Published in final edited form as:

Sci Signal. ; 4(175): pe28. doi:10.1126/scisignal.2002153.

Cancer Cells Exploit the Eph-Ephrin System to Promote Invasion and Metastasis: Tales of Unwitting Partners

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Abstract

The Eph subfamily of receptor tyrosine kinases and their membrane-anchored ephrin ligands mediate cell-cell contact signaling and are versatile regulators of cell migration and tissue patterning, which are often exploited by cancer cells during tumor progression. New evidence shows that prostate cancer cells use EphA2 and EphA4 receptors and ephrin-As to mediate homotypic contact inhibition of locomotion while co-opting ephrin-B2 on stromal cells through EphB3 and EphB4 receptors to propel migration. These processes could enhance cancer cell scattering from the primary tumor mass and promote unimpeded migration and invasion through the stromal space. The results provide another example in which Eph receptors are converted into pro-oncogenic proteins, contrary to their often-described tumor suppressor roles in normal tissues.

Nearly 50 years after the initial description of the phenomenon of contact inhibition of locomotion (CIL) and the loss of CIL in tumor cells, one of the underpinning molecular mechanisms is now coming to light (1). The molecules involved are the Eph (erythropoietin-producing hepatoma) receptors and their membrane-anchored ephrin ligands, which regulate cell migration during embryonic development and in disease processes, including cancer.

CIL was first described by Abercrombie and Heaysman in 1953, and referred to the change in the direction of cell locomotion that occurs upon collision of two normal fibroblasts (2, 3). Later studies showed that the process entails collapse of membrane protrusions at the cell-cell contact regions, cessation of cell migration, and formation of new membrane protrusions at different sites, culminating in a change of direction of cell migration [see (4) for a recent review]. The potential implications of CIL in cancer biology became evident when sarcoma cells were found to display compromised CIL and readily invaded into normal fibroblast cultures (5, 6), suggesting that the defective CIL in cancer cells may facilitate tumor cell invasion into surrounding normal stroma. Intriguingly, cancer cells retain CIL when they collide with each other. However, the molecular mechanisms underlying CIL have remained obscure decades after its initial discovery. The finding that the Eph-ephrin system regulates both homotypic CIL between cancer cells and the heterotypic attraction between cancer and stromal cells provides needed molecular insight on CIL and its evasion by cancer cells (1).

The 16 vertebrate Eph receptors constitute the largest subfamily of receptor tyrosine kinases (7). Ligands for Eph receptors are ephrins, which are divided into glycosylphosphatidylinositol (GPI)-anchored ephrin-A and transmembrane ephrin-B ligands that generally bind to EphA and EphB receptors, respectively. Because both receptors

(Ephs) and ligands (ephrins) are membrane proteins, Eph-ephrin interactions occur upon cell-cell contact. The best-characterized function of the Eph-ephrin system is the guidance of growth cone and cell migration through mostly repulsive and sometimes adhesive responses. The cell guidance activities contribute to precise axon targeting and tissue boundary formation during embryonic development (8). Perturbations of Eph-ephrin systems have been documented in various human cancers (8–11). In keeping with the cell guidance role in development, Eph receptors also critically regulate adhesion and migration of cancer cells. PC-3 prostate cancer cells were among the first model systems extensively examined for effects of Eph receptors on cell adhesion and migration (12). EphA2 is abundant on PC-3 cells, and stimulation of these cells with ephrin-A1 causes rapid cell rounding and reduced cell adhesion and migration (12).

The repulsion of migrating cells by Eph receptors upon cell-cell contact is likened to CIL (4). Supporting this notion, Astin *et al.* (1) provided elegant in vitro evidence demonstrating how PC-3 cells may switch between homotypic repulsion and heterotypic attraction by exploiting different sets of Eph receptors and ephrins on cancer and stromal cells (1). Consistent with the original observations by Abercrombie and Heaysman in 1953 (2, 3), the authors found that normal prostate epithelial cells and nonmetastatic DU-145 cells display CIL when they collide with each other or with normal fibroblasts. Migration of invasive and metastatic PC-3 cells, on the other hand, is stimulated upon contact with fibroblasts and endothelial cells, although homotypic CIL is retained. Cellular and biochemical evidence from PC-3 cells establishes that EphA2–ephrin-A and EphA4–ephrin-A interactions mediate homotypic CIL. In keeping with earlier studies (12), stimulation of PC-3 cells with recombinant ephrin-A1 or ephrin-A5 caused rapid cell rounding and inhibited cell migration. An unexpected finding was that PC-3 cells have endogenous ephrin-As (1, 3–5) in addition to EphA2 and EphA4 receptors. When migrating PC-3 cells collide, EphA2 and EphA4 receptors are trans-activated by ephrin-As on the opposing cells. Mechanistically, the activated EphA2 and EphA4 receptors trigger the activation of RhoA, leading to the retraction of membrane protrusions and ultimately reinitiation of migration in a different direction, or CIL.

