A new key in breast cancer metastasis

Unlocking the mysteries of cell metastasis, a major cause of cancer mortality, is essential in the development of novel therapies. In this issue of Cancer Cell, Li et al. (2004) identify a link between HER2 and CXCR4, two receptors previously implicated in breast cancer progression and metastasis. HER2 enhances the expression of CXCR4 by stimulating CXCR4 translation and attenuating CXCR4 degradation. Importantly, coexpression of HER2 and CXCR4 occurs in ~22% of human breast tumors and correlates with poor survival of breast cancer patients.

HER2 (ErbB2, Neu) is a member of the epidermal growth factor receptor family and is activated either by homodimerization that occurs upon overexpression or by growth factors following formation of heterodimers with other members of the ErbB family. HER2 is overexpressed in a large number of cancers, including ~30% of breast cancers, and is an important target in breast cancer therapy where treatment with the anti-HER2 monoclonal antibody trastuzumab (Herceptin) has proven effective (Slamon et al., 2001). HER2 overexpression appears to play an important role in promoting metastasis, although the mechanism for this is poorly understood. Li et al. (2004) provide new insight into the mechanism of HER2-mediated metastasis by identifying a link between HER2 and CXCR4, a G protein-coupled receptor (GPCR) previously implicated in breast cancer metastasis (Muller et al., 2001).

Li et al. (2004) demonstrate that overexpression of HER2 in breast cancer cells increases expression of CXCR4, while inhibition of HER2 expression using trastuzumab or RNAi treatment results in a corresponding reduction in CXCR4 expression. A primary mechanism for the HER2-promoted increase in CXCR4 expression appears to involve the ability of HER2 to increase the translation of CXCR4 via activation of a phosphatidylinositol 3-kinase/Akt/mTOR signaling pathway (Figure 1). Previous studies have also demonstrated that HER2 can activate the NF-κB pathway (Zhou et al., 2000) and that NF-κB activation enhances CXCR4 expression (Helbig et al., 2003), although these two processes have not been linked in breast cancer cells. Moreover, recent studies suggest that von Hippel-Lindau tumor suppressor pVHL negatively regulates CXCR4 expression, a process that is dysregulated under hypoxic conditions (Staller et al., 2003). Thus, multiple mechanisms appear to coordinate regulation of the expression of CXCR4.

Interestingly, HER2 also appears to regulate the rate of CXCR4 degradation in breast cancer cells (Li et al., 2004). Previous studies have demonstrated that CXCR4 degradation involves ligand-dependent ubiquitination of the receptor by the E3 ubiquitin ligase AIP4 (Marchese et al., 2003). CXCR4 activation by the ligand CXCL12 (also called stromal cell-derived factor or SDF-1) regulates receptor endocytosis, while CXCR4 ubiquitination promotes the subsequent sorting of the endocytosed receptor to the lysosome where it is degraded. Interestingly, HER2 appears to inhibit CXCR4 degradation by inhibiting CXCL12-promoted ubiquitination of the receptor (Li et al., 2004). Although the mechanism of this inhibition is unclear, the requirement for HER2 tyrosine kinase activity suggests that HER2-promoted signaling may regulate the mechanisms involved in targeting CXCR4 for ubiquitination. While the detailed mechanism of CXCL12-promoted ubiquitination of CXCR4 has not been elucidated, it likely involves phosphorylation of the receptor by a G protein-coupled receptor kinase (GRK), binding of an arrestin, and subsequent targeting of the receptor for ubiquitination by AIP4. It is possible that the initial steps in this process are disrupted.
ed by HER2 overexpression, although GRK6 and arrestin-3 appear to play stimulatory roles in CXCL12-promoted migration of lymphocytes (Fong et al., 2002). The ubiquitination machinery is another potential target for inhibition by HER2 overexpression, although little is presently known about whether AIP4 activity is regulated. Interestingly, overexpression of AIP4 completely inhibits the ability of HER2 to enhance CXCR4 expression (Li et al., 2004). This may reflect an enhanced rate of CXCR4 degradation but also suggests that AIP4 might play an inhibitory role in the enhanced translation of CXCR4, perhaps by promoting ubiquitination of components in the mTOR or NF-κB pathways.

