

A review on Eph/ephrin, angiogenesis and lymphangiogenesis in gastric, colorectal and pancreatic cancers

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Abstract

Erythroprotein-producing human hepatocellular carcinoma receptors (Eph receptors) compose a subfamily of transmembrane protein-tyrosine kinases receptors that takes part in numerous physiological and pathological processes. Eph family receptor-interacting proteins (Ephrins) are ligands for those receptors. Eph/ephrin system is responsible for the cytoskeleton activity, cell adhesion, intercellular connection, cellular shape as well as cell motility. It affects neuron development and functioning, bone and glucose homeostasis, immune system and correct function of enterocytes. Moreover Eph/ephrin system is one of the crucial ones in angiogenesis and lymphangiogenesis. With such a wide range of impact it is clear that disturbed function of this system leads to pathology. Eph/ephrin system is involved in carcinogenesis and cancer progression. Although the idea of participation of ephrin in carcinogenesis is obvious, the exact way remains unclear because of complex bi-directional signaling and cross-talks with other pathways. Further studies are necessary to find a new target for treatment.

Keywords: Eph/ephrin system; lymphangiogenesis; angiogenesis; gastric cancer; colon cancer; pancreatic cancer

Submitted Apr 27, 2017. Accepted for publication Jun 30, 2017.

doi: 10.21147/j.issn.1000-9604.2017.04.03

View this article at: <https://doi.org/10.21147/j.issn.1000-9604.2017.04.03>

Eph/ephrin structure, classification and signaling

Erythroprotein-producing human hepatocellular carcinoma receptors (Eph receptors) compose a subfamily of transmembrane protein-tyrosine kinases receptors. Those receptors consist of an extracellular part, combining ligands [Eph family receptor-interacting proteins (ephrins)], a transmembrane part and an intracellular part with kinase domain (1,2). Ephrins are ligands for those receptors. Ligands and receptors are bound into a structure which is responsible for bi-directional communication between cells, morphology cell changes, maturation and differentiation of stem cells, proliferation and cell survival (3). Signaling is done in two directions — “forward” and “reverse”, which enables different, sometimes opposite

effects resulting from receptor and ligand connection (4).

There are nine EphA receptors in the human genome which promiscuously bind five ephrin-A ligands and five EphB receptors which promiscuously bind three ephrin-B ligands. Besides bi-directional signaling, receptors are not specific for ligands and there is a cross-talk causing various effects.

As tyrosine kinases receptors, they are widespread within cell membrane and they take part in many cellular as well as intercellular processes. Therefore, they may be a good target for therapy.

As mentioned above, depending on ligand subclass and transmission direction, the same receptor may cause a different effect (5). Moreover, Eph and ephrins may also cooperate with different transmembrane systems. As an example, one may refer to the binding between epithelial

growth factor (EGF) and EphA2, which results in increased cell motility and proliferation independent of ephrin stimulation. Other interactions between Eph system and fibroblast growth factor (FGF) (6), Wnt receptor (7), E-cadherin (8) and claudins were also proven.

Receptor ligands (ephrins) may also be divided into two subclasses: glycosylphosphatidyl inositol (GPI) linked ephrin-A (5 members) and transmembrane ephrin-B (3 members). Most commonly ligands A bind with receptors A whereas ligands B with receptors B. However, some cross-talks between A and B subclasses are also possible.

Function of Eph/ephrin in physiology

Eph/ephrin system has wide and various impacts on cytoskeleton activity, cell adhesion, intercellular connection, cellular shape as well as cell motility (9). They affect, among others, proliferation, survival, differentiation and secretion of cells. There are many processes, in which ephrins act as mediators.

