Assessment of Contrast Perfusion, Blood Clotting, and Ulceration in Eotaxin-Secreting Tumors in Mice by T2-Weighted and Contrast Enhanced MRI

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Purpose

An important theme that is emerging in cancer research is the interaction between tumor cells and the host stroma. There is now increasing evidence that inflammatory cells may play a critical role in promoting rather than retarding tumor progression(1-2). One inflammatory cell that is commonly seen in the stroma of many human cancer is the eosinophil(3), a rare type of granulocyte that also appears to play a critical role in tissue remodeling associated with wound healing(4). Because eosinophil granules also contain a number of powerful cytotoxic compounds, some investigators have proposed that eosinophils may suppress tumor growth by mediating a cytotoxic response to tumor cells(5). At this time, however, the precise role of eosinophils in promoting or retarding the growth of cancers remains uncertain(6-7). In order to explore the role of eosinophils in modulating tumor growth in more detail, we developed an experimental model consisting of murine B16 melanoma cells that were transfected to secrete high levels of eotaxin, a potent and selective chemoattractant and activator of eosinophils. In-vivo tumor growth rates between the wild type tumors and transfectants were compared. T2-weighted and contrast enhanced MRI studies were performed to observe any evidence of blood clot and to measure the perfusion status of these two tumor models. The results were compared to the microscopic analysis of the tumors.

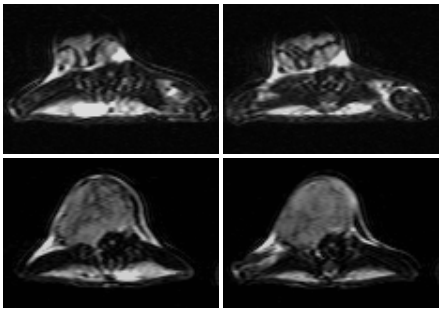
Methods

The production of eotaxin by the transfected cell line was confirmed by various techniques including RT-PCR, immunoprecipitation, ELISA, and Western blotting. Wild-type tumor cells or eotaxin-secreting tumor cells were then injected subcutaneously at a dose of 5 x 10^5 cells into the dorsal skin of groups of 10 C57Bl/6 mice. MRI studies were performed on day 20 after cell inoculation. The animals were imaged in pairs using a 4.0 Tesla scanner equipped with Philips acquisition console. T2-weighted anatomical images were acquired using a FSE pulse sequence (TR=5500 ms, TE=105.6 ms, 8 echo train, FOV= 8 cm, slice thickness =2 mm, and 256 x 256 image matrix) covering across the tumor. Then a fast 3-D T1-weighted gradient echo pulse sequence was applied for dynamic acquisition, with TR/TE = 18/3.63 ms, and flip angle=20°, acquisition matrix 256x128, and Δt = 24.1 sec. Forty acquisition were prescribed. The contrast agents Gd-DTPA-BMA (Omniscan 0.2 mmol/kg) was injected during the scan. Three animals also received another medium size blood pool agent Gadomer-17 (0.1 mmol/kg, provided by Schering AG, Berlin, Germany) one hour after the Omniscan injection. After the MRI study was finished the animal was sacrificed, and the tumors and normal organs were removed for measurement of mass and routine histologic studies.

Results

The RT-PCR, immunoprecipitation, and Western blotting studies confirmed the production of eotaxin mRNA and protein by the transfected cell line but not by the parental cell line. In cell culture, the eotaxin-secreting cells had a much slower growth rate than the wild type cells. Twenty days after the cells were implanted in animals, the average mass of the eotaxin-secreting tumors (750 ± 280 mg, n=10) was statistically identical to the average mass of the wild-type tumors (780 ± 290 mg, n=10). On the T2-weighted images it was observed that ulceration was commonly seen in eotaxin-secreting tumors (Fig.1a), and in contrast the wild type tumors might not ulcerate even if the tumor was very big (Fig.1b). Evidence of blood clot (very dark signal intensity on T2-weighted images) was clearly demonstrated in Fig.1a, and that might be associated with ulceration. The contrast enhancement kinetics measured by Gd-DTPA-BMA in these two tumors are shown in Fig.2. The wild type tumor had a much higher contrast enhancement, indicating a higher perfusion. The mean Gd-DTPA-BMA enhancement kinetics measured from the 8 wild type tumors and 7 transfectants (eotaxin-secreting tumors) are shown in Figure 3. The wild type tumors had a much smaller variation (12%) compared to transfectants (35%). The difference was significant after 12 minutes in the figure (197 ± 24 [n=8] for wild-type tumors vs. 145 ± 59 [n=7] for transfectants, p=0.04 by two-tailed, unpaired t-test). Gadomer-17 enhancement kinetics was measured from 3 tumors, and the results were consistent with those measured by Gd-DTPA-BMA. One tumor which had the lowest vascular perfusion and a high interstitial space among the three showed the lowest early enhancement followed by continuous increasing enhancements in the kinetics measured by both agents. Figure 4 shows a histological slide from one eotaxin-secreting tumor. There was extensive eosinophilia, blood clotting, and granulation tissue within the tumors produced by subcutaneous implants of eotaxin-secreting tumor cells.

(a) Transfectant



(b) Wild Type

Figure 1: The T2-weighted images from one wild type tumor and one transfectant showing severe blood clotting and ulceration (top missing).

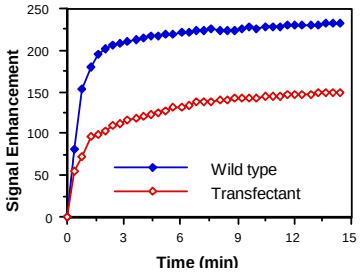


Figure 2: The Gd-DTPA-BMA enhancement kinetics from the two tumors shown in Figure 1. The blood clot in transfectant may cause lower enhancements.

Discussion

On the basis of the histologic and MRI studies, we conclude that eosinophils mediate a procoagulant effect within tumors that is also associated with increased ulceration. Despite the much slower in-vitro grow of the eotaxin-secreting cells, the in vivo growth rate was comparable to the wild type cells. The results suggest that eosinophils and eotaxin are more likely to mediate a wound-healing response to tumors than to mount an effective cytotoxic response against the tumor cells that has been previously proposed.

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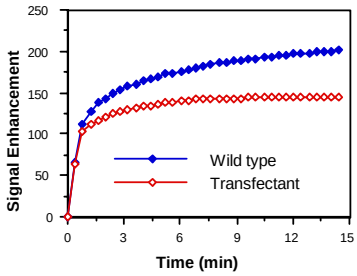

Figure 3: The mean Gd-DTPA-BMA enhancement kinetics measured from 8 wild type and 7 transfectants.

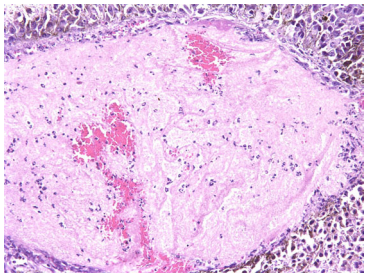

Figure 4: The histological slide showing evidence of clotting in one transfectant.

Acknowledgement

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