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

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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