Eph Receptors and Ephrins in Cancer: Common Themes and Controversies

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Introduction

Ephrins and Eph receptors are key regulators of physiologic and pathologic processes in development and disease. Expression of Eph receptors is often elevated in many types of malignant tumors, yet their precise role in cancer is not well-understood. The purpose of the first "Eph/Ephrins and Cancer" meeting, held in Winston-Salem, North Carolina on June 25 to 26, 2008, was to discuss the common themes and controversies of Eph/ephrins in tumor initiation and progression. We highlight here most recent discoveries in the areas of structural biology, tumor biology, signaling, and novel therapeutics discussed at the meeting and directions for future research.

Structural Basis of Ephrin-Eph Interaction

Recent studies revealed the structural basis of the interactions between Eph receptors and ephrins or their inhibitors. Dimitar Nikolov (Memorial-Sloan-Kettering Cancer Center, New York, NY) reported the three-dimensional structure of EphA2/ephrinA1 receptor-ligand complex in a 1:1 molecular ratio. He proposed that whereas interaction between EphB2 and ephrin-B2 uses an induced fit mechanism, binding of EphA2 to ephrin-A1 resembles a key-lock configuration. Peter Kuhn (The Scripps Research Institute, La Jolla, CA) presented an example of an Eph receptor-antagonist structure. The crystal structure of the EphB4-TNYL-RAW complex revealed that the antagonistic peptide inhibits ephrin binding to the receptor by steric hindrance. These studies lay foundation for future structure-based therapeutic strategies. A summary of the structural studies on Eph/ephrins is presented in Supplementary Table S1.

Dual Role of Eph Receptors in Tumor Promotion and Tumor Suppression

Emerging evidence points to a dual role for Eph receptors in both tumor promotion and tumor suppression, as illustrated by tumor phenotypes in Eph/ephrin mutant mice (Supplementary Table S2). Jin Chen (Vanderbilt University, Nashville, TN) spoke on the effects of EphA2 deficiency in mouse mammary tumor virus (MMTV) driven Neu transgenic breast cancer model. Loss of EphA2 impairs both tumor initiation and lung metastasis. Reciprocal transplantation analyses further revealed cell type–specific roles of EphA2. In tumor cells, interaction between EphA2 and ErbB2 promotes cell proliferation through activation of the Ras-mitogen-activated protein kinase (MAPK) pathway, and enhances cell motility through increased RhoA GTPase activity. In vascular endothelial cells, EphA2 promotes tumor recruitment of blood vessels and tumor cell intravasation into the blood circulation, contributing to both tumor growth and metastasis. Angela Hess (Bloomburg University, Bloomburg, PA) discussed the roles of EphA2 in promoting tumorigenesis and angiogenesis, as well as vascular mimicry, a process in which tumor cells trans-differentiate into endothelial-like cells and incorporate into tumor vessels. Specific knockdown of EphA2 in melanoma cells reduces vascular mimicry and tumor cell invasiveness and proliferation.

Bingcheng Wang (Case Western Reserve University, Cleveland, OH) discussed a role for EphA2 in tumor suppression. Increased tumor number, tumor burden, and invasiveness were observed in EphA2-deficient gene-trap mice when these animals were induced to develop skin and liver cancer. There is increased MAPK and Akt phosphorylation in EphA2-deficient tumor cells, suggesting a role of EphA2 in inhibition of tumor cell proliferation. Elena Pasquale (Burnham Institute, La Jolla, CA) presented data on a role for EphB4 in tumor suppression in a xenograft model. Systemic delivery of ephrin-B2-Fc inhibits the growth of MDA-MB-435 tumor xenografts. EphB4 forward signaling apparently activates the Abl/Crk pathway to inhibit tumor cell growth and motility. To reconcile these differences, Jin Chen proposed a working model of dual roles of Eph receptors in tumor promotion and tumor suppression (Supplementary Fig. S1).

