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REVIEW ARTICLE

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Proteolytic cleavage of membrane proteins by membrane type-1 MMP regulates cancer malignant progression

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Abstract

Strategies to develop cancer therapies using inhibitors that target matrix metalloproteinases (MMPs), particularly membrane type-1 MMP (MT1-MMP), have failed. This is predominantly attributed to the specificity of MMP inhibitors and numerous functions of MMPs; therefore, targeting substrates with such broad specificity can lead to off-target effects. Thus, new drug development for cancer therapeutics should focus on the ability of MT1-MMP to break down substrates, such as functional cell membrane proteins, to regulate the functions of these proteins that promote tumor malignancy. In this review, we discuss the mechanism by which proteolysis of cell surface proteins by MT1-MMP promotes progression of malignant tumor cells. In addition, we discuss the two protein fragments generated by limited cleavage of erythropoietin-producing hepatoma receptor tyrosine kinase A2 (EphA2-NF, -CF), which represent a promising basis for developing new cancer therapies and diagnostic techniques.

KEYWORDS
EphA2, membrane proteins, MT1-MMP, proteolytic cleavage, receptor tyrosine kinase (RTK)

1 | INTRODUCTION

1.1 | Insight from basic studies of membrane type-1 matrix metalloproteinase and clinical trials for cancer therapy

In biological tissues, the cells that comprise the basal lamina and interstitial tissues are surrounded by an extraculluar matrix (ECM).[1] The ECM is composed of collagens, fibronectins, and laminins, among other factors, which help maintain homeostasis while providing physical strength.[2] However, when cancer cells proliferate, infiltrate, and metastasize, they break down basement membranes (BMs), which are the physical barriers generated by the ECM.[3] MMPs play key roles in ECM degradation; membrane-type-1 MMP (MT1-MMP) is particularly important for the progression of progression of malignant tumor cells.[4] Although the substrate specificity of MMPs has been analyzed in many studies, effective cancer therapeutics targeting MT1-MMP have not been developed.[5]

Low-molecular-weight hydroxamic acid compounds, which exhibit broad-range inhibitory activity against numerous MMPs, are effective as cancer therapeutics.[6–10] Although these studies showed that these compounds were remarkably effective in basic

Abbreviations: BM, basement membrane; EGFR, epidermal growth factor receptor; EphA2, erythropoietin-producing hepatoma receptor tyrosine kinase A2; HCC, hepatocellular carcinoma; MT1-MMP, membrane type-1 matrix metalloproteinase.

Kazuki Ikeda and Ryo Kaneko, contributed equally.

research using cells and mice, their effectiveness in clinical trials has not been demonstrated.[5–10] This may be because the MMP family has numerous conserved active sites, making it difficult to develop low-molecular-weight compounds with high specificity for MT1-MMP.[5–10] This highlights the potential for developing novel therapeutics targeting membrane proteins, which play a crucial role in cancer development and progression of malignant tumor cells activated by MT1-MMP cleavage.[11–14] Several substrates are cleaved by MT1-MMP, including the ECM; however, very few membrane proteins serve as substrates of MT1-MMP.[13–18] In a previous proteomic study, we broadly identified the membrane proteins that interact with MT1-MMP in cancer cells, as well as approximately 150 types of MT1-MMP-interacting proteins[14,17] (Figure 1A–C), including membrane, cytoplasmic, secreted, and unknown proteins. Of these, over 25% of the identified proteins were classified as membrane proteins[14,17] (Figure 1C). Furthermore, many of these membrane proteins were cleaved when they were co-expressed with MT1-MMP in cells, and half of the proteins were found to be substrates of MT1-MMP (Figure 1D). We found that the proteolytic cleavage of membrane proteins plays a crucial role in the progression of malignant tumor cells.[14,16–19] In this review, we describe the mechanism by which proteolysis of cell surface proteins by MT1-MMP promotes tumor growth activity.[13] We also discuss the clinical applications of specific fragments generated by proteolysis, including that of the erythropoietin-producing hepatoma receptor tyrosine kinase A2 (EphA2), which is a substrate for MT1-MMP.[20]

2 | ROLES OF MEMBRANE TYPE-1 MATRIX METALLOPROTEINASE IN VIVO

Matrix metalloproteinases are metallopeptidases involved in breaking down ECM components such as interstitial tissue and BMs.[2] In humans, there are 24 families of MMPs. Among them, MT1-MMP cleaves collagen types I–III, fibronectin, and proteoglycan, which are principal components of interstitial tissues, as well as laminin-332, which comprises the BMs.[12] MT1-MMP also activates proMMP-2 to the active form for BM degradation.[21] MT1-MMP led to the formation of severe phenotypes in MMP knockout mice;[21] thus, it is thought to serve vital physiological functions. MT1-MMP accumulates at the leading edge of cancer cells, where it breaks down the surrounding ECM, giving cancer cells space for migration, invasion, and metastasis.[11,22] In addition, normal epithelial cells tend to show suppressed growth activity when cultured in 3D collagen gel; however, enforced MT1-MMP expression in cells enhanced cell growth.[23] This observation suggests that MT1-MMP plays a role in growth activity through collagen degradation.[11,23] Moreover, MT1-MMP is involved in regulating cell migration and adhesion outside the ECM by breaking down ECM receptors expressed in cell membranes, such as CD44, integrin, and Lutheran.[3,14,16–18,24] Furthermore, MT1-MMP has various cell regulatory functions, such as cleavage of cell surface signal molecules that transmit signals into the cell from external stimuli.[4,12,25] We previously showed that MT1-MMP promotes the malignant potential of cancer by cleaving heparin-binding epidermal growth factor (EGF)-like growth factor, which reduces its heparin-binding affinity.[15,19] Recently, MT1-MMP was found to be a causative factor in non-cancer illnesses such as obesity, arteriosclerosis, and sepsis through limited proteolysis of the GDNF family receptor alpha-like, GFRAL, low-density lipoprotein receptor-associated protein, insulin receptor, and Tie2.[26–29]

