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## EFNA1 in gastrointestinal cancer: Expression, regulation and clinical significance

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### Abstract

Ephrin-A1 is a protein that in humans is encoded by the *EFNA1* gene. The ephrins and EPH-related receptors comprise the largest subfamily of receptor protein-tyrosine kinases which play an indispensable role in normal growth and development or in the pathophysiology of various tumors. The role of *EFNA1* in tumorigenesis and development is complex and depends on the cell type and microenvironment which in turn affect the expression of *EFNA1*. This article reviews the expression, prognostic value, regulation and clinical significance of *EFNA1* in gastrointestinal tumors.

**Key Words:** *EFNA1*; Expression; Tumorigenesis; Clinical implication; gastrointestinal cancer

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**Core Tip:** Ephrin-A1, a protein that in humans is encoded by the *EFNA1* gene, is the ligand of EphA2. Studies have shown that the EphA2 receptor and its ligand ephrin-A1 are expressed in a variety of malignant tumors and the interaction between the two promotes the migration of tumor vascular endothelial cells. In addition, studies have shown that *EFNA1* widely affects tumor growth through enhancing tumor angiogenesis, malignant cell events and invasiveness. *EFNA1* is also up-regulated in gastrointestinal tumors and is closely related to the prognosis of gastrointestinal tumors. Therefore, this article reviews the expression, prognostic value, regulation and clinical significance of *EFNA1* in gastrointestinal tumors.

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## INTRODUCTION

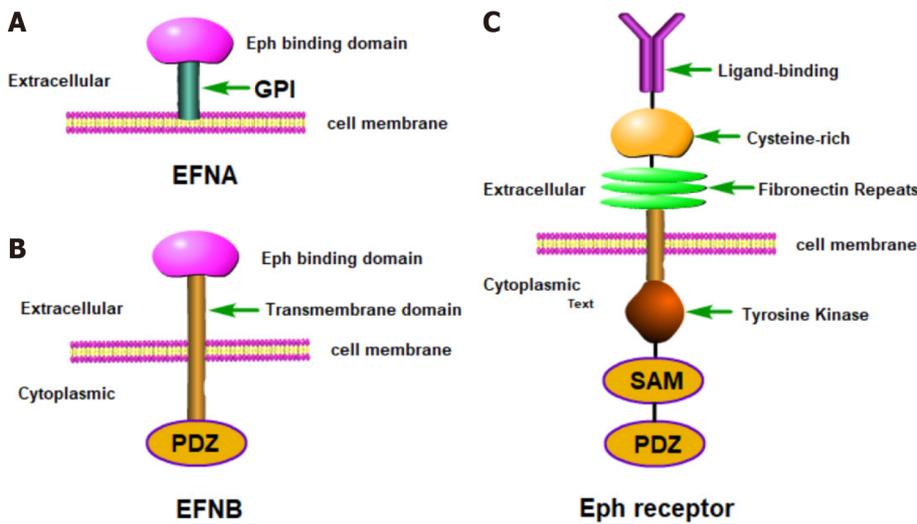
Current theory suggests that a tumor is an "organ" that contains a diverse collection of cells. Different cells sense changes in the external environment through signaling molecules on the surface of cell membrane or plasma membrane. It regulates a series of biological behaviors, such as tumor occurrence, development, invasion and metastasis[1]. Receptor tyrosine kinases (RTKs) can directly transmit external information to the nucleus and are key molecules in the signal transduction pathways through which cells convert external stimuli into biological behavior. The Eph (erythropoietin-producing hepatoma-amplified sequence) receptor family is the largest known family of RTKs[2]. By interacting with its ephrin ligands, Eph receptors regulate physiological and pathological processes, including the formation of tissues and organs, signal transmission of the nervous system, angiogenesis and cell-to-cell adhesion[3]. Studies have shown that the EphA2 receptor and its ligand ephrin-A1 are expressed in a variety of malignant tumors and the interaction between the two promotes the migration of tumor vascular endothelial cells[4]. Therefore, in recent years, the role of ephrins in the occurrence and development of tumors has become a hot topic in cancer research.

Studies have shown that *EFNA1* widely affects tumor growth through enhancing tumor angiogenesis [5,6], malignant cell events[7,8] and invasiveness[9-11]. It is up-regulated in gastrointestinal tumors (such as esophageal cancer (EC)[12], colorectal cancer (CRC)[13], and hepatocellular carcinoma (HCC) [14]) and is closely related to the prognosis of gastrointestinal tumors[12-16]. This article summarizes the research progress on *EFNA1* in terms of gene composition, protein structure, expression, regulation and biological effects. On this basis, the role of *EFNA1* in tumors and its regulatory mechanisms are described in detail as well as its potential clinical significance in gastric cancer (GC), HCC, CRC, EC and some common gastrointestinal cancers.

## THE EPHRIN FAMILY AND STRUCTURAL CHARACTERISTICS

The Eph family contains 14 tyrosine kinase receptors[17] and is the largest known RTK family. The Eph receptor is located on the cell membrane and can directly receive stimulation from the external environment. Eph receptors can also be divided into two categories: A and B, where EphA is comprised of 8 members and EphB is comprised of six members. Eph receptors contain a typical transmembrane structure and belong to transmembrane proteins[18,19]. The typical Eph family receptor structure involves an extracellular domain consisting of a globular domain, a unique cysteine-rich motif and two fibronectin type III motifs. The extracellular domain and the intracellular domain are connected by a short transmembrane domain. The intracellular membrane region is relatively conserved and includes the domain with tyrosine kinase activity, a sterile alpha motif domain and a C-terminal postsynaptic density protein, discs large, zonula occludens (PDZ) domain[20]. Ephrin ligands are divided into two subclasses according to the way they attach to the membrane. Type A ephrins are firmly anchored to the cell membrane with the aid of glycosylphosphatidylinositol (GPI) and include five members (ephrins A1-A5). Type B ephrins are transmembrane proteins[18,19] and include three members (ephrins B1-B3). Ephrin-B contains a PDZ-binding region and there is also a conserved tyrosine residue that can be phosphorylated. Ephrin-A is rather special in that it only contains a receptor-binding region which is coupled to the cell membrane through a GPI anchor. This structure also leads to the specificity of ephrin-A signal transduction (Figure 1).

