E-Cadherin: Context-Dependent Functions of a Quintessential Epithelial Marker in Metastasis

Cao Fang[1,2] and Yibin Kang[1,2]

Loss of E-cadherin expression has been well known as a hallmark of epithelial–mesenchymal transition (EMT), which is linked to increased risk of cancer metastasis. However, it was less clear whether E-cadherin and its downstream signaling pathways are functionally involved in driving EMT and the prometastatic phenotype. A study by Onder and colleagues in 2008 discovered that E-cadherin loss not only helps tumor cells detach from each other by breaking down cell–cell junctions but also elicits intracellular signaling events to confer a mesenchymal cell state and metastatic phenotype. This study established E-cadherin as an important global regulator, rather than just a marker, of EMT. The discovery inspired further investigation in the following decade that significantly deepened our understanding of E-cadherin and its diverse functions and more broadly of cellular plasticity in different stages and contexts of cancer metastasis.

See related article by Onder and colleagues, Cancer Res 2008;68: 3645–54

Epithelial–mesenchymal transition (EMT) has been regarded as a critical step in cancer metastasis, where cancer cells lose their epithelial characteristics and gain mesenchymal features to facilitate their detachment from the primary sites and gain motility. Initially described in developmental biology, EMT was increasingly linked to tumor invasion and metastasis by the early 2000s. Several master EMT transcription factors such as Twist, Snail, and Zeb family members have been shown to promote cancer metastasis (1). In these studies, E-cadherin was frequently used as the hallmark of epithelial cells and its loss, when combined with the altered expression of other marker genes, signaled the occurrence of EMT. However, it was unclear whether E-cadherin loss is simply a consequence of EMT or if it has functional importance in propagating or maintaining the EMT status. Furthermore, it had not been determined whether the functional impact of E-cadherin loss is mediated only through breaking down cell–cell junctions or additionally through eliciting distinct intracellular signaling pathways in cancer cells.

To address these questions, Onder and colleagues used two strategies to disrupt E-cadherin function in normal and malignant human mammary epithelial cells (2). One approach is using short hairpin RNAs (shRNA) to specifically reduce E-cadherin expression to a minimal level. The second approach utilized a truncated form of E-cadherin (DN-Ecad), which lacks the ectodomain but retains the cytoplasmic domain. This DN-Ecad is unable to mediate the formation of intercellular junctions and also acts in a dominant-negative manner by competitively binding to the cytoplasmic proteins associated with adherens junctions. Therefore, cells expressing DN-Ecad had diminished cell–cell junctions but retained some of intracellular signaling mediated by wild-type E-cadherin (2). Comparing the phenotype of cells with E-cadherin knockdown (shEcad) or expressing DN-Ecad allowed the authors to assess the relative contributions of E-cadherin–dependent intercellular adhesion and intracellular signaling in EMT phenotype and metastasis.

Both shEcad and DN-Ecad resulted in the loss of cell–cell contacts and scattering of cells. However, only cells with shEcad exhibited an EMT-like fibroblastic morphology, upregulated mesenchymal proteins, and acquired significantly increased lung metastasis potential. Additional functional analyses revealed that shEcad, but not DN-Ecad, resulted in increased cell motility, invasiveness, and anoikis resistance (2). These findings indicated that E-cadherin loss works by more than disrupting cell–cell junctions, rather, it facilitates an intracellular signaling cascade to enable invasion and metastasis.

At the molecular level, the global gene expression profiles of shEcad revealed greater differential expression of genes than DN-Ecad as compared with control cells, although significant overlap did exist between these two types of E-cadherin disruption. Consistent with the EMT phenotype in shEcad cells but not DN-Ecad cells, the induction of mesenchymal marker genes, including N-cadherin, vimentin, fibronectin, and collagens, occurred in shEcad cells but not in DN-Ecad cells (2). Among transcription factors involved in EMT, 19 were upregulated in shEcad, while only 2 were also upregulated in DN-Ecad cells, underscoring the importance of E-cadherin cytoplasmic domain in restraining EMT. The authors further validated that Twist, an EMT transcription factor, and β-catenin, which dissociated from adherens junctions and became transcriptionally active after E-cadherin knockdown, mediated the EMT phenotype in shEcad cells. Because Twist is known to transcriptionally repress E-cadherin expression, this constitutes a feed-forward mechanism to enforce low E-cadherin expression once EMT is induced (**Fig. 1**).

Since the publication of this study, the functional impact of E-cadherin loss in driving cancer metastasis has been validated in other cancer types as well, such as gastric cancer. For example, Till and colleagues determined the effects of E-cadherin in gastric cancer metastasis by generating a spontaneous gastric cancer mouse model based on gastric parietal cell lineage-specific expression of oncogenic $Kras^{G12D}$, coupled with genetic deletion of p53 and E-cadherin (3). These triple-conditional mice invariably developed mixed-type gastric tumors with metastasis to the lymph nodes, lung, and liver. In contrast, despite $Kras^{G12D}$, $Trp53^{-/-}$ mice that are heterozygotes for E-cadherin

[1]Department of Molecular Biology, Princeton University, Princeton, New Jersey. [2]Ludwig Institute for Cancer Research Princeton Branch, Princeton University, Princeton, New Jersey.

