Developmental Cell

CellPress

Previews

Recording EMT Activity by Lineage Tracing during Metastasis

Pauline Vieugué[1] and Cédric Blanpain[1,*]
[1]Laboratory of Stem Cells and Cancer, WELBIO, Université Libre de Bruxelles (ULB), 808 route de Lennik, Bat GE, G2 4.205, 1070 Bruxelles, Belgium
*Correspondence: Cedric.Blanpain@ulb.ac.be
https://doi.org/10.1016/j.devcel.2020.07.011

In this issue of *Developmental Cell*, Li et al. develop a novel lineage tracing system to record EMT activity during lung metastasis of mammary tumors. Using EMT-tracer mouse models, they reveal that N-cadherin is transiently expressed by most metastasis-initiating cells and demonstrate its functional importance during the metastatic cascade.

Epithelial to mesenchymal transition (EMT) is a dynamic, transient, and reversible cellular process by which epithelial cells lose their epithelial characteristics and acquire mesenchymal features such as a spindle-shaped fibroblast-like morphology, increased motility, and expression of mesenchymal markers such as N-cadherin, FSP1, or vimentin (Nieto et al., 2016; Yang et al., 2020). EMT plays important roles during tumorigenesis, including invasion, metastasis, and resistance to therapy (Nieto et al., 2016; Puisieux et al., 2014). During metastasis, EMT has been proposed to promote tumor cell dissemination by allowing tumor cells to leave the primary site, migrate, reach the blood stream, and colonize distant organs. Supporting this notion, downregulation of EMT transcription factors inhibits metastasis (Krebs et al., 2017; Nieto et al., 2016; Yang et al., 2020; Yang et al., 2004). Primary tumor cells and circulating tumor cells expressing mesenchymal markers have been reported in various cancers (Pastushenko and Blanpain, 2019). Moreover, tumor cells presenting EMT or partial EMT characteristics present higher metastatic potential when injected intravenously (Pastushenko and Blanpain, 2019; Pastushenko et al., 2018). At the metastatic sites, most tumor cells display epithelial characteristics, suggesting that EMT is a dynamic and reversible process during metastasis and that mesenchymal to epithelial transition could be important for metastatic outgrowth. Despite the evidence supporting EMT's role in initiating metastasis, it is still a matter of intense debate as to whether EMT is required for metastasis (Diepenbruck and Christofori, 2016). Direct evidence by lineage tracing, demonstrating unambiguously that EMT is associated with spontaneous metastasis in mouse cancer models, is still lacking. In this issue of *Developmental Cell*, Li et al. developed a novel lineage tracing approach to investigate the role of EMT during lung metastasis of mammary tumors (Li et al., 2020).

A potential technical hurdle in EMT tracing experiments is that EMT may only occur transiently during the metastatic cascade. Lineage tracing by genetic recombination is usually based on the expression of a recombinase enzyme, such as Cre or Dre, by a cell-specific or a tissue-specific promoter that induces the expression of a reporter gene such as fluorescent proteins. Recombinases can be fused to the estrogen receptor (ER) and are therefore only activated and translocated into the nucleus following estrogen analog (tamoxifen) administration to temporally control the expression of the reporter gene in the population of interest. However, the use of this technology requires precise knowledge of the time frame during which the promoter driving the recombinase expression is active in the cells of interest in order to administrate tamoxifen in the appropriate temporal window, which potentially limits the sensitivity of this technique to track and monitor a transient process such as EMT.

To overcome this issue, Li et al. (2020) generated an elegant, novel *in vivo* genetic approach combining two recombination systems (Cre/Lox and Dre/rox) to perform lineage tracing analysis of cells undergoing EMT, using either N-cadherin-Dre or vimentin-Dre (EMT tracer), two well-known EMT markers. The EMT tracer needs to be activated by the expression of Cre recombinase in the cells of interest to ensure specificity in tumor cells. The authors used a Kit-CreER, which is expressed in ER-negative mammary luminal cells, to activate the expression of the vimentin-Dre or N-cadherin-Dre in MMTV-PyMT mice that develop mammary tumors with lung metastases. To monitor Cre- and Dre-mediated recombination, the authors used a dual reporter system, which expressed green fluorescent protein following Cre-mediated recombination and red fluorescent protein (td-Tomato) following Dre-mediated recombination. They show that Kit-CreER labeled primary tumor cells in MMTV-PyMT mice. The labeled cells became more abundant as the mammary tumor progressed and gave rise to lung metastasis, showing that KitCreER is appropriate to label tumor- and metastasis-initiating cells with green fluorescent protein and the activation of the EMT tracer (Figure 1). The EMT tracer did not give rise to red fluorescent labeled cells in the normal mammary gland, suggesting that EMT does not occur under normal physiological conditions.

