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Role of human epidermal receptor targeted therapies in chemo-sensitization of oesophageal adenocarcinoma cells

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Background: Despite recent improvements in the survival of oesophageal cancer (EC) patients with the use of combination chemotherapy, chemotherapy resistance represents a major challenge in this disease. Sensitisation of EC cells to chemotherapy may therefore be an important anticancer strategy. In this study, we examined the effect of the epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI) gefitinib and dual EGFR/human epidermal receptor 2 (HER2)-TKI lapatinib on the sensitivity of human oesophageal adenocarcinoma cells (OE19, OE33, SEG-1, FLO-1, BIC-1) to chemotherapy (5-FU; cisplatin). Furthermore, we investigated the underlying mechanism of these interactions.
Methods: Cell viability was assessed using MTT assay. Apoptosis was measured by Flow Cytometry, PARP, caspase 8, and caspase 3 cleavage. EGFR, HER2, HER3 and Akt expression/activity were determined by Western blotting.
Results: All EC cell lines were relatively resistant to 5-FU and cisplatin with IC_{50} doses ranging from 20 μM to 50 μM and from 40 μM to 100 μM respectively. A significant increase in apoptosis was observed when chemotherapy was combined with gefitinib or lapatinib in OE19, OE33 and SEG-1 cell lines, correlating with a dose dependent increase in EGFR, HER2, HER3 and Akt activation following chemotherapy treatment. Lapatinib treatment resulted in more potent inhibition of chemotherapy-induced EGFR, HER3 and Akt activation than gefitinib and this was associated with a more pronounced interaction between lapatinib and chemotherapy compared with combined gefitinib/chemotherapy treatment. Using the EGFR monoclonal antibody panitumumab and gene specific siRNA directed against ADAM17, we found strong inhibition of CDDP-induced EGFR activation in SEG-1 cell line, indicating that increased EGFR activity following chemotherapy may be regulated by ADAM17-mediated shedding of ligands for EGFR.
Conclusions: Our data suggest that the EGFR-HER3/PI3K axis plays an important role in chemotherapy resistance in oesophageal adenocarcinoma. Moreover, dual inhibition of EGFR and HER2 may have therapeutic potential for sensitizing oesophageal tumours to chemotherapy treatment. We have identified ADAM17 as potential mediator of this anti-apoptotic stress response and we are currently further investigating the relevance of the ADAM17-EGFR signalling cascade following chemotherapy treatment.

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Manipulating prostate cancer cell susceptibility to docetaxel and novel titanocene analogues induced apoptosis

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Background: Docetaxel is the standard treatment strategy for androgen-independent metastatic prostate cancer, but only provides a short survival advantage. This indicates a need to both increase the sensitivity of cancer cells to docetaxel and to identify new chemotherapeutic options.
Id-1 (inhibitor of differentiation) is over expressed in many human cancers and provides protection against TNF-α-induced apoptosis in prostate cancer cells. Id-1 is associated with resistance to JNK-induced apoptosis and may represent a target for manipulation. The IAPs (Inhibitors of Apoptosis Proteins) are a family of proteins which are up regulated in androgen-independent prostate cancer and inhibit caspase activity. Bcl-2 over-expression is frequently reported in prostate cancer and inhibits mitochondrial mediated apoptosis.
Novel Titanocene Analogues synthesised by the Centre of Synthesis and Chemical Biology have shown promising cytotoxic activity against a range of tumour types and may be utilised in the triggering of apoptosis in prostate cancer.
The objectives of this study were to investigate if down-regulation of Id-1, the IAPs and Bcl-2 in androgen-independent prostate cancer cells will increase their apoptotic sensitivity to the novel titanocene analogue and the standard treatment Docetaxel.
Methods: PC-3 cells were cultured in supplemented RPMI medium with and without 10% fetal bovine serum. PC-3 cells were assessed for apoptosis and viability using propidium iodide DNA staining by flow cytometry following treatment with docetaxel and titanocene analogues. Id-1, IAPs and Bcl-2 knockdown was achieved using siRNA and confirmed by western blotting. JNK phosphorylation was assessed by western blotting.
Results: Docetaxel and the novel titanocene analogues induced apoptosis and JNK phoshorylation in a time and dose dependent manner in PC-3 cells. Direct (siRNA) and indirect (serum depletion) down-regulation of Id-1 expression increased apoptotic susceptibility to the titanocene analogues but not to docetaxel. However JNK inhibition only inhibited Docetaxel induced apoptosis but not that by the titanocene analogues. Triple IAP knockdown (cIAP-1, cIAP-2 and xIAP) resulted in an increased sensitivity to apoptosis induced by the titanocene analogues as did Bcl-2 knockdown but neither sensitized to docetaxel induced apoptosis.
Conclusions: Novel titanocene analogues induce apoptosis in androgen-independent prostate cancer cells independent of JNK phosphorylation. Manipulation of specific survival proteins sensitizes androgen-independent prostate cancer to these novel titanocence analogues but not Docetaxel which has important implications to increasing patients response to chemotherapeutic strategies.

