Regulation of Cancer Cell Survival, Migration, and Invasion by Twist: AKT2 Comes to Interplay

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Abstract

Metastasis, the foremost cause of mortality in cancer patients, is increasingly recognized as a coordinated biological process. The multistep process of metastasis posts difficulty in studying its mechanism and molecular basis. Recent works have shown that the basic helix-loop-helix transcriptional factor Twist and the serine/threonine kinase AKT play pivotal roles in tumor development and progression. Our recent study has shown that AKT2 is a transcriptional regulatory target of Twist and acts downstream of Twist to promote cancer cell survival, migration, and invasion. Functional convergence of Twist and AKT2 underscores the importance of this signaling pathway in tumor development and progression as a potential therapeutic target. [Cancer Res 2008;68(4):957–60]

Introduction

Understanding of the cellular and molecular basis of tumor metastasis inevitably faces the challenge of the fact that metastasis is a complex multistep biological process most likely controlled by distinct genes and signaling pathways in each step. It is well recognized that metastasis consists of distinct steps: (a) emigrating of tumor cells from the primary tumor, a step requiring the migrating cells to undergo epithelial-mesenchymal transition (EMT) to enable them detaching and migrating away from the primary tumor site; (b) invading to neighboring tissue and penetrating through basement membrane and entering blood or lymphatic vessels, the latter of which is referred to as invasations. In addition to migratory ability, proteolytic enzymes such as metalloproteinases are thought to be critical in this step, (c) surviving the condition of anoikis while detached from the tumor mass and in circulation, a process requiring anchorage-independent survival, which seems to be a common property of cancer cells; (d) exiting blood or lymphatic vessels, or extravasations, at a distant organ; (e) formation of micrometastatic nodule; and (f) adaptation and reprogramming of the surrounding stroma, reversion of EMT or mesenchymal-epithelial transition to reassume the epithelial nature, and formation of macrometastasis. The factors regulating the later steps are not clear. The question remains open if extravasation is a passive or active process. The factors determining the tissue specificity of metastasis are not completely clear either. However, chemokine and chemokine receptor interaction as well as preconditioning of the premetastatic organ site by factors secreted by the tumor cells has been implicated (1). Different approaches have been used to explore the basis of tumor metastasis including gene array, mouse model, and their combination. Each has its own merit and limitation. Comparison of gene profiles in noninvasive tumor and metastatic lesions yielded potentially useful diagnostic and prognostic gene markers. Complication of this method is, in part, due to the heterogeneous nature of the tumor mass, which consists of not only tumor cells proper but also other types of cells including stroma, vascular, and inflammatory cells. Diversity in histology and development of individual tumors even from the same histologic type adds to the complexity. This method could be refined by laser-capture microdissection to purify the tumor cells. Comparison of a pair or pairs of in vitro or in vivo derived cancer lines with distinct invasive ability has also been widely used and yielded very interesting results. The question here is whether those invasive lines faithfully mimic the selection and evolution of metastatic cells in human malignancy. For example, in the metastasis derived by tail vein injection of cancer cells in mouse models, the steps of initial EMT, local invasion, and invasations are bypassed; besides, the number of tumor cells introduced i.v., usually in millions, greatly deviates from the spontaneous release of tumor cells in real life. All the above approaches focus on the end-stage metastatic cells and thus are likely to miss the essential genes and pathways that operate in transient nature during the process of metastasis. It is conceivable that genes and signaling pathways required for the early steps of metastasis, including EMT, migration, invasion, and invasation, may have been subsequently inactivated and replaced by a new spectrum of genes and signaling pathways needed for the establishment of metastasis at a new organ site. Nevertheless, with proper model systems and functional assays, combination of gene arrays and in vivo assays has proved to be quite powerful in identifying metastasis relevant genes. These are exemplified by the identification of RhoC in melanoma cancer cells earlier (2) and the recent identification of a basic helix-loop-helix (bHLH) transcriptional factor Twist in breast cancer cells (3). This later study used cDNA array analysis with a series of isogenic tumor lines with increasing metastatic capability in a syngeneic mouse model to identify Twist as a regulatory gene for metastasis. The involvement of Twist in human tumor development and progression has been confirmed by examination of clinical tumor specimens in the original report (3) as well as in numerous subsequent studies by other laboratories.