Corresponding Author: Yibin Kang, Department of Molecular Biology, 255 Lewis Thomas Laboratory, Princeton University, Washington Road, Princeton, NJ 08544-1014. Phone: 609-258-8834; Fax: 609-258-2340; E-mail: ykang@princeton.edu

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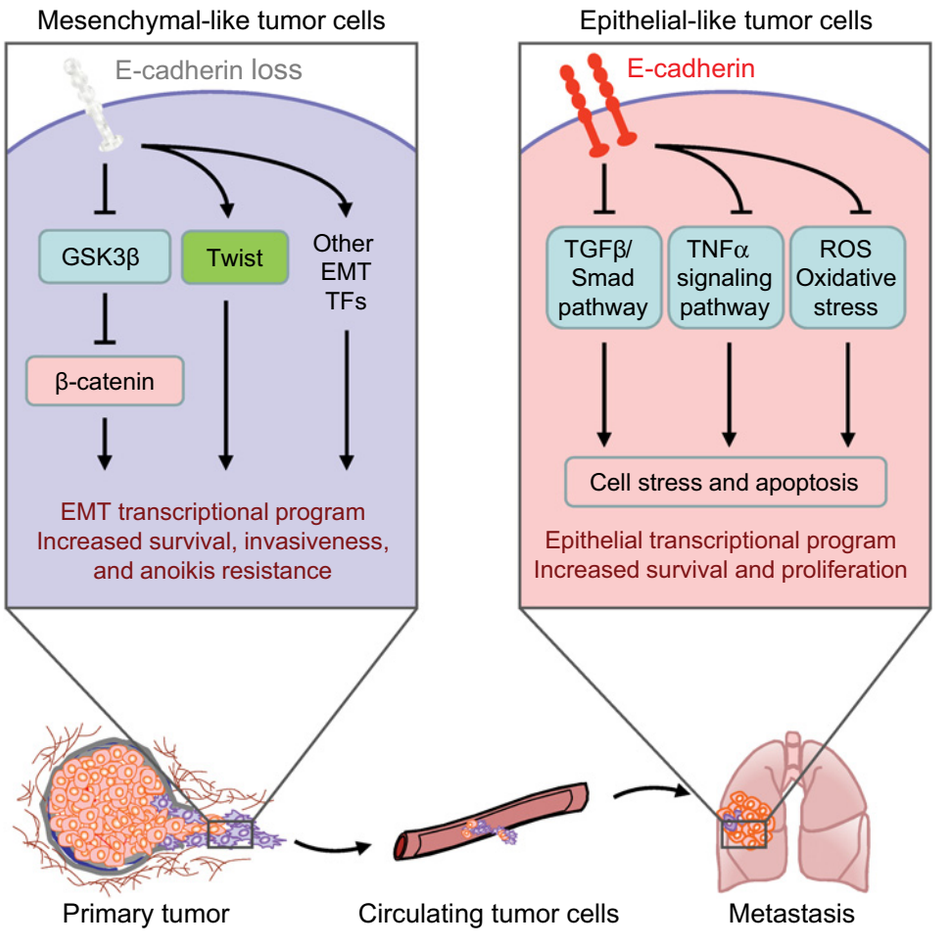

Figure 1.

Context-dependent functions of E-cadherin in different stages of metastasis. Left, during invasion and dissemination from the primary tumor, E-cadherin loss promotes EMT by repressing GSK3β–mediated phosphorylation and degradation of β-catenin, increasing the expression and transcriptional activity of Twist and other EMT-related transcription factors (TF), and eventually leading to increased invasiveness and resistance to anoikis. A partial EMT status of the cells, where E-cadherin expression is partially retained during extravasation, promotes cell cluster formation and collective migration. Formation of CTC clusters also confers resistance to immune surveillance by natural killer cells during their transition in blood circulation. Right, at the colonization site, regaining E-cadherin expression confers survival advantage to metastatic tumor cells by suppressing cellular stress and apoptotic pathways mediated by TGFβ and TNFα signaling pathways and oxidative stress driven by reactive oxygen species (ROS).

