Friday, 24 October 2008 08:00–09:45

PLENARY SESSION 8

RNA based technologies for target identification, validation and treatment

456 INVITED

Development of practical delivery systems for therapeutic small RNAs

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Since its discovery 10 years ago, RNA interference (RNAi) has become an almost standard method for the knockdown of any target gene of interest in vitro. Since it relies on a catalytic mechanism and targets mRNAs rather than proteins, it is particularly efficient and also allows the silencing of gene products which are 'non-druggable' by other approaches. Thus, beyond in vitro knockdown for functional analyses, RNAi can be exploited for the downregulation of pathologically relevant genes which are aberrantly expressed in a given disease, offering novel therapeutic approaches.
RNAi is mediated by small interfering RNAs (siRNA), and since all other components of the RNAi machinery are provided by the cell, the efficiency of RNAi in vitro and in vivo is determined by the intracellular presence and efficacy of these specific siRNA molecules. Due to their instability and physicochemical properties, the development of strategies and formulations for siRNA protection, cellular uptake, correct intracellular localization, endosomal release and absence of unwanted side effects is of critical importance. In vivo, favourable pharmacokinetic properties, preferential delivery to the target organ and high biocompatibility/absence of toxicity are other major issues. In fact, the in vivo delivery of siRNAs represents the probably most relevant challenge for the establishment of therapeutic RNAi. Delivery approaches include the encapsulation in lipids, the complex formation with a variety of liposomes or cationic polymers, the chemical conjugation of siRNAs for example to peptides, aptamers or antibodies as well as other formulations.
This presentation will discuss non-viral strategies for the delivery of therapeutic siRNAs to induce RNAi, based on different siRNA formulations and various modes of administration. An overview of siRNA applications in various animal models covering different pathologies (cancer, viral infections, other diseases) will be given, highlighting promising developments in the field. Finally, as a representative example the presentation will include a more in-depth description of one approach relying on cationic polymers, polyethylenimines (PEI), which have been used successfully for the therapeutic knockdown of cancer-relevant genes in mouse tumor models.

457 INVITED

Identifying mechanisms of drug resistance using large scale genetic screens

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Background: Unresponsiveness to therapy is remains a significant problem in the treatment of cancer, also with the new classes of targeted therapeutics. In my laboratory, we use functional genetic approached identify biomarkers that can be used to predict responsiveness to clinically-relevant cancer therapeutics. We focus on the well-established targeted cancer drugs such as Trastuzumab. This drug targets a specific molecule (HER2) that is over-expressed or in breast cancer. Nevertheless, it remains poorly explained why a significant number of tumors, which express the drug target, do not respond to the therapy. We aim to elucidate the molecular pathways that contribute to unresponsiveness to targeted cancer therapeutics using a functional genetic approach. This will yield biomarkers that can be used to predict how individual patients will respond to specific drugs. Furthermore, this work may allow the development of drugs that act in synergy with the established drug in the treatment of cancer.
Material and Methods: To identify biomarkers that control tumor cell responsiveness to cancer therapeutics, we use both genome-wide gain-of-function genetic screens (with cDNA expression libraries) and genome wide loss-of-function genetic screens (with RNA interference libraries) in cancer cells that are sensitive to the drug-of-interest. We search for genes whose over-expression or whose down-regulation in cultured cancer cells confers resistance to the drug-of-interest. Once we have identified such genes, we ask if their expression is correlated with clinical resistance to the drug-of-interest using tumor samples of cancer patients treated with the drug in question, whose response to therapy is documented.
Results: We have used BT474 human breast cancer cells (HER2 amplified), to find genes whose suppression confers resistance to Transtuzumab. We found that of 8,000 genes surveyed, only loss of PTEN caused resistance to Trastuzumab. In a cohort of 55 breast cancer patients, both loss of PTEN and mutation of the PIK3CA gene (which is controlled by PTEN) were predictive for poor response to Trastuzumab.
Conclusion: This study illustrates the power of genetic screens to identify biomarkers useful for predicting treatment response in the clinic. Our data demonstrate that activation of the PI3K pathway (caused either by loss of PTEN or by activating mutations in the PIK3CA gene) is predictive for poor responses to Trastuzumab-based therapy. Assessment of PI3K pathway activation in HER2+ breast cancer may help identify those patients that may benefit from drugs that inhibit the HER2 signaling pathway more downstream, e.g. by using PI3K inhibitor drugs or mTOR inhibitor drugs.

458 INVITED

High-throughput RNAi screening using cell microarray technology

O. Kallioniemi[1]. [1]*Haartman Institute, University of Helsinki, Helsinki, Finland*

We have developed an ultra-high density cell-microarray screening system for genome-scale loss-of-function RNA interference screening. The cell array technology has up to 100-fold screening throughput as compared to 384-well-based assays. In this technology, siRNAs and transfection agents are first printed as a microarray with up to 10–20,000 spots per array. Cultured cells are then allowed to adhere on top of the array, where they undergo transfection with the siRNAs in a spatially confined manner. Cell phenotypes resulting from knockdown of specific genes are read with HTS and HCS instrumentation using up to 4 parameters at a time. We have used the cell microarray technology for the identification of genes whose knockdown causes specific cell phenotypes in prostate cancer, such as induction of apoptosis and activation of oncogenic signalling. Examples from screening of epigenetic endpoints will also be shown.

