

The CCL5/CCR5 axis promotes metastasis in basal breast cancer

Marco Velasco-Velázquez¹ and Richard G. Pestell^{2,3,*}

¹Departamento de Farmacología; Facultad de Medicina; Universidad Nacional Autónoma de México; México DF, México;

²Kimmel Cancer Center; Thomas Jefferson University; Philadelphia, PA USA; ³Department of Cancer Biology; Thomas Jefferson University; Philadelphia, PA USA

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Recently, we have shown that the CCL5/CCR5 axis is active in patients affected by an aggressive basal subtype of breast cancer. Using preclinical models, we have demonstrated that CCR5 promotes breast cancer invasiveness and metastatic potential, while CCR5 inhibition abrogates them. Thus, CCR5 antagonists may constitute an alternative therapeutic approach for patients affected by metastatic basal breast cancer.

We have recently analyzed the expression of the chemokine CCL5 and its receptors in human breast cancer samples as well as in the normal breast epithelium.¹ We found that CCL5/CCR5 signaling is preferentially active in basal and ERBB2⁺ disease subtypes. Analyzing a combined microarray database including 2,254 samples from 27 independent studies,² we demonstrated that 58% or more patients affected by the basal and ERBB2⁺ subtype of breast cancer significantly overexpress both CCL5 and CCR5. In contrast, when a similar analysis was performed in non-neoplastic breast samples, no correlation between CCL5 and CCR5 expression levels was found, indicating that CCL5/CCR5 signaling may be preferentially activated during the development of specific breast cancer subtypes. In line with this notion, the invasiveness of human MCF-10A breast cancer cells transduced with *Neu-T*, *H-RAS* or *c-SRC* oncogenes increased in response to CCL5, suggesting that CCL5/CCR5 signaling is activated by oncogenic events to promote an aggressive phenotype.

Patients affected by basal breast carcinomas are at increased risk of metastasis and exhibit poor survival rates.^{3,4} Thus, we evaluated the importance of the CCL5/CCR5 axis in invasion and metastasis. By using human breast cancer cell lines exhibiting a basal phenotype

and molecular signature, we demonstrated that the increased expression of CCR5 we had observed in tissue microarray might be caused by the existence of a CCR5⁺ cell population. Phenotypic analyses demonstrated that a small fraction of cells within breast cancer cell lines express CCR5 and functional analyses showed that such CCR5⁺ cells respond to CCL5. Furthermore, basal breast cancer cells expressing CCR5 display an increased invasiveness and metastatic potential. Metastatic tumors generated by the intravenous injection of breast cancer MDA-MB-231 cells transduced with a lentiviral vector coding for luciferase fused to an enhanced variant of the green-fluorescent protein (Luc2-eGFP) displayed an 8-fold increase in the proportion of CCR5⁺ cells as compared with the same cell line maintained in vitro. This indicates either that the invasive population is enriched in CCR5⁺ cells or that the microenvironment at metastatic sites promotes a CCR5⁺ phenotype. In an additional series of experiments, we showed that CCR5⁺ cells isolated from basal breast cancer cell lines are approximately 40-fold more invasive in vitro than their CCR5⁻ counterparts, indicating that CCR5 expression correlates with a pro-invasive phenotype.

The importance of CCR5 in HIV infection led to the development of

drugs that specifically target this receptor. CCR5 antagonists, which were originally developed to prevent the interaction between CCR5 and the gp120 glycoprotein of HIV-1, can efficiently block CCR5 activity at nanomolar concentrations.^{5,6} We therefore examined the possibility that CCR5 inhibition may reduce the invasive and metastatic potential of breast cancer cells. The CCR5 antagonists Maraviroc and Vicriviroc (both employed at a concentration of 100 nM) impaired CCL5-stimulated cytoplasmic calcium waves and reduced the invasiveness of basal breast cancer cells. We also evaluated Maraviroc effects in vivo, using a mouse model of metastatic breast cancer. The intravenous injection of Luc2-eGFP-expressing MDA-MB-231 breast cancer cells indeed generates pulmonary metastasis that can be easily followed by bioluminescence imaging. In this setting, the administration of Maraviroc at the clinically relevant dose of 8 mg/kg (twice daily) efficiently reduced pulmonary tumor burden (Fig. 1). Histological analyses performed on animals treated with Maraviroc for five weeks confirmed a significant reduction of both the number and the size of pulmonary metastases (Fig. 1). Only 50% of Maraviroc-treated mice exhibited detectable neoplastic lesions and mean tumor size was reduced by > 65% as compared

