THE POTENTIAL ROLE OF LIVIN, A REGULATOR OF APOPTOSIS PROTEIN, IN CANCER THERAPY

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The Inhibitor of Apoptosis protein Livin is unique among IAPs due to its dual role in cancer development. Similar to other IAPs, Livin inhibits cell death by binding to and inhibiting the activity of specific Caspases. However, we discovered that following strong apoptotic stimuli Livin is cleaved by effector caspases to produce a truncated protein (tLivin) with a paradoxical pro-apoptotic activity.

We found that tLivin induces distinct forms of cell death, necrosis or apoptosis, in cells of different origin, and can induce more than one form of cell death in the same cell type by activation of an alternative form of cell death. We demonstrated that tLivin activates the JNK pathway and that tLivin-induced cell death is partially mediated by JNK activity.

We identified the domains involved in the pro-apoptotic activity of tLivin and the minimal sequence in tLivin that is required for its activity. mini-tLivin (mtLivin) is a potent, pro-apoptotic 70 aa derivative of tLivin, which is as potent as tLivin.

Our findings regarding tLivin suggest unique therapeutic modalities. It might serve as a novel therapeutic avenue against cancer that will overcome apoptosis resistance in malignancy. However, the exploitation of its therapeutic potential requires the development of novel, effective delivery strategies for tLivin that will enhance stability, bioavailability, intracellular uptake and efficacy to targeted cancerous cells.

We are developing mtLivin-conjugated, targeted nanoparticles that will induce cell death even of resistant tumors. The targeting ability of the nano-delivery system will be achieved by conjugating specific ligands that recognize specifically receptors overexpressed in cancer cells, particularly in hematological malignancies. Furthermore, to enhance the efficacy of the delivery system, the potential incorporation of doxorubicin within the NPs will be investigated.

The final objective of this project is the implementation of tLivin-based nano-delivery strategies targeting the cargo to the circulating cancerous cells and combining chemotherapy into the same nanoparticles for resistant cancer cells eradication.

RNA THERAPEUTICS: NEW ERA IN MOLECULAR MEDICINE?

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RNA based approaches have greatly contributed to better understanding of gene expression and function *in vitro*. The capability to apply these strategies *in vivo* in order to validate the role of specific genes in normal or pathological conditions, and to induce therapeutic gene silencing or upregulate a specific protein expression, opened new avenues for utilizing RNA as a novel therapeutic modality. However, the translation of RNA from an effective genomic tool into a novel therapeutic modality has been hindered by the difficulty to deliver RNA molecules into specific target tissues by systemic administration, especially to hematopoietic cells and brain tumors. In this presentation, I will describe some of the challenges and opportunities in modulating leukocytes response using RNA and discuss adverse effects such as immuno-toxicity. Special emphasize will be made on delivery strategies that target glioma cells and Mantle Cell Lymphoma (MCL) with novel therapeutic targets that were recently found to increase the survival of mice these tumors.

In addition, I will describe the discovery of exosomes from MCL that home into B cells and detail the opportunities in utilizing these natural carriers as RNA vehicles for personalized therapeutics.

TUMOR - MICROENVIRONMENT INTERACTIONS: THE BIG PICTURE

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The following topics will be briefly discussed:

- ✓ The tumor microenvironment
- ✓ The major microenvironmental factors that play a role in tumor formation and its progression to metastasis
- ✓ Mechanisms by which microenvironmental factors shape the malignancy phenotype of tumor cells
- ✓ Metastasis and micro-metastasis
- ✓ Drugs that manipulate tumor-microenvironment interactions
- ✓ Quo Vadis tumor microenvironment?

CHRONIC INFLAMMATION INDUCED IMMUNOSUPPRESSION: UNDERLYING MECHANISMS AND CLINICAL IMPLICATIONS IN CANCER

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It is well established that chronic inflammation and cancer are associated; chronic inflammation in many cases predisposes an individual to cancer, and developing tumors have the ability to induce micro and macro chronic inflammatory environments. Under both circumstances, an immunosuppressive milieu ensues, enabling escape of the tumor from immune surveillance and disrupting success of immune interventions. In the course of our studies we proved chronic inflammation and associated myeloid derived suppressor cells as the causative link for the induced immunosuppression described in various tumors and explored the underlying mechanisms. We demonstrate that such an environment suppresses not only the host's immune system but also newly administered immune cells and vaccination-based therapies, limiting the success of cancer immunotherapies. In addition, our results point at the diverged effect of chemotherapeutic drugs on the immunosuppressive environment by using novel biomarkers.

In aiming at extending our understanding of the mechanisms underlying chronic inflammation induced immunosuppression and its impact on tumor development and spreading, we seek to discover modalities/drugs that neutralize the immunosuppressive environment and increase the efficacies of given chemo- and immune-based therapies. We are also establishing a high-fidelity detection system using unique biomarkers for monitoring the host's immune status modified by chronic inflammation that could predict success of given therapies and disease regression. To this end, we are using mouse models for a pathology-free chronic inflammation, inflammatory bowel disease and inducible colorectal cancer, all driven by chronic inflammation.

