anti-apoptotic mediator in cancer cells. Furthermore, ADI causes arginine depletion enzymatically, and thus ADI is an inhibitor of NO synthesis by arginine substrate deprivation. However, the mechanisms of NO in ADI treated cell lines have not been previously elucidated. Here, we analyzed the mechanisms of NO in an ADI treated cell line.
We selected the Ramos human lymphoma cell line, a known ADI sensitive cell line. Having determined the optimum ADI concentration for experimentation, the cells were divided into several groups based on SNP (an NO donor) treatment levels (i.e., a ADI and SNP untreated control, ADI without SNP, ADI with 10 μM/ml SNP, ADI with 50 μM/ml SNP, and ADI with 100 μM/ml SNP). The MTT assay was used to determine cell survival fractions, nitric oxide assays to determine NO levels, and Western blot analysis to determine the expressions of NO mediators, such as NFκB and *Bcl-X_L* antibody.
The optimal ADI experimental concentration was 0.001 U/mL. Surprisingly, SNP treatment reversed ADI induced cell growth inhibition. Furthermore, we found that NFκB and *Bcl-X_L* expressions were induced by SNP.
We believe that ADI-induced Ramos cell growth inhibition is reversed by the NO donor SNP, and that this is mediated by NFκB and *Bcl-X_L*.

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Phase I of intermittent chronomodulated oral therapy with capecitabine in patients with advanced and/or metastatic cancer

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Background: Capecitabine is an orally administered pro-drug of 5-fluorouracil (5-FU) which goes through the intestinal mucosal membrane as an intact molecule. It is subsequently activated by a cascade of three enzymes resulting in the preferential release of 5-FU at the tumor site. In phase I study capecitabine was administered twice daily as outpatient therapy, each cycle administered for 2 weeks followed by 1 week of rest. The recommended phase II dose was 2510 mg/m^2 daily. The rational of capecitabine administration especially in nocturnal hours, as performed in the present report, is just based on the attempt to mime 5-FU chronomodulated infusion.
Material and Methods: The aim of this study was to determine the maximum tolerated dose (MTD) of capecitabine when administered in a chronomodulated way according to the following schedule: 1/4 dose at 8:00 a.m.; 1/4 dose at 6:00 p.m. and 1/2 dose at 11:00 p.m. each day for 14 consecutive days followed by 1 week of rest. A total of 24 pts (17 female, 7 male), aged 49–88 yr (median 75) with a variety of solid tumors (11 breast, 7 colorectal, 2 pancreas, 1 gastric, 1 renal, 1 hemangiopericytoma and 1 unknown primary) have been treated. The starting dose level in our phase I trial was 1500 mg/mq daily. The subsequent dose levels were: 1750, 2000, 2250, 2500, 2750 mg/m^2 daily. The capecitabine dose was escalated when almost 3 patients in a cohort had completed two cycles of treatment. Dose-limiting toxicities (DLT) were determined on the basis of toxicity from the first two cycles. The MTD was defined as the dose level at which no more than one of six patients experienced a DLT. The MTD represents the dose recommended for further studies. All patients except five had been pretreated for cancer.
Results: No DLT occurred at doses of 1500, 1750, 2000, 2250 and 2500 mg/m^2 in any of the almost 3 patients included at each level. At 2750 mg/m^2, 1 of 6 patients experienced DLT (fatigue grade 4 and diarrhoea grade 3). Another 6 patients are being evaluated at the capecitabine 2750 mg/m^2 level, with an ongoing evaluation of cumulative (all cycles) toxicity and efficacy. The other toxicities have been generally mild or moderate in nature with only one case of severe hand–foot syndrome being observed at the fourth cycle in one patient. All these toxicities resolved upon treatment interruption with patients restarting on the chronomodulated schedule where appropriate. The recommended dose for further studies is 2750 mg/m^2 daily for 14 consecutive days followed by 1 week of rest. In terms of response, we have observed 5 PR in breast cancer and 2 PR in colorectal cancer.
Conclusions: In conclusion, chronomodulated capecitabine treatment seems to be a feasible approach which has demonstrated promising clinical activity.

