396 POSTER

Sphingosine 1-phosphate (S1P) differently modulates migration of gastric cancer cell

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Cell migration is important in pathological processes as local tumor invasion and distant metastasis. In addition to the chemokines or cytokines, lipid mediators serve as chemoattractant that is closely associated with metastasis.

S1P is known to be capable of inducing diverse cellular responses through the EDG family G protein-coupled receptors. Among the 8 different EDG receptors, five receptors (Edg-1/$S1P_1$, Edg-3/$S1P_3$, Edg-5/$S1P_2$, Edg-6/$S1P_4$, Edg-8/$S1P_5$) are known to be activated by S1P. However, the functions or relevance of S1P in tumor biology have not been satisfactory investigated. In the present study, we examined the effects of S1P on human gastric cancer cell lines and characterized its functions according to the expression subtypes of S1P receptors.

The expression patterns of S1P receptor mRNAs in MKN1, HGC-27 and AZ-521 were evaluated using northern blot analysis. MKN1 expressed significant level of $S1P_3$ and relatively low level of $S1P_2$. HGC-27 cells also expressed also these two receptors, but $S1P_2$ expression was relatively high as compared with $S1P_3$ level. AZ-521 cells exclusively expressed $S1P_2$. Expression level of $S1P_1$ mRNA were very weak in these cell lines.

Migration of cells in response to a gradient of S1P was measured in a modified Boyden chamber. MKN1 cells exhibited little spontaneous migration without S1P stimulation, but the addition of S1P to the lower chamber markedly induced MKN1 cells migration in a dose dependent manner up to a concentration of 100 nM (225% of control). The S1P dose response curve was typical bell-shaped with a reduction of migration at supramaximal concentrations. In marked contrast, AZ-521 cells exhibited spontaneous migration without S1P and S1P markedly inhibited cell migration in a dose dependent manner down to a concentration of 10 nM (19% of control). S1P stimulated HGC-27 cells migration in a dose dependent manner up to a concentration of 10 nM. However, at a concentration of 100 nM, HGC-27 cells migration was drastically reduced to the level below control.

Our results indicate that S1P is a potent regulator for gastric cancer cell motility. The degree of the effects was different between three gastric cancer cells due to the expression pattern of S1P receptors.

397 POSTER

Aurocrine interleukin-8 signalling in prostate cancer cells promotes translation of cyclin D1

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The aim of this current study was to elucidate the importance of autocrine interleukin-8 (IL-8) signalling to the progression of prostate cancer. Our hypothesis proposed that elevated IL-8 signalling may confer a survival or proliferative advantage to prostate cancer cells. PC3 cells were stimulated with recombinant-human IL-8 (rh-IL-8) and the resulting effect on known survival and mitogenic signalling pathways determined using either whole cell activity assays or immunoblotting on protein lysates with phospho-specific antibodies. In these experiments, rh-IL-8 induced a rapid potentiation in the activation of phospholipase D increasing the intracellular levels of phosphatidic acid (PA) in PC3 cells. This was accompanied by rapid activation of phosphatoidyl-inositol-3 kinase activity (PI3K), detected by the increased phosphorylation of Akt following stimulation with rh-IL-8. Since phospho-Akt and PA are known stimulators of mTOR activity, we examined the effects of rh-IL-8 on the phosphorylation of known mTOR substrates. Immunoblotting demonstrated that rh-IL-8 stimulation induced time-dependent phosphorylation of the translation inhibitory protein 4E-BP1 and phosphorylation of the Thr389 residue of p70 S6 kinase (mTOR-dependent). In addition, time-dependent phosphorylation of the Thr421/Ser424 residues and Thr229 residues in response to rh-IL-8 were observed to be consistent with the hierarchical activation of p70 S6 kinase catalytic activity. This was demonstrated in a further western blot analysis demonstrating phosphorylation of the ribosomal S6 protein and given the concurrent phosphorylation of ribosomal S6 protein and 4E-BP1 in response to rh-IL-8 PC3 cells, suggested a role for IL-8 signalling in regulating the translation of key oncogenes. This was confirmed in further immunoblotting experiments in which rh-IL-8 potentiated the expression of cyclin D1 in PC3 cells, inducing peak increases in cyclin D1 expression at 20–30 min post-stimulation. This response was attenuated by treatment with cycloheximide but not by actinomycin D further supporting IL-8 dependent regulation of cyclin D1 expression at the level of translation. In addition to supporting the mitogenic effect of IL-8 in prostate cancer, our data implicate IL-8 signalling in regulating the translation of cellular oncogenes and suggest that the aberrant regulation of protein synthesis is one mechanism by which IL-8 signalling may underpin the tumourigenesis and metastasis of prostate cancer.