3 | REGULATION OF MEMBRANE TYPE-1 MATRIX METALLOPROTEINASE PROTEOLYTIC ACTIVITY

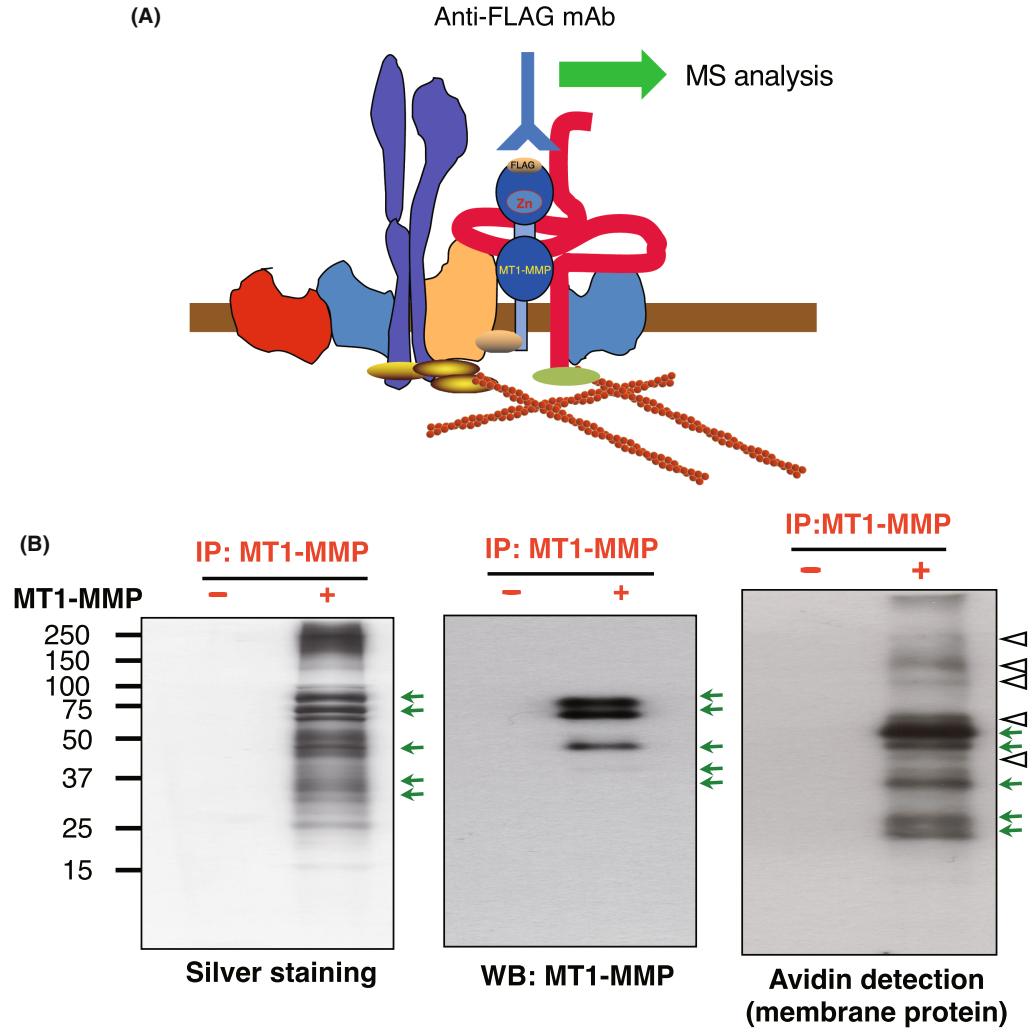
Membrane type-1 matrix metalloproteinase proteolytic activity is generally regulated at the expression level both genetically and epigenetically.[4,30,31] Human MT1-MMP promoter activity is strongly influenced by Sp1.[32] Compared to other MMPs, MT1-MMP does not have a TATA box. This distinctive structure may be involved in cancer-specific expression of MT1-MMP.[32,33]

Furthermore, the expression of the MT1-MMP gene is also regulated by DNA methylation and histone acetylation, chromatin remodeling, and DNA methylation in the promoter region of transcriptional factors such as SP1.[30,31]

It is also regulated by the balance between the levels of MT1-MMP and natural MMP inhibitors, known as tissue inhibitor of metalloproteinases (TIMPs).[34]

Moreover, MT1-MMP activity is regulated by its cytoplasmic and extracellular interacting proteins.[4,29] For example, MTCBP-1 suppresses tumor invasion by binding to the intracellular domain of MT1-MMP.[35] MTCBP-1 expression is inversely correlated with MT1-MMP expression, and MTCBP-1 appears to contribute to the clearance and downregulation of MT1-MMP.[36] In addition, MT1-MMP activity is regulated by clathrin-coated pit-mediated endocytosis on cell membranes.[25] Therefore, serum-starved cancer cells, such as on the invasive front of tumors, induce MT1-MMP accumulation in

FIGURE 1 Schematic image of FLAG-tagged membrane type-1 matrix metalloproteinase (MT1-MMP) interacting with cytoplasmic and membrane proteins at the cell surface in human melanoma A375 cells. (A) The MT1-MMP interacting molecules were extracted from the membrane using lysis buffer containing mild detergent Brij-98, which did not disturb the interacting proteins with MT1-MMP.[14] (B) FLAG-tagged MT1-MMP and its interacting proteins were immunoprecipitated using anti-FLAG monoclonal antibody and detected by silver staining (left panel). MT1-MMP (green arrows) was detected at 50 kDa by an anti-MT1-MMP monoclonal antibody (1D8, center panel). Moreover, biotinylated-membrane proteins (white arrow heads) were detected using avidin (left panel). (C) Precipitants were subjected to nano-liquid chromatography–tandem mass spectrometry analysis, and MT1-MMP interacting proteins were classified as membrane, cytoplasmic, secretory, and unknown proteins. (D) Identified MT1-MMP-interacting proteins were transfected into HEK293 cells expressing Myc-tagged MT1-MMP. CD9, CD98, EphA2, and IL-13Ra2 were cleaved and identified as substrates of MT1-MMP.[14]

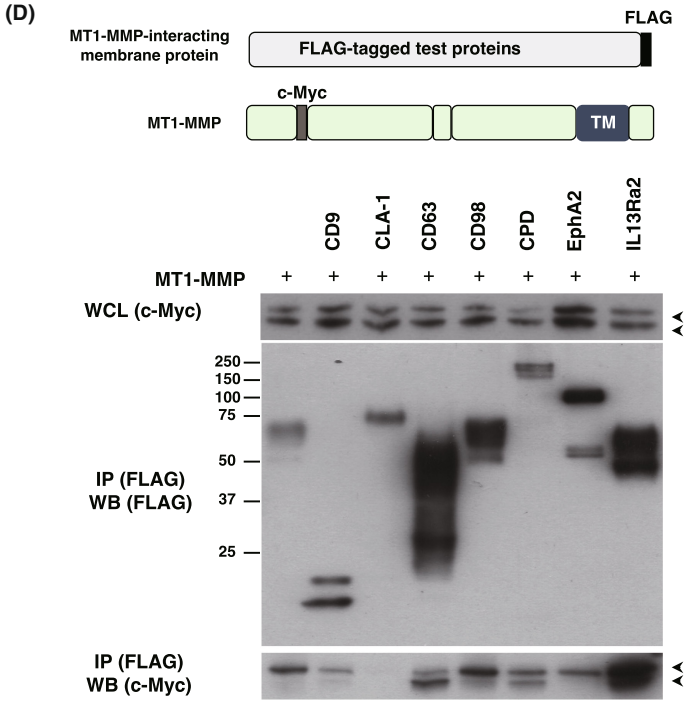


(C)

