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EPS8, an Adaptor Protein Acts as an Oncoprotein in Human Cancer

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http://dx.doi.org/10.5772/54906

1. Introduction

A spectrum of cellular activities including proliferation, differentiation and metabolism are controlled by growth factors and hormones. The effects of many growth factors are mediated and achieved via transmembrane receptor tyrosine kinases [1,2]. Following ligand binding, the intrinsic catalytic activity of the growth factor receptor tyrosine kinases (RTKs) is augmented. The autophosphorylated RTKs then transmit signals through their ability to recruit and/or phosphorylate intracellular substrates. Thus, identification and characterization of proteins that associate with and/or become tyrosyl phosphorylated by RTKs become critical to delineate RTKs-mediated signaling pathways. A variety of methodologies have been developed and applied to search for substrates of tyrosine kinases including RTKs [3-8]. Here, we focus on <u>E</u>GFR <u>p</u>athway <u>s</u>ubstrate no. <u>8</u> (Eps8), a putative target of epidermal growth factor (EGF) receptor (EGFR), and discuss its biological function as well as its implication in human cancers. Given Eps8-elicited effects contribute to neoplasm, its potential as a therapeutic target for cancer treatment is anticipated.

1.1. The identification of Eps8

To dissect EGFR-mediated signaling, Di Fiore's laboratory took advantage of immuno-affinity purification of an entire set of proteins phosphorylated in EGF-treated EGFR-overexpressing cells, followed by generation of antisera against the purified protein pool. With these antisera, bacterial expression libraries were immunologically screened. Several murine cDNAs (*eps* clones) representing genes encoding substrates for EGFR were obtained [7,8]. One of them was designated *eps8* [9,10]. The human *eps8* locus was mapped to chromosome 12p12-p13 via fluorescence in situ hybridization (FISH) [11] and confirmed by a computer search of a genomic DNA database utilizing human *EPS8* cDNA sequence (GeneBank accession number U12535) [12]. Similarly, the murine *Eps8* genomic DNA

sequences are defined on chromosome 6G1 [12]. The computer analysis also revealed three genes (designated as *Eps8R1*, *Eps8R2*, and *Eps8 R3*) that are highly homologous to both human and murine *eps8* [12]. Due to space limitation, studies on these Eps8Rs will not be described in this review.

Proteins with molecular weights of 97 kDa and 68 kDa were recognized by Eps8 antibodies and referred to as the two Eps8 isoforms (p97[Eps8] and p68[Eps8]) [12,13]. The exact nature (an alternatively spliced or a proteolytic product) of p68[Eps8] was still elusive and remained to be established. By contrast, p97[Eps8] has been well characterized and is the only isoform detected in human cancer cells so far and hereinafter is called Eps8.

Eps8 contains 821 amino acids and exhibits several features of interest: a split pleckstrin homology (PH) domain, a putative nuclear targeting sequence, an Src homology 3 (SH3) domain, several proline-rich regions, and a degenerated SH2 (dSH2) domain at the N-terminus (amino acids 55-96) (Figure 1). The proline-rich Eps8 has been demonstrated to interact with the SH3 domain of Src [13] and the SH3 of Eps8 was reported to associate with Shb [14], Shc [15], RN-tre [16], and Abi1 (also known as E3b1) [17]. The SH3 domain of Eps8 binds to a consensus sequence of proline-X-X-aspartate-tyrosine (PXXDY) instead of the canonical X-proline-X-X-proline (XPXXP) consensus, implicating the existence of a novel family of SH3-containing proteins [18]. Furthermore, two Eps8 proteins were demonstrated to form an interwound dimer with their SH3 domains being located at the interface [19]. In addition to the aforementioned Eps8-interacting proteins, Eps8 was also shown to bind directly to EGFR [20], Sos1 [21], and IRSp53 [22,23].
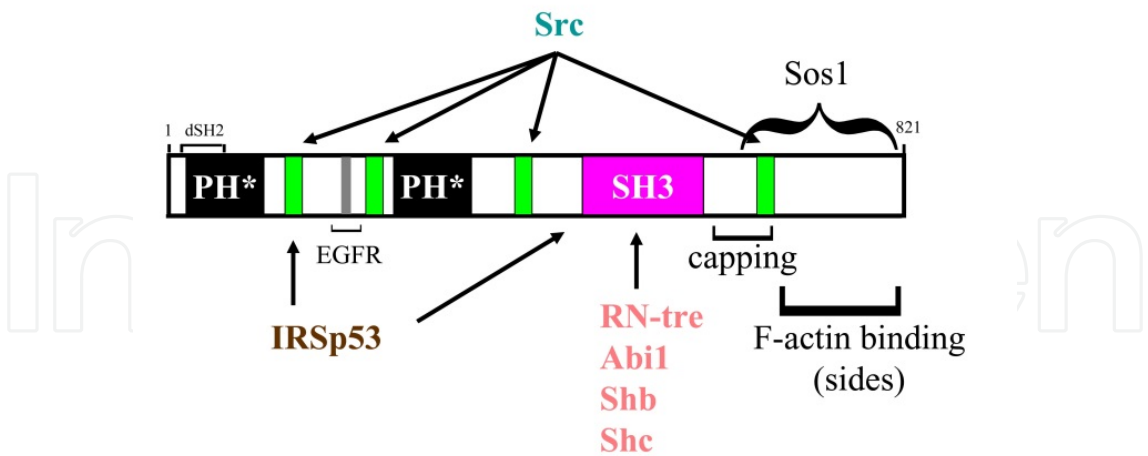

