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Somatostatin modulates Ku 70/86 DNA binding activity

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Somatostatin is a peptide hormone (SST) that exerts antisecretory and antiproliferative activities on some human cancer including breast cancer, pancreatic cancer, small cell lung carcinoma and neuroblastoma. The Ku 70/86 heterodimer acts as regulatory subunit of the DNA dependent protein kinase and its activity is crucial to mantain the genetic integrity of the genome. In particular the Ku 70/86 heterodimer is the regulatory subunit of the DNA-dependent protein kinase (DNA-PK) and its DNA-binding activity mediates DNA double-strand breaks repair. The activation of the heterodimer regulates cell cycle progression and the activation of nuclear transcription factor involved in cell proliferation. Moreover Ku 86 behaves as a somatostatin receptor for the growth inhibitory tetradecapeptide, somatostatin. In order to elucidate the involvement of SST in the DNA cell cycle progression and DNA repair machinery, we investigated the Ku 70/86 DNA binding activity in a colon carcinoma cell line (Caco) treated with the somatostatin peptide. Therefore we studied, at different times of culture, the effect of somatostatin treatment on DNA binding activity and the influence of the growth regulator factor on p70 and p86 levels in different cell compartments. The results demonstrate that somatostatin treatment to a colon carcinoma cell line (Caco) inhibits cell growth, at same time, strongly modulates the activation of Ku70 and Ku 86 gene products and induce a 2 times increase of p86 level after 4 hours of stimulation without affecting p70 expression. Our findings are consistent with the hypothesis that somatostatin regulates cell cycle progression and DNA repair by influencing the Ku70/86 activity in the nucleus and in the cytoplasm affecting Ku 86 expression and activation.

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Evidence of variation of poly(ADP-ribose) reactions in benign and malignant prostate cell lines

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Poly(ADP-ribosyl)ation plays important roles in cellular DNA repair mechanims as well as in cellular proliferation and genomic stability. It has been theorized that individual differences in repair ability and activity of poly(ADP-ribose) polymerase (PARP) could be used as biomarkers for cancer progression susceptibility. Two carcinoma cell lines, LNCaP and MatLu, and one prostate epithelial cell line, PNT1A, were tested using the H10 antibody to poly(ADP-ribose) by Western activity blotting, FACS analysis and immunofluoresence for basal PARP and activated (10 mM H2O2) PARP activity. In Western blotting, FACS analysis and immunofluoresence, high levels of basal polymer were present in the LNCaP cell line but not in the prostate epithelial cell line or the MatLu cell line. LNCaP cells demonstrated greater than 90% positive analyzed cells in FACS analysis both before and after treatment with H2O2. MatLu cells demonstrated approximately 50% postive cells after treatment with H2O2, but were negative before treatment. PNT1A epithelial cells also demonstrated polymer presence only after treatment with H2O2. Prostate tumor progression most likely occurs through the accumulation of genetic changes. Microsatellite instability due to increased oxidative damage in tumor cells has been proposed as a means for this accumulation. Detection of this increased amount of poly(ADP-ribose) may indicate a high level of oxidative damage occurring in the tumor cells, which contributes to higher levels of genetic instability and a greater propensity towards progression.

DNA interactive agents

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Radiosensitisation of cultured cells by brostallicin - a brominated minor groove binding DNA ligand