Signaling Mechanisms of Eph Receptors in Cancer

Soluble versus membrane-bound ephrins. Functional ephrin-Eph interactions are thought to require cell-cell contact or the clustering/oligomerization of an extracellular soluble ephrin. Jill Wykosky (Debinski's laboratory, Wake Forest University, Winston-Salem, NC) reported that a soluble ephrin-A1 monomer is capable of not only inducing Eph receptor phosphorylation but also of suppressing Ras-MAPK signaling and inhibiting tumor cell growth and motility. Soluble monomeric ephrin-A1 is detected in the medium of a number of tumor cell lines and is released from glioblastoma cells by metalloprotease cleavage upstream of the GPI-anchor. These data suggest a paracrine role for soluble monomeric ephrin-A1 in cancer and have important implications for the design of ephrin-A1–based cancer therapeutics.

Ligand-dependent versus ligand-independent signaling. Eph receptor expression has been associated with both tumor suppression and promotion. One possible reason for this paradox may reside in whether ligand activation is required. Bingcheng Wang presented data showing endogenous ephrin-EphA2 forward signaling inhibits Ras/MAPK in multiple tumor cell types. His laboratory also reported inhibition of Akt phosphorylation by EphA2 receptor signaling in liver cancer cells. Melanie Richter from the Pasquale laboratory reported that activation of the EphA4 receptor by ephrin in neuronal cells inhibits the activity of the Rap1

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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small GTPase through a PDZ domain-mediated interaction with the GTPase activating protein SPAR/E6TP1. Prominent Rap1 inactivation is also observed in melanoma and other cancer cell lines stimulated with ephrin-A ligands, potentially decreasing integrin-dependent cell adhesion.

A possible mechanism for ligand-independent Eph function in tumor promotion is that high levels of Eph receptors enable crosstalk with other oncogenic receptor tyrosine kinases (RTK), Jin Chen reported that the EphA2 receptor physically and functionally interacts with the ErbB2 RTK in MMTV-Neu breast cancer cells, promoting tumor cell proliferation, invasion, and motility through the Ras/MAPK pathway and the RhoA GTPase. Paola Chiarrugi (University of Florence, Florence, Italy) showed that overexpression of the EphA2 receptor in melanoma cells converts cell migration from a mesenchymal to amoeboid-like movement, a process that is independent of integrin and matrix metalloproteinase but dependent on RhoA GTPase activity. She proposed a model in which elevated levels of EphA2 in melanoma cells activate the RhoA GTPase through LMW-PTP–mediated inhibition of p190Rho-GAP, similar to the effects of EphA2 overexpression in mammary epithelial cells.

**Kinase-dependent versus kinase-independent signaling.** Rudiger Klein (Max Planck Institute, Munich, Germany) used his EphA4 knock-in models to illustrate that Eph receptor signaling has kinase-dependent as well as kinase-independent components. Mice expressing kinase-dead EphA4 had numerous neuronal targeting defects, suggesting that Eph kinase activity is important for neuronal development. Constitutively activated EphA4 mutant, however, was able to recapitulate wild-type activity in certain contexts but not others, indicating that both kinase-dependent and kinase-independent functions of Eph receptors are required for neuronal development. Further studies of kinase-active versus kinase-dead Eph receptor knock-in animal models will be required to determine the importance of Eph RTK activity for in vivo tumor progression.

