Track 7, 8, 11 & 12

7 : Gene Therapy for Autoimmune Diseases & Epigenetics
8 : Applied Molecular Biology
11: Foreground Advancements of Gene Therapy and its Applications
12: Genetic Mapping and Linkage Analysis

Session Chair
Jan Oxholm Gordeladze
University of Oslo, Norway

Session Co-Chair
Girish V. Shah
University of Louisiana, USA

Session Introduction

Title: **Interaction between T-cells and engineered osteoblasts and chondrocytes: Implications for tissue repair**
Jan Oxholm Gordeladze, University of Oslo, Norway

Title: **Adenoassociated virus-mediated anti-calcitonin ribozyme therapy inhibits growth and metastasis of prostate cancer**
Girish V. Shah, University of Louisiana, USA

Title: **Use of random and targeted genome editing of the RP disease gene *Cngb1* to study and rescue rod photoreceptor structure and function**
Steven J. Pittler, University of Alabama at Birmingham, USA

Title: **Multi-tiered strategy for an optimization of therapeutic siRNA compositions and their delivery**
Ancha Baranova, George Mason University, USA

Title: ***In vivo* transfection efficiencies and pharmacokinetics of mRNA delivered in naked and nanoparticle format**
Kyle Phua, Duke University, USA

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Interaction between T-cells and engineered osteoblasts and chondrocytes: Implications for tissue repair

Jan Oxholm Gordeladze
University of Oslo, Norway

MicroRNAs are small RNAs, 21–25 nt long, encoded in the genome, and exert important regulatory roles. In chondrocytes from growth plates, 30 microRNAs were preferentially expressed, most of which were virtually nonexistent in osteoblasts. It has been demonstrated that several microRNAs (e.g. miRs 16, 24, 29, 125b, 149, 328, 339, 133, and 135) are differentially expressed in osteoblasts and chondrocytes derived from mesenchymal stem cells (MSCs). Furthermore, we have shown that some microRNAs (e.g. miRs 150, 20a, 30d, 17, 19b, 638, 663, 923, and 155) constitute a discriminating signatures for CD4+ T cells. Since the miRs 923, 638 and 663 are all abundantly expressed in differentiating chondrocytes, and the miRs 16, 586, and 923 are all targeting Runx2, one might speculate that activated T cells, apart from influencing cartilage/bone turnover in inflamed joints, also may affect chondrocytes and osteoblasts by the microRNAs they secrete (in exosomes) into the synovial fluid.

The results of permutations of the experimental approach taken indicated that osteochondral phenotypes derived from MSCs and adipose stem cells were jeopardized when exposed to "artificial" synovial fluid containing a cytokine mixture, exosomes shredded from Th-17 cells, or certain pre-miR species. Furthermore, both cell phenotypes enhanced the induction and activity of osteoclasts derived from peripheral blood monocytes (PBMCs). Reinforcing the osteochondral phenotypes by manipulating the levels of microRNA species, transcription factors, and growth on scaffolds yielded better osteochondral cells in terms of wanted phenotypes and stability (resilience towards osteoclast activation) when exposed to growth conditions supporting inflammatory processes.

Biography

Jan O. Gordeladze, PhD (born 25th of April, 1950), holds a triple professor competence (medical biochemistry, physiology, and pharmacology), and is presently working as a professor at the Department of Biochemistry, Institute of Basic Medical Science, University of Oslo, Norway. He has previously been employed as the medical director of MSD, Norway, serving two years as a Fulbright scholar at the NIH, Bethesda, Maryland, USA, and from 2006-2009 being employed as associate professor at the University of Montpellier, France. He has published more than 100 scientific articles, reviews/book chapters and presented more than 250 abstracts/posters/talks at conferences world wide.

j.o.gordeladze@medisin.uio.no

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Adenoassociated virus-mediated anti-calcitonin ribozyme therapy inhibits growth and metastasis of prostate cancer

Girish V. Shah
University of Louisiana, USA

Gene therapy for cancer offers a possibility of targeted destruction of tumor cells in patients. Moreever, gene therapy can be used as a tool to identify new targets or investigate the role of specific gene(s) in carcinogenesis or cancer progression. Most gene therapy studies have used adenoviral vectors and to a lesser extent, retroviral vectors to deliver genes at the targeted site. However, recent studies suggest that AAV-based vectors can serve as potentially powerful delivery vehicle for cancer gene therapy.

