Introduction

Chemokines, small chemotactic cytokines, are immune regulatory molecules known to direct the trafficking of immune cells via binding to their cognate receptor on the surface of target cells. Chemokines expressed at sites of infection direct immune cells to that area in the classic view of proinflammatory chemokines. Other chemokines are constitutively expressed and play other important cell trafficking roles. The constitutive chemokine ligand, CXCL12, and its receptor CXCR4 are evolutionarily conserved and essential for life in mice. Thus, CXCL12 and CXCR4 are considered the primordial chemokine axis. Recently, a great deal of research has been focused on the role of CXCR4 signaling in carcinoma cells. This research has lead to a current paradigm that CXCR4 expression on metastatic cells enables these cells to home to organs expressing CXCL12. In fact, several studies suggest that CXCR4 expression on tumor cells correlates with increased metastasis and decreased clinical prognosis. The expression of CXCL12 in carcinoma cells is much less well understood. Previously, we have shown that CXCL12 is epigenetically silenced in colorectal carcinoma by DNA hypermethylation, and that this silencing event has a dramatic impact on the ability of those cells to metastatically invade the liver, an organ of high CXCL12 expression. Here we extend this epigenetic mechanism of CXCL12 silencing to mammary carcinoma. Further, using in vivo imaging we show that by restimulating endogenous CXCL12 in mammary cancer cells we can inhibit metastasis to the lung, another organ of high CXCL12 expression. Finally, we show evidence to suggest that by silencing their own expression of CXCL12 mammary carcinoma cells have increased CXCR4 signaling sensitivity in response to exogenous CXCL12 stimulation.

Figure 1. CXCL12 expression is absent in mammary carcinoma cell lines. CXCL12 is expressed in normal in vivo mammary epithelial cells as assessed by immunohistochemistry (A). Six of eight in vitro mammary epithelial cells lines lack expression of CXCL12 by RT-PCR. CXCR4 is consistently expressed (B). Primary mammary tumor stained for CXCL12. In contrast, to normal epithelium CXCL12 is not expressed by all cells (C).

Figure 2. CXCL12 is silenced by promoter DNA hypermethylation. All cell lines that lack CXCL12 expression are hypermethylated in the promoter as assessed by methylation specific PCR (A). Methylation of known essential transcription factor binding sites in the CXCL12 promoter occurs specifically in cell lines that lack expression of the gene (B). CXCL12 promoter methylation was detected in primary tumor but not matched normal tissue. Methylation was detectable in six of 15 tumors examined (40%) (C). MDA-MB-231 cells express CXCL12 when DNA methylation is inhibited by treatment of cells with 5-aza in both a dose and time dependent manner (D).

Figure 3. Functional re-expression of CXCL12 in MDA-231 cells. Single and double stable 231-luc cells express similar amounts of luciferase (A). Double stable cell lines specifically express either CXCL12 or eGFP as a control (B). CXCL12 expressed by 231-luc cells can stimulate CXCR4 mediated, U937 chemotaxis.

Figure 4. MDA-MB-231 cells that lack CXCL12 expression more effectively form lung metastases. MDA-231-luc cells (2X10^6) were injected into the lateral tail vein of SCID-mice and imaged ~30min later. Mice were then imaged weekly to monitor metastasis formation (A). Averaged photon readings expressed as percent of original reading upon injection (n=5 for both groups) showing a decrease in metastatic invasion by CXCL12 expressing 231-luc cells (B). Survival curve showing mice injected with CXCL12 expressing cells survived longer than control cells (C).

Figure 5. Mammary carcinoma cells that lack endogenous CXCL12 have increased sensitivity to exogenous ligand stimulation. 231-luc cells when grow to ~75% confluence were loaded with Fluo-4 AM and stimulated with CXCL12 (100ng/ml). FITC fluorescence was assayed as a measure of intracellular calcium flux (A). Max flux, calculated as maximum FITC fluorescence subtracted from background fluorescence was determined for each cell line in response to increasing concentrations of CXCL12 (B).

SUMMARY

•CXCL12 expression is silenced in mammary carcinoma by DNA hypermethylation while CXCR4 is consistently expressed.
• Endogenous re-expression of functional CXCL12 in mammary carcinoma cells reduced their in vivo lung metastasis.
• Reduced metastasis correlated with a desensitization in response to exogenous CXCL12 stimulation.
• These data are consistent with our previous findings that CXCL12 is also silenced in coloninial carcinoma, and that this epigenetic event increases the metastatic potential of carcinoma cells.
• These findings are consistent with and expand upon previous data concerning the role of CXCR4 signaling in carcinoma cell metastasis. Our results, together with recent findings emphasizing the importance of CXCR4 signaling in cancer cell migration and invasion, represent a unique observation that loss of autocrine CXCL12 signaling plays a role in the increased metastasis of cancer cells.

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Epigenetic silencing of the chemokine CXCL12 in mammary carcinoma enhances metastasis

Michael K. Wendt, Michael B. Dwinell
Department of Microbiology and Molecular Genetics, Medical College of Wisconsin, Milwaukee, WI