissue with abnormal metabolism such as tumors. The interest for its clinical application is even larger for the detection and the monitoring of the metastatic process. While the metabolic reprogramming of cancer cells is well identified, little is known about the metabolic profile of the disseminated tumor cells leading to metastasis. In this study we have used DNP-enhanced 13C MRS method to probe the metabolism of the primary tumors and metastatic lymph nodes in a breast cancer xenograft model using 1-13C hyperpolarized pyruvate. MDA-MB-231 human breast cancer cells were injected into the mammary fat pad of immunodeficient nude mice. The primary tumor and the contralateral inguinal LN were monitored by 1H MRI at 4.7T. Arrays of 13C MRS spectra were recorded on both sites by a 3mm 13C surface coil following the intravenous injection of hyperpolarized 13C-pyruvate. Histopathology confirmed the presence/absence of LN metastasis. Lactate production from injected pyruvate was observed in both tissues. The highest lactate/pyruvate signal was observed for a metastatic LN (1.14; n=1) while the mean lactate/pyruvate signal from the primary tumors and the non-metastatic LN were 0.50 (± 0.2, n=7) and 0.62 (± 0.2; n=6) respectively. The dynamic data also enabled the extraction of pyruvate-to-lactate rate constant (k-rate). The highest k-rate was observed for the metastatic LN (0.06 s-1), which was twice the mean of the ones calculated from primary tumor spectra (0.03 s-1; ± 0.008). This work, still under progress, presents an unprecedented effort to characterize the metabolism of both the primary tumor cells and their metastatic spread to regional LN, that once successful might be translated to a clinical application.

NOVEL METHODS FOR THE DETECTION OF OCCULT METASTATIC DISEASE IN THE CSF OF CHILDREN WITH MEDULLOBLASTOMA

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Medulloblastoma is the most common malignant brain tumor in children, and one of the deadliest. Patients with tumor dissemination have a low recovery rate. Moreover, imaging and cytology are not always sensitive enough to detect early metastatic spread. Given the importance of early metastatic disease detection and the lack of efficient clinical solutions, there is a need for developing new diagnostic tools. The purpose of this study is to combine bioinformatics with experimental approaches and fluorescence-lifetime imaging microscopy (FLIM) to ameliorate detection and the monitoring of minimal residual disease in the cerebrospinal fluid (CSF) in a personalized manner. DNA extracted from tumor and blood was analyzed using whole exome sequencing, to identify tumor specific chromosomal aberrations. In addition, cells from the CSF were studied for these aberrations, indicating the existence of metastatic cells. Slides with cells from the CSF were studied by DAPI nuclear staining and the fluorescence lifetime was analyzed using FLIM technology. FLIM from 9 patients revealed 3 populations of cells in the CSF: benign, inflammatory and metastatic. One of these patients was clinically thought to have a localized disease only. Our results exhibit the potential of identifying tumor specific structural variations and of FLIM analysis to improve the detection of occult metastatic disease in the CSF at diagnosis and during follow-up.

CELLULAR SENESCENCE IN HODGKIN'S LYMPHOMA

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Hodgkin's lymphoma (HL), a B cell originated malignancy of the immune system, is one of the most frequent lymphomas in the Western world. Although the rate of cure is high, about 20-35% of patients relapse, and about half of them eventually die of the disease or treatment-related late toxicities and secondary malignancies. Conceptually novel treatment strategies are thus needed, particularly for this category of patients. The malignant cells, called Hodgkin and Reed-Sternberg (HRS) cells, only make up 1-2% of the total tumor cellularity; the remaining mass comprising a mixed infiltrate population is thought to be recruited to the lymph node by HRS-driven pro-inflammatory signals. The origin of the pro-inflammatory signals remains elusive, and represents a critical gap in our knowledge of the pathogenesis of HL. We propose that a sub-population of HRS cells, which we call herein large RS cells, have characteristics of senescent cells, and, thus produce large amounts of inflammatory mediators (the so-called "senescence-associated secretory phenotype", or SASP). Senescence is characterized by permanent cell cycle arrest and loss of proliferative capacity, despite continued viability and metabolic activity. We show that the senescence markers the cell cycle inhibitors p16INK4a and p21Cip1 are expressed in large RS cells in all HL biopsies examined. Moreover, the large RS cells are negative for Ki-67 staining, demonstrating that these cells have ceased to proliferate. We also show that large, 'RS-like' cells in a HL-derived line, L428, stained for the characteristic senescence marker β-galactosidase (β-gal). Oxidative stress and chemotherapy increased the proportion of β-gal positive large RS cells. Furthermore, we show that the large RS cells secrete high levels of cytokines. We suggest that these senescent RS cells may be responsible, at least in part, for creating a pro-inflammatory microenvironment, promoting HL pathogenesis, and mediating chemo-resistance in relapsed disease. Understanding the pathways important for the establishment of senescence in HRS cells, as well gaining insight into targetable mechanisms for the eradication of these cells, will provide new therapeutic approaches for HL patients with recurrent or chemo-resistant disease.

COMBINED EFFECT OF MORINGA OLEIFERA AND IONIZING RADIATION ON THE SURVIVAL AND METASTATIC ACTIVITY OF PANCREATIC CANCER CELLS

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Background: We have reported previously the anti-tumor activity of Moringa Oleifera, grown in the Dead Sea valley, Israel. Radiotherapy is considered as an important treatment for various malignant tumors including pancreatic cancer. One of the principal mechanisms of radiation therapy efficacy is induction of apoptosis. Radio-resistance is a major obstacle in the cancer treatment. The enchantment of radio-responsiveness of tumors by using radio-sensitizers is suggested to be a promising strategy to improve radiotherapy efficacy. Aims - The goal of this study was to evaluate the anti-cancer activity of Moringa oleifera aqueous leaf extract (moringa) alone and in combination with radiation both in vitro and in vivo, and to investigate the underlying mechanism(s) of moringa anti-cancer effect.

Materials and Methods: The survival of PANC-1 cells was estimated using XTT and Clonogenic assays. The mode of the interaction between moringa and radiation in the combined treatment was analysed by Calcusyn software. The effect of moringa on cell migration and invasion was determined using standard Transwell cell migration and cell invasion assays. The effect of moringa on cell cycle and induction of apoptosis was tested using FACS analysis. The expression of pro-apoptotic and NF-kB related proteins was measured using Western blot analysis. Anti-tumor effect of moringa was evaluated in in vivo study using subcutaneous tumors generated by PANC-1 cells in athymic CD-1 nude mice. Immunohistochemistry of developed tumors was performed in order to detect the localization and the expression of proteins such as CD 31, Ki 67, P53 and BCL-2.

Results and Conclusions: The treatment of PANC-1 cells with moringa or radiation resulted in the dose dependent inhibition of cell survival evaluated by XTT and Clonogenic assays. The combination of moringa and radiation inhibited cell survival more significantly. According Calcusin analysis the combined effect was additive. Both moringa and radiation induced apoptosis and decreased migration and invasion of PANC-1 cells. The combined effect on the development of apoptosis in PANC-1 cells and their metastatic activity was more pronounced. Moringa treatment of tumor bearing mice resulted in the inhibition of tumor growth in a dose dependent manner. Further studies are ongoing to characterize the biological activity and the mechanisms of Moringa anti-cancer efficacy.

Keywords: Moringa oleifera, Radiation, Pancreatic cancer, PANC-1 cells, apoptosis.

ELUCIDATING THE EFFECT OF PHOTODYNAMIC TREATMENT IN COMBINATION WITH 3, 3' –DIINDOLYLMETHANE ON THE GROWTH OF HUMAN EPITHELIAL CANCER CELLS

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In current study we were elucidate the effect of photodynamic treatment (PDT) mediated by a promising photosensitizer mTHPC (Foscan) in combination with well-known phytochemical 3,3'- Diindolylmethane (DIM) on the growth of human head and neck squamous cell carcinoma (HNSCC) FaDu cell line. PDT is a regulatory-approved modality used for the treatment of various oncologic lesions. The most important aspects of PDT are the processes of light absorption and energy transfer. It involves a tumor localizing photosensitizer (PS) that induces cytotoxicity when activated by light of an appropriate wavelength. mTHPC is approved in Europe for the palliative treatment of head and neck cancers, it is a second generation PS that concerns to porphyrin derivate class. Indole-3-carbinol (I3C), a common phytochemical in our diet, it presents in all members of the cruciferous vegetable family. DIM is a major acid-catalyzed condensation product of I3C, which is produced in the stomach after consumption of cruciferous vegetables. DIM inhibits invasion and metastasis of nasopharyngeal carcinoma cells by regulation of epithelial mesenchymal transition (EMT). Key players of endotheliod characteristics of HNSCC that enable angiogenesis and invasion are Ve-cadherin (CDH5), Vimentin (VIM) and E-cadherin (CDH1). In present study under applied conditions, mTHPC mediated PDT in combination with Dim was found significantly more cytotoxic in comparison with either single treatment. Enhanced apoptotic cell death after the combined treatment in comparison with either single treatment was detected by Annexin-V/PI staining. Gene expression patterns were analyzed at 24h post-treatment by qRT-PCR. Significant down-regulation of CDH5 and subsequent up-regulation of CDH1 were observed following Dim treatment alone and mTHPC-combined treatment, while VIM exhibited no significant expression changes.

THE BETA SUBUNIT OF HEMOGLOBIN SUPPRESSES BONE MARROW AND LUNG METASTASIS

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Background: Metastatic disease may occur years or even decades after a successful treatment of the cancer patient. This disease progression is due to the propagation of micrometastases to clinically detectable metastasis. We previously showed that soluble factors derived from mouse lungs keep lung micrometastatic cells in check, inhibiting their viability and thus their progression to overt metastasis. Here we isolate and characterize the lung-derived inhibitory factor.

Methods: Reversed-phase HPLC followed by LC-MS/MS isolated and identified the lung-derived inhibitory factor to be the beta subunit of murine hemoglobin (HBB2). Synthesis of 14 peptide segments of 15 amino acids each of the beta subunit of human hemoglobin (HBB) enabled the identification of a short C-terminal region of HBB (designated Metox) as being responsible for the inhibitory activity.

Results: HBB2 and HBB exert growth arrest and apoptosis of human lung-metastasizing tumor cells in-vitro. In-vivo therapy experiments employing Metox which exerts growth arrest and apoptosis of tumor cells in-vitro, inhibits the development of adrenal neuroblastoma tumors in xenografted mice and the development of spontaneous lung and bone marrow metastasis.

Conclusions: In addition to its known functions, the beta subunit of hemoglobin operates as an innate anti-tumor resistance factor restraining the proliferation of cancer cells and keeping them in a state of dormancy. HBB2 is expressed by mouse lung cells and is up-regulated in mice bearing human neuroblastoma micrometastases. HBB may thus be developed into a novel drug and may serve as a biomarker signaling the presence of clinically undetectable micrometastasis.

UNIQUE MICRORNA EXPRESSION PATTERNS IN HUMAN MELANOMA METASTASES TO DIFFERENT ORGANS IN A XENOGRAFT MOUSE MODEL

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MicroRNA molecules emerge as a powerful novel tool for the understanding and diagnosis of melanoma progression. While the microRNA expression patterns of primary and metastatic melanoma were studied, no data prevail on the differences of microRNA expression between melanoma metastases to different organs. We decided to address this issue by using a NOD/SCID murine xenograft with A375 human melanoma. Serial injections of A375 cells to the heart yielded brain, lung, liver and bone metastases. Using the nCounter system and Nanostring technology, expression profiles of tissue (organ) specific microRNA were obtained. Exclusion of values ≤5-folds negative controls and of fold expression difference of <2 between tissues yielded a list of 14 microRNA with unique expression in one tissue as compared to the three others. The most prominent microRNA differences were up-regulation of has-miR-1246 in the brain, has-miR-204 in the bone and has-miR-4443 in the liver. These distinguished patterns of microRNA expression may help delineate the mechanisms underlying melanoma metastasis to different organs.

APOPROTEINS MODULATE TUMOR ANGIOGENESIS AND METASTASIS FORMATION VIA DIFFERENTIAL REGULATION OF VEGF RECEPTORS

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Angiogenesis correlates with the onset of tumor progression and is widely regarded as an attractive target for controlling cancer. Several years of study have uncovered key genetic players controlling the induction of tumor-related vessels. In contrast, the regulation of tumor angiogenesis by metabolic cues, has received much less attention. In particular, the putative role that lipoproteins might play in controlling tumor vascularization has remained controversial. Here we show that lipoprotein levels directly affect tumor-angiogenesis and metastasis formation. Using two genetic mouse models of hyperlipidemia, in combination with human cultured ECs we demonstrate that the protein components of lipoproteins-APOB100 and APOE-, and not their lipid moieties, control different features of endothelial cell behavior. Further we show that while increased levels of APOB100 lead to vessel normalization and drastic reduction of metastasis formation, Simvastatin treatment reverts these phenotypes restoring tumor-angiogenesis to normal levels. Mechanistically, we identify VEGFR1 and VEGFR2 as downstream targets of apoproteins within endothelial cells, and show that endothelial-specific deletions of these receptors in vivo, is sufficient to abolish the anti-angiogenic phenotypes induced by lipoproteins. Our results uncover a novel mechanism of regulation of tumor-angiogenesis and metastasis formation by apoproteins, and provide the first characterization of their downstream targets. On a broader sense these findings could aid in strategizing future anti-cancer therapy.

MACROPHAGES CONTRIBUTE TO METASTASIS IN TUMORS EXPOSED TO LOCAL RADIATION

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A major therapeutic obstacle in clinical oncology is intrinsic or acquired resistance to therapy, leading to subsequent relapse. We have previously shown that systemic administration of different cytotoxic drugs can induce a host response that contributes to tumor angiogenesis, regrowth and metastasis. Here we characterize the host response to local radiation, and its contribution to tumor progression and metastasis. We show that plasma from locally irradiated mice increases the migratory and invasive properties of colon carcinoma cells. Furthermore, locally irradiated mice intravenously injected with CT26 colon carcinoma cells succumb to pulmonary metastasis earlier than their respective controls. Consequently, orthotopically implanted SW480 human colon carcinoma cells in mice that underwent radiation, exhibited increased metastasis to the lungs and liver compared to their control tumors. The irradiated tumors exhibited an increase in the colonization of macrophages compared to their respective controls; and macrophage depletion in irradiated tumor-bearing mice reduces the number of metastatic lesions. Finally, the anti-tumor agent, Dequalinium-14, in addition to its anti-tumor effect, reduces macrophage motility, inhibits macrophage infiltration of irradiated tumors and reduces the extent of metastasis in locally irradiated mice. Overall, this study demonstrates the adverse effects of local radiation on the host that result in macrophage-induced metastasis.

IDENTIFICATION, VALIDATION AND THERAPEUTIC POTENTIAL OF KEY MICRORNAS INVOLVED IN THE PROGRESSION AND TUMOR-HOST INTERACTIONS OF OSTEOSARCOMA

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The presence of dormant, microscopic cancerous lesions possesses a major obstacle for the treatment of metastatic and recurrent cancers. While it is well-established that microRNAs play a major role in tumorigenesis, their involvement in tumor dormancy has yet to be fully elucidated. We developed a human osteosarcoma dormancy model of a pair of cells originating from the same parental tissue; one that remains avascular and non-palpable a year following inoculation into mice and another that generates vascularized palpable tumors one month following inoculation. Using this model of cell lines generating dormant or fast-growing osteosarcomas, we identified three novel regulators of osteosarcoma dormancy: miR-34a, miR-93 and miR-200c. This is the first time to show that loss of these three microRNAs occurs during the switch from dormant avascular into fast-growing angiogenic phenotype. Furthermore, we validated their downregulation in patients' tumor samples compared to normal bone. Reconstitution of these microRNAs into Soas-2 and MG-63 cells, which generate fast-growing osteosarcomas, reduced the levels of their targets, MET proto-oncogene, hypoxia-inducible factor 1α, and moesin, critical to cancer angiogenesis and cancer cells' migration. We further demonstrate that these miRNAs attenuate the angiogenic capabilities of fast-growing osteosarcoma in vitro and in vivo. Moreover, treatment with each of these microRNAs using our novel polyglycerol dendritic nanocarrier significantly prolonged their dormancy period. Taken together, these findings suggest that miR-34a, miR-93 and miR-200c have a key role in osteosarcoma progression, and provide the rationale for the development of novel diagnostic and therapeutic tools for osteosarcoma and other malignancies.

DRUG DESIGN AND THERAPY

HARNESSING THE POWER OF MYRIAD HETEROGENEOUS EXPRESSION PROFILES TO CLASSIFY DISEASES AND PROPOSE DRUG TARGETS

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Over the past fifteen years, numerous studies investigated diseases using gene expression profiles. Substantial progress was made in disease classification and biomarker discovery. However, such biomarkers typically suffered from poor reproducibility, robustness, and interpretability. The accumulation of thousands of studies spanning hundreds of diseases has great potential to remedy these shortcomings. Here we address this challenge by forming, manually annotating and analyzing a large compendium of over 14,000 profiles spanning 48 diseases and 18 expression platforms. We show that when studying a particular disease, judicious utilization of profiles from other diseases improves classification quality, provides more realistic evaluation of that quality, and enhances disease-specific biomarker discovery. By using such data alongside multiple studies for the target disease and the established disease ontology, we identify disease-specific biomarkers for 24 different diseases. We combine these biomarkers with large-scale interaction, mutation and drug target data, forming a highly valuable disease summary that suggests novel directions in disease understanding and drug repurposing. Finally, we give estimates for the amount of samples required to reach a desired biomarker stability level. Our methods, biomarkers and compendium can greatly improve the exploitation of the mountain of expression profiles for better disease analysis.

T-CELLS ENGINEERED TO EXPRESS A PD1/28 CO STIMULATORY CONVERTER DISPLAY ENHANCED ANTI-TUMOR ACTIVITY

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Adoptive transfer of T-cells genetically-modified to express cancer-specific receptors can mediate impressive tumor regression in terminally-ill patients. However, T-cell function and persistence over time could be hampered by the activation of inhibitory co stimulatory pathways, such as PD1/PDL1, leading to T-cell exhaustion and providing tumor cells with an escape mechanism from immunosurveillance. In addition, the lack of positive co stimulation at the tumor site can further dampen T-cell response. We previously showed that it is possible to generate and express in T-cells a PD1/CD28 chimeric molecule (termed PD1/28) that caused enhanced cytokine secretion and proliferation of tumor-specific T-cells. In the present study, we have extended the use of co-stimulatory converters in conjunction with different antigen-specific receptors including CARs (chimeric antigen receptors). In the case of the latter, we also observed enhanced cytokines secretion by PD1/28-CAR T-cells. We also demonstrate that PD1/28-CAR T-cells can upregulate the activation marker 41BB as well as significant anti tumor cytotoxicity, upon co-culture with tumor cells . To test the relevance of our results in a mouse model, we designed and optimized a murine version of PD1/28. Murine T-cells, isolated from OT1 transgenic TCR mice and transduced to express a PD1/28 chimeric molecule were adoptively transfer into B16-tumor bearing C57/B6 hosts. Mice treated with PD1/28-transduced splenocytes demonstrated a statistically significant delay in tumor growth compared to mice treated with control vector-transduced cells. Thus, PD1/28 can effectively enhance T-cell anti-tumor activity both in vitro and in vivo settings. Overall, we propose that engineering T-cells with a costimulatory converter bears important implications for the improvement of T cell-based immunotherapy.