MT1-MMP-interacting protein

Localization

Cellular Localization	%
Membrane proteins	28
Cytoplasmic proteins	57
Secretory proteins	4.0
Unknown proteins	11



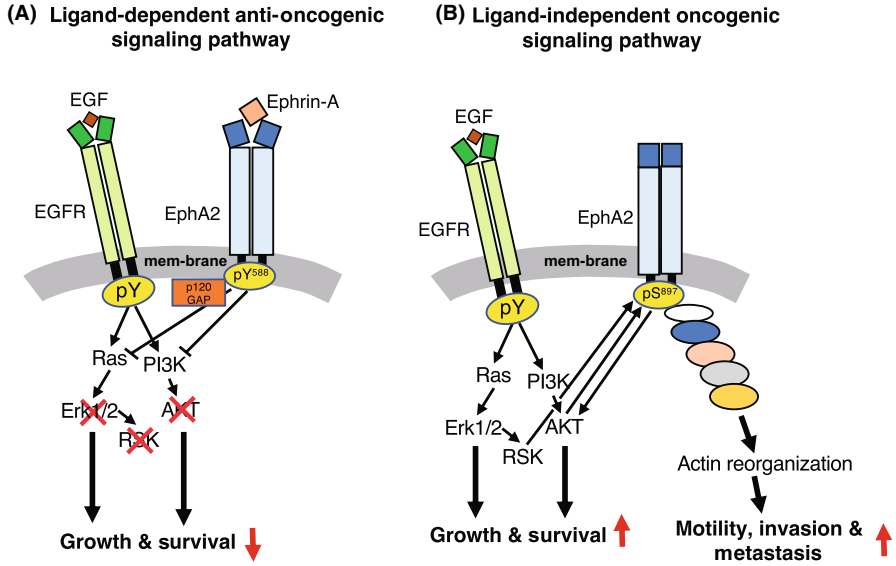


FIGURE 2 Bifunctional EphA2 signaling with/without its Ephrin-A ligand. (A) Ligand-dependent EphA2 phosphorylation at EphA2-Y^{588} recruits RAS-GAP^{120} to inhibit EGFR signaling pathways as an anti-oncogenic agent. (B) Ligand-free EphA2 interacts with EGFR downstream signaling molecules, such as AKT and RSK, activate EphA2-S^{897} phosphorylation. pS^{897}-EphA2 acts as a prooncogenic signal, facilitating small GTPase activation to increase motility, invasion, and metastasis

clathrin-coated pits by suppressing mTOR and promoting degradation of the surrounding ECM.[25]

4 | EPHA2 CLEAVAGE BY MEMBRANE TYPE-1 MATRIX METALLOPROTEINASE PROMOTES MALIGNANT TUMOR PROGRESSION

4.1 | .EphA2

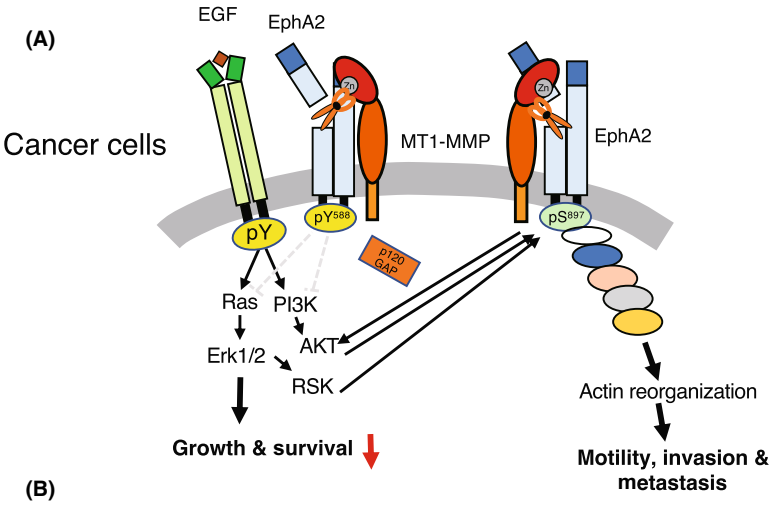
EphA2, a member of the large family of Ephrin receptor tyrosine kinases, recognizes ligands (such as Ephrin-As, which are located on the membranes of epithelial cells) and plays important roles in maintaining homeostasis in epithelial structures.[23,24] EphA2 is weakly expressed in various types of normal epithelial tissues but highly expressed in various cancers, with its expression correlated with cancer malignancy and prognosis.[37,38] Furthermore, overexpression of EphA2 in vitro accelerates the growth of malignant cells.[39,40] Enforced overexpression of EphA2 induces tumorigenicity of normal mammary epithelial MCF-10A cells in subcutaneously injected mice,[40] indicating a close relationship between EphA2 and tumor malignancy. Considering the importance of EphA2 in cancers, researchers have attempted to develop treatment methods to suppress EphA2 expression using antibodies, small molecule inhibitors, or oligonucleotide therapeutics, such as small interfering RNA, but none have been successful.[41–43]

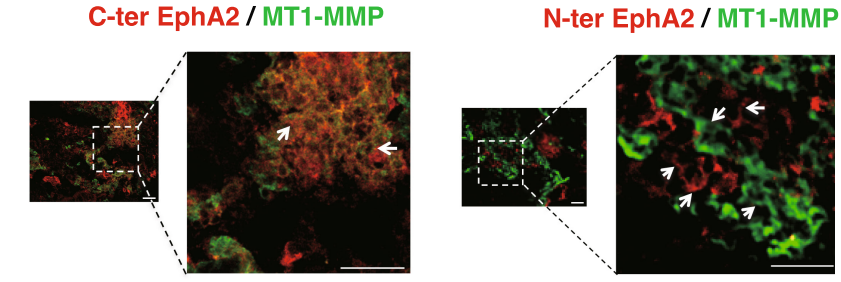
4.2 | Ligand-dependent and independent EphA2 signaling