Recent retrospective studies performed in the lab show the first proof of concept in humans; analyses of blood samples from cancer patients for the expression of the biomarkers that provide indications for the host's immune status prior to and following chemo- and immune-based therapies could predict therapy efficacies and success. Such studies could facilitate the designing of innovative combinatorial strategies for cancer therapies and immune system monitoring towards the establishment of optimal personalized cancer treatments.

EOSINOPHILS PROMOTE COLORECTAL CANCER THROUGH EXPRESSION OF S100A8 AND S100A9

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Eosinophils are bone marrow-derived cells that have been largely implicated in Th2-associated diseases. However, recent data highlight key roles for eosinophils in non-classical Th2 settings (e.g. metabolism, thermogenesis and tissue regeneration). Quite surprisingly, despite the fact that the gastrointestinal (GI) tract is the largest eosinophil reservoir in the body, the roles of GI eosinophils have been largely understudied especially in chronic GI inflammation and subsequent tumorigenesis.

We now report that eosinophils are a bona-fide cellular compartment of the tumor microenvironment in colorectal cancer (CRC). Substantial eosinophilic infiltration was observed in three independent models of CRC, representing the genetically driven and inflammation-driven CRC models (i.e. Apc(min/+) model and AOM+DSS treatment, respectively), as well as in colonic orthotopic injection of a tumor epithelial cell line. Eosinophil recruitment was accompanied with significant increase in CCL11, the key eosinophil chemokine and decreased blood eosinophilia, suggesting active recruitment to the colon. AOM+DSS-treated eosinophil-deficient mice (ΔdblGATA mice) displayed decreased epithelial cell proliferation, decreased collagen deposition and decreased expression of matrix metalloproteinases, tissue inhibitors of matrix metalloproteinases and TNF-α. Consequently, tumor load was dramatically reduced in the colons of AOM+DSS-treated and orthotopically transplanted ΔdblGATA mice.

Microarray analysis of primary colonic eosinophils that were sorted at various stages of the tumorigenic process (naïve-, inflammatory-, tissue repair- and tumor associated-eosinophils) revealed dynamic regulation of colonic eosinophil mRNA expression during carcinogenesis. Interestingly, the pro-tumorigenic and clinically relevant genes s100a8 and s100a9 were strikingly and kinetically increased in colonic eosinophils (250-fold and 90-fold, respectively). Indeed, local and systemic expression of s100a8 and s100a9 were nearly diminished in AOM+DSS-treated ΔdblGATA mice, and re-constituted upon adoptive transfer of eosinophils into the colon.

These data establish a key and unforeseen pro-tumorigenic role for eosinophils in CRC. Furthermore, our results suggest that eosinophils can promote carcinogenesis via expression and secretion of s100a8 and s100a9.

TUMOR RELATED NEUTROPHILS - A NEW CHALLENGE IN CANCER IMMUNOLOGY

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Neutrophils are the most abundant leukocyte in the human circulation, and a significant portion of the inflammatory cell infiltrate in many models of cancer. After many years of relative under-estimation, it is becoming clear that neutrophils play an important role in cancer biology. Over the past several years we and others have published several studies challenging the limited view of neutrophils as short-acting phagocytic cells. We demonstrated that Tumor associated neutrophils (TAN) can have a dual role in tumor biology, polarized by the tumor microenvironment to have either anti-tumorigenic ('N1') or pro-tumorigenic ('N2') functions. Circulating neutrophils in tumor-bearing mice and cancer patients have been shown to be capable of preventing the metastatic process by direct tumor cytotoxicity.

Using a transcriptomics approach in mice, we found that N1/N2 TAN differ from each other, from naïve neutrophils (NN) and from the granulocytic fraction of MDSC (G-MDSC), with many immune-related genes and pathways up-regulated in TAN. Furthermore, TAN and systemic neutrophils change during tumor progression, acquiring more pro-tumorigenic properties with time. In a new recently published study, we identified a heterogeneous subset of circulating low-density neutrophils (LDN) that appear transiently in self-resolving inflammation but accumulate continuously with cancer progression. LDN display impaired neutrophil function and immunosuppressive properties, characteristics that are in stark contrast to those of mature, high-density neutrophils (HDN). Importantly, LDN consist of both immature MDSC as well as mature cells that are derived from HDN, in a TGFβ dependent mechanism. These findings provide a mechanistic explanation to mitigate the controversy surrounding neutrophil functions in cancer.

In additional work we found several links between tumor neutrophils and the adaptive immune system affecting the immune microenvironment. We showed that TAN are capable of recruiting regulatory T-cells into the tumor by secretion of CCL17, and more recently we found and are investigating their capability in advanced tumor stages to induce apoptosis and inhibit the proliferation of $CD8^+$ cytotoxic T-cells.

Proper understanding of the effect of tumors on neutrophils, as well as the way these cells support or fight cancer and affect tumor immune microenvironment, will help us develop strategies to direct the immune system against the tumor. Our long term goal is to promote cancer treatment in general, and specifically immunotherapy using manipulated neutrophils as a novel tool to treat advanced cancer and metastases.

