

Original Article

Decreased expression of receptor tyrosine kinase of EphB1 protein in renal cell carcinomas

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Abstract: Receptors tyrosine kinase of Eph superfamily plays an important role in human cancers. We previously found that EphB1 subtype is down-regulated in gastric cancer, colorectal cancer and ovary serous carcinoma. Fore the more, the decreased expression of EphB1 is related to invasion and metastasis in cancers. Although EphB1 has been revealed as an important receptor in cancers, our understanding of its roles in renal cell carcinoma (RCC) is limited. In the present study, using specific anti-EphB1 polyclonal antibody and immunohistochemistry, we evaluated EphB1 protein expression levels in RCC specimens surgically resected from 82 patients (including 62 conventional clear-cell RCC, 10 papillary, and 10 chromophobic RCC cases). We found EphB1 protein is positively expressed in the epithelium of renal tubules. Decreased expression of EphB1 was found in all RCC carcinomas compared with expression in the normal epithelium of renal tubules. EphB1 protein moderately expressed in chromophobic RCC, weakly expressed in clear-cell RCC and negatively expressed in papillary RCC. Our results indicate that EphB1 may be involved in carcinogenesis of RCC, the molecular mechanisms of down-regulation of EphB1 including genetic and epigenetic alterations and the dedicated roles of EphB1 in occurrence and progress of RCC need to be explicated in next step.

Keywords: EphB1, renal cell carcinoma, down-regulation

Introduction

Renal cell carcinoma (RCC) has the highest mortality rate of the genitourinary cancers and the incidence of RCC has risen steadily. There are 63,920 new cases of kidney cancer are diagnosed in United States of 2014 [1]. RCC is the most common form of adult kidney cancer and accounts for 2-3% of all adult malignancies globally. RCC is heterogeneous and comprises several histological subtypes according to the differences in genetics, biology and behavior. The most common histological type is clear cell carcinoma, also called conventional RCC, which represents 75-80% of RCC. Papillary (10-15%), Chromophobe (5%) and other more rare forms such as collecting duct carcinoma (<1%) comprise the remainder. RCC is thought to arise from a variety of specialized cells located along the length of the nephron [2]. Both clear cell and papillary RCC are thought to arise from the epithelium of the proximal tubule. Chromophobe RCC is believed to arise from the distal neph-

ron, probably from the epithelium of the collecting tubule. Each type has differences in genetics, biology and behavior.

Clear cell RCC can be sporadic or familial. Chromosome 3p deletion and inactivation of the von Hippel-Lindau (VHL) suppressor gene is the most common genetic alteration [3-5]. Almost all familiar clear cell RCC arise from an inherited mutation in VHL tumor suppressor gene. The second allele of VHL has been shown to be inactivated by deletion and by promoter hypermethylation or rearrangement in the RCC. Papillary RCC (pRCC) are the most common non clear cell RCC. The MET proto-oncogene is a cell surface receptor tyrosine kinase for the ligand hepatocyte growth factor. Germline mutations of the MET oncogene at 7q31 have been detected in patients with hereditary type I pRCC and in 13% of sporadic type I pRCC [6].

The erythropoietin producing hepatocellular carcinoma (Eph) subfamily of genes encoding

receptor tyrosine kinases are structurally characterized by the juxtaposition of a vestigial immunoglobulin-like domain, a single cysteine-rich region, and two FN III domains in the extracellular region. The Eph receptors comprise eight EphA and six EphB receptors based on the similarity within each group of the extracellular domain sequences and on the affinity for binding ephrin-A and -B ligands. Eph receptors and their ligands of ephrin form a large family of receptor tyrosine kinases that involved in several physiological and pathological processes. Receptors of the Eph family and their ligands mediate developmental vascular assembly and direct axonal guidance [7-9]. Eph receptors and their ligands play important roles in tumorigenesis, cancer progression and invasion [10-12].

In our previous study on the Eph family, we have found that the reduced expression of EphB1 in colorectal cancer more often occurred in poorly differentiated and mucinous adenocarcinomas and showed more invasive power [13]. Interestingly, we also have found that underexpression of EphB1 protein is significantly associated with invasive, advanced stage and metastasis in gastric cancer [14]. We examined the expression of EphB1 protein in a series of surgically treated serous carcinomas of ovary. Loss of expression of EphB1 protein was associated with higher tumor grade, metastasis and high proliferative index Ki67 expression, but not with FIGO stage, age at diagnosis, and diameter of carcinoma [15].