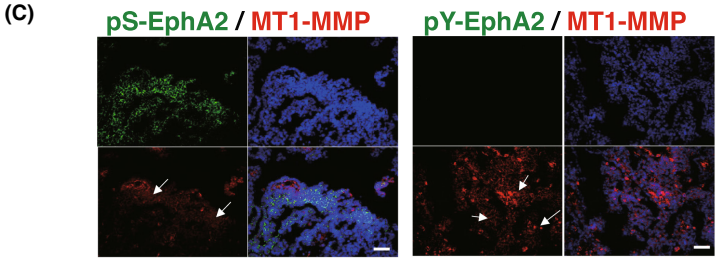
Similarly, in other tyrosine kinases receptors such as EGF receptor (EGFR), EphA2 binds to Ephrin-As, a ligand in the extracellular ligand-binding domain, which leads to autophosphorylation of tyrosine residues within cells (pY^{588}-EphA2) (Figure 2A).[44,45] In contrast, EphA2 overexpression in tumors leads to reduced expression of ligands such as Ephrin-A.[44,46] Because ligand-dependent EphA2 signaling inhibits the Ras-MAPK signaling pathways induced by EGFR activation,[45,47,48] it has been suggested that EphA2 signaling pathway(s) other than pY^{588}-EphA2 contribute to the acquisition of anti-oncogenic EphA2 functions[44,45] (Figure 2A).

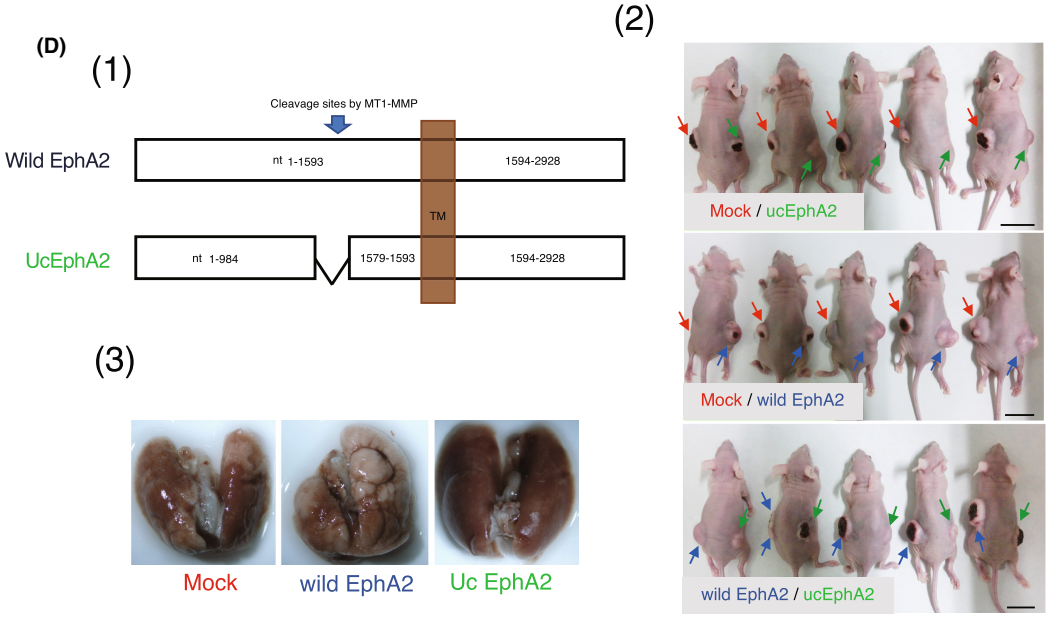
Miao et al. reported that EphA2 is phosphorylated at serine residue S^{897} (pS^{897}-EphA2) by AKT, which is activated by EGF, in glioblastoma and prostate cancer cells.[49] The authors showed that this phosphorylation increased cell motility (referred to as ligand-independent phosphorylation)[49] (Figure 2B). Zhou et al. subsequently analyzed a breast cancer cell line and showed that the RSK is also

FIGURE 3 EphA2 cleavage by membrane type-1 matrix metalloproteinase (MT1-MMP) promotes ligand-independent (A) EphA2 prooncogenic signaling. Schematic image of ligand-independent EphA2 signaling induced by MT1-MMP cleavage. The EphA2 cleavage fragment at the C-terminus (EphA2-CF) cannot bind to Ephrin-A ligand, whereas EphA2-CF may be a potent substrate for ATK and RSK, the downstream signals of EGFR. (B) MT1-MMP expression (green) was colocalized with the EphA2-C-termus (red, left panel) but not with the EphA2-N-termius (red, right panel) in ovarian carcinoma tissues. Scale bar, 50 μm. (C) MT1-MMP expression (red) was colocalized with the ligand-independent EphA2 prooncogenic signal (pS897-EphA2, green, left panel) but not with the ligand-dependent EphA2 anti-oncogenic signal (pY588-EphA2, green, right panel) in ovarian carcinoma tissues. Scale bar, 50 μm. (D-1) Schematic representation of EphA2 (wild-type EphA2) and an uncleavable EphA2 deletion mutant (ucEphA2) lacking the cleavage site. nt, nucleotide number. (D-2) Human carcinoma A431 cells (5 × 106 cells) transfected with mock (red), wild-type EphA2 (blue), or ucEphA2-expression plasmids were inoculated into nude mice subcutaneously. Photograph was taken at 28 days after inoculation. (D-3) Transfected A431 cells (1 × 106 cells) were injected into the tail veins of 7-week-old mice, and lung metastasis was analyzed after 60 days. Macroscopic observation of murine lungs


(A)
EGF
EphA2
Cancer cells
MT1-MMP
EphA2
pY
pY588
pS897
p120 GAP
Ras
PI3K
AKT
Erk1/2
RSK
Growth & survival
Actin reorganization
Motility, invasion & metastasis


(B)
C-ter EphA2 / MT1-MMP
N-ter EphA2 / MT1-MMP


(C)
pS-EphA2 / MT1-MMP
pY-EphA2 / MT1-MMP


(D)
(1)
Cleavage sites by MT1-MMP
Wild EphA2
nt 1-1593
1594-2928
TM
UcEphA2
nt 1-984
1579-1593
1594-2928
(2)
Mock / ucEphA2
Mock / wild EphA2
wild EphA2 / ucEphA2
(3)
Mock
wild EphA2
Uc EphA2

responsible for pS^{897}-EphA2.[50] The mechanism governing the role of pS^{897}-EphA2 in promoting tumor malignancy may involve pS^{897}-EphA2 binding to the guanine nucleotide exchange factor Ephexin-4, in which Ephexin-4 moves to cell membranes and activates RhoG, thereby activating Rac1[51] (Figure 2B). This mechanism is thought to increase cancer motility and invasiveness. Moreover, feedback activation of AKT by pS^{897}-EphA2 increases cell survival and malignant growth activity.[46] Harada et al. reported increased apoptosis in HeLa cells in suspension culture following knockdown of Ephexin-4 and treatment with PI3K/AKT inhibitors.[52] Therefore, pS^{897}-EphA2 may activate Ephexin-4, RhoG, and PI3K/AKT to increase cell survival (Figure 2B), suggesting that ligand-independent pS^{897}-EphA2 contributes to the acquisition of pro-oncogenic EphA2 functions.

