EXPERIMENTAL CELL RESEARCH 315 (2009) 707–716

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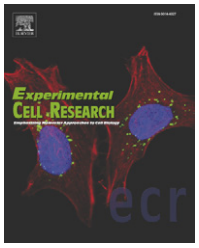

Review

ErbB receptors and cell polarity: New pathways and paradigms for understanding cell migration and invasion

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ARTICLE INFORMATION

Article Chronology:
Received 2 October 2008
Revised version received
19 October 2008
Accepted 20 October 2008
Available online 5 November 2008

Keywords:
ErbB
Cell polarity
Migration
Invasion
Metastasis
Signaling
Cell architecture

ABSTRACT

The ErbB family of receptor tyrosine kinases is involved in initiation and progression of a number of human cancers, and receptor activation or overexpression correlates with poor patient survival. Research over the past two decades has elucidated the molecular mechanisms underlying ErbB-induced tumorigenesis, which has resulted in the development of effective targeted therapies. ErbB-induced signal transduction cascades regulate a wide variety of cell processes, including cell proliferation, apoptosis, cell polarity, migration and invasion. Within tumors, disruption of these core processes, through cooperative oncogenic lesions, results in aggressive, metastatic disease. This review will focus on the ErbB signaling networks that regulate migration and invasion and identify a potential role for cell polarity pathways during cancer progression.

Contents

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doi:10.1016/j.yexcr.2008.10.034

Introduction

Dysregulation of oncogenes and tumor suppressors drives the malignant processes underlying human tumors. Disruption of normal cellular and tissue architecture and acquisition of poorly differentiated states are frequently associated with malignancy. How oncogenes and tumor suppressors interact with tumor cell architecture and microenvironment to induce invasive activity and metastatic spread remains largely unexplored. One of the most widely studied families of oncogenic proteins is the human epidermal growth factor receptor (EGFR) family. The EGFR family of type I receptor tyrosine kinases is comprised of four members: EGFR/HER1/ErbB1, Neu/HER2/ErbB2, HER3/ErbB3 and HER4/ErbB4 [1]. ErbB receptors have been implicated in the initiation and progression of various cancers, including those of the breast, ovary, lung, stomach, brain and bladder, where ErbB amplification and overexpression have been correlated with aggressive clinical outcome [2–4].

Activation of the ErbB receptors is accomplished through ligand binding to the extracellular domain, followed by receptor homo- or heterodimerization and tyrosine transphosphorylation of the C-terminal tail [5]. There are over 12 ligands that bind ErbB receptors and induce dimerization of distinct functional receptor pairs. Among these, epidermal growth factor (EGF), transforming growth factor alpha (TGF-α), and amphiregulin (AR) selectively bind to ErbB1 and primarily induce formation of ErbB1/ErbB1 homodimers and ErbB1/ErbB2 heterodimers [6]. Betacellulin (BTC), heparin-binding EGF (HB-EGF) and epiregulin (EPR) comprise a second class of ligands that bind both ErbB1 and ErbB4 [7]. The neuregulins (NRG1–4) encompass the third class of ligands and activate ErbB3 and ErbB4 [8–10]. Currently, no known ligands have been identified for ErbB2. However, ErbB2 is the preferred heterodimerization partner of the other ErbB family members and ErbB2-containing heterodimers have the strongest signaling output [11,12]. The great diversity of ligands and receptor dimer pairs allows the activation of numerous signaling pathways that coordinately regulate complex processes including developmental growth control and adult homeostasis.

In the inactive state, the extracellular domains of the ErbB receptors exist in a closed inhibited form [13]. However, ligand binding induces a conformational change in the extracellular domain that allows receptor dimerization and subsequent autophosphorylation of the tyrosine residues located in the cytoplasmic tail. Interestingly, ErbB2 is constitutively present in the active state and capable of forming heterodimers with ligand-bound ErbB1, 3 or 4 receptors for downstream signaling. ErbB3 lacks a functional kinase domain and must be paired in a heterodimeric fashion [14]. The phosphorylated ErbB receptors serve as molecular integrators through either direct phosphorylation of target molecules or by serving as scaffolds for adaptor proteins [15]. Activation of ErbB receptors leads to initiation of the mitogen activated protein kinase (MAPK) cascade, activation of phospholipase C gamma (PLCγ) and phosphatidylinositol 3 kinase (PI3K), as well as induction of the small GTPases Rho, Rac and Cdc42, among many other effectors [16]. Several reports have demonstrated a role for these pathways in ErbB-induced cell migration.

