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The Study of Efficacy and Safety of Recombinant Toxin Subunit Vaccines Against Swine Progressive Atrophic Rhinitis

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Backgrounds:

Progressive atrophic rhinitis (PAR) is an important upper respiratory disease in swine. The clinical symptoms of PAR are characterized by degeneration nasal turbinate atrophy, twisting of the snout and growth retardation, which cause economic loss. Previous studies have shown that PAR is caused by toxigenic Pasteurella multocida (P. multocida) and virulent Bordetella bronchiseptica strains. P. multocida toxin (PMT) produced from P. multocida is the major virulence factor of the PAR in swine. In 1991, Petersen et al. have reported that recombinant PMT derivatives induce effective protection against P. multocida infection. Therefore, PMT has been considered as a good candidate for subunit vaccine development. The efficacy of a subunit vaccine is determined by immunogenic epitopes.

Materials and Methods:

Several PMT fragments designed by computer software were fused with molecular adjuvant as novel plasmids. Novel antigens were highly expressed in E. Coli expression system. The protective efficacy of novel vaccines upon toxin challenge was examined in a mouse animal model. Enzyme-linked immunosorbent (ELISA) and neutralizing antibody assay were carried out for the measurement of anti-PMT antibody and neutralizing antibody titer. In addition, mice and guinea pigs were used for safety assay.

Results:

The results revealed that this novel subunit toxin vaccine protects mice from toxin challenge and the survival rate increased significantly when compared to the control group. Moreover, high anti-PMT antibody and neutralizing antibody titers are detected post-vaccination, which indicates that the novel vaccine induces humoral immune responses. This vaccine is also harmless to mice and guinea pigs in safety assay.

Conclusion:

This novel recombinant toxin subunit vaccine shows highly potential to against progressive atrophic rhinitis in swine.

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Irisflorentin Attenuates The Maturation and Function of Mouse Bone Marrow-Derived Dendritic Cells through Blocking of IKK/NF-κB and MAPK Activities

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Backgrounds:

Dendritic cells (DCs) are key antigen presenting cells in the immune system. One active field of study is the handling of DCs as pharmacological targets to descovery novel biological modifiers for the treatment of inflammatory and autoimmune diseases.

Materials and Methods:

Irisflorentin (IFT), an isoflavone component derived from the roots of *Belamcanda chinensis* (L.) herbs which have been used for the treatment of inflammatory diseases in traditional Chinese medicine. In this study, we tested the potential of IFT to modulate lipopolysaccharide (LPS)-stimulated activation of mouse bone marrow-derived DCs.

Results:

Our results revealed that treatment with up to 10 μM IFT does not cause cytotoxicity in cells. IFT inhibited the production of TNF-α, IL-6, and IL-12p70 by LPS-stimulated DCs. The expression of LPS-induced MHC class II, CD40, and CD86 on DCs was also decreased by IFT, and the endocytic capacity of LPS-stimulated DCs was restored by IFT. In addition, LPS-stimulated DC-elicited allogeneic T-cell proliferation was blocked by IFT, and the migratory ability of LPS-stimulated DCs was reduced by IFT. Moreover, our results confirmed that IFT impairs the responses of LPS-stimulated activation of DCs through suppression of IκB kinase and mitogen-activated protein kinase activities. Coadministration of IFT with 2,4-dinitro-1-fluorobenzene prevented 2,4-dinitro-1-fluorobenzene-induced delayed-type hypersensitivity.

Conclusion:

IFT may be a new potent immunosuppressive agent and could be used in the prevention and treatment of inflammation, and autoimmunity through the blockage of DC maturation and function.

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Investigating the roles of Influenza A virus NEP protein and its functional interaction with the F1α/β subunits of mitochondria ATP synthase for viral egression

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Background:

Influenza A virus (IAV) is an enveloped virus with a genome made up of 8 segmented RNAs, which encode 11 viral proteins. The IAV Nuclear Exporting Protein (NEP) has been reported to involve in virus ribonucleoprotein (vRNP) nuclear export through Nuclear Export Signal (NES)-independent interaction with hCRM1, and can negatively regulate viral genome transcription/ replication. However, the functional relevance of NEP in virus life cycle remains largely unknown. Our previous results demonstrated that NEP interacts with mitochondria ATP synthase *in vivo* and may enter into mitochondria. Interestingly, while NEP affected the cellular ATP level and type-I IFN response, it did not induce apoptosis, cause cellular Reactive Oxygen Species (ROS), and disrupt mitochondria membrane potential. Because IAV, unlike many RNA viruses, lacks self-equipped motors similar to the α subunit of F1 to pack its genomes, we surmise that NEP is not only a multifunctional protein but also has a role in viral egression by interacting with the F1α/β ATP synthase. In this study, we report the *in vivo* and *in vitro* interaction relationship between NEP and the subunits of ATP synthase F1α and F1β, and aim to identify their interaction domain(s) critical for their functional association and viral egression.

Materials and Methods:

E.coli expression plasmids expressing truncated ATP synthase F1α or β domain in 3 different lengths with 6xHis tagged at the N-terminus were constructed to map the NEP-interacting domain(s) of ATP synthase. These plasmids were transformed into BL21(DE3) and the respective recombinant proteins were purified to study their interaction site(s) with a recombinant GST-tagged NEP by a GST pull-down assay.

Results:

We have confirmed our previous result that NEP affected the cellular ATP level and type-I IFN response, but did not induce apoptosis, cause cellular Reactive Oxygen Species (ROS), and disrupt mitochondria membrane potential. We also found that the *in vivo* interaction of ATP synthase with NEP requires RNA in the association, because the treatment of RNAse disrupts the interaction. Functional characterization of their association in IAV infectivity may be important to understand the role of ATPsynthase in viral egression.

Conclusion:

This study provides a significant insight on the roles of IAV NEP protein and its functional interaction with mitochondria ATP synthase α or β in IAV egression. Identification of the interactive domain(s) of the ATP synthase α or β subunits to NEP-RNA complex may help to design competitive peptides for therapeutic application.

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Cytoprotective Effect of Fisetin on the Hypoxia-induced Cell Death in PC12 Cells

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Backgrounds:

Hypoxia plays a major role on the promotion of neuronal cell damage in stroke, heart failure and neurodegenerative diseases. Therefore, cell adaptation to hypoxia is an effective way for neuroprotection. Fisetin (3,7,3,4-tetrahydroxyflavone), a flavonoid widely distributed in fruits and vegetables, is known to exhibit several biological activity including neuroprotection. In this study, we aim to investigate the neuroprotective effect and mechanism of fisetin against hypoxia-induced cell death in PC12 cells.

Materials and Methods:

PC12 cells were treated with cobalt chloride ($CoCl_2$), which serves as a hypoxia-induced agent, and cell survival was examined by MTT assay. The effect of fisetin on the ROS scavenging was performed in the flow cytometry. The quantitative real-time PCR and Western blot analysis were used to determine the expression of HIF-1α and the signaling molecules involved in the fisetin-mediated cytoprotection in PC12 cells.

Results:

We found that fisetin could significantly rescue $CoCl_2$-induced cell death in PC12 cells. It has been reported that $CoCl_2$ induces reactive oxygen species (ROS) and leads to cell death. However, our flow cytometric result showed that ROS scavenging was not involved in the fisetin-mediated cytoprotection. Hypoxia induced factor 1 alpha (HIF-1α) is a known protein against hypoxia; we next investigated the effect of fisetin on HIF-1α expression. The quantitative real-time PCR and Western blot analysis showed that fisetin promoted the HIF-1α gene expression. This result implied that HIF-1α might contribute to fisetin-mediated cytoprotection of PC12 cells. Furthermore, to investigate which signaling pathways are involved in the fisetin-mediated neuroprotection, the selectively inhibitors for specific kinase including MAPK/ERK 1/2 (MEK1/2), JNK, p38 MAPK and PI3/Akt were used. Our data showed that the protective effect of fisetin was abolished by inhibition of MAPK/ERK-, p38-, and PI3/Akt-dependent pathways in PC12 cells. Further investigation on the activation of these pathways by fisetin and their roles on the fisetin-mediated cytoprotection of PC12 cells are in progress.

Conclusion:

In conclusion, HIF-1α may contribute to fisetin-mediated neuroprotection in PC12 cells. Furthermore, modulation of signaling pathways such as MAPK are involved in channeling the fisetin stimulus for cell survival against hypoxia insults.