The defect in CXCR4 degradation in breast cancer cells overexpressing HER2 is also interesting in light of a recent study demonstrating that ligand-promoted degradation of the protease-activated receptor 1 (PAR1) is also defective in several breast cancer cell lines (Booden et al., 2004). Previous studies have implicated a role for increased expression of PAR1, a GPCR for the enzyme thrombin, in breast cancer cell invasion. Although the detailed mechanism of PAR1 degradation is unknown, it is intriguing that attenuation of the normal regulatory mechanisms that attenuate CXCR4 and PAR1 function appear to be dysfunctional in breast cancer cells.

The role of HER2 and CXCR4 in cell invasion was also investigated by Li et al. (2004). Interestingly, the HER2-promoted increase in CXCR4 expression was essential for CXCL12-promoted cell invasion, migration, and adhesion. Importantly, CXCL12-promoted invasion of BT474 cells (that have high endogenous levels of HER2) was effectively inhibited by treatment with either CXCR4 inhibitory antibodies or with trastuzumab, thus providing a clear link between HER2 and CXCR4 in breast cancer cell invasion. Indeed, previous studies have implicated a role for CXCR4 in breast cancer metastasis (Muller et al., 2001) as well as metastasis of colon and prostate cancer (Kucia et al., 2004). To further establish a link between HER2 and CXCR4 in breast cancer, Li et al. (2004) also developed a retroviral RNAi to inhibit CXCR4 expression. This retrovirus effectively inhibited CXCR4 expression and attenuated basal and CXCL12-promoted invasion of HER2-expressing cells. A breast cancer mouse model was also used to evaluate the effect of reduced CXCR4 expression on breast cancer cell metastasis to the lung. These studies clearly demonstrate the important role of CXCR4 overexpression in breast cancer cell metastasis, a finding previously observed by Muller et al. (2001). A link between HER2 and CXCR4 expression was also observed in human breast tumors with ~22% of the tumors found to coexpress high levels of HER2 and CXCR4. Importantly, high expression of CXCR4 also correlated with a poor overall survival rate.

The study by Li et al. (2004) identifies a clear link between HER2 and CXCR4 and demonstrates that CXCR4 overexpression plays a central role in HER2-mediated metastasis. So how does CXCR4 regulate metastasis? The prevailing theory is that the ability of chemokine receptors to mediate cancer metastasis is similar to their normal function in regulating cell migration (Moore, 2001). CXCR4 controls the migration of multiple cell types, including hematopoietic stem cells, germ cells, lymphocytes, and neurons, to sites that express CXCL12. The high levels of CXCL12 in the bone marrow, lung, liver, and regional lymph nodes provides a likely mechanism as to why these tissues are the primary sites for metastasis of cancers that have been linked to CXCR4 overexpression such as breast, colon, and prostate. CXCL12 binding to CXCR4 activates multiple pathways that function to regulate cell invasion and migration. These include increased expression of adhesion molecules such as VLA4 and LFA-1 as well as the matrix metalloproteinase MMP9, thus enhancing the ability of these cells to cross endothelium and basement membrane (Moore, 2001). Multiple signaling pathways that function to promote cell migration are also activated by CXCR4, including stimulation of actin polymerization and pseudopodia formation. The central role of CXCR4 in cancer metastasis also raises the question as to whether CXCR4 can serve as an important diagnostic and/or target in the detection and treatment of cancer. Given the importance of CXCR4 in development and normal immune function, it is likely that complete inhibition of CXCR4 activity would be detrimental. However, the ability of CXCR4 to promote cancer cell metastasis appears to be linked to its overexpression in cancer cells. Thus, CXCR4 overexpression in cells that normally have low levels of this receptor might prove to be a useful diagnostic to identify cells that have the potential to metastasize. It is also important to further establish the mechanisms that result in increased CXCR4 expression and potentially target such pathways in cancer treatment. Thus, understanding the mechanisms that normally regulate CXCR4 expression and function should prove useful in the treatment and prevention of cancer metastasis.

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