**Forward versus reverse signaling.** A distinct feature of Eph receptors and ephrins is that they are capable of bidirectional signaling in both Eph receptor–expressing cells and ephrin ligand–expressing cells. Ira Daar (National Cancer Institute, Frederick, MD) discussed how studies in Xenopus reveal that ephrin-B1 reverse signaling regulates cell-cell adhesion. It seems that the amount of ephrin-B1 is critical: down-regulation of ephrin-B1 using morpholinos or overexpression of ephrin-B1 disrupts tight junction formation. He presented evidence that ephrin-B1 competes with the small GTPase Cdc42 for binding to Par6, a scaffold protein that regulates tight junctions. Phosphorylation of ephrin-B1 at tyrosine residue 310 by reverse signaling or through crosstalk with constitutively active fibroblast growth factor receptor disrupts ephrin-B1 binding to Par6, resulting in stabilization of cell-cell adhesion. This mechanism is likely to also be used by mammalian epithelial cells. Indeed, loss of ephrin-B1 or EphB in intestinal epithelial cells leads to intermingling of EphB and ephrin-B–expressing cells in the intestinal villi and to invasion of EphB-positive tumor cells into surrounding EphB–positive regions. Interestingly, these effects may be due not only to EphB-dependent repulsive signals in tumor cells but also to weakening of cell-cell junctions in the surrounding EphB-positive cells.

**Bidirectional signaling in tumor angiogenesis.** In contrast to the complex effects of Eph signaling in tumor cells, Eph-ephrin bidirectional signaling in the vascular endothelial cells promotes tumor angiogenesis. Jin Chen reported the mapping of phosphorylated tyrosine residues of EphA2 in vascular endothelial cells. Ephrin-A1–induced phosphorylation of Y587 and Y593 of the EphA2 receptor recruits the Vav2 and Vav3 guanine nucleotide exchange factors, whereas phosphorylation of Y734 provides a docking site for the p85 regulatory subunit of phosphatidylinositol-3-0H kinase. EphA2-null endothelial cells reconstituted with EphA2 mutants lacking these binding sites fail to activate Rac1 GTPase are defective in cell migration and assembly in vitro and are unable to incorporate into tumor vasculature in vivo. These results suggest a critical role for these tyrosine phosphorylation sites in transducing EphA2 forward signaling in angiogenesis. In support of this notion, Roberta Noberini in Pasquale’s laboratory has discovered two closely related small molecule compounds that bind specifically to the EphA2 and EphA4 receptors and inhibit receptor phosphorylation. These compounds also suppress EphA4-mediated growth cone collapse in retinal neurons and inhibit EphA2-mediated capillary-like tube formation in human umbilical vascular endothelial cells (HUVEC).

Reverse signaling through ephrin-B2 has long been known to regulate vascular remodeling during embryonic development. The Pasquale’s laboratory has now discovered a peptide, TNYL-RAW, which competes with ephrin-B2 for binding to the EphB4 receptor and blocks ephrin-B2/EphB4 bidirectional signaling. TNYL-RAW also inhibits capillary-like tube formation in HUVECs, demonstrating the potential of this peptide to inhibit tumor angiogenesis.

**Signaling and endocytosis.** Endocytosis of ligand-activated receptors has traditionally been considered a mechanism to attenuate or terminate signaling. However, a growing body of evidence suggests that many receptors signal in the endosome, linking endocytic membrane trafficking to intracellular signaling. Rudiger Klein has shown that engagement of ephrin and Eph receptors between two adjacent cells induces bidirectional endocytosis. Activation and phosphorylation of both ligand and receptor molecules are important for bidirectional internalization, as cytoplasmic truncation mutants of either ligand or receptor block endocytosis.

Ephrin-A1–induced EphA2 receptor endocytosis has been observed in tumor cells. Jill Wykosky and Dowdy Jackson (MedImmune/AstraZeneca, Gaithersburg, MD) reported that prolonged treatment of cancer cells with either ephrin-A1 or an activating anti-EphA2 monoclonal antibody induces EphA2 receptor internalization and degradation. It remains to be determined, however, whether internalized EphA2 receptor signals within endosomes and whether signaling strength and duration influence the decision of the internalized receptor to recycle to the membrane or to degrade. Another way to regulate EphA2 receptor signaling and degradation is through the Hsp90 molecular chaperone. Jennifer Isaccs (Medical University of South Carolina, Charleston, SC) reported that EphA2 forms a complex with Hsp90 and that chaperone activity is required to maintain the receptor in a signaling-competent state. Hsp90 inhibitors impair EphA2 signaling and promote receptor degradation, suggesting that Hsp90 inhibitors may be used therapeutically to antagonize the tumor-promoting effects of EphA2 in a variety of cancers.

