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Mycolic acid biosynthesis in *Rhodococcus erythropolis*

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Oil contamination is one of the most hazardous environmental pollutions. There were numerous oil spills in the last three decades. Oil tanker accidents and oil-rig catastrophes are still reported. Oil spills had great impact on the wildlife as well as on the economy by cutting down the agriculture, food industry and tourism.

Surfactants are useful weapons in the war against oil pollution. They are suitable to solubilize hydrophobic materials, consequently to clean oil tanks and pipes. In addition they can be used for emulsification of animal fats in food industrial and housekeeping wastewaters. Many bacteria can produce substantial amount of biosurfactants which can promote the solubilization of hydrophobic hydrocarbons, thus these bacteria themselves or the native microflora can utilize the unctuous pollutants. An additional advantage of the biosurfactants over the synthetic surface active molecules is that these compounds are easily biodegradable, they don't persist in the environment.

A special biosurfactant group is composed of mycolic acids which are basically α-alkyl, β-hydroxy fatty acids. Mycolic acids are the most characteristic components of the cell wall of the so called mycolata bacterial group. This group belongs to the Actinomycetales and contains the genera *Mycobacterium*, *Corynebacterium*, *Nocardia*, *Rhodococcus* and others.

A Gram+ bacterium, Rhodococcus erythropolis MK1 strain has been isolated from polluted soil in our lab. The cells grown in the precence of hydrocarbons produced cell-wall-bound surfactants in order to make oils accessible. Based on the literature and preliminary gas chromatographic analysis, this surface active compound seemed to be trehalomycolate. Although, the mycolic acid biosynthesis was already characterized in the pathogenic Mycobacteria, the biosynthetic routes of trehalomycolate in Rhodococcales are less known. Therefore, we aimed to map the mycolic acid biosynthesis pathway in Rhodococcus erythropolis strains. First, we sequenced the genome of our strain by SOLID™ next generation DNA sequencer. The reads were mapped on the R. erythropolis PR4 genome in the NCBI database. We searched for rhodococcal homologs of the known mycobacterial and corynebacterial genes involved in mycolic acid biosynthesis. We found conserved regions in the genome which are likely responsible for the biosynthesis of mycolic acids. Nevertheless, differences in the biosynthetic pathways can also be recognized. The ongoing comparative whole genome transcript analysis discloses the genes really necessary for the anabolism of trehalomycolates. This knowledge will be used for constructing strains for biotechnological applications.

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Chronic hypertriglyceridemia induces early tau hyperphosphorylation and impaired long term potentiation in apoB-100 transgenic mice

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Alzheimer's disease (AD) is the commonest form of dementia and affects more than 32 million people worldwide. Epidemiological studies confirmed the importance of vascular dementia as the second most common cause of dementia in the elderly, representing 15–20% of all cases of dementia. These figures threaten to rise as the population ages unless efficient preventative measures are introduced.

The role of hyperlipidemia in the development of vascular dementia and cognitive decline is still controversial. Recent studies indicate that ApoB-100-induced hyperlipidemia and atherosclerosis are not only implicated in the pathogenesis of cardiovascular diseases but may also affect the cerebrovascular system thus contribute to the development of neurodegenerative disorders. Other studies have shown that AD is accompanied by an elevation in apolipoprotein B concentration in the serum. It was also demonstrated, that progressive cognitive impairment in AD is accompanied by neurovascular dysfunction.

To further clarify the possible role of hyperlipidemia in the development of neurodegeneration and dementia we have generated transgenic mice overexpressing the human ApoB-100 protein. Transgenic mice fed a regular chow diet show elevated serum triglyceride levels, fed a cholesterol rich diet show hypercholesterolemia leading to coronary and systemic atherosclerosis by the age of 6 months. Previously, we have shown that microcapillary density significantly decreases in the cortex of hyperlipidemic (hypertriglyceridemic and hypercholesterolemic) transgenic mice.

