molecular etiology of HCC are limited. The Nrf2 signaling pathway can protect cells from a variety of toxicants and carcinogens by increasing the expression of a number of cytoprotective genes.The idea of using G. lucidum for cancer treatment is based on numerous laboratory and preclinical studies with cancer and immune cells as well as animal models demonstrating various biological activities in vitro and in vivo. The aim of our study is to evaluate the potential antioxidant role of G. lucidum in HCC. For this purpose, analysis of Nrf2 levels and cell cycle arrest were done. The most effective concentration of G. lucidum were found at 1/5 dilution. The changes in cytoplasmic/nuclear Nrf2 protein levels following G. lucidum extracts (1:5 or 1:10) treatments for 24, 48 or 72 h were observed. 1/10 and 1/5 diluted of G. lucidum induced G0/G1 cell cycle arrest in HCC. Consistently, the cells distributed in S phase were significantly reduced.In conclusion, our findings suggest that G. lucidum has a potential as an anticancer agent and. our data support the importance of the clarification of the molecular mechanisms of phytochemicals-induced Nrf2 activation.

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Crosstalk between insulin resistance and oxidative stress in the development of Alzheimer-like neurodegeneration

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Keywords: down syndrome; insulin signaling; alzheimer disease; protein oxidation

Down Syndrome (DS) individuals by the age of 40ys develop a type of dementia that has the same characteristics as Alzheimer disease (AD). Previous studies in DS and AD brain suggest common neurodegenerative pathways including mitochondrial dysfunction, oxidative stress (OS) and reduced glucose metabolism. In addition, several studies suggest a link between insulin resistance and cognitive dysfunction in AD.

The present study aims to analyze the crosstalk between the onset of brain insulin resistance (BIR) and OS as possible contributing factors to the neurodegenerative process in tg mouse model of DS (Ts65Dn).

We longitudinally analyze (at 1–3-9–18 months) changes of i) IR/IRS1/ERK1/2/Akt levels and activation state iii) oxidative stress markers and iii) biliverdin reductase-A (BVR-A), SIRT1 and PTEN protein levels and activation, in the cortex of Ts65Dn mice. In parallel, changes of APP/Abeta levels have been analyzed.

Our results show the mutual interaction between increased OS and BIR in Ts65dn, which does not correlate with Abeta levels. We found that OS negatively impacts the activation of insulin cascade since postnatal age that also persists with age. These findings highlight the role of BIR in the onset of AD-like neurodegeneration and suggest that aberrant insulin signaling strongly contributes to cognitive decline also in DS.

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HyPer biosensor to monitor intracellular hydrogen peroxide in skeletal muscle cells

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Keywords: HyPer; hydrogen peroxide; myoblast; myotubes; skeletal muscle fibres

Hydrogen peroxide (H_2O_2) is one of the reactive oxygen species (ROS) that seems to play an essential role in cellular signalling pathways coupled to frequent pathophysiological processes. However, using traditional methodology it is virtually impossible to identify and quantify H_2O_2 flux in cells.

We have developed methodological approaches based on the use of a hydrogen peroxide biosensor, HyPer, in skeletal muscle cells: myoblasts and myotubes C2C12, and individual matured muscle fibres isolated from the mouse muscle. Using transfection techniques with chemical agents and microinjection/electroporation techniques, we have achieved the expression of HyPer biosensor in those cells. In combination with live cell fluorescence microscopy image analysis we have monitored the intracellular flow of H_2O_2 in situ and in real time in those skeletal muscle cells that expressed HyPer. The HyPer fluorescence emitted by cells was registered during a period in which cells were exposed either to extracellular hydrogen peroxide or to a reducing agent, dithiothreitol, in the medium.

Conclusion: i) it is possible the expression of hydrogen peroxide biosensor HyPer in myoblasts and myotubes C2C12 and in single isolated skeletal muscle fibres, and ii) HyPer biosensor is functional and detects changes in the intracellular concentration of hydrogen peroxide in situ and in real time in skeletal muscle cells.

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