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The investigation of phosphatidylinositol 3-kinase (PI3k) isoforms which express by human prostate cancer cell lines, PC-3 and DU-145

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Matrix metalloproteinases (MMPs) are the most important enzymes which not only degrade basement membranes but also involve in angiogenesis and neovascularization; making possible cellular migration. Phosphatidylinositol 3-kinase (PI3K) is involved in modulating MMPs activities. PTEN is a tumor suppressor gene whose primary function is lipid phosphatase. By dephosphorylating phosphatidyl inositol 3, 4, 5-triphosphate, PTEN antagonizes the PI3K activity. Prostate cancer is one of the most prevalent cancers all over the world. Two highly invasive and metastatic cell lines from prostate cancer, PC3 and DU145, are not the same in respect to PTEN expression status. While DU145 express PTEN mRNA and its protein, PC3 is null for PTEN gene. Nevertheless PC3 is also invasive and metastatic, it was isolated from prostate cancer metastasis to the bone marrow. It was interesting for us to know if there was any difference in PI3K isoforms expression patterns between these two cell lines. For this reason the mRNA content from the cells was analyzed using RT-PCR method.
Surprisingly our data showed that both of the cell lines express identical isoforms. Here, we introduce P110α catalytic subunit and P85 adapter protein from classIA, PI3K-C2 from classII and Vps34p from classIII of PI3K super family as PI3K isoforms which expresse by PC3 and DU145 cells. Now to address any inequality in PI3K isoforms expression, using Real-Time RT-PCR we are going to quantify each isoform mRNA individually.

219 PUBLICATION

Expression and activity of signal transducer and activator of transcription (STAT) pathways in gastric adenocarcinoma

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Objective: Gastric cancer is initiated and progressed through a number of signaling pathways. In the present study, we investigated the expression and activity of signal transducers and activators of transcription (Stat) in gastric cancer cell line as well as in tissues and their relationship with clinicopathological parameters.
Methods: We have obtained 62 tissue specimens from 16 patients of surgically resected gastric adenocarcinoma and AGS gastric cancer cell line. Western blotting of gastric cancer tissues, adjacent normal tissues and AGS cell line were used to detect the expression of Stat1, Stat3 and Stat5. The expression intensity of phosphorylated STAT protein in gastric cancer tissues and adjacent normal tissues were measured by immunohistochemical stains.
Results: Of 16 patients with gastric adenocarcinoma, 14 were male and 2 were female, and median age was 66 years (range, 37 to 80). Eleven patients were at stage III or IV without distant metastasis, while 5 were at stage I or II. Activations of Stat1, Stat3, and Stat5 were observed in AGS cells, gastric cancer tissues and adjacent normal tissues. No significant difference in Stat activity was found between gastric cancer tissues and adjacent normal tissues. Furthermore, STAT activity did not correlate with stage, tumor penetration and nodal spread.
Conclusions: Expressed in gastric cancer tissues and adjacent normal tissues, Stats may play a critical role for development and adjacent penetration in gastric adenocarcinoma.

220 PUBLICATION

Bisphosphonates down-regulate the GAPDH gene expression in prostate and breast cancer cell culture: is the GAPDH a housekeeping or a new target gene?

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The employment of the RT-PCR method has been widely used for the analysis of gene expression in many systems, including tumor samples. The GAPDH (Glyceraldehyde-3-phosphate dehydrogenase) has been commonly considered as a constitutive housekeeping gene to normalize the specif gene expression. However the GAPDH has been shown to be upregulated in cancer. Bisphosphonates (BPs), synthetic analogs of pyrophosphate, are potent inhibitors of bone resorption and recently an antitumor effect has been shown *in vitro* and in animal models by inhibition of the mevalonate pathway. Furthermore BPs has been shown to modulate many gene expression not only in osteoclasts but also in cancer cells.
The aim of this study was to evaluate GAPDH gene expression by real time RT PCR (Applied Biosystems) in different breast (MCF-7 and T-47D) and prostate cancer cell lines (PC-3 and DU-145) lines (purchased from ATTC Rockville, MD, USA). treated with amino and non-amino bisphosphonates (clodronate, pamidronate, alendronate and zoleronate) to exclude, if any,