Ephrin-A1 was first discovered in 1990 as a soluble protein produced by human umbilical vein endothelial cells (HUVECs) in response to treatment with tumor necrosis factor (TNF). However, it was not confirmed until 1994 to be a ligand for EphA2 which had been considered an independent RTK



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**Figure 1 Ephrin-A signal transduction.** A: Structure of ephrinA ligands; B: Structure of ephrinB ligands; C: Eph/Ephrin interaction map. GPI: Glycosylphosphatidylinositol; PDZ: Postsynaptic density 95-Discs large Zonula occludentes-1-protein; SAM: Sterile alpha motif.

kinase before then[21,22]. Ephrin-A1 is a single-chain protein molecule containing 205 amino acid residues, has a molecular weight of 22-KD and is a membrane-coupled ligand protein. The *EFNA1* gene, encoding ephrine-A1, is located on chromosome 1q22[20,23]. *EFNA1* is 7024 bp in length and contains 5 exons (Table 1 and Figure 2). The length of exon 1 is 194 bp and includes the entire 5' untranslated region (5'UTR). Exons 2 and 3 are 295 bp and 65 bp, respectively, and encode most of the amino acid sequence of the central junction domain. The C-terminus of ephrin-A1 is encoded by exon 4 and the first half of exon 5 (the latter half is the 3' untranslated region (3' UTR)). As early as 1996, a study by Daniel *et al*[24] found that soluble ephrin-A1 can induce HUVECs cultured *in vitro* to form capillary-like structures, suggesting that ephrin-A1 has the potential to promote angiogenesis.

The binding of Eph receptor to ephrin ligand is very complicated. The same Eph receptor can bind different ephrins and the same ligand can also interact with multiple Eph receptors. EphA2 is the most common receptor for ephrin-A1. The signal transduction by the EphA2 receptor and ephrin-A1 is unique in that they can mediate two-way signal transmission. They can act as receptors or ligands for each other and transmit signals to the cells in which they are located. At present, the signal transmitted by the EphA2 receptor is usually referred to as forward signaling, and the intracellular signal transduction mediated by ephrin-A1 is called the reverse signaling[25,26]. For EphA2 to be activated by ephrin-A1, it must form oligomers in a ligand-dependent manner, indicating that the activation of the EphA2 receptor depends on the interaction between it and the ephrin-A1 Ligand[27]. When EphA2 is activated through ephrin-A1 binding, the tyrosines in their intracellular regions are phosphorylated to form a binding site for another protein, ultimately resulting in the signal transduction complex.

## EFNA1 AND GASTROINTESTINAL CANCERS

### Expression and prognostic value of ephrin-A1 in gastrointestinal cancers

Ephrin is up-regulated in various subtypes of tumor tissues and the up-regulation is closely related to tumor growth[28]. Among the ephrins, ephrin-A1 is highly expressed in human gastrointestinal cancers such as GC, CRC, and EC, as well as HCC. The degree of up-regulation of its expression is closely related to the malignancy of the tumor, metastatic potential and prognosis of the patient[13,29]. We summarize the expression of ephrin-A1 in gastrointestinal cancers and its prognostic value in Table 2.

### Gastric cancer

As a tumor-related secreted protein, ephrin-A1 is highly expressed in most GC tissues and cells. Further studies have found that there is a positive correlation between the expression level of *EFNA1* and the degree of malignancy of GC[30-41]. *EFNA1* is highly expressed in GC tissues but is low or not expressed in benign GC lesions, and its expression surges with increases in malignancy[30]. Overexpression of ephrin-A1 in GC tumors was reported for 57% of patients in one study and 72.7% of patients in another study, and the overexpression of ephrin-A1 was significantly related to TNM staging and lymph node metastasis[31]. Studies by Miyazaki *et al*[32] found that *EFNA1* is highly expressed in GC, and its high expression may be related to the occurrence, development, invasion and metastasis of GC. *EFNA1* expression increases with both clinical stage and lymph node metastasis and decreases in the degree of

**Table 1** *EFNA1* gene information

Gene name (known as)	Position and length	Exon number	Encoding mRNA and protein	5'UTR	CDS	3'UTR
<i>EFNA1</i> (ephrin-A1; B61; EFL1; GMAN; ECKLG)	1q22; 7024bp	5 (1..194, 3464..3759, 5682..5693, 5855..5905,6082..7024)	NM_004428.3, 1552bp, NM_182685.2, 1486 bp; NP_004419.2 , 205aa, NP_872626.1, 183 aa	1..103	103..194, 3464..3759, 5682..5693, 5855..5905,6082..7024	6192..7024

CDS: Coding DNA Sequence.

tissue differentiation, which indicates the malignant degree of GC. Yuan *et al*[33] studied 176 cases of human GC and found that *EFNA1* mRNA and protein are highly expressed in GC, suggesting a pre-transcriptional regulatory mechanism in GC. In addition, the study also found that *EFNA1* is greatly expressed in the highly invasive cancer cell line AGS compared with moderately invasive cancer cell lines, suggesting that high expression of ephrin-A1 is related to a more aggressive behavior. These results suggest that *EFNA1* plays an important role in progression and metastasis after human GC resection.

Genetic variation of miRNA binding sites may change the susceptibility of individuals to many cancers. Li *et al*[34] selected 525 GC patients and 501 controls, and selected 3 miRNA binding-site single nucleotide polymorphisms (SNPs) from 30 untranslated regions (UTRs) of GC-related genes to study their relationship with GC susceptibility. It was found that rs12904 in the *EFNA1* gene was significantly related to the risk of GC. In addition, luciferase detection showed that *EFNA1* mRNA is the target of hsamiR-200c, and expression of the rs12904G>A isoform resulted in a change of luciferase expression. In summary, these findings indicate that the miR-200c binding site containing the SNP (rs12904G>A) can regulate the expression of *EFNA1* and is related to GC susceptibility[34-36]. Zhuo *et al*[37] found that a lncRNA, GMAN, was increased in GC tissues and was associated with GC metastasis and decreased survival rates. GMAN regulates the translation of *EFNA1* mRNA by competitively binding antisense GMAN RNA, thereby affecting the invasion and metastasis of GC cells; and up-regulation of GMAN is associated with a poor prognosis of GC.