How can a migrating PC-3 cell evade CIL when colliding with a stromal cell that is likely to also possess ephrin-As capable of activating EphA2 and EphA4? A hint came when Astin *et al.* noticed that exogenous ephrin-B2 promoted PC-3 cell migration through EphB3 and EphB4, both of which were present on these cells. Activation of EphB3 and EphB4 by ephrin-B2 induced formation of filopodia and lamellipodia, which are associated with Cdc42 activation. Knockdown of EphB3 and EphB4 or Cdc42 diminished the migration stimulated by ephrin-B2. Moreover, overexpression of ephrin-B2 in PC-3 cells abolished homotypic CIL, suggesting that CIL mediated by EphA2–ephrin-A and EphA4–ephrin-A interactions can be overridden by EphB3–ephrin-B2 and EphB4–ephrin-B2 signaling. Of the six ephrins examined, only ephrin-B2 was highly abundant on stromal cells compared with PC-3 cells. Ephrin-B2 on stromal cells would be expected to outcompete the less-abundant ephrin-As for activation of EphB3 and EphB4, which would in turn activate Cdc42 and promote invasive migration into the stromal space. Thus, specific combinations of Eph and ephrin on cancer as compared with stromal cells dictate a repulsive or an attractive outcome upon heterotypic collision (Fig. 1).

The discovery of the Eph-ephrin system in both the execution of homotypic CIL and the evasion of heterotypic CIL could have important in vivo implications in malignant progression (Fig. 1). It is conceivable that homotypic CIL dependent on EphA2–ephrin-A and EphA4–ephrin-A interactions between cancer cells could facilitate the scattering of tumor cells from the primary tumor mass. Subsequently, heterotypic attraction through activation of EphB3 and EphB4 by ephrin-B2 on stromal cells could enable invading cancer

cells to navigate through the surrounding tissues. Supporting this notion, invasive prostate cancer cells in human specimens show high abundance of EphB4 but little ephrin-B2, whereas stromal cells show high abundance of ephrin-B2 but little EphB4 (1). Previous studies demonstrate high surface abundance of ephrin-B2 on pericytes and smooth muscle cells (13) in addition to fibroblasts and endothelial cells (1), potentially providing permissive paths for the invading cancer cells.

Although the data by Astin *et al.* (1) have established a pro-migratory role of activation of EphB3 and EphB4 in PC-3 cells upon contact with ephrin-B2 on stromal cells, it appears to be an opportunistic adaption by a specific cancer cell type rather than the norm for EphB4. Noren *et al.* previously demonstrated that EphB4 activation by ephrin-B2 caused inhibition, instead of stimulation, of migration of multiple breast cancer cell lines through activation of the Abl-Crk pathway (14). Likewise, in a mouse colon cancer model, EphB4 plays a tumor suppressive and an antimigratory role (15), which is consistent with reduced abundance of EphB4 in human colorectal cancer (16). Thus, under most conditions, EphB4–ephrin-B2 interactions result in a repulsive response or CIL. Lastly, both in vivo and in vitro evidence has established EphB3 as a receptor that promotes repulsion in migrating noncancerous cells (17, 18).

The cunning ability of PC-3 cells to take advantage of differential presence and abundance of Eph and ephrin proteins to evade CIL in stromal space is the latest example in which the versatile functions of Eph receptors in regulating cell migration are exploited by cancer cells. EphA2 has ligand-dependent CIL-like functions on prostate cancer and glioma cells. However, in the absence of ligand, it can be converted into a pro-oncogenic protein to promote tumor cell migration and invasion upon phosphorylation by Akt on a serine residue (Ser⁸⁹⁷) (19). EphA2 phosphorylation at Ser⁸⁹⁷, a telltale sign that EphA2 is a partner of oncogenic Akt, is absent from normal human brain, elevated in low-grade astrocytomas, and abundant in glioblastoma. The latter shows increased abundance of EphA2, reduced ephrin-A1, and frequent Akt activation (19, 20). Therefore, a combination of factors in cancer cells and their surrounding microenvironment determine whether EphA2 functions as anti- or pro-migratory receptor. The pro-migratory effects of the crosstalk between EphA2 and Akt may steer invading cancer cells toward favorable growth and survival sites.

