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EphA2 Mediates Ligand-Dependent Inhibition and Ligand-Independent Promotion of Cell Migration and Invasion via a Reciprocal Regulatory Loop with Akt

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SUMMARY

Both pro- and antioncogenic properties have been attributed to EphA2 kinase. We report that a possible cause for this apparent paradox is diametrically opposite roles of EphA2 in regulating cell migration and invasion. While activation of EphA2 with its ligand ephrin-A1 inhibited chemotactic migration of glioma and prostate cancer cells, EphA2 overexpression promoted migration in a ligand-independent manner. Surprisingly, the latter effects required phosphorylation of EphA2 on serine 897 by Akt, and S897A mutation abolished ligand-independent promotion of cell motility. Ephrin-A1 stimulation of EphA2 negated Akt activation by growth factors and caused EphA2 dephosphorylation on S897. In human astrocytoma, S897 phosphorylation was correlated with tumor grades and Akt activation, suggesting that the Akt-EphA2 crosstalk may contribute to brain tumor progression.

INTRODUCTION

Chemotactic cell migration plays an important role in tumor invasion by directing the spread of tumor cells toward growth factors. Tumor cells can move while attached to each other, which often occurs at early stage of cancer progression. During malignant progression, tumor cells can undergo epithelial to mesenchymal transition and adopt fibroblast-like cell migration or amoeboid movement, by which they migrate as individual cells (Friedl and Wolf, 2003). Although distinct in many aspects, different migration modes share similar signaling mechanisms. Phosphoinositide 3-kinases (PI3Ks) and Rho family of GTPases have been identified as key molecules in regulating cell migration. By generating PI(3,4,5)P3 at the proximity of chemoattractant, PI3Ks activity defines the leading edge of the migrating cell (Charest and Firtel, 2006), whereas Rho GTPases regulate the cytoskeletal reorganization that drives cell translocation (Hall, 1998; Ridley et al., 2003; Charest and Firtel, 2007).

As a primary target of PI3Ks, Akt has well-documented roles in promoting cell survival, proliferation, and growth (Engelman et al.,

SIGNIFICANCE

Akt is frequently activated in human glioblastoma and prostate cancer because of loss of PTEN or activation of components in PI3K/Akt pathway. We report that EphA2 is both an upstream negative regulator and a downstream effector of Akt. Phosphorylation of EphA2 by Akt promotes cell migration and invasion. In contrast, EphA2 stimulation by ephrin-A1 ligand suppresses Akt activation and inhibits cell migration. Thus, activation of PI3K/Akt pathway coupled with the loss of ephrin-As convert EphA2 from a tumor suppressor into a partner with Akt in promoting malignant progression. The data have important implications in developing therapeutic strategies targeting EphA2 for treatment of malignant tumors where PI3K/Akt pathway is activated.

2006; Manning and Cantley, 2007; Vivanco and Sawyers, 2002; Shaw and Cantley, 2006). Recent cancer genome analyses revealed Akt activation in vast majority of glioblastoma multiforme (GBM) through inactivation of phosphatase/tensin homolog deleted on chromosome 10 (PTEN), activation of receptor tyrosine kinases (RTKs), or amplification of Akt (TCGA Research Network, 2008; Parsons et al., 2008). PTEN loss and Akt activation also frequently occur in human prostate cancer (Tomlins et al., 2006). Increasing evidence shows that Akt signaling also regulates migration of many types of cells (Kim et al., 2001; Ju et al., 2007; Park et al., 2001; Meng et al., 2006; Shukla et al., 2007; Vasko et al., 2004; Kim et al., 2005; Higuchi et al., 2001). Akt has been proposed to modulate cell migration through several mechanisms, including activation and recycling of several integrins (Li et al., 2005; Somanath et al., 2007) and phosphorylation of an actin cross-linking protein Girdin at the cell leading edge (Enomoto et al., 2005; Jiang et al., 2008).