Figure 1. Structural organization of Eps8. The N-terminal split PH domain (PH*) is important for Eps8 membrane recruitment. Regions in Eps8 responsible for association with Eps8 binding partners are indicated. Green boxes indicate proline-rich regions; gray box indicates the potential nuclear localization signal. dSH2 indicates the position of a degenerated SH2.

Strikingly, Eps8 is an actin capper whose barbed-end capping activity resides in its C-terminal effector domain and is regulated by protein-protein interaction with Abi1 [24,25].

In addition to lamellipodia [26], Eps8 also localizes to other actin-rich structures such as PIP2-enriched vesicles, phagocytic cups and comet tails behind intracellular pathogens [25]. Two Abi1-containing complexes were reported to have multiple roles in regulating dynamic actin turnover [27]. One contains Nap1, Sra (PIR125), Hspc and WAVE, the other contains Eps8, Sos1, and the p85 subunit of PI3K. While the former may activate the Arp2/3 complex, the latter may regulate Rac activation and enable Eps8 to cap actin filament barbed ends. Of note, Eps8 turns out to be a novel capping protein capable of side-binding and bundling actin filaments [28]. The C-terminal Eps8 region (aa648-aa821) encompasses five helices (H1-H5). The N-terminal amphipathic helix (H1) is largely responsible for Eps8 capping activity, while a compact, globular domain composed of H2-H5 is critical for filament bundling. Thus, Eps8, as a bifunctional actin remodeller, regulates actin-based mobility and endomembrane cellular trafficking through its capping activity, whereas it contributes to proper structural organization of gut microvilli via its mediated actin filament bundling [28].

1.2. Eps8 is an oncoprotein

Known as the first identified oncoprotein, the tyrosine kinase activity of Rous sarcoma virus (RSV)-encoded v-Src is essential for its mediated transformation. Under its influence, a spectrum of proteins has their phosphotyrosine content increased and are viewed as putative v-Src substrates [3,29]. Intriguingly, some of them including Eps8 turn out to be the substrates for EGFR as well [13]. Elevated expression and tyrosyl phosphorylation of Eps8 are observed in v-Src transformed cells [13]. Overexpression of Eps8 confers the ability of fibroblasts to form foci in culture and to grow tumors in mice [30]. Consistent with its oncogenic potential [30], Eps8 attenuation retards cellular growth of fibroblasts expressing v-Src [31].

Like SH2 and SH3, PH is a common motif shared by signaling molecules, which mediates protein-protein and protein-lipid interactions. Mounting evidence indicates that via PH-mediated binding to either phospholipids [32,33] or membrane-associated proteins [34,35], the association between PH-containing molecules and plasma membrane is accelerated. The prominent examples are Akt/PKB that possesses a compact PH and PLCγ that retains a split PH. Like PLCγ, Eps8 contains a split PH (Figure 1), which does not weaken its linkage to the cell membrane. Remarkably, an intact split PH is indispensable for Eps8 membrane targeting as well as its mediated oncogenesis. In contrast, Eps8 with truncated PH fails to be recruited to plasma membrane and confers transforming ability [30].

To confirm the involvement of Eps8 in the development of cancer, the correlation between its expression and cell proliferation of various human cancer cell lines were examined [36]. In human colon cancers, there is a positive correlation between Eps8 expression and mitogenesis, implicating the importance of Eps8 in colon cancer formation. Indeed, Eps8 attenuation in high Eps8 expressing cells (i.e. SW620 and WiDr) reduces cellular growth [36]. In contrast, ectopically expressed Eps8 in low Eps8 expressing cells (i.e. SW480) or Eps8-attenuated SW620 promotes proliferation. In addition, relative to controls, there is a significant (>50%)

suppression of anchorage-independent growth in *eps8* siRNA-expressing SW620, and reintroduction of Eps8 rescues these defects. Concurrently, attenuation (or overexpression) of Eps8 significantly reduces (or promotes) the growth of tumors inoculated into nude mice [36]. In addition to fibroblasts and colon cancer cells, Eps8 also plays a pivotal role in cervical cancer formation. This can be supported by reduced proliferation and tumorigenesis in Eps8-attenuated SiHa and HeLa cells cultured in dishes or inoculated in mice [37].

