Review Article

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

Julia Rudno-Rudzińska[1], Wojciech Kielan[1], Ewelina Frejlich[1], Krzysztof Kotulski[1], Wojciech Hap[1], Krzysztof Kurnol[1], Przemysław Dzierżek[1], Marcin Zawadzki[1,2], Agnieszka Hałoń[2]

[1]2-nd Department of General and Oncological Surgery; [2]Pathology Department, Wrocław Medical University, Borowska 213, 50-556 Wrocław, Poland

Correspondence to: Julia Rudno-Rudzińska, PhD. 2-nd Department of General and Oncological Surgery, Wrocław Medical University, Borowska 213, 50-556 Wrocław, Poland. Email: juliarudnorudzinska@gmail.com.

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 2α (HIF-2α) 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 ephprin-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.

References

1. Pasquale EB. Eph-ephrin promiscuity is now crystal clear. Nat Neurosci 2004;7:417-8.
2. Pasquale EB. Eph receptor signalling casts a wide net on cell behaviour. Nat Rev Mol Cell Biol 2005;6:462-75.
3. Lisabeth EM, Falivelli G, Pasquale EB. Eph receptor signaling and ephrins. Cold Spring Harb Perspective Biol 2013;5:a009159.
4. Campbell TN, Robbins SM. The Eph receptor/ephrin system: an emerging player in the invasion game. Curr Issues Mol Biol 2008;10:61-6.
5. Pasquale EB. Eph receptors and ephrins in cancer: bidirectional signalling and beyond. Nat Rev Cancer 2010;10:165-80.
6. Arvanitis D, Davy A. Eph/ephrin signaling: networks. Genes Dev 2008;22:416-29.
7. Arvanitis DN, Davy A. Regulation and misregulation

of Eph/ephrin expression. Cell Adh Migr 2012;6: 131-7.
8. Orsulic S, Kemler R. Expression of Eph receptors and ephrins is differentially regulated by E-cadherin. J Cell Sci 2000;113:1793-802.
9. Egea J, Klein R. Bidirectional Eph-ephrin signaling during axon guidance. Trends Cell Biol 2007;17: 230-8.
10. Dines M, Lamprecht R. The role of Ephs and ephrins in memory formation. Int J Neuropsychopharmacol 2016;19:pyw106.
11. García-Ceca J, Alfaro D, Montero-Herradón S, et al. Eph/ephrins-mediated thymocyte-thymic epithelial cell interactions control numerous processes of thymus biology. Front Immunol 2015;6:333.
12. Konstantinova I, Nikolova G, Ohara-Imaizumi M, et al. EphA-Ephrin-A-mediated beta cell communication regulates insulin secretion from pancreatic islets. Cell 2007;129:359-70.
13. Zuo C, Huang Y, Bajis R, et al. Osteoblastogenesis regulation signals in bone remodeling. Osteoporos Int 2012;23:1653-63.
14. Sancho E, Batlle E, Clever H. Live and let die in the intestinal epithelium. Curr Opin Cell Biol 2003;15: 763-70.
15. Kosinski C, Li VS, Chan AS, et al. Gene expression patterns of human colon tops and basal crypts and BMP antagonists as intestinal stem cell niche factors. Proc Nati Acad Sci U S A 2007;104:15418-23.
16. Bentley K, Chakravartula S. The temporal basis of angiogenesis. Philos Trans R Soc Lond B Biol Sci 2017;372:20150522.
17. Folkman J. Angiogenesis: an organizing principle for drug discovery? Nat Rev Drug Discov 2007;6:273-86.
18. Arroyo AG, Iruela-Arispe ML. Extracellular matrix, inflammation, and the angiogenic response. Cardiovasc Res 2010;86:226-35.
19. Eilken HM, Adams RH. Dynamics of endothelial cell behavior in sprouting angiogenesis. Cur Opin Cell Biol 2010;22:617-25.
20. Phng LK, Gerhardt H. Angiogenesis: a team effort coordinated by notch. Dev Cell 2009;16:196-208.
21. Tammela T, Zarkada G, Wallgard E, et al. Blocking VEGFR-3 suppresses angiogenic sprouting and vascular network formation. Nature 2008;454:656-60.
22. Jakobsson L, Franco CA, Bentley K, et al. Endothelial cells dynamically compete for the tip cell position during angiogenic sprouting. Nat Cell Biol 2010;12:943-53.
23. Pitulescu ME, Adams RH. Eph/ephrin molecules — a hub for signaling and endocytosis. Genes Dev 2010;24:2480-92.
24. Wang Y, Nakayama M, Pitulescu ME, et al. Ephrin-B2 controls VEGF-induced angiogenesis and lymphangiogenesis. Nature 2010;465:483-6.
25. Sawamiphak S, Seidel S, Essmann CL, et al. Ephrin-B2 regulates VEGFR2 function in developmental and tumour angiogenesis. Nature 2010;465:487-91.
26. Zheng W, Tammela T, Yamamoto M, et al. Notch restricts lymphatic vessel sprouting induced by vascular endothelial growth factor. Blood 2011;118: 1154-62.
27. Noren NK, Lu M, Freeman AL, et al. Interplay between EphB4 on tumor cells and vascular ephrin-B2 regulates tumor growth. Proc Nati Acad Sci U S A 2004;101:5583-8.
28. Martiny-Baron G, Holzer P, Billy E, et al. The small molecule specific EphB4 kinase inhibitor NVP-BHG712 inhibits VEGF driven angiogenesis. Angiogenesis 2010;13:259-67.
29. Merritt WM, Kamat AA, Hwang JY, et al. Clinical and biological impact of EphA2 overexpression and angiogenesis in endometrial cancer. Cancer Biol Ther 2010;10:1306-14.
30. Wykosky J, Debinski W. The EphA2 receptor and ephrinA1 ligand in solid tumors: function and therapeutic targeting. Mol Cancer Res 2008;6:1795-806.
31. Shao Z, Zhang WF, Chen XM, et al. Expression of EphA2 and VEGF in squamous cell carcinoma of the tongue: correlation with the angiogenesis and clinical outcome. Oral Oncol 2008;44:1110-7.
32. Goel S, Fukumura D, Jain RK. Normalization of the tumor vasculature through oncogenic inhibition: an emerging paradigm in tumor biology. Proc Nati Acad Sci U S A 2012;109:E1214.
33. Jain RK. Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy. Science 2005;307:58-62.
34. Crawford Y, Ferrara N. VEGF inhibition: insights from preclinical and clinical studies. Cell Tissue Res 2009;335:261-9.