**Colorectal cancer**

*EFNA1* is highly expressed in most CRC tissues and cells. In recent years, studies based on the relationship between *EFNA1* and CRC have shown that it plays an important role in CRC cell growth, invasion, metastasis and angiogenesis[42-52]. Potla *et al*[42] found that overexpression of *EFNA1* can promote the growth of HT29 CRC cells. Ephrin-A1 activates EphA2 to weaken the connections between tumor cells, resulting in increased adhesion of tumor cells to the extracellular matrix (ECM) and enhanced invasion into the matrix. All of these are important characteristics of tumor cells for acquiring the ability to invade and metastasize. Shi *et al*[43] selected 14 genes through a literature analysis and compared their expression in rectal cancer tissues and para-cancerous tissues, as well as rectal adenomas and cancer tissues. Among them, the gene copy number and mRNA expression of *EFNA1* increased in the progression from adenoma to cancer, indicating that *EFNA1* may be a driving gene to promote rectal cancer. Studies have also evaluated the genetic association between *EFNA1* polymorphisms and susceptibility to CRC. The results showed that, compared with the normal control group, expression of *EFNA1* in CRC is increased, suggesting that *EFNA1* is involved in the occurrence of CRC and may be used as a diagnostic biomarker for CRC. In addition, it was also found that the rs12904G/A variant is significantly associated with a lower risk of CRC compared with the AA genotype[44,45]. A study by Rosenberg *et al*[46] showed that the CRC epithelial cell line Caco-2 simultaneously expresses ephrin-A1 (B61) and its receptor EphA2 (Eck). The ephrin-A1 and EphA2 are co-localized in the same cell and play a role in the development, migration and barrier function of CRC epithelial cells helping to maintain the homeostasis and continuity of the epithelial barrier.

Kataoka *et al*[47] detected the expression of *EFNA1* in CRC specimens and found that 62.5% (25/37) expressed ephrin-A1 to a greater extent which correlated with low survival rate and poor prognosis. Overexpression of *EFNA1* in CRC stages I and II is more significant than in stages III and IV, and overexpression in tumors < 5 cm is greater than that in tumors > 5 cm. This data suggests an importance of *EFNA1* in the early stages of CRC progression. However, the prognostic role of *EFNA1* in CRC patients is still controversial. Robertis *et al*[48] reported that low expression of *EFNA1* in CRC cells is indicative of poor patient prognosis, including poor disease-free survival, cancer-specific survival and progression-free survival. However, two other gene chip analyses showed that the prognosis of patients with high *EFNA1* expression is worse than that of patients with low expression[49,50]. In addition, multivariate analysis showed that *EFNA1* expression is an independent prognostic factor of CRC[49,50]. Therefore, a large sample, multi-center clinical study is needed to verify the prognostic value of *EFNA1* in CRC.

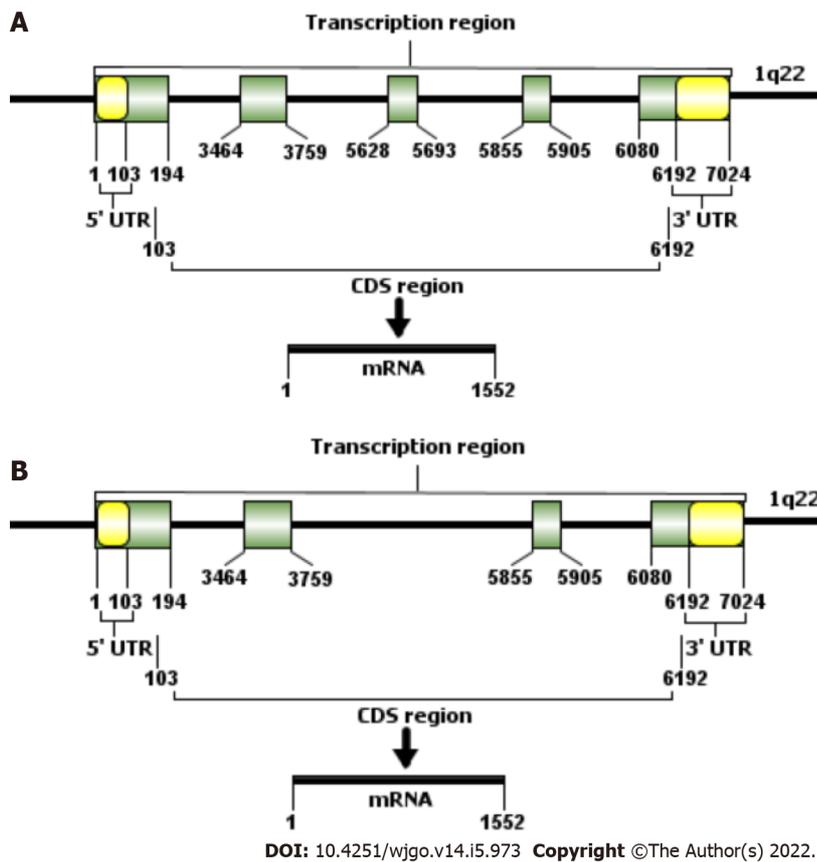
**Table 2** *EFNA1* prognostic value in gastrointestinal cancers