1.3. Eps8-interacting proteins

As an adaptor, Eps8 intreracts with a variety of signaling proteins such as EGFR, Src, Abi1, RN-tre, Shb, and IRSp53 to exert its biological functions. The detail and implication of the interaction between Eps8 and each of its above-mentioned partners are described below.

1.3.1. EGFR

Via serial deletion mutants of Eps8 and EGFR, Castagnino et al. [20] determined the minimal region of Eps8 (aa298-aa362) and EGFR (juxtamembrane region, aa648-aa688) is required for Eps8-EGFR interaction. Obviously, this interaction is not via classical pTyr-SH2 or proline-rich region-SH3 binding manner. Interestingly, the EGFR-binding region in Eps8 is rich in basic amino acids while multiple glutamic acid residues are found in the juxtamembrane region of EGFR. Although overexpression of Eps8 enhances EGF-mediated mitogenesis, the underlying mechanisms are not yet resolved. Nevertheless, the intimately correlated transforming ability of EGFR mutants and the level of tyrosyl phosphorylated Eps8 suggested the importance of tyrosyl phosphorylation of Eps8 in cellular transformation [38].

1.3.2. Src

Oncogenic Src not only enhances Eps8 expression but also its tyrosyl phosphorylation [13]. In GST-pull down experiments, fusion proteins with Src SH3, but not SH2 domain directly interact with Eps8, presumably via its proline-rich sequences. Through *in vitro* Src kinase reactions, Eps8 was further confirmed to be directly phosphorylated by Src. Notably, simply augmenting Eps8 expression in murine C3H10T1/2 fibroblasts can not elevate its tyrosyl phosphorylation and promote cell proliferation despite these cells being tumorigenic. Given Eps8 attenuation reduced cell growth in v-Src transformed cells [31], Src-mediated Eps8 phosphorylation might be important for cell proliferation. To date, the residues on Eps8 mediated by Src are still elusive. Whether Eps8 retains the same receptor (i.e. EGFR)- and nonreceptor (i.e. Src)-mediated sites becomes an interesting issue.

1.3.3. Abi1

ABI1 (also known as E3b1) was identified as an Eps8-binding protein by screening a human embryonic fibroblast (M426) cDNA expression library with Eps8 SH3 [17]. It contains a proline-rich sequence at its C-terminus followed by an SH3 domain. The PXXDY consensus is identified at aa389-aa393 [17]. In addition to Eps8, it also interacts with Abl tyrosine

kinase and is a human homologue of previously identified murine Abl-interactor 1 (Abi1) [39]. Both Abi1 and Abl contain SH3 and proline-rich sequences. These two proteins associate through the SH3 domain of Abi1 and the proline-rich region of Abl [17,39]. Overexpression of Abi1 decreased cell proliferation in NIH3T3-based EGFR overexpressors [17] while overexpression of the murine Abi1 suppressed v-Abl transforming activity [39]. Abi1/Eps8 associate with Sos1 and enable the latter protein to act as a guanine nucleotide exchange factor (GEF) of Rac to facilitate membrane ruffling in response to the activation of Ras and PI-3 kinase [21,40]. Strikingly, PI3K was also present in this multi-protein complex containing Abi1/Eps8/Sos1 to activate Rac [41]. It is noteworthy that alternatively spliced Abi1 fused with MLL has been identified in a human acute myeloid leukemia patient, suggesting the role of Abi1 in leukemogenesis [42].

1.3.4. RN-tre

RN-tre (Related to the N-terminal of *tre*) was originally identified by utilizing Eps8 SH3 as a probe to fish out its binding protein(s) in a bacterial cDNA expression library generated from NIH3T3 cells [16]. RN-tre shares a homology domain (TrH) with *tre* oncoprotein at its N-terminus and contains an extended proline-rich sequence at the C-terminus. The PXXDY consensus is present at aa725-aa729 (GeneBank database, accession number D13644; [16]). Overexpression of full-length RN-tre in NIH3T3 cells did not cause cell transformation [16]. However, NIH3T3 cells transfected with a plasmid encoding RN-tre C-terminal truncated mutant (lacking aa463-aa828) exhibited growth advantage in both soft agar and in low serum (1%) cultured medium [16]. The TrH domain in RN-tre possesses a Rab5 GTPase-activating protein (GAP) activity [43]. RN-tre overexpression suppressed EGF internalization via its Rab5 GAP activity [43]. Interactions with Grb2 [44] and/or Eps8 [43] are required for RN-tre inhibiting EGF receptor endocytosis. The association between RN-tre and Eps8 inhibits the ability of Eps8 to complex with Abi1 and Sos1 and attenuates EGF-mediated Rac activation [43].