NON-THERMAL EFFECTS OF MILLIMETER WAVES ON HUMAN LUNG CANCER CELLS

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Lung cancer is lethal as well as resistant to chemo- and radiotherapy. Development of new and effective methods of treatment is currently an unmet need. Here, we suggest a novel method of treatment that is based on low-intensity millimeter waves (MMW- 75-110 GHz (2.7-4 mm wavelengths). Cells were irradiated in continuous sweeping regimes with different exposure times. The effects of irradiation on cell death, survival and morphology are described for human lung cancer adenocarcinoma cells. We show that cells irradiated for 2 and 4 minutes survived while cells irradiated for 10 minutes died a few days later. In addition to morphological changes, we observed two general effects in the surviving populations: cell mortality and senescence. These effects were specific for cancer cells and were not observed in the non-tumorigenetic human epithelial cells, MCF-10A, indicating that MMW irradiation could be a selective method for treating cancer. In view of very low power of irradiation (≈ 1 mW in total), these effects appear to have non-thermal characteristics. The presented method suggests a possible application in therapeutics.

THE IMPACT OF NEXT-GENERATION SEQUENCING ON CLINICAL DECISIONS IN LUNG CANCER

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Background: In the last decade, important advances have been made in understanding genetic and molecular mechanisms of Non-Small Cell Lung Cancer (NSCLC) tumor development. This has led to the creation of innovative, targeted drugs that significantly prolong survival in advanced patients. Recent data shows that 63% of NSCLC tumors harbor at least one activating driver mutation, including treatable mutations such as RET, HER2 and ROS1 gene mutations, besides the regularly screened ALK and EGFR genes that account for 23% of the patients. Clinical cancer genomic profiling tests based on Next Generation Sequencing (NGS) technologies are capable to reveal clinically actionable genetic alterations in up to three times the number of actionable alterations detected by current diagnostic tests. However, there is no data regarding the true impact of these tests on clinical decisions in lung cancer. In this study, our objective is to evaluate the impact of NGS-based genetic profiling tests on treatment strategy in NSCLC patients in the real life setting, considering the additional diagnostic tests performed. Based on clinical experience from Israel, NGS-based tests actively change treatment plans, but the effect size is unknown and merits further investigation.
Methods: In this retrospective study, data is collected from patient files at the Thoracic Cancer Unit of Davidoff Cancer Center, Rabin Medical Center, Israel. The current study population is 78 NSCLC patients who performed an NGS-based genetic profiling test.
Results: Out of 78 patients, 58 patient files have already been reviewed and 6 were excluded. Treatment decision change rate after NGS testing was 33% (17 out of 52 patients were treated with a targeted therapy - 24% of the current study population). Interestingly, 9 patients became EGFR and ALK positive by NGS after the previous standard local molecular testing was negative. Based on the RECIST criteria of response evaluation, 41% of the patients had a partial response after switching to targeted therapy, 23% had a complete response, 18% experienced progressive disease and 18% were not evaluated yet. Survival rates will be calculated further in the study based on data availability.
Conclusions: The use of NGS for tumor classification and treatment planning holds a great potential for improving patient life quality and survival. In this study, we aimed to determine its clinical impact in the real life setting in the treatment of lung cancer. Our partial results show that in addition to performing standard molecular testing for NSCLC, almost a quarter of the patients can be identified having an actionable driver mutation and switched to targeted therapy. Most of these patients showed a positive response to treatment. Although this topic needs to be further assessed in large randomized controlled trials, these positive results emphasize the importance of upfront multiplex testing or suggest such technology as a reflex test in places where the primary kits are done first in sake of cost-benefit.

SYNTHESIS AND CHARACTERIZATION OF POLYMERIC NANOTHERANOSTICS FOR REAL-TIME NON-INVASIVE OPTICAL IMAGING OF DRUG RELEASE

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Theranostics is a relatively new term, introduced in 2002 (1) that describes any material for applications combining both therapy and diagnostics. The great challenge for future personalized therapy in oncology is exploring improved methodology for (i) early detection of localized and disseminated tumor cells in patients and (ii) monitoring drug release at the target site in order to evaluate the treatment's efficacy. The determination of both is critical to success of cancer therapy and improvement of patients' survival rates. A theranostic nanosystem composed of nanocarrier, drug and Turn-ON probe is an ideal platform to address these challenges. In this study, we designed, synthesized and characterized a theranostic nanomedicine based on N-(2-hydroxypropyl) methacrylamide (HPMA) copolymer. The diagnostic system consists of self-quenched Cy5 and the therapeutic system is based on the anticancer agent paclitaxel (PTX). Both diagnostic and therapeutic moieties were conjugated to HPMA copolymer through a Gly-Phe-Leu-Gly (GFLG) linker, cleaved by cathepsin B, a lysosomal cysteine protease overexpressed in several tumor types such as lung, colon, prostate, melanoma and breast cancers. Our systems enable site-specific release of the drug concomitantly with the fluorophore activation to its Turn-ON state upon enzymatic degradation (2). HPMA copolymer-PTX conjugate inhibited the proliferation of breast cancer cells. Furthermore, our conjugate demonstrated anti-angiogenic properties inhibiting endothelial cells proliferation and migration. Our preliminary results with the diagnostic nano-conjugate HPMA copolymer-Cy5 present its potential use as a novel probe for sensing real-time drug release from the polymeric nanocarrier. This approach of co-delivery of two complementary systems serves as a proof-of-concept for non-invasive real-time deep tissue intravital orthotopic monitoring that may potentially be exploited as a theranostic nanomedicine in the clinic.

EXOSOME SECRETION IN OVARIAN CARCINOMA

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The purpose of this study is to elucidate the mechanism of exosome secretion in Ovarian Carcinoma (OC). Exosomes, 30-100 nm vesicles, contain various lipids, proteins, and nucleic acids, exhibit paracrine bioactivities as well as distant transfer of regulatory messages to other cells. In our lab, the presence of OC derived exosomes accelerated cancerous pathologies in mice, therefore a worthy target for OC therapy. The precise mechanism of the biogenesis and release of exosomes has yet to be elucidated; however, several studies have revealed the involvement of NSMASE2, TSAP6, RAB27A/B and ARF6 genes in these processes. OC, the leading cause of death from gynecological cancers in western countries, is characterized by primary solid tumors, solid metastases, and effusions to the peritoneal and pleural cavities as the tumor progresses. In order to develop a model for solid tumors and effusions in vitro we utilized 3D Macro-porous alginate scaffolds and 3D ES2 cell line spheroids respectively. In this study, we examined the mRNA and protein expression of the above mentioned genes in human ovarian cancer samples derived from primary, metastatic lesions and from effusion derived OC cells and compared the expression profile to that of cell lines in our 3D model. Our results show that in OC samples, NSMASE2, TSAP6 and RAB27A mRNA expression are significantly higher in effusions vs solid tumors (p<0.026, p<0.0001 and p<0.02, respectively). Surprisingly, the protein content of these genes are significantly lower in effusions vs. the solid samples in these very same samples (p<0.001-0.0001). This is explained by the presence of NSMASE2, TSAP6 and RAB27A proteins in effusion fluid-derived exosomes. ARF6 is also expressed in our samples. Clinical analysis shows that elevated NSMASE2 and TSAP6 mRNA expression correlates with poor survival (p<0.036) and less favorable response to chemotherapy, respectively (p<0.027). Furthermore, RAB27A protein is lower in pleural effusions vs peritoneal effusions (p<0.013). In vitro, mRNA analysis of ES2 cells grown on alginate scaffold and spheroids show a higher expression of TSAP6 and RAB27A in spheroid cultures vs. scaffold cultures, emulating the gene profile in effusions vs. solid tumors in OC. To the best of our knowledge, this is the first attempt to develop a 3D in vitro model for the various forms of OC that will enable research on the exosome secretion mechanism in this disease, hopefully leading to a fuller understanding of the exosomes secretion process and to the development novel therapies to address this disease.

TREATMENT RESPONSE ASSESSMENT MAPS (TRAMS) CALCULATED FROM DELAYED-CONTRAST MRI FOR ASSESSMENT OF RESPONSE TO BEVACIZUMAB (BEV)

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Current imaging is unable to differentiate tumor from treatment-effects in brain tumor patients. We have recently applied delayed-contrast MRI for calculating TRAMs clearly differentiating tumor/non-tumor tissues. Histological validation from 51 resected patients confirmed that regions of contrast clearance >1hr post contrast injection represent tumor while regions of contrast accumulation represent non-tumor tissues with 100%/93% sensitivity/PPV to active tumor. The role of BEV in treating HGG is controversial. Here we studied the TRAMs added value in assessing response to BEV. 24 patients with recurrent HGG were scanned before/during BEV. Tumor volumes were calculated from the TRAMs (regions of contrast clearance), T1-Gd (enhancing regions) and perfusion-MRI (rCBV>1.8). Patients were divided into responders/non-responders (PFS>6/PFS<6) using standard radiological reports. 6 responders had PFS/OS=16.5±2.8months at the time of analysis. 18 non-responders had PFS/OS=3±0.4/7.4±0.6months. All patients had significant baseline tumor volumes in the TRAMs (responders/non-responders: 15.6±8.9ml/15.8±2.2ml) suggesting BEV-induced anti-neoplastic effects in the responding patients. The TRAMs tumor volumes increase was 29% higher than the T1-Gd increase at progression (95% CI: 9%-68%, p<0.02), and the decrease was 24% stronger at response (95% CI: 2%-45%, p<0.05), suggesting higher sensitivity of the TRAMs to response/progression. Tumor volume change in the 1st month of treatment was found to be a strong predictor of response: the sensitivity/PPV of the TRAMs, T1-Gd and rCBV to response was 100%/75%, 67%/57% and 75%/75%. Wilcoxon test confirmed the significance of these predictors for PFS (p<0.002, 0.05 and 0.05 for TRAMs, T1-Gd and rCBV) and OS (p<0.001, 0.01 and 0.05). 7 patients were re-irradiated under BEV. In the TRAMs tumors decreased to 62±10% while treatment effects increased to 296±72%, in agreement with longer survival (73 days, p<0.02). Standard MRI showed no response: T1-Gd decreased to 92±21%, FLAIR increased to 155±18%. In summary, the TRAMs provide increased sensitivity to response/progression in BEV-treated patients with various possible applications.

ERYTHROPOIETIN IN 5T33 MURINE MULTIPLE MYELOMA: OPPOSING IMMUNE AND SKELETAL OUTCOMES

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Erythropoietin (Epo) is the major hormone that promotes erythropoiesis. Clinical introduction of recombinant human Epo (rHuEpo) has been a breakthrough in treating patients with anemia, mainly those with chronic kidney failure or chemotherapy. Epo was found to have immune-mediated anti-cancer effects as shown in multiple myeloma (MM) patients and mouse models. In that respect, we have found that Epo treatment for anemia in MM patients and mouse models was associated with improved immunological functions and general outcome. We have recently shown that Epo treatment or overexpression, stimulates bone resorption in mice (Hiram-Bab et al., 2015). We thus set to determine the mechanism of action of Epo on the immune and skeletal systems in the 5T33 MM mouse model. Epo administration to MM mice attenuated disease progression as demonstrated by a decrease in MM pathological κ light chain, expression of IL-6 and RORγt (a hallmark for Th17), all pathological markers for MM. IFN-γ transcript levels and number of macrophages (F4/80+CD11b+) in the bone marrow (BM) were increased 1.7 and 1.4-fold, respectively in the Epo- versus vehicle-treated MM mice. In vitro, Epo stimulated phagocytosis of 5T33MM cells (1.3 fold) by purified BM-derived macrophages. High-resolution microCT analysis of the distal femurs revealed Epo-associated bone loss in both healthy and 5T33MM mice, which correlated with a 50% increase in transcript levels of the osteoclastogenic nuclear factor-kappa B ligand (RANKL) in BM. In MM, Epo may thus act as a double-edged sword, by stimulating the immune response while accelerating bone resorption. The current study highlights administration of targeted bone protective treatment alongside Epo in MM patients, to attenuate the anemia and MM progression, while preventing bone damage. This study was supported by a grant from the Multiple Myeloma Research Foundation and by the FP7 European commission grant; 282551 EpoCan.

CHIMERIC PROTEINS FOR TARGETING EGFR OVER-EXPRESSING CANCERS

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The Epidermal Growth Factor Receptor (EGFR) is over-expressed in multiple types of human cancer and is associated with aggressive disease and low survival rate. Therefore, EGFR is a popular target for many antitumor strategies. Current anti-EGFR therapies are only partially successful, mostly because the receptor is not absolutely essential for the survival of the cancer cell in which it is over-expressed. Herein, we propose a new therapeutic approach, where the over-expression of EGFR is utilized to selectively introduce long synthetic dsRNA, poly Inosine/Cytosine (polyIC), into tumor cells. We hypothesize that targeted delivery of polyIC into cancerous cells will lead to the activation of dsRNA-dependent pathways, and consequently induce tumor cell apoptosis and the secretion of cytokines and chemokines. Finally, the secreted compounds will recruit immune cells to the tumor area, thus causing a "bystander effect", i.e. killing neighboring tumor cells, even those that do not over-express EGFR. We have developed recombinant chimeric proteins which comprise the dsRNA binding domain of human PKR (dsRBD) fused to an EGFR binding molecule (EGF or EGFR affibody-Z1907). These chimeras are able to selectively introduce polyIC into EGFR over-expressing cell lines. Treatment with the chimera/PolyIC complexes of MDA-MB-468 cells, which highly express EGFR, induced approximately 90% cell killing. The chimera/PolyIC did not promote apoptosis in cell lines that do not over-express EGFR.

ACTIVATION OF SYSTEMIC ANTI–TUMOR IMMUNITY BY IN SITU ABLATION OF BREAST CARCINOMA BY INTRATUMORAL 224RA-LOADED WIRES

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Introduction: It has been demonstrated that aggressive in situ tumor destruction (ablation) could lead to the release of tumor antigens, which can stimulate anti-tumor immune responses. We developed an innovative method of tumor ablation based on intratumoral alpha irradiation, Diffusing Alpha-emitters Radiation Therapy (DaRT), which efficiently ablates local tumors and enhanced anti-tumor immunity. In this study, we investigated the anti-tumor potency of a treatment strategy, which combines DaRT tumor ablation with two approaches for an enhancement of anti-tumor reactivity: i) neutralization of immunosuppressive cells such as regulatory T cells (Tregs) and myeloid derived suppressor cells (MDSC); ii) boost the immune response by the immunoadjuvant CpG.

Materials and Methods: Mice bearing weakly immunogenic DA3 mammary cancer cells were treated by DaRT. Tregs were inhibited by low-dose cyclophosphamide (CP) and MDSCs were inhibited by the PDE-5 inhibitor, Sildenafil. The immune system was stimulated by the immunoadjuvant, CpG.

Results: Combination of all four therapies led to growth retardation of 7 out of 9 tumors and 2 of the tumors were completely eliminated. The treatment also reduced lung metastases. Only 30% of such treated mice carried metastases compared with 100% in the untreated controls. The treatment with DaRT and Treg or MDSC inhibitors (without CpG) also resulted in a significant reduction in tumor size, reduced the lung metastatic burden and extended survival compared to the corresponding controls. Tumors treated with DaRT+Treg inhibitor were 1.3-2.3 times smaller than those in the control groups, and 50% of the mice in the DaRT + Treg inhibitor treated group survived 119 days post treatment, whereas all mice in the DaRT or low dose cyclophosphamide groups died.

Conclusion: We suggest that the therapy with DaRT combined with the inhibition of immunosuppressive cells and CpG enforced both local and systemic anti-tumor immune responses and displayed a significant anti-tumor effect in tumor-bearing mice.

NOVEL COMBINATORIAL TREATMENT OPTION FOR METASTATIC UVEAL MELANOMA

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Purpose: To date, chemotherapy for metastatic uveal melanoma (mUM) is limited to dacarbazine (DTIC) and fotemustine. We tested the effect of the common chemotherapeutic drug doxorubicin (DOX) on cell mortality in order to expand the chemotherapeutic arsenal for mUM.
Methods: We examined the effect of both DITC and DOX in five different uveal melanoma cell lines – originating from both metastases (OMM1, OMM2.3 and OMM2.5) and from primary tumors (92.1 and MEL270) and performed dose response tests using both drugs. Based on our previous results, we hypothesized that combining DOX and knockdown of CREB will increase cellular death. To test our hypothesis, we infected cells with replicative competent retroviruses (RCR) expressing shRNA against CREB to create a continuous infective knockdown of CREB.
Results: Both chemotherapeutic drugs induced cell death in a dose dependent manner. Knockdown of CREB in these cells increased the effect of DOX on cell mortality.
Conclusions: Treatment with DOX is at least as efficient and in some cases even more efficient than DTIC in inducing UM cell mortality in vitro. Moreover, the ability of combining CREB knockdown and DOX treatment to achieve the same amount of cell death in lower concentrations of DOX may result in lower side effects from DOX. This combination is a possible new treatment for metastatic uveal melanoma.

IN VIVO MOLECULAR IMAGING OF HIF-1ALPHA AND SEPTIN 9 INTERACTION BY BIMOLECULAR FLUORESCENCE COMPLEMENTATION

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Hypoxia is a common feature of many solid tumors that promotes tumor progression and leads to radiation and chemotherapy resistance. Hypoxia-inducible factor 1 (HIF-1), as one of the major mediators of the hypoxic response, has been shown to activate hypoxia-responsive genes, which are involved in multiple aspects of tumorigenesis and cancer progression. We have previously described an interaction between septin 9 isoform 1 (SEPT9_i1) protein and HIF-1a, the oxygen regulated subunit of HIF-1. SEPT9_i1 is a member of the conserved family of septins. The interaction of SEPT9_i1 with HIF-1a increases protein stability and HIF-1 transcriptional activity in vitro and promotes proliferation, tumor growth and angiogenesis in vivo. Recent data indicated that SEPT9_i1 was also involved in trafficking of cytoplasmic HIF-1a into the nucleus by direct interaction to the nuclear transporter importin-alpha. Herein, we utilized split YFP bimolecular fluorescence complementation (BiFC) methodology to monitor HIF-1a/SEPT9_i1 interactions in vivo. First, we generated split YFP protein chimeras fused to HIF-1a and to SEPT9_i1 on both their amino and carboxyl termini. Different pairs of HIF-1a and SEPT9_i1 chimeras were tested for functional complementation of the split YFP fragments using transient transfection in PC-3 human prostate cancer cells. The complemented proteins capable of fluorescence were identified as SEPT9_i1-YN and YC-HIF-1a. This pair was selected for further studies in PC-3 cells. YFP complementation fluorescence derived from these chimeras was increased in the presence of hypoxia mimicking agents, such as CoCl2 and the iron chelators deferoxamine (DFO) and dibenzoylmethane (DBM). The chimera, YC-ΔHLH-HIF-1a lacking the HLH domain that is essential for the interaction with SEPT9_i1 gave a significant reduction in BiFC signal. Likewise, expression of SEPT9_i1 252-379aa fragment, the domain essential for the interaction with HIF-1a, abolished almost completely the BiFC signal. Furthermore, using this transient split YFP system, we were able to show a reduction of the BiFC signal when cells were treated with DBM together with 17-N-allylamino-17-demethoxygeldanamycin (17-AAG), and a dramatic increase of the BiFC signal when cells were treated with forchlorfenuron (FCF). Subsequently, we created a stable split YFP system in PC-3 cells that stably expressed SEPT9_i1-YN and a Tet-On induced YC-HIF-1a. Upon induction, these cells showed a significant increase in the BiFC signal. These results reconfirm HIF-1a/SEPT9_i1 interactions in vivo and make this system attractive for identifying new compounds capable of disrupting this complex and to inhibiting tumor growth and angiogenesis.

NUPHARIDINE INHIBITS NF-KB ACTIVITY, HAS SYNERGISTIC CYTOTOXIC ACTIVITY WITH CISPLATIN AND ETOPOSIDE AND INDUCES APOPTOSIS

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Introduction: The nuclear factor-kappa B (NF-kB) family of transcription factors plays a pivotal role in inflammation, proliferation, and prevention of apoptosis. Due to its central role in many physiological and pathological processes, including cancer, they constitute attractive targets for therapy. The use of plant extracts to alleviate inflammatory diseases is centuries old and continues to this day, therefore we screened thirty-four methanolic plant extracts for inhibition of constitutive NF-kB activity by a NF-kB-luciferase reporter gene assay.