Here we show, that adult transgenic mice (6 months old) present significantly elevated cerebral level of triglycerides and ApoB-100 indicating the dysfunction of blood-brain barrier. Moreover, in aging transgenic mice (10 months old) triglyceride-rich lipid-droplets in the cortex can be detected. The level of ApoE, the major lipid transporter molecule in the brain was also significantly increased in adult transgenic brains. Under pathological conditions (e.g. AD) the Tau protein becomes hyperphosphorylated, which leads to dynamic instability and disintegration of microtubular network and eventually to the formation of neurofibrillary tangles (NFT). Tau phosphorylation patterns in the brain of 3 and 6 month old transgenic animals were investigated using several phosphosite-specific antibodies (Ser^{199}, $Ser199/^{202}$, Ser^{262},

Ser^{396} and Ser^{404}) which are commonly used in the molecular diagnosis of AD. We demonstrate here, that Tau protein is hyperphosphorylated at sites Ser^{199}, Ser^{202}, Ser^{262}, Ser^{396} and Ser^{404} in the cortex of 6 month old hypertriglyceridemic transgenic mice. Neuronal apoptosis was monitored in transgenic brains using FluoroJade staining. Significantly increased degenerated neurons were counted in the cortical and hippocampal regions of adult transgenic versus wild-type mice. Furthermore, using electrophysiological recordings (long-term potentiation and paired pulse facilitation) we demonstrated severe impairment in presynaptic function of transgenic brains.

These results indicate that chronic hypertriglyceridemia can induce neurodegeneration possibly via hyperphosphorylation of tau protein. Beyond of theoretical observations we aim to develop a novel mouse model of age-related neurodegeneration, providing a useful tool for the development of efficient therapies against neurodegenerative diseases.

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Examination of real-time effects of different cytotoxic and cytoprotective compounds with a novel cell-microelectronic sensing technique

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Real-Time Cell Analyzer (RTCA) DP is a novel cell migration and invasion assay system that uses the Boyden Chamber principle but does not involve any fixation, labeling or counting of the cells. The core of the system is the CIM-Plate device, composed of an upper chamber and a lower chamber. The upper chamber has 16 wells that are sealed at the bottom with a micro-pore-containing polycarbonate or polyester membrane. The membrane contains microelectronic sensor arrays that are integrated on its bottom surface. Migration of cells will occur through these electrodes, which changes impedance, and will increase cell index. The more cells migrate the higher the cell index will be. RTCA SP is also a microelectronic cell sensor method, where microlectrodes are integrated in the bottom of a microtiter plate (96-well E-plate) and measures adhesion, proliferation or cytoxicity. The real-time measurement can detect changes continuously, which means that the system can give information at any stages of the experiment.

We examined the effects of cytotoxic compounds on the migration and proliferation properties of human glioblastoma, liver carcinoma and melanoma cells with a novel cell microelectronic sensing technique. We tested the migration potential of several tumor cell lines in a trans-well migration system. In addition we examined the effects of neuro/cytoprotective compounds on the viability of primary rat neuronal cultures with RTCA-SP system. During the cytotoxicity and cytoprotection screenings *in vitro* cytotoxicity or cytoprotectivity was elicited by numerous compounds synthesized by Avidin Ltd.

In our experiment we examined the migration properties of different tumor cells. GBM3 human glioblastoma cells migrated the fastest. A549 human adenocarcinomic lung cancer cells also showed a relatively high cell index increase, probably due to its small size, enabling it to pass easily through the membrane. Ac929 treatment decreased migration ability of Hep3B hepatoma cells dose-dependently. The highest concentration used (1 μM) resulted the lowest cell index. U87-MG human glioblastoma-astrocytoma cells were treated with Ac1041and Ac915 compounds. The treatment caused dose-dependent decrease of cell index, where 500 nM and 1 μM concentrations were ineffective. 5 μM showed a slight change in migration, and higher doses (20-50 μM) were cytotoxic. Cell index data were calculated 24 hours after treatment. The experiments clearly shows the dose-dependent effect of Ac-compounds. Effects of Ac1041, Ac929 and Ac915 were validated by the RTCA SP system.

During the cytoprotective screenings in vitro cytotoxicity was elicited by hydrogen peroxide in primary rat neuronal cultures. Cells were either pre-treated 5 min before oxidative stress or post-treated at 30 min with novel cytoprotective compounds. Cell Index of Q2 treated cells started to rise as high as absolute control and remained elevated for hours, showing a long-term cytoprotective effect. Vehicle-control cells (which received H_2O_2 and vehicle, but no treatment with cytoprotective compounds), showed a rapid decline of cell index. Pre-treatment with compound 9791 or post-treatment of the cells with the fatty acid derivative 9528 prevented the cells from the toxic effects of oxidative stress dose-dependently.

The cell-microelectronic sensing technique (RT-CES) method is suitable for the screening of molecular libraries to identify molecules or molecule combinations that attenuate oxidative stress-induced cell damage and can also be useful for screening of agents with antitumor properties.

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