mitochondrial kinases responsive to oxidative stress communicate to the rest of the cell.

Using pharmacological and immunochemical methods we tested the role of mitochondrial permeability transition (MPT) and the Bcl-2 proteins in oxygen-dependent radiosensitivity. Treated or untreated cervical cancer HeLa, breast cancer MCF-7 and melanoma MeWo cell lines were irradiated at 6.2 Gy under normoxic and hypoxic conditions (<0.2% O2 x 1h) then allowed to proliferate for seven days. Reduction of resazurin to resorufin was used as an index of cell growth.

MPT blocker cyclosporin A (2μM) strongly protected HeLa but not the other two lines against oxygen-dependent radiosensitivity. By contrast, bongkrekic acid (50μM) had only marginal effect and calcineurin inhibitor FK-506 (0.1 μM) had none. Nor was evidence found for MPT modulation by Bax/Bcl-2 signaling, mitoKATP channels or mitochondrial Ca2+ uptake.

Calcineurin-independent protection by cyclosporin A suggests that MPT but not mitoKATP or the mitochondrial apoptosis pathway plays a causal role in oxygen-dependent radiosensitivity of HeLa cells. Targeting MPT may therefore improve the effectiveness of radiotherapy in some solid tumours.

We gratefully acknowledge Aid Cancer Treatment, Cork, for financial support.

07 July 2008 08:00 - 08:50

EDUCATIONAL LECTURE

Angiogenesis / Hypoxia

232

The von Hippel-Lindau protein: insights into hypoxic signaling and cancer

W. Kaelin Jr[1]
[1]*Dana-Farber Cancer Institute and Harvard Medical School, Howard Hughes Medical Institute, Boston, USA*

Inactivation of von Hippel-Lindau (VHL) tumor suppressor gene plays an important role in clear cell renal carcinoma, hemangioblastoma, pheochromocytoma, as well as some other tumors. Individuals with germline VHL mutations (VHL disease) are at increased risk for these tumors in an allele-specific manner (genotype-phenotype correlation). The VHL gene product (pVHL) has multiple functions including serving as the substrate recognition subunit of an E3 ubiquitin ligase that targets the alpha subunits of the heterodimeric transcription factor HIF (Hypoxia-inducible Factor) for destruction. HIFα must be hydroxylated on one (or both) of two conserved prolyl residues by members of the EglN family (also called PHD or HPH family), which are oxygen-dependent enzymes that also require reduced iron, 2-oxoglutarate, and ascorbic acid, in order to bind to pVHL. Under low oxygen conditions, or in cells lacking wild-type pVHL, HIFα accumulates and activates 100-200 genes involved in adaptation to hypoxia. Deregulation of HIFα (especially HIF2α) appears to play a causal role in clear cell renal carcinoma and almost certainly contributes to the development of hemangioblastomas, which are blood vessel tumors. Loss of pVHL might explain why clear cell renal carcinomas are high angiogenic, overproduce the HIF-responsive gene product VEGF, and are particularly sensitive (among solid tumors) to VEGF inhibitors. We are also conducting "synthetic lethal" screens in search of kinases that are particularly important for the survival of VHL-/- tumor cells compared to pVHL-proficient cells. In theory inhibitors of such kinases would kill VHL-/- tumor cells while sparing normal cells. In addition, we are using high density SNP arrays, gene expression profiling, and siRNA functional screens to identify mutations that cooperate with VHL loss in renal carcinogenesis. It is hoped that these studies will identify additional 'drugable' targets in renal carcinoma.