1.3.5. Shb

Shb is an adaptor protein containing amino-terminal proline-rich sequences and a C-terminal SH2 domain [45]. Unlike Abi1 and RN-tre, there is no PXXDY consensus found in Shb sequences. Shb interacts with Eps8 SH3 domain and phosphorylated PDGFβ-receptor and FGF receptor-1 (via Shb SH2 domain) [14]. Although the role of Eps8-Shb interaction in cancer biology was not defined yet, overexpression of Shb reduced Eps8 expression [46] and induced apoptosis of NIH3T3 cells cultured in low serum [47]. Markedly, while Shb overexpression reduced tumor growth of PC3 prostate cancer cells [48], Shb attenuation sensitized SVR endothelial tumor cells to apoptotic agents such as cisplatin and staurosorine [49].

1.3.6. IRSp53

IRSp53 (Insulin/IGF-1 Receptor tyrosine kinase Substrate of 53 kDa; [50]) was also designated as brain-specific angiogenesis inhibitor 1-associated protein 2 (BAIAP2) [51,52].

Its interaction with Eps8 was originally observed from a GST-IRSp53 SH3 pull-down experiment [22] and confirmed by a yeast two-hybrid screening utilizing N-terminal Eps8 sequences as a bait to search for Eps8 binding partners [23]. IRSp53 contains an N-terminal I-BAR (inverse-Bin-Amphiphysin-Rev) domain, followed by a CRIB, an SH3, a WW-binding sequence (WWB), and a PDZ (post synaptic density 95, disc large, zonula occludens-1) domain. The I-BAR domain might form dimers and induce membrane curvature depending on the shape of I-BAR dimer (reviewed in [53,54]). In addition to the originally identified 53-kDa protein IRSp53S (designated IRSp53 from now on); three other IRSp53 isoforms (designated IRSp58M, IRSp53T, and IRSp53L) were identified in human cells. In contrast, only three murine homologues (i.e. mIRSp53S, mIRSp58M, and mIRSp53T) were detected [55]. Interaction between IRSp53 SH3 domain and Eps8 N-terminal proline-rich sequences (aa207-aa221) activates Rac and results in cell motility and invasion of cancer cells [22]. Interestingly, in addition to the SH3 domain, PPPDY (aa468-aa471; GeneBank accession number NP_001138360) within the IRSp53 C-terminal WWB domain also participates in the association with Eps8 SH3 [23]. The interplay between Eps8 and IRSp53 is important for Src-mediated STAT3 activation that leads to cell proliferation in cancer cells [23].

1.4. Eps8-mediated signal transduction

Mechanistic studies reveal that serum-induced ERK activation is involved in Eps8-mediated transformation since coexpression of dominant negative MEK1 blocks its induced oncogenesis. Consistent with the PH domain of Eps8 being critical for its oncogenic potential and membrane targeting, PH-truncated Eps8 is unable to trigger ERK activation in response to serum. These data corroborate the importance of the PH domain of Eps8 for its membrane association, ERK activation and its ability to transform cells [30].

Src becomes activated when its SH3 and/or SH2 are occupied. Given Eps8 interacts with Src SH3 *in vitro* [13] and the Src SH3-binding region of Eps8 resides in its multiple proline-rich containing sequences, Eps8 is thereby speculated to increase Src enzymatic activity. In agreement with the mechanism underlying Src activation, Eps8 does elevate Src activity. This can be verified by diminished Eps8, which reduces Src activation as reflected by decreased Src Pi-Y416, which can be restored by ectopically expressed Eps8 [36]. Remarkably, Eps8 also regulates the activity and the expression level of FAK. While activation of FAK can be achieved by Src-mediated phosphorylation [56], its elevated expression relies on Akt/mTOR/STAT3 Pi-S727 pathway, which also modulates cyclin D1 expression [36].

Expressing small interfering RNA of *eps8* in HeLa and SiHa cells impedes G1-phase progression. In addition to cyclin D1, attenuated Eps8 also reduces expression of cyclins D3 and E, elevates accumulation of p53 and p21$^{Waf1/Cip1}$, and inhibits hyperphosphorylation of Rb. Reintroduced siRNA-resistant *eps8* into Eps8-attenuated HeLa and SiHa cells reverses the described alteration, indicating that the effect of Eps8 on the mentioned cell cycle modulators is specific. Eps8 facilitates p53 degradation and decreased levels of Eps8 block this process and cause p53 accumulation. Studies of the turnover rate of p53 reveal that Eps8

attenuation significantly increases the half-life of p53 in HeLa cells from ~12 min to ~40 min [37]. It is noteworthy that via accelerated degradation of p53 as well as increased activation of Src and Akt, Eps8 enables cervical cancer cells to be resistant to chemotherapeutic agents.

