8th Student Symposium
on Molecular Medicine

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New Approaches in Molecular Medicine

April 18, 2015
Symposium Program

Morning Session

08:30	**Introduction**	
08:30 - 09:00	**Dr. Christian Riedel** *Ulm University*	Studying bacterial tumor-targeting in 3D cell culture
09:00 - 09:15	**Annika Osswald** *Ulm University*	
09:15 - 10:00	**Dr. Henoch Hong** *CureVac GmbH, Tübingen*	Messenger RNA-based immunotherapy as novel treatment for cancer
10:00 - 10:30	**Coffee Break**	
10:30 - 11:00	**Prof. Dr. Christian Plank** *ethris GmbH, Munich* *Technical University Munich*	Nucleic Acid Delivery – From Academic Discovery to Drug Development
11:00 - 11:15	**Mehrije Ferizi** *Technical University Munich*	Strategies to improve stability and translational capacity of chemically modified mRNAs
11:15 - 11:45	**Prof. Dr. Renko de Vries** *Wageningen University, NL*	Artificial viruses for drug delivery
11:45 - 12:00	**Rob de Haas** *Wageningen University, NL* *Uppsala University, SE*	mRNA transfection with artificial viruses
12:00 - 12:15	**Student representatives** *Ulm University*	Master Program and International Graduate School in Molecular Medicine

Afternoon Session

12:15 - 13:30	**Lunch Break & Poster Session**	
13:30 - 13:55	**Prof. Dr. Kerstin Otte** *University of Applied Sciences Biberach*	Cell line engineering to boost biopharmaceutical production
13:55 - 14:15	**Simon Fischer** *University of Applied Sciences Biberach*	Tiny but mighty – Harnessing microRNAs for next-generation cell engineering of biopharmaceutical production cells
14:15 - 15:00	**Prof. Dr. Günter Tovar** *Fraunhofer IGB, Stuttgart* *Stuttgart University*	Processing of tailor-made biomaterials by additive manufacturing – on the way to 3D structured personalized elastic implants
15:00 - 15:15	**Coffee Break**	
15:15 - 16:00	**Dr. Moritz von Heimendahl** *Boehringer Ingelheim GmbH, Biberach*	Optogenetics: What a new tool can do for neuroscience and drug discovery
16:00 - 16:15	**Poster Award Ceremony**	
16:15 - 16:45	**Prof. Dr. Tobias Moser** *University Medical Center Göttingen*	Optogenetic stimulation of the auditory pathway for research and future prosthetics
16:45 - 17:00	**Daniel Keppeler** *University Medical Center Göttingen*	
17:00	**Socializing with Cake and Champagne**	

Contact Information

Symposium Organization

www.molmed-symposium.de

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Studying bacterial tumor-targeting in 3D cell culture – bifidobacteria as an example

Dr. Christian Riedel | Annika Osswald, M.Sc.
Institute for Microbiology and Biotechnology, Ulm University

Hypoxic regions of solid tumors have higher therapeutic resistance compared to oxygenated tumor tissues. Several studies demonstrated that anaerobic bacteria of the genera Bifidobacterium, Salmonella or Clostridium specifically target hypoxic areas in tumors in mice and are thus discussed as live vectors to deliver therapeutic genes to inhibit tumor growth. Tumor therapy using anaerobic bacteria has been studied in different in vivo mouse models. We generated three-dimensional HT-29 multicellular tumor spheroids (MCTS) under aerobic cell culture conditions and evaluated their potential to investigate tumor targeting with obligate anaerobic bacteria.

Cryosections of HT-29 tumor spheroids resembled solid tumors and displayed relevant features including proliferating areas as well as hypoxic and apoptotic regions inside the tumors. Upon co-culture with HT-29 MCTS, obligate anaerobic B. bifidum S17 and the genetically rendered anaerobic S. typhimurium YB1 selectively localized to the hypoxic areas inside of tumor spheroids and were able to survive. Furthermore, spores of the obligate anaerobe C. sporogenes germinated in hypoxic areas of MCTS. In a first attempt to generate recombinant bifidobacteria for tumor therapy, chromate reductase was cloned and expressed in B. bifidum S17. The recombinant strain metabolized the prodrug CB1954 into a toxic substance which inhibits DNA synthesis. Moreover, growth of HT-29 MCTS was markedly reduced upon co-culture with this strain in the presence of CB1954.

