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Established optical fusion protein (mPlum-IFP1.4) for in vivo imaging based on near-infrared property

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Backgrounds:

Nowadays, utilizing the visibly fluorescent protein became an optical reporter monitoring in the cells level condition was not difficult already. But monitoring in the deep tissue and tumorgenesis of mice was been handicapped by poor penetration of emission light. In the previous study, infrared fluorescent proteins (IFPs) with the excitation/emission peaks maxima of 684/708nm and showed well fluorescent expression in the cell and mice which more increased fluorescence intensity by biliverdin. However, IFPs still had some limitations should be overcomed such as monitoring more deeper tissue that penetrated emission fluorescent light or before in vivo imaging needed intravenous biliverdin that not very convenient for each times.

Materials and methods:

Here we cloned the mPlum (Far red) and IFP1.4 fluorescent proteins to become an optical fusion protein (mPlum-IFP1.4) based on foster resonance energy transfer (FRET). Olympus confocal microscopy was used to characterize mPlum-IFP1.4 based on FRET and defined by acceptor photobleach technique. Both of IFP1.4 and mPlum-IFP1.4 fusion protein fluorescence were quantitative by In vivo imaging system (IVIS).

Results:

We expected that mPlum-IFP1.4 fusion protein not only increased the IFP1.4 fluorescent intensity but also more convenient operated during in vivo imaging that meaning unnecessary biliverdin. Each of cells level *in vitro* and mice level in vivo imaging, all the results showed mPlum-IFP1.4 fusion protein had stably expression fluorescence intensity than native IFP1.4 that based on FRET property and brightness intensity unaffected by biliverdin.

Conclusion:

Taken together, the mPlum-IFP1.4 fusion protein exhibits improved brightness compared to native IFP1.4, and it would be useful for in vivo optical imaging of deep tissue, cancer or stem cell viability via gene expression.

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The Effects of Diosgenin in Fibrosis Regulation of Renal Proximal Tubular Epithelial cells

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BACKGROUND:

Fibrosis is the important pathway of many diseases such as end-stage renal failure. Recent study has demonstrated that high concentration of glucose is the most important fibrogenesis-inducing and propagating cytokine. Diosgenin has been shown to be cancer-chemopreventive and and anti-inflammatory. In this study, renal proximal tubular epithelial cells (designated as HK-2) were treated with high concentration of glucose (HG, 27.5 mM) to determine whether the Diosgenin reduces fibrosis in vitro.

METHODS:

Cells were cultured in high concentration of glucose (HG, 27.5 mM) for 48 hours. Different concentrations of Diosgenin (0.1 1 10 μM) were added to cells for the last 24 hours. Cells were trypsinized and subjected to the following assays. ELISA was used to evaluate the secreted protein, such as fibronectin and Signal transducer EMT initiator (e.q Snail). Immunofluorescence staining was used to assay the in situ expression of proteins (e.q. fibronectin and Snail).

RESULTS:

We found that the 10 μM of Diosgenin exert optimal inhibitory effects on high glucose-induced fibronectin in HK-2 cells. Diosgenin markedly inhibited HG-induced increase in α-smooth muscle actin and snail. Whereas HG-induced decrease in E-cadherin expression was reversed as well.

CONCLUSION:

Diosgenin has the potential to inhibit high glucose-induced renal tubular fibrosis possibly through EMT pathway.

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Prior Manipulation Therapy induced Primary Osteosarcoma Metastasis – From Clinical to Basic Research

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Backgrounds:

Our previous clinical study demonstrated that manipulation therapy (MT) on osteosarcoma (OS) patients prior to diagnosis resulted in poor prognosis after surgical treatment. This study was aimed to provide the evidence from clinical to basic for MT-induced metastasis in primary osterosarcoma.

Materials and Methods:

Eight-week-old male GFP-labeled human OS cells-transferred nude mice were randomly allocated into 2 groups, namely, MT (+) and MT (-) groups. MT was conducted with repeated massage on tumor site twice a week for 7 or 15 weeks. The parameters evaluated were x-ray diagnosis, micro-PET/CT scan, histopathology and serum metalloproteinase 9 (MMP9) level.

Results:

The results showed that MT (+) mice showed a decreased body weight (30.5±0.65g) and an increased tumor volume (8.3±1.18 mm3) compared to MT (-) group with body weight (35·8±0·40g, p<0·0001) and tumor volume (3.9±1.34mm3, p=0.038), respectively. There was an increased signal intensity over lymph node region of hind limb by micro-PET/CT and the GFP-labeled human OS cells were detected in the lung and bilateral lymph nodes in MT (+) group, while there were no such findings in MT (-) group. The serum MMP9 level was higher in MT (+) group (27 1±1.29 ng/ml) than in MT (-) group (17.8±1.97 ng/ml, p=0.048).

Conclusion:

Taken previous clinical observation and the present in vivo evidence together, we conclude that physicians should pay more attention on those patients who seek MT before diagnosis or during treatment for osteosarcoma.

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GTP-binding Protein Involves in the Regulation of Axin Degradation

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Backgrounds:

Wnt are secreted lipoglycoproteins that function as signaling molecules to regulate embryonic development at different stages and tissue homeostasis. Without Wnt stimulation, Axin serves as a platform to form a protein complex with MACF1 GSK3β, CK1 and β-catenin in the cytoplasm. GSK3β and CK1 will phosphorylate β-catenin and target β-catenin for proteasome degradation. Upon Wnt stimulation, Wnt binds to its receptor Frizzled and co-receptor LRP5/6, and MACF1 helps Axin complex translocation from the cytoplasm to the cell membrane, then Axin is degraded and in some way β-catenin is accumulated in the cytoplasm for transducing Wnt signaling. So far, how Axin stability is regulated by Wnt stimulation remains elusive and is the focus of current study.

Materials and Methods:

Human embryonic kidney 293T cells and African green monkey kidney fibroblast-like COS7 cells were treated with several inhibitors to G protein-mediated signaling in control-conditioned medium or Wnt3a-conditioned medium, and Western blotting, immunofluorescence staining and TCF-mediated luciferase activity assay were performed to examine the involvement of G protein in the proteolysis of Axin.

Results:

By Western blotting, compare to control treatment, Axin was degraded and beta-catenin was accumulated in Wnt stimulated cells. When cells were treated with different concentrations of inhibitors to G protein-mediated signaling, Axin degradation was inhibited and beta-catenin accumulation was reduced. Immunofluorescence staining data showed that, as compare to control treatment, beta-catenin was accumulated in the cytoplasm and translocated into the nucleus in the Wnt-stimulated cells. This phenomenon was completely blocked by treatment with these inhibitors. We then performed the beta-catenin/TCF-mediated luciferase activity to examine the effects of these inhibitors on Wnt signaling. As compare to control treatment, the luciferase activity increased after Wnt stimulation. Treatment of cells with different concentrations of inhibitors decreased the luciferase activity.

Conclusion:

We conclude that the involvement of G proteins and the underlying signaling pathway in regulating Axin degradation.