However, the interaction between ErbB signaling and cell polarity pathways and its role in migration and invasion are poorly understood. In polarized epithelial cells, ErbB receptors are present in the basolateral surface, along with E-cadherin, gap junctions and desmosomes [17]. ErbB receptors are neither seen in the apical surface, containing glycoproteins and microvilli, nor in tight junctions, containing occludins and claudins. This spatial asymmetry is lost during initiation and progression of cancer, and in particular, during metastasis. In this review we will discuss a potential role for cell polarity pathways during progression of ErbB-driven tumors.

ErbB receptor function in cancer progression

Overexpression or mutations in ErbB receptors have been identified in numerous human tumors and cancer cell lines [18]. ErbB1 was first cloned and its sequence determined from A431 epidermoid carcinoma cells, facilitated by its amplification and mRNA overproduction [19–22]. Subsequent analysis has demonstrated that ErbB1 is amplified in 40% of glioblastomas [23]. More recently, mutations in ErbB1 have been detected in non-small cell lung carcinomas, medulloblastomas and ovarian cancers [24,25]. Activated ErbB1 can also function as a tumor promoter through autocrine signaling in lung, prostate and gastrointestinal stromal tumors coexpressing TGF-α [26–28]. Kinase inhibitors (Erlotinib, Gefitinib) and monoclonal antibodies (Cetuximab, Panitumumab) targeting ErbB1 are currently in clinical use against non-small-cell lung cancer, colorectal cancer, pancreatic cancer and squamous-cell carcinoma of the head and neck [29].

Since the discovery of *neu* as an oncogene in a rat carcinogen-induced tumor model in 1981 [30], several lines of evidence demonstrate that this gene product possesses oncogenic properties. *Neu* was subsequently shown to be similar to the v-erbB oncogene from the avian erythroblastosis virus and the mammalian EGFR [31,32]. One of the strongest pieces of evidence for its role in cancer came from the observation that *neu is* amplified in human breast and ovarian cancers and cancer-derived cell lines [2,33]. These studies laid the foundation for the development of targeted therapeutic strategies against *neu/ErbB2/HER2*. Today, an antibody against ErbB2, Herceptin (Trastuzumab), and a kinase inhibitor, Lapatinib (Tykerb), are used in the clinic for the treatment of ErbB2-positive breast cancers [34].

Several mouse models expressing *neu* in the mouse mammary gland have allowed us to gain critical insights into the mechanisms by which ErbB2 induces mammary tumor initiation and progression. Expression of activated *neu* (*neuT*; a V664E mutation that promotes spontaneous receptor dimerization) in the mammary glands of mice under the direction of the mouse mammary tumor virus (MMTV) promoter led to the formation of mammary adenocarcinomas [35,36]. These mice developed multifocal tumors involving the entire mammary gland, suggesting that additional genetic changes were not required for *neuT*-induced tumorigenesis. Strikingly, mice expressing wild-type *neu* in the mammary epithelium developed focal mammary tumors after long latency, with a low penetrance of lung metastasis [37]. The presence of transgene-positive regions of normal mammary epithelium surrounding the tumors supported the hypothesis that overexpression of wild-type *neu* alone is not sufficient for tumor development. Further investigation into the mechanism of tumor formation in these transgenic mice showed that additional genetic changes were necessary for tumo-

rigenesis, including novel activating mutations in the *neu* gene itself [38].

