DCE MRI Detected Differential Response to ZD6126 of Metastatic versus Indolent Human Melanoma

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Introduction Previous studies suggest that a metastatic human melanoma tumor may employ a different mechanism to make its blood vessels to cope with the fast growth [1] compared to its indolent counterpart. The vasculature of the metastatic tumors may be highly immature (e.g., not surrounded by pericytes), which should make them more susceptible to ZD6126, which targets the microtubular cytoskeleton of endothelial cells, is shown to induce vascular shutdown leading to tumor necrosis [2, 3]. Non-invasive DCE MRI techniques could play a critical role in detecting the differential responses of the two melanomas to ZD6126 treatment thereby providing further insight about the radical difference in vasculature between the two tumors.

Methods Metastatic (C8161) or indolent (A375P) human melanoma cells (2 million) were subcutaneously implanted into the flanks of nude mice; imaging studies were performed when tumor reached volumes between 5 x 5 x 3 mm^3 to 10 x 11 x 5 mm^3. The DCE MRI protocol consists of acquisition of a T_{10} map followed by acquisition of 180 dynamic images; after 20 precontrast images were acquired, about 0.2 mL of Gadodiamide (Nycomed, diluted to 20 mM in saline) was injected as a bolus. The arterial input function was obtained by imaging the left ventricle of the heart, which was in the same FOV as the tumor. A cardiac-gated saturation recovery sequence was used to obtain the dynamic images with a birdcage coil interfaced to a 4.7T/30 cm scanner equipped with a Varian INOVA console [4]. The DCE images were analyzed by the BOLus Enhanced Relaxation Overview method [5] (using a MATLAB program), which accounts for equilibrium transcytolemmal water exchange and yields K_{trans} and v_e (extravascular extracellular volume fraction). After completion of the baseline DCE protocol, ZD6126 (200 mg/kg) was injected i.v. and 40-60 min later, the DCE MRI protocol was repeated. Histology was performed on tumors that were flash frozen in liquid nitrogen immediately after imaging. Immunostaining for CD31 was performed on 5 μm cryosections (rat anti-mouse CD31 as 1st antibody followed by flourescein conjugated secondary antibody). Fluorescent images of tumor sections were captured and analyzed by ImageJ software; the number of CD31 positive pixels was estimated and normalized to the total number of pixels contained in the tumor for each section; 3 sections (from different levels of the tumor) were used for the estimation of the microvasculature density.

Results The K_{trans} values of C8161 (n = 5) and A375P (n = 4) are 0.45 (±0.29) min^{-1} and 0.15 (±0.11) respectively. However, they are not statistically different. Immunostaining for CD31 shows that C8161 has higher microvessel density than the A375P tumor (4.5% vs 2.2%, P<0.05). A significant reduction of K_{trans} (to 0.16 min^{-1} from the pretreatment value of 0.45 min^{-1}, P < 0.05) in C tumors was observed within an hour after injection of ZD6126, whereas K_{trans} was reduced only slightly in A tumors (P = 0.1) in response to ZD6126 treatment (Fig 1).

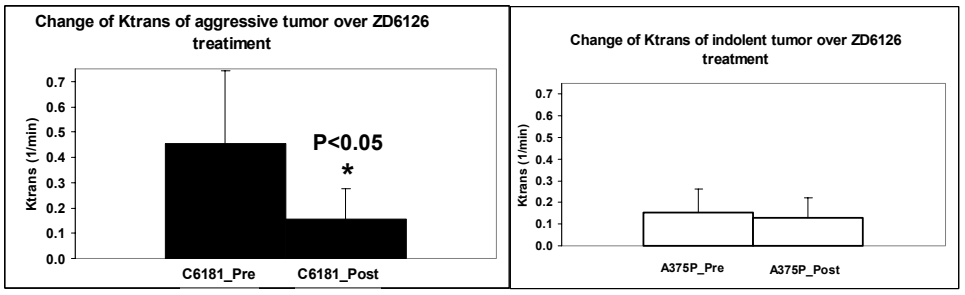

Fig.1. Acute responses of metastatic and indolent tumors to ZD6126 treatment (200 mg/kg). Significant reduction of K_{trans} was observed in C8161 but not in A375 melanomas.

Discussion Prior studies demonstrated that ZD6126 selectively induced apoptosis of CD31 positive tumor endothelial cells but spared normal tissue endothelium [6]. The differential response of the metastic versus indolent tumor to ZD6126 treatment suggests that there is a radical difference in vasculature between the two tumors. Apparently, the metastatic C tumors are more angiogenic (more CD31 positive staining) than the A tumors; the nacent blood vessels may be intrinsically sensitive to disruption by agents affecting the endothelial cell cytoskeleton such as ZD6126. A reduction in K_{trans} suggests a decrease in vascular permeability and/or flow; both factors contribute to the K_{trans} value and are not distinguished in the current DCE MRI analysis.

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References **1.** Nat Rev Cancer, 2003. **3**: p. 411-21. **2.** Cancer Res, 2002. **62**: p. 7247-53. **3.** Clin Cancer Res, 2005. **11**: p. 835-42. **4.** Magn Reson Med, 2004. **52**: p. 248-57. **5.** Magn. Reson. Med., 2003. **50**: p. 1151-69. **6.** Cancer Res, 2002. **62**: p. 3711-5.