Breast cancer metastasis driven by ErbB2 and 14-3-3ζ
A division of labor

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Metastasis remains the leading cause of cancer morbidity and mortality. ErbB2, a metastasis-promoting oncoprotein, is overexpressed in 50–60% of non-invasive ductal carcinoma in situ (DCIS). However, only 25% of invasive breast cancer (IBC) overexpress ErbB2, indicating that ErbB2 alone is not sufficient to drive metastasis and additional risk factors are necessary for the progression of ErbB2-overexpressing DCIS to IBC. A recent study published in Cancer Cell identified 14-3-3ζ as a risk factor aiding the transition of ErbB2-overexpressing DCIS into IBC. Furthermore, the study elucidated molecular mechanisms by which ErbB2 and 14-3-3ζ co-overexpression drives metastasis. Namely, ErbB2 promotes cell motility and migration via the activation of Src, while 14-3-3ζ induces epithelial-mesenchymal transition by activating TGFβ pathway to reduce cell adhesion. On the other hand, two studies recently published in British Journal of Cancer and Oncogene provide mechanistic insight into how ErbB2 signalling is transduced via Src, focal adhesion kinase and Ste20-like kinase to regulate focal adhesion turnover and modulate cell motility and migration. Taken together, these studies reveal that metastasis engages a variety of players that must show team spirit to win the game of spreading.

Summary

About 90% of deaths from cancer are due to metastasis, a complex process in which tumor cells escape the primary local tumor, invade through surrounding tissues to spread and colonize distant organs. Although the molecular and genetic events underlying tumor metastasis are still not well understood, intense investigation into this process has led to the notion that molecules involved in cell adhesion and migration are critical in tumor invasion and metastasis. A new study recently published provide strong support to this notion by demonstrating that invasive breast cancer is driven by the combinational overexpression of ErbB2 and 14-3-3ζ, which promotes cell migration and reduces cell adhesion, respectively. Two additional studies illustrate how ErbB2 impacts focal adhesion turnover to modulate cell motility and migration in invasive breast cancer cells.

Introduction

While the cancer research field is still dominated by exhaustive investigation into the genetic and epigenetic abnormalities underlying tumor initiation, given the fact that metastatic cancer accounts for most of cancer-associated death, the past several years have witnessed an ongoing shift of focus to secondary tumor formation, a process known as metastasis. Perhaps the most ominous aspect of cancer is its ability to spread, or metastasize. To metastasize, cancer cells have to escape the primary local tumor, invade through surrounding tissues to spread and colonize distant organs. Cell adhesion system provides a powerful safeguard mechanism to prevent cancer cells from metastasizing. Noteworthy is the E-cadherin cell adhesion molecule which performs important function to maintain adherent junctions between cells and prevent the metastatic behavior of cells. Cancer cells employ
multiple strategies to circumvent the body’s defense to facilitate their spreading. They frequently undergo epithelial-mesenchymal transition (EMT), a process whereby cells downregulate the epithelial specific adherens junction proteins including E-cadherin and re-express mesenchymal makers such as vimentin and N-cadherin. As a result of this transition, the mesenchymal cells migrate from the epithelial layer where they are originated and invade distant areas of the body.\(^2\)

Ductal carcinoma in situ (DCIS) is the most common type of non-invasive breast cancer. At this early stage of breast cancer, cancer cells are found in the lining of breast ducts and have not spread outside of the ducts to other normal surrounding tissues in the breast. DCIS isn’t life-threatening since it is 98% curable, but it is a precursor of invasive breast cancer (IBC), which causes over 90% of breast cancer-related death. Overexpression of ErbB2 (HER-2/neu), a well known oncoprotein, is found in about 25% of IBC and is associated with poor patient survival. In contrast, ErbB2 is overexpressed in more than half of noninvasive DCIS. The more frequent expression of ErbB2 in DCIS compared with IBC has led to a debate on the clinical implication of ErbB2 expression in the progression of DCIS to IBC. A recent study revealed that the risk for women whose DCIS overexpress ErbB2 to develop invasive cancer is 6.4-fold higher than women whose DCIS do not overexpress the protein.\(^3\) These data suggest that ErbB2 overexpression is crucial for the transition from in situ cancer to invasive cancer. Nevertheless, previous data indicate that ErbB2 alone is not sufficient and additional risk factors are necessary for the progression of ErbB2-overexpressing DCIS to IBC. In this context, Yu and his colleagues identified the risk factor or “second hit” that cooperates with ErbB2 to drive the progression of DCIS to IBC and the novel findings were reported in a recent paper in Cancer Cell.\(^4\)