In human breast cancer, amplification of ErbB2 correlates with a decrease in time to disease relapse and overall patient survival, with highly expressing tumors predictive of worse clinical outcome [2]. Despite the clinical relationship between ErbB2 expression and poor patient outcome, several lines of evidence suggest that amplification of ErbB2 is an early event in tumorigenesis, and not solely associated with invasive disease. As discussed previously, mouse models of wild type ErbB2 fail to transform the entire mammary epithelium and form only focal tumors after long latency that rarely metastasize, requiring the acquisition of secondary genetic events. Additionally, we demonstrated that forced dimerization of ErbB2 in three dimensional (3D) mammary acinar structures induced proliferation and lumen filling, but did not lead to degradation of the basement membrane or invasive behavior [39]. More than 45% of human non-invasive breast carcinomas can posses amplified and over-expressed ErbB2, suggesting that additional genetic events are required for metastatic progression of ErbB2-positive tumors [40]. It is likely that understanding the genetic and biochemical events that cooperate with ErbB2 to induce metastatic disease will provide novel insights to control the spread of ErbB2-positive tumors.

ErbBs regulate epithelial cell migration and invasion

The ability of growth factors of the EGF family to stimulate epithelial cell migration has been appreciated for over 20 years. In an early experiment, EGF was shown to stimulate migration of untransformed small intestine cells through gelatin-coated filters [41]. This induced movement was directional and specific for EGF and not other growth factors. Similarly, EGF and TGF-α were found to increase cell migratory capacity of epidermal keratinocytes [42]. In renal carcinoma-derived cell lines, EGF can induce production of matrix metalloproteinase-9 (MMP-9) and *in vitro* invasion [43]. In addition, stimulation of glioma-derived cells with TGF-α or breast cancer-derived cells with heregulin induces invasive progression, suggesting that multiple EGF ligands and their receptors are likely to be involved in metastatic disease [44,45].

ErbB receptors are also known to play critical roles during migration and invasion of cancer-derived cell lines *in vitro* and *in vivo*. Prostate carcinoma cells expressing ErbB1 injected into athymic mice formed tumors that are capable of metastasizing to the lung [46]. However, treatment of these mice with an inhibitor of ErbB1-mediated cell motility (through inhibition of phospholipase Cγ), but not proliferation, resulted in tumors with decreased invasiveness. Similarly, inhibition of ErbB1 in a mouse model of pancreatic cancer attenuated tumor volume and liver metastasis through a mechanism involving inhibition of tumor-associated angiogenesis [47]. Tumor cells with decreased ErbB1 signaling produced fewer proangiogenic molecules, resulting in increased endothelial cell apoptosis. In a breast cancer model utilizing injection of mammary adenocarcinoma cells into the mammary fat pad, ErbB1 or ErbB3 overexpression was found to increase intravasation and metastasis to the lungs, while having minimal impact on tumor growth [48,49]. These studies have highlighted, both *in vitro* and *in vivo*, the crucial roles of ErbB receptors in metastatic cancer progression.

Signaling pathways downstream of ErbB receptors and cell migration

Several signaling cascades are activated in response to specific ErbB homo- or heterodimer pairs, resulting in the cytoskeletal reorganization and gene transcription necessary for migration and invasion of epithelial cells [16]. These involve stimulation of PLCγ, small G-proteins of the Ras superfamily, PI3K and Src. How these proteins coordinately regulate epithelial cell migration and invasion in response to ErbB activation is critically important for understanding the metastatic process, and can lead to novel targets for therapeutic intervention.