In early stages of tumorigenesis, primary tumors derived from the vimentin-tracer model only displayed green cells. In contrast, a small percentage of tumor cells (2%) became tomato positive at later stages, showing that only a small fraction of primary tumor cells in the model upregulate vimentin expression. Most lung metastatic cells were composed of green cells

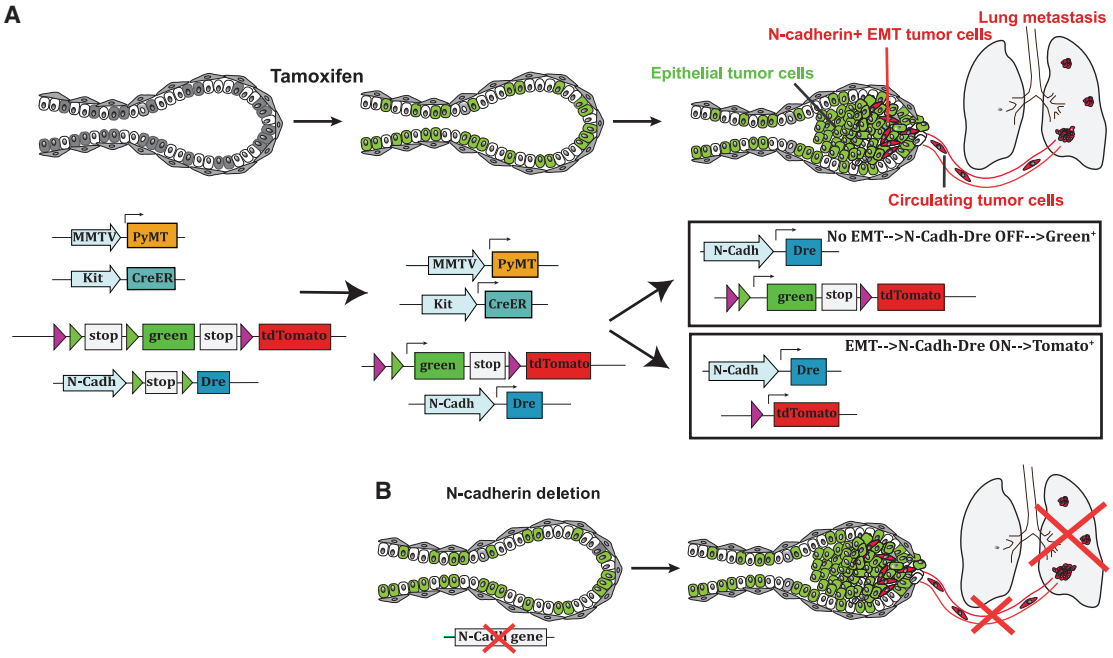

Figure 1. N-Cadherin-Dependent EMT Is Required for Lung Metastasis of Mammary Tumors

(A) To record the activation of the N-cadherin EMT-dependent program during the MMTV-PyMT-induced mammary tumorigenesis, a dual recombinases-based fate mapping system reporting the sequential activity of Cre and Dre recombinase is used. Upon Tamoxifen injection, CreER activates the expression of the green fluorescent protein in the mammary gland and mammary tumors, while the expression of Dre, if N-cadherin is expressed, switches the expression of green to tomato fluorescent protein. Lung metastasis expressed tomato, supporting that EMT program is expressed during the metastatic cascade.
(B) Deletion of N-cadherin dramatically decreases lung metastasis.