Neuron development and regeneration are one of the well documented processes, in which Eph and ephrins take part. Ephrin system participates in communication between neurons as well as between neurons and glial cells. Moreover, it takes part in neuron connection development process, synapses development and their conduction direction as well as in the formation of stromal cells in the embryonic period. It influences transduction within synapse transmitters N-methyl-D-aspartate (NMDA) and alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA). Ephrins are present in an adult brain during remodeling processes due to external factors (in hippocampus, where quantity and size of the synapses are changing during processes corresponding to learning), whereas mutations connected with ephrins cause disorders such as Alzheimer disease and anxiety. Drugs antagonizing Eph/ephrin system may be a cure in these diseases (10).

Ephrins also play a role in the immunological process such as development of thymocytes into lymphocytes T in thymus and their differentiation within perimeter. The role of EphB6 was characterized the most. EphB, among others, modulates lymphocytes T answer, causes their proliferation, is responsible for gamma interferon level increase and stimulates cytotoxic lymphocytes activity (11).

Glucose homeostasis is the following process in which EphA is involved. Forward signaling inhibits insulin secretion whereas reverse signaling acts opposite — increase insulin secretion. Receptors are located on not

only the cell membrane level but also graininess with insulin inside the cell (12).

Homeostasis in adult bones depends on osteoblasts and osteoclasts activity. EphB/ephrin-B system adjusts activity of the above-mentioned cells. The lack of proper EphB/ephrin-B system during development due to gene mutation causes cleft palate, skull Vault and craniosynostosis. Mutations within Eph signaling cause also disorders in intercellular communication, which results in disorders in osteoblasts development and delays calvarial bones osteosis. Dysfunction of this system may lead to disturbed bone homeostasis and osteoporosis (13).

Proper function of the intestinal epithelium (absorption, active ingredients absorption, mucus secretion, antimicrobial protection) also depends on the Eph system. Intestinal epithelium is constantly regenerating through proliferation and apoptosis. Stem cells are capable of dividing and migration along the crypts. EphB/ephrin-B takes part in the differentiation and motility of cells by using intercellular repulsion (14). Moreover, changing the “gradient” and Eph type as well as their ligands were noted. Overexpression of EphB 1, 2, 3 and 4 is present in cells at the bottom of the intestinal crypts, whereas EphB6 expression is dominating in surface cells. Analogical differences concerning distribution were shown in the second subclass. Expression of EphA 1, 4 and 7 prevails at the bottom, while 2, 5 and 1 near the surface (15). Therefore, the Eph/ephrin class system participates in differentiation, maturation and apoptosis of regular enterocytes. It is likely that in the case of quantity imbalance and overexpression of one or more factors, neoplasia may occur.

Vessels formation (angiogenesis and lymphangiogenesis) and Eph/ephrin system

Physiological growth and development, similar to expansion of cancer, largely depend on blood and lymphatic vessel formation. Tumor can grow without vessels only to 2 mm in diameter. Cancer induces angiogenesis process, which enables their growth despite improper construction of new vessels. Angiogenesis is a very dynamic, multicellular and environmental process. Those vessels enable local growth and development of the neoplasm. Formation of new blood vessels is by “sprouting” new vessels from pre-existing ones. This process starts from hypoxia of cancer tissue (16). New blood vessels are also responsible for the process of

metastasis and generalization of neoplastic process. Similar “production” is caused by inflammation and some eye diseases (17). Formation of improper vessels or lack thereof causes such diseases as: ischemic heart disease, stroke, neurodegenerative diseases or diseases associated with obesity.

Cancer neoangiogenesis is induced by hypoxia in the environment due to the growing tumor mass. Hypoxia results in proangiogenic factors secretion. Consequently, endothelial cells (ECs) become mobile, which starts vessel formation process. Cell motility is enabled once microenvironment is changed and matrix metalloproteinase-1 (MMP-1) concentration is increased which leads to basal membrane degradation. It is accompanied by decreasing of plasminogen activator inhibitor 1 (PAI1) level, whereas increased MMP level results in concentration incensement of proangiogenic factors (18), and angiopoietin-2 (ANG2) is one of them. It is responsible for loosening of intercellular connection and release of ECs. Process of changing and separation of ECs is controlled by Notch pathway (19). During the angiogenesis process (blood and lymph vessels), growth factors, vascular endothelial growth factors (VEGF), as well as their receptors, vascular endothelial growth factors receptors (VEGFR), are engaged, creating together with Notch pathway an integrated regulatory system. VEGF, secreted upon hypoxia, using VEGFR2 induces EC morphology change and formation of filopodia (20). VEGFR3 is present in embryonic cells, and in its later life, this receptor is involved in lymph vessel formation (21). VEGFR1 is present only within transformed cells and limits new cell formation as a feedback (22). Next, ECs are directed to the vessel formation destination place.