Recently, advancement in understanding of the VHL and MET gene pathway has produced pharmaceutical outcomes based on specific molecular targets that have changed the treatment landscape for patients with RCC [6, 16-23]. Unfortunately, the vast majority of treated patients with RCC eventually develop progressive disease due to acquired resistance. Hence, a better understanding of mechanisms involved in the pathogenesis of RCC and more effective therapeutic approaches are urgently required.

Materials and methods

Patients and tissues

RCC tumor and adjacent normal kidney specimens were analyzed from a total of 82 patients (54 males, 28 females, average age = 57.7 years; range = 33-82 years old at the time of

resection) with consent under the Jinling Hospital Ethic Committee-approved protocols. All patients were treated by radical or partial nephrectomy and rendered disease-free. Of the 82 RCC tumors evaluated, 62 were diagnosed as conventional clear cell RCC, 10 as papillary RCC, and 10 as chromophobe RCC.

Immunohistochemistry

Sections from surgical specimens had been fixed in 10% formalin and embedded in paraffin and they were used here for immunohistochemical staining according to a standard method. Briefly, each 4- μ m tissue section was deparaffinized and rehydrated. After rehydration through a graded ethanol series, the sections were autoclaved in 10 mM citrate buffer (pH 6.0) at 120°C for 2 min for antigen retrieval, then cooled to 30°C and washed with phosphate-buffered saline (PBS, pH 7.3). After endogenous peroxidase had been quenched with aqueous 3% H₂O₂ for 10 minutes and washed with PBS, the sections were incubated at 4°C overnight with an EphB1 polyclonal antibody (Abgent, San Diego, CA, USA) at a 1:100 dilution in antibody diluent solution (Zymed, Invitrogen) and then washed with PBS. Next, the sections were incubated with secondary antibody (Dako REAL EnVision Detection System, Dako, UK) for 30 min at room temperature. Color development was performed with 3, 3'-diaminobenzidine (DAB). Nuclei were lightly counterstained with hematoxylin. Two pathologists independently assessed the immunostained slides. Any difference in immunohistochemical scores was resolved by a consensus. Immunohistochemical staining of cancer cells was semi-quantitatively assessed according to the staining intensity of positive cells. EphB1 expression was assessed for intensity (0 = no staining, 1 = weak, 2 = moderate, 3 = strong). Here, we used colorectal cancer and normal mucosa tissues that showed negative and positive expression of EphB1 as controls. The specificity of EphB1 antibody was investigated by using blocking peptide as we previously reported.

Results

EphB1 is positively expressed in the epithelium cells of the proximal tubule

All RCC samples were pathologically diagnosed by two pathologists. There were 82 RCC tumors

