receptor and IGF-1 receptor). Here we evaluated the effects of statins on IGF-1R signaling, and growth of normal and transformed breast epithelial cells.
Methods: Breast cancer cell lines (MCF-7, MDA-231), non-transformed cell line (MCF-10A), and normal mammary epithelial cells (HMEC) were treated for 3 days with fluvastatin or atorvastatin in presence or absence of mevalonic acid. Proliferation assay was performed by MTT. IGF-IR levels were determined in different treatment conditions by Western blot and flow cytometry and compared with the effect of IGF-1R siRNA. IGF-IR downstream signaling pathway was also analyzed.
Results: Statins reduced proliferation of all cell lines in a dose-dependent manner. In general, this growth inhibition could be rescued by exogenous mevalonic acid, which would be expected to compensate for HMG-CoA inhibition. We observed a reduction IGF-IR expression when cells were treated with statins, but for all cell lines, IGF-IR siRNA treatment had a stronger effect on IGF-1R levels than statins. IGF-1R expression reduction by statins was accompanied by a decrease in phosphorylation of AKT, p70S6K, and MAPK. Despite this, for most cell lines, maximal growth inhibition achieved by statins was greater than that achieved by IGF-1R siRNA. This suggests that either (a) IGF-1R siRNA was insufficient to completely abolish IGF-1R signaling or (b) effects of statin on growth inhibition involve important additional IGF-1R-independent mechanisms. We observed that fluvastatin repressed the farnesylation of $p21^{WAF1}$ in the cancer cell lines but not in the normal breast epithelial cells.
Conclusion: These data provide evidence for an effect of statins on IGF-1R levels, but suggest that additional mechanisms are involved in statin induced growth inhibition.

425 POSTER

Biological role of the CREB/COX-2 pathway in the regulation of human pancreatic cancer proliferation and survival.

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Cyclooxygenase-2 (COX-2) is a pro-inflammatory enzyme expressed in the majority of human primary pancreatic carcinomas. Its expression is regulated by the cyclic AMP response element binding protein (CREB) and a large body of evidence has implicated the CREB/COX-2 pathway in the suppression of apoptosis, induction of proliferation, angiogenesis and tumor metastasis. The L3.6pl human pancreatic cancer cell line, which displays constitutive CREB phosphorylation and COX-2 overexpression, was exposed for 24 hours to increasing concentrations of H-89 (a selective PKA inhibitor), AH-6809 (an EP_2 receptor inhibitor), and a small interfering RNA against CREB (siRNA CREB). Both H-89 and AH-6809 inhibited DNA synthesis in a dose-dependent manner as measured by ^{3}[H]-thymidine incorporation. DNA synthesis was completely abrogated with 25 μM H-89, whereas a 50% reduction was observed with 50 μM AH-6809. A dose-dependent increase in apoptosis-associated DNA fragmentation as measured by propidium iodide staining and FACS analysis was observed only in cells treated with H-89 (50% apoptosis at 30 μM), whereas no apoptosis was observed with AH-6908 at all concentrations tested. Transient transfection of L3.6pl cells with a CREB-specific siRNA construct (siRNA CREB) under conditions that induced $\geqslant$ 80% reduction in CREB protein expression minimally affected apoptosis induction, as compared with a non-specific siRNA construct (siRNA NS). However, the levels of DNA fragmentation were consistently higher in the siRNA CREB-transfected L3.6pl cells exposed to stimuli that activate CREB via a Ca2+-dependent mechanism, such as the endoplasmic reticular Ca2+ ATPase inhibitor thapsigargin (5 μM, 38% vs 19% apoptosis) or the Ca2+ ionophore A23187 (0.5 μM, 27% vs 17% apoptosis). In contrast, no differences in apoptosis induction were observed in the siRNA CREB- and siRNA NS-transfectants exposed to 250 nM staurosporine, 5 μg/ml brefeldin A, or 5 μg/ml tunicamycin, which do not activate CREB. Our results clearly demonstrate that constitutively active CREB suppresses apoptosis, possibly via COX-2 upregulation, in human L3.6pl pancreatic cancer cells, suggesting that it might be an important new target in the treatment of such a deadly, and currently incurable, disease.