Control over actin dynamics allows ErbB receptors to induce cell shape changes required for cell movement, either through direct actin binding or modulation of actin-regulatory proteins [50]. Upon EGF stimulation, ErbB1 phosphorylates PLCγ1, inducing enzyme activation and subsequent production of the second messengers inositol trisphosphate (IP_3) and diacylglycerol (DAG) from phosphatidylinositol 4,5-bisphosphate (PIP_2) [51]. Inhibition of PLC, or a dominant-negative PLCγ1 fragment attenuates EGF-induced migration in NR6 fibroblasts demonstrating that PLCγ1 activation plays a critical role during cell migration [52]. Experiments performed in invasive prostate and breast tumor cells lines confirmed the requirement of PLCγ1 in migration, showing that chemical inhibition of PLCγ1 reduced invasion through Matrigel [53]. PLCγ is also involved in ErbB heterodimer-induced migration and invasion [54]. Whereas ErbB1 and ErbB2 homodimers were unable to induce invasion in mammary epithelial cells, dimerization of ErbB1 and ErbB2 led to PLCγ-dependent invasion. The hydrolysis of PIP_2 allows dissociation of several actin-binding proteins from the plasma membrane, including cofilin, profilin and gelsolin, which then function to regulate actin dynamics and cell motility [55]. In response to EGF, mammary adenocarcinoma cells display increased actin nucleation within extending lamellipodia through recruitment and activation of cofilin [56,57] in a PLCγ1 dependent manner [58,59].

PI3K, composed of regulatory (p85) and catalytic (p110) subunits, binds to ErbB receptor family members, and functions in growth factor signaling pathways crucial for proliferation and migration. Inhibition of PI3K blocks EGF-induced DNA synthesis [60]. Upon EGF stimulation, PIP_3 levels are rapidly increased, with a coordinate short-lived PI3K activation. Inhibition of Ras attenuates the EGF-induced PIP_3 production, as well as lamellipodia formation, suggesting that PI3K is downstream of Ras in the process of EGF-mediated migration [61]. In breast cancer cells, PI3K activity is required for HRG-mediated cell migration and actin reorganization. Furthermore, PI3K is necessary for HRG-induced p21-activated kinase 1 (PAK1) activity, as well as formation of an ErbB2/actin/PAK1 signaling complex [62]. PAK1 controls several aspects of epithelial cell invasion, including stress fiber formation, focal adhesion maintenance, MAPK and JNK signaling cascades, and vascular endothelial growth factor (VEGF) expression [63–65]. PAK1 regulates actin dynamics by phosphorylating LIM-kinase, which in turn phosphorylates cofilin, leading to decreased depolymerization of F-actin [66]. PI3K signaling is also required for EGF and HRG-mediated upregulation of β1-integrin, an adhesion protein important for regulation of epithelial cell polarity and implicated in tumor cell migration and invasion [67]. Several small molecule PI3K inhibitors showing

anti-tumoral effects have recently entered clinical trials for various solid tumors [68].

The non-receptor tyrosine kinase Src is overexpressed and highly activated in a number of human cancers, including those of the breast, lung and colon [69]. Although overexpression of Src alone appears insufficient for tumor initiation, Src is required for EGF-induced proliferation. Src directly interacts with the catalytic domain of ErbB2 and ErbB2-induced mammary tumors show increased levels of Src activation [70–72]. In a panel of human colon cancer cell lines, ErbB1 and ErbB2-regulated Src activity was associated with highly metastatic cell populations [73], suggestive of a role for Src in the invasive phenotype. Inhibition of Src kinase activity blocked invasion and lung metastasis of ErbB2-overexpressing breast cancer cells, suggesting a role for Src in ErbB2-mediated invasiveness [74]. In non-tumorigenic human mammary epithelial cells grown in 3D culture, co-overexpression of ErbB1 and Src induced aberrant proliferation and disrupted polarity, leading to increased migration and invasion [75]. Several downstream effectors of Src in response to ErbB activation have been identified, including focal adhesion kinase (FAK) and protein kinase C alpha (PKCα) [76,77]. FAK co-localizes with activated ErbB2/3 receptors at cell protrusions, and is required for focal adhesion complex formation, cell transformation and invasion. In addition, FAK and ErbB2 are co-expressed in 50% of circulating tumors cells in the peripheral blood from breast cancer patients, suggesting a role for these proteins in migration and malignant progression [78]. PKCα, a Src substrate, is required for ErbB2-induced upregulation of urokinase-type plasminogen activator receptor (uPAR) and cell invasion [79].