BONE MARROW-DERIVED CANCER ASSOCIATED FIBROBLASTS ARE A FUNCTIONALLY DISTINCT POPULATION IN THE MICROENVIRONMENT OF BREAST CANCER AND LUNG METASTASES

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Cancer Associated Fibroblasts (CAFs) support tumorigenesis by stimulating angiogenesis, cancer cell proliferation, and invasion. We previously demonstrated that CAFs also orchestrate tumor-enhancing inflammation in multiple cancers, including mouse and human breast tumors. Although breast cancer is one of the major tumor types where CAFs were shown to be tumor promoting, there is no detailed analysis of the dynamic changes in CAFs characteristics and function in correlation with tumor progression and metastasis. We therefore set out to characterize the dynamic changes in CAFs during progression of mammary carcinogenesis in a transgenic mouse model of human breast cancer. We analyzed the various sub-populations of fibroblastic cells during progression of mammary carcinoma and lung metastasis by morphometric analysis with multiple known mesenchymal markers and found a gradually increasing population of activated fibroblasts that were αSMA+ PDGFRα-. This population was evident also in spontaneous lung metastases, but not in normal mammary glands or lungs. Adoptive bone marrow transplantations with labeled cells revealed that this subpopulation of CAFs is specifically recruited from the BM to mammary tumors and lung metastases and are differentiated fibroblastic cells of mesenchymal origin. Utilizing multi-transgenic mice where all fibroblasts are fluorescently labeled, combined with a mouse model of breast cancer, we were able to isolate resident and BM-derived CAFs from breast tumors and from lung metastases and profile their inflammatory transcriptome. Analysis of expression profiling revealed that BM-derived CAFs exhibit a unique pro-inflammatory gene signature, implying a distinct function from resident CAFs. Moreover, the pro-inflammatory profile of BM-derived CAFs was location, rather than origin dependent: BM-derived lung metastases-associated CAFs were not similar to BM-derived CAFs isolated from the primary tumor, indicating organ specific modifications in their function. Thus, CAF populations in primary breast tumors and in lung metastases are dynamic in their origin and gene expression, and co-evolve with tumor progression.

THE TRANSLATION APPARATUS IN THE CANCEROUS CELL

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Cancerous cells must modify their gene expression programs to support their needs. In doing so, they appear to adapt a modified protein translation program that is better geared towards translation of genes that carry cell autonomous functionalities. In this talk I will discuss our characterization of the transcription rearrangements that take place in cancer, and how they support such reprogrammed translation. I will then present our recent experimental results that suggest means to lower proliferation of aggressive cell lines by manipulation of their "proliferation-oriented" translation program.

THALIDOMIDE DISTINCTLY AFFECTS THE PRODUCTION OF AUTOCRINE FACTORS IN OVARIAN CARCINOMA CELLS

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Background: Cytokines are pleotropic factors, involved in the regulation of growth, proliferation, and functions of cells from different tissues and organs under normal and pathological conditions. Over-expression of different cytokines was demonstrated in ovarian cancer tissues and cells. Thalidomide, which was initially marketed as a sedative-hypnotic drug with anti-emetic activity against morning sickness of early pregnancy, was withdrawn from the market as it was found to cause severe fetal malformations. Thalidomide inhibited TNF-α production in lipopolysaccharide-stimulated monocytes.

Aim of The Study: To evaluate the effect of thalidomide on TNF-α, IL-6 and MMP secretion in epithelial ovarian carcinoma cells.

Materials and Methods: Ovarian normal and carcinoma tissues were used in the present study. SKOV-3 cells and primary epithelial ovarian carcinoma cells were cultured in the presence of various concentrations of thalidomide. Cytokine and MMP localization and levels were determined in ovarian tissues and cells using immunohistochemical staining, RT-PCR analysis, ELISA and gelatin zymography (for MMPs activity), respectively. Cell proliferation was examined by MTT proliferation assay.

Results: Our results showed high levels of the examined cytokines (IL-1, IL-6, TNF and MMPs) in cancer ovarian tissues and cells compared to normal. TNFR2 mRNA levels were significantly higher in ovarian carcinoma tissues than in normal ovarian tissues, whereas TNFR1 mRNA levels were similar. TNFR1 and TNFR2 were mainly localized in the epithelial neoplastic cells of the tumor. Knocking-down TNF-α activity with anti-TNF antibodies altered ovarian carcinoma cell morphology (with more branches) in vitro. Thalidomide did not significantly affect the proliferation and growth of ovarian cancer cells in vitro. However, it decreased significantly the capacity of these cells to secrete TNF-α, IL-1 family but not IL-6. Thalidomide also significantly decreased the capacity of SKOV-3 cells, but not primary epithelial ovarian carcinoma cells, to secrete MMP-9 and MMP-2.