*Correspondence to: Richard G. Pestell; Email: richard.pestell@jefferson.edu

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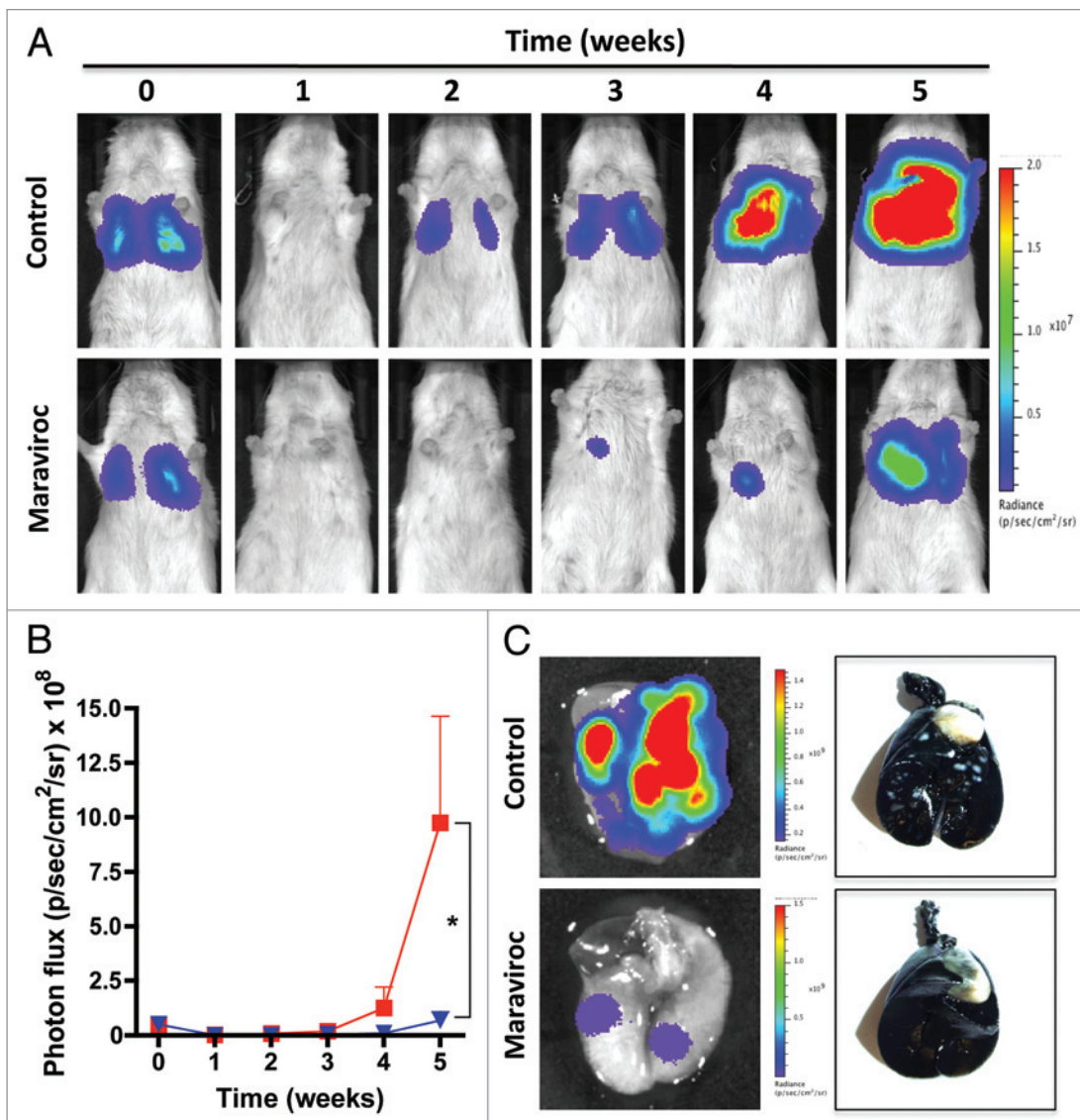