With a genome-wide screen, matrix metalloproteinase 9 (MMP9) along with the forkhead transcription factor FOXM1 and a cohort of its target genes encoding the cell cycle mediators and the chemokine ligands (i.e. CXCL5 and CXCL12) are upregulated by Eps8 through a PI3K/Akt-dependent mechanism [57]. Through degradation of extracellular matrix components as well as processing of cytokine and growth factors, Eps8-elicited MMP9 plays a critical role in the migratory and invasive phenotype of squamous cell carcinoma (SCC) [58].

It is well established that Eps8 forms a complex with Sos1 and Abi1 to transmit signals to Rac from receptor tyrosine kinases [21,26,40] and PI3K [41]. By its N-terminal region, Abi1 is recruited to the tips of filopodia and lamellipodia in motile cells [59], and associates to WAVE-1 [60], the actin regulatory protein, further supporting its importance in actin remodeling. Notably, Eps8 also interacts with RN-tre (a specific Rab5 GAP) to modulate Rab5 activity, inhibit receptor internalization and prolong receptor signaling at the cell membrane [43].

IRSp53 is an adaptor protein that plays an important role in actin cytoskeleton reorganization. By pull-down assays, Eps8 is demonstrated as an IRSp53-binding protein. Through its N-terminal proline-rich sequence, Eps8 directly associates with the SH3 domain of IRSp53. This Eps8/IRSp53 complex reinforces the formation of a trimolecular Rac-GEF complex (i.e. Eps8/Abi1/Sos1) to synergistically activate Rac and contribute to cell motility [22]. Through an independent yeast two-hybrid screening, IRSp53 was identified as an Eps8-interacting protein. In addition, its C-terminal SH3/WWB-containing domain (aa376-aa521) was essential and sufficient for Eps8 association [23]. Strikingly, Eps8 modulates IRSp53 expression in cells transformed with v-Src and attenuation of IRSp53 results in reduced cell proliferation in culture and reduced tumor formation in mice, which can be partly rescued by ectopically expressed IRSp53. Src drives the formation of Eps8/IRSp53 complex, which leads to activation of Akt, ERK, STAT3 and enhancement of cyclin D1. This signaling event not only occurs in v-Src transformed cells but also in EGF-stimulated cells. Notably, Eps8/IRSp53 is important in both cell proliferation and cell mobility [23].

2. Prospective study of Eps8 as an anti-cancer drug targeting protein

As a signaling intermediate, Eps8 has been demonstrated to be critical in proliferation and control of actin dynamics that lead to increased mitogenesis and motility of various tumor cells (see below). Now, we will not only summarize the published reports regarding the role of Eps8 in human tumors, but also discuss its potential to become a novel tumor marker as well as a therapeutic target for cancer treatment.

2.1. Aberrant overexpression of Eps8 in human cancer

Eps8 overexpression confers the ability of murine fibroblasts to form foci in culture and to grow tumors in mice [30]. Its oncogenic potential invites the speculation that Eps8 might be

involved and contribute to development of human cancers. Indeed, following is the published reports concerning the role of Eps8 in various human tumors.

2.1.1. Breast cancer

Accumulated evidence indicates that gene amplification contributes to activation of oncogenes, and is often associated with tumor progression, acquired drug resistance and poor prognosis [61]. By an integration of serial analysis of gene expression with cDNA array comparative genomic hybridization, Yao et al. [62] aimed to identify and characterize amplicons and their targets in both *in situ* and invasive breast carcinomas. Their characterization of the 12p13-p12 amplicon identified four putative oncogenes including Eps8. Compared with a panel of normal mammary epithelial cells, Eps8 was confirmed to be overexpressed in breast tumors with 12p13 amplification by quantitative RT-PCR as well as fluorescence *in situ* hybridization.

2.1.2. Pancreatic cancer

Pancreatic ductal adenocarcinoma (PDAC) is an extremely aggressive maliganacy and characterized by early invasion and metastasis [63]. In pancreatic ductal cells, Eps8 colocalizes to the tips of F-actin filaments, filopodia, and the leading edge of cells. Its knockdown alters actin-based cytoskeletal structures and cell shape, impairs cell-cell junctions and protrusion formation.