We used recombinant adeno-associated virus (rAAV) to target the expression of a neuroendocrine peptide calcitonin (CT) in prostate cancer cells. This is because the expression of CT and its receptor (CTR) is frequently elevated in prostate cancers (PCs), and activation of CT–CTR axis in non-invasive PC cells induces an invasive phenotype. In contrast, inactivation of CT-CTR axis diminishes tumorigenicity and abolishes the ability of highly invasive PC-3M prostate cancer cells to form distant metastases. We employed rAAV to deliver anti-CT ribozymes (RZ) in PC cells in culture as well as in mouse models of PC. Efficacy of rAAV-anti-CT ribozymes (rAAV-RZ) was assessed either by the measurement of calcitonin secretion in conditioned media or CT immunohistochemistry of tumors. rAAV-RZ demonstrated high functional efficacy as indicated by greater than 90% decline in CT secretion from cultured cells or CT immunostaining of tumors. CT silencing led to a dramatic decline in angiogenic activity as assessed by tube formation under in vitro as well as in vivo conditions. Moreover, administration of rAAV-RZ not only abrogated the growth of pre-implanted tumors in nude mice, but also significantly reduced the growth of spontaneous tumors in LPB-Tag mice. These results demonstrate that rAAV can be successfully used to selectively silence genes associated with cancer progression. The rAAV vector offers several additional advantages over other vectors such as the absence of wt genes, relatively easier incorporation of desired gene or shRNA sequences, relatively easier production of high-titer, contamination-free virus with low immunogenicity, and the ability to infect targeted cells in culture or whole animals.

shah@ulm.edu

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Use of random and targeted genome editing of the RP disease gene *Cngb1* to study and rescue rod photoreceptor structure and function

Steven J. Pittler
University of Alabama at Birmingham, USA

The *Cngb1* locus encodes the rod photoreceptor (PR) cGMP-gated cation channel β-subunit and two soluble glutamic acid rich proteins, GARP1 and GARP2. The GARP proteins are generated by alternative splicing towards the 5'-end of the gene. Defects in the α- or β-subunit genes are a cause of retinitis pigmentosa (RP), a heterogeneous hereditary blinding disorder. A recent study has shown that the channel could be a promising new target for RP disease intervention. Knockout of all three proteins in rods leads to disorganized rod outer segment (ROS) structure and an attenuated photoresponse. Transgenic mice expressing a truncated β-subunit missing most of the GARP 5' region exhibit significant rescue of ROS structure and function. When the transgene is placed on a β-subunit knockout background that retains soluble GARP expression, near complete recovery is observed. To further understand the role of GARPs in ROS we have used zinc finger nuclease genome editing technology to selectively remove a GARP2 unique exon from the mouse genome. Two knockout alleles were established in mice, one that removes the entire exon and one that removes only the 3'-UT region that may be a hypomorph expressing lower levels of GARP2. Recent studies on PR GARP-containing proteins have established that the β-subunit and soluble GARPs are required for both normal structure and function. We are now analyzing the mechanism of action of the GARP proteins and moving towards developing treatments for RP that directly involves *Cngb1* mutation or indirectly in related disorders that exhibit elevated cGMP in ROS.

Biography

Pittler completed his Ph.D. at age 30 from Michigan State University and postdoctoral studies at Baylor College of Medicine. He is a Professor of Vision Sciences, Ophthalmology, and Biochemistry and Molecular Genetics and Director of the UAB Vision Science Core Facilities. He has published more than 50 papers in journals such as Cell, PNAS, Nature Genetics, JBC, JCB, JCS and others and serves on the editorial board of Molecular Vision, Open Ophthalmology, Eye and Brain, and Cell Health and Cytoskeleton. He was the 1995 international Association for Research in Vision and Ophthalmology Cogan Awardee recognizing outstanding vision scientists.

pittler@uab.edu

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Multi-tiered strategy for an optimization of therapeutic siRNA compositions and their delivery