Materials and Methods: There was strong inhibition of NF-kB activity by Nuphar lutea L. SM. (Nuphar), leaf and rhizome extracts. An active fraction containing a mixture of dimeric sesquiterpene thioalkaloids was purified by solvent extraction, pH adjustment silica gel chromatography and HPLC. One- and two-dimensional NMR spectroscopy indicated the presence of nupharidine, 6-hydroxythionuphlutine as a major component.

Results and Discussion: Nupharidine showed a dose dependent inhibition of NF-kB activity by luciferase reporter gene assay as well as reduction of nuclear NF-kB subunits expression as tested by western blots and immunohistochemistry. Diminution of DNA binding was demonstrated by Electro Mobility Shift Assay (EMSA). Nupharidine inhibited both inducible and constitutive NF-kB activation and affected the canonical and alternative pathways. Suppression of NF-kB was not cell type specific. Induction of apoptosis by Nupharidine was demonstrated by time and dose dependent cleavage of procaspase-9 and PARP. Synergistic cytotoxicity of nupharidine with cisplatin and etoposide was demonstrated in vitro as well as in vivo with cisplatin, diminishing experimental B16 melanoma lung metastases.

Conclusion: Based on these results, we propose that Nupharidines may be further developed as possible “sensitizer” in anticancer treatment.

PROMIS-QUAN: A NOVEL PROTEOMIC METHOD FOR PLASMA BIOMARKER QUANTIFICATION

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Uncovering potential biomarkers in the blood, towards the development of non-invasive diagnostic/prognostic tests, is one of the holy grails in cancer research. However, plasma proteomics research has been hampered by the tremendous dynamic range in the plasma, which leads to the masking of 'tissue leakage' proteins that comprise of potential biomarkers by the highly-abundant core plasma proteins. To overcome this challenge we developed PROMIS-Quan: PROteomics of MIcroparticles with Super-SILAC Quantification, which provides a high throughput unbiased approach for deep coverage of plasma microparticle proteomes, combined with relative and absolute protein quantification. Based on this method we reached an unprecedented depth of over 3,000 plasma microparticle proteins in single MS runs. We further tested PROMIS-Quan by comparing plasma samples of prostate cancer patients with healthy individuals' samples. In total we identified 5,374 plasma proteins and revealed a predictive signature comprised of 3 proteins that were higher in the patient specimens. Additionally, we determined the absolute quantitative changes upon treatment of prostate specific antigen (PSA), a well-known prostate cancer biomarker. Altogether we propose PROMIS-Quan as an innovative platform for biomarker discovery, validation and quantification in both the biomedical research and in the clinical worlds (Harel et al., MCP 2015).

SOMATOSTATIN RECEPTORS IN PANCREATIC ADENOCARCINOMA: EXPRESSION PROFILING AND IDENTIFICATION OF A NOVEL RECEPTOR-DIRECTED SMALL MOLECULE

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Introduction: Somatostatin is a peptide hormone involved in the regulation of hormone secretion from various endocrine cells as well as in the regulation of cell growth and apoptosis. Somatostatin acts via five G-protein coupled somatostatin receptor subtypes (SSTR1–5). SSTRs are overexpressed in various malignancies, and treatment with long-acting synthetic analogs of somatostatin is the mainstay of treatment of gastrointestinal neuroendocrine tumors. Currently available analogs target mainly SSTR2 and may not be useful for the treatment of tumors expressing predominantly other SSTRs. Recent in vitro data suggest the ability of various SSTRs to mediate growth inhibition of pancreatic cancer cells, and others have noted expression of SSTR2 in pancreatic adenocarcinoma (PDAC). Therefore, our first aim was to characterize SSTRs expression pattern in clinical samples of PDAC. To this aim we conducted immunohistochemistry (IHC) analysis of the five SSTR subtypes in 93 PDAC samples. Our analysis revealed a significant SSTR4 overexpression in the vast majority of these tumors (69.2%). SSTR2 was highly expressed in 62.6% of patients. Expression of SSTR1, 3, and 5 was noted in 26.4%, 36.3% and 4.4% of cases, respectively. These results suggest that development of an SST analog with high specificity to SSTR4, 1 and 3 may be of clinical significance to PDAC patients. We are currently developing a novel SST analogue, PTR-86, and testing its activity, pharmacodynamics and binding properties in vivo and in vitro and in various malignancies and in cell line models. Therefore, our next aim was to characterize the binding affinity of this PTR-86. We examined binding affinity in HEK-293 cells over-expressing the five different SSTR subtypes, compared to empty-vector transfected cells. PTR-86 is conjugated to FITC and thus we monitored, using live imaging PTR-86 binding and internalization into cells. Binding analysis data indicated that PTR-86 binding affinity is highest in HEK-293 cells expressing SSTR3, SSTR4 and SSTR5, lower in SSTR2 expressing cells and almost undetectable in SSTR1 transfected cells. Specificity was confirmed using octreotide, a specific SSTR2 agonist. Importantly, these results were confirmed using flow cytometry of the HEK-293 transfected cells. In conclusion, we show, for the first time, high expression of SSTR4 in the majority of PDAC samples. This data emphasizes the need for analogs with high binding affinity to SSTR4 in order to serve as a novel treatment for this cancer. The binding properties of PTR-86 make it an excellent candidate for further studies.

PLASMA-SHLA BOUND PEPTIDES IN ACUTE MYELOID LEUKEMIA AS PREDICTORS OF INDIVIDUAL RESPONSE AND THEIR APPLICATION IN IMMUNOTHERAPY

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Introduction: Myeloid leukemia cell population is heterogeneous and composed of different immunophenotypically sub-populations. These leukemic sub-populations may also vary in their sensitivity to chemotherapy. During treatment, inherent chemo-resistant sub-populations survive and are becoming a dominant clone. It has shown that soluble HLA (sHLA) -bound peptides molecules present in the plasma of cancer patient are mostly derived from the disease cells and thus allow the identification of sHLA peptidomes. Here we analyzed and compared differences in the expression of sHLA peptides between BM plasma at: diagnosis, during chemotherapy induction and remission as well as normal healthy population, to find a peptide model which will be used to measure the response of each patient to the treatment and map out disease-specific peptide spectrum, clarify the possible pathogenesis, resistance mechanism, and disease prognosis.

Materials and Methods: Plasma from BM aspirates samples derived from AML patients will be extracted during the follow time points: at diagnosis (D1), fifth day (D5) and day fourteen (D14) from induction therapy. sHLA and peptides purification from plasma: samples are loaded sHLA class I molecules with their bound peptides loaded on pretreated monoclonal antibody W6/32. Elution is done with Trifluoroacetic acid and the sHLA is separated from their peptides on C18 Micro TipColumn with acetonitrile. The peptides fraction is concentrated by vacuum centrifugation until complete evaporation and are examined in Liquid chromatography coupled mass spectrometry (LC-MS/MS).

Results: Peptides repertoire bound to sHLA-plasma were identified by LC-MS. The peptides from diagnosis (D1), during chemotherapy treatment (D5) and remission (D14) was compared in eleven AML patients. We found that during chemotherapy (D5) there is an elevation of 25 peptides related to a variety of cancers genes and to proteins involved in different cell biological processes. These peptides were correlated with patients who did not respond to chemotherapy treatment.

Conclusions: In this study we found peptides unique to AML which were not existed in healthy donors, and also found a difference in their appearance during chemotherapy treatment compared with pre-treatment. Furthermore, we found a correlation between specific peptides and patients' response to treatment.

TUMOR ABLATION AND INDUCTION OF ANTI-TUMOR IMMUNITY BY ELECTROCHEMICAL TREATMENT

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Background: Tumor ablation is a nonsurgical method to eradicate solid tumors. This strategy combines the advantage of removing the primary tumor with the option to immune the body against distant tumor cells by exposing the body to large amount of tumor antigen. Pulsed Electric Current Tumor Ablation (PECTA) is an intratumoral treatment developed in our lab, which destroys the tumor tissue by a number of different chemical and physical mechanisms, mainly pH changes and free radicals formation. In this study we examined the effects of PETCA treatment on primary tumor destruction, tumor recurrence, stimulation of anti-tumor immunity and survival. Moreover, we examined the effects of combining the treatment with either immuno-adjuvants or inhibitors of immuno-suppressor cells on enhancing the immune response.

Methods: Balb/c and C57BL/6 mice bearing subcutaneous tumors of murine breast adenocarcinoma (DA3), fibrosarcoma (BLC25) or melanoma (B16) cells were treated by PECTA or surgery. PECTA was applied by intratumoral electrodes delivering 50-100 coulombs per electrode per cm3 (C/E/cm3) of tumor tissue and compared to surgical removal. The efficacy of ablation (elimination of the primary tumor and recurrence) and the development of anti-tumor responses of mice cured by PECTA or surgery were evaluated by survival monitoring and challenging the mice with secondary tumor cells. The effects of combining the treatment with the immunoadjuvant, CpG, or the suppressor cell inhibitors; Sildenafil, APCP and Cimetidine were examined as well.

Results: A direct correlation between the amount of charge delivered and the rate of primary tumor ablation was found in treated DA3 and B16 bearing mice. The charge of 100C/E/cm3 was found as optimal for maximal eradication of primary tumors in DA3 model, where in B16 the optimal charge was 75C/E/cm3. DA3 bearing mice cured by PECTA combined with CpG, APCP or Sildenafil were more resistant to the growth of a tumor cell challenge and survived longer than surgery treated mice. As for B16, PECTA combined with CpG had also prolonged the survival and augmented the immune response against secondary tumor development, but surgery was more effective in eradicating the primary tumors.

Conclusions: The findings introduce a novel method that efficiently eradicates DA3/B16 tumors and stimulates a protection against recurrence and distant metastases development.

A SYSTEM FOR COMPUTATIONALLY EVALUATING DRUG PRIORITIZATION METHODS

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Introduction: Any cancer therapeutic approach tested to date benefits only a part of the treated population. Personalized medicine calls for more full consideration of the biological and molecular characteristics of the tumor. One approach for prioritized drugs is to devise methods, or algorithms, that score the predicted value of drugs for a given patient, and to choose off the top of the list the therapies most likely to benefit the patient. However, as more and more schemes for drug prioritization emerge, there is a growing need for a system that can a compare different scoring schemes, allowing to choose between different, or even slightly modified, scoring schemes without clinical trial.
Methods: (1) RNASeq-based expression profiles were gleaned from the TCGA, choosing only profiles for which matched-normal profiles are available. For each pair of profiles, the WINTHER1.5 score was used to generate a drug prioritization list. (2) lists of drugs or therapies known to be effective for different cancer types were downloaded from the NCI web site. (3) For each list of drugs, the average number of highly ranked chemicals corresponding with the list of drugs from (2), i.e. the drugs compatible with a particular cancer type, were calculated.
Results: Drugs known to be effective were high ranking more often in patients with the same type of cancer in 2 out of 3 tumor types tested. For breast cancer (BRCA), drugs from the BRCA effective drugs list were high ranking more often than LUAD or KIRC (5.2±1.6 vs 4.6±1.4 and 2.4±1.1, correspondingly; p<0.001). Similarly, drugs from the KIRC list were high ranking most frequently in KIRC patients (1.0±0.4 vs 0.3±0.6 and 0.3±0.4 for BRCA and LUAD ; p<0.001). Interestingly, drugs effective for LUAD were more frequently high ranking with KIRC patients then with LUAD patients (1.1±0.5 vs 0.4±0.5; p<0.001).
Conclusions: We present a novel approach for evaluating the precision of cancer medicine scoring schemes. Further research is required to optimize the score, but the score is already sufficiently useful to compare the impact of algorithm changes on the accuracy of drug prioritization schemes.

MAGRET NANOPARTICLES: AN IRON OXIDE NANOCOMPOSITE PLATFORM FOR GENE SILENCING FROM MICRORNAS TO LONG NON-CODING RNAS

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Silencing of RNA to knock-down genes is currently one of the top priorities in gene therapies for cancer. However, the main obstacle in using this powerful gene silencing tool lies in the ability of siRNAs to be efficiently delivered to cells. In this study, we developed a highly innovative nanoscale reagent based on functional superparamagnetic maghemite (γ-Fe2O3) nanoparticles (NPs) that are surface-doped by coordinating lanthanide Ce3/4+ cations using high-power sonochemistry (CAN-γ-Fe2O3). Thereafter, via the unique coordinative chemistry enabled by doped [CeLn]3/4+ cations/complexes, a polycationic polyethyleneimine (PEI) polymer phase was bound to the maghemite NP core resulting in an efficient binding of siRNA molecules. These NPs, termed magnetic reagent for efficient transfection (MagRET), are amenable to silence mRNA, microRNA and long non-coding RNAs (lncRNA) without any toxicity. The NPs penetrate and silence a variety of human cancer cell lines, such as pancreatic, ovarian, lung and osteosarcoma cancers, and also hard-to-transfect cells which are reluctant to standard transfection methods, such as human leukemic cells and primary megakaryocytes. Intraperitoneal injection of these NPs, carrying specific siRNA against the overexpressed PLK-1 kinase in an ovarian cancer model, decreased tumor progression and prolonged the mice lifespan. This work is financially supported by the RTD FP7 EU project SaveMe (FP7-NMP-2010-LARGE-4 area) and Magnet Rimonim.

COVALENT DOCKING OF LARGE CHEMICAL LIBRARIES FOR THE DISCOVERY OF NEW INHIBITORS

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Small molecules are invaluable tools for the investigation of biology. However, discovering new molecules to specifically modulate a target protein is still one of the biggest challenges of chemical biology. Molecules that are able to form a covalent bond with their target often show enhanced selectivity, potency and utility for biological studies, but are yet harder to discover, as they are typically expunged from high throughput screening libraries. Computational methods can help bridge this gap. I developed a covalent docking method for the discovery of covalent probes. Applying this method prospectively to several protein targets we were able to discover potent covalent inhibitors (typically with <50nM IC50), with chemotypes not previously explored. The docking predictions were confirmed by crystallography, the inhibitors displayed marked selectivity and were active in cellular assays. This approach should be applicable for a broad range of protein targets.

TUMOR ASSOCIATED NEUTROPHILS (TAN) REDUCE CD8+ ANTI-TUMOR CYTOTOXIC T-LYMPHOCYTES (CTLS), INCREASING TUMOR GROWTH IN LUNG CANCER AND MESOTHELIOMA

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Background: The role in cancer of neutrophils (TAN), a major cell type in the innate immune system, has not been extensively studied as has been the case for other cells of the immune system. In the last years we and others have studied the different ways by which these cells affect directly and indirectly tumor growth in thoracic malignancies. It has been shown that TAN interact in different ways with the immune microenvironment in cancer, mainly with cells of the adaptive immune-system (e.g. lymphocytes).

Methods and Results: In the current work we evaluated the effects of TAN on CD8+ cytotoxic T-cells (CTLs). Using flow cytometry (Annexin V/PI staining) we found that neutrophils isolated from flank mesothelioma (AB12) or lung cancer (LKR & LLC) tumors induce significant apoptosis of CD8+ CTLs. This effect was found to be contact-dependent, and mediated by iNOS. Furthermore, this effect was markedly reduced in TNFα knock-out mice, suggesting that TNFα is needed for this effect to occur. We next showed by the CFSE method that TAN also inhibit the proliferation of CD8+ CTLs. Surprisingly, TAN mildly increased the activation of CD8+ T-cells as evaluated using activation markers (e.g. CD69), and intracellular cytokines (e.g IFN-gamma). Using a modified Winn assay we demonstrated that the net effect of TAN was, at-least partly, to prevent initial growth-inhibition induced by splenic CD8+ T-cells co-injected with tumor cells.

Conclusions: Our results suggest a new indirect mechanism by which TAN may inhibit anti-tumor immune activity in cancer, thus promoting tumor growth. Proper understanding of the effects of neutrophils, a major effector of the immune system, in cancer, and the ways these cells support or fight cancer will help us develop strategies to direct the immune system against the tumor, potentially improving cancer treatment in general and immuno-gene-therapy in particular.

ROLE OF BENZISOTHIAZOLONE DERIVATIVES 1, 2 ON THE INHIBITION OF NFΚB IN HODGKIN'S LYMPHOMA CELLS

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The NF-κB family of heterodimeric transcription factors (Rel proteins) plays an important role in determining cell survival during immune, inflammatory, and stress responses. NF-κB typically exists as a heterodimeric complex composed of the Rel family proteins p50 and p65. It usually resides in the cytoplasm in an inactive form as a result of its association with its inhibitor IκB. In normal cells, NF-κB is strictly regulated, whereas it is often constitutively activated to high levels in cancer cells. More importantly, NF-κB activation in cancer cells has been shown in many studies to be one of the major causes of resistance to chemotherapy. Since NF-κB represents an important and attractive therapeutic target for treating a variety of diseases, a great deal of attention has been focused on the identification of compounds that selectively interfere with this pathway. Heterocyclic compounds containing sulfur and nitrogen atoms are known to exhibit interesting biological properties. Among these, isothiazolones and benzisothiazolones (BIT) constitute an important class due to the presence of electrophilic sulfur as part of the ring. The aim of the present study was to investigate the NF-κB inhibitory role of BIT derivatives (1 & 2) against Hodgkin's Lymphoma cells lacking IκB. BIT's shows dose dependent NF-κB inhibitory activity in the preliminary reporter gene assay and also shows cytotoxicity in L428 cells. Furthermore, we show synergistic activity with standard chemotherapy drugs such as Etoposide and Doxorubicin. The scratch assay also confirms that the BIT derivatives inhibit the A549 cell migration. In addition, the western blot study proves that the BIT (1 & 2) suppresses the NF-κB subunits p50 and p65 both in cytoplasmic and nuclear extracts in concentration dependent manner as well as the elimination of p50 and p65 proteins from the nucleus by the confocal microscopic studies. The results confirm that the BIT derivatives inhibit the NF-κB against Hodgkins' lymphoma cells.

MICRORNA-BASED NANOMEDICINE FOR GLIOBLASTOMA

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Introduction: Glioblastoma multiforme (GBM) is an aggressive primary neoplasm of the brain that exhibit notable refractivity to standard treatment regimens. Recent large-scale molecular profiling has revealed deregulated molecular networks as potential targets for therapeutic development. MicroRNAs (miRNAs) are non-coding RNA molecules which act as post-transcriptional regulators of specific messenger RNA transcripts. miRNAs play major roles in normal developmental processes, and their deregulation significantly contributes to various aspects of carcinogenesis. Nevertheless, in vivo delivery of small interfering RNA (siRNA) and miRNA remains a crucial challenge for their therapeutic success. siRNAs and miRNAs on their own are not taken-up by most mammalian cells in a way that preserves their activity.

Results and discussion: In order to circumvent these limitations, we developed a cationic carrier system, which can strongly improve its stability, intracellular trafficking and silencing efficacy. Polyglycerol (PG)-Amine, a water-soluble polyglycerol-based hyperbranched polymer accumulates in the tumor microenvironment due to the enhanced permeability and retention (EPR) effect, and therefore, represents an ideal nanocarrier for antitumor oligonucleotides. Using our novel nanocarrier, we have studied the expression targets and functional effects of miR-34a in several human glioblastoma cell lines and human tissue samples. miR-34a levels inversely correlated to their target gene levels measured in the same cell lines or tissue. Transient transfection of PG-NH2-miR-34a polyplex into glioblastoma cells strongly inhibited cell proliferation, cell cycle progression, and cell migration. Consequently, we performed an in vivo experiment and achieved a significant tumor growth inhibition following treatment with PG-NH2-miR-34a polyplex in a human glioblastoma mouse model. We further characterized the synergistic effect of combining PG-NH2-miR-34a polyplex with chemotherapy and achieved promising results.