Tumor type	Sample type	Expression	Methods	Prognosis value	Notes	Ref.
Gastric cancer	Tissues	Increased	RT-PCR	(-)	<i>EFNA1</i> expression is related to GC stage, depth of invasion, lymph node metastasis and recurrence	Nakamura <i>et al</i> [30], 2005
	Tissues	Increased	IHC	Poor DSS	(-)	Miyazaki <i>et al</i> [32], 2013
	Tissues	Increased	IHC, RT-PCR	Poor DSS	<i>EFNA1</i> expression is related to TNM and lymph node metastasis	Yuan <i>et al</i> [33], 2009
	Tissues	Increased	RT-PCR	(-)	SNP (rs12904G>A) can regulate the expression of <i>EFNA1</i> and is related to GC susceptibility	Li <i>et al</i> [34], 2014
	Tissues	Increased	(-)	(-)	<i>EFNA1</i> expression increase the susceptibility of GC	Zhu <i>et al</i> [35], 2015
	Tissues	Increased	(-)	(-)	<i>EFNA1</i> expression may be related to GC susceptibility	Lee <i>et al</i> [36], 2015
	Tissues	Increased	IHC, RT-PCR	Poor DSS	GMAN up-regulates the expression of <i>EFNA1</i> and promotes the transfer of GC	Zhou <i>et al</i> [37], 2019
Colorectal cancer	Cells	(-)	(-)	(-)	<i>EFNA1</i> overexpression can inhibit the growth of HT29 cells	Potla <i>et al</i> [42], 2002
	Tissues	Increased	IHC, RT-PCR	(-)	The expression of <i>EFNA1</i> promotes the development of rectal adenocarcinoma to rectal cancer	Shi <i>et al</i> [43], 2012
	Tissues	Increased	(-)	(-)	<i>EFNA1</i> may be used as a diagnostic biomarker for the characteristics of CRC. In addition, the rs12904G/A variant is related to the susceptibility to CRC	Mao <i>et al</i> [44], 2013
	Cells	(-)	(-)	(-)	Eck and B61 are co-expressed in the same cell, suggesting the existence of an autocrine loop	Rosenberg <i>et al</i> [46], 1997
	Tissues	Increased	RT-PCR	Poor DSS	Decreased survival	Kataoka <i>et al</i> [47], 2004
	Cells	Reduced	(-)	Poor DSS	<i>EFNA1</i> can be used as a prognostic marker for CRC	Robertis <i>et al</i> [48], 2017
	Tissues	Increased	RT-PCR	Poor DSS	<i>EFNA1</i> is an independent prognostic factor for CRC	Yamamoto <i>et al</i> [49], 2013
	Serum	Increased	IHC, QRT-PCR	Poor DSS	<i>EFNA1</i> may be used for the identification of CRC	Lip <i>et al</i> [50], 2008
Hepatocellular carcinoma	Tissues	Increased	RT-PCR	Poor DSS	The high expression of <i>EFNA1</i> protein is related to histological differentiation, portal vein tumor thrombus and lymph node metastasis	Zhang <i>et al</i> [54], 2007
	Tissues	Increased	RT-PCR	Poor DSS	<i>EFNA1</i> is an independent prognostic factor of HCC	Wada <i>et al</i> [55], 2014
	Tissues	Increased	IHC	Poor DSS	<i>EFNA1</i> is involved in the mechanism of AFP induction in HCC	Lida <i>et al</i> [57], 2005
	Tissues, Serum	Increased	IHC, RT-PCR	Poor DSS	The expression of <i>EFNA1</i> is positively correlated with AFP	Cui <i>et al</i> [58], 2010
Esophageal cancer	Tissues	Increased	IHC, RT-PCR	Poor DSS	Decreased survival	Xu <i>et al</i> [59], 2005
	Tissues	Increased	(-)	Poor DSS	Decreased survival	Chen <i>et al</i> [60], 2019
	Cells	Increased	RT-PCR	(-)	High expression of <i>EFNA1</i> decreased the viability of ESCC cells	Yang <i>et al</i> [61], 2015

AFP: Alpha-fetoprotein; CRC: Colorectal cancer; DSS: Disease free survival; ESCC: Esophageal squamous cell carcinoma; GC: Gastric cancer; HCC: Hepatocellular carcinoma; IHC: Immunocytochemistry; RT-PCR: Reverse transcription-polymerase chain reaction; SNP: Single nucleotide polymorphism.

**Hepatocellular carcinoma**

*EFNA1* is widely expressed in HCC tissues[53-58]. Its expression is lowest in normal liver tissues, increases in liver cirrhosis tissues and is further increased in HCC tissues[54,57,58]. Existing studies have shown that the expression of *EFNA1* is related to HCC tissue differentiation and lymph node



**Figure 2** Schematic representation of the *EFNA1* chromosomal gene. A: mRNA (NM\_004428.3) schematic; B: mRNA (NM\_182685.2) schematic.

metastasis. In addition, overexpression of *EFNA1* indicates poor prognosis[14,54]. Cox multivariate analysis showed that *EFNA1* is an independent prognostic factor of HCC, suggesting that the expression of *EFNA1* may be a useful indicator for predicting the high risk of recurrence after radical resection of HCC[55].

In HCC, ephrin-A1 is closely related to expression of alpha-fetoprotein (AFP) and can indicate poor prognosis in patients with AFP[57,58]. A study by Lida *et al*[57] showed that ephrin-A1 induces the expression of genes related to the cell cycle (p21), angiogenesis, and cell-cell interaction (Rho, integrins, and matrix metalloproteinases) in HCC cells, and these ephrin-A1-induced genes are also activated in HCC tissues overexpressing AFP. Part of the reason for the poor prognosis of HCC patients with AFP is the expression of ephrin-A1 which induces the expression of tumor cell growth, angiogenesis, invasion and metastasis-related genes. In addition, Cui *et al*[58] found that the frequency of *EFNA1* expression in HCC tissues is higher than that of AFP (91% and 45%, respectively). In HCC cell lines and tissues, ephrin-A1 is positively correlated with AFP expression. In terms of secreted proteins, ephrin-A1 is detected in the supernatant of most primary HCC cell lines and it was clearly found that serum ephrin-A1 Levels in HCC patients are elevated. This suggests that *EFNA1* can be used as a useful serum marker to measure the development and progress of HCC.