Studies of the expression of Eps8 in cell lines derived from various tumor stages demonstrate that the levels of Eps8 are higher in cell lines derived from metastases and ascites as compared to those from primary tumors [64]. These results suggest that Eps8 plays a critical role in the metastatic potential of PDAC.

2.1.3. Thyroid cancer

Papillary thyroid carcinoma (PTC) is a common thyroid maliganacy whose biological behavior varies widely. By studying the expression profiles of eight matched pairs of PTC and normal throid tissues, Eps8 was identified as one of the overexpressed genes in PTC [65]. Notably, Griffith et al. [66] performed a meta-review of thyroid cancer biomarkers from a large number of published studies, identified twelve candidate diagnostic biomarkers, including Eps8. Unfortunately, no follow-up study, even at the RNA level, has confirmed Eps8 upregulation in thyroid lesions. Thus, inclusion of Eps8 as a diagnostic marker for thyroid cancer requires further investigation.

2.1.4. Oral cancer

Oral squamous cell carcinoma (OSCC) is a common maliganacy. Its local invasion and regional lymph node metastases usually cause early death. Using expression microarrays, the *eps8* gene was identified as being overexpressed in OSCC cell lines compared with normal oral keratinocytes. Despite attenuation of Eps8 in VB6, BICR56, and CA1 OSCC cells

does not inhibit cell proliferation, but it does impair the cell spreading and migration toward fibronectin. Not surprisingly, Eps8 was upregulated in a subset of OSCCs where it correlated significantly with lymph node metastasis. Knockdown of Eps8 suppressed αVβ6-, and α5β1-integrin-dependent Rac1 activation and inhibited tumor cell invasion in an organotypic model of OSCC [67].

2.1.5. Ovarian cancer

Among gynecologic cancers, ovarian cancer has the greatest motality rate due to its metastasis [68]. Not relying on the vasculature for metastasis as seen in solid tumors, ovarian maligancy is confined within the abdominal cavity and extends to adjacent organs and/or disseminate throughout the peritoneal cavity [69]. Lysophosphatidic acid (LPA), a growth factor-like phospholipid, retains migration-stimulating potential and is present at high levels in ascites of ovarian cancer patients. LPA-mediated cell motility is confirmed to play an important role in ovarian cancer metastasis, and the integrity of Sos1/Eps8/Abi1 tricomplex is essential for LPA-induced Rac activation, cell migration and metastatic colonization. Strikingly, only coexpression of the three members of the tricomplex correlates with advanced stages and shorter survival of ovarian cancer patients [70]. For ovarian cancer metastasis, these findings not only indicate the tricomplex is a reliable marker, but also suggest targeting the tricomplex can be developed as a therapeutic approach.

2.1.6. Colorectal cancer

Colorectal cancer (CRC) is the most common gastrointestinal cancer and one of the leading causes of cancer mortality worldwide. Preferentially increased Eps8 in the advanced stage of human CRC specimens is detected. Intriguingly, simultaneous up-regulation of Eps8, Src and FAK in CRC is observed and these three proteins are positively correlated as indicated by Spearman rank correlation. This is in agreement with Eps8 modulating the expression of FAK via mTOR/STAT3 pathway [35].

2.1.7. Cervical cancer

Cervical carcinoma evolves slowly from intraepithelial neoplasia to invasive carcinoma and is the second most common malignancy among women [68]. Through clinicopathologic examination and immunohistochemical staining, an intimate correlation between Eps8 abundance and the aggressiveness (local lymph node metastasis or parametrium invasion) of early-stage cervical cancer is established. Concurrently, Eps8 expression inversely correlates with the survival rate of cervical cancer patients [36].

2.1.8. Esophageal cancer

A thorough analysis of both esophageal squamous cell carcinoma and esophageal adenocarcinoma revealed 4-6 fold increase in expression of Eps8 in esophageal cancers compared to adjacent normal tissues. Notably, unlike colon cancer, higher Eps8 expression

in tumors as compared to their nearby normal tissue was independent of grade of esophageal cancer [72].

2.1.9. Pituitary tumor

Comprising some of the most common intracranial neoplasms, pituitary tumors can either be detected clinically (i.e. acromegaly and amenorrhea) or become clinically silent such as those of the gonadotrope lineage [73]. By DNA microarrays, *eps8* is identified as an overexpressed transcript (5.9-fold) in pituitary tumors compared with normal controls. Xu *et al*. [74] demonstrated that overexpression of Eps8 in gonadotrope pituitary cells results in activation of ERK and Akt, which provide proliferative stimulation and antiapoptotic protection respectively. Remarkably, the above mentioned signaling components are upregulated in human pituitary tumor tissues suggesting a functional significance of Eps8 in human pituitary tumorigenesis.