4.3 | EphA2 cleavage promotes the progression of malignant tumor cells

Cancer cells must selectively use EphA2 ligand-dependent tumor suppressor and cancer-promoting signals for tumor progression.

The availability of EphA2 ligands is expected to reduce during tumor progression. However, EphA2 ligands, such as Ephrin-A1, are abundantly expressed in many tumor types.[53] Furthermore, soluble monomeric Ephrin-A1 is detectable in glioblastoma patient sera and hepatocellular carcinoma,[54,55] and monomeric Ephrin-A1 can induce EphA2 tyrosine phosphorylation.[54] Therefore, it remains unclear how the conversion of EphA2 signaling from a ligand-dependent to an independent state is switched in cancer cells.

Previously, we identified EphA2 as an MT1-MMP substrate through systematic proteomic analysis (Figures 1D, 3A).[14,17] In addition, immunohistochemical staining of various cancer tissues revealed that the extracellular N-terminal end of EphA2 was not co-localized with MT1-MMP when the intracellular C-terminal of EphA2 was co-localized with MT1-MMP (Figure 3B left and right, respectively).[13] Therefore, EphA2 is likely cleaved by MT1-MMP in vivo. Previous research revealed that MT1-MMP cleaves multiple sites on EphA2[13,56]; however, in every case, the extracellular ligand-binding domain of EphA2 was cleaved to produce an N-terminal-truncated form. According to immunostaining of various types of cancer tissues (e.g., ovarian, head and neck, and stomach cancers), when pS^{897}-EphA2 was co-localized with MT1-MMP, pY^{588}-EphA2 was not co-localized with MT1-MMP[13] (Figure 3C, left and right, respectively).

Therefore, the MT1-MMP-cleaved EphA2 receptor becomes insensitive to ligands during tumor progression and no longer suppresses oncogenic signaling pathways even in the presence of the Ephrin-A ligand (Figure 3A). When tumor cells expressing an EphA2 deletion mutant with the cleavage sites removed to resist MT1-MMP-cleavage were injected subcutaneously into nude mice (Figure 3D-1), the tumor size was significantly reduced compared to that following injection of cells expressing wild-type EphA2[13] (Figure 3D-2); lung metastasis was also reduced[13] (Figure 3D-3).

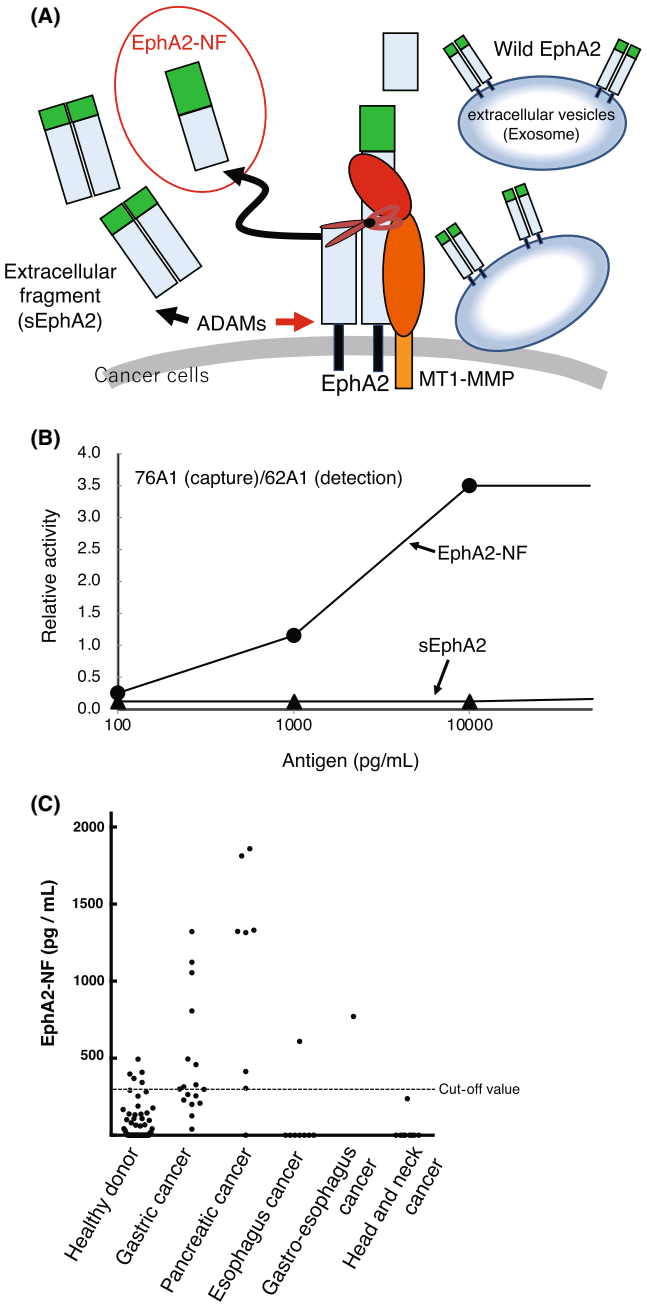


FIGURE 4 Application of EphA2-NF in clinical diagnosis. (A) Metalloproteinases such as a disintegrant and metalloproteinases ADAMs and membrane type-1 matrix metalloproteinase (MT1-MMP) cleave EphA2 into fragments that are released from the cell surface. Moreover, extracellular vesicles such as exosomes contain intact EphA2 (wild-type EphA2) secreted from cancer cells. (B) A specific ELISA detection method using antibodies (76A1 and 62A1) against EphA2-NF released by MT1-MMP cleavage was established in our laboratory, as reported previously.[20] (C) Serum levels of EphA2-NF in some patients with carcinoma and healthy donors. The cutoff value of 350 pg/mL was determined as the mean plus two standard deviations

These results suggest that cleavage of EphA2 by MT1-MMP triggers activation of ligand-independent EphA2 oncogenic signaling, leading to progression of malignant tumor cells (Figure 3A).