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Brostallicin is a potent DNA-targeted cytotoxic drug currently in phase II clinical trials. The interaction of brostallicin with ionizing radiation was explored initially with two cell lines, SQ20B (derived from a radiation-resistant human squamous cell carcinoma) and A431 (human vulval carcinoma). More extensive studies focused on the latter cell line because of the intention to proceed to *in vivo* studies, and the abundant comparative data available for the A431 xenograft model. For all studies, the cytotoxicity endpoint was clonogenic survival. In the initial experiments, brostallicin and cisplatin were compared using a single radiation (^{137}Cs-gamma) dose, iso-effective ($\sim$10% survival) for both cell lines (7.2Gy for A431; 11Gy for SQ20B). The total drug exposure time was 2hrs in all cases, within which the irradiation period (10.6 or 16.1 min) started one hour after addition of the drug. The drug concentrations used were chosen to produce drug-alone survivals of 80% (C_{80}) and 20% (C_{20}) (for Brostallicin: 50 and 350 ng/ml for SQ20B, 17 and 90 ng/ml for A431; for cisplatin: 0.4 and 6.4 mM for SQ20B and 0.8 and 5 mM for A431). The results were expressed in terms of a sensitization ratio (SR): the survival fraction for the drug plus radiation treatment, divided by the product of the survivals for drug-alone and radiation-alone. SR values of <1 are indicative of super-additivity. The mean data are given as pairs of SR values, corresponding to C_{80} and C_{20} drug concentrations. For brostallicin, drug-radiation interaction was observed for both cell lines; SR 0.85, 0.50 for SQ20B; 0.62, 0.37 for A431, whereas for cisplatin, there was little evidence for interaction for A431 cells; SR 0.96, 0.97, compared to SQ20B; 0.80, 0.77. The extended studies involve more than six different drug concentrations and five radiation doses, and the total drug exposure time has been extended to 4 hours (irradiation after 3hrs). The available results indicate super-additivity of brostallicin and radiation. To more conclusively establish super-additive interaction between brostallicin and ionizing radiation, isobologram analysis will be applied to the complete set of data. Possible explanations for the interaction between brostallicin and ionizing radiation are that drug treatment increases the levels of radiation-induced DNA damage, and/or inhibits DNA repair.

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A phase 1 trial of the sulfonylhydrazine prodrug VNP40101M, a novel alkylating agent for the treatment of cancer

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VNP40101M is a new alkylating agent with expected specificity for the O6 position of guanine in DNA. VNP40101M also releases methyl isocyanate, which inhibits the DNA repair enzyme O6 alkylguanine-DNA alkyl transferase (AGT). VNP40101M displayed broad anti-tumor activity in animal models including cures of L1210 leukemia resistant to BCNU, cyclophosphamide, and melphalan, respectively, and was shown to penetrate across the blood-brain barrier (Cancer Research 61, 3033, 2001).

Objectives: To determine the safety, MTD, and pharmacokinetics of VNP40101M administered IV over 15-minutes every 4 weeks.

Design: One patient is entered per dose level until the first instance of grade 2 toxicity; subsequently, cohorts are expanded to 3-6 patients. Intrapatient dose escalation is permitted if courses are associated with less than grade 2 toxicity. Results: A total of 16 patients, 11 male, 5 females, median age 68 (range 42-82) with metastatic solid tumors (6 gastrointestinal origin, 3 prostate, 7 others) have been entered to the trial. Dose levels in mg/m^2 and number of patients treated at each dose: 3 (1), 6 (2), 12 (2), 24 (2), 40 (4), 60 (4), 80 (3), 100 (3), 125 (1), 155 (6), 195 (1). Patients treated through the 155 mg/m^2 dose level are evaluable for toxicity. One patient receiving 2 cycles of 100 mg/m^2 developed an asymptomatic grade 2 drop in DLCO whose relationship to treatment is unclear. At 155 mg/m^2, 1 patient developed grade 2 nausea and vomiting. No other drug-related hematologic or non-hematologic toxicity $>$ grade 1 has been observed with the exception of mild reduction in platelet counts (but grade 0).

Pharmacokinetics: VNP40101M exhibited kinetics that best fit a two-compartment model. Peak plasma levels and AUC through 100 mg/m^2 have been linear and consistent with data from rodent studies. The half-life of the initial and terminal phases is 4.2 $\pm$ 4.0 and 36.8 $\pm$ 11.1 minutes, respectively. The clearance is 0.69 $\pm$ 0.27 L/min/m^2, and the volume of distribution at steady state is 26.0 $\pm$ 8.0 L/m^2.

Conclusions: To date, VNP40101M has been very well tolerated and the MTD has not been reached. Based on body surface area, dose levels reached to date without toxicity are above the curative doses in alkylating agent resistant L1210 leukemia models, and are above doses given on a q4d $\times$ 6 schedule in mice that produced broad solid tumor activity. Phase 1 trials in relapsed leukemia, and of weekly $\times$ 3 administration in solid tumors, are planned.