that did not activate vimentin expression, and only a few cells localized at the periphery of metastases were tomato positive, suggesting that vimentin becomes expressed in the lung as metastases grow. Deletion of vimentin did not impair metastasis formation, showing that vimentin expression was not required for tumor cell dissemination and lung colonization. Similar to the vimentin-EMT tracer, the N-cadherin tracer revealed that the tumor cells in early stages of tumorigenesis did not activate N-cadherin expression. However, at a later stage, about 10% of the primary tumor cells were tomato positive. Very interestingly, the vast majority (about 70%) of the lung metastases were tomato positive, strongly suggesting that metastasis-initiating cells underwent EMT during the metastatic cascade. Importantly, deletion of N-cadherin strongly decreased the number of lung metastases, demonstrating that, in addition to marking metastasis-initiating cells, N-cadherin is functionally required during lung metastasis.

To assess whether the N-cadherin expression in tumor cells is associated with bona fide EMT programs and not EMT-independent genetic program, the authors investigated the expression of key EMT transcription factors (e.g., Zeb1, Twist2, and Snai1) and other mesenchymal markers (e.g., N-cadherin and vimentin), as well as epithelial markers (E-cadherin) in green and red tumor cells. They found that EMT markers were upregulated and E-cadherin was decreased in tomato positive cells, supporting the notion that the N-cadherin tracer labels tumor cells undergoing EMT within the primary tumors. Finally, intravenous injection of green tumor cells gave rise to lung metastasis without activating N-cadherin, as shown by the lack of a switch to red fluorescence. This indicates that N-cadherin expression is required during the early stages of dissemination rather than during extravasation and lung colonization.

Altogether, this study describes a powerful system to perform lineage tracing of transient and reversible EMT states during lung metastasis of mammary tumors. Li et al. (2020) reveal that N-cadherin, but not vimentin, labels metastatic-initiating cells in the MMTV-PyMT mouse model and demonstrate the essential role of N-cadherin during lung metastasis. As partial EMT states are more metastatic as compared to full EMT (Pastushenko et al., 2018), the discrepancy in the metastatic capacity between N-cadherin- and vimentin-traced cells suggests that N-cadherin labels a more partial EMT state as compared to vimentin-expressing cells, similar to FSP, another mesenchymal marker that also fails to trace lung metastasis in this model (Fischer et al., 2015). The non-inducible tracing method to record EMT activity developed in this study is very sensitive as it is always active. However, an inducible tracing method would be more suitable to precisely define the timing of the requirements of EMT during the metastatic cascade. It would also be important to assess, using this method, the requirement of EMT for the metastasis in other metastatic sites such as in lymph node metastatic and in other cancers that are more mesenchymal as compared to MMTV-PyMT such as genetic skin cancer, metaplastic mammary tumors, or pancreatic tumors. It would also be important to assess the requirement of N-cadherin in the metastasis of cancers that are thought to be independent of EMT, such as colorectal

cancer. It will also be possible to define the intrinsic and extrinsic mechanisms regulating the different steps of EMT, as well as their functions during metastasis, using this technique. Finally, this innovative technique can be used to trace other transient states and follow their fates during development, regeneration, or other aspects of tumorigenesis, such as therapy resistance.

REFERENCES

Diepenbruck, M., and Christofori, G. (2016). Epithelial-mesenchymal transition (EMT) and metastasis: yes, no, maybe? Curr. Opin. Cell Biol. *43*, 7–13.

Fischer, K.R., Durrans, A., Lee, S., Sheng, J., Li, F., Wong, S.T., Choi, H., El Rayes, T., Ryu, S., Troeger, J., et al. (2015). Epithelial-to-mesenchymal transition is not required for lung metastasis but contributes to chemoresistance. Nature *527*, 472–476.

Krebs, A.M., Mitschke, J., Lasierra Losada, M., Schmalhofer, O., Boerries, M., Busch, H., Boettcher, M., Mougiakakos, D., Reichardt, W., Bronsert, P., et al. (2017). The EMT-activator Zeb1 is a key factor for cell plasticity and promotes metastasis in pancreatic cancer. Nat. Cell Biol. *19*, 518–529.

Li, Y., Lv, Z., Zhang, S., Wang, Z., He, L., Tang, M., Pu, W., Zhao, H., Zhang, Z., Shi, Q., et al. (2020). Genetic Fate Mapping of Transient Cell Fate Reveals N-Cadherin Activity and Function in Tumor Metastasis. Dev. Cell *54*, this issue, 593–607.