Conclusions: Together, our findings show that PG-NH2 efficiently delivers anticancer miRNAs to glioblastoma cells and suppresses brain tumor growth. These results suggest that our polyplex could serve as a potential nanomedicine for glioblastoma.

ABERRANT GLYCOSYLATION AS A POSSIBLE THERANOSTICS TARGET

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Cells are typically coated with a thick sugar layer. It is made up of glycolipids, glycoproteins and free glycans and can facilitate immune recognition processes. In cancer, this cell surface glycosylation is frequently modified due to distorted expression of glycan-modifying enzymes. This often leads to aberrant expression of sialic acids (Sia) that cap glycan-chains. The two major Sia forms in mammals are N-acetylneuraminic acid (Neu5Ac) and its hydroxylated form, N-glycolylneuraminic acid (Neu5Gc). Humans cannot synthesize Neu5Gc due to a specific inactivation of the CMAH gene encoding CMP-Neu5Ac hydroxylase. Nevertheless, this non-human Sia metabolically incorporates in human cells as 'self', apparently originating from dietary Neu5Gc-rich foods (e.g. red meat). Neu5Gc is present at low levels on cell surfaces of human epithelia and endothelia, but especially accumulates in carcinomas. Consequently, it is recognized as foreign by the human immune system and result in broad anti-Neu5Gc antibodies response. Low levels of these antibodies promote weak chronic inflammation facilitating tumor progression that can be suppressed by anti-inflammatory drugs. However, at higher concentrations, these antibodies suppress growth of Neu5Gc-expressing tumors. Concurrently, some anti-Neu5Gc antibodies hold potential for novel targeted immunotherapy as they could promote complement- or antibody-dependent cellular cytotoxicity (CDC/ADCC) on related human cancer cells. A sialoglycan-microarray containing the most common naturally-occurring sialic acids can be used as a high-throughput discovery platform. Using such an array, a unique anti-Neu5Gc IgG was discovered as a novel carcinoma biomarker. Altogether, these findings highlight the immune recognition of incorporated dietary non-human sugar in humans and its theranostics potential.

DEEP PROTEOMIC PROFILING OF BREAST CANCER PROGRESSION

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Breast cancer is the most common malignancy in women worldwide. Nearly all breast cancer-related deaths occur as a result of metastasis, mainly to the liver, lungs and bones. Regional lymph nodes are the first place the cancer is likely to spread to, and the lymph node status (positive or negative for cancer cells) is tightly related to prognosis and survival rates. It remains unclear whether the metastatic capability is acquired at the primary site, and also to which extent it is possible to determine the metastatic potential in early stages of cancer development. Here we use a large-scale proteomic approach to elucidate changes in protein expression profiles along the gradient of cancer progression. The recently developed super-SILAC mix for quantification of human tissue has allowed us to analyze an array of clinical human luminal tumor samples, including healthy breast duct epithelia, lymph node negative and positive primary tumors, and lymph node metastases. By quantifying more than 10,000 proteins with high accuracy, we highlight key proteins and processes involved in tumorigenicity. Furthermore, we use support vector machines algorithms to extract a protein signature that segregates between lymph node-negative and lymph node-positive patients, which may aid in early detection of aggressive cancer and contribute to disease management.

CENTROSOME DEPLETION IN OVARIAN CANCER SENSITIZES CELLS TO DNA DAMAGE

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The STIL gene encodes a 150 KD cytosolic mitotically regulated phospho-protein which is expressed exclusively in proliferating cells. Increased expression of STIL characterizes cancer types and was shown to be required for cancer cell survival and to be associated with increased risk of metastasis. Recently, STIL was shown to be essential for centriolar biogenesis and duplication. Knockdown of STIL leads to loss of centrioles and results in block at mitotic entry and P53 independent apoptosis in many cancer cell lines. The requirement of STIL for cell survival is relatively selective for cancer cells as fibroblasts and embryonic stem cells lacking STIL slow their entrance to mitosis without undergoing apoptosis. A link between the centrosome over-duplication and the DNA damage response has been suggested. To examine whether the centrosomal defects caused by STIL knockdown are associated with increased DNA damage we have silenced its expression using siRNA in ovarian cancer cell lines. We demonstrate that STIL knockdown reduces centrosome number, significantly increases gamma H2AX foci in cells nucleus and dramatically enhances their response to DNA damaging chemotherapy and radiotherapy. Importantly, cells silenced for STIL expression are not more sensitive to Paclitaxel, a microtubule stabilizing agent, indicating that the increased sensitivity to DNA damage causing agents is specific. SiRNA depletion of SAS6, another protein involved in centrosomal biogenesis, enhanced the response to cisplatin and radiotherapy in a similar manner to STIL siRNA, indicating that the enhanced sensitivity to DNA damage is due to centrosomal defects and is not specific to STIL silencing. We have extended these in-vitro observations to an in-vivo xenograft orthotropic ovarian cancer model. After establishment of peritoneal human ovarian tumors, mice treated with cisplatinum and siRNA against STIL, packed in DOPC-nanoliposomes, had significantly smaller tumors compared to control untreated tumors or each of the single therapies. These results indicate that targeting STIL and/or centrosomes enhances sensitivity to DNA damaging anti-cancer therapies.

TOWARD PRECISION CANCER MEDICINE: A SIMPLIFIED INTERVENTIONAL MAPPING SYSTEM (SIMS) FOR THE SELECTION OF COMBINATIONS OF TARGETED TREATMENTS IN NON-SMALL CELL LUNG CANCER

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Non-small cell lung cancer (NSCLC) is a leading cause of death worldwide. Targeted monotherapies produce high regression rates, albeit for limited patient subgroups, who inevitably succumb. We present a novel strategy for identifying customized combinations of triplets of targeted agents, utilizing a simplified interventional mapping system (SIMS) that merges knowledge about existent drugs and their impact on the hallmarks of cancer. Based on interrogation of matched lung tumor and normal tissue using targeted genomic sequencing, copy number variation, transcriptomics, and miRNA expression, the activation status of 24 interventional nodes was elucidated. An algorithm was developed to create a scoring system that enables ranking of the activated interventional nodes for each patient. Based on the trends of co-activation at interventional points, combinations of drug triplets were defined in order to overcome resistance. The use of this methodology will inform a prospective trial to be conducted by the WIN consortium ('SPRING'), aiming to significantly impact survival in metastatic NSCLC and other malignancies. The SPRING trial, as well an existing trial ('WINTHER'), will be used to highlight some of the difficulties of testing precision cancer medicine, and will offer complementary means of addressing such difficulties.

A COMPARATIVE STUDY OF FOLATE RECEPTOR-TARGETED DOXORUBICIN DELIVERY SYSTEMS: DOSING REGIMENS AND THERAPEUTIC INDEX

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Ligand-receptor mediated targeting may affect differently the performance of supramolecular drug carriers depending on the nature of the nanocarrier. In this study, we compare the selectivity, safety and activity of doxorubicin (Dox) entrapped in liposomes versus Dox conjugated to polymeric nanocarriers in the presence or absence of a folic acid (FA)-targeting ligand to cancer cells that overexpress the folate receptor (FR). Two pullulan (Pull)-based conjugates of Dox were synthesized, (FA-PEG)-Pull-(Cyst-Dox) and (NH2-PEG)-Pull-(Cyst-Dox). The other delivery systems are Dox loaded PEGylated liposomes (PLD, Doxil®) and the FR-targeted version (PLD-FA) obtained by ligand post-insertion into the commercial formulation. Both receptor-targeted drug delivery systems (DDS) were shown to interact in vitro specifically with cells via the folate ligand. Treatment of FR-overexpressing human cervical carcinoma KB tumor-bearing mice with three-weekly injections resulted in slightly enhanced anticancer activity of PLD-FA compared to PLD and no activity for both pullulan-based conjugates. When the DDS were administered intravenously every other day, the folated-Pull conjugate and the non-folated-Pull conjugate displayed similar and low antitumor activity as free Dox. At this dosing regimen, the liposome-based formulations displayed enhanced antitumor activity with an advantage to the non-folated liposome. However, both liposomal formulations suffered from toxicity that was reversible following treatment discontinuation. Using a daily dosing schedule, with higher cumulative dose, the folated-Pull conjugate strongly inhibited tumor growth while free Dox was toxic at this regimen. For polymeric constructs, increasing dose intensity and cumulative dose strongly affects the therapeutic index and reveals a major therapeutic advantage for the FR-targeted formulation. All DDS were able to abrogate doxorubicin-induced cardiotoxicity. This study constitutes the first side-by-side comparison of two receptor-targeted ligand-bearing systems, polymer therapeutics versus nanoparticulate systems, evaluated in the same mouse tumor model at several dosing regimens.

A NOVEL NANOPARTICLE-BASED CT IMAGING TECHNIQUE: IN VIVO CELL TRACKING TO STUDY ADOPTIVE T-CELL IMMUNOTHERAPY

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Cell-based therapy is defined as the transplantation of living cells for the treatment of diseases and injuries. This therapeutic strategy offers a promising solution for the treatment of various pathologies and development of medical procedures ranging from organ transplantation to cancer immunotherapy. In that regard, in vivo cell tracking could help providing essential knowledge regarding trafficking patterns and poorly-understood mechanisms underlying the success or failure of cell therapy. Thus, the development of an accurate and quantitative imaging technique which will allow for non-invasive in vivo cell tracking is required. To answer this need, we designed a novel methodology for longitudinal and quantitative in vivo cell tracking. We surmised that uniting the superior visualization abilities of classical X-ray CT with state-of-the-art nanotechnology would represent a major development for high-resolution cell tracking. In this project, we labeled with GNPs primary human T-cells that were transduced to express a melanoma specific TCR. After calibration of the amount of GNPs as well as the labeling time, we studied the function of these T-cells. In vitro studies revealed that GNP-labeling did not hampered T-cell function, as demonstrated in cytokine release and proliferation assays. Labeled cells were adoptively transferred to melanoma-bearing immunodeficient mice. Whole body imaging using computed tomography (CT) enabled us to examine the distribution and the migration and persistence of T-cells in the tumor vicinity. This new method offers a valuable tool for research and more importantly for clinical applications to study the fate of T-cells in cancer immunotherapy and transplantation.

MITOCHONDRIAL INDUCTION AS A POTENTIAL RADIOSENSITIZIER IN LUNG CANCER

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The unique metabolism of most solid tumors, including lung cancer, stems from remodeling mitochondrial functions to produce a glycolytic phenotype (Warburg effect). The reduction in the mitochondrial activity may contribute to a strong resistance to apoptosis. There is a growing body of evidence indicating that mitochondrial activation may be a target for cancer therapeutics. Potential mechanisms include increase sensitivity to apoptosis and release of oxidative stress. Cancer specific remodeling can be reversed by a small molecule named dichloroacetate (DCA), which promotes mitochondrial activation by increasing the influx of pyruvate. Sodium oxamate, another molecule that interferes with cells metabolism, inhibits the formation of lactate-the end product of glycolysis by inhibiting the enzyme Lactate Dehydrogenase (LDH) Here, we tested whether mitochondrial induction (by DCA and/or sodium oxamate may induce cell' death and increase the sensitivity of non-small cell lung cancer (NSCLC) cells to radiation alone or in combination. Methods: Two representative NSCLC cell lines (A549 and H1299) were tested for their sensitivity to radiation with and without pre-exposure to DCA and sodium oxamate. The treatment efficacy was evaluated using a clonogenic survival assay. An extracellular flux analyzer was used to assess the effect of DCA on cellular oxygen consumption as a surrogate marker for mitochondrial activity. Results: DCA increases the oxygen consumption rate in both A549 and H1299 cells by 60 % ($p = 0.0037$) and 20 % ($p = 0.0039$), respectively. Pre-exposure to DCA one hour before radiation increased the cytotoxic death rate 4-fold in A549 cells (55 to 13 %, $p = 0.004$) and 2-fold in H1299 cells (35 to 17 %, $p = 0.28$) respectively, compared to radiation alone. Sodium Oxamate radisosensitized H1299 cells as well (44% to 26%). Double treatment with DCA and Sodium Oxamate enhances the radiosensitivity of H1299 cells 5 folds. Conclusion Mitochondrial activation may serve as a radio-sensitizer in the treatment of non-small cell lung cancer. Inhibition of the end stage of glycolysis by sodium oxamate increases the effect of mitochondrial induction (by DCA).

STABLE KNOCKDOWN OF CREB HIF-1 AND HIF-2 BY REPLICATION COMPETENT RETROVIRUSES ABROGATES THE RESPONSES TO HYPOXIA IN HEPATOCELLULAR CARCINOMA

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The fast proliferation of tumor cells develops faster than the vasculature, resulting, in most malignant tumors, in generation of hypoxic regions. Hypoxia renders solid tumors more resistant to ionizing radiation and chemotherapeutic drugs while providing opportunities for tumor-selective therapies that exploit tumor hypoxia as a treatment target. In this work we exploit two properties of tumors: propagation of tumor cells and ongoing generation of hypoxic regions, to construct a system that preferentially leads to the death of tumor cells and thus hinders tumor growth. We constructed Murine Leukaemia virus (MuLV) replication competent (RCR) viruses that infect only propagating cells. These viruses express shRNAs targeting CREB, HIF-1 or HIF-2 individually or all three together. These viruses efficiently infected in vitro human hepatocellular carcinoma (HepG2) cells and established persistence of the virus and knocked down the expression of the regulators of the hypoxia responding genes. Knockdown of either HIF-1 or CREB or both in hypoxia reduced the expression of the HRE and CRE mediated gene expression, diminished cell proliferation and increased caspase-3 activity. We did not detect any significant effect of the efficiently knocked down HIF-2 on any of the functions tested in-vitro. Moreover, SCID mice implanted subcutaneously with HepG2 stably infected with recombinant RCRs showed reduction of tumor growth and VEGF expression, and no hypoxia guided neovascularization. Combined treatment (RCRs+doxorubicin) improves efficacy in the context of in vitro hypoxia. This synergistic effect may lead to an improved efficacy and safety profile of the treatment that may result in fewer side effects.

COMBINING CHEMOTHERAPY WITH IMMUNOTHERAPY FOR THE TREATMENT OF GLIOBLASTOMA

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Glioblastoma multiforme (GBM) is a deadly type of brain cancer. Most Glioblastoma patients face grim prognosis, therefore, better targeted therapeutic treatments, such as immunotherapy are needed. Potentially useful sources of tumor antigens for development of Glioblastoma immunotherapy are the pools of HLA-bound peptides (the HLA peptidome or immunopeptidome). One group of antigens that can be used for immunotherapy is tumor antigens that are expressed only in tumor cells, embryonal and immune-privileged tissues, such as the testis, placenta and ovaries. Many of these cancer antigens and their derived HLA peptides can be epigenetically up-regulated in tumors' cells, following exposure to DNA demethylating chemotherapy agents, such as 5-aza-2'-deoxycytidine (Decitabine). This project aims to study the HLA peptidomes of human Glioblastoma tumors and cultured cells to identify tumor specific peptides for development of Glioblastoma immunotherapy. We are focus on identifying an up-regulated or newly synthesized HLA peptides that are presented by Glioblastoma cells following treatment with Decitabine chemotherapy. We specifically search for antigens that are derived from tumor-testis genes, aiming to combine such chemotherapy with a cancer immunotherapy composed of the HLA peptides that are induced by the chemotherapy treatment. We hope that such treatment will enhance killing of tumor cells by T cells directed against the cancer-testis antigens. Such strategy can be rapidly implemented for treating patients in the clinic. Our preliminary results show significant and consistent changes in the HLA peptidome of the cancer cells' HLA peptidomes after the treatment with this chemotherapeutic and include identification of many HLA peptides that are derived from proteins that are not normally expressed in healthy tissues of the body.

THE HPV E6 PROTEINS OF MUCOSAL AND CUTANEOUS HPV TYPES COOPERATE WITH E6AP TO STIMULATE AND AUGMENT WNT SIGNALING

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Certain cutaneous human papilloma viruses (HPVs) which belong to the β-PV genus have been associated with the development of non-melanoma skin cancer (NMSC). The mechanisms by which the viruses contribute to the of skin cancer are presently being investigated. Our previous studies showed that E6 protein of HPV16, a mucosal α HPV type, is capable to cooperate with the ubiquitin ligase E6AP, to enhance the oncogenic Wnt/β-catenin pathway. HPV16 E6 increased Wnt/β-catenin signaling by both stimulation and augmentation of the signal, through different mechanisms. In the present study, we investigated the ability of E6 proteins of several β and α cutaneous HPV types to potentiate Wnt signaling. We show that E6 proteins from cutaneous β (8, 24, 38, 49) and α (10) HPV types were capable to augment Wnt signaling. E6AP increased the activity of E6 by stabilizing the E6 proteins. Both wild type and mutant E6AP that lacks the ubiquitin ligase activity were capable to stabilize E6 and augment Wnt/β-catenin transcription. The ability of E6AP to elevate β-catenin/TCF transcription correlated with the ability of E6AP to associate with E6, showing higher ability with the α E6 proteins. The E6 proteins of the cutaneous HPVs were also capable to stimulate Wnt signaling. The stimulation was associated with stabilization of β-catenin with the α types exhibiting higher activity in stabilization of β-catenin and stimulation of Wnt/β-catenin transcription. This study revealed the role of cutaneous HPV types in potentiating the Wnt/β-catenin signaling pathway, which may contribute to skin carcinogenesis.

RNF20 LINKS HISTONE H2B UBIQUITYLATION WITH INFLAMMATION AND INFLAMMATION-ASSOCIATED CANCER

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Factors linking inflammation and cancer are of great interest. We report that the chromatin-targeting E3 ubiquitin ligase RNF20 modulates inflammation and associated cancer in mice and humans. With its partner RNF40, RNF20 drives histone H2B monoubiquitylation (H2Bub1). Downregulation of RNF20 and H2Bub1 decreases the heterochromatin mark H3K9me3 on a subset of NF-κB target genes, favoring recruitment of p65-containing NF-κB dimers over repressive p50 homodimers and augmenting transcription. Concordantly, RNF20+/- mice are predisposed to colonic inflammation and associated colorectal cancer. Notably, ulcerative colitis patient colons tend to underexpress RNF20 and RNF40. Furthermore, colorectal tumors exhibit reduced RNF20/RNF40 expression and a progressive decrease in H2Bub1. Reduced RNF20 may promote cancer also by upregulating myeloid-derived suppressor cells that quench antitumoral T cell activity. Our study highlights non cell-autonomous functions of H2Bub1 and suggests that inherently low H2Bub1 might predispose to inflammatory diseases and cancer, while further H2Bub1 downregulation might accelerate cancer progression.

THERAPEUTIC GENE SILENCING IN MANTLE CELL LYMPHOMA

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Mantle Cell Lymphoma (MCL) is a rare and incurable B-cell malignancy bearing a unique genetic profile: It is defined by a chromosomal translocation that repositions the proto-oncogene cyclin D1 downstream of the immunoglobulin heavy chain gene promotor. This genetic aberration leads to the constitutive over-expression of cyclin D1, a gene that is not detected in healthy B-lymphocytes. To date, MCL therapy relies on general features of cancer or B-lymphocytes (chemotherapy cocktails, anti-CD20 mAbs, etc.), but unfortunately all patients eventually relapse and die of the disease, raising an urgent need for new therapeutic approaches. We focused on utilizing the well-defined genetic footprint of MCL, cyclin D1 overexpression, as a starting point for a new therapeutic strategy. We previously demonstrated using an electroporation that siRNA-mediated gene silencing of cyclin D1 induce cell death in MCL cell lines, highlighting cyclin D1 as a potential therapeutic target. However, in order to transform these findings into a therapeutic modality in vivo, it is imperative to develop a potent siRNA delivery strategy targeted towards MCL cells.
Herein, using an advanced form of Lipid Nanoparticles (LNPs) encapsulating siRNA, we were able to obtain efficient gene silencing in MCL cell lines. Next, we covalently coated the LNPs with a MCL-specific targeting moiety by attaching an anti-CD38 monoclonal antibody. Using these CD38-LNPs, we succeeded to specifically target MCL cells in vitro in MCL cell lines, ex vivo in samples extracted from MCL patients, and in vivo in tumor bearing mice, as well as to induce therapeutic gene silencing in these various experimental settings. These results open new avenues in utilizing RNAi for therapeutics in MCL and ultimately might become a new therapeutic modality in other hematological malignancies.