Functional Role of Twist-AKT2 Signaling in Invasive Breast Cancer

In view of the complexity of the biological process of metastasis and intrinsic limitation of the approaches, we rationalized that a well-defined system coupled with proper functional assays and study of clinical relevancy is required to identify physiologically relevant genes and pathways for metastasis (4). We hypothesized that if functional selection for invasive capability from distinct
cancer cell lines repeatedly identifies association with the same gene or pathway, then this gene or pathway is likely to be functionally relevant to this biological process. We chose a normal human breast epithelial line, AB589, and two breast cancer lines, MCF-7 and MDA-MB-435 (3), which all displayed low invasive ability, for selection of invasion capability by Boyden chamber assay. After four successive cycles of selection, stable highly invasive lines were obtained and named respectively the I4 lines for 4th cycle selected invasive cells. Both cDNA array and targeted gene and signaling pathway analyses were undertaken to identify potential invasion relevant genes and pathways. Among them, Twist and AKT2 were found to be elevated in all three I4 lines compared with their respective parental lines. Because both genes have been reported to be involved in cancer development and metastasis, we then focused on the role of these genes in cell invasion. Because Twist is known as a transcriptional factor, we inquired if the expression of AKT2 was regulated by Twist. Indeed, knockdown of Twist by small interfering RNA in the I4 cells led to a significant decrease of AKT2, whereas ectopic expression of Twist in the low AKT2 expressing parental cells resulted in a dose-dependent increase of AKT2 at protein and mRNA levels. Subsequently, Twist was shown to be able to bind to a specific E-box in the AKT2 promoter and activate its transcriptional activity. Thus, AKT2 is a target gene of Twist-mediated transcriptional regulation. To inquire about the functional link between Twist and AKT2, Twist was introduced into parental MCF-7 cells and shown to promote migration, invasion, and drug resistance, and these Twist-mediated functions were significantly inhibited by AKT2 small interfering RNA. Conversely, Twist was knocked down from the I4 cells with concurrent decrease in migration, invasion, and drug resistance, which could be significantly rescued by introduction of AKT2 into the cells. Those observations established the functional link between Twist and AKT2, and Twist acts upstream of AKT2. To assess the clinical significance of this functional link, expression of Twist and AKT2 in different stages of breast cancer was examined. About 69% coexpression of Twist and AKT2 was observed in stage III/IV breast cancer, but only 13% in stage 0/II breast cancer. Overall, those observations suggest that Twist functions through AKT2 in the regulation of cancer cell migration, invasion, and drug resistance (Fig. 1).

**Twist-AKT2 Signaling in Cancer Development and Progression**

Accumulating evidence indicates that Twist and AKT have a wide-spectrum involvement in human malignancy. Twist, a bHLH transcriptional factor, is well known for its regulatory role in development during neurogenesis, osteogenesis, and myogenesis.
In vertebrate, there are two Twist genes, Twist-1 and Twist-2, and their encoded proteins share the bHLH domain but differ in the NH2-terminal region. Twist-1 null is embryonic lethal at E10.5 due to abnormal head mesenchyme development that results in a failure of cranial neural tube closure (5). The absence of Twist-2 results in normal development till birth, but leads to perinatal death due to overexpression of proinflammatory cytokines, which cause severe cachexia, failure to thrive, and depletion of glycogen and fat reservoir (6). Germ-line mutation in human Twist-1 results in Saethre-Chotzen syndrome characterized by abnormalities of limbs, asymmetrical head and face, and premature fusion of cranial sutures (7). Twist fulfills several criteria as a potential oncogene. These include its overexpression in a variety of cancers as well as its role in antiapoptosis, drug resistance, angiogenesis, EMT, and invasion (Fig. 1). Several recent excellent reviews have touched on these subjects (8, 9). Thus, they will be only briefly described here. For antiapoptosis, Twist was shown to inhibit c-Myc-induced apoptosis via suppression of the ADP ribosylation factor (ARF)/murine double minute 2/p38 pathway, allowing c-Myc–mediated cell proliferation to proceed (10). Twist inhibits the p53 pathway both transcriptionally and posttranslationally. Twist interacts with HOXA5, a potent transactivator of p53, and negatively modulates its activity (11). Twist reduces ARF expression leading to destabilization of p53 and prevention of phosphorylation of the essential activating Ser20 of p53 (10, 11). Thus, Twist contributes to tumor development by shutting down the apoptotic response. Twist was shown to signal through AKT to mediate Taxol resistance in nasopharyngeal carcinoma cells (12); in agreement, our study showed that this could occur via AKT2 in breast cancer cells (4). For angiogenesis, it was reported that overexpression of Twist in MCF-7 breast cancer cells resulted in higher vascular volume and permeability in the xenograft tumors (13). Finally, as mentioned above, Weinberg’s laboratory initially identified Twist as a key mediator in EMT, invasion, and metastasis of breast cancer cells (3). Twist was shown to inhibit E-cadherin and induce N-cadherin and marker proteins related to EMT. All those observations clearly showed the important role of Twist in regulating the hallmark properties of cancer cells. How is Twist regulated, and through which mediators does Twist manifest its function? Figure 1 depicts the current view of the signaling network of Twist. Twist expression has been documented to be responsive to Wnt-1, insulin-like growth factor I (IGF-I), and nuclear factor-κB signaling. Undoubtedly, additional upstream regulators of Twist exist depending on the type of cancer and context of tissues and cells. Aside from being a direct positive or negative transcriptional regulator for its target genes, very few active downstream mediators of Twist are known, among them are AKT2, FOXC2, and Cbl (4, 12, 14, 15). In osteoblast, Twist seems to negatively regulate phosphatidylinositol 3-kinase and AKT signaling via transcriptional regulation of Cbl expression, an E3 ligase mediating the degradation of phosphatidylinositol 3-kinase (15). It is not known which specific AKT isoform is involved in this process, but as the author discussed, the Twist-Cbl-phosphatidylinositol 3-kinase/AKT pathway was unlikely to be involved in the survival because they found increased survival in mutant Twist-harboring osteoblasts in Saethre-Chotzen syndrome patients (15). The negative regulation of the phosphatidylinositol 3-kinase/AKT pathway has a significant effect on osteoblasts proliferation or differentiation, but not survival.