Figure 1. The CCR5 antagonist Maraviroc inhibits lung metastases in vivo. MDA-MB-231 cells expressing luciferase fused to an enhanced variant of the green-fluorescent protein (Luc2-eGFP) were injected into the tail vein of NOD/SCID mice and the bioluminescent signal was quantified weekly in vivo. Representative images of mice receiving vehicle or 8 mg/Kg Maraviroc (every 12 h) are shown in (A) Quantitative data on bioluminescence imaging (means \pm SEM, n = 6) are reported in panel (B) (control mice = red line; treated mice = blue line). Statistical comparisons (*p = 0.048) were by means of Student's t-tests coupled to Welch's correction for heterogeneous variances. (C) The presence of pulmonary tumors and the differences between experimental groups were corroborated by ex vivo imaging (left panels) and India ink staining (right panels).

with vehicle-treated mice. These results demonstrate (1) that basal breast cancer cells are sensitive to pharmacological CCR5 inhibition, and (2) that the pro-invasive and pro-metastatic activities of CCR5 can be abrogated by using specific antagonists.

CCR5 has also been shown to alter the proliferation of luminal breast cancer^{7,8} and prostate cancer cell lines.⁹ However, the impact of CCR5 on the proliferation of basal cancer cells is controversial. The results obtained with our model of

metastatic breast cancer did not allow us to understand whether CCR5 influences cell proliferation or tissue colonization. We therefore analyzed the role of CCR5 on tumor cell proliferation by three different strategies. We observed that (1) Maraviroc as well as Vicriviroc do not affect the viability and proliferative potential of MDA-MB-231 cells in vitro; (2) CCR5 overexpressing cells display the same proliferation rate as cells transfected with an empty vector; and (3) the administration of Maraviroc does not alter the

growth of pre-established pulmonary metastases in vivo. Taken together, these results indicate that CCR5 activation does not promote the proliferation of basal breast cancer cells and that the anti-metastatic effect of CCR5 antagonists does not stem from effects on tumor growth.

To further clarify the mechanisms by which CCR5 promotes metastasis, we evaluated the effects of Maraviroc on the homing of breast cancer cells to the lungs. Of note, Maraviroc reduced the number of cancer cells that could be detected in the

lungs of mice 24 h after intravenous injection by 40%. Our results are in agreement with previous findings demonstrating that the CCL5/CCR5 signaling axis plays a crucial role in the metastatic cascade as it is involved in cancer cell extravasation.¹⁰ Blocking the homing of cancer cells to metastatic sites is a highly desirable feature for a bona fide anti-metastatic drug.

Our recent paper¹ demonstrates for the first time that the expression of CCR5 and CCL5 correlates with a metastatic

phenotype of basal breast cancer, both in clinical samples and in cell lines. We were also the first to report the anti-metastatic effects of a CCR5 antagonist that has already been licensed by FDA for use in humans.

Our data suggest that CCR5 antagonists may be used to reduce the risk of metastasis in patients bearing basal breast cancer. Given the aggressive course of the disease and the lack of targeted therapies, the use of CCR5 antagonists as an

adjuvant therapy may constitute a major improvement in the clinical management of basal breast cancer patients.

Disclosure of Potential Conflicts of Interest

R.G.P. holds minor (< \$10,000) ownership interests in, and serves as CSO/Founder of the biopharmaceutical companies ProstaGene, LLC and AAA Phoenix, Inc. R.G.P. additionally holds ownership interests (value unknown) for several submitted patent applications.

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