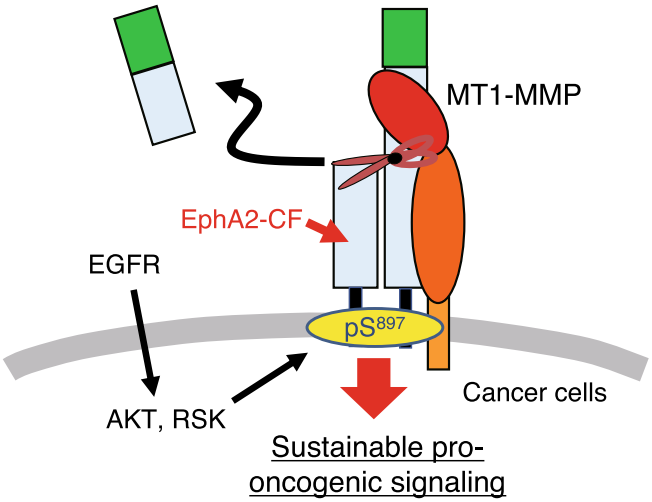


FIGURE 5 Schematic image of EphA2 cleavage by membrane type-1 matrix metalloproteinase (MT1-MMP) producing the ligand-unbound fragment (EphA2-CF). EphA2-CF no longer acts as a transducer for ligand-dependent anti-oncogenic signaling. In contrast, EphA2-CF may be the ligand-independent EphA2 pro-oncogenic signal that is constitutively activated as a substrate for AKT and RSK in epidermal growth factor (EGFR) downstream. EphA2-CF may be a key molecule regulating the malignant progression of tumor cells and an important candidate target in tumor molecular targeted therapy

5 | EPHA2 CLEAVAGE FRAGMENTS AS A DIAGNOSTIC AND THERAPEUTIC TARGET FOR CANCER

Membrane type-1 matrix metalloproteinase and EphA2 are closely related to cancer progression, as each molecule is thought to be a therapeutic target for cancer therapy.[42,57–63] Therefore, the two N- and C-terminal cleavage fragments of EphA2 formed through interactions of a protease and its substrate are specifically produced in cancers and may be useful as biomarker and/or therapeutic targets for cancer therapy.[13,20,56]

The EphA2-N terminal fragments (EphA2-NF) freed from cells are cancer biomarkers. However, several studies reported that EphA2-related proteins are released extracellularly.[13,64,65] For example, wild-type EphA2 can be released from cells as extracellular domains through ectodomain shedding by a disintegrin and metalloproteinase family protease or as vesicles[44,64,65] (Figure 4A). Therefore, two monoclonal antibodies (76A1, 62A1) were developed that specifically recognize EphA2-NF produced by MT1-MMP cleavage in ELISA[20] (Figure 4B). When serum EphA2-NF in patients with various cancers was measured using sandwich ELISA with the specific antibody, high levels of serum EphA2-NF were observed in patients with pancreatic carcinoma[20] (Figure 4C). Currently, EphA2-NF is being clinically evaluated as a new serum biomarker for pancreatic cancer diagnosis.

In contrast, because the C-terminal EphA2 fragment (EphA2-CF) left behind following MT1-MMP cleavage cannot bind to Ephrin-A, it is thought to escape uptake into the cell caused by ligand stimuli; therefore, this fragment can be stably expressed on cancer cell membranes (Figure 5A) [13] Many pathological studies reported that cancer tissues exhibit higher expression of EphA2 compared with normal tissues[37,39,42,44]; however, these studies used a polyclonal antibody to recognize the C-terminal cytoplasmic domain, which suggested that the N-terminally truncated EphA2 is expressed in many cancer tissues.[44,57] Furthermore, compared with wild-type EphA2, EphA2-CF functions as a pro-oncogenic signaling molecule to convey ligand-independent pro-oncogenic signals from AKT or RSK[66] (Figure 5). In fact, EphA2 is highly expressed in aggressive hepatocellular carcinoma (HCC) cells with acquired resistance to sorafenib, a molecular targeted drug for HCC.[67] In addition, statistical analysis of 17 HCC cell lines with different levels of malignancy, which was performed to quantify EphA2, EGFR, and its downstream signaling pathways, revealed pAKT and pS^{897}-EphA2 as key molecules for regulating malignant growth in highly metastatic HCC.[66] Moreover, AKT inhibitors strongly suppressed HCC proliferation and motility in vitro.[66] These findings suggest that in contrast to previous cancer molecule-targeting drugs that target wild-type EphA2, EphA2-CF, which is generated by MT1-MMP cleavage, should be targeted for HCC therapeutics.

AUTHOR CONTRIBUTIONS

Ikeda K, Kaneko R, Tsukamoto E, and Funahashi N contributed to the concept and drafted the manuscript, and N Koshikawa contributed to the concept and design and approved the final version of the manuscript.

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CONFLICT OF INTEREST

NK received research funds from Abbott Japan LLC. The other authors have no potential conflicts of interest.

ETHICAL APPROVAL

Approval of the research protocol by an Institutional Reviewer Board: N/A.

Informed Consent: N/A.

Registry and the Registration No. of the study/trial: N/A.

Animal Studies: N/A.