A different strategy is used by LK63 leukemia cells to overcome the EphA3 activation–induced CIL upon ligand stimulation (21, 22). LK63 leukemia cells have high abundance of protein tyrosine phosphatases (PTPases), which prevent phosphorylation and activation of EphA3 upon contact with ephrin-A5–presenting cells. In human embryonic kidney (HEK) 293 cells, EphA3 activation is required for cleavage of ephrin-A5 in the EphA3–ephrin-A5 complex by the metalloprotease Adam10 at the cell-cell contact region, enabling separation of two cells after initial contact (23). Lack of EphA3 activation in LK63 cells causes failed cell separation, resulting in attraction rather than repulsion after cell collision, a phenotype that is rescued by inhibition of PTPase activity (21, 22). Conceivably, the loss of CIL in leukemia cells could facilitate their dissemination to sites where they would have been repelled had CIL remained intact.

The report by Astin *et al.* (1) and two examples above illustrate distinct schemes that different cancer cells use to exploit the Eph-ephrin system to evade CIL and propel tumor cell dissemination. However, the examples may only represent a fraction of different strategies that can be adopted by cancer cells. In addition to these schemes, tumor cells often can directly blunt the anti-migratory functions of the Eph-ephrin system by decreasing the expression of the genes encoding either the ligands or the receptors (14, 24, 25). Thus, despite the seemingly complex and often paradoxical roles of the Eph-ephrin system in tumor cell migration and invasion, a unifying theme is emerging in which an evolving

cancer cell either directly ablates anti-migratory effects of the activated Eph receptors or co-opts Eph receptors into oncogenic partners that promote migration and invasion instead. Cancer cells harboring such alterations in the abundance or function of Ephs and ephrins can have a survival advantage and therefore are selected for during tumor progression.

Data by Astin *et al.* from in vitro studies and analyses of human specimens suggest a model depicted in Fig. 1 (1). A key question remains as to whether the scenarios actually occur in vivo. It will be important to determine whether EphB3 and EphB4 knockdown in PC-3 cells will lead to diminished invasiveness and metastatic potential in an appropriate xenograft system. Metastatic mouse prostate cancer models, including the prostate-targeted *PTEN* knockout (26) combined with stromal *Efnb2* deletion (13), will help elucidate whether ephrin-B2 can indeed promote invasion and metastasis. Similar xenograft and genetically engineered mouse models can also be used to interrogate the contribution of the Eph-ephrin system in other tumor types (14, 19, 21, 22). It is apparent that despite advances in the past two decades, we are still at the early stages in our understanding how Eph receptors and ephrins regulate tumorigenesis. The increasingly powerful animal model systems now make it possible to provide rigorous in vivo evidence to validate many of the suspected and often conflicting roles of Eph receptors and ephrins to tumor etiology and progression. Further progress in this emerging field could lead to new strategies to curb tumor cell development and dissemination by targeting the Eph-ephrin system.

Acknowledgments

I thank J. Schelling, L. Bruggeman, and members of my group for comments on this essay. **Funding:** Work from my laboratory is supported by grants from NIH (CA152371 and DK077876) and awards from Flight Attendant Medical Research Institute (FAMRI) and Prayers From Maria (PFMF) Foundation.

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Fig. 1.

In prostate cancer, cells exploit the Eph-ephrin system for invasion and metastasis. **(A)** Homotypic CIL. EphA2 and EphA4 activation by ephrin-As on opposing cells triggers repulsion of two colliding PC-3 cells through RhoA activation. **(B)** Loss of heterotypic CIL. EphB3 and EphB4 activation on PC-3 cells by stromal ephrin-B2 stimulate invasive migration of PC-3 cells through Cdc42 activation. For clarity, EphB–ephrin-B proteins in homotypic CIL and EphA–ephrin-A proteins in heterotypic CIL are not shown.