**14-3-3\(^{ξ}\)** Promotes the Transition of ErbB2-Overexpressing DCIS into IBC

The highly conserved 14-3-3 protein family is involved in various vital cellular processes due to their specific phospho-serine/threonine binding properties. 14-3-3\(^{ξ}\) is a member of 14-3-3 family postulated to have oncogenic function based on its overexpression in a wide range of cancers as well as its gene localization to a chromosome region (8q23) that is frequently amplified in metastatic cancer.\(^7\) In view of their prior data on the increased expression of 14-3-3\(^{ξ}\) in DCIS, Lu et al. hypothesized that 14-3-3\(^{ξ}\) may serve as the additional risk factor for the progression of DCIS to IBC. First, the authors examined clinical samples from 25 DCIS patients for the protein level of 14-3-3\(^{ξ}\) and ErbB2. Strikingly, they found that 17 DCIS patients whose tumors did not overexpress both proteins never developed distant metastasis, whereas 4 out of 8 patients whose tumors overexpressed both proteins had disease recurrence with distant metastasis. These data demonstrate that co-overexpression of 14-3-3\(^{ξ}\) and ErbB2 in DCIS is associated with high metastasis potential.\(^3\) To substantiate this finding, the authors then employed MCF10A 3D culture system as an in vitro DCIS model to explore the functional synergy between 14-3-3\(^{ξ}\) and ErbB2 in DCIS progression to IBC. MCF10A cells overexpressing ErbB2 form highly proliferative but noninvasive DCIS-like structures, while those overexpressing 14-3-3\(^{ξ}\) develop into abnormal acinar structures with no lumen formation. In contrast, MCF10A cells overexpressing both ErbB2 and 14-3-3\(^{ξ}\) escape from acini and invade surrounding matrix. Additionally, the authors observed the loss of basement membrane integrity in these cells, whereas the integrity is kept in cells overexpressing ErbB2 or 14-3-3\(^{ξ}\) alone.\(^4\) Taken together, these results confirm that only the combination of ErbB2 and 14-3-3\(^{ξ}\) overexpression can promote the invasive behaviours of mammary epithelial cells.

Next, the authors used the MCF10A derived cell lines to dissect molecular mechanism by which ErbB2 and 14-3-3\(^{ξ}\) co-overexpression confer cell invasiveness. They found that increased cell motility was contributed by ErbB2 but not 14-3-3\(^{ξ}\) overexpression and it could be specially inhibited by Src kinase inhibitor, suggesting that ErbB2 mediates cell motility and migration via the activation of Src. Interestingly, 14-3-3\(^{ξ}\) but not ErbB2 overexpression contribute to the loss of cell-cell adhesion and EMT phenotype.\(^7\) Thus ErbB2-mediated increase of cell migration and 14-3-3-mediated decrease of cell adhesion cooperatively drive the invasion of cells co-overexpressing ErbB2 and 14-3-3\(^{ξ}\). The author then demonstrated that 14-3-3\(^{ξ}\) modulates EMT by upregulating TGFβ/Smads pathway and found a correlation between 14-3-3\(^{ξ}\) and TGFβ receptor I (TβRI) expression in DCIS samples. Furthermore, the authors experimentally established the TβRI/14-3-3/Smads/ZFHX1B/E-cadherin axis to explain how 14-3-3\(^{ξ}\) contributes to EMT and decrease of cell adhesion.\(^4\) Binding of 14-3-3\(^{ξ}\) to TβRI inhibits proteasome-mediated TβRI degradation and the stabilized TβRI activates TGFβ/Smads pathway, promoting Smad2/3 translocation into the nucleus where Smads drive the expression of ZFHX1B, which is a transcriptional repressor of E-cadherin, consequently leading to decreased cell adhesion (Fig. 1).