Loss of expression of EphB1 in RCC

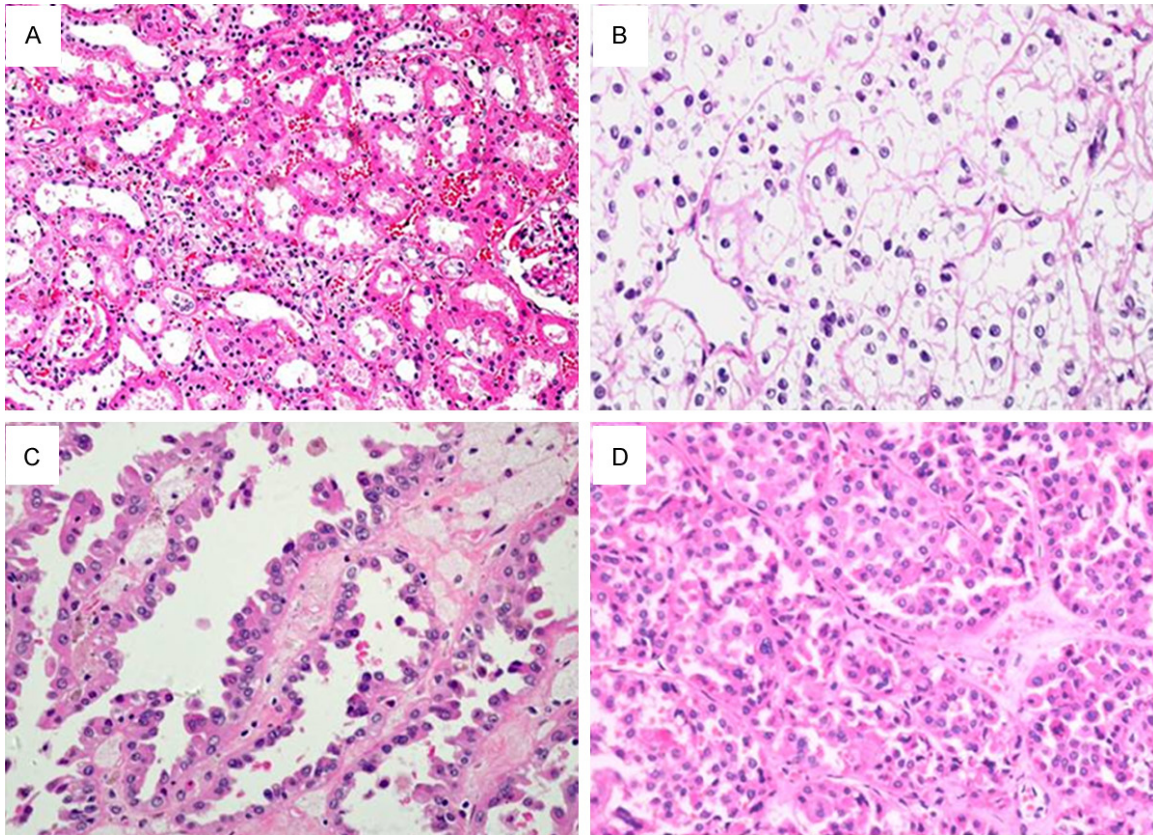


Figure 1. Representative illumination of different type of RCCs with H&E stain. A: Normal renal tissue. B: Clear cell RCC. C: Papillary RCC. D: Chromophobic RCC.

evaluated, which including conventional clear cell RCC, papillary RCC, and chromophobe RCC (**Figure 1**). The EphB1 protein was localized in the cytoplasm of the epithelial cells. The cytoplasm of normal epithelium cells of the proximal tubule are stained strongly for EphB1 (**Figure 1**). There is no staining of EphB1 found in the epithelium cells of distal nephron and the collecting tubule. No staining of EphB1 was found in epithelium cells of renal glomerulus.

Expression of EphB1 protein is decreased in renal cell carcinomas

Decreased expression of EphB1 was found in all RCC carcinomas compared with expression in the normal epithelium of renal tubules. EphB1 protein moderately expressed in chromophobic RCC, weakly expressed in clear-cell RCC and negatively expressed in papillary RCC (**Figure 2**).

Discussion

The Eph gene family is the largest subfamily of receptor tyrosine kinase. Roles of the Eph in

normal physiology and oncogenesis have been well-established. Eph receptor genes are primarily considered to be classical oncogenes. EphA2 is overexpressed in numerous epithelial-type carcinomas and associated with metastatic lesions. Herrem *et al* assessed EphA2 expression in archived renal cell carcinoma tissues and found that regardless of histopathologic subtype, RCC lesions expressing higher levels of EphA2 [24]. Higher levels of EphA2 tended to be a higher grade, larger and more highly vascularized tumors. The degree of EphA2 overexpression seemed predictive of short-term disease-free interval and of overall survival. These data suggest that EphA2 expression level may serve as a useful prognostic tool in RCC. Nagano *et al* analyzed the expression of EphA10 at the mRNA and protein level in clinical breast cancer tissues and evaluated the relationship with clinicopathological parameters. Their results show that lymph node metastasis and stage progression were significantly correlated with EphA10 expression at the mRNA and protein level [25]. However, the role of Eph receptors and Ephrin ligands in