Formation of blood vessels has to evolve into veins or arteries. Eph/ephrin system is involved in that process. Ephrin-B2 expression is present within arteries, whereas EphB4 expression results in vein vessel formation. Both factors regulate creation of a regular “product”. Should one of them is missing a disorder in differentiation process is occurring (23). It has been shown that Ephrin-B2 is responsible for VEGFR3 and VEGFR2 internalization process and therefore it controls lymphangiogenesis and angiogenesis process (24,25) as well as reconstruction of lymphatic vessels during pathological processes of tumor and inflammations. It can also be a target for cancer treatment. Blocking ephrin-B2 suppresses VEGF-related lymphangiogenesis (26). Moreover, EphB4 in combination with ephrin-B2 causes angiogenesis and vessels

reconstruction within tumors (27). EphB4 forward signaling induces VEGF-dependent angiogenesis and it was proven that inhibiting EphB4 inhibits VEGF-dependent angiogenesis (28). EphA2 forward signaling stimulates angiogenesis and it is known as a poor prognostic factor (29,30). In addition, EphA2 is related to angiogenesis through VEGF (31).

Blood vessels created during cancer neoangiogenesis do not have a regular, physiological structure and function. They do not create a regular vascular grid either. This results in hypoxia area and microenvironment changes. It is a part of more aggressive tumor cell clone selection. Disorder in nutrients distribution leads to distribution disorder of antineoplastic agents, whereas hypoxia causes deterioration of radiotherapy effects (32,33).

Research concerning the use of the above-mentioned phenomenon in vessel formation process, inhibition of vessel formation in eye diseases (age-related macular degeneration) or neoplasm processes was conducted (34). However, no satisfactory results were obtained. Antiangiogenic treatment, used and registered, is based on antibodies against VEGF like bevacizumab, in colon cancer or glioblastoma therapy (35). Unfortunately, as the therapy based on angiogenic drugs is applied, tumor immunity occurs. Angiogenic therapy based on ephrin system may be an alternative in the case of drug resistance and may be another line of anticancer treatment (36-38). Dasatinib, EphA2 receptor inhibitor, is a good example of an antiangiogenic therapy, currently used in chronic leukemia treatment. Nowadays, there are ongoing attempts using this drug in solid tumors treatment (39,40). Moreover, there are attempts using blood and lymphatic vessel concentration level in order to assess treatment effectiveness (41).

Role of Eph/ephrin in cancers

In accordance with complex function and variety of bindings between ligands and receptors as well as two-way signaling, the role of Eph/ephrin is not clear and well known. It is known that these receptor tyrosine kinases (RTKs) system is involved in cancer progression. Moreover, based on complex relations network and cross-talks, RTKs system inhibits carcinogenesis and it also takes part in neovascularization process. Eph receptors as well as ephrins are present practically within all kinds of neoplasm cells. Their expression may be stimulated by various processes such as hypoxia or proinflammatory cytokines.

However, it is needless to say that increased or decreased expression may stimulate as well as inhibit cancer progression. It has long been known that both neoplasm cells and tumor environment show Eph receptor expression, interfering with proper communication between cells, particular tumor compartments and between environment and cells and cell migration (42-44).