CANCER-ASSOCIATED FIBROBLASTS AS A "CELL STATE" OF DIFFERENT CELL TYPES IN THE TUMOR MICROENVIRONMENT OF TONGUE CARCINOMA

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Objectives: Cancer-associated fibroblasts (CAFs) share a mesenchymal morphology and a tumor-promoting function, but are heterogeneous in their origin. Therefore, it was proposed to refer to CAF as a "cell state" rather than a "cell type" in order to encompass the diversity of the origins of these cells. CAFs in the tumor microenvironment (TME) of tongue carcinoma were found to be an independent prognostic factor for poor survival. We aimed to examine presence of different cells that show a "cell state" of CAFs in tongue carcinoma and investigate how these relate to clinical outcomes.

Methods: Study group comprised of CAF-poor (n=24) and CAF-rich (n=28) tongue carcinoma cases as defined in a previous study (Dayan et al, Cancer Med, 2012;1:128-40). Serial sections were double-immunostained with α-smooth muscle actin (SMA, CAF marker) combined with Nanog (mesenchymal stem cell marker), CD133 (hematopoietic/endothelial stem cell marker), and with CD80 and CD86 (myeloid/monocytic-derived cell markers). Density of cells co-expressing each of these combinations was assessed as 0 – no double-stained cells, 1 – isolated positive cells, 2 – considerable positive cells.

Results: The double immunostains showed that stromal CAF-like cells (αSMA+) commonly co-expressed CD80 or CD86. Increased density of CD86+αSMA+ stromal cells was related to CAF-rich rather than to CAF-poor tumors (p<0.05). Almost no Nanog-αSMA cells were found and those expressing CD133+αSMA+ were found only in association with blood vessels. Abundance of αSMA+CAFs was associated with disease recurrence and poor survival (p<0.05). Presence of CAFs expressing CD86+αSMA+ was found to be associated with poor survival (p<0.05). Conclusion: The myeloid/monocytic cells in a CAF "cell state" constituted the main sub-population of CAFs in the tumor microenvironment of tongue carcinoma and had a negative impact on survival. Further multicenter studies are needed to define the profile of CAFs cell state in tongue carcinoma for developing better treatment approaches.

HEPARANASE INHIBITORS AND NEUTRALIZING ANTIBODIES ATTENUATE LYMPHOMA GROWTH

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Heparanase activity is strongly implicated in the progression of many carcinomas, and anti-heparanase inhibitors are currently evaluated in phase I clinical trials. While a role of heparanase was revealed in multiple myeloma, its significance in human lymphomas has not been so far examined. We found that heparanase expression is induced in 50% of patients with B-cell lymphoma. Notably, the heparanase inhibitor PG545 efficiently attenuated the growth of tumor xenografts produced by lymphoma cells. This was associated with decreased tumor angiogenesis (CD31 staining) and increased apoptosis (cleaved caspase-3). In addition, PG545 markedly inhibited metastatic dissemination of lymphoma and myeloma cells. In-vitro, treatment with PG545 results in increased apoptosis measured by elevated staining with Propidium Iodide and Annexin V, and higher levels of cleaved caspase 3 and PARP-1. Furthermore, PG545 treatment resulted in decreased phosphorylation and expression of lymphoma-associated signaling molecules such as Akt, Src, IkB, Myc, and Bcl-6, as well as decreased histone methylation, and alteration of the cell cycle evident by FACS analysis. Notably, heparanase-neutralizing polyclonal antibody also effectively attenuated lymphoma growth by inhibition of angiogenesis and increased tumor necrosis. However, heparanase neutralizing monoclonal antibodies 9E8 and 1023 were not as efficient in restraining tumor growth as single agents but showed modest tumor inhibition once combined. These results tie heparanase with human lymphoma and suggest that anti-heparanase strategies may be applied in hematological malignancies.

NOVEL TREATMENT MODALITY TARGETING HER-2 POSITIVE BREAST INDUCES CANCER APOPTOSIS AND IMMUNE CELL ACTIVATION

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Breast cancer is currently the most common cancer in women and the second leading cause of cancer-related deaths in females. Overexpression of HER-2 occurs in 20-30 % of breast cancer and is associated with increased aggressiveness and significantly shortened disease-free and overall survival. Trastuzumab is the most commonly used therapy for HER-2 positive breast cancer. However, resistance often develops, due to cellular heterogeneity and failure of immunosurveillance and the overall response rate remains modest. Herein, we aim to develop a novel targeted therapy to eradicate HER-2 overexpressing breast cancers by inducing both cancer cell apoptosis and activation of the immune system selectively against tumor cells, while sparing the non-cancerous cells. We have recently shown that targeted delivery of synthetic dsRNA, Poly Inosine Poly Cytosine (pIC), a known activator of pro-apoptotic and pro-inflammatory processes, into EGFR overexpressing cancers induced tumor cell killing and the expression of cytokines in EGFR-overexpressing tumors both in vitro and in vivo. In the current study we have designed a novel chemical vector composed of linear polyethylenimine-polyethyleneglycol and a high affinity targeting moiety, HER-2 Affibody, (PPHAffibody), to deliver pIC to HER-2 overexpressing breast cancer cells. We demonstrated that targeted delivery of pIC into HER-2 overexpressing breast cancer cells in vitro selectively induced apoptosis in HER-2 overexpressing cells while cells that express lower levels of HER-2 or do not express the receptor remained unaffected. Confocal microscopy confirmed the selectivity of pIC/PPHAffibody binding and internalization into HER-2 overexpressing cells. Furthermore, targeted delivery of pIC using HER-2 targeting vector induced the secretion of pro-inflammatory cytokines including Type I IFN, IP-10 and RANTES and promoted peripheral blood mononuclear cells (PBMC) chemotaxis. Additionally, using co culture experiments we found that targeted delivery of pIC promotes immune cell activation and further eradication of HER-2 overexpressing tumor cells. Significantly, the HER2 targeted pIC strongly inhibits the growth of HER2 overexpressing tumors in nude mice and much more strongly the growth of these tumors in immune competent mice. The ability of pIC to restore immune surveillance and induce targeted breast cancer cell apoptosis provides significant advantages over current therapies and is expected to open a new avenue in cancer therapy.

THE IMPACT OF MULTIPLE DRUGS OR MUTATIONS ON BIOLOGICAL SYSTEMS: A MECHANISM-FREE PREDICTIVE FORMULA

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Treating cancer with combinations of drugs may play an important role in controlling the disease and making it chronic but bearable. High order combinations, such as combinations of four or more drugs in a cocktail may eliminate acquired resistance, be effective at low doses minimizing side effects, and may provide the freedom to rationally design special cocktails for different pathologies and individual patients. However, screening all combinations for every disease is impossible because the number of experiments needed grows exponentially with the number of drugs and doses. To address this, we scanned a wide range of mathematical formulae which predict the effects of three or more anti-cancer drugs based on the measurements of single drugs and drug pairs, without need for mechanistic information. We show that a single formula obeys all requirements to predict high order cocktails effects on cancer cells, and this formula has high explained variance when measurements were compared with different models. Remarkably, we find that the same formula also applies to other biological perturbations: the effect of multiple antibiotics on bacterial growth, and the effect of multiple mutations on a phenotype such as enzyme activity. This suggests a deep connection between the ways that high-order perturbations add up to affect different biological systems. The formula opens a new way to estimate the epistatic effects of multiple mutations in DNA sequences, a major obstacle in understanding human genetics, and to search for multi-drug combinations, using a small number of experiments.

GENETIC ALTERATIONS

MIR-377 TARGETS E2F3 AND ALTERS THE NF-KB SIGNALING PATHWAY THROUGH MAP3K7 IN MALIGNANT MELANOMA

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Background: The incidence of cutaneous malignant melanoma continues to rise, and once the disease metastasizes it is almost inevitably fatal. We were the first to report that a large micro-RNA (miRNA) cluster on human chromosome 14q32, implicated in many types of cancers, is significantly down-regulated in melanoma, and have been studying this 'tumor-suppressor miRNA cluster' for several years now. MiR-377, one of the miRNAs located within this cluster, was studied in this work.

Methods: qRT-pCR was used to quantify miR-377 levels in melanoma cell lines and samples. Melanoma cell lines ectopically expressing miR-377 were generated by stable transfection and mRNA expression was assessed using mRNA arrays. Potential targets of miR-377 were identified through luciferase reporter assays. Cellular proliferation, migration and soft-agar colony formation were monitored in control and miR-377-expressing cells using cell biology techniques and protein expression was assessed by western blot.

Results: miR-377 is expressed in normal melanocytes but not in melanoma cell lines or samples. Its ectopic stable expression in melanoma cell lines decreased their proliferative and migratory capacity and their colony-forming capability. mRNA arrays of melanoma cells over-expressing miR-377 pointed to several down-regulated mRNAs that have putative binding sites for miR-377 in their 3'UTR, of which both E2F3 and MAP3K7 were found to be direct targets of miR-377. E2F3, a potent transcriptional inducer of cell-cycle progression, was found to be elevated in melanoma cell lines, but decreased following ectopic expression of miR-377. Ectopic miR-377 also led to a decrease in the activity of a reporter plasmid containing three E2F DNA-binding sites linked to a luciferase cDNA sequence, demonstrating that miR-377 down-regulates E2F3-induced transcription. MAP3K7, a serine/threonine kinase along the MAPK signaling pathway, was over-expressed in melanoma but decreased following ectopic expression of miR-377. MAP3K7 is known to be involved in the activation of NF-κB. MiR-377 over-expression led to decreased activity of a reporter plasmid containing two NF-κB DNA-binding sites and to decreased output along the NF-kB signaling pathway.

Conclusion: Our results suggest that miR-377 is an important negative regulator of E2F and of the MAP3K7/NF-kB signaling pathway in melanoma cells. The NF-kB signaling has been implicated in the acquisition of resistance to B-Raf inhibition. It is tempting to speculate that silencing of mir-377 in melanoma promotes the tumorigenic and metastatic potential of the cells through activation of these pathways. More research is currently underway to study the cross talk between mir-377 and drug sensitivity in melanoma.

CONTRIBUTION OF ENHANCER DNA METHYLATION TO TUMOR PROGRESSION, CANCER CELL PLASTICITY AND PATIENT MORTALITY

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Background: The role of DNA methylation at promoters has been widely studied in cancers, however, other genomic regions have been overlooked. **Results:** Using methylation data from thousands of patients with multiple cancer types, we found highly variable methylation patterns at enhancers. These distal regulatory regions were enriched for markers of strong enhancers, and were highly organ-specific. We integrated RNA-seq expression data and identified altered enhancer-associated genes and microRNAs that promote cancer progression. Additionally, tracing methylation patterns of melanoma, a highly metastatic cancer, revealed that methylation plasticity of enhancers was significantly correlated with poor survival rates, and suggests that DNA methylation of enhancers is a strong prognostic indicator in cancer patients. Conclusions: Dysregulation of methylation of enhancers in cancers is widespread, and is closely related to altered expression of genes supporting tumor initiation and progression. Enhancer methylation likely contributes to cancer progression by increasing cancer plasticity and ultimately patient mortality. This study could be of benefit to the development and improvement of epigenetic anticancer therapies.

A NOVEL BRCA1 FRAME SHIFT MUTATION IN WOMEN OF KURDISH JEWISH DESCENT

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Hereditary cancer comprises more than 10% of all breast cancer cases. In patients with a family history suggestive of a hereditary component, a mutation is often identified in the high penetrant genes BRCA1 and BRCA2. Several founder mutations have been detected in some Jewish communities, yet no BRCA1/2 founder mutation had been known in Kurdish Jews. Here we describe the validation of a 22 hereditary cancer gene panel and a new BRCA1 mutation found in 4 women from 2 unrelated Kurdish Jewish families utilizing this gene panel. A panel spanning the coding sequences of 22 familial cancer related genes was planned. The panel validation included 25 cancer patients with previously identified mutations. All previously identified mutations in the BRCA1, BRCA2, MLH1 and PMS2 genes were detected by this dedicated panel. The panel did not test for large deletions or insertions. Using the validated panel, 40 cancer patients of Kurdish Jewish descent who had been diagnosed with cancer before the age of 50 years were tested. Libraries built using the panel and genomic DNA were sequenced using the Ion Torrent PGM machine. We identified a novel BRCA1 mutation, c.224_227delAAAG, in 2 unrelated Jewish Kurdish families. The probands were diagnosed with cancer at a young age and had significant family history, suggesting a founder mutation in this population. We suggest testing Kurdish Jewish women with a personal or family history of breast and or ovarian cancer for this mutation.

MICRORNA EXPRESSION AS POTENTIAL BIOMARKERS FOR MEDULLOBLASTOMA SUBGROUP CHARACTERIZATION

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Medulloblastoma (MB) is a common malignancy with high heterogeneity in the pediatric population. Physicians currently diagnose MB patients based on clinical features such as histological variance, metastatic stage, extent of resection and age. MB was recently divided into four tumor subgroups with distinct profiles: WNT, SHH, Group 3, and Group 4. These subgroups represent distinct clinical, biological and genetic molecular entities. The ability to easily differentiate between MB classes as well as understanding the biological mechanisms behind their pathology are important for clinical practice and targeted treatment should be designed accordingly. In this study we investigated miRNA expression in MB subtypes in order to identify novel MB miRNA subgroup biomarkers. These biomarkers uniquely represent each subtype, and their mRNA targets belong to known signaling pathways that are typically used for characterizing MB classes. Integrated analysis with paired mRNA expression from the same MB tumors may faithfully depict regulation links between miRNAs and their targeted genes. MiRNAs are clinically relevant in a diagnostic prospective for classification purposes and may add new possibilities for treating MB.

IF YOU CAN'T BEAT THEM CHANGE THEM: A NEW WAY OF THINKING ABOUT TUMOR THERAPY - LESSONS FROM C. ELEGANS

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A major challenge in the tumor therapy field is the development of new strategies to eliminate tumors and cancer cells. Whereas most of the current therapeutic strategies are based on apoptosis induction in the tumor cells, the effectiveness of these approaches is limited due to acquired apoptosis resistance. Thus, alternative therapies need to be developed in order to overcome the escape of tumor cells from the cell death machinery. Here, we studied a C. elegans germline tumor model which recapitulates several aspects of human tumors. We discovered that although the original germline tumor is apoptosis–resistant, its' transformation into a teratoma restored its' sensitivity to apoptosis. Transformation of the tumorous germ cells into apoptosis-prone somatic cells and their subsequent cell death was achieved by inflicting ER stress. This, in turn, suppressed the tumor. Interestingly, we found that ER stress acts cell non-autonomously from the soma, rather than from within the germline tumor, to dictate the transformation and cell death events. In summary, our results imply a novel approach for dealing with aggressive and apoptosis resistant tumors by transforming them into a different type of tumor, a process that can be accompanied by restoration of apoptosis sensitivity.

IDENTIFICATION OF BREAST CANCER SUBTYPES USING HIGH-THROUGHPUT GENOMIC DATA

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Breast cancer is a heterogeneous disease composed of several biologically different subtypes, exhibiting distinct responses to treatment. In the past decade, gene expression profiling enabled the identification of four "intrinsic subtypes" of breast cancer, and microarray based predictors such as PAM50 have been introduced. Despite their advantage over the traditional histopathology approach, precise identification of breast cancer subtypes remains a challenge, hopefully to be met with newer high-throughput genomic technologies. In this study, we applied unsupervised clustering analysis on hundreds of breast cancer samples using RNA-Seq and DNA methylation data. The resulting sample clusters were compared to PAM50 labels and characterized using the available clinical data. Gene enrichment analysis performed on subtype differential genes shed light on the biological themes underlying the new sample groups. Our analysis of the expression data revealed two biologically distinct subgroups of Luminal-A samples, exhibiting differential expression of immune related genes. Analysis of the methylation data identified a cluster of patients with poor survival prognosis, composed of a mixture of Luminal-A, Luminal-B and Her2 labels. By further characterizing these and other novel subtypes we hope to advance our understanding of cancer heterogeneity and also promote the development of subtype specific diagnosis and treatment.

DEFINING PATTERNS OF TUMOR EPIGENETIC HETEROGENEITY

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Intra tumor phenotypic heterogeneity is recognized as a major obstacle to successful treatment of cancer, especially at advanced stages. Various properties of tumor sub-populations, such as self- renewal and differentiation capacity, are correlated with the proliferation, metastatic potential and treatment sensitivity of tumors. DNA methylation is an epigenetically stable marker that can be readily quantified at single allele resolution. This has potential applications for characterizing tumor heterogeneity defining tumor sub populations, resistance patterns and evolutionary history. Typical Formalin Fixed Paraffin Embedded (FFPE) tumor samples, contain a mixture of heterogeneous tumor cells as well as stroma, immune cells and more. Characterization of DNA methylation from such samples therefore requires careful consideration of epigenetic heterogeneity within and between tumor and normal cell populations. We develop experimental strategies and computational tools to facilitate such analysis. Our techniques combine unique molecular identifiers (UMIs) with bisulfite sequencing and algorithms for identifying loci with strong statistical support for intra-population heterogeneity. We also implemented techniques for modelling data from multiple samples from within the same tumor, and for inference of normal vs. tumor heterogeneous methylation patterns based on such data. Application of these methods to cancer samples identify hundreds of loci with strong evidence for epigenetic heterogeneity, and suggest different tumors are highly variable in their degree of epigenetic coherence. UMI-RRBS based analysis of DNA methylation distributions in tumor populations is likely to become an effective tool for analysis of FFPE samples in a variety of clinical applications.

TUMOR MICROENVIRONMENT

ROLE OF MYOSIN, MICROTUBULES AND ACTIN NETWORK ON THE BIOMECHANICAL INTERACTION OF BREAST CANCER CELLS AND THEIR MICROENVIRONMENT DURING INVASION

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Metastasis requires cells to change morphology and apply forces during their invasion through extracellular matrix and neighbouring cells. We evaluated invasion-related force application mechanisms by identifying specific cell elements affecting the mechanical interactions of cancer cells with their substrate. Metastatic breast cancer cells (MDA-MB-231) were used to determine the mechanical role of myosin II, microtubules and nuclear actin network in force application, as defined through invasive forces applied by cells indenting an impenetrable gel. Myosin II isoforms were inhibited by blebbistatin, microtubules were disrupted by applying nocodazole and nuclear actin network assembly was disrupted by using small molecule inhibitor of formin-mediated actin assembly (SMFIH2). Following treatment, we have observed changes in mechanical interaction (e.g. indentation depth, force, and cell area), which revealed the relative impact of acto-myosin, microtubules and actin network assembly in cell invasive force application. Myosin II inhibition decreased the force applied by the MDA-MB-231 cells by 30%, while microtubule disruption effect is 20%. SMIFH2 reduces the indentation depth, force and changes the morphology. We relate the reduction in applied force to changes in cell morphology and the way the cells interact with the substrate.

