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Study of PFKFB2 isoforms in pancreatic duct cells transformed with mutant K-Ras

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6-phosphofructo-2-kinase/fructose-2,6-bisphospatase (PFKFB) family of enzymes are responsible for the conversion of fructose-6-phosphate (F6P) to fructose-2,6-bisphosphate (F2,6BP) and *vice versa*, and F2,6BP is an allosteric activator of phosphofructokinase-1 (PFK1), a rate-limiting enzyme of glycolysis. Among the four identified PFKFB isozymes (PFKFB1-4), PFKFB2 is the least studied isozyme in human cancers. There exists two different splice variants of PFKFB2, variant-1 and variant-2, coding two different isoforms, isoform a and b, respectively.

In this study, we first analyzed the effect of K-Ras(G12D)-induced oncogenic transformation on PFKFB2 expression in pancreatic duct cells. We found that oncogenic K-Ras induction in immortalized pancreatic duct cells (iPDE) was associated with decreases in total PFKFB2 mRNA and protein expressions (mRNA; iPDE: 1 ± 0.15; iPDE+KRas: 0.78 ± 0.24 and protein; iPDE: 1 iPDE+KRas: 0.55). We then, checked individual expressions of splice variants and observed that while PFKFB2 splice variant-1 (P2-v1) expression was reduced by K-Ras induction (iPDE:100; iPDE+KRas:81.50), PFKFB2 splice variant-2 (P2-v2) expression was increased (iPDE:100; iPDE+KRas:125.70). Then, we checked effects of P2-v1 and P2-v2 on glycolytic phenotype of iPDE and iPDE+KRas cells. Over-expression of PFKFB2 variants increased F2,6BP concentration (P2-v1: 1.96; P2-v2: 1.72 fold; compared to empty vec), glucose uptake (P2-v1: 16%; P2-v2: 30%) and glycolysis (P2-v1: 20%; P2-v2: 30%) in iPDE+K-Ras cells.

We next analyzed the subcellular localizations of PFKFB2 isoforms and observed that both PFKFB2 isozymes localize to the nucleus, with more prominent nuclear localization of P2-v1 compared to P2-v2. Also, nuclear localization ratio of P2-v2 increases after oncogenic transformation with mutant K-Ras.

Taken together, these results suggest that PFKFB2 may have a role in the glycolytic phenotype of pancreatic cancers characterized with hyperactive K-Ras signaling.

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Effects of p38 MAP kinase inhibitors on MDA-MB-231 cell line

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Introduction: p38 MAPK phosphorylates serine and/or threonine residues of the target proteins. The activation of p38 MAPK leads to cell growth, differentiation, survival or apoptosis. In this study, we tested the effect p38 MAPK SB203580 and SB202190 on MDA-MB-231 cells to further elucidate the controversial role of p38 MAPK on cell proliferation or cell migration.

Materials and Methods: MDA-MB-231 cancer line was cultured in RPMI-1640 supplemented with 10% FBS. The cytotoxic and cell migration effects of SB203580 and SB202190 inhibitors were tested by MTT assay and wound assay, respectively. The effects of both inhibitors on proliferation and adhesion of MD-MB-231 cells were determined by iCELLigence system.

Results: It was found that SB202190 p38 MAP kinase inhibitor was more effective than SB203580. However, no significant effects of low doses of 1 μM and 5 μM of both inhibitors were seen on cell proliferation as compared to the DMSO-treated control cells for up to 96 hours as determined by iCELLigence system. On the other hand, both SB203580 and SB202190 significantly prevented cell proliferation at a concentration of 50 μM. Both SB203580 and SB202190 significantly reduced cell migration in a time-dependent manner at a concentration of 50 μM. Then, we tested whether each p38 MAPK inhibitors have any effect on cell adhesion during a treatment period of 3 hours using iCELLigence system. Only 50 μM concentration of SB202190 reduced cell adhesion for about 1.5 hour ($p < 0.001$).

Conclusion: p38 MAPK inhibitors SB203580 and SB202190 differentially affect cell proliferation, survival and migration.

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Mutagenicity of a series efficacious benzoxazine derivatives – a new approach to evaluate ames test data

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Testing safety of drug candidates is as crucial as evaluating their efficacy in early drug development. We previously synthesized a series of 1,4-benzoxazine-3-one derivatives showing significant antimicrobial, *in vitro* anticancer, topoisomerase I inhibitory activities and studied their several mechanisms of action. In this present study, we have evaluated mutagenic activities of these compounds and their potential metabolites. Moreover, we aimed to develop a new statistical algorithm available for structure-activity relationship analysis to identify the regions responsible for the activity.

To evaluate mutagenicity of the compounds, Ames *Salmonella*/microsome test was used. *Salmonella typhimurium* TA98 and TA100 strains were used to detect for frameshift and base-pair substitution mutagens, respectively. Additionally, mutagenicity of potential metabolites of them were evaluated by adding metabolic activation system (S9) which was prepared from a pool of male *Sprague Dawley* rats. Results were evaluated with Student's-T test. Following regression model estimation analysis, we detected minimum mutagenic doses of all tested compounds for generating a 3D-common features pharmacophore model with HipHop method.

According to the results, only BS12, BS13, BS16 and BS17 exhibited strong mutagenic effects on both strains in the presence and absence of S9. Additionally BS10, BS7, BS1 and BS15 (in the absence of the S9), BS18, BS4 and BS7 (in the presence of the S9) showed weak mutagenic effects on TA98. HipHop analysis results revealed that mutagenicity was increased in the presence of aromatic desactivating groups which might form hydrogen bonds at the position of R3 and hydrophobic groups at the position of R2 of the benzene ring in the structure of benzoxazine.