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REFERENCES

1. Theocharis AD, Skandalis SS, Gialeli C, Karamanos NK. Extracellular matrix structure. *Adv Drug Deliv Rev*. 2016;97:4-27.
2. Lu P, Takai K, Weaver VM, Werb Z. Extracellular matrix degradation and remodeling in development and disease. *Cold Spring Harb Perspect Biol*. 2011;3(12):a005058.
3. Liotta LA. Cancer cell invasion and metastasis. *Sci Am*. 1992;266:54-59. 62–53.
4. Turunen SP, Tatti-Bugaeva O, Lehti K. Membrane-type matrix metalloproteases as diverse effectors of cancer progression. *Biochim Biophys Acta Mol Cell Res*. 2017;1864:1974-1988.
5. Barbolina MV, Stack MS. Membrane type 1-matrix metalloproteinase: substrate diversity in pericellular proteolysis. *Semin Cell Dev Biol*. 2008;19:24-33.
6. Winer A, Adams S, Mignatti P. Matrix metalloproteinase inhibitors in cancer therapy: turning past failures into future successes. *Mol Cancer Ther*. 2018;17:1147-1155.
7. Shalinsky DR, Brekken J, Zou H, et al. Marked antiangiogenic and antitumor efficacy of AG3340 in chemoresistant human non-small cell lung cancer tumors: single agent and combination chemotherapy studies. *Clin Cancer Res*. 1999;5:1905-1917.
8. Gatto C, Rieppi M, Borsotti P, et al. BAY 12-9566, a novel inhibitor of matrix metalloproteinases with antiangiogenic activity. *Clin Cancer Res*. 1999;5:3603-3607.
9. Poulaki V. BMS-275291. Bristol-myers squibb. *Curr Opin Investig Drugs*. 2002;3:500-504.
10. Gingras D, Boivin D, Deckers C, Gendron S, Barthomeuf C, Beliveau R. Neovastat—a novel antiangiogenic drug for cancer therapy. *Anticancer Drugs*. 2003;14:91-96.
11. Itoh Y, Seiki M. MT1-MMP: a potent modifier of pericellular microenvironment. *J Cell Physiol*. 2006;206:1-8.
12. Osenkowski P, Toth M, Fridman R. Processing, shedding, and endocytosis of membrane type 1-matrix metalloproteinase (MT1-MMP). *J Cell Physiol*. 2004;200:2-10.
13. Koshikawa N, Hoshino D, Taniguchi H, et al. Proteolysis of EphA2 converts it from a tumor suppressor to an Oncoprotein. *Cancer Res*. 2015;75:3327-3339.
14. Tomari T, Koshikawa N, Uematsu T, et al. High throughput analysis of proteins associating with a proinvasive MT1-MMP in human malignant melanoma A375 cells. *Cancer Sci*. 2009;100:1284-1290.
15. Koshikawa N, Mizushima H, Minegishi T, et al. Proteolytic activation of heparin-binding EGF-like growth factor by membrane-type matrix metalloproteinase-1 in ovarian carcinoma cells. *Cancer Sci*. 2011;102:111-116.
16. Niiya D, Egawa N, Sakamoto T, et al. Identification and characterization of Lutheran blood group glycoprotein as a new substrate of membrane-type 1 matrix metalloproteinase 1 (MT1-MMP): a systemic whole cell analysis of MT1-MMP-associating proteins in A431 cells. *J Biol Chem*. 2009;284:27360-27369.
17. Egawa N, Koshikawa N, Tomari T, Nabeshima K, Isobe T, Seiki M. Membrane type 1 matrix metalloproteinase (MT1-MMP/MMP-14) cleaves and releases a 22-kDa extracellular matrix metalloproteinase inducer (EMMPRIN) fragment from tumor cells. *J Biol Chem*. 2006;281:37576-37585.
18. Mori H, Tomari T, Koshikawa N, et al. CD44 directs membrane-type 1 matrix metalloproteinase to lamellipodia by associating with its hemopexin-like domain. *EMBO J*. 2002;21:3949-3959.
19. Koshikawa N, Mizushima H, Minegishi T, Iwamoto R, Mekada E, Seiki M. Membrane type 1-matrix metalloproteinase cleaves off the NH2-terminal portion of heparin-binding epidermal growth factor and converts it into a heparin-independent growth factor. *Cancer Res*. 2010;70:6093-6103.
20. Koshikawa N, Minegishi T, Kiyokawa H, Seiki M. Specific detection of soluble EphA2 fragments in blood as a new biomarker for pancreatic cancer. *Cell Death Dis*. 2017;8:e3134.
21. Holmbeck K, Bianco P, Yamada S, Birkedal-Hansen H. MT1-MMP: a tethered collagenase. *J Cell Physiol*. 2004;200:11-19.
22. Sabeh F, Shimizu-Hirota R, Weiss SJ. Protease-dependent versus -independent cancer cell invasion programs: three-dimensional amoeboid movement revisited. *J Cell Biol*. 2009;185:11-19.
23. Hotary KB, Allen ED, Brooks PC, Datta NS, Long MW, Weiss SJ. Membrane type I matrix metalloproteinase usurps tumor growth control imposed by the three-dimensional extracellular matrix. *Cell*. 2003;114:33-45.
24. Kajita M, Itoh Y, Chiba T, et al. Membrane-type 1 matrix metalloproteinase cleaves CD44 and promotes cell migration. *J Cell Biol*. 2001;153:893-904.
25. Colombero C, Remy D, Antoine-Bally S, et al. mTOR repression in response to amino acid starvation promotes ECM degradation through MT1-MMP endocytosis arrest. *Adv Sci (Weinh)*. 2021;8:e2101614.
26. Chow CFW, Guo X, Asthana P, et al. Body weight regulation via MT1-MMP-mediated cleavage of GFRAL. *Nat Metab*. 2022;4:203-212.
27. Alabi A, Xia XD, Gu HM, et al. Membrane type 1 matrix metalloproteinase promotes LDL receptor shedding and accelerates the development of atherosclerosis. *Nat Commun*. 2021;12:1889.
28. Idowu TO, Etzrodt V, Seeliger B, et al. Identification of specific Tie2 cleavage sites and therapeutic modulation in experimental sepsis. *Elife*. 2020;9:e59520.
29. Guo X, Asthana P, Gurung S, et al. Regulation of age-associated insulin resistance by MT1-MMP-mediated cleavage of insulin receptor. *Nat Commun*. 2022;13:3749.
30. Chernov AV, Sounni NE, Remacle AG, Strongin AY. Epigenetic control of the invasion-promoting MT1-MMP/MMP-2/TIMP-2 axis in cancer cells. *J Biol Chem*. 2009;284:12727-12734.
31. Jiang D, He Z, Wang C, et al. Epigenetic silencing of ZNF132 mediated by methylation-sensitive Sp1 binding promotes cancer progression in esophageal squamous cell carcinoma. *Cell Death Dis*. 2018;10:1.
32. Lohi J, Lehti K, Valtanen H, Parks WC, Keski-Oja J. Structural analysis and promoter characterization of the human membrane-type matrix metalloproteinase-1 (MT1-MMP) gene. *Gene*. 2000;242:75-86.
33. Gifford V, Itoh Y. MT1-MMP-dependent cell migration: proteolytic and non-proteolytic mechanisms. *Biochem Soc Trans*. 2019;47:811-826.
34. Cabral-Pacheco GA, Garza-Veloz I, Castruita-De la Rosa C, et al. The roles of matrix metalloproteinases and their inhibitors in human diseases. *Int J Mol Sci*. 2020;21(24):9739.
35. Uekita T, Gotoh I, Kinoshita T, et al. Membrane-type 1 matrix metalloproteinase cytoplasmic tail-binding protein-1 is a new member of the Cupin superfamily. a possible multifunctional protein acting as an invasion suppressor down-regulated in tumors. *J Biol Chem*. 2004;279:12734-12743.
36. Qiang L, Cao H, Chen J, et al. Pancreatic tumor cell metastasis is restricted by MT1-MMP binding protein MTCBP-1. *J Cell Biol*. 2019;218:317-332.
37. Wykosky J, Gibo DM, Stanton C, Debinski W. EphA2 as a novel molecular marker and target in glioblastoma multiforme. *Mol Cancer Res*. 2005;3:541-551.
38. Zhuang G, Brantley-Sieders DM, Vaught D, et al. Elevation of receptor tyrosine kinase EphA2 mediates resistance to trastuzumab therapy. *Cancer Res*. 2010;70:299-308.
39. Thaker PH, Deavers M, Celestino J, et al. EphA2 expression is associated with aggressive features in ovarian carcinoma. *Clin Cancer Res*. 2004;10:5145-5150.
40. Zelinski DP, Zantek ND, Stewart JC, Irizarry AR, Kinch MS. EphA2 overexpression causes tumorigenesis of mammary epithelial cells. *Cancer Res*. 2001;61:2301-2306.