JDP2 EXPRESSION IN BONE MARROW CELLS INHIBITS TUMOR GROWTH AND PROMOTES METASTASIS

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The activating protein AP-1 is a transcription factor composed of multiple proteins with an important role in cellular processes such as proliferation, differentiation and apoptosis. The c-Jun dimerization protein 2, JDP2, is a bone fide member of the AP-1 family. JDP2 is able to either repress or activate transcription of AP-1 target genes depending on its protein partner. Whereas much has been identified regarding the role of JDP2 in cancer cells, little is known about its role in the tumor microenvironment. Here we studied murine tumor growth in JDP2 knockout (KO) and their wild type (WT) C57Bl/6 counterpart mice, using the syngeneic Lewis Lung Carcinoma and breast cancer induced by polyoma middle T-antigen (PyMT) tumor models. We found a delayed LLC tumor growth implanted in WT mice when compared to JDP2-KO mice. Conversely, analysis of lung metastases revealed a reduced number of metastatic lesions in JDP2-KO mice when compared to their WT counterpart mice. Comparable results in primary tumor growth and its metastasis lesions were observed in lethally irradiated WT mice transplanted with JDP2-KO BM cells. These results indicate that JDP2 expression in BM cells account for aggressive tumor phenotype. Overall, our findings suggest that JDP2 expression has a double-edge sword role in host cells. On one hand, it inhibits primary tumor growth, but on the other, it promotes metastasis. Revealing the mechanism of the action of JDP2 in host cells may pave the way for new therapeutic modalities for treating tumor cell growth and more importantly metastasis.

PKCH CHEMOTHERAPY PROMOTES SENESCENCE INDUCED BY OXIDATIVE STRESS AND

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Senescence is characterized by permanent cell cycle arrest despite continued viability and metabolic activity, in conjunction with the secretion of a complex mixture of extracellular proteins and soluble factors known as the senescence-associated secretory phenotype (SAPS). Cellular senescence has been shown to prevent the proliferation of potentially tumorigenic cells, and is thus generally considered a tumor suppressive process. However, some SASP components may act as pro-tumorigenic mediators on premalignant cells in the microenvironment. A limited number of studies indicated that PKC plays a role in senescence, with different isoforms having opposing effects. It is therefore important to elucidate the functional role of specific PKCs in senescence. Here we show that PKCh an epithelial specific and anti-apoptotic kinase, promotes senescence induced by oxidative stress and DNA damage. We further demonstrate that PKCh promotes senescence through its ability to upregulate the expression of the cell cycle inhibitors p21Cip1 and p27Kip1 and enhance transcription and secretion of IL-6. Moreover, we demonstrate that PKCh creates a positive loop for reinforcing senescence by increasing the transcription of both IL-6 and IL-6 receptor, whereas, the expression of IL-8 is specifically suppressed by PKCh. Thus, the presence/absence of PKCh modulates major components of SASP. Furthermore, we show that the human polymorphic variant of PKCh 374I, that exhibits higher kinase activity in comparison to WT-374V, is also more effective in IL-6 secretion, p21Cip1 expression and the promotion of senescence, further supporting a role for PKCh in senescence. As there is now considerable interest in senescence activation/elimination to control tumor progression, it is first crucial to reveal the molecular regulators of senescence. This will improve our ability to develop new strategies to harness senescence as a potential cancer therapy in the future.

TUMOR PROMOTION AND SUPPRESSION IN A SINGLE MOUSE MODEL: TUMOR DEVELOPMENT IN PPM1A-DEFICIENT MICE

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Using Protein phosphatase metal dependent 1 A (PPM1A) knockout mice, created in our laboratory, we identified PPM1A as a major regulator of the microenvironment, down regulating the inflammation response (Dvashi et al, 2014). In the PPM1A-ablated mice, wound-healing process goes awry and culminates in uncontrolled inflammation and angiogenesis. As these features are comprised among the Hallmarks of Cancer, reflecting the microenvironment response to tumor cell's growth, we investigated the role of PPM1A in cancer using multiple mouse models e.g. chemical carcinogenesis, tumor cell injection, etc. Surprisingly, the absence of PPM1A could be either tumor promoting or tumor suppressive depending on the tumor initiation protocol. Our preliminary studies demonstrate that the absence of PPM1A had a major impact on the tumor microenvironment including stromal stem cells, fibroblasts, immune cells etc. The hypothesis that PPM1A operates at niche critical for the maintenance of tissue homeostasis modulating immune system functions will be discussed.

MOLECULAR SIGNATURE OF BRAIN-METASTASIZING MELANOMA CELLS: THE ROLE OF ANGPTL4 IN MELANOMA PROGRESSION

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The progression of cancer towards metastasis is driven by autonomous traits of the tumor cells as well as by interactions of the cancer cells with non-tumor cells in their vicinity and with soluble factors released or secreted from them. The tumor evolves into an increasing malignant entity and at the same time programs microenvironmental cells to promote tumor progression. Signals delivered by such cells may direct the tumor towards one or several possible molecular evolution pathways; many leading to metastasis. Brain metastases occur frequently in melanoma patients with advanced stage disease whereby the prognosis is dismal. The mechanisms underlying development of melanoma brain metastasis are not well understood. Our study aims to uncover these mechanisms and the interactions between melanoma and brain cells. We developed melanoma xenograft models encompassing cutaneous, brain macro-metastatic and brain micro-metastatic melanoma variants all originating from single melanoma tumors. Using these models we identified a set of melanoma brain metastasis signature genes, including angiopoietin-like 4 (ANGPTL4), expressed more highly in brain metastasizing melanoma cells than in cutaneous cells. To investigate the involvement of ANGPTL4 in melanoma malignancy, we have established cutaneous and brain metastasizing melanoma cells that overexpress ANGPTL4 by viral infection. We have shown that increasing ANGPTL4 expression in cutaneous and brain metastatic melanoma cells altered the malignancy phenotype of melanoma cells in a differential manner, depending on tumor stage. In vivo, ANGPTL4 significantly enhanced tumor growth only in mice inoculated with the cutaneous variant. In vitro, ANGPTL4 altered melanoma cell adhesion to brain endothelial cells (BECs), MMP2 secretion, migration through collagen coated transwells, and transmigration through BECs towards astrocytes. Stimulation of BECs with conditioned medium of mock vs. ANGPTL4 transfected cells has also induced differential regulation of the expression of genes involved in angiogenesis and cell adhesion (IL8, CLDN1, etc.). Our results suggest that the expression and roles of ANGPTL4 may be context and tumor stage dependent. This study was supported by the Dr. Miriam and Sheldon G. Adelson Medical Research Foundation (Needham, MA, USA).

BORTEZOMIB PROMOTES MULTIPLE MYELOMA CELL AGGRESSIVENESS VIA PRO-INFLAMMATORY MACROPHAGES IN MICE

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Multiple myeloma (MM) is a chronic progressive malignancy of plasma cells. Although treatment with the novel proteasome inhibitor, bortezomib, significantly improves patient survival, some patients fail to respond due to the development of de novo resistance. Previous studies revealed that chemotherapy induces pro-tumorigenic host-mediated effects which could explain tumor re-growth and metastasis. Here we show that plasma from bortezomib-treated mice significantly increases migration, viability and proliferation of human MM cells in vitro, compared to plasma from control untreated mice. Comparable results were demonstrated with plasma obtained from patients with MM treated with bortezomib. Additionally, bortezomib induces the mobilization of pro-angiogenic bone marrow cells. Mice treated with bortezomib and subsequently intravenously injected with MM cells succumb to MM aggressiveness earlier than mice treated with the vehicle control. We show that pro-inflammatory macrophages contribute to MM cell aggressiveness in response to bortezomib treatment, in part by secreting interleukin-16(IL-16). Blocking IL-16 in conditioned medium obtained from bortezomib-treated macrophages generated reduced viability of MM cells in vitro. Accordingly, co-inoculation of MM cells with pro-inflammatory macrophages from bortezomib-treated mice accelerates MM disease progression. Taken together, our results suggest that, in addition to the known effective anti-tumor activity of bortezomib, this drug can induce host-driven pro-tumorigenic effects that may promote MM aggressiveness.

CD38 INHIBITION DECREASES MELANOMA EXPANSION AND AMELIORATES METASTATIC BURDEN

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Melanoma has the highest propensity to metastasize to the brain and in many reports is the third most frequent cause of metastatic brain tumors. The tumor microenvironment contains different types of cells including cancer-associated fibroblasts (CAFs). Numerous studies showed that CAFs support the growth and angiogenesis of various types of cancers including melanoma. CD38 is a multifunctional ectoenzyme that uses NAD+ as a substrate to generate second messengers. We have recently shown that targeting CD38 in the tumor microenvironment may serve as a novel approach to treat glioma. Using the syngeneic B16-F10 model of melanoma progression in wild-type and CD38 null mice, we show that CD38 deficiency or treatment with the novel CD38 inhibitor K-rhein, which was identified by us, significantly attenuates melanoma expansion both subcutaneous and in the brain, and prolonged the survival of melanoma bearing mice. This reduction in tumor growth was associated with decreased necrotic area and angiogenesis in Cd38-/- tumors. CD38 deficiency inhibited also primary tumor volume and pulmonary and brain spontaneous metastases in Ret melanoma injected mice When B16-F10 cells were co-injected with WT or Cd38-/- primary fibroblasts tumor volumes were affected mostly and significantly by the genotype of the co-injected fibroblasts, regardless to the hosts genotype; i.e. the tumors grown in Cd38-/--enriched microenvironment were significantly smaller. Our results thus suggest that CD38 participates in the tumor-supporting action of the tumor microenvironment and that targeting CD38 might be a potential therapeutic approach for melanoma treatment.

ROLE OF HEPARANASE IN MAMMARY GLAND DEVELOPMENT AND CANCER PROGRESSION

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Compelling evidence tie heparanase with human cancer, but the timing of its induction and the significance of heparanase in the early phases of tumor initiation and development are largely obscure. Recently, we have shown that over expression of heparanase or its C-terminal domain (8C) that mediate signaling properties of heparanase, by human breast MCF10A cells results in larger and asymmetrical acinar-like structures. In order to explore the role of heparanase in mammary gland development we have targeted heparanase and 8C expression to the mammary gland of transgenic mice utilizing the regulatory elements of the mouse mammary tumor virus (MMTV). Specific targeting of heparanase or the 8C variant to the mammary epithelium at relatively low levels was sufficient to enhance mammary gland development evident by thicker end-buds and more branch alveolar structures that densely fill the mammary fat pad. Enhanced mammary branching morphogenesis was associated with increased STAT5 phosphorylation in heparanase transgenic strains, while decreased STAT5 phosphorylation was detected in Hpa-KO mice. Furthermore, high levels of heparanase/8C expression are maintained during the involution phase of the mammary gland, associating with delayed involution. Notably, orthotopic implantation of EMT6 cells in the mammary gland of MMTV-HPA mice resulted in bigger tumors vs control, indicating that heparanase provided by the microenvironment enhanced tumor growth. Taken together, these results show that over-expression of heparanase in the mammary gland of transgenic mice enhance mammary gland development, delay involution and support tumor growth.

FIBROBLAST-SECRETED CHI3L1 ENHANCES TUMOR GROWTH AND ANGIOGENESIS

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Breast cancer continues to be one of the leading causes of cancer related death in women. Breast tumors are characterized by an extensive desmoplastic stroma, abundantly populated by fibroblasts. Cancer-Associated Fibroblasts (CAFs) are an activated sub-population of stromal fibroblasts, which have different characteristics in different tumor types and tissue locales. CAFs were shown to facilitate tumor growth by supporting tumor cell growth, enhancing angiogenesis and remodeling the extracellular matrix (ECM). During breast carcinogenesis, fibroblasts are reprogrammed to express various factors that facilitate tumor progression, but many of them remain unknown. We found that CHI3L1, also known as YKL-40, is highly upregulated in CAFs isolated from invasive mammary tumors and from their pre-metastatic lungs, as compared with normal mammary and lung fibroblasts, respectively. CHI3L1 is a secreted heparin-binding glycoprotein, induced specifically during the course of inflammation. In addition, CHI3L1 was shown to be expressed and secreted by several types of solid tumors. Although CHI3L1 plays a pivotal role in exacerbating the inflammatory processes and in promoting angiogenesis and remodeling of the extracellular matrix, its functional role in cancer-related inflammation is still largely unknown. We show that CHI3L1 could induce in breast cancer cells the expression of pro-inflammatory and pro-tumorigenic pathways. In vivo, tumors supplemented with CHI3L1 were more vascularized and were more infiltrated with macrophages than control tumors. Furthermore, orthotopic transplantation of breast cancer cells mixed with fibroblasts in which the expression of CHI3L1 was knocked-down resulted in attenuated tumor growth, indicating the functional contribution of CAF-derived CHI3L1. Interestingly, CHI3L1 also increased macrophage migration and upregulation of a pro-invasive gene signature. Taken together, our findings implicate fibroblast-derived CHI3L1 as a key player in the crosstalk between tumor cells and their microenvironment, and deepen our understanding of the contribution of CAFs to tumor progression and metastasis.

BONE MARROW MESENCHYMAL STEM CELLS' CONDITIONING BY MULTIPLE MYELOMA CELLS: TRANSLATION INITIATION AS THE PLAYING FIELD

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Background: Myeloma cells' (MM) interaction with the bone marrow (BM) microenvironment is integral to disease progression and drug resistance. Mesenchymal stem cells (MSCs) are an important component of BM niche. Protein syntheses mostly regulated at the initiation phase is critically important to the cells' phenotype. Previously we showed that MM cells affect MSCs' translation initiation (eIF4E, eIF4GI). Here, we assessed the time line of this phenomenon and its reversibility.

Methods: MSCs were isolated and propagated from BM of normal donors (ND). ND-MSCs were co-cultured with U266 or ARP1 MM cell lines (MMcond-MSCs)(1-3 days). MMcond-MSCs were counted and assayed for death (trypan blue), viability (WST1), and expression of eIF4E, eIF4GI, their regulators (Mnk1/2, 4EBP), and targets (cyclin D1, NFkB, for eIF4E; SMAD5, for eIF4GI; c-Myc, HIF1-α for both)(immunoblotting). MMcond-MSCs were re-cultured alone (1-14 days) then harvested and immunoblotted for eIF4E, eIF4GI to test reversibility.

Results: MMcond-MSCs (U266 and ARP1) displayed increased total/dead cell counts and viability after 1 day of co-culture (↑>130%; p<0.01) that persisted through 3 days of co-culture. Elevated eIF4E/eIF4GI expressions were already observed after 1 day of co-culture with U266 (↑>140%; p<0.05) and 2 days with ARP1 (↑>190%; p<0.05). The expressions continued to rise in the proceeding day and retained that level for the next day as well (↑>190%, p<0.05). MMcond-MSCs displayed increased levels of eIF4E/eIF4GI regulators in the 1st and 2nd days (↑>220%, p<0.05) and targets on the 3rd day (↑>250%; p<0.05). Preliminary results show eIF4E/eIF4GI modulation by co-culture with MM is reversible.

Discussion: These results demonstrate that crosstalk between MM cells and BM-MSCs affects BM-MSCs phenotype and translation initiation status in a time dependent manner and that this effect is contingent on the cell populations' proximity. Future studies need to address the mechanism by which the cell populations communicate and the possibilities of blocking this crosstalk.

FIRST TRIMESTER PLACENTAL FACTORS INDUCE BREAST CANCER CELL AUTOPHAGY

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Purpose: Placental factors, progesterone included, facilitate breast cancer cell line (BCCL) motility and thus may contribute to the advanced breast cancer found during pregnancy. Cancer and placental implantations are similar; the last is accompanied by extravillous trophoblast cell invasion and autophagy which are interlinked. We aimed to analyze the effect of first trimester human placenta on BCCL autophagy.

Methods: BCCLs (MCF-7/T47D) were cultured with placental explants (60hr) or placental supernatants (24hr). Following cultures, BCCLs were sorted out for RNA/protein extraction. RNA served for microarray/qPCR (BNIP3) and protein for western-blot (HIF1α, LC3BII) analyses. Inhibitors were added to the placenta-MCF-7 coculture or placental supernatants (autophagy inhibitor-3MA, progesterone receptor (PR) inhibitor-RU486 and HIF1α inhibitor-Vitexin) in order to evaluate their effects on BCCL motility and LC3BII/HIF1α expression.

Results: LC3BII (an autophagy marker) expression was elevated in BCCLs following placental explant coculture and exposure to placental supernatants. The autophagy inhibitor (3MA) repressed the placenta induced MCF-7/T47D migration, establishing a connection between BCCL autophagy and migration. Microarray analysis of MCF-7 following placenta-MCF-7 coculture showed that "HIF1α pathway", a known autophagy facilitator, was significantly manipulated. Indeed, placental factors elevated HIF1α and its target BNIP3 in the BCCLs, verifying array results. Lastly, PR inhibitor reduced HIF1α expression and both PR and HIF1a inhibitors reduced MCF-7 LC3BII expression and motility, suggesting involvement of the PR-HIF1a axis in the autophagy process.

Conclusion: Placental factors induced BCCL autophagy that is interlinked to their motility. This suggests that autophagy related molecules may serve as targets for therapy in pregnancy associated breast cancer.

INFLAMMATORY CANCER-ASSOCIATED FIBROBLASTS: ORCHESTRATING THERAPY RESPONSE

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Breast tumors are characterized by an extensive desmoplastic stroma, abundantly populated by fibroblasts. Cancer-associated fibroblasts (CAFs) are the most abundant cell type in tumor stroma. CAFs are an essential component of the tumor microenvironment involved in many processes that promote tumor progression. Inflammation is now considered a hallmark of cancer and CAFs were shown to be mediators of tumor-promoting inflammation. Recently, inflammatory cells in the tumor microenvironment were implicated in conferring drug resistance that frequently hinders cancer therapy. However, the role of CAF-mediated inflammatory signaling in affecting tumor response to cytotoxic therapy is unknown. We show that the expression of a pro-inflammatory gene signature by CAFs isolated from mammary tumors is upregulated upon exposure to chemotherapeutic agents. Moreover, CAFs isolated from mammary tumors promote breast cancer cells survival upon chemotherapeutic treatment in a co-culture system, suggesting that CAFs may be able to promote chemo-resistance. In addition, we demonstrate that an inflammatory signature is acquired by normal lung fibroblasts when treated with chemotherapeutic agents, suggesting that systemic chemotherapy may promote drug resistance by instigating an inflammatory response of fibroblasts at distant sites upon systemic cytotoxic treatment, which may promote tumor cells spread and metastasis. We are characterizing the functional role of CAF-mediated inflammatory signaling in acquiring chemo-resistance by utilizing a transgenic mouse model of spontaneous breast carcinoma and lung metastasis (MMTV-PyMT). Uncovering specific molecular pathways that contribute to the reciprocal interactions between inflammatory CAFs and cancer cells following chemotherapy will hopefully uncover a new mechanism by which CAFs affect tumor response to chemotherapy.

THE RELATIONSHIP BETWEEN A NEW INDEX OF VAGAL NEUROIMMUNOMODULATION AND SURVIVAL IN TWO FATAL CANCERS

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The vagus nerve may slow tumor progression possibly since it inhibits inflammation. This study examined the relationship between a new vagal neuroimmuno-modulation (NIM) index and survival in two fatal cancers. Sample 1 included 202 patients with advanced pancreatic cancer (PC), while sample 2 included 71 patients with non-small cell lung cancer (NSCLC). We retroactively derived markers of vagal activity indexed by heart-rate variability (HRV), specifically RMSSD, from patients' electrocardiograms near diagnosis. In sample 1 we examined overall survival while in sample 2, we focused on survival time in deceased patients. The NIM-index was the ratio of RMSSD to C-reactive protein levels (RMSSD/CRP). In a multivariate cox regression, controlling for confounders (e.g., metastasis location), the NIM-index had a protective relative risk (R.R) and 95% confidence interval (95% CI) of R.R = 0.65, 95% CI: 0.52-0.80, p < 0.001 in PC. PC patients with higher NIM survived 106.6 days compared to those with lower NIM (51.05 days, p < 0.001). In NSCLC patients, the NIM-index was positively correlated with survival time (r = 0.32, p < 0.01), independent of confounders (e.g., age, treatments). Again, patients with a higher NIM index survived more days (475.2) than those with lower NIM (285.1; p < 0.05). These results show that a NIM-index which may reflect vagal modulation of inflammation predicts longer survival in patients with two fatal cancers. Though based on correlational evidence, these results help to understand neuro-modulation of tumors, provide a new estimation of cancer prognosis and propose testing effects of vagal nerve activation on cancer patients' prognosis in experimental randomized controlled trials.