developed hyperplastic growth, and only 20% of them progressed to invasive gastric adenocarcinoma with no gross lung or liver metastasis. In metastatic E-cadherin null tumors, increased RAS activity and MAPK signaling were observed, driving the aggressive growth and metastatic phenotypes. In contrast to these well-established tumor- and metastasis-suppressive functions of E-cadherin, recent experiments in multiple breast cancer models revealed a surprising role of E-cadherin in promoting metastatic colonization (4). By labeling E-cadherin$^+$ and E-cadherin$^-$ PyMT mouse mammary tumor cells with different fluorescent markers in the same primary tumor, Padmanaban and colleagues investigated the E-cadherin expression pattern in cells that successfully accomplished distal metastasis. They found that almost none of the macrometastases were E-cadherin$^-$, suggesting that E-cadherin might be essential for successful metastatic colonization (4). In this model system, loss of E-cadherin increased invasion but reduced cancer cell proliferation and survival by activating the TGFβ, TNFα, oxidative stress, and apoptosis pathways (**Fig. 1**). Taken together, these diverse roles of E-cadherin in different cancer types and different stages of metastasis contributed to an emerging theme that E-cadherin plays important but context-dependent roles in cancer progression and metastasis. This notion is also consistent with the clinical observation that E-cadherin is frequently mutated in a few subsets of cancers, such as gastric cancer and lobular carcinoma of the breast, but is more often flexibly regulated at the epigenetic level in cancer cells and often retains its expression in metastases (4).

The biphasic role of E-cadherin in suppressing migration and promoting metastasis is echoed in other key regulators of EMT. In the same year of the Onder and colleagues study, the miR-200 family miRNAs were discovered to be strong enforcers of the epithelial state by targeting ZEB-1 and ZEB-2, which are transcriptional repressors for E-cadherin and strong inducers of EMT. Just like E-cadherin, sustained expression of miR-200 suppresses migration and invasion of breast cancer but promotes metastatic colonization in the lungs (5). Mechanistically, the lung metastasis–promoting effect of the miR-200s is mediated by targeting Sec23a-dependent secretion of metastasis suppressive extracellular proteins such as Tinagl1 and Igfbp4 (5). The dynamic temporal-spatial requirement of cellular plasticity during different phases of metastasis was further illustrated by another study published in 2012 using an elegant genetic mouse model of skin cancer by Tsai and colleagues (6). Specifically, Twist induction promotes EMT and local invasion at the primary sites, while its inactivation and mesenchymal–epithelial transition are required for metastatic colonization in distant organs.

Subsequent research in the field has led to a model that EMT is not only a temporary and reversible process, but also exists as a spectrum instead of being a binary event (1). Several studies have identified cancer cells in a hybrid epithelial–mesenchymal state, in which they express both epithelial and mesenchymal markers and have enhanced cancer stemness, tumorigenic, and metastatic properties (1, 7). In recent years, cell cluster formation and collective migration of cancer cells has been linked to increased risk of metastasis and found to be

closely related to partial EMT. Staining of circulatory tumor cells (CTC) revealed that CTC clusters retained E-cadherin expression at cell–cell junctions while single CTCs did not (8). Using a multicolor lineage tracing method, Cheung and colleagues discovered that most metastases were derived from polyclonal seeding, in contrast to the conventional model that each metastasis comes from a single tumor cell (9). Such multi-cell clusters existed across different stages of metastasis, and experimentally aggregating tumor cells into clusters substantially increased formation of metastasis (9). Interestingly, those cell clusters frequently expressed another epithelial marker Keratin14, which was subsequently validated to play a functional role in driving cluster formation and metastasis (9). The formation of cell clusters by cancer cells maintaining epithelial features has also been shown to confer resistance to natural killer cell killing, which allows cancer cells to escape immune surveillance during circulation in the bloodstream (10). These findings further strengthen the concept that although EMT facilitates early steps of metastasis, maintaining at least partial epithelial features is critical for later steps of metastasis, such as collective migration, cluster formation, immune evasion, and metastatic colonization.

The research by Onder and colleagues in 2008 not only recategorized E-cadherin as a functional molecule rather than merely an epithelial marker, but also elicited broader exploration of EMT, which redefined EMT as a reversible process conferring cell plasticity and better survival advantage in different contexts rather than a permanent transformation of cells in a single direction. It has become increasingly clear that tumor cells utilize cellular plasticity to deal with a variety of stresses they face during different stages of tumorigenesis and metastatic progression. Transitioning between different epithelial–mesenchymal states and cell fates also gives tumor cells the flexibility to interact productively with their surrounding microenvironment and to survive various therapeutic challenges. Identifying tumor-intrinsic and stromal-dependent factors that drive and sustain cellular plasticity in different cancers will pave the way toward potential novel therapeutics. As cellular plasticity is also critically important for embryonic and postnatal development as well as normal tissue homeostasis and damage repair, potential adverse effects of plasticity targeting agents on normal tissues need to be carefully evaluated. With the implementation of cutting-edge technologies that allow cell fate mapping, lineage tracing and recording, and multiomic analysis of both tumor cells and the surrounding stromal populations at a single-cell resolution, we can expect continued evolution of our understanding of cellular dynamics during cancer metastasis, with the hope of applying such knowledge to improve preventative measures as well as effective treatment of metastatic cancers.

Authors' Disclosures

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