41. Carles-Kinch K, Kilpatrick KE, Stewart JC, Kinch MS. Antibody targeting of the EphA2 tyrosine kinase inhibits malignant cell behavior. *Cancer Res*. 2002;62:2840-2847.
42. Wilson K, Shiuan E, Brantley-Sieders DM. Oncogenic functions and therapeutic targeting of EphA2 in cancer. *Oncogene*. 2021;40:2483-2495.
43. Duxbury MS, Ito H, Zinner MJ, Ashley SW, Whang EE. EphA2: a determinant of malignant cellular behavior and a potential therapeutic target in pancreatic adenocarcinoma. *Oncogene*. 2004;23:1448-1456.
44. Tandon M, Vemula SV, Mittal SK. Emerging strategies for EphA2 receptor targeting for cancer therapeutics. *Expert Opin Ther Targets*. 2011;15:31-51.
45. Miao H, Wei BR, Peehl DM, et al. Activation of EphA receptor tyrosine kinase inhibits the Ras/MAPK pathway. *Nat Cell Biol*. 2001;3:527-530.
46. Macrae M, Neve RM, Rodriguez-Viciana P, et al. A conditional feedback loop regulates Ras activity through EphA2. *Cancer Cell*. 2005;8:111-118.
47. Chang Q, Jorgensen C, Pawson T, Hedley DW. Effects of dasatinib on EphA2 receptor tyrosine kinase activity and downstream signalling in pancreatic cancer. *Br J Cancer*. 2008;99:1074-1082.
48. Irie N, Takada Y, Watanabe Y, et al. Bidirectional signaling through ephrinA2-EphA2 enhances osteoclastogenesis and suppresses osteoblastogenesis. *J Biol Chem*. 2009;284:14637-14644.
49. Miao H, Li DQ, Mukherjee A, et al. EphA2 mediates ligand-dependent inhibition and ligand-independent promotion of cell migration and invasion via a reciprocal regulatory loop with Akt. *Cancer Cell*. 2009;16:9-20.
50. Zhou Y, Yamada N, Tanaka T, et al. Crucial roles of RSK in cell motility by catalysing serine phosphorylation of EphA2. *Nat Commun*. 2015;6:7679.
51. Katoh H, Hiramoto K, Negishi M. Activation of Rac1 by RhoG regulates cell migration. *J Cell Sci*. 2006;119:56-65.
52. Harada K, Hiramoto-Yamaki N, Negishi M, Katoh H. Ephexin4 and EphA2 mediate resistance to anoikis through RhoG and phosphatidylinositol 3-kinase. *Exp Cell Res*. 2011;317:1701-1713.
53. Hao Y, Li G. Role of EFNA1 in tumorigenesis and prospects for cancer therapy. *Biomed Pharmacother*. 2020;130:110567.
54. Wykosky J, Palma E, Gibo DM, Ringler S, Turner CP, Debinski W. Soluble monomeric EphrinA1 is released from tumor cells and is a functional ligand for the EphA2 receptor. *Oncogene*. 2008;27:7260-7273.
55. Cui XD, Lee MJ, Yu GR, et al. EFNA1 ligand and its receptor EphA2: potential biomarkers for hepatocellular carcinoma. *Int J Cancer*. 2010;126:940-949.
56. Sugiyama N, Gucciardo E, Tatti O, et al. EphA2 cleavage by MT1-MMP triggers single cancer cell invasion via homotypic cell repulsion. *J Cell Biol*. 2013;201:467-484.
57. Zhao P, Jiang D, Huang Y, Chen C. EphA2: a promising therapeutic target in breast cancer. *J Genet Genomics*. 2021;48:261-267.
58. Xia XD, Alabi A, Wang M, et al. Membrane-type I matrix metalloproteinase (MT1-MMP), lipid metabolism, and therapeutic implications. *J Mol Cell Biol*. 2021;13:513-526.
59. Sachdeva A, Hart CA, Kim K, et al. Non-canonical EphA2 activation underpins PTEN-mediated metastatic migration and poor clinical outcome in prostate cancer. *Br J Cancer*. 2022;127:1254-1262.
60. Volz C, Breid S, Selenz C, et al. Inhibition of tumor VEGFR2 induces serine 897 EphA2-dependent tumor cell invasion and metastasis in NSCLC. *Cell Rep*. 2020;31:107568.
61. Paraiso KH, Das Thakur M, Fang B, et al. Ligand-independent EPHA2 signaling drives the adoption of a targeted therapy-mediated metastatic melanoma phenotype. *Cancer Discov*. 2015;5:264-273.
62. Fattet L, Jung HY, Matsumoto MW, et al. Matrix rigidity controls epithelial-mesenchymal plasticity and tumor metastasis via a Mechanoresponsive EPHA2/LYN complex. *Dev Cell*. 2020;54(3):302-316.
63. Feng J, Lu SS, Xiao T, et al. ANXA1 binds and stabilizes EphA2 to promote nasopharyngeal carcinoma growth and metastasis. *Cancer Res*. 2020;80:4386-4398.
64. Wei Q, Wei L, Zhang J, Li Z, Feng H, Ren L. EphA2enriched exosomes promote cell migration and are a potential diagnostic serum marker in pancreatic cancer. *Mol Med Rep*. 2020;22:2941-2947.
65. Gao Z, Han X, Zhu Y, et al. Drug-resistant cancer cell-derived exosomal EphA2 promotes breast cancer metastasis via the EphA2-Ephrin A1 reverse signaling. *Cell Death Dis*. 2021;12:414.
66. Asakura N, Nakamura N, Muroi A, et al. Expression of cancer stem cell markers EpCAM and CD90 is correlated with anti- and pro-oncogenic EphA2 signaling in hepatocellular carcinoma. *Int J Mol Sci*. 2021;22(16):8652.
67. Chen CT, Liao LZ, Lu CH, et al. Quantitative phosphoproteomic analysis identifies the potential therapeutic target EphA2 for overcoming sorafenib resistance in hepatocellular carcinoma cells. *Exp Mol Med*. 2020;52:497-513.

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