MRS assessment of lactate in dedifferentiated liposarcoma models treated with chemotherapy

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Introduction: Well-differentiated/dedifferentiated liposarcomas occur mainly in the abdomen, and surgery is the mainstay of treatment. However, the local recurrence rate is high even if margins are negative and de-differentiation is predictive of poor outcome. The lack of effective chemotherapy of de-differentiated liposarcoma (DDLS) leaves non-surgical candidates with few options. Recent investigations have revealed genes abnormally expressed in DDLS which are potential therapeutic targets (1,2). For example, CEBPα, which is a transcription factor involved in adipocyte differentiation, is under expressed in DDLS. Administration of the SN-38 prodrug CPT11 (irinotecan) caused increased CEBPα expression and growth delay in DDLS cells and xenografts (3). A non-invasive marker reflecting the effect of such agents could be quite valuable in pre-clinical drug evaluations. Furthermore, in the clinic, an early marker of response/non-response could permit the physician to discontinue ineffective treatment without delay. Lactate, an end-product of glycolysis has the potential to be a biomarker of prognosis and treatment effect. The goal of the current study was to assess the change in lactate levels in a human DDLS tumor xenograft (DDLS BWH) implanted in mice in response to CPT-11. A second cohort was treated with doxorubicin (Adriamycin), an anthracycline used to treat sarcomas.

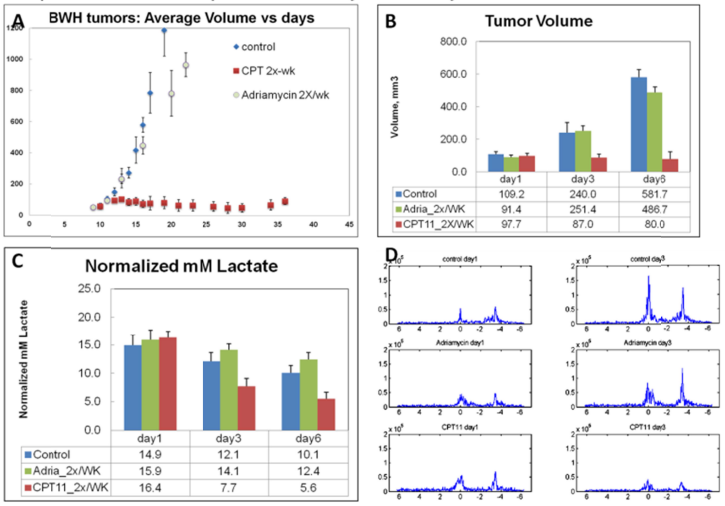

Figure 1: A. BWH tumor growth curves, B. Average tumor volumes at days 1, 3 and 6. C. Average normalized lactate at days 1, 3 and 6. D. Representative MR spectra from days 1 and 3 for control, Adriamycin and CPT-11 treated mice.