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A high throughput screen and secondary assays for the identification and evaluation of histone methyltransferase inhibitors

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Chromatin is a complex between DNA and histone proteins allowing the compaction of DNA into the nucleus. Chromatin modifications such as acetylation, phosphorylation and methylation on histone N-terminal tails constitute part of the histone code and can determine whether chromatin conformation is heterochromatic (transcriptionally repressive) or euchromatic (transcriptionally active). Chromatin modifying enzymes are emerging as interesting therapeutic targets in cancer. It is hypothesised that in the same way that inhibition of chaperone proteins such as HSP90 causes multiple signalling blockades, inhibition of chromatin modifying enzymes can simultaneously affect many pathways by changing chromatin structure and thus gene transcription. Histone lysine and arginine methylation can signify both heterochromatic and euchromatic domains depending on the residue modified and the combinations of modifications in the local chromatin environment. Histone lysine methyltransferases (HKMTs) are mostly SET domain containing proteins which are divided into four families SET1, SET2, EZH and RIZ and several HKMTs have been shown to be deregulated in cancer. Due to the increasing evidence implicating these enzymes in tumourgenicity, we have run a high throughput screen against SET7/9. This enzyme is a SET1 HKMT, homologous to other cancer-related enzymes, that mono-methylates histone H3 K4 *in vitro* and also p53 *in vivo*. A FlashPlate® assay measuring incorporation of tritiated acetyl coenzyme A into histones was used. In total 64,000 compounds were screened ($Z' = 0.6$, CV% = 9.1). S-adenosylornithine was used as a positive control (7.3 ± 1.4 μM). In addition, a virtual high throughput screen of >100,000 compounds has been carried out against the substrate and co-factor binding site using the published crystal structure. For downstream compound evaluation, two secondary cell-based assays have been developed to confirm HKMT inhibition by measuring changes in mono-methylation at specific sites (i.e. H3 K4). A time-resolved fluorescence immunoassay (TRF-Cellisa) has been validated for this purpose and also provides a format suitable for phenotypic screening of compound libraries. An electro-chemiluminescent assay using the Meso Scale Discovery platform has also been developed. This will be a useful assay to identify changes in mono-methylation as a mechanistic and pharmacodynamic marker in the later stages of drug discovery.
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Preclinical development of xiapuradamib therapy for lung cancer

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Evasion of apoptosis allows malignant cells to proliferate, and resist response to chemotherapy, radiotherapy or immune surveillance. The ultimate effectors of apoptosis are a family of intracellular cysteine proteases termed caspases, which are activated by various cell death stimuli. X-linked inhibitor of apoptosis protein (XIAP) is one of eight IAPs that selectively inhibit caspase-3, -7, and -9, preventing apoptosis. The observations that overexpression of XIAP confers resistance to chemotherapy, and that suppression of XIAP with siRNA or antisense oligonucleotide restores chemosensitivity in pre-clinical models has heightened interest in XIAP as a potential therapeutic target. Notably, temporary inhibition of XIAP does not appear toxic to normal cells. We are evaluating a small molecule inhibitor of XIAP, xiapuradamib, on lung cancer cell lines *in vitro*. Using a short-term cell viability assay (SRB) we have determined ED50 values for xiapuradamib, in comparison with clinically relevant chemotherapeutic agents. Results from our studies of the combinational effects of xiapuradamib with standard cytotoxics have demonstrated synergism that is most marked with vinorelbine over 24, 48 and 72 hour time courses, and with cisplatin over 48 and 72 hours, in non-small-cell lung cancer cells using the Chou-Talalay and Pritchard & Shipman methods. Synergism is less marked with taxanes. Exposure of cells to xiapuradamib prior to cisplatin or vinorelbine treatment results in greater synergy compared with exposure to the cytotoxic followed by xiapuradamib. Experiments to determine the timing of apoptosis in relation to xiapuradamib treatment as a single agent and in combination will be reported.