Conclusion: Our study suggests the possible involvement of cytokines in the pathogenesis of ovarian carcinoma. Thalidomide distinctly affected TNF-α, IL-6 and MMPs secretion by an ovarian carcinoma cell line (SKOV-3) and primary ovarian cancer cells. This might suggest a different susceptibility of these two types of cells to thalidomide, and/or that the mechanisms of secretion of the factors examined are differently regulated in these cells. Our results may deepen our understanding the mechanism/s of action of thalidomide in ovarian carcinoma cells. The results might have important implications in future therapeutic strategies that will incorporate thalidomide and other cytokine inhibitors in the treatment of epithelial ovarian carcinoma.

CAN THE UREA CYCLE ENZYMES REGULATE PH IN CANCER CELLS?

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The mammalian urea cycle takes place in the liver where it converts access nitrogen in the form of ammonia into urea which can be secreted in the urine. In extra hepatic tissues, only part of the urea cycle is active for the synthesis of arginine which is further utilized for the generation of multiple metabolites. A germ-line mutation in the urea cycle enzyme Argininosuccinate synthetase 1 (ASS1) causes a urea cycle disorder called Citrullinemia. Patients with Citrullinemia present with high blood glutamine levels and high ammonia (hyperammonemia) levels. Interestingly, in multiple cancers ASS1 has been shown to be down regulated. Since cancer cells generate high levels of lactate and because ammonia and glutamine can elevate pH, we hypothesized that ASS1 silencing in cancer may benefit cancer survival by helping the cells to buffer the acidic pH.

We first tested our hypothesis by performing a non-biased computational analysis in which we found that three first urea cycle's enzymes have decreased activity in a low intracellular pH in cancer, as compared to the healthy state. In agreement with published data, we next showed that under hypoxic conditions, ASS1 is down regulated in an osteosarcoma cell line while HIf1α is up regulated. Furthermore, when we silenced ASS1 in the osteosarcoma cell line, cells with ASS1 downregulation showed higher proliferation rate and higher production of lactate, even in bicarbonate and CO2 free conditions which prevent the medium from buffering the pH. Surprisingly, despite higher secreted lactate levels, the pH was less acidic in the medium of the cells with ASS1 deficiency and the glutamine uptake was lower, as compared to the control cell line.

Our results suggest a new role for ASS1 downregulation in cancer as a possible pH regulator of intra cellular pH. Further studies are needed to measure the intracellular pH following changes in ASS1 levels. Dissecting the contribution of ASS1 to cancer metabolism can potentially identify new targets for treatment modulation in cancers with ASS1 deficiency.

KAPOSI'S SARCOMA-ASSOCIATED HERPESVIRUS: FROM HEALTH CONCERN TO MOLECULAR INSIGHTS

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Kaposi's sarcoma-associated herpesvirus (KSHV) is aetiologically linked to Kaposi's sarcoma, primary effusion lymphoma, and plasmablastic multicentric Castleman's disease. The KSHV genome is notable for molecular piracy and encodes an extensive array of regulatory genes with similarity to cellular genes. These genes, probably pirated from the host during evolution, harbor unique functions. For example, the virus-encoded Bcl-2 homolog blocks cellular apoptosis and autophagy pathways, but has additional regulatory function which is critical during the infectious cycle. Like all other herpesviruses, primary infection with KSHV precedes lifelong infection involving two distinct cycles of viral DNA replication and gene expression: latent and lytic. Only few viral genes are expressed and no viral particles are produced during latency, whereas extensive viral DNA replication and a well-controlled array of viral-gene expression characterize the lytic phase which may end in the release of new viral particles. Latency is reversible and certain conditions reactivate hidden latent virus to enter the lytic phase. The lytic cycle is crucial for virus spread between hosts, for the maintenance of lifelong infection and for the progression and pathogenesis of KSHV-related neoplasms. Accordingly, compromised immunological surveillance, coupled with increased loads of KSHV, is strongly associated with the development of KSHV-related diseases. The epidemiology of KSHV as well as insights into unique properties of viral gene products and signaling pathways involved in lytic virus reactivation will be discussed.

POST-TRANSLATIONAL MODIFICATION (PTM) PROFILING -
A NOVEL TOOL FOR MAPPING THE PTM LANDSCAPE IN CANCER

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Aberrations in various components of the ubiquitin system have been implicated in the pathogenesis of cancer. In the past decade high-throughput analyses have profoundly broadened our understanding of the processes underlying cancer development and progression. Yet, most of the analyses focused on genomic and transcriptional changes while proteomics and the protein modification landscape remained relatively untouched. Partly this is because of the difficulties in analyzing changes in post-translational modifications, such as ubiquitin in biological samples. I will describe a platform for PTM profiling which allows the detection of protein modifications in various cellular and physiological conditions. Using this approach, we have revealed a novel role for the ubiquitin-like modifier, FAT10, in cancer.

