BIOMOLECULES & THERAPEUTICS

Review
Biomol Ther 31(4), 359-369 (2023)

Caspase Cleavage of Receptor Tyrosine Kinases in the Dependence Receptor Family

Gyu Hwan Park[1,†], Yoo Kyung Kang[2,†], Seung-Mann Paek[2], Chan Young Shin[3] and Sun-Young Han[2,*]

[1]College of Pharmacy, Research Institute of Pharmaceutical Sciences, Kyungpook National University, Daegu 41566,
[2]College of Pharmacy and Research Institute of Pharmaceutical Sciences, Gyeongsang National University, Jinju 52828,
[3]Department of Neuroscience and Pharmacology, School of Medicine, Konkuk University, Seoul 05029, Republic of Korea

Abstract

Dependence receptors are a group of receptor proteins with shared characteristics of transducing two different signals within cells. They can transduce a positive signal of survival and differentiation in the presence of ligands. On the other hand, dependence receptors can transduce an apoptosis signal in the absence of ligands. The function of these receptors depends on the availability of their ligands. Several receptor tyrosine kinases (RTKs) have been reported as dependence receptors. When cells undergo apoptosis by dependence receptors, the intracellular domain of some RTKs is cleaved by the caspases. Among the RTKs that belong to dependence receptors, we focused on eight RTKs (RET, HER2, MET, ALK, TrkC, EphA4, EphB3, and c-KIT) that are cleaved by caspases. In this review, we describe the features of the receptors, their cleavage sites, and the fate of the cleaved products, as well as recent implications on them being used as potential therapeutics for cancer treatment.

Key Words: Dependence receptor, Cleavage, Receptor tyrosine kinase, Caspase

INTRODUCTION

Receptor tyrosine kinases (RTKs) are essential for signal transduction as they relay signals from the outside to the inside cells. Conventionally, ligands bind to the extracellular domain of RTK, activate tyrosine kinase inside the cells, and transduce signals for diverse cellular responses such as proliferation, differentiation, and migration. Signal transductions other than kinase activation are present in cells, although phosphorylation is the central part of the RTK function. One of the unique methods of regulation is the cleavage of the RTK intracellular domain by various enzymes such as caspases (Huang, 2021). The proteolytic cleavage of RTK by caspases is closely related to the dependence receptor (DR) concept.

The DR paradigm is based on the observation that some receptors trigger apoptosis in the absence of their ligand while activating signals in the presence of ligands. Without cognate ligands, receptors are not only just in an "off" state, but can also induce apoptosis. Since the discovery of the p75 neurotrophin receptor (p75NTR) as the first DR (Rabizadeh *et al*., 1993), more than twenty DRs have been reported. DRs have been found in various receptor families without shared structural features (Brisset *et al*., 2021). Representative DRs include deleted in colorectal carcinoma (DCC), uncoordinated five homologs (UNC5H (A-D)), NOTCH3 receptors, and several receptor tyrosine kinases (Negulescu and Mehlen, 2018).

The mechanism of the apoptosis-inducing effects of DRs has yet to be clearly elucidated. The cleavage of some receptors belonging to DRs has also been observed. The intracellular domain of DRs can be cleaved by caspases without ligands (Fig. 1). Several RTKs classified in DRs have also exhibited the cleavage of intracellular tyrosine kinase domain by caspases. Eleven RTKs have been identified as DR until now: RET, HER2, MET, ALK, TrkA, TrkC, EphA4, EphB3 c-KIT, insulin receptor, and IGF-1 receptor (Negulescu and Mehlen, 2018). The proteolytic cleavage of TrkA, insulin receptor, and IGF-1 receptor has also been reported (Huang, 2021). However, proteases other than caspases are involved, and cleavage does not appear to be related to the DR function. The intracellular domain of the RTKs can be cleaved in one or two sites by caspases (Fig. 2). The location of cleavage sites is diverse: Some are within the tyrosine kinase domain, while others are outside the tyrosine kinase domain (Fig. 2). In this review, the cleavage of eight RTKs by caspases will be

Open Access https://doi.org/10.4062/biomolther.2022.133

Received Oct 24, 2022 **Revised** Jan 19, 2023 **Accepted** Feb 14, 2023
Published Online Mar 15, 2023

***Corresponding Author**
E-mail: syhan@gnu.ac.kr
Tel: +82-55-772-2423, Fax: +82-55-772-2429
[†]The first two authors contributed equally to this work.

