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Targeting the protein translation factor eIF4E for cancer therapy

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Eukaryotic Translation Initiation Factor 4E (eIF4E) plays a pivotal role in cellular mRNA translation, binding the cap structure at the 5′ end of cellular mRNAs and delivering these mRNAs to the eIF4F translation initiation complex. A substantial body of evidence has accumulated in the past 18 years implicating enhanced eIF4E activity in cellular transformation, tumorigenesis and metastatic progression. In human cancers, eIF4E expression is commonly elevated with disease progression in many tumor types including lymphomas as well as cancers of the head and neck, breast, colon, bladder and lung. We now show that eIF4E activation is universally and significantly increased in human and experimental prostate cancers. In human cancers, elevated eIF4E activation is significantly associated with reduced patient survival.

In experimental models, eIF4E overexpression can drive cellular transformation, tumorigenesis, invasiveness and metastases by selectively and disproportionately enhancing the translation of select mRNAs that code for the critical proteins that promote and sustain the phenotypes necessary for malignancy-uncontrolled growth (c-myc, cyclin D1), angiogenesis (VEGF), survival (BCL-2, survivin), and invasion (MMP-9). In addition, eIF4E overexpression facilitates autocrine growth and survival via activation of both the ras and AKT signaling pathways. Reduction of eIF4E expression in highly metastatic, ras-transformed experimental cancer models effectively blocks tumor growth and invasiveness as well as spontaneous and experimental metastasis, suppressing the expression of MMP-9, CD44v6 and ODC and restoring expression of the metastasis suppressor nm-23. These data clearly implicate eIF4E as an attractive anti-cancer therapeutic target.

Exploiting advances in antisense oligonucleotide (ASO) chemistry, we have developed eIF4E-specific ASOs with the tissue stability and nuclease resistance necessary for systemic, anti-cancer therapy. These ASOs specifically target the eIF4E mRNA for RNAse-H mediated destruction, repressing expression of eIF4E and the eIF4E-regulated proteins VEGF, cyclin D1, survivin, c-myc, and Bcl-2. In multiple human cancer cell lines, the 4EASO robustly induces apoptosis independent of cell cycle phase and, in endothelial cells, directly blocks the formation of vessel-like structures. Most importantly, intravenous administration selectively and significantly reduces eIF4E expression in human tumor xenografts, significantly suppressing tumor growth. As in cultured cells, systemic 4EASO administration significantly induced apoptosis in xenograft tissue (8X vs. control) and significantly reduced the number of Ki-67+ cells within the xenograft tumors as well. Because these ASOs also target murine eIF4E, we assessed the impact of eIF4E reduction in normal tissues. Despite reducing eIF4E levels by 80% in mouse liver, eIF4E-ASO administration did not affect body weight, organ weight or liver transaminase levels. Collectively, these data therefore provide the first direct, *in vivo* evidence that tumor tissues would be more sensitive to the effects of eIF4E inhibition than normal tissues, a differential effect consistent with the conceptual understanding that eIF4E activity is elevated in, and required by, tumor tissue to sustain the expression of key growth and survival factors that contribute to malignancy. These data have now prompted eIF4E-ASO clinical trials for the treatment of human cancers.

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Exploring translation initiation as a therapeutic target

