The role of VEGF in growth of brain metastases from single cells as visualized by contrast enhanced MRI

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Introduction: Brain metastasis occurs in 20-40% of patients with cancer, including lung cancer, breast cancer, melanoma, and prostate cancer. Patients with brain metastasis have a very limited life expectancy. The factors that contribute to the development of brain metastasis are poorly understood. There is evidence for the role of angiogenesis in brain metastasis and that over-expression of VEGF contributes to metastasis growth. However, there are no longitudinal studies monitoring the effects of angiogenesis on tumor growth from a single cell. Recently we have demonstrated that single cells labeled with micron sized particles of iron oxide (MPIOs) could be detected in hepatocytes in liver (1) and in single cancer cells in the brain (2). Thus, it should be possible to monitor tumor growth from a single cell metastasis by using MRI and metastatic cells labeled with MPIOs. The purpose of this study is to determine if inhibition of VEGF activity alters angiogenesis in the brain and affects the growth of established tumors.

Methods: Ras activated DU145 cells were labeled with 1.6 micron MPIOs (Bangs Inc.) for 24 hours (2), and then those cells were injected into the left cardiac ventricle of male nude mice. Three days later the mice were anesthetized with 1.5% isofluorane and mounted in a 7 Tesla, horizontal Bruker Avance scanner using a steriotaxic holder and centered in a 72/25mm volume (transmit)/surface (receive) coil ensemble. The body core temperature was maintained at 37^{0}C using a circulating water pad. Using scout images, 3-D gradient echo images (parameters: TR/TE= 30/8 ms, 2 averages, total scan time= 37 minute, isotropic resolution = 100 µm) were acquired encompassing the whole brain. Two weeks post-inoculation, half of the mice received daily treatments of the VEGF receptor antagonist, AZD2171. The other mice received vehicle as a control. The progression of brain metastasis was monitored weekly by MRI. Tumors were assumed to be spherical, and their volumes were calculated by measuring the tumor's largest diameter.

Results: After the cells were injected, the average number of visible single cells in each mouse was 53 +/- 16 cells. The MRI allowed for the following of tumor growth from a single cell (Fig.1). Before AZD2171 treatment began, there was one brain metastasis in each group of three mice. After treatment began, the untreated group developed eight more brain metastases, and the treated group developed two more metastases. Also, the treated group developed smaller metastases (Fig.2). The average tumor volume in the untreated group was 1.78mm^3 and the average volume in the treated group was 0.30 mm^3.

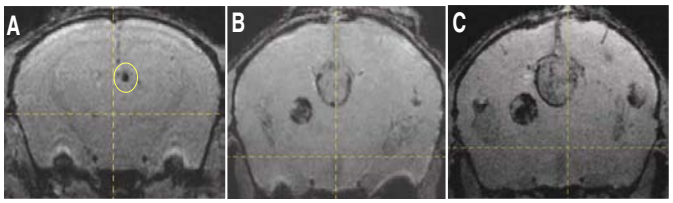

Fig.1. MRI images following the progression of brain metastasis in an untreated mouse 2 weeks (A), 4 weeks (B) and 5 weeks (c) after tumor cell inoculation.

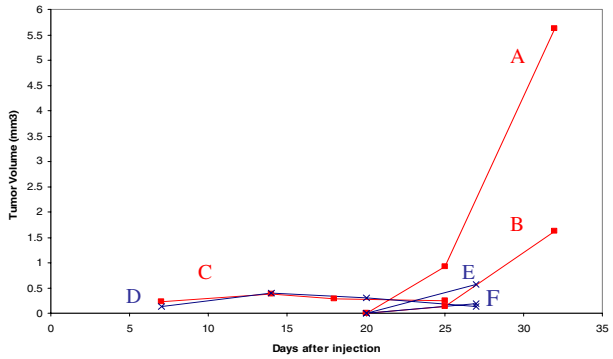

Fig.2. Graph of weekly tumor growth. Untreated mice are in red, and treated mice are in blue. Large tumors developed in the untreated group (A and B). Smaller tumors developed in the treated group (E and F). Some metastases remained dormant in both of the groups (C and D).

Discussion: Using MRI, we were able to follow the progression of single metastatic cells to tumors. We found that treatment with AZD2171 of mice with established brain metastases resulted in both fewer and smaller brain metastases than in mice who received no treatment. Future studies will investigate the effects of angiogenesis on the initiation of metastasis and tumor cell dormancy.

References:

1) Shapiro, E., Sharer, K., Skrtic, S. and Koretsky, A. Magn Resonan. Med in press.
2) Yin, J., Chock, M., Munasinghe, J., Shapiro, E., Koretsky, A., and Kelly, K. Book of Abstracts, Soc. Mol. Imag., Abst # 221, p. 50, Sept 7-10, 2005, Cologne, Germany.
3) Shapiro, E., Skrtic, S., and Koretsky, A. Magn Reson.Med. 53 (2):329-338, 2005.