Nieto, M.A., Huang, R.Y., Jackson, R.A., and Thiery, J.P. (2016). EMT: 2016. Cell *166*, 21–45.

Pastushenko, I., and Blanpain, C. (2019). EMT Transition States during Tumor Progression and Metastasis. Trends Cell Biol. *29*, 212–226.

Pastushenko, I., Brisebarre, A., Sifrim, A., Fioramonti, M., Revenco, T., Boumahdi, S., Van Keymeulen, A., Brown, D., Moers, V., Lemaire, S., et al. (2018). Identification of the tumour transition states occurring during EMT. Nature *556*, 463–468.

Puisieux, A., Brabletz, T., and Caramel, J. (2014). Oncogenic roles of EMT-inducing transcription factors. Nat. Cell Biol. *16*, 488–494.

Yang, J., Mani, S.A., Donaher, J.L., Ramaswamy, S., Itzykson, R.A., Come, C., Savagner, P., Gitelman, I., Richardson, A., and Weinberg, R.A. (2004). Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. Cell *117*, 927–939.

Yang, J., Antin, P., Berx, G., Blanpain, C., Brabletz, T., Bronner, M., Campbell, K., Cano, A., Casanova, J., Christofori, G., et al.; EMT International Association (TEMTIA) (2020). Guidelines and definitions for research on epithelial-mesenchymal transition. Nat. Rev. Mol. Cell Biol. *21*, 341–352.

Buckling Up from the Bottom

Marija Matejčić[1,*] and Xavier Trepat[1,2,3,4,*]
[1]Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute for Science and Technology (BIST), 08028 Barcelona, Spain
[2]Facultat de Medicina, Universitat de Barcelona, 08036 Barcelona, Spain
[3]Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain
[4]Centro de Investigación Biomédica en Red en Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), 08028 Barcelona, Spain
*Correspondence: mmatejcic@ibecbarcelona.eu (M.M.), xtrepat@ibecbarcelona.eu (X.T.)
https://doi.org/10.1016/j.devcel.2020.08.010

In this issue of *Developmental Cell*, Trushko et al. (2020) develop a bottom-up approach to understand the physics underlying confined epithelial monolayer folding. Investigating this process is currently unattainable *in vivo* but is essential to our understanding of tissue formation from the gastrulating blastula to the developing nervous system.

While sculpting our tissues during development, epithelia morph from smooth sheets into folded structures. In the complex *in vivo* environment, many factors can simultaneously influence this folding process, making it difficult to assess individual contributions in intact embryos. Moreover, folding an epithelial sheet is in its essence a mechanical process, but no current method enables us to measure absolute forces driving this process in complex *in vivo* environments. In this issue of *Developmental Cell*, the Roux lab makes a U-turn away from the conventional top-down approaches based on tinkering with *in vivo* biology to study morphogenetic processes such as folding. Establishing a bottom-up approach, Trushko et al. (2020) construct a minimal epithelial system to unravel stresses and cell properties that drive fold formation.

Multiple mechanisms can induce a fold in an epithelial sheet. Most studies have focused on the widely spread and conserved process of active tissue bending driven by actomyosin differentials, such as apical constriction (Pearl et al., 2017). An alternative to such active deformations is sheet buckling, a mechanism of compressive origin implicated in fold formation in the lung, brain, and gut (Nelson, 2016). Mechanically, buckling is a sudden out-of-plane collapse of a material caused by an increasing in-plane compressive load. Such load can arise from contraction of surrounding muscle tissues (Shyer et al., 2013), compaction of the surrounding mesenchyme (Hughes et al., 2018), different regional growth rates (Tozluoğlu et al., 2019), or regional instabilities such as mitotic rounding (Kondo and Hayashi, 2013), among others. If we knew the material properties and forces acting during these different biological processes, we could build a unifying mechanical view to identify which morphogenetic processes may indeed be driven by buckling.

Aiming to grow an epithelium with access to mechanical measurements, Trushko et al. (2020) further developed their method to produce alginate-based three-dimensional confinements (Alessandri et al., 2016; Figure 1A). Using a