### Esophageal cancer

At present, there are few studies on *EFNA1* in EC. Existing studies have confirmed that *EFNA1* is highly expressed in esophageal squamous cell carcinoma (ESCC) tissues and cells, and is indicative of a relatively poor prognosis[59-61]. Xu *et al*[59] used immunohistochemistry and reverse transcription-polymerase chain reaction (RT-PCR) to analyze the expression of *EFNA1* protein and mRNA in ESCC tissue. The results showed that 84.4% (146/173) sample positively expressed and 15.6% (27/173) sample negatively expressed *EFNA1*. In addition to overall survival, *EFNA1* protein expressions were significantly associated with histological grade, number of lymph node metastasis and clinical stage for patients with ESCC in the univariate analysis. In addition, studies have also shown that ephrin-A1 and EphA2 often co-localize in the tumor area and vascular endothelial cells in ESCC, and their expression is related to co-localization[59]. A study by Chen *et al*[60] showed that the expression level of *EFNA1* in ESCC tissues is higher than that in normal tissues. Survival analysis showed that *EFNA1* expression is associated with shorter overall survival. Regarding the expression of *EFNA1* in ESCC and its prognostic role, more studies are needed to further confirm these results.

### **Role of *EFNA1* in gastrointestinal cancers**

*EFNA1* is differentially-expressed in many gastrointestinal cancers and high expression of *EFNA1* may have an important function in the formation of the malignant phenotype of gastrointestinal cancers[28-61]. The effects of differential *EFNA1* expression on gastrointestinal cancers are mainly manifested in the following aspects.

### **Regulation of gastrointestinal cancer cell growth**

Ephrin-A1 exerts an inhibitory effect on the growth of GC, CRC, HCC and ESCC cells. Both anchorage-dependent and anchorage-independent growth of tumor cells overexpressing EphA2 was observed to be reduced by treatment with ephrin-A1-Fc, an ephrin-A1 fused to the Fc domain of IgG[30,62]. The EphA2 receptor is activated by its ligand ephrin-A1, triggering the down-regulation of the total expression of EphA2 in GC cells resulting in a net inhibition of the proliferation of GC cells[33]. Potla *et al*[42] found that in three-dimensional spheroid cultures of HT29 colon cancer cells, an increase of *EFNA1* expression reduces the growth of tumor cells. Shi *et al*[43] reported that the expression of *EFNA1* mRNA increases in the progression from rectal adenoma to rectal cancer. In addition, a recent study conducted by Yamamoto *et al*[49] showed that *EFNA1* is an independent prognostic factor for CRC and its loss of function is related to decreased proliferation, invasion and migration of CRC cell lines.

Eph/Ephrin can also regulate the effects of other growth factors on cell growth. Miao *et al*[63] reported that when EphA2 is activated by ephrin-A1, the Ras/Erk pathway can be inhibited to reduce cell growth induced by platelet-derived growth factor, vascular endothelial growth factor (VEGF) and epidermal growth factor. In addition, the overexpression of *EFNA1* is related to the growth and proliferation of gastrointestinal cancer cells and may play the role of a cell growth factor or growth promoting factor[64]. Therefore, in a sense, *EFNA1* can be considered as a potential growth factor[65] and its abnormal expression in cancers can affect tumor growth and formation.

### **Regulation of gastrointestinal cancer cells adhesion**

Malignant tumor cells often exhibit low cell adhesion which can be due to a lack of cadherin function. Ephrin-A1 has been shown to recruit the Src family kinase Fyn into lipid rafts which is followed by redistribution of vinculin, activation of the mitogen-activated protein kinase pathway, protein tyrosine phosphorylation and increased cell-substrate adhesion[66,67]. In addition, studies have shown that the amount of ephrin-A1 determines the extent of EphA2-dependent, integrin-mediated cell adhesion[68]. In cancer cells lacking cadherin, cell-to-cell contact is reduced. Therefore, EphA2 cannot bind to ephrin-A1 attached to the adjacent cell membrane and cannot undergo tyrosine phosphorylation which facilitates cancer cell detachment from surrounding cells leading to cancer cell spread and increased invasion.

Studies have shown that cadherin can significantly affect the expression and subcellular localization of ephrin-A1/EphA2, and ephrin-A1/EphA2 in turn can also regulate the function of cadherin[69]. EphA2 promotes tumor growth by enhancing the adhesion of tumor cells to the extracellular matrix increasing anchorage-independent growth and angiogenesis[70]. The specific mechanism may be related to the dysfunction of the cadherin glycoprotein in the phosphorylation or distribution of EphA2 at the sites of cell contact[71].

### **Regulation of gastrointestinal cancer cells migration**

*EFNA1* not only plays a role in normal physiological processes but also plays an important role in pathological processes such as tumor formation[72,73]. It has been reported that ephrin-A1 and EphA2 are up-regulated in most gastrointestinal tumors and this up-regulation is related to tumor formation and tumor migration[73-75]. Microarray analysis of 220 CRC samples and RT-PCR analysis of 146 CRC samples showed that loss of ephrin-A1 after siRNA knockdown decreases cell proliferation, invasion and migration. Expression of *EFNA1* is a high-risk indicator for predicting recurrence and cancer-related death after radical resection of CRC[49]. Leguchi *et al*[76] showed that when tumor cells treated with PBS or ephrin-A1-Fc are injected into mice, tumor cells in the lungs can be detected, but that ephrin-A1-Fc treatment increased lung permeability and enhanced tumor metastasis, whereas neutralization by anti-ephrin-A1 antibody reduced the effect.

The regulation of Eph/Ephrin on cancer cell migration is mainly through its influence on the function of integrins. Miao *et al*[77] showed that when EphA2 is activated, it can inactivate integrin function, inhibit cell spreading, migration and integrin-dependent cell adhesion. They also found that when EphA2 is activated with ephrin-A1, EphA2 can quickly recruit the tyrosine phospholipase SHP2, which can dephosphorylate focal adhesion kinase (FAK) and paxillin, leading to the dissociation of the EphA2 and FAK complex[77,78]. Other data also indicate that the activation of ephrin-A1 can generally increase the adhesion of cells to the extracellular matrix and promote cell migration[79-81].