Higher metazoans, including people, have three EglN family members (EglN1, EglN2, and EglN3). We generated a conditional EglN1 mouse (EglN1-/- embryos are not viable) and confirmed cell culture experiments that suggested EglN1 is the primary HIF prolyl hydroxylase. Our recent studies suggest that EglN2 and EglN3 play roles in control of cell proliferation and apoptosis, respectively. We found, for example, that the genes that, when mutated, cause familial paraganglioma and pheochromocytoma define a pathway that is activated in sympathetic neuroblasts during embryological development by growth factor withdrawal. Interestingly, this pathway impinges upon EglN3, which is both necessary and sufficient for apoptosis in this setting. In an unbiased screen for shRNAs that confer protection against EglN3-induced apoptosis, we identified an shRNA directed against KIF1Bβ, which maps to 1p36.2. This region of the genome is frequently deleted in a variety of tumors, including neuroblastoma. Notably, this gene is also one of only 6 annotated genes located within a 500 kB homozygous deletion in a neuroblastoma line. Restoration of KIF1Bβ function in this line induces apoptosis and we have identified germline loss of function KIF1Bβ mutations in some neuroblastoma and pheochromocytoma patients, arguing that KIF1Bβ is a potential tumor suppressor gene. Preliminary data suggest that KIF1Bβ haploinsuffiency is sufficient to protect from apoptosis, which might account for the observation that many 1p deleted tumors retain a wild-type KIF1Bβ allele.

07 July 2008 08:00 - 08:50

EDUCATIONAL LECTURE

Drug targets screening

233

An in vitro systems approach to predicting and understanding clinical responses to molecularly targeted therapeutics

P. Spellman[1], D. Das[1], W.L. Kuo[1], S. Bhattacharya[1], N.J. Wang[1], H.S. Feiler[1], L. Jakkula[1], A. Wyrobek[1], J.W. Gray[1]
[1]*Lawrence Berkeley National Laboratory, Life Sciences Division, Berkeley, USA*

Background: We are developing methods that allow targeted treatment of individual cancer patients by using in vitro models of response to identify molecular signatures that predict clinical utility. Materials and Methods: We use a well-characterized panel of more than 50 breast cancer cell lines to model the clinical responses of breast cancers to molecularly targeted and traditional anti-cancer agents. The panel of cell lines reflects the substantial heterogeneity of breast cancers at the genomic and transcriptional levels. Traditional growth assays are used to assess cell line responses to individual agents and are then compared to the mutational spectra, copy number aberrations, and transcriptional profiles to identify predictors of response. These predictors can then be deployed clinically to determine which patients are likely to benefit from a given agent. Results: We have created molecular signatures that predict cell line response for more than 20 therapeutic agents including traditional chemotherapeutics (i.e. carboplatin) and targeted agents. Several of our predictors from the cell line system make implicit biological sense (i.e. ErbB2 expression level is an excellent predictor of response for ErbB2 targeting agents, or mutations in the Akt pathway are excellent predictors of response for at least one anti-Akt agent). We have successfully validated some of these signature in both tissue culture and clinical materials using the Quantigene gene expression platform that allows multiplex mRNA level measurements for ~100 genes from a single 10 micron tumor section and with no purification of RNA. Conclusion: The process of identifying patients who might benefit from particular therapeutic regimens is unlikely to be solved by clinical trials. Additionally, as molecularly targeted therapeutics target ever-smaller subsets of the patient population it is necessary for clinical trials to enrich for patients that are likely to respond. In vitro systems can be effectively utilized to identify predictive signatures that can either help clinical trials achieve success or identify therapeutic regimens of approved drugs for patients.

07 July 2008 08:00 - 08:50

EDUCATIONAL LECTURE

Antibody engineering

234

Vascular targeting antibodies: from the bench to the clinic

D. Neri[1]
[1]*Institut für Pharmazeutische Wissenschaften, Chemistry and Applied Biosciences, Zurich, Switzerland*

BACKGROUND: One avenue towards the development of more selective anti-cancer drugs consists in the targeted delivery of bioactive molecules (drugs, cytokines, procoagulant factors, photosensitizers, radionuclides, etc.) to the tumor environment by means of binding molecules (e.g., human antibodies) specific to tumor-associated markers. In this context, the targeted delivery of therapeutic agents to newly-formed blood vessels ("vascular targeting") is particularly attractive, because of the dependence of tumors on new blood vessels to sustain growth and invasion, and because of the accessibility of neo-vascular structures for therapeutic agents injected intravenously. MATERIALS AND METHODS: Human