35. Ferrara N, Hillan KJ, Gerber HP, et al. Discovery and development of bevacizumab, an anti-VEGF antibody for treating cancer. Nat Rev Drug Discov 2004;3:391-400.
36. Ireton RC, Chen J. EphA2 receptor tyrosine kinase as a promising target for cancer therapeutics. Curr Cancer Drug Targets 2005;5:149-57.
37. Noren NK, Pasquale EB. Paradoxes of the EphB4 receptor in cancer. Cancer Res 2007;67:3994-7.
38. Casanovas O, Hicklin DJ, Bergers G, et al. Drug resistance by evasion of antiangiogenic targeting of VEGF signaling in late-stage pancreatic islet tumors. Cancer Cell 2005;8:299-309.
39. Chang Q, Jorgensen C, Pawson T, et al. Effects of dasatinib on EphA2 receptor tyrosine kinase activity and downstream signalling in pancreatic cancer. Br J Cancer 2008;99:1074-82.
40. Karaman MW, Herrgard S, Treiber DK, et al. A quantitive analysis of kinase inhibitor selecivity. Nat Biotechnol 2008;26:127-32.
41. Tolaney SM, Boucher Y, Duda DG, et al. Role of vascular density and normalization in response to neoadjuvant bevacizumab and chemotherapy in breast cancer patients. Proc Nati Acad Sci U S A 2015;112: 14325-30.
42. Michaelis UR. Mechanisms of endothelial cell migration. Cell Mol Life Sci 2014;71:4131-48.
43. Castaño J, Davalos V, Schwartz S Jr, et al. EPH receptors in cancer. Histol Histopathol 2008;23: 1011-23.
44. McCarron JK, Stringer BW, Day BW, et al. Ephrin expression and function in cancer. Future Oncol 2010;6:165-76.
45. Ahmed Z, Bichnell R. Angiogenic signalling pathways. Methods Mol Biol 2009;467:3-24.
46. Yamashita T, Ohneda K, Nagano M, et al. Hypoxia-inducible transcription factor-2α in endothelial cells regulates tumor vascularisation through activation of ephrin A1. J Biol Chem 2008;283:18926-36.
47. Hafner C, Becker B, Landthaler M, et al. Expression profile of Eph receptrors and ephrin ligands in human skin and downregulation of EphA1 in nonmelanoma skin cancer. Mod Pathol 2006;19:1369-77.
48. Pasquale EB. Eph receptors and ephrins in cancer: bidirectional signalling and beyond. Nat Rev Cancer 2010;10:165-80.
49. Li RX, Chen ZH, Chen ZK. The role of EPH receptors in cancer-related epithelial-mesenchymal transition. Chin J Cancer 2014;33:231-40.
50. Hänze J, Henrici M, Hegele A, et al. Ephitelial mesemchymal transition status is associated with anti-cancer responses towards receptor tyrosine-kinase inhibition by dovotinib in human bladder cancer cells. BMC Cancer 2013;13:589.
51. Jolly MK, Ware KE, Gilja S, et al. EMT and MET: necessary or permissive for metastasis? Mol Oncol 2017;11:755-69.
52. Hasegawa J, Sue M, Yamato M, et al. Novel anti-EPHA2 antibody, DS-8895a for cancer treatment. Cancer Biol Ther 2016;17:1158-67.
53. Landen CN, Kinch MS, Sood AK. EphA2 as a target for ovarian cancer therapy. Expert Opin Ther Targets 2005;9:1179-87.
54. Dobrzanski P, Hunter K, Jones-Bolin S, et al. Antiangiogenic and antitumor efficacy of EphA2 receptor antagonist. Cancer Res 2004;64:910-9.
55. Miao H, Burnett E, Kinch M, et al. Activation of EphA2 kinase suppresses integrin function and causes focal-adhesion-kinase dephosphorylation. Nat Cell Biol 2000;2:62-9.
56. Nakada M, Niska JA, Tran NL, et al. EphB2/R-Ras signaling regulates glioma cell adhesion, growth, and invasion. Am J Pathol 2005;167:565-76.
57. Kittles RA, Baffoe-Bonnie AB, Moses TY, et al. A common nonsense mutation in EphB2 is associated with prostate cancer risk in African American men with a positive family history. J Med Genet 2006;43:507-11.
58. Huusko P, Ponciano-Jackson D, Wolf M, et al. Nonsense-mediated decay microarray analysis identifies mutations of EPHB2 in human prostate cancer. Nat Genet 2004;36:979-83.
59. Kumar SR, Masood R, Spannuth WA, et al. The receptor tyrosine kinase EphB4 is overexpressed in ovarian cancer, provides survival signals and predicts poor outcome. Br J Cancer 2007;96:1083-91.
60. Yang NY, Pasquale EB, Owen LB, et al. The EphB4 receptor-tyrosine kinase promotes the migration of melanoma cells through Rho-mediated actin cytoskeleton reorganization. J Biol Chem 2006;281: 32574-86.
61. Ciasca G, Papi M, Minelli E, et al. Changes in cellular