The 16 members of vertebrate Eph kinases constitute the largest subfamily of RTK superfamily. Interaction of Eph receptors and their membrane-bound ligands, called ''ephrins,'' leads to contact-dependent bidirectional signaling into the opposing cells, which regulates diverse developmental and physiological processes (Kullander and Klein, 2002; Miao and Wang, 2008; Pasquale, 2008; Poliakov et al., 2004). Perturbation of Eph/ephrin systems has been documented in different types of human cancer (Nakamoto and Bergemann, 2002; Pasquale, 2005; Pasquale, 2008). The exact role of Eph kinase in tumor etiology and progression has remained controversial. A case in point is EphA2 kinase, which is among the most frequently affected Eph kinases in human cancer. It is overexpressed in a variety of human malignancies and is associated with poor prognosis in several different tumor types, including GBM and cancers of prostate, kidney, and lung (Ireton and Chen, 2005). In several studies, overexpression of EphA2 has been linked to malignant progression (Fang et al., 2005; Zelinski et al., 2001). Paradoxically, activation of EphA2 kinase on tumor cells can trigger signaling events that are more consistent with a tumor suppressor. Thus, ligand stimulation of EphA2 inhibits integrin signaling, Ras/ERK pathway, and Rac GTPase activation, which is correlated with inhibition of cell proliferation and migration (Miao et al., 2000; Miao et al., 2001; Miao et al., 2003). Furthermore, EphA2 is found to be a target gene for p53 family of proteins and causes apoptosis when overexpressed (Dohn et al., 2001). Recent data also show that EphA2 is a key mediator of UV-induced apoptosis independent of p53 (Zhang et al., 2008). Further supporting a tumor suppressor role of EphA2, we recently report dramatically increased susceptibility to skin carcinogenesis in *EphA2* KO mice (Guo et al., 2006). The seemingly conflicting role of EphA2 kinase in the literature, either as an oncoprotein or a tumor suppressor, is an outstanding dilemma in cancer research today.

It is reported recently that EphA2 overexpression is frequently accompanied by the loss of its cognate ligands (Dodelet and Pasquale, 2000; Hafner et al., 2004; Wykosky et al., 2005; Macrae et al., 2005). In human breast cancer and mouse skin tumors, for example, there is an inverse relationship between EphA2 and ephrin-A1 expression (Macrae et al., 2005; Guo et al., 2006). This unbalanced expression pattern of EphA2 and ligands led us to investigate whether the unligated EphA2 can be selected during tumor progression as the result of a pro-oncogenic role.

RESULTS

Ligand-Independent Stimulation and Ligand-Dependent Inhibition of Cell Migration by EphA2 Kinase

We investigated how EphA2 overexpression may regulate chemotactic cell migration and invasion in the absence and presence of its ligand ephrin-A1 using glioma cells as a model system. Examination of public microarray database also revealed overexpression of EphA2 at mRNA levels in GBM (www.oncomine.org). To mimic the in vivo situation, we overexpressed EphA2 in U373 glioma cells. Figure 1A shows that U373 cells express moderate levels of endogenous EphA2 (Figure 1A), and infection with EphA2-expressing retrovirus increased the level by about one fold. In keeping with the lack of ligand expression, both endogenous and ectopic EphA2 in U373 cells showed low basal activation. Stimulation with exogenous ephrin-A1 caused rapid EphA2 activation, which was followed by degradation of the receptor itself, characteristic of most RTKs including Eph kinases.

Overexpression of EphA2 alone, in the absence of ligand stimulation, significantly enhanced serum-induced migration of U373 cells in a Boyden chamber cell migration assay (Figure 1B). In contrast, activation of EphA2 with its ligand ephrin-A1 significantly inhibited the chemotaxis of both vector control and EphA2-overexpressing cells. These data suggest that EphA2 has both ligand-independent stimulatory effects and ligand-dependent inhibitory effects on chemotactic cell migration. The diametrically opposite properties of EphA2 in regulating cell migration were also observed in other cell types, including HEK293, U87, A172, and PC-3M cells (see below).

In a reverse experiment, shRNA knocking down of EphA2 expression in U373 cells (Figure 1C) led to a significant reduction in ligand-independent chemotaxis toward serum (Figure 1D). The residual EphA2 on U373 cells was still able to mediate inhibition of cell migration upon ligand stimulation (Figure 1D). Similarly, shRNA knockdown of EphA2 in PC-3M prostate cancer cells significantly reduced ligand-independent chemotaxis (Figures 1E and 1F), whereas overexpression of EphA2 promoted it (see below).

Extensive previous investigations have established that a major function of Eph kinases is the ligand-dependent repulsion of migrating cells and axons both in vitro and in vivo (Pasquale, 2005). Our data demonstrate that EphA2 receptor can also promote cell migration in cooperation with growth factors in a ligand-independent manner.

EphA2 Is Both a Substrate and a Negative Regulator of Akt

In exploring the molecular mechanisms that mediate ligand-independent promotion of chemotaxis by EphA2, we screened several signaling pathways. Among them, the PI3K/Akt pathway stood out by robustly responding to serum in migrating U373 cells. As shown in Figure 1G, Akt became highly phosphorylated at both T308 and S473 sites upon serum stimulation. Cotreatment with ephrin-A1 completely blocked Akt activation. To test possible direct crosstalk between Akt and EphA2, we precipitated EphA2 and probed it with an antibody recognizing the consensus Akt substrate sites