INTRAVITAL MICROSCOPY OF CANCER INVASION, METASTASIS AND THERAPY RESPONSE

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The tumor microenvironment supports both cancer cell invasion and growth/survival programs, with impact on response to therapy and prognosis. Using intravital near-infrared/infrared multiphoton microscopy, we have established preclinical sarcoma and melanoma models for spontaneous cancer cell invasion and distant metastasis to lymph nodes and lungs. Using multi-parameter 3D detection with subcellular resolution, we identify the tissue niches enabling growth and invasion, as well as therapy resistance. Interstitial dissemination away from the primary lesion occurred along blood vessels, myofibers, nerves, adipocytes and collagen bundles, as guided migration along preformed multi-interface conduits. Once populated by cancer cells, these invasion niches enabled tumor cell survival and relapse of disease even after high-dose hypofractionated radiotherapy. This invasion-associated resistance was sensitive to combined anti-ß1/ß3 integrin and radiation therapy, which resulted in complete eradication and relapse-free survival. Despite its radiation-resistance, the invasion niche was particularly sensitive to adoptive CTL-based therapy, as consequence of particularly high local CTL accumulation, robust CTL-mediated apoptosis induction in tumor cells, and eradication of invasion strands. This establishes tumor-stroma interaction niches of overlapping invasion and resistance programs which can be exploited by combining conventional with integrin- and CTL-targeted therapy.

TUMOR MICROENVIRONMENT: EXOSOMES IN OVARIAN CARCINOMA AS A PARADIGM

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The metastatic process involves the manipulation of the tumor microenvironment to optimize the conditions for local growth and to enable dissemination of the tumor cells through out the patient's body.

One of the components of the microenvironment are the exosomes, small (30-100 nm) vesicles secreted continuously by both, normal and diseased cells. Exosome cargo reflects the molecular and cellular content of the cell of origin such as growth factors, receptors, proteases, coding and non-coding RNA and certain lipids. They also reflect dynamic changes that are occurring in health and at different stages of a disease.

In the present study we analyzed the expression profile of miRNAs in exosomes isolated from the effusion fluid of OC patients and compared them to the cellular profile of miRNAs in those patients. Our results indicated on specific miRNA population that correlated with the survival of the patients. Further, the same population of miRNAs increased the invasive potential of OC cells in vitro and in vivo models.

Examination of the secretory mechanism of exosomes revealed the expression of nSMmase2, Tsap6 and Rab27a mRNA in our tumor 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 ($p<0.027$).

Understanding the precise mechanism of exosome secretions opens a new avenue for potential treatment of this yet incurable disease.

IDENTIFYING MOLECULAR SIGNATURES FOR TUMOR DORMANCY AS A BASIS FOR THE DEVELOPMENT OF THERANOSTIC NANOMEDICINES

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Tumor progression is dependent on a number of sequential steps, including initial tumor-vascular interactions and recruitment of blood vessels (i.e., the "angiogenic switch"), as well as an established interaction of tumor cells with their surrounding microenvironment and its different immune, endothelial and connective cellular and extra-cellular components. Failure of a microscopic tumor, either primary, recurrent or metastatic, to complete one or more of these early stages may lead to delayed clinical manifestation of the cancer (i.e., tumor dormancy). Micrometastasis, dormant tumors, and residual tumor cells – referred to as minimal residual disease, contribute to the occurrence of relapse, and constitute fundamental clinical manifestations of tumor dormancy that together are responsible for the vast majority of cancer deaths. However, although the tumor dormancy phenomenon has critical implications for early detection and treatment of cancer, it is one of the most neglected areas in cancer research and the associated biological mechanisms are still mostly unknown.

We have created several models of patient-derived xenografts mimicking pairs of dormant vs fast-growing, primary vs metastatic and drug-sensitive vs resistant cancers. We investigated the molecular and cellular changes in tumor-host interactions that govern tumor dormancy. Those led to the discovery of novel targets and provided important tools for cancer theranostics (therapy and diagnostics). Based on the acquired knowledge, we designed a new strategy to improve treatment outcomes of patients with bone neoplasms, glioblastoma, brain metastases, melanoma, breast and prostate cancers. We have identified molecular signatures that, following selective delivery into their target cells, can potentially induce a dormant-like phenotype. This goal was achieved by utilizing polymeric nanomedicines and guidance by high resolution, intravital non-invasive imaging techniques.

A better understanding of tumor dormancy and the availability of relevant markers will most likely change the way we diagnose and treat the disease using novel combined theranostic nanomedicines.

OPTIMIZING PDX MODELS FOR NEXT GENERATION PRECISION MEDICINE

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Background: The inability to distinguish patient populations that are most likely to respond to different treatments is a continuing challenge for clinical oncologists. Individualized approaches developed from molecular analysis of tumors have only become a reality for a small percentage of patients. Technologies that can be broadly applied to all patients, which enable multiple therapeutic regimens to be evaluated simultaneously to identify the ones most likely to be clinically beneficial, are needed. Preclinical screening in patient-derived xenografts (PDXs) is a viable solution. In this study we examined the capacity of PDXs to replicate patient outcomes across a variety of solid tumors and treatments and report performance metrics highlighting the clinical utility of this tool.