mechanical properties during onset or progression of colorectal cancer. World J Gastroenterol 2016;22: 7203-14.
62. Batlle E, Henderson JT, Beghtel H, et al. β-Catenin and TCF mediate cell positioning in the intestinal epithelium by controlling the expression of EphB/ephrinB. Cell 2002;111:251-63.
63. Lugli A, Spichtin H, Maurer R, et al. EphB2 expression across 138 human tumor types in a tissue microarray: high levels of expression in gastrointestinal cancers. Clin Cancer Res 2005;11:6450-8.
64. Guo DL, Zhang J, Yuen ST, et al. Reduced expression of EphB2 that parallels invasion and metastasis in colorectal tumours. Carcinogenesis 2006;27:454-64.
65. Batlle E, Bacani J, Begthel H, et al. EphB receptor activity suppresses colorectal cancer progresssion. Nature 2005;435:1126-30.
66. Jubb AM, Zhong F, Bheddah S, et al. EphB2 is a prognostic factor in colorectal cancer. Clin Cancer Res 2005;11:5181-7.
67. Chiu ST, Chang KJ, Ting CH, et al. Over-expression of EphB3 enhances cell-cell contacts and suppresses tumor growth in HT-29 human colon cancer cells. Carcinogenesis 2009;30:1475-86.
68. Cortina C, Palomo-Ponce S, Iglesias M, et al. EphB-ephrin-B interactions suppress colorectal cancer progression by compartmentalizing tumor cells. Nat Genet 2007;39:1376-83.
69. Kumar SR, Scehnet JS, Ley EJ, et al. Preferential induction of EphB4 over EphB2 and its implication in colorectal cancer progression. Cancer Res 2009;69: 3736-45.
70. Peng L, Tu P, Wang X, et al. Loss of EphB6 protein expression in human colorectal cancer correlates with poor prognosis. J Mol Histol 2014;45:555-63.
71. Maru Y, Hirai H, Takaku F. Overexpression confers an oncogenic potential upon the eph gene. Oncogene 1990;5:445-7.
72. Herath NI, Doecke J, Spanevello MD, et al. Epigenetic silencing of EphA1 expression in colorectal cancer is correlated with poor survival. Br J Cancer 2009;100:1095-102.
73. Dong Y, Wang J, Sheng Z, et al. Downregulation of EphA1 in colorectal carcinomas correlates with invasion and metastasis. Mod Pathol 2009;22:151-60.
74. Wu BO, Jiang WG, Zhou D, et al. Knockdown of EPHA1 by CRISPR/CAS9 promotes adhesion and motility of HRT 18 colorectal carcinoma cells. Anticancer Res 2016;36:1211-9.
75. Lu C, Shahzad MM, Wang H, et al. EphA2 overexpression promotes ovarian cancer growth. Cancer Biol Ther 2008;7:1098-103.
76. Merritt WM, Kamat AA, Hwang JY, et al. Clinical and biological impact of EphA2 overexpression and angiogenesis in endometrial cancer. Cancer Biol Ther 2010;10:1306-14.
77. Saito T, Masuda N, Miyazaki T, et al. Expression of EphA2 and E-cadherin in colorectal cancer: correlation with cancer metastasis. Oncol Rep 2004;11:605-11.
78. Dunne PD, Dasgupta S, Blayney JK, et al. EphA2 expression is a key driver of migration and invasion and a poor prognostic marker in colorectal cancer. Clin Cancer Res 2016;22:230-42.
79. Sjöblom T, Jones S, Wood LD, et al. The consensus coding sequences of human breast and colorectal cancers. Science 2006;314:268-74.
80. Bardelli A, Parsons DW, Silliman N, et al. Mutational analysis of the tyrosine kinome in colorectal cancers. Science 2003;300:949.
81. Oshima T, Akaike M, Yoshihara K, et al. Overexpression of EphA4 gene and reduced expression of EphB2 gene correlates with liver metastasis in colorectal cancer. Int J Oncol 2008;33: 573-7.
82. Hafner C, Schmitz G, Meyer S, et al. Differential gene expression of Eph receptors and ephrins in benign human tissues and cancers. Clin Chem 2004;50:490-9.
83. Iiizumi M, Hosokawa M, Takehara A, et al. EphA4 receptor, overexpressed in pancreatic ductal adenocarcinoma, promotes cancer cell growth. Cancer Sci 2006;97:1211-6.
84. Liu C, Huang H, Wang C, et al. Involvement of ephrin receptor A4 in pancreatic cancer cell motility and invasion. Oncol Lett 2014;7:2165-9.
85. Duxbury MS, Ito H, Zinner MJ, et al. Ligation of EphA2 by Ephrin A1-Fc inhibits pancreatic adenocarcinoma cellular invasiveness. Biochem Biophys Res Commun 2004;320:1096-102.
86. Boyd AW, Lackmann M. Signals from Eph and

