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Fluoxetine selectively inhibits glioblastoma invasion and induces excitotoxicity through AMPAR

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Backgrounds: Glioblastoma multiforme (GBM) is one of the most common and lethal primary brain tumor in adult. The median survival time of GBM patients receiving active treatment is only about 12~15 months. Unfortunately, most chemotherapeutic drugs commonly used for cancer treatments have limited therapeutic potential for GBM, due to their incapability to pass through blood-brain barrier and distinguish between normal and tumor cells. As much evidence suggests that antidepressants decrease cancer incidence and improve patients' quality of life, we therefore attempted to investigate the potential for fluoxetine to be used to treat GBM and its possible underlying mechanism. **Materials & Methods:** By cell-based assays, we analyzed the expression level of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) and elucidated the mechanism of fluoxetine-induced apoptosis in glioma. The intracellular calcium ion concentration was measured by Fura-2 indicator. We then determined whether fluoxetine directly interact with AMPAR by computer modeling and surface plasmon resonance binding assay. The inhibition of cell invasion by fluoxetine on glioma was evaluated by trans-well assay. We also carried out the intracranial glioma xenografts animal model to further confirm the therapeutic effect of fluoxetine. **Results:** We found that fluoxetine-induced glioma cell death is activated by AMPAR evocation and calcium ion influx. AMPAR are excessively expressed in glioma tissues, and thus fluoxetine specifically executes glioma cells. Fluoxetine-induced calcium overloading damaged the mitochondrial membrane and triggered apoptosis pathway in glioma. Moreover, low dose fluoxetine treatment shrunk the cell volume and diminished the glioma invasion. In vivo study revealed that fluoxetine suppressed the growth of glioblastoma in the Nu/Nu mice brain, an effect similar to that given by Temozolomide (TMZ), a first-line GBM chemotherapy drug. Fluoxetine, however, had less toxic effects on the liver as compared to the effects of TMZ. **Conclusion:** Our analysis indicated that fluoxetine directly binds to AMPAR and evokes calcium influx in glioma cells. It not only inhibits the glioma invasion ability but also, in high dose, specifically executes the glioma cells. Those findings suggest that fluoxetine could be a safe and practical approach to treat GBM.

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Improvement of Docetaxel Induced Side Effects by Fungal Immunomodulatory Proteins

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Backgrounds: Docetaxel (Taxotere®) is a potent antitumor agent that is commonly used in cancer therapy. The most common side effects of docetaxel treatment are neutropenia and anemia. Human granulocyte colony stimulating factor (hG-CSF) recovers the inhibition of white blood cells and hematopoietic stem cells by chemotherapeutic agents for cancer patients. FIP-*fve* and FIP-*gts* are Fungal immunomodulatory proteins (FIPs) from *Flammulina velutipes* and *Ganoderma tsugae*, respectively. In the previous studies, FIP-*fve* and FIP-*gts* shows anti-cancer and anti-metastasis activity. However, little is known the improvement of chemotherapeutic side effects. **Materials and Methods:** RT-PCR was used to investigate hG-CSF and IFN-γ expression. Complete blood count (CBC) was performed to assess effects of FIP-*fve* and FIP-*gts* on Docetaxel-induced hematologic adverse events. The Hematoxylin and eosin stain were performed to investigate whether FIPs protect from Docetaxel-inducing damage in small intestinal and bone marrow. Micro Computed Tomography Imaging System (Micro CT) and Dual-energy X-ray absorptiometry (DEXA) were used to detect the morphology and density of bone. The effects of FIP-*fve* and FIP-*gts* on osteoblast differentiation were investigated by ALP staining and ALP activity assay. **Results:** FIP-*fve* and FIP-*gts* induced hG-CSF and IFN-γ mRNA expression. This results suggested the protective potential of FIP-*fve* and FIP-*gts* on Docetaxel-induced neutropenia. However, only FIP-*gts* reversed the Docetaxel-decreased white blood cell (WBC) count. FIP-*fve* and FIP-*gts* significantly mitigated the bone marrow adipogenesis and intestinal villus injury following Docetaxel treatment *in vivo*. Furthermore, Docetaxel obviously induced bone loss in mice, but both FIPs did not decrease this bone damage. On ALP stain and ALP activity assay, FIP-*fve* and FIP-*gts* inhibited the osteoblast differentiation induced by Vit C and β-Glycerophosphate disodium salt hydrate (BGP) in preosteoblast MC3T3-E1. **Conclusion:** FIP-*gts* exhibited more potent for Docetaxel-mediated bone marrow adipogenesis and intestinal villus damage than FIP-fve. Further, FIP-*gts* recovered neutropenia induction by Docetaxel. In addition, Docetaxel-induced osteoporosis is an important issue and needs further investigation. This study may prove that FIP-*gts* are potential chemotherapeutic adjuvants.