Methods: Tumor tissue from 495 cancer patients was engrafted into immunodeficient mice to generate PDX models. Of these, 65 were screened against the treatments received by the corresponding patient. Patient clinical responses and model drug responses were assessed and correlated using RECIST criteria, with parameters, including sensitivity, specificity, and predictive values, calculated to determine the capacity of PDX responses to capture patient responses .

Results: Both positive (complete/partial response and stable disease) and negative (progressive disease) patient outcomes to treatment were accurately replicated by PDXs, regardless of tumor type or treatment. Based on 96 correlations in 65 models, we calculated a sensitivity of 99% and specificity of 70% for our PDX drug screens, as well as predictive values of 91% (positive) and 94% (negative). PDX models generated from the first resection retained the ability to reproduce clinical outcomes to treatments used for recurrent disease, despite patient exposure to interim lines of chemotherapy.

Conclusions: PDXs accurately reflect tumor responses in patients and have strong potential to correctly guide an oncologist to treatments most likely to yield a favorable patient response, whilst avoiding those that would not. Integration of PDX technology into routine cancer care will re-shape clinical decision-making paradigms in oncology and optimize patient outcomes.

SURGICAL EXCISION OF A PRIMARY TUMOR ENHANCES SPONTANEOUS METASTASIS OF BREAST CANCER THROUGH COX-2 AND BETA-ADRENERGIC PATHWAYS

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Evidence suggests that the surgical removal of a primary tumor in cancer patients elicits processes that promote the outbreak of pre-existing micrometastases and the initiation of new metastases. Indeed, the peri-operative period has been suggested to be pivotal in determining long-term cancer outcomes, despite of its relatively short duration in the course of cancer progression. Recent findings have pinpointed peri-operative surgical stress responses and inflammation as potential pro-metastatic mediators. Specifically, the excess secretion of catecholamines and prostaglandins was shown to suppress immunity and to affect the tumor and its microenvironment to acquire pro-metastatic characteristics. In this study we aimed at assessing the simultaneous blockade of catecholamines and prostaglandins during the perioperative period, addressing the clinical significance of this approach, as well as elucidating underlying mechanisms of the effects of surgery and of our drug treatment. To this end, we employed a human luciferin-labelled breast cancer xenograft (MDA-MB-231) injected orthotopically to the mammary fat pad of nude mice. Primary tumor and metastatic progression were monitored in vivo employing a high-sensitive bioluminescent imaging. Once metastatic foci were detected, the primary tumor was excised, and half of the mice were subjected to laparotomy, simulating a more extensive surgical procedure. Additionally, mice were treated peri-operatively for 30 hrs with both a non-selective b-adrenergic blocker and a COX-2 inhibitor (propranolol and etodolac), or with vehicle. Our results indicate that an extensive surgical procedure significantly increases metastatic burden, and that our combined drug treatment completely abolish this deleterious effect of surgery. As several interrelated mechanisms most likely underlie these effects, we are now examining immune involvement, as well as alterations in the pro-metastatic profile of tumor cells and their microenvironment. Natural killer cells may have a role, as surgery was shown to reduce their cytotoxicity through elevating catecholamines and prostaglandins, and as NK-depletion in nude mice significantly elevated lung colonization by MDA-MB-231 cells. Based on these and previous findings, a similar treatment protocol is now initiated in breast cancer patients.

SENESCENT CELLS COMMUNICATE VIA INTERCELLULAR PROTEIN TRANSFER

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Mammalian cells mostly rely on extracellular molecules to transfer signals to other cells. However, in stress conditions more robust mechanisms might be necessary to facilitate cell-cell communications. Cellular senescence, a stress response associated with permanent exit from the cell-cycle and the development of an immunogenic phenotype, limits both tumorigenesis and tissue damage. Paradoxically, the long-term presence of senescent cells can promote tissue damage and ageing within their microenvironment. Soluble factors secreted from senescent cells mediate some of these cell non-autonomous effects. However, it is unknown whether senescent cells impact neighboring cells by other mechanisms. Here we show that senescent cells directly transfer proteins to neighboring cells and that this process facilitates immune surveillance of senescent cells by NK cells. We found that transfer of proteins to NK and T cells is increased in murine pre-neoplastic pancreas, site where senescent cells are present in vivo. Proteomic analysis and functional studies of the transferred proteins revealed that the transfer is strictly dependent on cell-cell contact and CDC42-regulated actin polymerization, and is mediated, at least partially, by cytoplasmic bridges. These findings reveal a novel mode of intercellular communication by which senescent cells regulate their immune surveillance and might impact tumorigenesis and tissue ageing.