Several other cellular factors cooperate with ErbB receptors to induce migratory and invasive phenotypes. A cDNA expression screen for inducers of migration in growth-arrested 3D mammary acini containing regulatable-ErbB2 uncovered transforming growth factor β (TGF-β) as a mediator of invasion [80]. ErbB2 and TGF-β cooperate to activate extracellular signal-related kinase (ERK), which is required for the migratory phenotype. Furthermore, mice co-expressing ErbB2 and TGF-β in the mammary epithelium develop more lung metastasis than those expressing ErbB2 alone, and TGF-β induces motility and invasiveness in an ErbB2-dependent manner [81,82]. Recently, several specific ErbB2 phosphorylation events have been found to be required for TGF-β-induced cancer cell invasion. These sites bind to the adaptor protein ShcA. A dominant-negative ShcA molecule blocks migration and invasion induced by TGFβ; however, the signaling pathway responsible for the cell movement downstream of ShcA remains unclear [83].

Small G-proteins of the Ras superfamily regulate cell shape and motility in response to extracellular signals. Upon EGF stimulation, fibroblasts undergo Rho-dependent actin stress fiber reorganization and Rac-dependent membrane ruffling [84,85]. Similarly, Cdc42 is required for EGF-induced lamellipodia protrusion and migration in breast cancer cells [86]. In A431 cells, EGF stimulates an invasive phenotype dependent upon AP-1 transcription factor activation. Inhibition of AP-1 activity blocks EGF-induced Rho and Rac activation and cell motility [87]. EGF also induces Rac-dependent migration through activation of MAPK and induction of metalloproteinase ADAM10. ADAM10 cleaves the adhesion molecule CD44, thereby increasing migratory ability of cells [88]. ErbB2 induces invasion in breast cancer cells by Rac-dependent downregulation of α4 integrin [89]. Together, these results suggest a critical role for small G-proteins and cytoskeletal regulators in the processes of migration and invasion.

ErbB receptors, cell polarity and invasion

Changes in cellular cytoarchitecture have been strongly associated with migration and invasion of cancer cells. For example, loss of the epithelial state and acquisition of a mesenchymal phenotype (through an epithelial–mesenchymal transition, EMT) is thought to play a critical role during the processes of invasion and migration. Changes in the normal apical–basal polarity of epithelial cells are thought be necessary for the acquisition of a mesenchymal state. Overexpression or activation of ErbB receptors has been shown to disrupt normal polarity and lead to loss of cell–cell junctions in epithelial cells. The precise mechanism by which ErbB receptors deregulate normal epithelial architecture to promote invasion remains to be understood. However, the relationship between ErbB receptors and cell polarity signaling are beginning to be unraveled in several model organisms, including *Drosophila*, *C. elegans* and mammalian cells in culture.

Molecular regulators of cell polarity

Cell polarity is a highly conserved evolutionary process. Genetic analysis in *Drosophila* and *C. elegans* has identified several genes that are involved in establishment and maintenance of apical–basal polarity in epithelial cells. These determinants are conserved in mammals and can be broadly grouped into three classes: the Par complex, the Scribble complex and the Crumbs complex. The Crumbs complex, containing Crumbs (Crb), PALS1 and PATJ, acts to specify the apical domain, while the Scribble complex, consisting of Scribble (Scr), Discs large (Dlg) and Lethal giant larvae (Lgl), opposes the function of apical proteins to specify the basolateral domain [90]. The Par complex consists of Par3, Par6 and atypical protein kinase C (aPKC) and functions to establish and maintain the stability of the apical region and the apical–basal border [91]. The aPKC activity associated with the Par complex plays a critical role in defining the apical and basolateral domains. During the early stages of the polarization process the kinase activity of aPKC is kept low by its interaction with Par6, which in turn is bound to members of apical and basolateral complexes such as Crb and Lgl. As the polarization process continues, binding of Cdc42-GTP to Par6 promotes activation of the Par6-associated aPKC kinase activity and induces phosphorylation and release of the members of the Crumbs and Scribble polarity complexes. For example, aPKC phosphorylates Crb and directs Crb to the apical surface while phosphorylation of Lgl results in release of Lgl from the apical membrane and restricts it to the basal domain [91,92]. Release of Lgl from the Par6 complex is accompanied by recruitment of Par3 to form the functional Par complex that defines the apical–lateral border and the formation of tight junctions [91,92]. aPKC also phosphorylates another serine/threonine kinase, Par1, to release it from the apical–lateral border and localize it to the basolateral membrane where it is thought to promote assembly of E-cadherin junctions [92–94]. Par1 at the lateral membrane phosphorylates Par3 and prevents its interaction with Par6/aPKC to restrict assembly of the Par complex at the lateral membrane. Thus, the scaffolding and kinase functions of the members of the polarity