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Recruitment of the 40S ribosomal subunit and associated factors (43S pre-initiation complex) to eukaryotic mRNAs during translation initiation is catalyzed by several initiation factors: the eukaryotic initiation factor (eIF) 4F complex, eIF4A, eIF4B, eIF4H, and the poly (A) binding protein (PABP). The eIF4F complex binds to the 5' cap structure and delivers eIF4A, an RNA helicase thought to be required to unwind secondary structure and facilitate access of the 43S pre-initiation complex to mRNA templates. eIF4B and eIF4H stimulate translation by increasing the processivity of eIF4A, whereas PABP interacts with the poly(A) tail and the eIF4G subunit of eIF4F to circularize the mRNA during translation. The ribosome recruitment step of translation initiation is rate-limiting and under regulation of the PI3K/Akt/mTOR signaling axis in normal cells – a target for interdiction by several viruses during replication, and deregulated in many cancers. Small molecule modulators of the ribosome recruitment phase of initiation would help to better delineate the role played by individual factors in this process. Until recently, the only compounds capable of blocking eIF4F activity were cap analogues, such as m7GDP. These act by preventing binding of the eIF4E subunit to mRNA cap structures and only work at high concentrations in vitro. A small molecule, called 4EGI-1, has been reported to be capable of blocking the interaction between eIF4E and eIF4G. To expand the repertoire of compounds that target translation initiation, our lab has established several high-throughput assays that score for ribosome recruitment, eIF4E:eIF4G interaction, eIF4H-RNA and PABP-RNA interaction. From screens utilizing these assays, we identified and characterized three natural products that modulate eIF4A activity and affect translation initiation. We will discuss the characterization of the biological properties of these compounds, as well as results from more recent high throughput screening campaigns.

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Small molecule inhibitors of the human MDM2-p53 interaction as anticancer agents

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Background: p53 is a powerful tumor suppressor and is an attractive cancer therapeutic target since it can be functionally activated to eradicate tumors. The p53 gene is mutated or deleted in half of human cancers, which inactivates its tumor suppressor activity. In the remaining cancers with wild-type p53 status, its function is effectively inhibited through direct interaction with the human MDM2 oncoprotein. Design of small-molecule inhibitors to blocking the MDM2-p53 interaction to reactivate the p53 function is a promising cancer therapeutic strategy.

Materials and Methods: Using a structure-based strategy, we have designed and developed a class of potent, specific and orally bioavailable small-molecule inhibitors of the MDM2-p53 interaction. We have investigated their mechanism of action and therapeutic potential in vitro using human cancer cell lines, *ex vivo* using patient samples and *in vivo* in xenograft models of human cancer.

Results: Our studies showed that our designed MDM2 inhibitor MI-219 binds to MDM2 protein with an affinity 1000-times higher than the natural p53 peptide and is highly selective for blocking the MDM2-p53 interaction over other protein–protein interactions, including the MDMX-p53 interaction. MI-219 disrupts the cellular MDM2-p53 interaction, activates the p53 pathway in cells with wild-type p53, and leads to induction of cell cycle arrest in all cells and selective apoptosis in tumor cells. MI-219 stimulates rapid but transient p53 activation in established tumor xenograft tissues, resulting in inhibition of cell proliferation and induction of apoptosis, and complete tumor growth inhibition. MI-219 activates p53 in normal tissues with minimal p53 accumulation, is not toxic to animals. Using chronic lymphocytic leukemia samples from patients, we further showed that activation of p53 by MDM2 inhibitors is highly effective in induction of cell death and p53 status is the major determinant factor for the activity of MDM2 inhibitors.

Conclusions: Our present study provides compelling evidence that pharmacological activation of p53 by blocking the MDM2-p53 interaction is a promising cancer therapeutic strategy and MI-219 warrants clinical evaluation as a new cancer therapy.

Thursday, 23 October 2008

Poster Sessions

Apoptosis, necrosis, autophagy

255 POSTER

Cytostatic effect induced by 2-chloroadenosine sensitises PC3 cells to docetaxel

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Background: 2-Chloroadenosine (2-CADO) is an adenosine analog capable of inducing apoptosis in several cell lines by acting either via adenosine receptors or via uptake that is followed by metabolic transformations leading to nucleotide analogs. DNA-directed nucleotide analogs are antimetabolites effective in the treatment of a variety of malignancies. Docetaxel-based chemotherapy is the only treatment that demonstrated an overall survival benefit in men with hormone refractory prostate cancer.