LYSYS OXIDASE OVEREXPRESSED IN MICE THAT UNDERGO SURGERY MAY PROMOTE METASTASIS

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Tumor resection is one of the major treatment modalities for cancer. It is sometimes combined with chemo-radiation in order to reduce the risk of tumor re-growth or metastasis spread from existing residual disease. Yet, patients who undergo surgery may exhibit metastatic spread. Here we show that the host in response to radical surgery is vulnerable to metastasis seeding. Non-tumor bearing mice, which undergo surgery, succumb to LLC or EMT/6 lung metastasis earlier than control mice in an experimental lung metastasis assay. Similarly, mice injected with plasma from mice which underwent surgery were prone to metastasis seeding more than mice injected with plasma from control mice. Changes in primary tumor growth, angiogenesis and the colonization of bone marrow derived cells at the primary tumor site were documented following surgery. Importantly, increased LOX activity in the lungs of mice that underwent surgery resulted in lung extracellular matrix modulation which may account for tumor cell seeding. Consequently, the blockade of LOX family members by BAPN or by neutralizing antibodies reduced metastasis spread in the lungs of mice following surgery and increased their survival. Taken together, our results emphasize the modulation of the extracellular matrix in the pre-metastatic microenvironment induced by surgery, and suggest that LOX may contribute to surgery-induced metastasis. The study also offers new therapeutic intervention in combination with surgery to reduce risks of metastasis.

FULL LENGTH SEMAPHORIN-3C IS AN INHIBITOR OF TUMOR LYMPHANGIOGENESIS AND METASTASIS

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Semaphorins play important regulatory roles in diverse processes such as axon guidance, angiogenesis and immune responses. We find that semaphorin-3C (sema3C) induces the collapse of the cytoskeleton of lymphatic endothelial cells (LEC) in a neuropilin-2, plexin-D1 and plexin-A1 dependent manner, while most other semaphorins, including anti-angiogenic semaphorins such as sema3A do not. Sema3C is cleaved, like other class-3 semaphorins, by furin like pro-protein convertases (FPPC). Cleaved sema3C (p65-Sema3C) was unable to induce the collapse of the cytoskeleton of LEC. FPPC are strongly up-regulated in tumor cells. In order to examine the effects of full length sema3C on tumor progression we therefore generated an active point mutated furin cleavage resistant sema3C (FR-sema3C). FR-sema3C inhibited potently proliferation of LEC and to a lesser extent proliferation of umbilical vein derived endothelial cells (HUVEC). FR-sema3C also inhibited VEGF-C induced phosphorylation of VEGFR-3, ERK1/2 and AKT. Expression of recombinant FR-sema3C in metastatic, triple negative LM2-4 breast cancer cells did not affect their migration or proliferation in-vitro. However, tumors derived from FR-sema3C expressing LM2-4 cells implanted in mammary fat pads developed at a slower rate, contained a lower concentration of blood vessels and lymph vessels, and metastasized much less effectively to lymph nodes. Interestingly, p65-Sema3C, but not FR-sema3C, rendered A549 lung cancer cells resistant to serum deprivation suggesting that previously reported pro-tumorigenic activities of sema3C may be due to p65-Sema3C produced by tumor cells. Our observations suggest that FR-sema3C may be further developed into a novel anti-tumorigenic drug.

THE PROTEIN INTERACTION MAP OF APOPTOSIS AND AUTOPHAGY: FROM BASIC SCIENCE TO A THERAPEUTIC VISION

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Apoptosis and autophagy are distinct biological processes, each driven by a different set of protein-protein interactions, including some direct points of interface between apoptotic and autophagic proteins. Our laboratory studies the overall landscape of these proteomic maps and subsequently zooms into novel biochemical pathways discovered by the systems level approaches. To measure the global profile of protein interactions in cells, we have adapted the Protein fragment Complementation Assay (PCA), which monitors binding between proteins fused to complementary fragments of a luciferase reporter. A library encompassing 63 proteins from the basic machineries of autophagy and apoptosis, and some of their regulatory proteins, was constructed for the analysis of ~3600 protein-pair combinations. This generated a detailed landscape of the apoptotic and autophagic modules and the points-of-interface between them, identifying 46 previously unknown interactions. Two of these novel interactions were further investigated in details. One deals with DAPK2, a Ser/Thr kinase that promotes cell death and autophagy. It was found here that DAPK2 interacts with 14-3-3t, resulting in inhibition of DAPK2 dimerization and suppression of its biochemical and cellular activities. Another autophagic Ser/Thr kinase, ULK1, was found to interact with WIPI2b, thus providing a direct link between a regulatory process and the basic machinery of autophagosome formation. This proof-of-concept underscores the power of the PCA platform for the discovery of novel biochemical pathways within the cell death network. The emerging landscape of the global map including the connectivity between apoptosis and autophagy is currently used for identifying the cell death signature of individual tumor cells. Novel platforms that measure the global functionality of the cell death network are being developed to follow the diversity among individual tumors, and identify alternative cell death pathways that are still active in the tumor.