459 INVITED

microRNA and therapeutic applications

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No abstract received

Friday, 24 October 2008 10:15–12:00

PLENARY SESSION 9

Imaging molecular targets

460 INVITED

Update on imaging tumor proliferation with PET

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PET has been in development for a number of years yet only in the last decade has it become an accepted technique for the routine evaluation of oncology patients. When combined with fluorodeoxyglucose (FDG), PET is regularly used in the staging of cancer. PET has been found to complement cross-sectional imaging with CT and MR and alter the course of patient treatment. While FDG PET has gained clinical acceptance, work must continue to validate its use in less common tumor types and in measuring treatment response. The expanding use of PET needs to be accompanied by the development of new tracers to measure different aspects of tumor metabolism and proliferation. Tumors differ from normal tissues in terms of increased biosynthesis, particularly of DNA. Initial work was done to validate imaging tumor growth with labeled thymidine. Other tracers were needed to make imaging proliferation practical, given the rapid biologic degradation of thymidine along with the short half-life of 11C. This led to the development of 3′-deoxy-3′-fluorothymidine (FLT), which resists degradation, can be labeled with 18F, and tracks cellular proliferation through retention by thymidine kinase 1. Pilot studies with FLT have been done in a number of centers and FLT appears promising in its ability to detect tumors and may find use in measuring treatment response. Using FLT one can obtain reproducible measurements of tumor retention using either simplified approaches, such as the standardized uptake value (SUV), or full dynamic modeling. FLT uptake within hours of treatment may also reflect pharmacodynamic changes in some situations. Imaging tumor

apoptosis may complement images of energetics and proliferation. One may also be able to image cell stress using FMAU [1-(2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)-thymine] which tracks thymidine kinase 2. The best use of this array of tracers will require detailed studies to determine the best approach for different tumors and treatments. This is particularly important given the advent of many newer targeted drugs, which require new ways to determine their efficacy.

461 INVITED

Hypoxia imaging

K.A. Krohn[1]. [1]*University of Washington Medical Center, Radiology, Seattle, USA*

Hypoxia, a condition of insufficient O2 to support metabolism, occurs when the blood supply is inadequate. It occurs when a tumor grows so fast that immature blood vessels supply the tumor. Increased diffusion distances to tumor cells and reduced O2 transport exacerbate this problem. When tumor cells become hypoxic they adapt by up-regulating the production of numerous proteins that promote their survival. These proteins slow the rate of growth, switch the mitochondria to glycolysis, stimulate growth of new vasculature, inhibit apoptosis and promote metastatic spread, processes that enable tumor cells to survive or escape the O2-deficient environment. The consequence is that hypoxia represents a significant challenge to the curability of human tumors; patients with hypoxic tumors invariably experience overall diminished therapeutic response, malignant progression, increased recurrence, loco-regional spread and distant metastases. Strategies are being developed to surmount the cure-limiting consequences of hypoxia but methods are needed to select patients most likely to benefit from these approaches. Even though hypoxia is a common tumor phenotype, it is by no means universal and is often heterogeneous within a patient. This report considers several novel methods for imaging regional hypoxia and how information about the oxygenation of tumors might impact treatment. Tumor hypoxia can be detected by invasive and non-invasive techniques. Regional measurement of O2 partial pressure can be made using electrodes placed under CT guidance. While this provides an absolute measure of PO2 (mm Hg), it is inherently invasive and can only be applied to accessible sites. Nuclear medicine approaches have been developed with radiopharmaceuticals that accumulate with an inverse relationship to PO2. 18F-MISO and Cu-ATSM-PET, and BOLD-MRI are the lead contenders for human application based on their non-invasive nature, ease of use, robustness, validity, ability to demonstrate heterogeneity and general availability. This presentation will discuss where developments are required for hypoxia imaging to become clinically useful and explore potential new uses for hypoxia imaging. In conclusion, invasive methods to measure regional PO2 are now being replaced by noninvasive imaging methods using radionuclides or magnetic resonance detection. The most widely studied imaging procedure, FMISO PET, has been shown to have independent predictive value for outcome. The clinical role of hypoxia imaging will probably be less to detect disease and more to improve radiation treatment planning and selection of appropriate patients for treatment with hypoxia-selective cytotoxins. Hypoxia imaging has become an important success story over the last decade and provides some important lessons for development of new imaging probes or biomarkers.