machinery coordinate the highly intricate interactions between the Scr, Crb and Par protein complexes during establishment of apical–basal polarity (Fig. 1). These processes are further coordinated with changes in cytoskeletal reorganization, reorientation of intracellular organelles and activation of small G-proteins, protein kinases and phosphatases, resulting in diverse biological outcomes during development. It is becoming clear that we have just begun to scrape the surface in our understanding of the mechanisms that regulate establishment of apical–basal polarity and there remains much to be understood about the how polarity regulators interact with each other and with the cell's cytoskeletal machinery.

In addition to their role in the establishment of apical–basal polarity, polarity pathways have been strongly implicated in the asymmetric cell division process and organ morphogenesis in *C elegans* and *Drosophila* [95]. In mammals, polarity proteins are known to regulate asymmetric cell division in T cells and cell migration during neural tube closure and heart tube formation [96,97]. The complex molecular mechanisms by which polarity proteins signal to downstream effector molecules to establish and maintain polarity during organogenesis in mammals is an area of intense interest.

Polarity proteins as regulators of cell migration and invasion

Multiple lines of evidence in *Drosophila* suggest that inactivation of polarity proteins can promote cancer formation and progression, suggesting that polarity pathways can function as tumor suppressors. *Drosophila* epithelia lacking Scr, Lgl or Dlg display increased cell proliferation, in addition to loss of cellular organization [98]. However, when cells lacking Scr are surrounded by normal epithelia, the ectopic cell proliferation induced by loss of Scr function is balanced by increased apoptosis through a Jun N-terminal kinase (JNK)-dependent process. The increased cell death is inhibited by coexpression of oncogenic Ras or Notch [99] demonstrating a cooperation between polarity proteins and oncogenes in regulating tumorigenesis in *Drosophila*. Similarly, a screen designed to identify promoters of metastatic growth in a noninvasive tumor model driven by activated Ras in *Drosophila* detected numerous polarity proteins, including Scr, Dlg and Lgl [100]. Recently, cooperation between Ras and Scr has been demonstrated in mammalian cells, a process regulated by MAPK signaling [101]. In humans, Scr and Dlg are targeted for degradation by the E6 oncoprotein of high-risk human papilloma virus, suggesting that Scr and Dlg play a role in human papillomavirus (HPV)-induced cervical cancer [102,103]. Most notably, several polarity proteins show reduced expression in human cancers. In colon neoplasias, expression of Scr and Dlg are downregulated in regions of disrupted cell polarity and disorganized tissue architecture [104]. Expression of *Hugl-1*, the human homologue of Lgl, is diminished in malignant melanoma, as well as solid tumors of the breast, prostate, lung, ovary and colon [105–107]. Furthermore, loss of *Hugl-1* expression in endometrial cancer was shown to correlate with increased lymph node metastasis as well as poor clinical outcome [108]. Together, these observations strongly suggest a role for polarity proteins during initiation and progression of cancer in humans.