ephrin proteins: a developmental tool kit. Sci STKE 2001;2001:re20.
87. Kobayashi H, Kitamura T, Sekiguchi M, et al. Involvement of EphB1 receptor/EphrinB2 ligand in neuropatic pain. Spine (Phila Pa 1976) 2007;32: 1592-8.
88. Lu Z, Zhang Y, Li Z, et al. Overexpression of the B-type Eph and ephrin genes correlates with progression and pain in human pancreatic cancer. Oncol Lett 2012;3:1207-12.
89. Wang J, Dong Y, Wang X, et al. Expression of EphA1 in gastric carcinomas is associated with metastasis and survival. Oncol Rep 2010;24:1577-84.
90. Nakamura R, Kataoka H, Sato N, et al. EPHA2/EFNA1 expression in human gastric cancer. Cancer Sci 2005;96:42-7.
91. Yuan W, Chen Z, Wu S, et al. Expression of EphA2 and E-cadherin in gastric cancer: correlated with tumor progression and lymphogenous metastasis. Pathol Oncol Res 2009;15:473-8.
92. Yuan W, Chen Z, Chen Z, et al. Silencing of EphA2 inhibits invasion of human gastcric cancer SGC-7901 cells *in vitro* and *in vivo*. Neoplasma 2012;59:105-13.
93. Xi HQ, Wu XS, Wei B, et al. Aberrant expression of EphA3 in gastric carcinoma: correlation with tumor angiogenesis and survival. J Gastroenterol 2012;47: 785-94.
94. Oki M, Yamamoto H, Taniguchi H, et al. Overexpression of the receptor tyrosine kinase EphA4 in human gastric cancers. World J Gastroenterol 2008;14:5650-6.
95. Yu G, Gao Y, Ni C, et al. Reduced expression of EphB2 is significantly associated with nodal metastasis in Chinese patients with gastric cancer. J Cancer Res Clin Oncol 2011;137:73-80.
96. Kataoka H, Tanaka M, Kanamori M, et al. Expression profile of EFNB1, EFNB2, two ligands of EPHB2 in human gastric cancer. J Cancer Res Clin Oncol 2002;128:343-8.
97. Tanaka M, Kamata R, Takigahira M, et al. Phosphorylation of ephrin-B1 regulates dissemination of gastric scirrhous carcinoma. Am J Pathol 2007;171: 68-78.
98. Tanaka M, Kamata R, Yanagihara K, et al. Suppression of gastric cancer dissemination by ephrin B1-derived peptide. Cancer Sci 2010;101:87-93.

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