HEPATIC MACROPHAGES AS NOVEL TARGETS OF ERYTHROPOIETIN

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Erythropoietin (EPO) synthesized by the kidney and by the fetal liver is the major hormone that drives mammalian erythropoiesis. It is thus used in treatment of anemia, associated with chronic renal failure, as well as for alleviating chemotherapy or radiotherapy induced anemia in certain cancer patients. The well-known physiological role of EPO is to regulate red blood cells production via its receptor, EPO-R. However, EPO-R was found also in non-erytroid cells, such as bone marrow derived macrophages (BMDM) as we have previously reported, suggesting that EPO may have pleiotropic activities. In this study demonstrate EPO effects on hepatic macrophages. Using C57BL/6 wt mice, injected with 180U of recombinant human EPO (rHuEPO) we found that cells displayed a 2 fold increase in the percentage of mononuclear-F4/80+/CD11b+ double positive cells (p=0.03), as compared to their untreated littermates. Similar results were observed in an experimental murine model of multiple myeloma (5T33MM). Furthermore, we found that C57BL/6 wt mice, injected with 180U of rHuEPO, displayed a 1.4 fold increase (p=0.02) in the number of CD45+/F4/80 low/CD11b high cells (liver monocyte-derived macrophages) and a 1.6 fold increase (p=0.05) of CD45+/F4/80 high/CD11b low cells (Kupffer cells). We thus questioned whether Kupffer cells are direct targets of EPO, utilizing the rat Kupffer cell line (KCL-2). We demonstrate that these cells express EPO-R mRNA and cell surface EPO-R, as detected by our novel EPO-R antibody, termed GM1012 (Br J Haematol 2015). Following treatment of KCL-2 cells with EPO, EPO-R mRNA levels increased by 1.5 fold (p=0.02) and surface EPO-R levels decreased by 2 fold (p=0.01). Stimulation of the KCL-2 cells with EPO led to activated MAPK signaling, induced a 50% increase (p<0.005) in cell migration, a 40% increase (p=0.05) in phagocytosis of microbeads and a 13% increase (p=0.03) in phagocytosis of E.coli. A 3 fold increase (p=0.04) in the levels of the CCL-2 mRNA and a 15% increase (p=0.05) in the chemokine secretion levels following treatment with EPO, points to a possible mechanism by which monocytes are recruited to the liver, where they differentiate into hepatic macrophages. This is in line with our in vivo observations. Our findings strongly suggest new function of EPO, in the healthy liver as well as in pathological conditions.

HEPARANASE 2 ATTENUATES HEAD AND NECK TUMOR VASCULARITY AND GROWTH

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Heparanase is the only functional endoglycosidase capable of cleaving heparan sulfate (HS) in mammals, activity that is highly implicated in cell dissemination associated with tumor metastasis and inflammation. The clinical significance of heparanase activity critically emerges from numerous recent publications describing induced heparanase expression in human hematological and solid tumors, and its inverse correlation with post operative patients' survival, thus encouraging the development of heparanase inhibitors. Based on amino acid sequence, cloning of heparanase homolog termed heparanase 2 (Hpa2) was reported. Unlike heparanase, Hpa2 lack of intrinsic HS-degrading activity, the hallmark of heparanase, but retain the capacity to bind heparin/HS. In fact, Hpa2 exhibits even higher affinity towards heparin/HS than heparanase, suggesting that Hpa2 may inhibit heparanase activity by competition for the HS substrate. Clinically, Hpa2 expression was markedly elevated in head and neck carcinoma patients, correlating with prolonged time to disease recurrence (follow-up to failure) and inversely correlating with tumor cell dissemination to regional lymph nodes, suggesting that Hpa2 function as a tumor suppressor. The molecular mechanism associate with favorable prognosis following Hpa2 over expression is unclear. In order to explore the molecular mechanism underlying the tumor suppressor property, we infected cancer cell lines with Hpa2 gene constructs. Hpa2 over expression by FaDu pharyngeal carcinoma cells was associated with a marked decrease in tumor volume and weight, while Hpa2 silencing resulted in nearly 2-fold increase in tumor volume. Notably, Hpa2 over expression resulted in decrease blood and lymphatic vessel density associating with reduced VEGF-A and VEGF-C levels, closely resembles the clinical results. Furthermore, we observed marked increase in collagen deposition in the tumors produced by Hpa2 overexpressing cells. This was associated with a noticable increase of lysyl oxidase (LOX) expression evident by immunostaining, immunoblotting and real-time PCR analyses.Notably, heparanase enzymatic activity was not impaired in cells over expressing Hpa2, suggesting that reduced tumor growth is not due to heparanase regulation. Moreover, growth of tumor xenografts produced by Hpa2 over-expressing cells was not affected by a newly generated monoclonal antibody that target the heparin binding domain of Hpa2, implying that Hpa2 functions in an heparanase-, and HS-independent manner.

CIRCULATING HTERT (HUMAN TELOMERASE) MRNA: IMPLICATIONS FOR TUMOR MICROENVIRONMENT AND POTENTIAL USE FOR EARLY DIAGNOSIS OF MALIGNANCY

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In contrast to current impressive advances in biological knowledge and achievements in the therapy of cancer, the field of early diagnosis lags behind and presents an unmet need. We hereby present the human telomerase as a potential tool for early diagnosis of cancer. Telomerase activation is a prerequisite for the perpetuation of the malignant clone during cancer progression as it elongates telomeres in each cell division. We established a method for the detection and quantification of hTERT mRNA products in exosomes derived from cancer cell lines growth media and human sera for future use as a diagnostic tool for the early detection of cancer. In four cancer cell lines, exosomal hTERT mRNA expression was detected and correlated with telomerase activity and intracellular hTERT mRNA expression, compared to a non-telomerase expressing cells. We studied the crosstalk between T cell leukemia derived exosomes and primary human fibroblast cells and revealed that the secreted exosomes transfer the hTERT mRNA from the "donor" cancer cell into the "recipient" non-telomerase expressing fibroblasts. These transcripts were successfully translated into a mature and fully active telomerase 24h post exposure. To establish a method for early diagnosis of malignancies, we screened sera from 130 patients with various malignancies and compared their exosomal hTERT mRNA levels to that of healthy volunteers. The results have shown that the expression of hTERT is variable among malignancies and between different patients with the same cancer type. To exclude some variables, such as drugs, that may influence exosome secretion on our chosen experimental cell lines, we examined the effect of the chronically administered drugs: aspirin, simvastatin and captopril. While simvastatin significantly decreased hTERT mRNA in exosomes derived from one cell line, neither aspirin nor captopril effected the secretion of exosomes, indicated by a similar hTERT expression in the relevant treated cells. Furthermore, no change in the intracellular hTERT expression and telomerase activity after drug exposure was evident. In light of these results we confirmed that exosomes derived from tumor cells can affect the surrounding microenvironment by exploiting the recipient cell mechanism and promoting the activation of telomerase in those cells. Understanding these mechanisms may have a strong impact on deciphering metastases formation. Exosomal hTERT may serve as a valid marker for the detection of malignancy, also in patients treated with commonly used medications. Hopefully, these results will be translated into the development of a new diagnostic tool for the early diagnosis of cancer.

IMPACT OF HEPARANASE RESIDING IN THE TUMOR MICROENVIRONMENT ON CANCER PROGRESSION

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Heparanase, an endoglycosidase that degrades heparan sulfate chains, is up-regulated in many types of cancers. In many cases, heparanase induction correlates with increased tumor metastasis, and vascular density, thus encouraging the development of heparanase inhibitors as anti-cancer drugs. We investigated the impact of host- vs. tumor- derived heparanase on cancer progression, emphasizing the cross talk between the epithelial, stromal and immune compartments of the tumor and its microenvironment. To this end, we utilized heparanase knockout (KO) and transgenic (Tg) mice to investigate tumor development following inoculation with cancer cells or exposure to carcinogen. We found that cancer cells produced bigger tumors in Tg vs. control mice. Likewise, smaller tumors were developed by those cells when inoculated in heparanase KO mice. To examine if the changes in tumors size are due to involvement of immune compartments, we inoculated LLC cells that over express MIP2 to KO and control mice. MIP2 over expression in LLC tumors results in reduced tumor weight in control but not in KO mice, associating with the recruitment of MΦ. In KO mice, the recruited MΦ arrest at the tumor periphery and are significantly less activate as indicated by lysosome 1 expression. Implantation of control, but not KO MΦ together with LLC cells caused to recruitment of MΦ, NK cells, T cells and dendritic cells that resulted in eradicate tumor growth. Furthermore, according to activity markers, MΦ, NK and dendritic cells activity in the tumors was elevated only when control MΦ were implanted. To characterize the differences between control and KO MΦ, control and KO mice were treated with Thioglycollate for 72 h and MΦ were collected from the peritoneum. The total number of MΦ that were collected was significantly lower in KO mice compared to control. Moreover, the invasion and migration capacity of the KO MΦ was reduced. Real time PCR analyses reveal that most of the cytokines are expressed at lower levels in the KO MΦ. Notably, comparable induction of cytokines expression was observed following the addition of heparanase to control or KO MΦ.

COMMON MECHANISMS IN BRAIN NEUROREGENERATION AND TUMOR METASTASIS

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Brain function in health and disease is largely controlled by the local environment. In the diseased brain, an altered cellular microenvironment, consisting of neurons, astrocytes and endothelial cells, plays a role in the progression and alternatively in the repair of diseases. After stroke, a regenerative niche controls the localization of immature neurons, and whether these cells survive and differentiate. The brain microenvironment also affects metastasis localization and, once there, whether a metastatic melanoma cell remains dormant or progresses towards metastasis. The main goal of our study is to find common mechanisms used in post-stroke neuroregeneration and in brain metastasis. More specifically, we focus on the question: can metastatic melanoma cells utilize post-stroke repair mechanisms for the establishment of brain metastasis? An oxygen-glucose depravation (OGD) treatment of astrocytes and brain endothelial cells (BECs) was used as an in-vitro model of stroke. Melanoma cell adhesion to "stroked" BECs and migration towards "stroked" BECs and astrocytes increased compared to control brain cells. The OGD treatment resulted in changes in the secretion of inflammatory cytokines from these "stroked" microenvironmental cells, including IFNγ, CD40L and IL-23. Melanoma cell adhesion and transmigration through BECs increased via an autocrine endothelial activation by CD40L. Melanoma cells treated with conditioned medium of "stroked" astrocytes exhibited changes in the expression of melanoma-brain metastasis-signature genes, previously characterized in our lab, including COX2, ANGPL4 and CYR61. Inoculation of melanoma cells into mice after applying a transient middle cerebral artery occlusion (MCAO) showed that metastatic melanoma cells preferentially localize to areas of tissue regeneration after stroke. This localization was not due to alterations in vascular flow to these peri-infarct areas, as preferential localization of inert particles was not observed in these areas. These results lead us to the hypothesis that melanoma metastatic cells are indeed able to "hijack" mechanisms involved in neural regeneration after stroke, for the establishment of brain metastasis. Uncovering these common mechanisms could lead to the detection of novel molecular targets that would manipulate cancer progression or neural repair. This study was supported by the Dr. Miriam and Sheldon G. Adelson Medical Research Foundation (Needham, MA, USA).

REDOX MODULATION OF ADJACENT THIOLS IN VLA-4 BY AS101 CONVERTS MYELOID LEUKEMIA CELLS FROM A DRUG-RESISTANT TO DRUG-SENSITIVE STATE

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Interactions between the integrin VLA-4 on acute myelogenous leukemia (AML) cells with stromal fibronectin is a decisive factor in chemotherapeutic resistance. In this study, we provide a rationale for a drug repositioning strategy to blunt integrin activation in AML cells and restore their sensitivity to chemotherapy. Specifically, we demonstrate that the non-toxic tellurium compound AS101, currently being evaluated in clinical trials, can abrogate the acquired resistance of AML. Mechanistic investigations revealed that AS101 caused redox inactivation of adjacent thiols in the exofacial domain of VLA-4 after its ligation to stromal fibronectin. This effect triggered cytoskeletal conformational changes that decreased PI3K/Akt/Bcl2 signaling, an obligatory step in chemosensitization by AS101. In a mouse xenograft of AML derived from patient leukemic cells with high VLA-4 expression and activity, we demonstrated that AS101 abrogated drug resistance and prolonged survival in mice receiving chemotherapy. Decreased integrin activity was confirmed on AML cells in vivo. The chemosensitizing activity of AS101 persisted in hosts with defective adaptive and innate immunity, consistent with evidence that integrin deactivation was not mediated by heightening immune attack. Our findings provide a mechanistic rationale to reposition the experimental clinical agent AS101 to degrade VLA-4-mediated chemoresistance and improve clinical responses in AML patients. Cancer Res. 2014 Jun 1;74(11):3092

ASTROCYTES FACILITATE MELANOMA BRAIN METASTASIS VIA SECRETION OF IL-23

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Melanoma is the leading cause of skin cancer mortality. The major cause of melanoma mortality is metastasis to distant organs, frequently to the brain. In order to treat or prevent metastasis, the interactions of disseminated tumor cells with the microenvironment at the metastatic organ have to be elucidated. Astrocytes are glial cells that function in repair and scarring of the brain following injury, in part via mediating neuroinflammation, but the role of astrocytes in melanoma brain metastasis is largely unresolved. Here we show that astrocytes can be reprogrammed by human brain-metastasizing melanoma cells to express pro-inflammatory factors, including the cytokine IL-23, which was highly expressed by metastases-associated astrocytes in vivo. Moreover, we show that the interactions between astrocytes and melanoma cells are reciprocal: paracrine signaling from astrocytes up-regulates the secretion of the matrix metalloproteinase MMP2 and enhances the invasiveness of brain-metastasizing melanoma cells. IL-23 was sufficient to increase melanoma cell invasion, and neutralizing antibodies to IL-23 could block this enhanced migration, implying a functional role for astrocyte-derived IL-23 in facilitating the progression of melanoma brain metastasis. Knocking down the expression of MMP2 in melanoma cells resulted in inhibition of IL-23-induced invasiveness. Thus, our study demonstrates that bidirectional signaling between melanoma cells and astrocytes results in the formation of a pro-inflammatory milieu in the brain, and in functional enhancement of the metastatic potential of disseminated melanoma cells.

CHARACTERIZING THE IMMUNE RESPONSE IN TUMOR MICRO ENVIRONMENT FOLLOWING TREATMENT WITH LPS AND C5A LOADED ON MICRO PARTICLES

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The immune cells are significant component in tumor micro environment (TME) which paradoxically depressants anti-cancer activities and allow the evasion of the immune response by the tumor. Is possible to alter the activity of immune cells in the TME and harness the innate immune activity to anti-cancer immune response. This can be done by Induced acute inflammatory response of the immune system cells in the TME by using inducers such as LPS(lipopolysaccharide) that bind to TLR-4 and C5a (complement 5a) that bind to C5aR. Induction of splenocytes by combination of soluble inducers LPS and C5a. stimulate proliferation of immune cells (in vitro). Covalently attached LPS and C5a to micro-particles injected directly into melanoma B16-F10. Inducted expression of pro-inflammatory anti-inflammatory cytokines gene in TME cells. The treatment enriched specific immune cells populations especially neutrophils cells in the TME and delay tumor development. In conclusion, this study demonstrates the possibility to change the immunological nature of tumor micro environment immune cell population and activity by intra-tumoral injection of LPS and C5a loaded together on micro-particles.

BREAST CANCER: COORDINATED REGULATION OF CCL2 SECRETION BY INTRACELLULAR GLYCOSAMINOGLYCANS AND CHEMOKINE MOTIFS

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The chemokine CCL2 (MCP-1) has been identified as a prominent tumor-promoting factor in breast cancer. The major source for CCL2 is in the tumor cells; thus, identifying the mechanisms regulating CCL2 release by these cells may enable the future design of modalities inhibiting CCL2 secretion and consequently reduce tumorigenicity. Using cells deficient in expression of glycosaminoglycans (GAGs) and shRNAs reducing heparan sulfate (HS) and chondroitin sulfate (CS) expression, we found that intracellular HS and CS (=GAGs) partly controlled the trafficking of CCL2 from the Golgi towards secretion. Next, we determined the secretion levels of GFP-CCL2-WT and GFP-CCL2-variants mutated in GAG-binding domains and/or in the 40s loop of CCL2 (45TIVA48). We have identified partial roles for R18+K19, H66 and the 45TIVA48 motif in regulating CCL2 secretion. We have also demonstrated that in the absence of R24 or R18+K19+45TIVA48, the secretion of CCL2 by breast tumor cells was almost abolished. Analyses of the intracellular localization of GFP-CCL2-mutants in the Golgi or the endoplasmic reticulum revealed particular intracellular processes in which these CCL2 sequences controlled its intracellular trafficking and secretion. The R24, 45TIVA48 and R18+K19+45TIVA48 domains controlled CCL2 secretion also in other cell types. We propose that targeting these chemokine regions may lead to reduced secretion of CCL2 by breast cancer cells (and potentially also by other malignant cells). Such a modality may limit tumor growth and metastasis, presumably without affecting general immune activities.

INFLAMMATION-DRIVEN TUMOR-MSC INTERACTIONS IN BREAST CANCER: REGULATION BY THE NOTCH PATHWAY

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Breast tumor cells interact with mesenchymal stem cells (MSC) that are recruited to the tumor and promote processes that aggravate disease course. Such tumor-MSC interactions may be mediated by cell-to-cell contacts and may be promoted by inflammatory cytokines that prevail at the tumor microenvironment (TME), leading to elevated angiogenesis, tumor growth and metastasis. Our recent findings indicate that the release of the inflammatory and metastasis-promoting chemokines CXCL8, CCL2 and CCL5 by breast tumor cells and bone-marrow-derived MSC is amplified when the two cell types are grown in co-cultures. The release of the inflammatory chemokines was further potentiated by stimulation of such co-cultures by the inflammatory cytokines TNFα and IL-1β, known to be highly prevalent at the TME in breast tumors. This amplified release the chemokines was partly mediated by soluble factors exchanged between the two cell types but in parallel significantly required direct tumor-MSC cell-to-cell contacts. Accordingly, confocal studies demonstrated that breast tumor cells position themselves in close proximity to MSC and make intimate contacts with them. These analyses also proposed the generation of tumor-MSC hybrids in culture. Using specific inhibitors, we identified distinct mechanisms that may regulate the release of the pro-inflammatory chemokines CXCL8, CCL2 and CCL5 in cytokine-stimulated tumor-MSC co-cultures. Our results revealed that contacts generated through the actin cytoskeleton were essential for CCL2 and CCL5 up-regulation, whereas, the Notch signaling pathway may tightly regulate CXCL8 and CCL2 (but not CCL5) induction in co-cultures. The potential roles of the Notch pathway in chemokine induction was further supported by qPCR analyses of different members of the Notch pathway and their ligands. These analyses suggest complex regulatory roles in the cytokine-induced, contact-dependent process of chemokine up-regulation by contacts that are formed through Notch-1 and Jagged-2 expressed in breast tumor cells and Notch-3 and Delta-like-1 expressed in MSC. Overall, our findings indicate that inflammatory networks regulate the tumor-promoting associations between tumor cells and stroma cells in their vicinity. Further elucidation of the impact of such process on tumor progression may provide a better basis for the design of new therapeutic strategies in breast cancer.