**Therapeutics**

Several therapeutic strategies targeting Eph receptors have been recently developed for cancer treatment. Dowdy Jackson reported that an anti-EphA2 activating human monoclonal antibody, 1C1, was able to induce rapid tyrosine phosphorylation, internalization,
and degradation of the EphA2 receptor, providing an ideal vehicle for targeted delivery of cytotoxins into tumor cells. Indeed, 1C1 conjugated with the microtubule polymerization inhibitor (1C1-mcMMAF) significantly inhibits tumor cell growth both in vitro and in vivo without adverse effects. Waldemar Debinski presented a novel cytotoxin composed of the ephrin-A1 ligand conjugated to a genetically modified bacterial toxin, Pseudomonas exotoxin A (PE38QQR). Ephrin-A1-PE38QQR exhibits extremely potent and specific killing of EphA2-expressing cancer cells.

Other approaches include siRNAs, antagonistic peptides, small molecular inhibitors, and immunotherapy. Pavel Levin (M. D. Anderson Cancer Center, Houston, TX) showed that neutral liposome-coupled EphA2 siRNA reduces both tumor growth and metastasis in gemcitabine-resistant pancreatic tumors that over-express EphA2. The Pasquale laboratory has developed an EphB2 antagonistic peptide, TNYL-RAW, as well as two isomeric small molecule compounds that selectively inhibit ephrin binding to EphA4 and EphA2, as discussed above. Hideho Okada (University of Pittsburgh, Pittsburgh, PA) presented an ongoing phase I/II clinical trial with dendritic cell-based vaccines loaded with the human leukocyte antigen-A2 binding peptides from EphA2, IL-13Rα2, YKL-40, and GP100, which are commonly overexpressed antigens in glioblastomas. To date, the vaccine regimen has induced type-I CTL responses in 2 of 3 participants. Tumor biopsy after vaccination revealed intensive infiltration of CD8+ T cells and macrophages. These preliminary results show that EphA2-derived epitopes may represent important candidate vaccines to be tested in clinical trials for the treatment of cancer.

Although targeting Eph receptors holds great promise for therapeutic intervention in cancer, devising effective therapeutic agents can also be complicated by the bidirectional signaling of the Eph/ephrin system. Therapies designed to either activate or block an Eph receptor may also alter the signaling function of the ligand in adjacent cells. Further research in dissecting bidirectional signaling of Eph-ephrin in cancer is essential for developing successful therapeutic strategies.

**Perspective**

Tremendous progress has been made in the past few years in both understanding the role of Eph receptors and ephrins in cancer and designing therapeutic strategies for cancer therapy. However, the results presented at this meeting illustrate that we are only beginning to understand how this family of receptors and their ligands work in cancer. Elena Pasquale, the keynote speaker at the meeting, summarized the field and outlined the most pressing and important issues to be addressed. Thus, further studies are required to address the differences between ligand-dependent and ligand-independent receptor signaling, kinase-dependent and kinase-independent signaling, the importance of receptor phosphorylation versus kinase activity, ligand-induced signaling versus degradation, the signaling through endocytosis, and reverse signaling in tumor cells. Functional antibodies, chemical genetics, and systems biology, in conjunction with mouse models carrying targeted mutations, are critical tools for dissecting the complex roles of Eph molecules in biology of cancer. Understanding the role of Ephs and ephrins in both host and tumor tissue will enhance our ability to exploit this family in the development of new rational antiangiogenic and anticancer therapies.

**Disclosure of Potential Conflicts of Interest**

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