Our results show that the 3D tumor spheroids are a suitable and reliable model to investigate tumor-targeting with anaerobic bacteria for cancer therapy in vitro. Furthermore, growth of cancer cells was reduced by incubation with recombinant bifidobacteria expressing chromate reductase and the prodrug CB1954.

Abstracts

Messenger RNA-based immunotherapy as novel treatment for cancer

Dr. Henoch S. Hong
CureVac GmbH, Tübingen

Over decades, messenger RNA (mRNA) was regarded as being unsuitable for medical applications because of its perceived instability and difficult handling. However, recent studies are uncovering the substantial clinical potential of mRNA. This has sparked the development of a novel class of drugs for various clinical needs, including prophylactic vaccines against pathogens and therapeutic cancer immunotherapy.
First approaches in the therapy of cancer focused on the ex vivo modification of antigen-presenting cells (APCs). In contrast, our pre-clinical experiments showed that intradermal-injection of self-adjuvanted optimized mRNA (RNActive®) is sufficient to induce boostable and balanced effector and memory immune responses resulting in strong anti-tumor effects. Only two intradermal injections were sufficient to induce substantial changes in the tumor microenvironment in mice.
Two clinical Phase I/IIa studies with RNActive® in prostate cancer and NSCLC – encoding four or five tumor (associated) antigens, respectively – demonstrated that mRNA vaccines are safe, well tolerated and support the induction of antigen-specific T cell and antibody responses. First results suggest a prolonged survival of multiple responders treated with this novel vaccination approach. The clinical efficacy of RNActive® is currently being evaluated in a randomized, double-blind, placebo-controlled phase IIb study in prostate cancer patients. In summary, our data show that RNActive® represents a novel, highly versatile treatment platform with great clinical promise.

Nucleic Acid Delivery – From Academic Discovery to Drug Development

Prof. Dr. Christian Plank
Institute for Molecular Immunology and Experimental Oncology, Technical University Munich
Ethris GmbH, Munich

Nucleic acids carry the building plans of living systems and fulfill numerous further functions in living cells. In theory, any cellular function may be influenced in a purposeful manner if an appropriate nucleic acid can be shuttled into a target cell in a precise enough manner. In nucleic acid therapy, nucleic acids are used to complement or repair damaged genes or to interfere with endogenous gene expression. For this purpose, nucleic acids are usually formulated as multifunctional nanoparticles which are designed to overcome numerous barriers on the way from an administration site to the target cells. We have developed various methods for using nucleic acids in medical applications. More recently, we have focused on “gene therapy without genes”, that is on using messenger RNA instead of genes for making patient cells produce their own therapeutic protein. This program is intended to enter first-in-man application in 2015. In this presentation, challenges encountered when developing nucleic acid drugs from bench to bedside will be discussed.

Abstracts

Strategies to improve stability and translational capacity of chemically modified mRNAs

Dipl.-Ing. (FH) Mehrije Ferizi
Institute for Molecular Immunology and Experimental Oncology, Technical University Munich

Messenger RNA has gained more importance over the last few years. It is known that mRNA stability and turnover is crucial for gene expression. In contrast to DNA, mRNA can be successfully transfected into slowly dividing primary cells without the risk of insertional mutagenesis, which makes it favorable for treating diseases. However, native mRNA is not very stable and externally administered mRNA triggers an immunogenic response. Therefore, scientists developed several strategies to conquer these obstacles. The incorporation of chemically modified nucleotides within the mRNA and insertion of untranslated regions (UTRs) into the mRNA sequence, have been shown to increase stability and enhance translational efficiency of exogenous mRNA. The aim of the current project is to optimize the mRNA structure by inserting a set of different UTR sequences. To achieve this, various cellular UTRs were selected and screened in two different cell lines. As a result, we could observe longer expression with some of the tested UTR combinations compared to control mRNA without UTRs. Further we tested the mRNA stability by conventional RT-PCR. Interestingly, the mRNA half-life itself was not stabilized. However, mRNA molecules furnished with UTRs revealed a higher protein expression normalized to the amount of delivered mRNA. Latest experiments with mRNA coding for hBMP-2, furnished with the best working UTRs, showed a better transgene expression compared to the control without UTRs.