Loss of expression of EphB1 in RCC

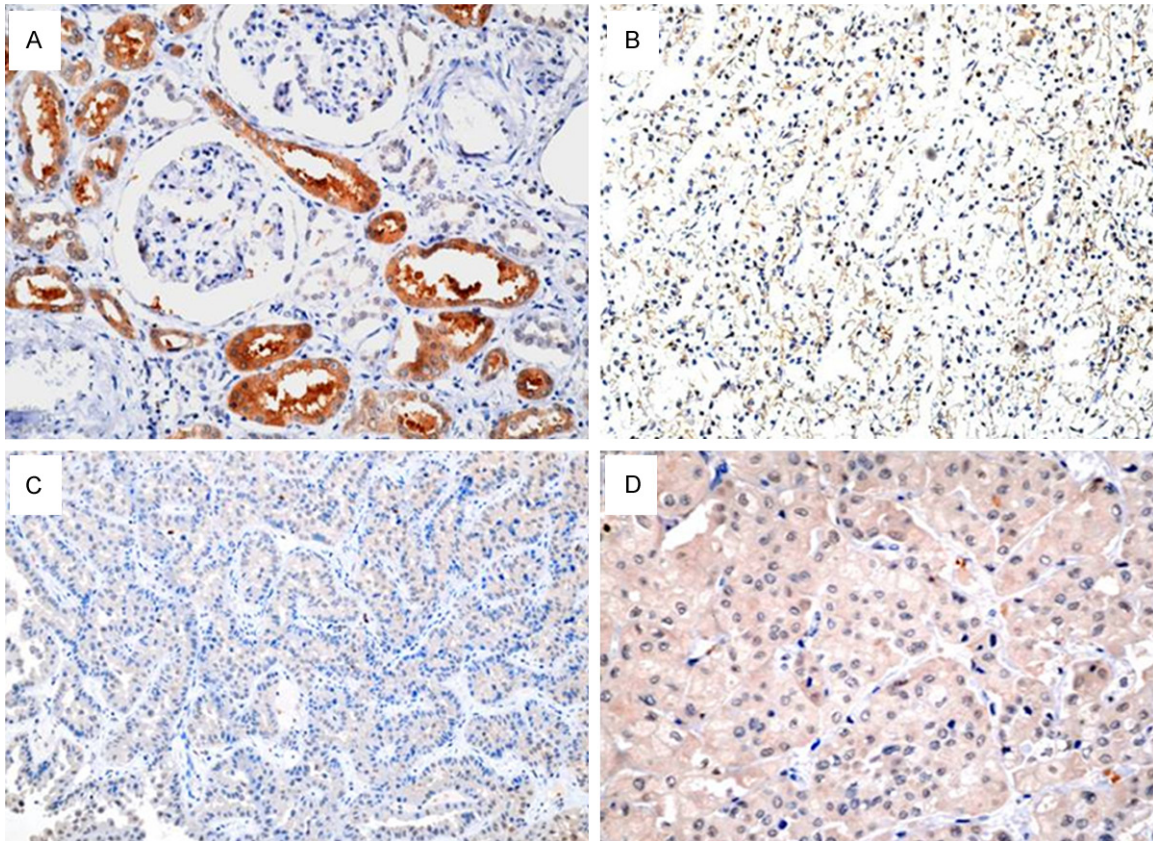


Figure 2. EphB1 protein is positively expressed in the epithelium of renal tubules (A). Decreased expression of EphB1 was found in all RCC carcinomas compared with expression in the normal epithelium of renal tubules. EphB1 protein is weakly expressed in clear-cell RCC (B), negatively expressed in papillary RCC (C) and moderately expressed chromophobic RCC (D).

oncogenesis appears to be complex and remains ill-defined. There are increasing conflicting data regarding Eph receptor genes in different cancer types, especially in regarding to the putative function of the genes that encode Eph receptors as oncogenes or tumor suppressor genes [26, 27]. We have previously reported that down-regulation of the EphA7 in colorectal cancers and gastric cancers [28, 29]. The down-regulation of EphA7 is secondary to hypermethylation of the 5' CpG island. Using quantitative real-time reverse transcriptase-polymerase chain reaction and/or immunohistochemical staining, we analyzed EphB1 expression in gastric cancer and serous carcinoma of ovary [14, 15]. We demonstrated that loss of expression of EphB1 protein associated with invasion and metastasis in gastric carcinoma and is associated with metastasis and poorer survival in patients with serous ovarian cancer. Our data indicate that EphB1 may have a tumor-suppressive role in cancers. These

results are consistent with that of other studies. Teng *et al* reports Ligand-dependent EphB1 signaling suppresses glioma invasion and correlates with patient survival [30]. In fact, the role of Ephs and Ephrins in cancer progression has been demonstrated for many of the family members and they have both tumor promoter and suppressor functions in different cellular contexts.

In the present study, we found EphB1 protein is positively expressed in the epithelium of renal tubules. Decreased expression of EphB1 was found in all RCC carcinomas compared with expression in the normal epithelium of renal tubules. EphB1 protein moderately expressed in chromophobic RCC, weakly expressed in clear-cell RCC and negatively expressed in papillary RCC.

Our results indicate that EphB1 may be involved in carcinogenesis of RCC, the molecular mech-

anisms of down-regulation of EphB1 including genetic and epigenetic alterations and the dedicated roles of EphB1 in occurrence and progress of RCC need to be explicated in next step.

Identification of additional and essential molecular determinant is imminent to designate alternative strategies to overcome resistance in RCC therapy. The Eph receptors and ephrin ligands are studied as novel therapeutic approaches of anticancer therapies in cancers [31-35].

Our findings indicated that EphB1 can be a potential biomarker in RCC diagnosis and a therapeutic target of RCC.

In conclusion, our results indicate that EphB1 loss in RCC, the molecular mechanisms of down-regulation of EphB1, the dedicated roles of EphB1 in occurrence, progress and therapy of RCC need to be explicated in the next step.

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Disclosure of conflict of interest

None

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