antibodies specific to markers of angiogenesis have been generated either by antibody phage display or by iterative colony filter screening. In this lecture, I will present data on antibodies specific to splice isoforms of tenascin-C and of fibronectin. Human antibody derivatives which are currently in clinical trials have been produced in mammalian cell expression systems. RESULTS: The human antibodies F8, L19 and F16 (specific to the alternatively spliced EDA and EDB domain of fibronectin, and to the A1 domain of tenascin-C, respectively) have extensively proven their ability to efficiently and selectively localize around tumor blood vessels, following intravenous injection in tumor bearing mice. In the case of L19, its tumor targeting ability in patients with cancer has been extensively demonstrated using scintigraphic techniques, following antibody radioiodination. Five of the most promising antibody derivatives (L19-131I, L19-IL2, L19-TNF, F16, 131I, F16-IL2) are currently being investigated in over ten clinical trials, while three derivatives of the F8 antibody should enter clinical trials for the therapy of cancer by the end of 2008. In my lecture, I will provide an overview about the preclinical and clinical therapeutic performance of these products. CONCLUSIONS: Vascular targeting antibody derivatives represent a promising class of novel anti-cancer biopharmaceuticals. Five products of this type, developed in my lab and in the lab of Luciano Zardi in collaboration with Philogen SpA and with Bayer Schering AG, are currently being investigated in clinical trials in several European centers.

07 July 2008 09:00 - 09:45

PLENARY LECTURE

Metabolism

235
Metabolism

No abstract received

07 July 2008 10:15 - 12:15

SYMPOSIUM

Invasion and metastasis

236
Cell adhesion and signalling

M. Frame[1], V. Brunton[1], J. Evans[2], O. Sansom[3]
[1]Edinburgh University, Edinburgh Cancer Research Centre, Edinburgh, United Kingdom; [2]Beatson Institute, Translational Cancer Research Laboratory, Glasgow, United Kingdom; [3]Beatson Institute, Animal models of cancer, Glasgow, United Kingdom

Cancer cell invasion and metastasis is a hallmark of the malignant phenotype that is responsible for most cancer deaths. Despite this, proteins involved in these processes are rarely considered as anti-cancer targets. One reason for this is the difficulty in devising optimal pre-clinical and clinical tests of the success of putative anti-invasive agents.

In recent years we have been devising ways of examining cancer cell invasion and metastasis in vitro and in vivo, and of testing the efficacy and mode of action of tyrosine kinase inhibitors that have anti-invasive activity and which are undergoing clinical evaluation. In particular, we are using genetically engineered mouse models of pancreatic and breast cancer, in which tumour cells arising also express GFP, to examine tumour development and progression in vivo by direct imaging. This is being done at the whole body- and single-cell levels in vivo.

Our results to date suggest that Src inhibitors might best be used as anti-invasive agents, and that invasion and metastasis is one role of both elevated Src itself and of focal adhesion kinase, Src's binding partner and substrate. This has implications for the use of these agents and for designing clinical trials that will examine their clinical usefuleness.

237
Signaling mechanisms of tumor cell migration and metastasis

R. Klemke[1], K. Stoletov[1], V. Montel[1], R. Lester[1], S. Gonias[1]
[1]University of California San Diego, Department of Pathology and Moores Cancer Center, La Jolla, USA