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Snail-induced Epithelial-Mesenchymal Transition Increases Nanog through Smad1/5-AKT-GSK-3β Pathways in Lung Cancer cell

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Backgrounds: The epithelial-mesenchymal transition (EMT), a crucial step in cancer metastasis, is important in transformed cancer cells with stem cell-like properties; however, the molecular mechanisms underlying the EMT stem cell-like transformation remains unclear. **Materials and Methods:** Here, we established a Snail-overexpressing EMT model using A549 cells that shows morphological change, migration potential enhancement, chemoresistance, and EMT biomarker expression. **Results:** Stem cell-like properties, such as an increase in the $CD24^{low}/CD44^{high}$ subpopulation, CD133 surface marker expression, and stemness gene (*Oct4, Nanog*, and *Sox2*) expression, have also been observed in these cells. Using this model system, the signals required for Snail-mediated cancer stem-like transformation were studied. Hyper-activation of Smad1/5 and AKT and inactivation of glycogen synthase kinase-3 beta (GSK-3β) were observed in both Snail-overexpressing A549 cells and in endogenous Snail-expressing CL1-5 cells. Pretreatment with SB431542 (transforming growth factor beta [TGF-β] receptor inhibitor) or LDN193189 (bone morphogenetic protein [BMP] receptor inhibitor) prevented Snail-induced Smad1/5 and Akt hyper-activation and reactivated GSK-3β. In addition, LY294002 pretreatment also prevented AKT hyper-activation and reactivated GSK-3β but without any changes in Smad1/5 activation. LY294002, SB431542, and LDN193189 pretreatment also inhibited Snail-induced Nanog expression, suggesting involvement of the Smad1/5-AKT-GSK-3β pathway in Snail-induced EMT and acquisition of cancer stem cell-like properties. **Conclusion:** These findings demonstrate the involvement of a novel signaling pathway in EMT and cancer stem cell-like transformation and may provide useful therapeutic targets for cancer stem cell elimination.

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Alteration in Adenylate Cycalse Activity Mediated by Coexpressed μ-, κ-Opioid and Nociceptin Receptors Following Buprenorphine Exposure

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Background: Buprenorphine is used in maintenance therapy for heroin addicts. It is a μ-opioid receptor (MOP) partial agonist and a potent κ-opioid receptor (KOP) antagonist as well as a nociceptin/opioid receptor-like 1 (NOP) agonist. **Materials and Methods:** In this study, we established an *in vitro* cell model overexpressing human MOP, KOP, and NOP receptors individually or simultaneously in human embryonic kidney (HEK) 293 cells; and then compared the effects of U-69593, DAMGO, nociceptin, and buprenorphine on adenylate cyclase (AC) activity in these cells (KOP, KOP+MOP, KOP +NOP, and KOP+MOP+NOP). **Results:** Saturation radioligand binding assay using [^{3}H]-diprenorphine was performed to verify surface expression of KOP. After acute exposure, U-69593 inhibited AC activity in all four stable clones, showing that KOP was successfully expressed. DAMGO and nociceptin could exert AC activity inhibition in cells expressing MOP and NOP receptors, respectively. Buprenorphine was able to inhibit AC activity to about 90% of the E_{max}, yet with lower potency, in cell expressing MOP, NOP and KOP receptors simultaneously compared with other three stable clones. **Conclusion:** The study demonstrated that buprenorphine is more efficient, but less potent, for AC inhibition when KOP receptor is coexpressed with MOP and NOP receptors.