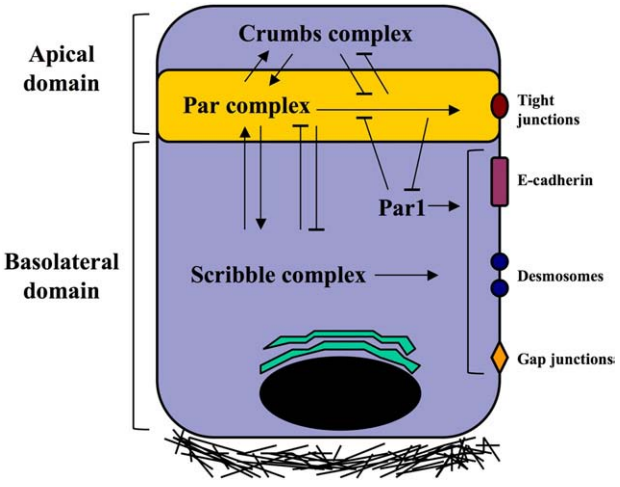

Fig. 1 – Reciprocal interactions between polarity complexes initiate and maintain apical–basal polarity in epithelial cells. The Scribble complex consists of Scribble (Scr), Discs large (Dlg) and Lethal giant larvae (Lgl). The Crumbs complex is made up of Crumbs (Crb), Pals1 (Protein associated with lin-7) and Pals1-associated tight junction protein (PATJ). The Par (portioning defective) complex consists of Par3, Par6 and atypical protein kinase C (aPKC).

Polarity pathways as mediators of ErbB signaling

We recently demonstrated direct cooperation between members of the ErbB family and polarity proteins during transformation of polarized mammary epithelial cells. ErbB2 disrupts apical–basal polarity and induces proliferation when activated in 3D mammary acinar structures [109]. ErbB2 interacts with the Par6/aPKC complex and this interaction is required for ErbB2-induced disruption of polarity and inhibition of apoptosis. This interaction is not required for ErbB2 induced cell proliferation. A detailed investigation into the direct substrates of the Par6/aPKC complex during ErbB2-induced disruption of 3D acini is likely to uncover novel drug targets for treatment of ErbB2-positive breast cancers.

ErbB2-induced changes in polarity are also regulated by the β4 integrins. Tumor onset and metastatic progression of ErbB2-induced tumors are delayed in mice lacking the intracellular signaling domain of the β4 integrin. The β4 integrin promotes ErbB2-driven mammary tumorigenesis through activation of the transcription factors c-Jun and signal transducer and activator of transcription 3 (STAT3) [110]. ErbB2 and β4 integrin cooperate by recruiting the tyrosine kinase Src, which acts to phosphorylate both proteins, enhancing ErbB2 kinase activity. Activation of the ErbB2/β4 integrin complex promotes STAT3-dependent transcription, which is required for disruption of cell polarity and the tight junction complex.

ErbB1 directly regulates tight junction assembly through phosphorylation of Par3 in a process requiring activation of the tyrosine kinases Src and Yes [111]. Phosphorylation of Par3 disrupts the Par3-LIM kinase 2 (LIMK2) interaction, allowing LIMK2 to inactivate cofilin and promote tight junction assembly. In bladder carcinoma cells, EGF stimulation creates a pool of free, uncomplexed tyrosine phosphorylated β-catenin that interacts less strongly with E-cadherin, resulting in decreased cell–cell adhesion

and increased migratory capacity [112]. EGF also disrupts cell–cell adhesion by inducing an epithelial–mesenchymal transition through depletion of E-cadherin, both at the level of caveolin-dependent endocytosis and Snail-mediated transcriptional repression [113]. The role polarity proteins play in ErbB-induced migration and invasion is an underexplored area of investigation that is likely to provide novel insights into how ErbB receptors regulate metastatic disease.

Polarity proteins as regulators of migration and invasion

Studies in other signaling systems have identified polarity proteins as critical regulators of migration and invasion. For example, TGF-β induces disruption of tight junctions and EMT in NMuMG mouse mammary epithelial cells [114] by interacting with the Par6 polarity complex. The type II TGF-β receptor phosphorylates Par6, resulting in recruitment of the E3 ubiquitin ligase, Smurf1, which in turn targets RhoA for proteasomal degradation and tight junction disassembly. In rat proximal epithelial cells, TGF-β induces disruption of apical–basal polarity by downregulating Par3 [115] suggesting that TGF-β interacts with the Par complex in multiple ways to disrupt cell polarity and promote migration.

