Gemcitabine uptake in transplanted and primary pancreatic ductal adenocarcinoma: A ^{19}F MRS study

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INTRODUCTION: Pancreatic ductal adenocarcinoma (PDA) is profoundly insensitive to treatment with a wide range of chemotherapeutic agents. Indeed, only limited clinical activity is demonstrated by treatment with gemcitabine, the most commonly employed chemotherapeutic agent for patients with advanced PDA. However, transplanted tumour (ectopic) models of PDA, whether subcutaneous or orthotopic, are consistently sensitive to treatment with gemcitabine, questioning the relevance of such models in therapeutic development. In efforts to clarify whether animal models can be informative for preclinical trials of therapies for PDA, Tuveson and colleagues[1] developed a genetically engineered mouse model of PDA (KPC mice) that recapitulates the cardinal pathophysiological and molecular features of the cognate human disease. Consistent with the human experience, mice with this primary pancreatic cancer are largely resistant to treatment with gemcitabine, whereas transplanted tumours grown from primary tumour cells show sensitivity. After uptake into the cell, gemcitabine (difluoro-deoxycytidine, dFdC), undergoes three stages of phosphorylation catalysed by cytosolic enzymes, forming the active drug compound dFdCTP, which breaks DNA strands and stops proliferation of the cancer cells. To determine whether intratumoural levels of gemcitabine can explain the differences in tumour response between the two types of models, we used ^{19}F MR spectroscopy to measure the levels of gemcitabine (dFdC) and its active compound dFdCTP in primary and transplanted PDA tumors.

METHODS: KPC mice harbour heterozygous conditional mutant alleles of Kras and p53 as well as a pancreatic-specific Cre recombinase, Pdx1-Cre. Mice bearing the Kras, p53 and Cre alleles develop a full spectrum of premalignant lesions that stochastically undergo loss of the remaining wild-type *Trp53* allele and culminate in overt invasive and metastatic PDA with a mean survival of 4.5 months[1]. For ectopic tumour models 1×10^6 cells (derived from KPC PDA cells) suspended in $100\mu L$ of PBS were injected subcutaneously into the flanks of immunocompetent mice (syngeneic). Gemzar™ (Eli Lilly) powder (a ~48% preparation of gemcitabine, difluoro-deoxycytidine, dFdC) was purchased (Hannas, Delaware) and resuspended in sterile normal saline at 5mg/mL dFdC. Mice were injected i.p. with 100mg/kg gemcitabine and sacrificed after four hours. Tissues were rapidly dissected and snap frozen in liquid nitrogen. Tissue specimens were maintained at -80ºC until subjected to nucleotide extraction. Samples were homogenized in a Tissue Lyser with a 5mm steel ball for 2 x 6 minutes at 25 KHz, in the presence of 4 volumes (w/v) of 6% perchloric acid, followed by a neutralization step with potassium hydroxide.

Freeze dried tissue/tumour extracts were re-suspended in 1ml of D_2O. $600\mu L$ of the sample was taken in a 5mm NMR tube (Wilmad) for ^{19}F NMR analysis on a Bruker 600 MHZ (14.1T) Avance NMR spectrometer using a QNP probe. Acquisition parameters included a 1D pulse sequence for ^{19}F observation and inverse-gated ^{1}H decoupling, spectral sweep width of 177 ppm (100000Hz), 4096 scans and 1.65sec of repetition time. Total acquisition time for each sample was about 1 hr 55 min. Trichloro-fluoro-methane ($CFCl_3$) was used for ^{19}F NMR chemical shift calibrations. Chemical shift assignments of gemcitabine (dFdC) at -116.23 and its active anti-cancer product dFdCTP at -116.67ppm in ^{19}F NMR spectra were determined by spiking pure samples of each. A broad hump observed in the baseline of the ^{19}F NMR spectra was removed by application of Linear-prediction (LP) back projection to the time domain data by using 2000 (number of LP) coefficients and 128 back-prediction points prior to Fourier transformation and phase correction.

RESULTS: Figure 1A shows the ^{19}F NMR spectra of metabolite extracts from liver (reference), spleen and pancreatic ductal adenocarcinoma (PDA) tissue. Liver and spleen tissue extracts from this mouse model show ^{19}F signals from gemcitabine and dFdCTP, whereas PDA tumour extracts show no ^{19}F signals, indicating there was no uptake and thus no action of gemcitabine in the tumours. In contrast to these results, ectopic tumour model tissues extracts of xenograft tumours showed (Figure 1B) ^{19}F NMR signals of gemcitabine and dFdCTP, as did the livers and spleens of these animals.

DISCUSSION: The absence of the dFdC and dFdCTP in KPC mouse PDA tumour tissue suggests that these tumours are not taking up or responding to gemcitabine. In contrast, liver and spleen show uptake of these compounds in this model, suggesting that the drug would be toxic to normal tissues. Ectopic tumours grown from KPC PDA cells but implanted subcutaneously responded to the gemcitabine treatment. These observations indicate that the KPC PDA tumour micro environment plays a vital role in the drug delivery and/or drug metabolism.

REFERENCES:

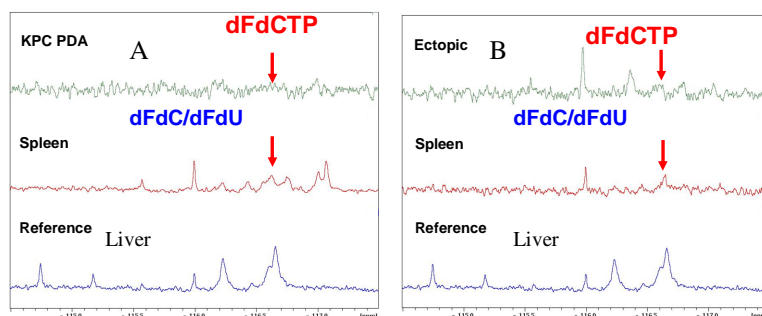1. Olive KP, Tuveson DA. *Clin Cancer Res* 2006; 12(18):5277-5287

Figure 1. ^{19}F NMR spectra from A) KPC mouse tissue B) Ectopic (transplanted) tumour mouse tissue.