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Anti-NLRP3 Inflammasome Activity of Stigmasterol

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Backgrounds:

Stigmasterol is an unsaturated plant sterol showing anti-inflammation, anti-cancer, anti-microbial, and anti-osteoarthritic; however, the effect of stigmasterol on inflammasome activation is unclear.

Materials and Methods:

Effect of stigmasterol on NLRP3 inflammasome activation in LPS- and ATP-activated macrophages was monitored by detecting IL-1β secretion and caspase-1 activation using ELISA and western blot respectively. LPS- and ATP-mediated signaling associated with NLRP3 inflammasome activation were measured by western blot.

Results:

Stigmasterol reduced IL-1β secretion and caspase-1 activation by inhibiting both priming signal and activation signal of NLRP3 inflammasome in LPS- and ATP-activated macrophages. The stigmasterol also reduced LPS-induced protein expression levels of NLRP3 and IL-1β precursor. The underlying mechanisms for the anti-NLRP3 inflammasome activity were demonstrated as reducing ATP-induced phosphorylation of ERK1/2, but not JNK1/2, PKC-α, and PKC-δ. Stigmasterol also inhibited the activation of NLRP1and NLRC4.

Conclusion:

These results demonstrate that stigmasterol inhibited NLRP3 inflammasome activation through reducing not only the priming signal, but also the activation signal, of NLRP3 inflammasome in LPS- and ATP-activated macrophages.

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Identification of IRES trans-acting factors (ITAFs) Involved in the Hypoxia-induced Up-regulation of FGF9 Protein Expression

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Background:

Hypoxia is a reduction in the normal level of tissue oxygen tension, and involved in many human diseases including acute and chronic vascular diseases, pulmonary diseases and cancers. Under hypoxia, canonical cap-dependent translation is generally inhibited, many hypoxia-responding genes initiate their translations with an alternative mechanism known as internal ribosome entry site (IRES)-mediated translation. The IRES-mediated translation requires RNA sequence to form a complex tertiary structure in the 5'untranslated region (5'UTR). It has been demonstrated that some proteins, named IRES trans-acting factors or ITAFs, are interacting with IRES-containing mRNA and recruiting ribosomes to the IRES element. Thus, these ITAFs directly involves in the initiation of IRES-dependent translation. Our previous study showed that FGF9 5'UTR contains an IRES element and FGF9 protein expression is up-regulated during hypoxia through this IRES-mediated mechanism.

Material and method :

Both cytosol and nuclear fractions were isolated from hypoxia-treated HEK293 cells and incubated with biotinylated-RNA probes, which were in vitro transcribed from different regions of FGF9 5'UTR. After pulled-down by streptavidin-agarose beads, the captured proteins were separated by SDS-PAGE and followed by silver stain. Specific bands were excised from the gels and subjected for protein identification by MALDI-TOF analysis. The results of mass analysis were confirmed by Western blot.

Result :

The results of RNA pull-down followed by mass analysis revealed that NF90 and hnRNP M bind to FGF9 IRES region. These results were confirmed by Western blot. In addition, screening the association between FGF9 mRNA 5'UTR and common ITAFs also identified hnRNP C1/C2 and HuR interacting with FGF9 mRNA 5'UTR.

Conclusion:

Our results demonstrated that NF90 and hnRNP M specifically bind to the FGF9 IRES region, while hnRNP A1,C1/C2, PTB, HuR bind to the other regions of FGF9 5'UTR. These proteins may form a protein network to regulate FGF9 hypoxia-induced IRES-mediated translation. In this study, we have identified FGF9 IRES interacting ITAFs and illustrate their role of this interaction in the regulation of FGF9 expression during hypoxia.

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The biological function of endothelial cells to natural polymers comprised of nanogold composites and the associated signaling pathway

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Backgrounds:

The biological function of endothelial cells (ECs) on two novel nanogold composites (fibronectin-nanogold composites, FN-Au) and (collagen-I-nanogold composites, Col-Au) containing smaller amount of nanogold (≈ 43.5 ppm) were used as a model system to study the molecular mechanisms that influenced endothelial cell migration and proliferation on biomaterial surfaces.

Materials and Methods:

The cellular behavior and signaling pathway of ECs on the FN-Au and Col-Au was evaluated by a series functional assay (such as flow cytometry, western blot, MMP zymography and migration rate test).

Results:

It was found that ECs had the highest migration rate on the FN-Au and Col-Au while containing 43.5 ppm of AuNPs. The high ECs migration rate was associated with increased levels of endothelial nitric oxide synthase (eNOS) and phosphorylated-Akt (p-Akt) expressed by ECscultured on FN-Au and Col-Au. The higher expression of α5β3 integrin for ECs on FN-Au and Col-Au was also demonstrated. Phalloidin staining showed that actin appeared as a circumferential band surrounding each cell on control group (glass), whereas on FN-Au and Col-Au , the cells had their margin spread out and extend processes with stress fibers in the protruding lamellipodia. The higher MMP-2 Protein expression on FN-Au and Col-Au was also observed.

Conclusion:

It was concluded that FN-Au and Col-Au activated α5β3 integrin/MMP-2 signaling pathway in ECs, leading to better proliferation and migration effect of ECs on these surfaces.

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The Effect of Novel Histone Deacetylase Inhibitors in Human Pancreatic Carcinoma

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Backgrounds:

Pancreatic carcinoma is one of the leading causes of cancer mortality. In the worldwide, either the prevalence or incidence of pancreatic cancer is increasing. Despite recent progress in chemoradiation therapy and improved systemic chemotherapeutic agents, the 5-year survival rate is still less than 10%. In many cancers, histone acetylation plays an important role in the epigenetic regulation of gene expression. Recent studies have suggested that histone deacetylase inhibitors could induce histone acetylation and resulted in chromatin remodeling as well as repression of tumor suppressor genes.

Materials and Methods:

The cell viability was measure by MTT assay. Caspase-3 activation was measure by flow cytometry and analyzed by WinMDI analysis software. The expression of apoptosis-related proteins, including members of Bcl-2 family and survivin, was determined by western blot. The expression of histone acetylation and p21 were also determined by western blot.

Results:

We investigated the effect of a novel phenylbutyrate-derived histone deacetylase inhibitor (HDACi) in human pancreatic cancer cell lines. Novel HDACi induced pancreatic cancer cells cytotoxicity and apoptosis. Novel HDACi also suppressed the expression of survivin. Conversely, the members of Bcl-2 family, including anti-apoptotic proteins Bcl-2, Bcl-xL, and pro-apoptotic proteins including Bak, Bid, were unchanged upon novel HDACi treatment. p21, which is a cyclin-dependent kinase inhibitor, was significantly induced by novel HDACi.

Conclusion:

In summary, novel HDACi induced cell apoptosis through repressing survivin expression as well as up-regulating the expression of p21, but not via Bcl-2 mitochondrial apoptotic pathway. Our results demonstrated that novel HDACi is a potential drug for cancer therapy.