Methods. Overview: 35 BWH tumors were grown in the flanks of SCID mice and monitored until the volume was approximately 100 mm^3. For tumor growth delay studies, tumor volume was monitored in 7 control, 5 Adria-treated, and 5 CPT-11-treated mice (V= (π/6)* L * W * D). MRI/MRSI was performed serially at day 1 (baseline), day 3 and day 6 in 7 control, 6 Adria-treated, and 5 CPT-11 treated mice. The mice were treated twice per week (Adria 0.9 mg/kg i.p., CPT11 100mg/kg i.p.) with the first treatment given immediately after the baseline MRS scan. **MR Experiments:** Mice were anesthetized with Isoflurane during the MR experiments on a Bruker 4.7-T Biospec Spectrometer. Two home built 2-turn solenoid coils with diameters of 10 mm and 14 mm were used. T2-weighted sagittal MR images were collected (slice thickness = 1 mm, number of slices=10, FOV =24 mm, TR = 3000ms, TE = 40 ms, matrix= 512 X 256, number of acquisitions = 4). Lactate detection was performed using the Selective Multiple Quantum Coherence (Sel-MQC) editing sequence (4,5). Lactate spectra were obtained from a 5-mm thick center slice with TR = 2 sec, number of excitations =512, 1024 data points, and spectral width of 2510 Hz. Peak fitting was performed in Matlab (Natick, MA). Quantitation of lactate was performed using the phantom substitution technique. **Histology:** Selected animals were injected with Pimonidazole hydrochloride (PIMO) (hypoxyprobe-1, HPI) and Hoechst 33342 (Sigma-Aldrich) at 60 and 40 mg/kg, respectively. These agents were dissolved in PBS and administered via the tail vein at 1 hour before sacrifice (PIMO) and 5 minutes pre-sacrifice (Hoechst 33342). Tumors were excised, embedded in cutting medium (OCT 4583, Sakura Finetek), snap-frozen and stored at −80°C. 10μm thick frozen sections were obtained using a cryostat microtome (Microm International GmbH). Sections were fixed in 4% paraformaldehyde then blocked in Superblock-PBS (Pierce, USA) followed by hematoxylin/eosin staining. **Data Analysis:** The lactate peak area was normalized by the slice volume and compared to the lactate content of a phantom section of known volume and concentration. **Results:** The non-treated tumors grew more rapidly than the CPT11-treated (Fig-1A). Adria had little effect on tumor growth. On day 3, the CPT11 treated group had lower lactate than the control and Adria groups (P = 0.001 for CPT11 vs control, P = 0.0006 for CPT11 vs Adria). At day 6, the CPT-11 treated tumors still had significantly lower lactate concentrations than the control and Adria groups, although lactate had decreased in these groups compared to baseline (P<0.0001 for CPT11 vs. control, P < 0.0001 for CPT11 vs Adria). Histologic and IHC analysis (Fig. 2) showed regions of reduced cell density, reduced vascularization (Hoechst) and hypoxia (PIMO) in the growing control and Adria-treated tumors at 1 week. In contrast, in the growth-halted CPT-11-treated tumors, the tissue appeared fairly homogeneous, well vascularized and free of hypoxia. The trend toward reduced lactate concentration at 1 week in the Adria and control groups is probably due to reduced cell density and/or the development of necrosis as seen on histology.

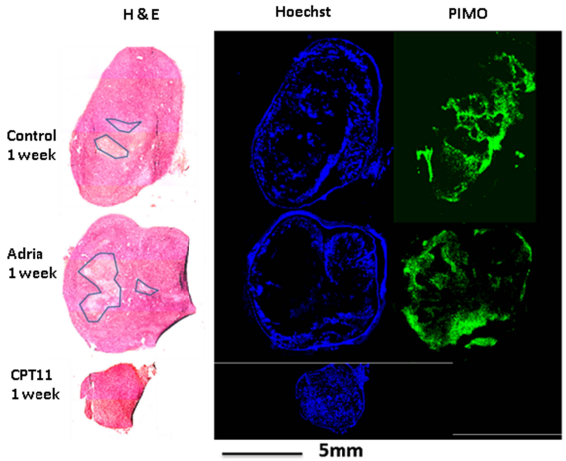

Figure 2: Histology and immunohistochemistry of DDLS-BWH xenografts. Macroscopic regions of necrosis are outlined on hematoxylin and eosin stained sections. Hoechst stain indicates functional vasculature and PIMO indicates hypoxia.

Discussion. As early as 3 days after treatment, a reduction in lactate was observed accompanied by growth arrest in CPT-11-treated BWH dedifferentiated liposarcoma tumors. The mechanism by which lactate concentration is reduced by CPT-11 is under investigation. One could speculate that CEBPα's role in promoting differentiation results in a conversion to a less glycolytic phenotype. In addition, CEBPα has been shown to induce cell growth arrest (6). CPT-11 is also known to inhibit the activity of topoisomerase I, resulting in double-strand DNA breaks and cell death. Adria is an antimitotic and cytotoxic agent which interferes with DNA repair by complexing with DNA and interfering with the topoisomerase II complex. While further investigation is needed, our data suggest that the CPT-11's induction of CEBPα expression may result in increased effectiveness compared to agents which interfere with DNA repair/replication.

References (1). 1. Brill E, Gobble R, **Angeles** C, et. al. Cancer Res. 2010 Sep 1;70(17):6891-901. Epub 2010 Aug 16. **2**. Gobble RM, Qin LX, et. al.. Cancer Res. 2011 Apr 1;71(7):2697-705. Epub 2011 Feb 18 **3**. Angeles CV, Laxa B, et. al. J Clin Oncol (Meeting Abstracts) 2010;28(15_suppl):10005. **4**. He, Q.,Shungu, D. C., et. al. J. Magn. Reson B. 106(3) pp. 203-11. **5**. Muruganandham M, Koutcher JA, et. al. Magn Reson Med 2004;52(4):902-906. 6. Timchenko, NA, Wilde, M., et. al. Genes Dev. 1996 Apr 1;10(7):804-15