MYELOID-DERIVED PROS1 PROTECTS FROM PRIMARY TUMORIGENESIS AND METASTASIS, THROUGH NEGATIVE REGULATION OF INFLAMMATION

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Tumor cells promote mobilization and recruitment of bone marrow cells into the tumor-microenvironment, often enhancing tumor aggressiveness by promoting invasion, angiogenesis, chemo-resistance and metastasis. Tyro3, Axl and MerTK (collectively termed TAMR) are proto-oncogenic receptor tyrosine kinases expressed by a wide variety of cells, but also serve as negative regulators of innate immunity. Recently, the anticoagulant Protein S (PROS1) was shown to function as a bona fide TAMR ligand in the mouse eye and vasculature. Here we test the hypothesis that PROS1 may be involved in tumorigenesis and metastatic colonization through modulation of the tumor microenvironment. Using our unique mouse model we genetically ablated PROS1 expression in myeloid cells. These mice (LMKO) were challenged with subcutaneous injection of Lewis Lung Carcinoma (LLC) cells, and followed tumor growth. Primary tumor and metastatic parameters in LMKO mice were significantly more severe than in control mice. Additionally, priming of LLC cancer cells with conditioned medium from LMKO macrophages increased their metastatic potential when injected into wild type mice. Finally, LMKO macrophages exhibited an increased inflammatory profile, suggesting inflammation as a mediator of this effect. Here we identify PROS1 as a negative regulator of tumor metastasis, implying macrophage-derived PROS1 is protective against tumorigenesis and metastasis. We speculate that deletion of myeloid-derived PROS1 contributes to an elevated inflammatory milieu, promoting cancer through rendering host cells permissive to metastatic colonization, and identify a novel role for PROS1 in cancer.

ADHERENCE RATES AND FORCE DEPEND ON METASTATIC POTENTIAL IN BREAST CANCER CELLS

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Cell adhesion plays an important role in the normal functions of cells, including contraction, spreading, crawling, and invasion. In cancer cells, adhesion regulates migration and invasiveness depending on the stiffness of the matrix surrounding a tumor. The rate of adhesion, changes in cell morphology and forces applied during adhesions likely vary between benign and cancer cells. Thus we suggest an approach to use measurements of those differences as an approach for determination of malignancy and metastatic potential (MP) cells. In this study, we evaluate the rate of attachment to a 2D substrate, concurrently through changes in cell morphology and the strength of adherence. We compare low and high metastatic potential breast cancer cells and use benign cells as a control. By using traction force microscopy on polyacrylamide gels (stiffness 4300±150 Pa and 7200±50 Pa) we monitor the time-dependent force applied by the cells to the gel through displacement of particles embedded in the gel surface. We observe that high metastatic cancer cells adhere more rapidly, we also show that high and low MP cells apply larger lateral forces than benign cells. In conclusion, we have shown that a there is a direct correlation between metastatic potential of cancer cells and the lateral forces applied by those cells during the adhesion process.

PHOX2B: A MICROMETASTASIS REGULATOR IN NEUROBLASTOMA

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Neuroblastoma is the most common extracranial solid tumor in children. Survival rates of patients with metastatic disease are poor despite extensive research and many clinical trials. We developed an orthotopic mouse model for human neuroblastoma metastasis, comprising local and metastatic variants originating from single tumors. Inoculation of these variants into a new set of nude mice generated two types of variants: lung macro-metastatic cells and lung micro-metastatic cells, the latter not generating overt lung metastases. In previous work, these macro and micro metastatic cells were characterized for tumorigenic and metastatic abilities, indicating a more malignant phenotype of the macro-metastatic cells. PHOX2B is a transcription factor that is used as a minimal residual disease marker in neuroblastoma patients. This protein turned out to be superior to other minimal residual disease markers such as Tyrosine Hydroxylase (TH) and GD2 synthase, in its specificity and sensitivity. In this work, higher expression levels of PHOX2B were indeed identified through qRT-PCR and western-blot analyses in micro as compared to macro-metastatic neuroblastoma cells. Having a common genetic background, we hypothesized that an epigenetic event had led to the differential expression of PHOX2B in the micro and macro-metastatic neuroblastoma cells. Indeed, examination of the methylation pattern of the PHOX2B promoter revealed that 25 CpG dinucleotides in the promoter region are 75-100% methylated in the macro-metastatic cells and only 37-75% are methylated in the micro metastatic cells. These results strongly imply that hyper-methylation could be the reason for PHOX2B lack of expression in the macro-metastatic cells. Macro-metastatic cells manipulated to overexpress PHOX2B were inoculated orthotopically to nude mice: these cells exhibited a lower capacity to metastasize to the lungs. That and more, an orthotopic inoculation of micro-metastatic cells in which PHOX2B was down-regulated, showed a significant difference in the cells tumorigenic and metastatic abilities. Larger primary tumors were seen in mice inoculated with the micro metastatic variant where PHOX2B was down-regulated, and 20 fold more human cells were detected in the lungs and bone marrow of these mice (compared with mice inoculated with micro-metastatic cells). These results strongly support the hypothesis postulating that PHOX2B can function as an inhibitor of primary tumor cells spreading and metastasizing. Deciphering the mechanism through which PHOX2B influences the tumorigenic and metastatic properties of micro and macro-metastases may lead to the development of targeted therapy for neuroblastoma patients. This study was supported by the Deutsche Forschungsgemeinschaft (DFG).

THE ROLE OF AUTOTAXIN - LYSOPHOSPHATIDIC ACID SIGNALING IN OVARIAN CARCINOMA

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Ovarian carcinoma ranks as the fifth most common cancer among women. This is attributed to late presentation of the patients. The disease is characterized by widespread intraperitoneal metastases and accumulation of effusion fluids mainly in the peritoneal cavity. Lysophosphatidic acid (LPA) is a bioactive phospholipid with mitogenic and growth factor-like activities affecting cell invasion, cancer progression and drug-resistance. It is produced by Autotaxin (LysoPLD) and acts on six specific G-coupled protein receptors, LPAR1-6. LPA has recently been implicated as a growth factor present in ascites of ovarian cancer patients. It has recently been implicated as a growth factor present in ascites of ovarian cancer patients The aim of our study was to examine the role of ATX-LPA signaling in the progression of serous ovarian carcinoma. A total of 230 samples of serous ovarian carcinoma from the three different anatomic sites of the disease, were used .Effusion fluids were frozen and stored separately. All specimens were submitted for routine diagnostic purpose to the Division of Pathology at the Norwegian Radium Hospital during the period 1998-2008. mRNA levels of the six different LPA receptors were measured by RT-PCR, protein levels of Autotaxin, ERK and phospho-ERK were measured by Western blotting. Results show that LPA2R is higher in effusion-derived tumor cells compared to solid lesions (p<0.001), while the opposite was found for LPA3R (p<0.001) and LPA6R (p<0.001). Clinico-pathological analysis showed that LPA3R levels are significantly higher in pre-chemo patients (p=0.038). LPA2R (p=0.021) and LPA5R (p=0.035) were found higher in cases with larger residual disease volume. LPA5R (p=0.032) was higher in cases with primary chemo-resistance and LPA1R (p=0.05) was marginally associated with better overall survival. Autotaxin protein levels were lower in effusion-derived tumor cells compared to solid lesions (p<0.001), yet higher levels of Autotaxin were found in peritoneal lesions compared to pleural ones (p=0.033). LPA3R was found positively related to ERK (p=0.001) and negatively related to LPA2R (p=0.006), where ERK (p=0.013) and phospho-ERK (p<0.001) levels were lower in effusions, yet cases with higher phospho-ERK levels in effusions were related to longer survival in patients (p=0.045) and in cases with pre-chemotherapy (p=0.015). In this study we showed significant changes in mRNA LPAR levels and Autotaxin protein levels with the progression of the disease that correlates to clinic-pathological parameters. To our best knowledge, this is the first description of the ATX-LPA signaling pathway in ovarian carcinoma with clinical relevance.

IDENTIFYING CCL2- AND CCL5-INDUCED LEUKOCYTE LANDSCAPE IN BREAST TUMORS AND ITS IMPACT ON TUMOR PROGRESSION AND ANGIOGENESIS

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The microenvironment of breast tumors is comprised of resident tissue cells, infiltrating cells and secreted factors such as cytokines and chemokines. The inflammatory chemokines CCL2 and CCL5 are extensively expressed in breast tumors and promote breast malignancy by a variety of manners and mechanisms. The most important of all is the ability of both chemokines to modify the type of leukocyte infiltrates in tumors so that the equilibrium is shifted towards the presence of cells that promote malignancy. Moreover, CCL2 has strong angiogenic activities which enhance tumor metastasis. The aim of our study was to characterize the landscape of immune/inflammatory cells that are recruited to breast tumors by CCL2 and CCL5 and the way the balance between different leukocyte subtypes affects different stages along tumor progression. In parallel, since certain leukocyte subtypes promote angiogenesis, we wish to determine the contribution of CCL2-induced immune/inflammatory landscape to elevated angiogenesis in breast cancer. To this end, we are using specific blockers of CCL2 and CCL5 activities, consisting of mutated chemokines. We have created a system of HEK-293 cells over-expressing the receptors of CCL2 (CCR2 - human and murine) and CCL5 (CCR1, CCR3, CCR5), in order to determine and validate the inhibitory potential of the mutants on cell migration in vitro. We also used human monocytic cell line which expresses all of the above receptors. So far, we found dose dependent effects of 3 mutated chemokines on cell migration that reaches up to ~ 99% of migration inhibition. To determine the inhibitory potential of the chemokine blockers on angiogenesis, we used human pulmonary endothelial cells. Under certain conditions, these cells form spheroids, which sprout as a response to angiogenic stimuli such as CCL2. We have observed a significant reduction in the number of sprouts and in their length when endothelial cells were treated with the inhibitors. These encouraging findings are at the basis of the work that will be performed in vivo. Following administration of the inhibitors to mice, we will determine tumor growth, metastasis and angiogenesis, and in parallel will identify the leukocyte landscape of tumors at different stages of progression. Such a study will provide accurate information on the contribution of different leukocyte sub-populations to different stages of breast cancer progression and will improve our understanding of the therapeutic potential of CCL2 and CCL5 competitors in this disease and in other malignancies alike.

BONE MARROW-DERIVED FIBROBLASTS ARE A FUNCTIONALLY DISTINCT STROMAL CELL POPULATION IN BREAST CANCER AND LUNG METASTASES

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Breast cancer is the most frequently diagnosed and one of the leading causes of cancer-related deaths in women in the western world. Breast tumors are characterized by an extensive desmoplastic stroma, abundantly populated by fibroblasts. Cancer-associated fibroblasts (CAFs) are a heterogeneous population of fibroblastic cells found in the microenvironment of solid tumors that were shown to promote tumor growth in various mechanisms, including stimulation of tumor cell proliferation and enhancement of angiogenesis. We previously uncovered a novel role for CAFs in mediating tumor-promoting inflammation in several tumor types, including breast cancer. Here we set out to characterize the various subpopulations of fibroblastic cells during progression of mammary carcinoma and lung metastasis utilizing a transgenic mouse model of breast carcinoma – the MMTV-PyMT model. Using adaptive bone-marrow (BM) transplantations of whole BM from MMTV-PyMT β-actin-GFP (PyMT-GFP) or from β-actin-GFP mice to PYMT and FVB/n mice, respectively, we identified two main populations of CAFs: resident mammary fibroblasts that express PDGFRα, previously shown to be a robust marker for normal fibroblasts, and PDGFRα- BM-derived fibroblasts, recruited specifically to the microenvironment of mammary tumors and to lung metastases but not to normal mammary glands and normal lungs. By breeding MMTV-PyMT female mice with Collagen-Iα (Col-Iα) –dsRED/YFP reporter mice we were able to generate an experimental system in which CAFs are fluorescently labeled. We utilized these mice in bone marrow transplantations, isolated the different CAF subpopulations from both the primary tumor and lung metastases and analyzed their immunological gene expression utilizing the nanostring nCounter® system. This analysis revealed distinct gene expression signatures of resident and BM-derived CAFs, as well as genes that are commonly expressed by both subpopulations. Moreover, the gene signature of BM-derived CAFs was affected more by their location than by their origin: resemblance was higher between CAFs in the same organ than CAFs from the same origin. Thus, CAF populations in primary breast tumors and in lung metastases are distinct in origin, cellular markers and immune-related function and co-evolve with tumor progression.

ASTROGLIOSIS IS INSTIGATED IN A NOVEL MOUSE MODEL OF SPONTANEOUS MELANOMA BRAIN METASTASIS

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Malignant melanoma is the deadliest of all skin cancers since it is highly metastatic. Melanoma brain metastasis confer poor prognosis, with a median survival of less than one year. Astrocytes are glial cells that maintain brain homeostasis, and constitute a central part of the brain microenvironment. Reactive astrogliosis is the primary response of astrocytes to brain insult, characterized by proliferation and migration to the injured site and extensive up-regulation of glial fibrillary acidic protein (GFAP). While much data have accumulated on the contribution of astrogliosis to neurodegenerative diseases, the involvement of astrogliosis in brain metastasis has only recently begun to be explored. However, the functional role of astrogliosis in promoting the growth of melanoma brain metastases is largely unknown. Utilizing a Ret-melanoma-derived cell line, we set out to establish a spontaneous melanoma brain metastasis model in immunocompetent mice, in which interactions between metastasizing melanoma cells and astrocytes can be studied. We show that mCherry-ret melanoma cells injected orthotopically into syngeneic mice form aggressive local tumors. 3-4 months after surgical excision of the primary tumor, 20-30% of the mice develop brain macrometastases. By utilizing a unique ex-vivo modeling system we detected brain micrometastases in 50-60% of the mice and quantified the metastatic load with a threshold of as few as 100 cells. Furthermore, we show that the presence of circulating melanoma cells in peripheral blood and the detection of melanoma transcripts in cerebrospinal fluid (CSF) are correlated with brain metastases. We next demonstrated that astrocytes activation is an early event and that astrocytes are recruited to metastatic brain lesions. In-vitro, paracrine signaling by melanoma cells activated normal astrocytes to up-regulate a 'gliosis gene signature' including CXCL10, SERPINA3N, SERPINE1 and LCN2. In turn, activated astrocytes enhance the invasion and proliferation of melanoma cells. Notably, these interactions are functionally important in vivo: co-injection of astrocytes with melanoma cells intra-cranially resulted in a 9-fold increase of tumor volume, accompanied by extensive up-regulation of gliosis markers. Collectively, we have established a novel mouse model of melanoma brain metastases in immunocompetent mice, and our newly established tools enable reliable intra-vital detection of brain micrometastases. Further understanding of the functional role of astrocyte-mediated gliosis in facilitating melanoma brain metastases may lead to the development of new therapeutics to control early brain metastatic disease.

TUMOR-DERIVED OSTEOPONTIN REPROGRAMS NORMAL MAMMARY FIBROBLASTS TO PROMOTE INFLAMMATION AND TUMOR GROWTH IN BREAST CANCER

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Breast tumors are characterized by an extensive desmoplastic stroma, abundantly populated by fibroblasts. Fibroblasts populating the neoplastic microenvironment have an important role in facilitating tumorigenesis. Cancer Associated Fibroblasts (CAFs) are an activated sub-population of stromal fibroblasts, which can have different characteristics in different tumor types. CAFs promote tumor growth by directly stimulating tumor cell proliferation and by enhancing angiogenesis and inflammation. However, the mechanisms through which normal tissue fibroblasts are reprogrammed to tumor-promoting CAFs are mainly obscure. Here, we show that mammary fibroblasts can be educated by breast cancer cells to become activated to a pro-inflammatory state that supports malignant progression. Proteomic analysis of breast cancer cell–secreted factors identified the secreted pro-inflammatory mediator Osteopontin, which has been implicated in inflammation, tumor progression, and metastasis. Osteopontin was highly secreted by mouse and human breast cancer cells, and tumor cell–secreted Osteopontin activated a CAF-like phenotypes in normal mammary fibroblasts in vitro and in vivo. Osteopontin was sufficient to induce fibroblast reprogramming and neutralizing antibodies against Osteopontin blocked fibroblast activation induced by tumor cells. The ability of secreted Osteopontin to activate mammary fibroblasts relied upon its known receptors CD44 and aVβ3 integrin. Strikingly, Osteopontin silencing in tumor cells in vivo attenuated stromal activation and inhibited tumor growth. Our findings establish a critical functional role for paracrine signaling by tumor-derived osteopontin in reprograming normal fibroblasts into tumor-promoting CAFs.

EXTRACTION OF IMMUNE STATUS FROM RNASEQ OF TUMOR TISSUES: A METHODOLOGY TO CORRELATE PROGNOSIS AND TUMOR-ASSOCIATED IMMUNOME

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Cancer-associated immunome is concomitant with cancer prognosis, yet evaluating expression of immune-associated genes within cancer biopsy is subjected to significant inconsistencies related to the methodology of sampling biopsies. Here we present immFocus, a method for extracting immune signals from total RNAseq of tumor biopsies, intended for immunity depiction and prognosis evaluation. The method is based on reducing the variation in apparent expression level of genes expressed specifically by immune cells due to the biopsy preparation. An immune index is employed to normalize the expression of every gene. We employed the immFocus method to analyze biopsies from Kidney Renal clear cell Carcinoma patients. We find that genes that become less variable due to normalization are preferentially immune genes, and that immune-related genes are more likely to become more prognostic. These results demonstrate, for the first time, that whole transcriptome sequencing can be used interrogation of cancer immunome and advancing immune-based prognosis.

FACTORS OF THE TUMOR MICROENVIRONMENT RE-SHAPE THE CCR7-DRIVEN METASTATIC SPREAD OF LUMINAL-A BREAST TUMORS

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Introduction: Organ-specific metastatic spread is regulated by axes of chemokines and their receptors. CCR7- and CXCR4-expressing tumor cells home to organs constitutively expressing the corresponding ligands CCL21 and CXCL12, primarily lymph nodes (LN) and remote metastatic sites, respectively. We investigated how factors of the tumor microenvironment (TME) impact the chemokine-induced metastatic spread of luminal-A breast tumors, in which despite overall good prognosis a considerable percentage of patients progress to metastatic phase.

Methods: Using the METABRIC dataset, we determined the expression levels of CCR7 and CXCR4 in different breast cancer subtypes, and associations with LN metastasis. Luminal-A MCF-7 cells were transduced to express functional CCR7 or CXCR4 and were stimulated by CCL21 or CXCL12. Cells were also pre-exposed to TME stimulation, representing the hormonal, inflammatory and growth stimulating arms residing in many primary breast tumors. Such "TME stimulation" was introduced by simultaneous exposure in culture to estrogen+Tumor necrosis factor α+Epidermal growth factor. Signaling in tumor cells, migration and formation of directional protrusions in a novel 3D hydrogel system were determined. Metastatic spread to LN and remote organs were studied using mouse models.

Results: Luminal-A breast cancer patients expressed low levels of CCR7 and CXCR4 and exhibited low incidence of LN metastasis. TME factors down-regulated the migration of CCR7- and CXCR4-over-expressing MCF-7 cells towards CCL21 and CXCL12, and inhibited formation of directional protrusions in response to CCL21. Focusing on the CCR7-CCL21 axis, we found that CCL21 could not promote any further MAPK and PI3K activation, which were potently activated by TME factors. In vivo, dissemination of luminal-A breast tumor cells to LN (constitutively expressing CCL21) was increased by TME stimulation alone or by CCR7-over-expression alone, but was not further increased when the two pathways were combined. Rather, when the TME stimulus was introduced to CCR7-over-expressing cells, the metastatic spread in bones was considerably elevated.

Conclusions: Microenvironment-driven signals, acting on luminal-A breast tumor cells, interfere with CCL21-mediated LN-homing cues and enable the cells to respond more effectively to signals directing their dissemination to remote organs, particularly bones. These findings provide a personalized context to the process of metastatic spread in breast cancer.