398 POSTER

Development of an LC/MS/MS method for the quantitative determination of the Akt inhibitor PIA5 in mouse plasma

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The phosphoinositide-3 kinase (PI3K)/Akt signaling pathway is frequently deregulated in cancer and many efforts are underway to discover and develop novel inhibitors of its components. In contrast to approaches that are directed against the ATP binding site of Akt, phosphatidylinositol ether lipid analogues (PIAs) were rationally designed to target the pleckstrin homology domain of this serine/threonine kinase. From screening a panel of twenty-four PIAs, five structurally similar compounds that selectively inhibited Akt translocation and activity and induced apoptosis in Akt-dependent cancer cell lines emerged. To evaluate the preclinical pharmacokinetics of one of these active compounds, a sensitive and selective analytical method for the determination of PIA5 in mouse plasma has been developed utilizing liquid chromatography (LC) coupled to tandem mass spectrometry (MS/MS). Plasma samples (100 μL) were prepared by protein precipitation with 3 volumes of methanol. Supernatants were dried under vacuum followed by the reconstitution of PIA5 and the internal standard (PIA18) in 60 μL of methanol. Chromatographic separation of PIA5 and PIA18 was performed on a Hypersil C18, 5 μm, 100×2.0 mm column using a gradient system of 20 to 100% methanol in 10 mM ammonium acetate over 1 minute. The sample injection volume was 20 μL and the total run time was 12 minutes. Detection was performed by multiple reaction monitoring (MRM(of the selected precursor and product ions using a MICROMASS® Quattro *micro*™ API mass spectrometer equipped with an electrospray source and operated in negative ion mode. The MRM transitions for PIA5 and PIA18 were m/z 597 → 239 and m/z 830 → 590, respectively. The limit of detection in plasma was 0.25 ng/mL and the response was linear from 5 to 1000 ng/mL. The accuracy and precision of the analytical method were determined to be within the acceptance criteria recommended in the FDA guidelines. In conclusion, a rapid, sensitive and selective method has been developed and validated for the determination of PIA5 in mouse plasma. This will be used to support preclinical pharmacokinetic studies of this compound, which are currently ongoing and will ultimately guide administration schedules in testing the *in vivo* efficacy of this series of novel Akt inhibitors.

399 POSTER

Constitutive erbB family phosphorylation in osteosarcoma as a target for CI 1033 inhibition

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The role of erbB tyrosine kinases, especially Her-2, in osteosarcoma has engendered intense debate. Some identified an association between low-level Her-2 expression, compared to none, and poor patient outcome. Others questioned the importance of apparent cytoplasmic expression of Her-2, since membranous overexpression is associated with poor outcome in carcinomas. We previously demonstrated that primary osteosarcoma cells express cell-surface EGFR, with the p80 isoform of Her-4 localized to the nucleus. We wished to determine if erbB kinases in osteosarcoma were phosphorylated, and if this was required for growth. We cultured primary osteosarcoma lines in the presence or absence of the pan-erbB inhibitor CI 1033 (Pfizer) and examined the phosphorylation of EGFR and Her-4 by immunohistochemistry, cell-based ELISA, flow cytometry and two dimensional Western blot. We also assessed CI 1033 impact upon osteosarcoma growth and survival *in vitro*. EGFR and Her-4 were constitutively phosphorylated in osteosarcoma. CI 1033 induced erbB receptor dephosphorylation, growth inhibition and apoptosis in a titratible fashion with as little as 1 μM concentrations. We conclude that erbB signaling provides essential growth and anti-apoptotic signals to osteosarcoma cells. This suggests that erbB overexpression is not required for erbB to promote malignancy, but rather that overexpression is one of several mechanisms that generates unregulated erbB signaling.