## EFNA1 AND TUMOR ANGIOGENESIS

Tumor angiogenesis is a common pathological phenomenon in carcinogenesis and directly regulates the pathological process of tumor growth, invasion and metastasis. Tumor angiogenesis can bring nutrients and oxygen necessary for tumor cell growth and discharge metabolic waste. At the same time, new blood vessels can be used as a metastasis channel to mediate distant metastasis of tumors[82]. Angiogenesis is regulated by a variety of pro-angiogenic factors and anti-angiogenic factors. Currently, five major protein families are considered to be key regulators of tumor angiogenesis, namely VEGF and its receptor family, angiopoietin and the TIE receptor family, Notch receptor family, Eph/ephrin family and Slit ligand/Robo receptor family[1,83]. Among them, ephrin-A1 and its main receptor EphA2, as the main members of the Eph/ephrin family, are not only significantly expressed in a variety of malignant tumors but are also closely related to normal and tumor angiogenesis.

### Role of EFNA1 in tumor angiogenesis

In 2000, Ogawa *et al*[84] first reported that ephrin-A1/EphA2 plays an important role in tumor angiogenesis, showing that overexpression of ephrin-A1 in tumor cells promotes tumor angiogenesis, whereas down-regulation of ephrin-A1 expression inhibits tumor cell-induced endothelial cell migration and reduces microvascular density. Functional changes such as migration of vascular endothelial cells, play a key role in tumor angiogenesis. Ephrin-A1 is mainly expressed in tumor cells while EphA2 is mainly expressed in tumor blood vessels. Therefore, it is speculated that tumor cells expressing ephrin-A1 have the effect of attracting endothelial cells expressing EphA2 leading to formation of new blood vessels and angiogenesis. EphA2 expressed on the surface of endothelial cells is a key component in the regulation of angiogenesis. Blocking EphA2 can limit the migration of endothelial cells, vascular reorganization and VEGF-induced angiogenesis.

EphA2 can promote the migration of tumor vascular endothelium and ephrin-A1 has been confirmed to act as a chemical inducer in the process of vascular remodeling[85], suggesting that the interaction between the two in tumor cells and vascular endothelial cells is jointly involved in tumor angiogenesis [85,86]. Combination of the two can promote the migration of tumor vascular endothelial cells and promote the formation of capillary-like structures in tissues and endothelial cells by affecting the cytoskeleton, matrix adhesion and/or cell adhesion. Inhibition of EphA2 activation also reduces tumor angiogenesis, further supporting an important role for EphA2 in tumor neovascularization, invasion and metastasis[85-87]. Pandey *et al*[85] confirmed that ephrin-A1, not fibroblast growth factors, specifically regulates TNF- $\alpha$ -induced angiogenesis in mice *in vivo*. This suggests that the induction of ephrin-A1 and subsequent activation of its receptor EphA2 may regulate angiogenesis mediated by TNF- $\alpha$ .

Ogawa *et al*[84] found that ephrin-A1 and EphA2 are stably expressed in some endothelial cells within gastrointestinal tumors including EC and CRC. In CRC, the expression of ephrin-A1/EphA2 is up-regulated in tumor areas with higher blood vessel density. In small volume CRC tumors (< 5 cm), the expression of ephrin-A1 and EphA2 is higher[47,88]. Liu *et al*[89] used the microvessel density (MVD) method to label tumor blood vessels with CD34 and directly observe and quantify tumor angiogenesis as well as observe tumor invasion and metastasis. The results of the study showed that MVD in GC tissue is higher than that in adjacent tissues and normal gastric mucosa. MVD increases with the decrease of GC differentiation and increases in infiltration depth, lymph node metastasis and tumor diameter and it is closely related to increased tumor malignancy and metastasis. It is also positively correlated with the expression of EphA2 and ephrin-A1. This suggests that ephrin-A1 may play a role in promoting vascularization and play an important role in the formation of blood vessels in GC.

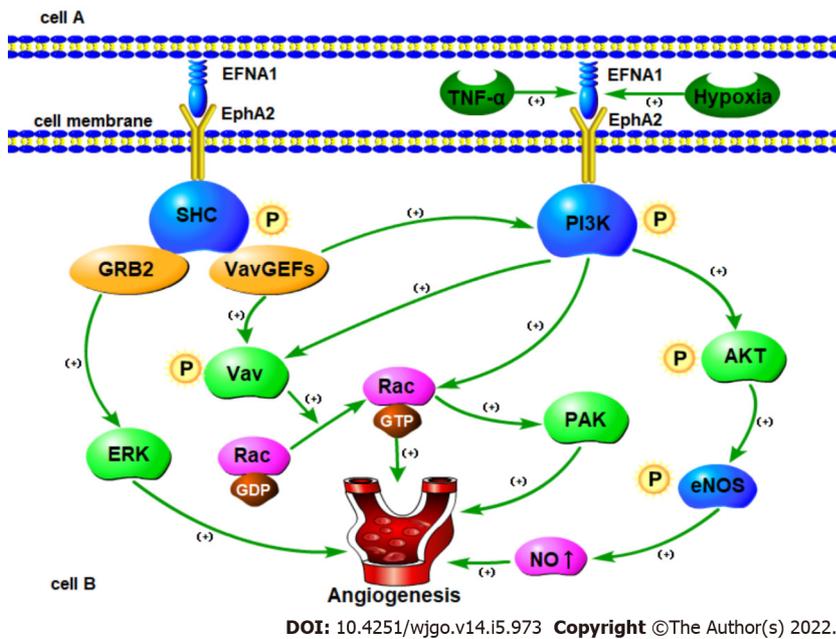
### Possible mechanisms of EFNA1-promoted tumor angiogenesis

There is sufficient experimental evidence to show that EphA2 activation on endothelial cells is necessary for ephrin-A1 to exert its angiogenic effect *in vitro* and *in vivo*[90]. The mechanism by which EFNA1 induces angiogenesis is not fully understood. So far, only a few studies have shed light on the molecular mechanism of ephrin-A1-induced angiogenesis. Based on this, we summarize the possible mechanism by which ephrin-A1 promotes tumor angiogenesis (Figure 3).

### Erk-associated signaling pathways

EFNA1 can be activated *via* the ERK1/2 pathway through EphA2 and promote the proliferation, migration and angiogenesis of HUVECs[91,92]. Activation of EphA2 by ephrin-A1 can promote the migration of endothelial cells and the formation of capillary structures by regulating the morphology, migration, adhesion and proliferation of vascular endothelial cells. Interaction between the two has also been confirmed to induce angiogenesis *in vivo*[93]. For example, ephrin-A1-Fc can increase the adhesion of HUVECs by activating integrins and promoting vascular function[94].