The Par complex has also been shown to regulate astrocyte migration [116]. Upon scratching through a monolayer culture, astrocytes at the wound edge extend protrusions perpendicular to the wound, reorient their microtubule cytoskeleton and Golgi, and begin directed migration. This process involves the activation of Cdc42, resulting in the recruitment of Par6 and aPKC to the leading edge. The Par6/aPKC complex interacts directly with glycogen synthase kinase 3β (GSK3β) to promote interaction of APC (adenomatous polyposis coli) with the plus ends of microtubules specifically at the leading edge [117] and regulate the polarity of cell migration. APC in turn can recruit Dlg1 at discrete puncta within the plasma membrane to promote microtubule polarization and cell migration [118]. In addition, Scr and the guanine nucleotide exchange factor βPIX regulate migration of astrocytes by promoting activation of Cdc42 at the leading edge [119]. The precise relationship between the Scr, Dlg and Par complexes during astrocyte migration remains to be understood.

Conclusions and perspectives

Cancer pathologists have long noted the striking clinical correlation between poorly differentiated primary tumors and less favorable patient prognosis. Poorly differentiated tumors display features including loss of glandular organization, loss of cell–cell adhesion and loss of polarity. Although very little is known about the processes underlying loss of polarity in human tumors, accumulating evidence from several model systems has demonstrated a role for regulation of cell polarity as a critical event during migration and invasion. In addition to the known pathways that control the cytoskeleton and cell adhesion, polarity pathways are an emerging class of regulators of cancer cell biology that warrants further study (Fig. 2). Detailed analysis into the mechanisms by which oncogenes and tumor suppressors regulate mammalian epithelial polarity is therefore a novel area of investigation that will deepen our understanding of cancer biology and uncover innovative paradigms for the development of strategies for therapeutic intervention.

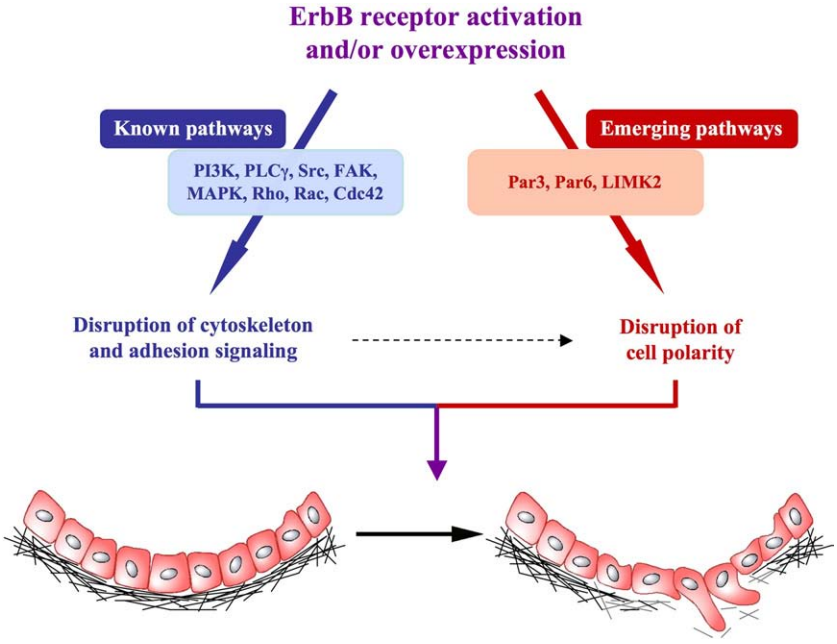

Fig. 2 – ErbB receptor activation and overexpression disrupt mammalian epithelial polarity through known and emerging pathways. ErbB-regulated cytoskeletal and adhesion signaling is mediated by numerous proteins, including phosphatidylinositol 3 kinase (PI3K), phospholipase Cγ (PLCγ), Src, focal adhesion kinase (FAK), mitogen activated protein kinase (MAPK), and the small molecular weight GTP-binding proteins Rho, Rac and Cdc42. Recently, ErbB receptors have been shown to disrupt cell polarity through Par3, Par6 and LIM domain kinase 2 (LIMK2).

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