2.2. The potential of Eps8 attenuation in cancer treatment

Accumulated evidence revealed the significance of Eps8 in tumor development and metastasis. According to the elucidated mechanisms underlying Eps8-mediated transformation, suppressed expression of Eps8 as well as disruption of the Eps8-containing signaling complex become two promising strategies to inhibit tumorigenesis.

2.2.1. Suppressing expression of Eps8

Considering Eps8 is an oncoprotein whose overexpression is closely linked to tumor formation, suppressing its expression might provide a means to treat and eradicate tumors. Strategies published to decrease Eps8 expression are described below.

2.2.1.1. Histone deacetylase inhibitors

Epigenetic modulation of gene expression is implicated in cancer development. Emerging evidences indicate the acetylation status of histones controls the acess of transcription factors to DNA and influences gene expression. Histone acetylation and deacetylation are mediated by histone acetyl transferases (HATs) and histone deacetylases (HDACs) respectively. HDAC inhibitors are well documented to promote differentiation, growth arrest and apoptosis of cancer cells with minimal effects on normal tissues. Notably, HDAC inhibitors not only decompact histone/DNA complex, but also influence acetylation status and function of nonhistone proteins [75]. A number of HDAC inhibitors have entered preclinical and early clinical studies. Although these compounds were chosen for their ability to inhibit histone deacetylation, they still had widely varying potency and HDAC isoenzyme specificity as well as different effects on acetylation of nonhistone proteins. To date, the prominent targets of HDAC inhibitors include HDACs, p21$^{WAF1/CIP1}$, p53, death receptor proteins (i.e. TNF-α, Fas and TRAIL receptors), HIF-1α, and VEGFR [75].

Trichostatin A (TSA), an antifungal agent, is a HDAC inhibitor. The reversal of v-Src-mediated transformation by TSA is attributable to its suppression of Eps8 expression. RT-

PCR and Northern analyses reveal the significant decrease of *eps8* transcripts in TSA-treated v-Src-expressing cells relative to control cells [31]. Similar reduction of Eps8 is also obtained when butyrate, another well known HDAC inhibitor is applied (unpublished result). These data indicate Eps8 can be added to the growing list of HDAC inhibitor targets and its downregulation contributes to the antineoplastic effects of HDAC inhibitors.

2.2.1.2. Mithramycin

Mithramycin (MIT, also known as mithracin, aureolic acid and plicamycin), a polyketide produced by various soil bacteria of the genus *streptomyces*, is an inhibitor that blocks the binding of the Sp-family transcription factors to the GC box [76]. To date, expression of several proto-oncoproteins such as Met, Myb, Myc, Ras and Src can be suppressed by MIT. Interestingly, in several cancer cell lines, MIT also reduces the protein and mRNA levels of Eps8 in dose- and time-dependent manners [77]. Considering the mechanistic action exerted by MIT, the promoter composition and the transcriptional regulation of *eps8* gene warrant further investigation.

2.2.1.3. Small interference RNA or short hairpin RNA methodology

Expressing either small interference RNA (siRNA) [31,36,37] or short hairpin RNA (shRNA) of *eps8* [58] in tumor cells efficiently inhibits the expression of Eps8. Decreased levels of Eps8 alters the behavior of cancer cells such as (1) suppressed v-Src-mediated transformation in fibroblasts [31], (2) reduced colonocyte proliferation and motility in colon cancer cells [36], (3) retarded cell cycle and decreased chemoresistance in cervical cancer cells [37], (4) impaired tumorigenicity of HNSCC cells in xenograft assays [58]. Hence, using siRNA (or shRNA) methodology to suppress Eps8 expression in tumor cells might block their progression, invasion and increase their chemosensitivity to anti-cancer drugs.

2.2.2. Disruption of the formation of Eps8-containing complexes

Eps8 exerts its effects through the formation of various complexes. The well-studied ones are Sos1/Eps8/Abi1 and Eps8/IRSp53. Formation of both complexes results in Rac activation and promotes cell proliferation and cell motility.

2.2.2.1. Disruption of Sos1/Eps8/Abi1 complex formation

Sos1/Eps8/Abi1 tricomplex is well established to mediate Rac activation and whose integrity is required for LPA-stimulated cell motility and metastatic colonization in ovarian cancer cells. Given coexpression of Sos1, Eps8 and Abi1, but not any one alone, correlates with advanced stages as well as shorter survival of ovarian cancer patients [70], silencing any member of Sos1/Eps8/Abi1 tricomplex and/or targeting this tricomplex can be developed as a therapeutic approach for tumor metastasis.