Pratt *et al*[95] have shown that ephrin-A1-mediated stimulation of EphA2 receptor tyrosine kinase can transmit signals from the cell membrane through MAP kinase. These signals are transmitted to the nucleus by inducing the transcription of Elk-1 and are transmitted back to the cell membrane through



**Figure 3 Possible molecular mechanisms by which *EFNA1* induces angiogenesis.** PI3K: Phosphatidylinositol 3-hydroxy kinase; eNOS: Endothelial nitric oxide synthase; TNF- $\alpha$ : Tumor necrosis factor  $\alpha$ ; PAK: p21-activated kinase.

the destabilization of the cell's attachment to the ECM. In addition, studies have shown that the biochemical mechanism of EphA2 signaling involves the activation-dependent interaction between tyrosine phosphorylation of EphA2 and SHC adaptor protein. SHC in turn bridges EphA2 to GRB2 which contributes to ERK kinase activation and nuclear translocation.

### Growth factors and cytokines mediated signaling pathways

In different types of cells, growth factors and cytokines can induce the expression of *EFNA* ligands. Ephrin-A1 was the first *EFNA* ligand identified and shown to be an immediate early gene product induced by TNF- $\alpha$  in cultured HUVECs[21]. Unlike other angiogenic factors induced by TNF- $\alpha$ [96,97], Cheng *et al*[98] showed that *EFNA1* induction does not require NF- $\kappa$ B or p42/44 MAPK signaling, but rather activation of the JNK and p38MAPK signaling pathways[99]. Both of these pathways have been shown to regulate actin reorganization and cell migration in endothelial cells[100,101]. Therefore, regulating the expression of *EFNA1* by p38 MAPK and JNK is consistent with the role of *EFNA1* in endothelial cell migration and blood vessel assembly. In addition, Hess *et al*[102] showed that TNF- $\alpha$  can up-regulate the expression of *EFNA1* by acting on HUVECs leading to increased phosphorylation of EphA2 resulting in increased angiogenesis and enhanced cell chemotaxis. Phosphorylation of EphA2 caused by ephrin-A1 can activate phosphatidylinositol 3-hydroxy kinase (PI3K) and up-regulate Rac1 activity thereby causing endothelial cell migration to increase and promote angiogenesis[102].

In addition to TNF- $\alpha$ , ephrin-A1 is also induced by lipopolysaccharide[103], interleukin-1 $\beta$ [21,103], and VEGF in HUVECs and microvascular endothelial cells[98]. The study of Cheng *et al*[98] showed that similar to TNF- $\alpha$ , VEGF induces ephrin-A1 as an immediate early gene product. Blocking EphA receptor signaling inhibits VEGF-induced endothelial cell survival, migration, *in vitro* sprouting and *in vivo* angiogenesis indicating that EphA receptor activation is necessary for VEGF-induced angiogenesis[98]. Ojima[104] and Chen *et al*[105] showed that soluble ephrin-A1-Fc can promote the tube formation and migration of HUVECs, while EphA2-Fc can antagonize the interaction between EphA2 and ephrin-A1 thereby reducing VEGF-induced endothelial cell migration and proliferation.

### Vav-mediated signaling pathways

Studies have shown that *EFNA1* stimulates endothelial cell migration and assembly in culture[84,106], while EphA2 receptor-dependent endothelial cell migration and assembly require activation of Rac1 GTPase[107]. In addition, Vav2 and/or Vav3 are required for ephrin-A1-induced endothelial cell migration/assembly and Rac1 activation[107,108]. Therefore, Hunter *et al*[108] studied ephrin-A1 and Vav and found that when ephrin-A1 binds to EphA2, EphA2 is phosphorylated by tyrosine. Activated EphA2 can directly recruit Vav-GEFs through the SH2 region so that the Vav protein can be phosphorylated and activated directly or indirectly. In addition, by recruiting p85, EphA2 receptors can also up-regulate phosphatidylinositol-3,4,5-trisphosphate levels through the PH domain and enhance Vav-GEF activity. The activated Vav-GEFs subsequently increase Rac1-GTP levels and promote endothelial cell migration and angiogenesis.

### **eNOS-mediated signaling pathways**

The promotion and inhibition of ephrin-A1 on the same signal pathway has also been observed in different cell or tumor types. It is well known that endothelial nitric oxide synthase (eNOS) and NO play a key role in endothelial cell migration and angiogenesis[109]. There is ample evidence that eNOS is mainly expressed in tumor vascular endothelial cells, and the NO produced by it plays a direct role in tumor angiogenesis induced by various angiogenic factors[110,111]. Hypoxia is one of the most common and important features in the tumor microenvironment which helps induce a variety of angiogenic factors[112].

Therefore, Song *et al*[113] explored the mechanism of *EFNA1* regulating angiogenesis by observing the effect of hypoxia on the expression and secretion of ephrin-A1 in tumor cells and the possible relationship between *EFNA1* and eNOS/NO in tumor angiogenesis. Studies have shown that the upregulation of membrane-bound ephrin-A1 induced by hypoxia may interact with EphA2 receptors on endothelial cells in the tumor microenvironment and induce eNOS phosphorylation and increase NO production through PI3K/AKT-dependent pathways thereby promoting tumor angiogenesis. These results show that the PI3K/AKT/eNOS signaling cascade may be a common pathway for hypoxia-induced ephrin-A1-dependent angiogenesis.

### **Rac-PAK signaling pathways**

Studies have shown that in the vasculature, stimulating vascular smooth muscle cells with ephrin-A1 can inhibit cell proliferation through the inactivation of Rac1 and p21-activated kinase (PAK)[107]. Therefore, ephrin-A1 stimulation leads to inactivation of Rac1 and inhibition of cell proliferation in smooth muscle cells of the blood vessel wall leading to a loss of blood vessels. On the contrary, ephrin-A1 activates Rac1 and induces cell migration and blood vessel assembly of endothelial cells and promotes the sprouting and branching of new capillaries from existing blood vessels[107,114].

