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.

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EDUCATIONAL LECTURE

Angiogenesis / Hypoxia

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

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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.

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EDUCATIONAL LECTURE

Drug targets screening

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

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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.

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EDUCATIONAL LECTURE

Antibody engineering

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Vascular targeting antibodies: from the bench to the clinic

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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