Changes concern tumor environment as well. Hypoxia and factors such as tumor necrosis factor α (TNF- α), VEGF-A and hypoxia inducible factor 2 α (HIF-2 α) lead to overexpression (upregulation) of ephrin-A1 within endothelium cells. Ephrin-B2 is upregulated by VEGF and hypoxia, whereas increased tissue tension results in its downregulation within endothelium cells. Same intensified tension process causes upregulation within endothelium precursor cells, inducing their differentiations, which stimulates angiogenesis and tumor growth (45). Moreover, Eph and ephrins expression within blood and lymphatic vessels was noticed (45,46).

EphA1 was one of the first discovered receptors present within neoplasm cells. Its decreased expression was noticed, among others, in skin cancer (47). Nowadays, there are many ongoing research concerning those receptors' roles in carcinogenesis (48).

Some research has proven that EphA2 overexpression through binding with E-cadherin is involved in cell morphology change and in gaining motility by neoplasm cell. EphA2 expression is controlled by E-cadherin level, which is responsible for cell adhesion. It is a part of the epithelio-mesenchymal transition (EMT) process during which a cell is changing its phenotype from epithelial into mesenchymal in order to separate from the tumor. It is also correlated, via focal adhesion kinase (FAK) trail, with MMP-2 level, which is responsible for tumor microenvironment change that enables metastasis process, again in EMT process (49,50). This process is responsible for metastasis (51). Taking the above into consideration, it becomes a target for a cancer treatment. Recent reports on mouse models show the effectiveness of anti-EphA2 antibody — DS-8895a therapy (52) or dovitinib (TKI-258) (50).

EphA2 upregulation is present within many cancers and is correlated with bad prognosis, for instance in ovarian cancer (53). Conducted research has also shown that signal blocking within EphA2 and EphA3 trail using Fc-fusion proteins results in the stop of cancer progression and neoangiogenesis (29,54). EphA1 adjusts EphA2 level through its phosphorylation and internalization. Therefore,

it also has influence on the process mentioned above and other (55).

In gastrointestinal cancers and gliomas, EphB2 overexpression was found, which is through R-ras trail and integrin reaction to stimulate invasion and progression of those cancers (56). Opposite, inhibitory effect on colon cancer and prostate cancer is observed in the case of EphB2 (57,58). Such variety of EphB2 activities is correlated with external cell environment and may serve as a good example of Eph/ephrin system complexity. EphB4 is mainly related to tumor progression in many cancers, including ovarian cancer (59) and melanoma (60).

Role of Eph/ephrin in colon, pancreatic and gastric cancers

Colon cancer

Tcf/ β -catenin transcription increase caused by adenomatous polyposis coli (APC) suppressor gene mutation is one of the possible ways for carcinogenesis to proceed (61). In the case of colon cancer, the same process that causes cell regeneration may be responsible for the initiation of neoplastic transformation. Assuming a regular process, during differentiation from progenitor cells, cells moving along crypt-villus axis are losing EphB2 expression. Neoplastic transformation is accompanied by the same process. Wnt/ β -catenin/Tcf trail activation results in the development of adenomatous polyps and colon cancer. Receptor tyrosine kinases EphB2 is directly related to Tcf/ β -catenin transcription in premalignant changes and its expression suppression is notified after neoplastic transformation (62). EphB2 was present in healthy cells and in polyps cells, whereas within cancer cells, reduced expression of EphB2 was noticed (63,64). In some cases of colon cancer and polyps, decreasing EphB2 expression was noted. It should be pointed out that an increase of that expression was a good promising factor (65,66). Therefore, it seems that the carcinogenesis process and colon cancer progression process are related with EphB2 expression inhibition (65).

In the case of EphB3, its overexpression is related to cancer development suppression and inhibition of epithelial to mesenchymal transition. This process occurs by activation of factors which are responsible for strict cell adherence [zonula occludens-1 (ZO-1), E-cadherin, plakoglobin]. In this way, EphB3/ephrin complex overexpression prevents against metastasis changes (67,68).