However, another study using rat vascular smooth muscle cells showed that ephrin-A1-mediated morphological changes are related to the inhibition of Rac1 and PAK1 activity and are antagonized by the expression of a constitutively-active Rac mutant[115]. The use of siRNA to inhibit the synthesis of Rac1 enhanced the ephrin-A1-induced inhibition of proliferation. Sphingosine-1-phosphate (S1P), a lipid mediator known to inhibit Rac activation in vascular smooth muscle cells, amplifies the effect of ephrin-A1. In conclusion, the authors emphasized the role of the Rac/PAK pathway in ephrin-A1-mediated cell proliferation inhibition. In this way, ephrin-A1 alone or in synergy with S1P can participate in vascular instability which is a prerequisite for angiogenesis[107,115].

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## **TARGETED THERAPY OF *EFNA1* IN GASTROINTESTINAL CANCERS**

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*EFNA1* is widely expressed in gastrointestinal cancer tissues, especially in highly aggressive cancer cells, suggesting that ephrin-A1 can be used as an important surface marker of gastrointestinal cancer cells and has potential diagnostic and prognostic value. The close relationship between *EFNA1* and the occurrence and development of gastrointestinal cancers has been confirmed which could represent a breakthrough in the search for new cancer treatment drugs.

Yang *et al*[61] found that *EFNA1* is involved in the resistance of ESCC cells to Photofrin-mediated photodynamic therapy (PDT). *EFNA1* is up-regulated in PDT-resistant ESCC cells and simultaneous incubation with oligomeric ephrin-A1 and soluble ephrin-A1 leads to significant resistance of ESCC cells to Photofrin-PDT[61]. These findings suggest that in ESCC, ephrin-A1 may be an attractive research direction and target for PDT resistance.

Studies have shown that in CRC, the combination of ephrin-A1-Fc and EphA2 can make EphA2 phosphorylated, and the complex formed moves into the cell and gradually degrades, thereby achieving the effect of inhibiting tumor progression[30]. In addition, the overexpression of EphA2 in CRC leads to resistance to chemotherapy[48] and the activation of EphA2 after ephrin-A1 treatment restores the efficacy of cetuximab against CRC cells[116]. These studies show that the combination of ephrin-A1 and cetuximab in tumor treatment provides a method for reversing CRC chemotherapy resistance but more preclinical and clinical studies are needed for confirmation.

Aiming at the specific binding between the G-H loop of ephrin-A1 and the ligand binding domain of EphA2[117], investigators have screened for small molecule antagonists that can selectively block Eph receptors thereby preventing the activation of EphA2[118]. For example, lithocholic acid (LCA), as a small molecule compound, can compete to hinder the binding of ephrin-A1 and EphA2. Its role is to interact with the G-H loop of ephrin-A1 and hinder the binding of ephrin-A1 to its receptor[119]. In addition, anti-EphA2 antibody and EphA2-Fc fusion protein have also been used to block the activation of EphA2, and significant anti-tumor angiogenesis effects have been observed *in vitro* and *in vivo*[120-122]. The activation of EphA2 receptors in tumor cells can block the activation of some important oncogenes[123,124] and ephrin-A1-Fc is currently the most widely used EphA2 receptor agonist. Duggineni *et al*[125] have designed and synthesized peptide molecules that can functionally bind to ephrin-A1 based on the characteristics of the ephrin-A1-binding domain. Such peptides can be expected to become new drugs for tumor suppression, targeted therapy and tumor imaging.

## CONCLUSION

In summary, *EFNA1* plays an important role in the occurrence, development and angiogenesis of gastrointestinal tumors and its mechanism of promoting angiogenesis has also been studied in depth. However, the research on *EFNA1* and pancreatic cancer is still in the initial exploration stage. In future work, the clinical application of *EFNA1* in pancreatic cancer still needs more experiments and clinical studies to conduct a comprehensive verification of the system. In addition, the specific molecular mechanism of *EFNA1* in tumor progression is still poorly understood, and many aspects remain to be explored.

Rac/PAK, PI3K/AKT, ERK and other pathways are involved in tumor angiogenesis mediated by *EFNA1*/EphA2. *EFNA1* is expressed in tumor cells and tumor-related blood vessels. Current research mainly focuses on the function and mechanism of *EFNA1* in tumor cells and vascular endothelial cells. Tumors are dependent on angiogenesis but there are few reports on whether ephrin-A1 on the surface of tumor cells is related to EphA2 receptors on the surface of vascular endothelial cells or how they interact.

Ephrin-A1 has always been considered a GPI-coupled membrane-coupled ligand and its activation requires cell-to-cell contact. However, in 2008, Wykosky *et al*[126] found that ephrin-A1 can be secreted from malignant glioma cells and breast cancer cells into the cell supernatant and still retain its ability to activate EphA2. This suggests that ephrin-A1 derived from tumor cells not only acts on adjacent vascular endothelial cells to induce angiogenesis through a paracrine mechanism, but may also act on distant blood vessels to promote angiogenesis.

Hypoxia and inflammation are two major characteristics of the tumor microenvironment. Accompanied by many pathological processes, such as tumor occurrence, development, invasion, metastasis and angiogenesis, they also regulate the expression and function of tumor-related proteins. Studies have found that in solid tumors with hypoxia due to ischemia, the expression of *EFNA1* can be significantly upregulated[127]. Vihanto *et al*[128] also found, using a rat skin hypoxia model, that the expression of ephrin and Eph receptors in skin epithelial cells increases under hypoxic conditions. If it is possible to clarify the effect of hypoxia on the expression of *EFNA1* in gastrointestinal tumor cells, especially the effect on the secretion of soluble *EFNA1*, it may further reveal the function of *EFNA1* in gastrointestinal tumors.

Research on *EFNA1* in gastrointestinal tumor formation, tumor cell apoptosis and angiogenesis are still in its infancy. Further analysis and study of its signal transduction mechanisms in gastrointestinal tumors will help clarify the mechanism of tumor progression, invasion and metastasis, and provide a more reliable theoretical basis for tumor therapy.

## FOOTNOTES

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