2.2.2.2. Disruption of Eps8/IRSp53 complex formation

Eps8/IRSp53 complex, occurs at the leading edge of motile cells, augments Sos1/Eps8/Abi1 trimolecular complex formation, and synergistically activates Rac. Inhibiting its formation

reduces the mobility and invasiveness of fibrosarcoma cells [22]. Strikingly, elevated activity of Akt, ERK, STAT3, and augmented expression of cyclin D1 are also dictated by Eps8/IRSp53 that can be reduced by SU6656, an inhibitor of Src family kinases [23]. Of note, through activation of Src, EGF induces the formation of Eps8/IRSp53 and activation of STAT3 in HeLa cells [23]. Since the association between Eps8 and IRSp53 is Src-dependent and might be a physiological event in relaying EGF signaling, strategies that interrupt the interaction between Eps8 and IRSp53 can be developed and applied for cancer treatment. Specifically designed peptides, naturally occurring or artificially synthesized chemicals that block the association between Eps8 and IRSp53 might fulfill the purpose and become novel cancer therapeutics.

3. Concluding remarks

Considering a spectrum of proteins bind to Eps8 and play important roles in cell proliferation and migration (Figure 2), Eps8 upregulation is thus expected to cause human carcinogenesis. Indeed, aberrant overexpression of Eps8 is closely linked to many types of human cancer. Although Eps8-mediated signal transduction is gradually being resolved, several important questions still remain unanswered. For instance, tyrosyl phosphorylation of Eps8 mediated by either Src or EGFR is not addressed yet. Where are these tyrosine residues located? How does their phosphorylation contribute to abnormal cell proliferation and motility in cancer cells? In addition, Eps8 possesses a nuclear localization signal (Figure 1). However, to date there are no reports regarding the role of nuclear Eps8 and how the nuclear localization of Eps8 is regulated? Tremendous work needs to be done before these questions get answers.
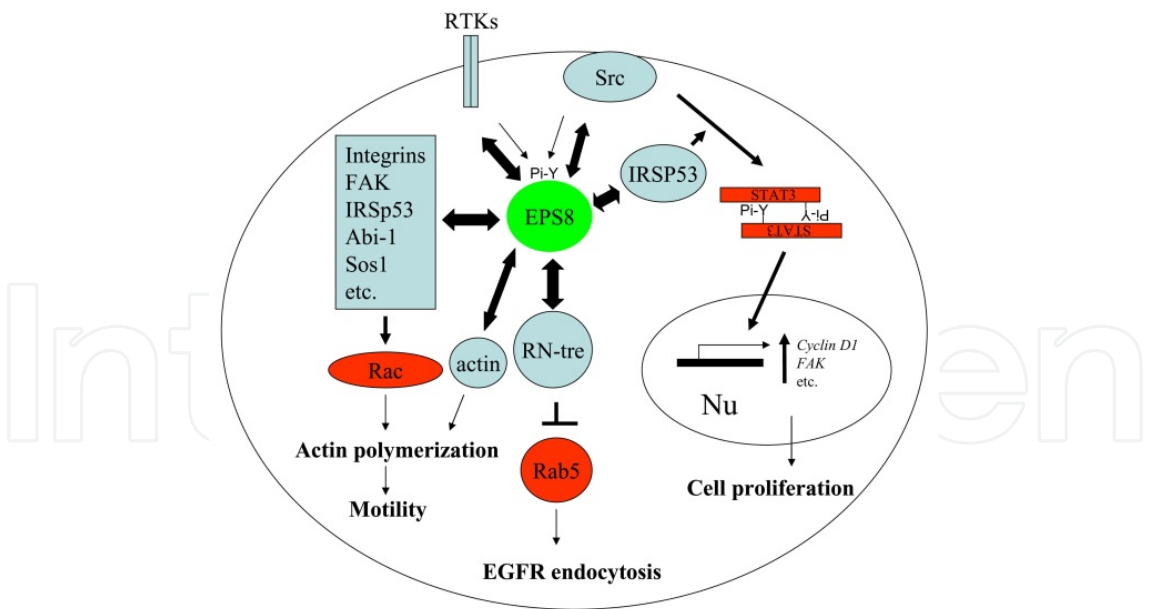

Figure 2. The signals transmitted by Eps8. Active EGFR and Src phosphorylate Eps8 and induce Eps8-IRSp53 interaction that facilitates Src-mediated STAT3 Pi-Y705 and dimerization, resulting in the increased transcription of *Cyclin D1* and *FAK* and in cell cycle progression. By binding to RN-tre, Eps8 reduces Rab5 activity and hinders EGFR endocytosis. In addition to its actin-capping activity, Eps8 interacts with proteins listed in the square box, activates Rac, promotes membrane actin polymerization, and increases motility. Nu: nucleus.

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Acknowledgement

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