EphB4 expression within a regular colon tissue was not confirmed, whereas such expression was noticed in cancer cells. Their expression was correlated with advanced stage (69).

EphB6 expression is reduced during neoplastic transformation. Low expression is related to bad differentiation, advanced disease and bad survival prognosis (70).

It was stated in the later research that EphB/ephrin-B positive cells, in accordance with communication between receptors and ligands, prevent against colon cancer cell invasion. Interaction between EphB positive colorectal cancer (CRC) cells and healthy colonocytes results in cancer cell invasion suppression. Forced interaction between cells causes compulsory adhesion (E-cadherin adhesion). This process inhibits colonization of further part of the colon, EMT process and CRC spreading (8).

EphA1 presence was proved in majority of cancer cells, including colon cancer cells (71). Research has revealed EphA1 upregulation and also downregulation in colon cancer. In some papers, the relation between decrease of EphA1 expression with worse prognosis (72), greater aggressiveness and tumor advance was shown (73). EphA1 protection mechanism relies on extracellular signal-regulated kinase (ERK) and c-Jun N-terminal kinase (JNK) trails deactivation, which results in cancer cell migration inhibition. In the case of lowering EphA1 expression, those trails are not inhibiting, and cancer cells have greater motility and disease prognosis is worse (74).

EphA2 overexpression was shown in numerous cases of cancer, including colon cancer (75,76). Ras/mitogen-activated protein (MAP) kinases promote EphA2 expression in prostate cancer. It was demonstrated that Eph signaling inhibits tumor activity in colon cancer, prostate cancer, breast cancer and skin cancer — *in vivo* and *in vitro*. According to their bi-directional signaling, it is suggested that opposite direction may promote tumor development. EphA2, adjusted by E-cadherin, is responsible for cell adhesion. Its overexpression is correlated with metastasis changes within liver, lymphatic vessel invasion or higher stage of cancer diseases (77). In the early stages, its overexpression is correlated with bad prognosis (78), whereas low expression of EphA2 is related to longer survival and better prognosis.

It has been proved that APC gene mutation, one of the most common mutations in colon cancer, modulates EphA3 function, which disturbs the ephrin balance and may result in a pro-oncogenic impact (79,80).

It was shown that EphA4 expression level is correlated

with metastasis presence in liver (81) and EphA6 and EphA7 were down-regulated in CRC (82).

Pancreatic cancer

The role of ephrin in pancreatic cancer has not been thoroughly studied as in the case of colon cancer. Increased EphA4 level within cancer cells was correlated with cell motility and cancer progression (83). Moreover, a relation between EphA4 level and matrix metalloproteinases (MMP-2 and MMP-4), E-cadherin and Snail levels was noticed. Those factors take part in changes in extracellular matrix and EMT process, which is related to metastasis (84).

Also EphA2 affects EMT process and progression of the tumor. Its overexpression results in FAK-dependent MMP-2 increase, changing cell environment and hence pancreatic cancer progression. Research has shown that EphA2 inhibition through ephrin A1-Fc antibody results in MMP-2 level decrease and may be a new aim for a targeted therapy (85).

Within Eph B/ephrin class, downregulation (silencing) of B1, 3, 4 and 6 as well as ephrin was noted (86). It is proved that Eph/ephrin system is correlated with neuropathic pain in pancreatic cancer and may be a therapeutic agent (87).

A relation between EphB2 overexpression and pancreatic cancer progression as well as pain in the abdomen and back was stated. Overexpression of EphB2 is related to metastasis within lymph nodes (N) and tumor infiltration (T), and it is correlated with disease-free survival (88).

