Investigating the Impact of a Primary Tumor on Metastasis and Dormancy Using MRI: New Insights into the Mechanism of Concomitant Tumor Resistance

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Introduction: Dormant cancer cells, also referred to as quiescent, slowly-cycling or 'nonproliferative' cells, are believed to contribute to tumor recurrence. Clinical dormancy is reflected by relapses at distant sites after the original primary cancer diagnosis. The time to transition between dormancy and active metastatic growth may be governed by local (tumor microenvironments) or systemic (concomitant tumor resistance, CTR) mechanisms. CTR is the ability of a primary tumor to restrict the growth of secondary metastases [Galmarini *et al*, Cancer Met Rev, 2014]. CTR has been described in human and animal systems and it can be generated by both immunogenic and non-immunogenic tumors. The relevance of CTR has been highlighted by numerous observations showing that the removal of human and murine tumors can be followed by an abrupt increase in metastatic growth, suggesting that a primary tumor may exert a controlling action on metastases. Three potential mechanisms are usually cited for CTR, the primary tumor may: (i) prime the immune system to assist clearance of metastatic cells, (ii) restrict the growth of distant metastases through production of anti-angiogenic molecules, limiting the necessary growth of vessels, or (iii) systemically deplete essential host factors preventing the growth of any other tumors.

Our lab has developed technology to track single iron-labeled cells over time [Heyn *et al*, Magn Reson Med, 2006] and has shown that the retention of iron oxide nanoparticles in nonproliferative cancer cells can be exploited to track this important cell population [Economopoulos *et al*., Transl Oncol, 2013]. In this paper we use these cell tracking technologies to provide new evidence that CTR may restrict the development of metastases by inducing tumor dormancy. This work puts forth a novel potential mechanism for CTR.

Methods: Two groups of female nude mice (n=12/per group) were studied (**Figure 1**). On day 0 mice in Group 1 received an injection of vehicle (HBSS) in the lower right mammary fat pad and mice in Group 2 received an orthotopic injection of 30,000 human breast cancer cells (231) to initiate a primary tumor in the mammary fat pad. Fifteen days were allowed to pass to permit the primary MFP tumors in Group 2 mice to grow, at which time mice in both groups received an intracardiac (IC) injection of 175,000 iron-labeled human, brain metastatic breast cancer cells (231BR) cells for delivery to the brain. Mouse brain images were acquired using a 3D-balanced steady state free precession (bSSFP) sequence. Scan time was 40 minutes. Image resolution was 100x100x200μm^3. Images were acquired on the day of the 231BR cell injection (day 15) to assess the arrest of iron-labeled 231BR cells in the brain. Images were acquired again 30 days after the 231BR cell injection (day 45) to assess brain metastases and residual signal voids. The total number of brain metastases in the whole brain was counted and the individual tumor volumes were measured. The number of signal voids was counted from 20 image slices spaced evenly in the midbrain for images acquired at day 15 and day 45.

Results/Discussion: Discrete signal voids, representing iron-labeled cells, were identified in bSSFP images in all scans. Brain metastases were visible in the final scan as areas of signal hyperintensity in all mice (**Figure 2**). The mean number of brain metastases that had developed by on day 45 was significantly decreased in Group 2 mice, indicating that the presence of a primary mammary fat pad tumor suppressed the development of secondary metastases (**Figure 3**). The mean tumor volume was not significantly different for the two groups. The mean number of signal voids counted in images acquired on day 15, the day of the cell injection, was not significantly different for Group 1 versus Group 2 (black bars, **Figure 4A,B**) indicating that the arrest of cancer cells in the brain was not different. However, the number of signal voids retained in the brain to day 45 was significantly increased in Group 2 compared to Group 1 (white bars, **Figure 4A,B**), suggesting that the presence of a primary tumor enhances cancer cell dormancy.

Significance: Tumor dormancy and recurrence are important clinical problems for cancer patients and their physicians, introducing years of uncertainty and questions about best ways to treat (but not over-treat) patients. Our work has suggested a possible natural regulator of dormancy and metastatic recurrence, i.e. enhancement of tumor dormancy by the presence of a primary tumor. This novel work is only possible because of our ability to track cancer progression using MRI, from the initial arrest of cancer cells in the brain through to the development of brain metastases, with the additional capability of distinguishing between proliferating and nonproliferating cancer cells.

Figure 1.

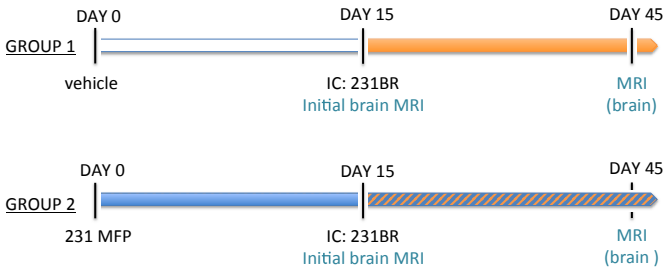


Figure 2.

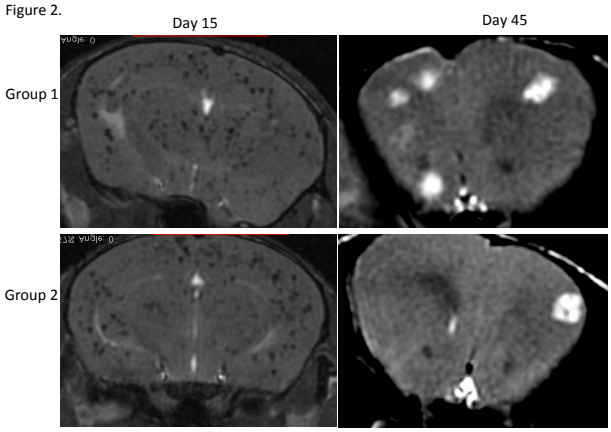


Figure 3.

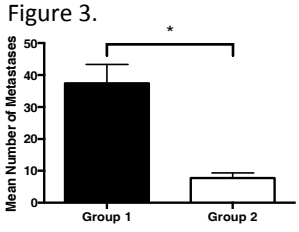


Figure 4A.

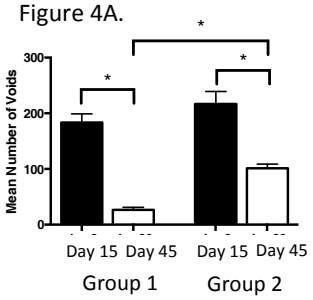
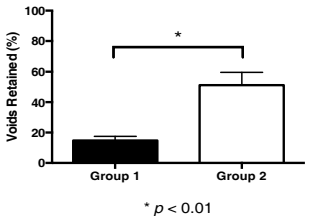


Figure 4B.



Group 1 – naïve
Group 2 – primary tumor