Ancha Baranova
George Mason University, USA

Silencing of gene expression by small interfering RNA (siRNA) is promising for drug target discovery and as a therapy. However, there are a number of impediments to the practical use of this technology, namely, an off-target effects of siRNA through its less-than perfect but still detrimental binding to partially matching mRNAs, an instability of siRNAs in the bloodstream due to their susceptibility to nucleases and undesired effects on immune system. To address these restrictions, we developed multi-tiered strategy of optimization of therapeutics siRNA compositions and their delivery. First, we designed Tree-SORT algorithm capable of top-down prediction of the human siRNA with minimized off-target hybridization and pre-computed a list of the best siRNA locations within each human gene ("siRNA seats"). To efficiently deliver siRNA, we designed a novel DNA/albumin-based vehicle that forms a “basket” surrounding the siRNA and provides both steric separation of siRNA from nucleases and local excess of the substrate for nuclease action. Importantly, basket-forming DNA molecules may be modified to stimulate an antioxidant response in target tissue that is achieved by increase in its 8-oxo-dG or GC content that is known to stimulate TLRs that could be desirable when treating certain human disorders. To study effects of these modifications to the standard siRNA delivery protocols further, we chose relatively uncomplicated model of substantial clinical value - a perfusion of isolated human organs before transplantation. Our preliminary data imply that DNA baskets may be a promising technology for further development as the delivery vehicle for siRNA therapeutics.

Biography

Ancha Baranova, a specialist in the area of functional genomics of complex human diseases, is an Associate Professor in the School of Systems Biology, College of Science, George Mason University in Fairfax, Virginia, USA. Dr. Baranova's major academic contributions are in the field of functional genomics, with emphasis on cancer and metabolic syndrome-related disorders. A significant part of Dr. Baranova’s efforts is dedicated to *in silico* analysis of the publicly available genomics and proteomics databases. Dr. Baranova directs a team of postgraduate and graduate associates that employs a multidisciplinary approach in order to broaden research perspective in the genetics of complex human diseases. Dr. Baranova has published about 50 manuscripts in international journals including Genomics, Hepatology, Clinical Cancer Research, FASEB Journal, FEBS Letter, Bioinformatics, Leukemia and Lymphoma, Oncogene, and others.

abaranov@gmu.edu, aancha@gmail.com

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In vivo transfection efficiencies and pharmacokinetics of mRNA delivered in naked and nanoparticle format

Kyle Phua
Duke University, USA

As an emerging class of genetic medicine, pharmacokinetics of messenger RNA (mRNA) has been poorly characterized. We evaluated transfection efficiencies of mRNA in naked and nanoparticle format *in vitro* and *in vivo*. mRNA nanoparticles transfected both primary human and mouse DCs highly efficiently *in vitro* while naked mRNA did not. *In vitro* expression peaks rapidly 5-7th hours and decays in a biphasic manner. Naked mRNA transfects much more efficiently than mRNA nanoparticles *in vivo* at subcutaneous sites and is pH dependent. On the other hand, only mRNA delivered in nanoparticle form transfected when administered through intranasal and intravenous routes. Gene expression is the most transient when delivered intravenously with an apparent half-life of 1.4hours and lasts for less than 1 day. In contrast, gene expression is the most sustained when delivered in naked format subcutaneously at the base of tail with an apparent half-life of 18hours and lasts for at least 6 days. Interestingly, consistent exponential decreases in gene expression are observed when mRNA is delivered in both naked and nanoparticle formats at all sites of administration. Our data suggest a niche for mRNA therapeutics delivered in nanoparticle format administered through intranasal and intravenous routes, and predictable *in vivo* mRNA pharmacokinetics.

Biography

Kyle holds a B.Eng (Chemical Engineering) from National University of Singapore and a M.S (Chemical Engineering) from University of Illinois-Urbana-Champaign. Kyle won an academic scholarship from the National University of Singapore to pursue a PhD in Biomedical Engineering at Duke University under the supervision of Dr Kam Leong. He is currently investigating mRNA delivery strategies to transfect, activate and enhance mRNA tumor vaccination.

kkp7@duke.edu