**ErbB2 Regulates Focal Adhesion Turnover and Cell Motility**

Complementing the conclusion that ErbB2 mediates cell motility and migration via the activation of Src reported in Cancer Cell, two studies recently published in British Journal of Cancer and Oncogene provide mechanistic insight into how ErbB2 signalling is transduced via Src, focal adhesion kinase and Ste20-like kinase to regulate focal adhesion turnover and modulate cell motility and migration.\(^6,7\)

Focal adhesion is complicated macro-molecular assemblies at sites of integrin adhesion to the extracellular matrix, which is established to regulate cell motility and migration. The highly dynamic nature of cell motility necessitates the rapid turnover of focal adhesion. Focal adhesion kinase (FAK), a non-receptor tyrosine kinase, acts as a multifunctional adaptor and signalling molecules to modulate focal adhesion formation and turnover and has been implicated in various aspects of tumor development.\(^8\) Based on their previous results that ErbB2-induced cell invasion depends on FAK signalling, Xu et al. went on to explore the molecular details of the modulation of focal adhesion by ErbB2. Using FAK knockout cells and Src/Yes/Fyn triple knockout cells, Xu et al. found...
that the regulation of focal adhesion turnover by ErbB2 is FAK and Src dependent. In addition, they employed the ErbB2 antibody Herceptin as a tool to confirm the connection between ErbB2, Src and FAK, signaling that they established. Herceptin inhibits Src binding to ErbB2, Src kinase activity and FAK activity. As a result focal adhesion turnover is inhibited and the stabilized focal adhesion leads to reduced cell motility and invasion. Thus this study revealed a novel mechanism of action of Herceptin in breast cancer therapy.

Ste20-like kinase (SLK), a serine/threonine kinase, has emerged as a novel regulator of focal adhesion turnover and cell migration. Roovers et al. made the first attempt to investigate whether SLK also mediates cell migration downstream of ErbB2 in breast cancer cells. First, they found that ErbB2 activation or overexpression induces SLK activity in breast cancer cell lines. Next they showed that SLK is necessary for ErbB2-mediated cell motility since catalytic inactive mutant of SLK or SLK shRNA inhibits the migration of ErbB2-overexpressing breast cancer cells. Furthermore, SLK knockdown impairs the invasion of mouse mammary epithelial cells expressing activated ErbB2 in matrigel invasion assay. By use of FAK-null cells and Src/Yes/Fyn-deficient cells, Roovers et al. further demonstrated that Src kinase and FAK are required for SLK activation downstream of ErbB2. SLK kinase activity is important to mediate ErbB2-stimulated focal adhesion turnover because kinase inactive SLK induces focal adhesion stabilization. Taken together, these results help define that SLK functions downstream of ErbB2, Src and FAK to modulate focal adhesion turnover which is essential for cell motility and migration (Fig. 1).

Conclusions and Perspectives

Collectively, the exciting findings discussed above provide novel insight into the process of transition from non-invasive DCIS to invasive breast cancer and support new concepts for the diagnosis and treatment of cancer metastasis. Metastasis involves functional cooperation of a diversity of molecules. As summarized in Figure 1, breast cancer cells have to simultaneously hijack the signalling cascades that control cell motility (ErbB2 pathway) and modulate cell adhesion (TGFβ pathway) to drive their invasion and metastasis. Therefore a cocktail therapy concept by targeting individual molecule involved in cell motility and cell adhesion will greatly reduce the risk of DCIS progression to IBC. The identification of 14-3-3ξ as the second hit for DCIS metastasis indicates that 14-3-3ξ represents a biomarker for selection of high-risk DCIS patients who need urgent treatment before deadly metastasis develop. The significance of 14-3-3ξ as an indicator of metastasis potential may be not limited to breast cancer, since 14-3-3ξ is also over-expressed in hepatocellular carcinoma, seminoma, pancreatic adenocarcinoma and lung carcinoma, and knockdown of 14-3-3ξ in spontaneously immortalized human skin keratinocytes could enhance cell-cell adhesion by upregulating the expression of adhesion proteins including E-cadherin.

Future studies are necessary to inspect exactly what signalling cascades collaborate with 14-3-3ξ overexpression to promote metastasis in these tumors. An earlier study demonstrated the collaboration of ErbB2 and TGFβ1/3 in the induction of invasion in mammary epithelial cells and proposed TGFβ as a pro-invasion factor in the progression of ErbB2-expressing breast cancer, thus it is worth investigating the potential of other TGFβ pathway components as biomarkers and therapeutic targets for metastasis in the future.

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