Material and Methods: Androgen-independent and -sensitive (PC3 and LNCaP) prostate cancer cells and non-neoplastic HECV cells were used in the study. Proliferation, cell cycle progression and apoptosis were analyzed by MTT assay and cytofluorimetric analysis. DNA damage was assessed by COMET assay. Invasive potential was investigated by soft agar assay and metastatic ability by adhesion assay. Interleukin 23 (IL-23) and Protease activated receptor-1 (PAR-1) expression were determined by real time PCR.
Results: 2-CADO inhibits the growth of PC3 cells, through a mechanism involving cellular uptake, by inducing apoptosis and accumulation of cells in the S-phase of the cell cycle. 2-CADO pre-treatment followed by docetaxel at subclinical dosage reduced the viability of either PC3 or LNCaP while it did not enhance docetaxel-induced cytotoxicity in adherent non-neoplastic HECV. The drugs reduced the invasive potential of PC3 cells by inducing apoptosis and blocking cell cycle progression. Down-regulation of PAR-1 gene expression resulted in a slightly lower metastatic potential, whereas up-regulation of IL-23 induced the activation of the immune system.
Conclusions: Pretreatment of PC3 cells with 2-CADO decreased the effective concentration of docetaxel, lowered the metastatic potential, and induced the production of cytokines known to stimulate the immune response against cancer. The treatment was effective for prostate cancer cells independently on their androgen sensitiveness.

256 POSTER

SiRNA-mediated Apollon gene silencing induces apoptosis in breast cancer cells via p53 stabilization and caspase-3 activation

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Aims: Apollon is a member of the Inhibitor of Apoptosis Proteins family that contains baculoviral-IAP-repeat and ubiquitin-conjugating enzyme domains. It inhibits apoptosis by promoting ubiquitination and proteasomal degradation of Smac/DIABLO and by interfering with caspase activity. In the present study, we proposed to analyze cellular and molecular effects consequent to Apollon gene knockdown in breast cancer cells using small interfering RNA (siRNA).
Materials and Methods: Three human breast cancer cell lines differing in p53 gene status were used in the study: ZR75.1 and MCF-7 cells (wild-type p53), and MDA-MB-231 (mutant p53). The knockdown phenotype arising after transfection of cells with 10 nM of Apollon siRNA was evaluated at different time points. The effects of the treatment on Apollon expression were determined by RT-PCR and Western blotting. Cell death was evaluated by fluorescence microscopy analysis of cells stained with propidium iodide and by monitoring the catalytic activity of caspase-9 and caspase-3. The expression pattern of mitochondrial apoptosis-related proteins was analyzed by Western blotting, and the release of cytochrome c from mitochondria was assessed by ELISA.
Results: In ZR75.1 cells, silencing of the Apollon gene resulted in a significant and time-dependent decline in cell proliferation and an increase in the rate of spontaneous apoptosis, which was associated with p53 stabilization and activation of the mitochondrial-dependent apoptotic pathway (up-regulation of Bax and Bad, marked release of cytochrome c, and activation of caspase-9 and caspase-3). Conversely, only a modest reduction in cell survival was observed in MDA-MB-231. Pre-incubation of ZR75.1 cells with p53-specific siRNA resulted in a partial rescue of the cell growth inhibition induced by Apollon knockdown. Furthermore, the activation of caspase-3 seemed to be essential for the induction of apoptosis mediated by Apollon silencing, since Apollon siRNA had no effect on the viability of caspase-3-deficient MCF-7 cells or ZR75.1 cells transfected with caspase-3-specific siRNA.
Conclusions: Our results indicate that p53 stabilization and caspase-3 activation concur to determine the apoptotic response induced by Apollon siRNA and suggest that targeting Apollon may have therapeutic potential for the treatment of breast cancer.

257 POSTER

Inhibition of autophagy significantly enhances the anticancer activity of SAHA by promoting ubiquitin-conjugated protein accumulation and oxidative stress in colon cancer cells