Cell metastasis is a highly dynamic process that occurs in multiple steps that include cell invasion, intravasation, survival in the circulation, extravasation, and cell growth at the metastatic site. Understanding this process has been limited by the inability to visualize tumor cell behavior in real time using animal models. This is especially true in regards to the early events of metastasis which involve cell invasion and vessel wall penetration during intravasation. We have utilized translucent, GFP vascular transgenic zebrafish, and high resolution intravital confocal microscopy to study how human cancer cells expressing DsRed or CFP invade tissues, induce angiogenesis, and interact with newly formed vessels. The optical clarity and fluorescent vessels of this new xenograft model allowed us to visualize how the human metastatic gene RhoC promotes cell invasion and intravasation during the early events of cancer cell metastasis with unprecedented resolution. We find that RhoC expression induces a primitive amoeboid-like cell invasion characterized by the formation of dynamic membrane protrusions and blebs. Surprisingly, these structures penetrate the blood vessel wall exclusively at sites of vascular remodeling and not at regions of existing intact vessels. This process requires tumor cells to secrete VEGF which induces vascular openings, which in turn, serve as portholes allowing access of RhoC expressing cells to the blood system. Our results support a model in which the early steps in intravasation and metastasis require two independent events: 1) dynamic regulation of the actin/myosin cytoskeleton within the tumor cell to form protrusive structures and 2) loss of vessel wall integrity as a result of VEGF-induced permeability and vascular remodeling. The integration of zebrafish transgenic technology with human cancer biology may aid in the development of novel cancer models that target specific organs, tissues or cell types within the tumors. Zebrafish could also provide a cost effective means for the rapid development of therapeutic agents directed at blocking human cancer progression and tumor-induced angiogenesis.

238
Individual and collective cancer cell invasion

P. Friedl[1]
[1]Radboud University Nijmegen Medical Centre, Microscopical Imaging of the Cell, Department of Cell Biology, NCMLS, Nijmegen, The Netherlands

Cancer cell dissemination and metastasis in vivo result from a diverse set of migration strategies including individual cells and multicellular strands and clusters, referred to as collective invasion. Using 3D collagen lattices and in vivo intravital microscopy of cancer cell invasion, we have resonstructed at high resolution the subcellular location of pericellular proteolysis during the migration process, the resulting ECM remodeling, and invasion mechanism. Proteolytic microtracks generated by single cells are subsequently filled and widened by following cells that form collective stands moving along expanding macrotracks. Collective invasion in vitro was confirmed using in vivo xenografts monitored by intravital multiphoton microscopy. The findings show how cell invasion and proteolytic ECM remodeling form a functional unit of collective cell invasion and the generation of aligned tissue structures. Using molecular interference, including anti-integrin and protease-inhibitor-based inhibition, collective invasion is abrogated yet converted towards amoeboid single-cell scattering (collective-amoeboid transition), suggesting novel compensation strategies of cancer cell dissemination.

239
Cell adhesion to the extra cellular matrix (ECM) in motility and metastasis

B. Geiger[1], S. Naffar Abu-Amara[1], S. Winograd-Katz[1], L. Nadav[1], B. Katz[2]
[1]Weizmann Institute of Science, Department of Molecular Cell Biology, Rehovot, Israel; [2] Tel-Aviv Sourasky Medical Center, Institute of Hematology, Tel-Aviv, Israel

The adhesive interactions of cells with their environment regulate a wide variety of cellular responses that affect multiple cellular features, including cell proliferation, survival, gene expression and migration. These signals are affected by a wide variety of environmental cues, including both chemical and physical properties of the adhesive surface. Thus, cells can differentially respond to different adhesive ligands, and can sense the geometry, rigidity, contractility and ligand density of the external surface. The complex information "gathered" by the cells via their matrix adhesion sites is processed and integrated, affecting a wide variety of cellular processes. Interestingly – this adhesion-mediated cross talk between the cells and the matrix is often perturbed in cancer cells, leading to many features of the transformed phenotype. In this lecture I will address the complex molecular interactions of cells with the extracellular matrix (ECM), focusing on the molecular complexity and diversity of the "integrin adhesome, and its multiple roles in regulating cell structure, migration and signaling. I will demonstrate that integrin adhesions can "sense" a wide variety of chemical and physical "environmental cues", including the nature