MACROLIDE INDUCED READ-THROUGH OF APC NONSENSE MUTATIONS IN FAMILIAL ADENOMATOUS POLYPOSIS

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Colorectal cancer (CRC) is the third most common cancer worldwide. Approximately 85% of all colorectal adenomas or carcinomas show Adenomatous Polyposis Coli (APC) gene, a classical tumor suppressor, loss of function. Familial adenomatous Polyposis (FAP) is caused by dominant germline mutations in the APC gene. In a subset of FAP patients, APC loss occurs due to a pre-mature termination codon (PTC) caused by a signal nucleotide substitution (nonsense mutation) leads to expression of a truncated, non-functional protein. It has been shown that various nonsense mutations can be ameliorated by treatment with aminoglycosides antibiotics or other compounds, which result in PTC read-through and expression of a full length, functional protein. We have recently reported that members of the macrolide antibiotic family can mediate read-through of APC nonsense stop mutations in tissue culture and in mice models. As compared to aminoglycosides, macrolides are safer and can be administrated for long periods. We have recently constructed a novel reporter system where the expression level of the blue fluorescent protein (BFP) correlates read-through levels of stop codon sequences. In this study we provide proof of concept for the ability of macrolides to induce APC PTC read-through in FAP patients. Using our system we can demonstrate that different types of macrolide antibiotics lead to read-through of various human APC nonsense stop mutations. We have tested 4 specific APC mutations from FAP patients and found various levels of read-through induction. Our preliminary experiments in different cell lines and in APCMin mice show that macrolide treatment of polyps caused by APC nonsense mutations lead to a reduction in both the number and size of such polyps. Gene arrays show that the treatment leads to differential gene expression. We are currently conducting a clinical trial in FAP patients with promising preliminary results.

TARGETING THE REPROGRAMMED ENERGY GENERATION SYSTEM OF CANCER CELLS

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The aspiration to achieve efficacious cancer targeted therapy involves intense global R&D efforts. These are aimed toward the development of selective inhibitors of cancer driving aberrant and mutated regulatory pathways, thereby leading to the elimination of malignant tumors. However, vastly accumulating evidence highlight the complexity and challenging nature of this goal. This reflects the genomic instability of malignant cells and their tendency to acquire resistance to therapeutic drugs. Thus, blockage of fundamental processes like the unique reprogramed metabolic and energy generation systems of malignant cells, should offer new tools for efficient interference with cancer progression.

The kinase Fer and its spermatogenic meiotic variant, FerT, are co-expressed in normal testes and cancerous tumors, but whether they exert related roles in spermatogenic and malignant cells has not been known.

Here we show that Fer and FerT reside in the mitochondria of spermatogenic cells and are harnessed to the reprogrammed mitochondria of colon carcinoma (CC) cells. Both kinases bound complex I of the mitochondrial electron transport chain (ETC) in spermatogenic and in CC cells and silencing of either Fer or FerT was sufficient to impair the activity of this complex. Directed mitochondrial accumulation of FerT in non-malignant NIH3T3 cells increased their ETC complex I activity, ATP production and survival, contingent upon stress conditions caused by nutrient and oxygen deprivation. Strikingly, directed mitochondrial accumulation of FerT endowed non-malignant cells with tumor-forming ability. Thus, recruitment of a meiotic mitochondrial component to cancer cell mitochondria highlights a pivotal role for reprogrammed mitochondria in tumorigenesis.

To translate these findings into a novel anti-cancer therapy we have developed a synthetic low molecular weight compound which binds and inhibits the kinase activity of both Fer and FerT. This compound termed E260 selectively perturbs mitochondrial functioning in malignant cells thereby imposing ATP depletion and necrotic death in cancer cells. The anti-cancer potency of E260 is also manifested in xenograft models in mice, thus portraying it as a new potential anti-cancer drug.

POPULATION SCREENING FOR MUTATIONS IN BRCA1 AND BRCA2 - ARE WE THERE YET?

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Among Ashkenazi Jews (AJ), 11% of breast cancer and 40% of ovarian cancer cases are due to three inherited founder mutations in the cancer predisposition genes BRCA1 and BRCA2. For carriers of these mutations, risk-reducing salpingo-oophorectomy significantly reduces morbidity and mortality. Population screening for these mutations among AJ women would be justifiable if cancer risks were high in carriers identified at the population level. We recently determined breast and ovarian cancer risks for BRCA1 and BRCA2 mutation carriers ascertained irrespective of personal or family history of cancer. (Gabai-Kapara et al., PNAS 2014). Families harboring BRCA1/BRCA2 mutations were ascertained by identifying mutation carriers among healthy AJ males. Female relatives of these male carriers were then tested. Among the female relatives found to be carriers, cumulative risk of developing either breast or ovarian cancer by age 60 and 80, respectively, were 0.60 (± 0.07) and 0.83 (± 0.07) for BRCA1 carriers and 0.33 (± 0.09) and 0.76 (± 0.13) for BRCA2 carriers. Risks were higher in recent vs. earlier birth cohorts (P = 0.006). High cancer risks in BRCA1/BRCA2 mutation carriers identified through healthy males provide an evidence base for initiating a general screening program in the AJ population. General screening would identify many carriers who are not evaluated by genetic testing based on family history criteria. Such a program could serve as a model of population screening for genetic predisposition to cancer in other populations. We are currently performing further studies to investigate implementation and outcomes of such a program.