Artificial viruses for drug delivery

Prof. Dr. Renko de Vries
Laboratory of Physical Chemistry and Colloid Science, Wageningen University, NL

Viruses are among the simplest biological systems and are highly effective vehicles for the delivery of genetic material into susceptible host cells. Modified viruses are widely used and studied as drug delivery vehicles and for vaccinations. While the idea of designing "artificial viruses" that could be used for similar purposes is not new, so far not much progress has been made with this approach. Inspired by the assembly of the well known Tobacco Mosaic Virus, we present a rational design for a self-assembling minimal viral coat protein based on polypeptide domains with very simple primary sequences. When mixed with nucleic acids, the artificial coat proteins assemble into rod-shape Virus-Like Particles (VLP) each encapsulating a single nucleic acid molecule. Inside the VLPs, the nucleic acids are protected against enzymatic attack. The VLPs are quite effective as non-viral gene transfer agents, both for pDNA and mRNA. Since the VLPs have an extremely well defined structure, they are unique scaffolds to start engineering newer generation of artificial viruses that do not only mimick the self-assembly, but also the cell attachment, endosomal escape, etc. of natural viruses.

Abstracts

mRNA transfection with artificial virus

Rob de Haas, B.Sc.
Laboratory of Physical Chemistry and Colloid Science, Wageningen University, NL
Uppsala Univeristy

By making use of a previously synthesized artificial virus coat protein, GFP coding mRNA (~1000nt) is encapsulated through a self-assembly process. This process is characterized through Atomic Force Microscopy (AFM). The resulting complexes are studied in transfection experiments in Hela cells and show great potential for further developments in the field of gene therapy.

Cell line engineering to boost biopharmaceutical production

Prof. Dr. Kerstin Otte
Institute for Applied Biotechnology, University of Applied Sciences Biberach

The most widespread mammalian production host for therapeutic proteins is the Chinese hamster ovary (CHO) cell system. Due to the rapidly growing demand for biopharmaceuticals like antibodies for cancer treatment, various strategies are currently being pursued to improve production cells in order to achieve higher product titers while maintaining maximal product quality. The potential of traditional approaches as well as novel strategies as the use of small non coding RNAs (miRNAs) to optimize biopharmaceutical production will be presented in the light of recent revelations as the CHO genome, transciptome and miRnome.

Abstracts

Tiny but mighty – Harnessing microRNAs for next-generation cell engineering of biopharmaceutical production cells

Simon Fischer, M.Sc.
Institute for Applied Biotechnology, University of Applied Sciences Biberach

The steady improvement of mammalian cell factories for the production of biopharmaceuticals is still a key challenge to meet increasing demands for therapeutic proteins. Furthermore, healthcare systems are already facing tremendous costs associated with the increasing demand for biological drugs. Therefore, novel strategies have to be implemented to enable cost-effective and efficient high-yielding production processes. Although mammalian cells still exhibit limitations regarding productivity and growth compared to prokaryotic expression systems, they are irreplaceable since correct post-translational modifications are fundamental to functionality and safety of the product. MicroRNAs (miRNAs) are key modulators of global gene expression in mammalian cells and are capable of regulating entire cellular pathways. Although miRNAs have been extensively studied for their role in fine-tuning gene expression in human disease research, their role as engineering tool for improving biopharmaceutical production cells still remained elusive.

Due to the undisputed relevance of the Chinese hamster ovary (CHO) cell line for the manufacturing of biopharmaceuticals, we performed a functional high-content miRNA screen in recombinant CHO production cells. Following high-throughput screening of 1,139 different miRNAs significant effects on cell proliferation, apoptosis, necrosis and recombinant protein expression were assessed. A remarkable number of impactful miRNAs could be identified to affect cellular behavior either in a positive or negative manner. Effects of impactful miRNAs could be successfully confirmed in various validation experiments giving rise to a functional 'miRNA catalogue' useful for innovative cell engineering strategies. Strikingly, re-screening of apoptosis-inducing miRNAs in different human cancer cell lines confirmed transferability of the developed methodology to human disease models. This might enable the development of novel therapeutic strategies for the treatment of cancer in the future.