Gastric cancer

While analyzing gastric cancer, EphA1 overexpression was pointed out. It was correlated with depth of invasion and short survival time in comparison to gastric cancer with low EphA1 expression (89). Overexpression of EphA2 was correlated with tumor infiltration (T) as well as metastasis in lymph nodes (N) and distant organs (M) (90,91), whereas decreased overexpression of EphA2 inhibits gastric cancer cell invasion. This encourages to further research concerning drugs that inhibit EphA2 in gastric cancer (92). The relation between EphA3 and VEGF, vessels concentration and patient survival time was confirmed (93). Research has revealed EphA4 level increase (94). Lower EphB2 level was correlated with higher gastric cancer stages, bad prognosis (95) and poor histological differentiation, same as increase of EphB1 level (96).

Decreased EphB1 level was correlated with invasion and cancer cell *in vitro* migration inhibition (97). High expression of ephrin-B1 is correlated with peritoneal dissemination. Its blocking may be a therapy in the fourth stage of gastric cancer (98).

Conclusions

Eph/ephrin system is examined by research with interest from many years. It is proved that it has impact on cancer disease at almost every stage. It can be a useful tool in screening, prognosis and treatment at last.

First stage cancer transformation is related to down-expression of EphB2. As was mentioned before, colonocytes change its phenotype. Examination of phenotype can be useful in colon cancer screening. Also in the next stage cancer progression, this system plays a role. In many research, it was proved that Eph expression was correlated with poor prognosis (for example overexpression of EphB2 is correlated with poor prognosis in gastric and pancreatic cancers), so they can be considered as prognostic factors. One of the most exciting processes is EMT. It is responsible for cancer cells spreading and together with opposite mesenchymal-epithelial transition (MET) process for cancer metastasis. The exact role is not yet well known, but it is proved that overexpression of EphA2 leads to EMT process, while EphB3 seems to inhibit it. As the consequence of EMT process distant metastases forms. And again it is established that some of Ephs are related to this stage, like EphA4 and ephrin-B1 in gastric cancer. So that the Eph/ephrin system is present at every stage of cancer which gives lots of treatment possibilities. There are few examples like blocking ephrin-B1 in peritoneal dissemination in gastric cancer, EphA2 inhibition through ephrin A1-Fc antibody which results in MMP-2 level decrease and may stop EMT process.

Eph/ephrin system also takes part in lymphangiogenesis and angiogenesis and it also seems to be an interesting therapeutic target. Dasatinib, an EphA2 receptor inhibitor, is used in antiangiogenic therapy in chronic leukemia.

Although some knowledge about Eph/ephrin is examined, the exact role of Eph/ephrin system in gastrointestinal cancer remains unclear. In some cases, it is possible to specify the impact of this system on cancers. Overexpression of EphA2 is correlated with the stage of gastric, colon and pancreatic cancers. Higher expression of EphA4 in these neoplasms has correlation with cancer cell motility and distant metastasis. Nevertheless, there are also

some discrepancies. In different cancers, disturbance of this system can lead to opposite effects. For example, EphA1 has opposite correlations in colon and gastric cancers. Overexpression in gastric cancer correlates with progression, whereas in colon cancer, it has protective impact. The same is with EphB2. In gastric and colon cancers, its down-regulation correlates with poor prognosis, whereas in pancreatic cancer, tumor progression and pain are correlated with its up-regulation. The cause of these discrepancies can be in bi-directional signaling and cross-talks between this and other systems and thus further investigations are required.

To summarize, Eph/ephrin system plays a role in every stage of cancer transformation and progression. It can be a useful tool in screening, prognosis and therapy, but because of complicated relation, bi-directional signaling and cross-talks with other systems, it needs to be further investigated.

Acknowledgements

I would like to express my very great appreciation to Jędrzej Kabarowski and Adam Skalski for their valuable and constructive suggestions during this research work.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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Cite this article as: Rudno-Rudzińska J, Kielan W, Frejlich E, Kotulski K, Hap W, Kurnol K, Dzierżek P, Zawadzki M, Hałoń A. A review on Eph/ephrin, angiogenesis and lymphangiogenesis in gastric, colorectal and pancreatic cancers. *Chin J Cancer Res* 2017;29(4):303-312. doi: 10.21147/j.issn.1000-9604.2017.04.03