AKT, a serine/threonine protein kinase and a key downstream mediator of the phosphatidylinositol 3-kinase pathway, has been well studied for its role in cell growth, survival, migration, and differentiation physiologically and pathologically. Three forms of AKT (AKT1, AKT2, and AKT3) exist in mammalian cells. Whereas they are known to share common substrates and functions, their specific role and function is always an intriguing and challenging question. Ectopic expression of wild-type AKT2 results in invasion and metastasis in human breast and ovarian cancer cells (16) whereas AKT1 promotes cell survival and limits breast cancer invasion via activation of HDM2 E3 ligase and degradation of NFAT (17). The migration/invasion inhibitory effect of AKT1 is in agreement with the finding of Irie et al. (18) who reported that silencing AKT1 expression increased migration of MCF10A cells overexpressing IGF-I receptor, whereas down-regulating AKT2 had no effect. They observed that AKT1 acts as a suppressor of extracellular signal–regulated kinase (ERK), which is required for migration, and thus down-regulation of AKT1 leads to ERK activation and migration. Those observations on AKT1 are corroborated by the in vivo evidence that expression of an activated AKT1 allele in ErbB2 transgenic mice resulted in a more pronounced tumor growth but fewer metastatic lesions in the transgenic mice (19). However, the migration inhibitory effect of AKT1 seems to be cell type specific. For examples, AKT1 was shown to increase invasion in HT1080 fibrosarcoma (20) and pancreatic cancer cells (21). Our observation of Twist-mediated induction of AKT2, but not AKT1, expression in promoting migration/invasion of breast cancer cells (4) is consistent with those of Bruggel’s and Toker’s laboratories (17, 18). However, both isoforms of AKT could be involved in survival in different types of cells.

EMT, although not always the case, is in general considered a prerequisite step during initial phase of metastasis. EMT is triggered by genetic and epigenetic alterations of the tumor cells and their interaction with the surrounding microenvironment including stromal cells and matrix components. A great deal has been learned in recent years about the mechanism of EMT largely from in vitro studies (1, 22). A pivotal step of EMT is the loss of E-cadherin, which results in destabilization of cell-cell interaction and cytoskeleton, as well as release of β-catenin from its anchoring at the cytoplasmic domain of E-cadherin. Translocation of β-catenin to nucleus and its association with lymphoid enhancer factor/T-cell factor lead to expression of EMT-related gene markers including fibroblast related matrix proteins such as fibronectin, vimentin, and integrin. Thus, any factor and signaling pathway that could modulate the expression of E-cadherin and EMT-related markers could have the potential to trigger this process. Various growth factors/receptors, transforming growth factor β (TGFβ), and their downstream pathways including phosphatidylinositol 3-kinase/AKT, ERK/mitogen-activated protein kinase, Smads, and Rho GTPases have all been shown to be able to induce EMT in different types of cells. TGFβ, in cooperation with various growth factors/receptors and oncogenes, has been shown to effectively induce down-regulation of E-cadherin and EMT. The mediating transcriptional factors negatively regulating E-cadherin include Snail, Slug, SIP1, E2a, and the recently shown Twist. Our finding of the up-regulation of Twist in invasive breast cancer cells and Twist as the upstream positive regulator of AKT2 suggests an enhanced effect of the Twist/AKT signaling in promoting EMT and its likely involvement in the subsequent steps of metastasis.

Closing Remarks

The finding of converged signaling of two important factors, Twist and AKT2, involved in cancer development and progression, underscores the importance of this pathway in human malignancy
and as a potential target for therapy. Potential upstream and downstream regulators of this pathway in different contexts of cancer cells could vary and are yet to be unveiled. They could all be potential therapeutic targets. Whether the role of Twist-AKT2 signaling is limited to the initial stage of cancer cell invasion or has a wider implication of metastasis is still unclear. Future study of metastasis will have to overcome the limitation of methodologic bypass of the intermediate phases. The ability to visualize the real-time process of invasive cancer cells will allow a better understanding of the basis for the distinct stages of metastasis, including target organ selection and stroma interaction, in the establishment of metastatic lesions.

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