Processing of tailor-made biomaterials by additive manufacturing – on the way to 3D structured personalized elastic implants

Prof. Dr. Günter Tovar
Institute for Interfacial Process Engineering and Plasma Technology IGVP, Stuttgart University
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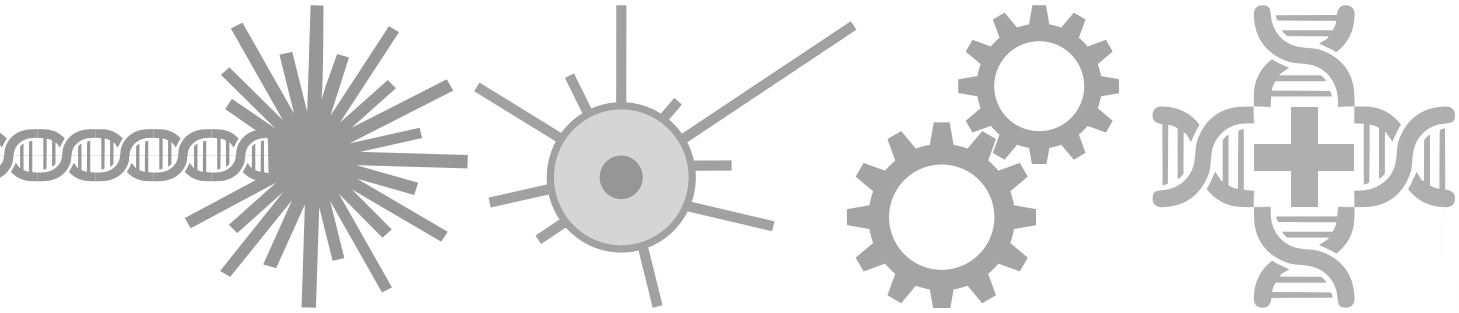

Optogenetics: What a new tool can do for neuroscience and drug discovery

Dr. Moritz von Heimendahl
Boehringer Ingelheim GmbH, Biberach

Great progress has been made in the last century in our understanding of the nervous system, but for some questions, progress has been difficult because of technical limitations. In animals, the rather old method of electrophysiology, which allows the recording of electrical signals or the stimulation of neuronal activity, has allowed key insights into the brain's functioning. It is, however, unable to accurately distinguish between different cells types. This makes the elucidation of microcircuits hard or impossible. The recent development of optogenetics allows manipulation of nervous cell activity in a way that is regionally restricted, cell-type specific and temporally precise. This is achieved through the artificial expression of light-sensitive ion channels in genetically targeted cells, and their subsequent activation with light. This new set of techniques has already allowed new experiments that would previously have been impossible, has led to interesting new discoveries, and holds great promise for a new approach to drug discovery in psychiatric diseases.

Optogenetic stimulation of the auditory pathway for research and future prosthetics

Prof. Dr. Tobias Moser | Daniel Keppeler, M.Sc.
Institute for Auditory Neuroscience, University Medical Center Göttingen

When hearing fails, speech comprehension can be restored by auditory prostheses. However, sound coding with current prostheses, based on electrical stimulation of auditory neurons, has limited frequency resolution due to broad current spread. Optical stimulation can be spatially confined and may therefore improve frequency and intensity resolution. We have established optogenetic stimulation of the auditory pathway using virus-mediated expression of channelrhodopsins to render spiral ganglion neurons and/or cochlear nucleus neurons light-sensitive. Optogenetic stimulation of spiral ganglion neurons activated the auditory pathway, as demonstrated by recordings of single neuron and neuronal population responses at various stages of the auditory system. We approximated the spatial spread of cochlear excitation by recording local field potentials in the inferior colliculus in response to suprathreshold optical and electrical stimuli, which suggested a better frequency resolution for optogenetic than for electrical stimulation. Moreover, we were able to restore auditory activity in deaf mice. In a collaborative effort we are involved in developing and characterizing flexible µLED-based multichannel intracochlear stimulators. The presentation will review recent progress in optogenetic stimulation of the auditory system and its potential for future application in research and hearing restoration.

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