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Cellular and molecular patterns associated with sensitivity and resistance to enzastaurin in human cancer cells

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Background: Enzastaurin (LY317615, HCl), an acyclic bisindolmaleimide, is a potent and selective competitive inhibitor of PKC beta. Enzastaurin was shown to suppress tumor-induced angiogenesis and to induce cell death in multiple human tumor cell lines by inhibiting PKC and PI3K/AKT signaling pathway. Enzastaurin is currently evaluated in phase III clinical trials as well as multiple phase II trials as a single agent and in combination with a variety of cytotoxic and targeted therapies. The aim of our study was to explore the molecular mechanisms of sensitivity/resistance to enzastaurin in the human solid tumor models.
Methods: The antiproliferative effects of enzastaurin were assessed in a panel of colon (Colo205, HT29, HCT116), breast (SKBR3, MCF7, MDA435), ovarian (OVCAR3, SKOV3, IGROV1), prostate (DU145, PC3), head and neck carcinoma (SCC61, SQ20B) and lung (HOP62, HOP92) cancer cells using MTT assay. In these cells, baseline PKC protein and mRNA expression levels were determined using western blots and RT-PCR.
Results: Enzastaurin displayed antiproliferative effects in a panel of human cancer cell lines with IC50s ranging 11–150 μM, SKBR3 and Colo205 being the most sensitive. In this panel, the spectrum of activity of staurosporine was different to that of enzastaurin. We evaluated the protein expression of PKCα, β, γ, δ, ε and η isoforms in our panel of cell lines. Although most cancer cells used in our panel were shown to express several isoforms of PKC, no clear correlation between PKC isoform expression and sensitivity to enzastaurin was detected. We further evaluated the cytotoxic effects of enzastaurin in a colon cancer cell line (Colo205-R) with acquired resistance to several PKC modulators including PMA, bryostatin, staurosporin, PEP005, and bistratene A. Colo205-R cell line was also resistant to enzastaurin with an IC50 of 50 μM. Resistance to PKC modulators in Colo205-R was associated with morphological changes, increased invasion capacities, and gene expression profile that suggested an epithelial-to-mesenchymal transition. Epithelial markers such as claudin-4, E-cadherin, connexin 32 were down-expressed in resistant cells, in contrast, mesenchymal markers including SNAIL, TWIST, N-cadherin, endothelin-1, vimentin and TGF β were overexpressed in Colo205-R. Significant correlation between endothelin-1 mRNA expression level and resistance to staurosporin was detected but in contrast, endothelin-1 expression was not correlated with resistance to enzastaurin in a panel of ten cancer cell lines, suggesting different mechanisms of sensitivity/resistance between these two compounds.
Conclusion: Sensitivity of human cancer cell lines to enzastaurin doesn't correlate with expression of PKC isoforms. Sensitivity and resistance to enzastaurin may be associated with changes in cell signaling that control epithelial-to-mesenchymal transition.