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Background: Autophagy is a degradation process responsible for the turnover of organelles and long-lived proteins by targeting them to lysosomes. The scope of the role that autophagy plays in cancer remains highly controversial as its induction has been proposed to both mediate cell death and promote cell survival in the face of metabolic stress. The histone deacetylase (HDAC) inhibitor, SAHA has been reported to induce autophagy, which may contribute to its anticancer activity. However, we have demonstrated that inhibitors of autophagy stimulate ubiquitin-conjugated protein accumulation, increase oxidative stress, and induce synergistic levels of apoptosis when administered in combination with SAHA.
Materials and Methods: The effects of autophagic inhibition on the anticancer activity of SAHA were investigated in the colon cancer cell lines, HCT8 and HT29. Autophagy was inhibited using Atg7 siRNA and the pharmacological inhibitors, chloroquine (CQ) and 3-methyladenine (3-MA). Apoptosis was measured by propidium iodide and active caspase-3 staining followed by flow cytometry. Oxidative stress was evaluated by using the dyes hydroethidine and DCF-DA and subsequent flow cytometric analysis. Ubiquitin-conjugated protein accumulation was measured by immunocytochemistry. The combination of CQ and SAHA was further investigated in vivo using HCT8 and HT29 xenograft models of colon cancer.
Results: Inhibition of autophagy resulted in the accumulation of ubiquitin-conjugated proteins and potently enhanced SAHA-induced apoptosis in the HCT8 and HT29 colon cancer cells. While SAHA disrupted bortezomib-induced aggresome formation, it enhanced ubiquitin-conjugated protein accumulation stimulated by CQ. The CQ+SAHA combination also produced a strong increase in the levels of the superoxide anion, which was determined to be critical for apoptosis induction. Furthermore, the CQ+SAHA combination significantly reduced tumor burden in both HCT8 and HT29 xenograft models.
Conclusions: Inhibition of autophagy by CQ in combination with SAHA stimulates ubiquitin-conjugated protein accumulation, oxidative stress, and apoptosis in the HCT8 and HT29 colon cancer models. This combination warrants further investigation for the treatment of colon cancer and other malignancies. A Phase 1 clinical trial evaluating the efficacy of the CQ/SAHA combination for the treatment of advanced solid malignancies is planned.

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Apoptosis and oxidative stress induced by 2-chloroadenosine in PC3 cell line

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Background: 2-Chloroadenosine (2-CADO) is an adenosine analog capable of inducing apoptosis in several cell lines by acting either via adenosine receptors or via uptake through nucleoside transporters that is followed by metabolic transformations leading to nucleotide analogs.
Material and Methods: Androgen-independent and -sensitive (PC3 and LNCaP) prostate cancer cells cells were used in the study. RT-PCR analysis was performed to determine the expression of nucleoside transporters and adenosine receptor subtypes. Proliferation, cell cycle progression and apoptosis were analyzed by MTT assay and cytofluorimetric analysis. DNA damage was assessed by COMET assay. Deoxy-and ribonucleoside triphosphate pools were determined by HPLC. The molecular mechanism was examined by assessing the involvement of DNA synthesizing enzymes in the cellular response. Reactive oxygen species (ROS) production and glutathione (GSH) concentration were analysed by spectrofluorimetric and spectrophotometric assay, respectively. Nuclear factor erythroid 2-related factor (Nrf2) nuclear translocation was assessed by Western blotting.
Results: 2-CADO treatment dramatically reduced the number of prostate cancer cells and permanently blocked cell-cycle progression in the S-phase. The block of prostate cancer cell proliferation is mediated by 2-CADO uptake through equilibrative nucleoside transporter, followed by sequential phosphorylations to 2-Cl-ATP that irreversibly inhibits several key-enzymes for DNA biosynthesis. 2-CADO treatment also induced ROS accumulation, GSH depletion and Nrf2 nuclear translocation.
Conclusions: 2-CADO caused oxidative stress and arrested DNA synthesis by irreversibly inhibiting purine/pyrimidine ribo-and 2-deoxyribonucleotides salvage enzymes.

259 POSTER

Histone deacetylase inhibitors restore caspase-8 expression and overcome TRAIL resistance in cancers with epigenetic silencing of caspase-8

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Resistance to apoptosis is a hallmark of human cancers and can be caused by epigenetic silencing of caspase-8, a key component of the death receptor pathway. Loss of caspase-8 correlates with poor prognosis in medulloblastoma, the most common primary malignant brain tumor in childhood. In search for novel strategies to restore defective