462 INVITED

Apoptosis imaging

H. Strauss[1], F. Blankenberg[2]. [1]*Memorial Sloan-Kettering Cancer Center, Nuclear Medicine Section, New York, USA;* [2]*Stanford University, Radiology, Stanford, USA*

Background: Over 4 decades ago John Kerr described an unusual form of cell death in rats undergoing experimental portal vein ligation. Kerr observed that some cells appeared to shrink and disappear without producing any inflammation. This observation contrasted with the findings of typical necrosis, where cells lose membrane integrity, spill their contents into surrounding tissues, and cause inflammation. Although it was clear that these cells were dying, the process was different from necrosis, since it was not associated with inflammation, the mitochondria and ribosomes remained intact, and extracellular bodies, occasionally occurring in clusters, suggested budding from the surface of the cells. Similar observations were made in histologic specimens of basal cell carcinoma. The incidence of 'shrinkage necrosis' was increased by treatment with radiotherapy. Professor Curry, while a visiting professor working with Kerr in Brisbane, described a similar observation made by his colleague Dr. Wyllie in the adrenal cortex of rats treated with prednisolone (to suppress ACTH). In a seminal article, Kerr, Wyllie and Curry coined the term apoptosis, derived from two greek words, 'apo' which means from and 'ptosis' which means falling, to describe this type of cell death.

Biochemical modifications of apoptotic cells include: (A) Activation of a series of cysteine protease enzymes (i.e. caspases), to crosslink and cleave specific intracellular proteins. Each of the caspases is associated with a specific inhibitor, allowing the system to be strictly regulated by a number of positive and negative feedback mechanisms. (B) DNA molecules are degraded into 50 to 300 kilobase-sized pieces. (C) Leakage of potassium and chloride from the intracellular environment, which results in loss of intercellular water and an associated decrease in cell size. Pieces of the cell undergoing apoptosis are packaged in small vesicles derived from the cell membrane. These cell pieces are called "apoptosomes" (D) Cells undergoing apoptosis signal their neighbors by expressing phosphatidylserine (PS) on the external leaflet of the cell membrane (as well as apoptosomes). PS is one of the 4 major phospholipids that make up the cell membrane (the other three are phosphatidylethanolamine, sphingomyelin, and phosphatidylcholine). Normal cell polarization confines PS to the inner leaflet of the cell membrane. Cells undergoing apoptosis lose this polarization, resulting in the expression of PS on the outer leaflet of the cell membrane. The expression of PS on the outer leaflet of the membrane signals neighboring cells that the expressing cell is undergoing apoptosis. A combination of macrophages and adjacent normal cells then phagocytize the remaining components of the cell carcass.

Annexin V (MW ~ 36,000) is an endogenous human protein that is widely distributed intracellularly, with very high concentrations in the placenta and lower concentrations in endothelial cells, kidney, myocardium, skeletal muscle, skin, red cells, platelets and monocytes. The precise physiologic function of annexin is uncertain. Annexin V binding to rafts of PS exposed on a cells surface with internalization via a newly discovered unique pathway of pinocytosis. Other investigations of annexin V binding have found that PS can be expressed at low levels in a reversible fashion under condition of cell stress that does not necessarily commit a cell to apoptotic cell death.

PET and SPECT Imaging with Radiolabeled Annexin V: Annexin has been radiolabeled with Iodine 125, Iodine 124, Fluorine 18, Technetium-99m and gallium-68. The half lives of the tracers limit the choices of imaging time. Typically, the tracer clears from the blood rapidly. When labeled with I-123, there was <4% residual in the blood 40 minutes after injection. However there is significant non-specific distribution in soft tissue, which has much slower clearance. Based on sequential imaging studies, once annexin binds to sites expressing PS, it appears that the signal persists, suggesting that imaging should be performed several hours after injection to maximize contrast while maintaining a reasonable count rate. Annexin imaging has been used to detect response to therapy in patients undergoing chemo and or radiotherapy.

463 INVITED

Applications of nanotechnology and molecular probes in cancer imaging

T.J. Meade[1]. [1]*Department of Chemistry, Northwestern University, Evanston, USA*

Fundamental biological and clinical questions have driven technological advances in a number of diagnostic techniques. In vivo molecular imaging has demonstrated the ability to profoundly change our understanding of developmental and pathological events. One technique that has been a powerful tool in both experimental and clinical settings is magnetic resonance imaging (MRI). The intrinsic contrast in acquired MR images can be augmented by the use of paramagnetic contrast agents in both clinical and experimental settings.

The direct observation of ongoing developmental events in living embryos, and the descendants of individual precursors in an intact embryo, has been dominated by optical microscopy. We have been developing MR probes to augment this analysis in whole animals. Since a complete time-series of high-resolution three-dimensional MR images can be analyzed forward or backward in time, it is possible to reconstruct the cell divisions and cell movements responsible for any particular descendant(s). Unlike previous methods, where labelled cells are identified at the termination of the experiment, this technique allows the entire kinship relationships of a clone to be determined.

In order to understand signal transduction mechanisms of gene expression in whole animals we have developed a library of molecular MR probes that are biochemically activated in-vivo. The lanthanide chelates modulate fast water exchange with the paramagnetic center, yielding distinct "strong" and "weak" relaxivity states. The modulation is triggered by two types of biological events: i. enzymatic processing of the contrast agent and, ii. the reversible binding of an intracellular messengers (e.g., Ca^{2+}, Zn^{2+}).