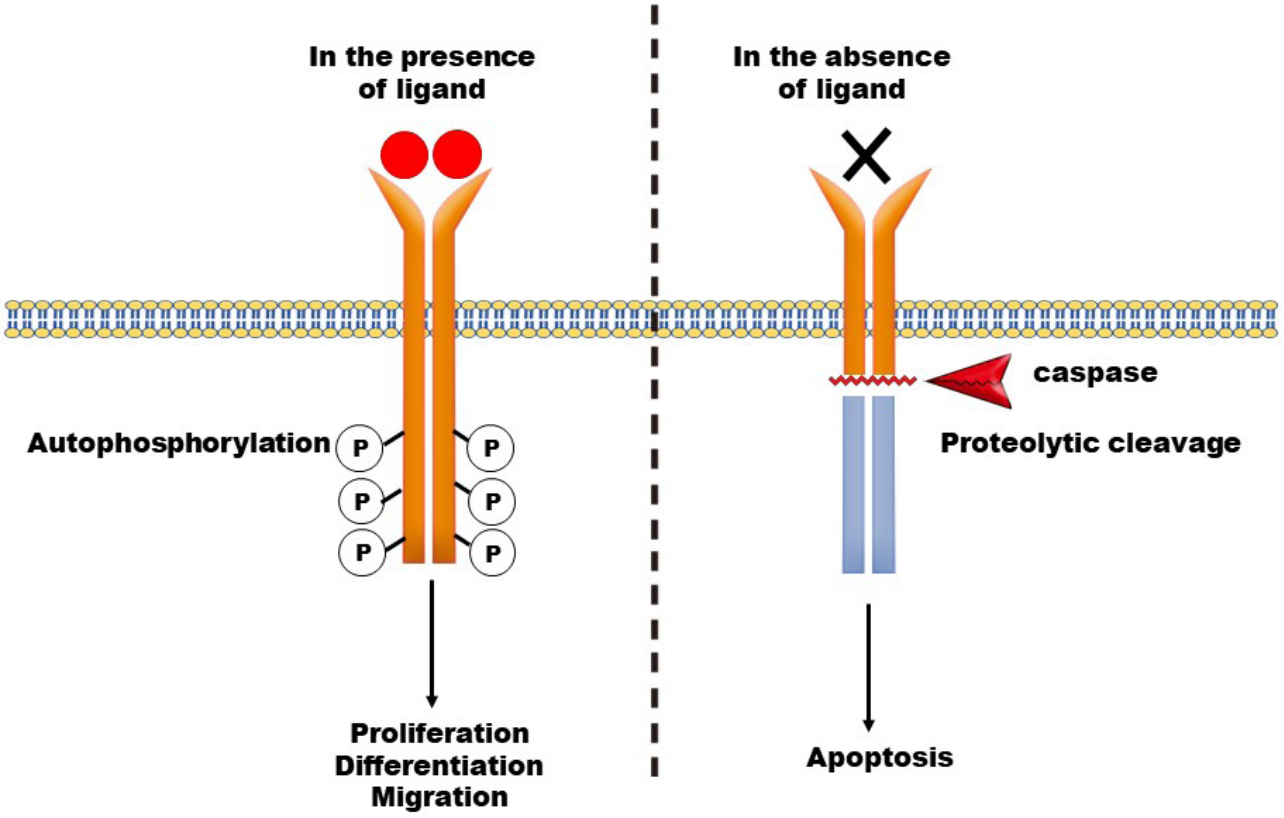

Fig. 1. Schematic representation of RTKs in the dependence receptor (DR) family. In the presence of ligands, RTKs are autophosphorylated by the intracellular tyrosine kinase domain and transduce signals for proliferation, differentiation, and migration (left panel). In the absence of ligands, RTKs are proteolytically cleaved by various enzymes such as caspases and induce apoptosis (right panel).

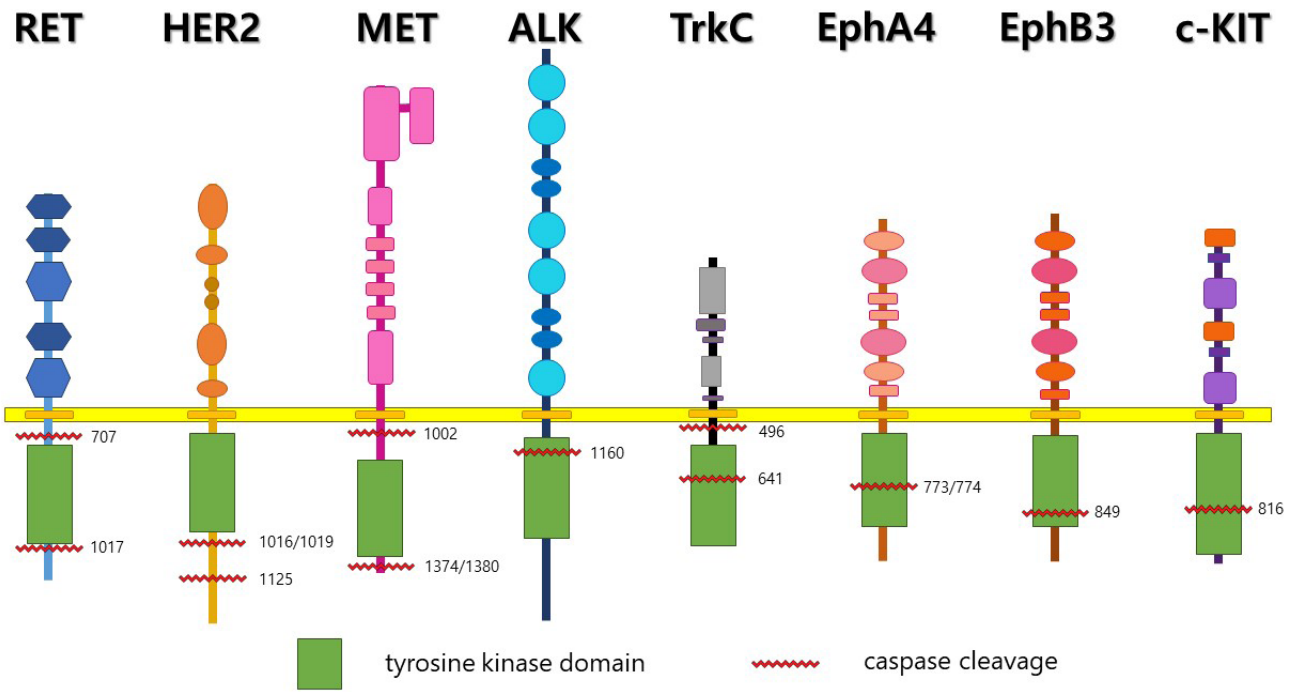

Fig. 2. Cleavage of eight RTKs by caspases. Caspase cleavage sites are indicated in the intracellular domain of RTKs.

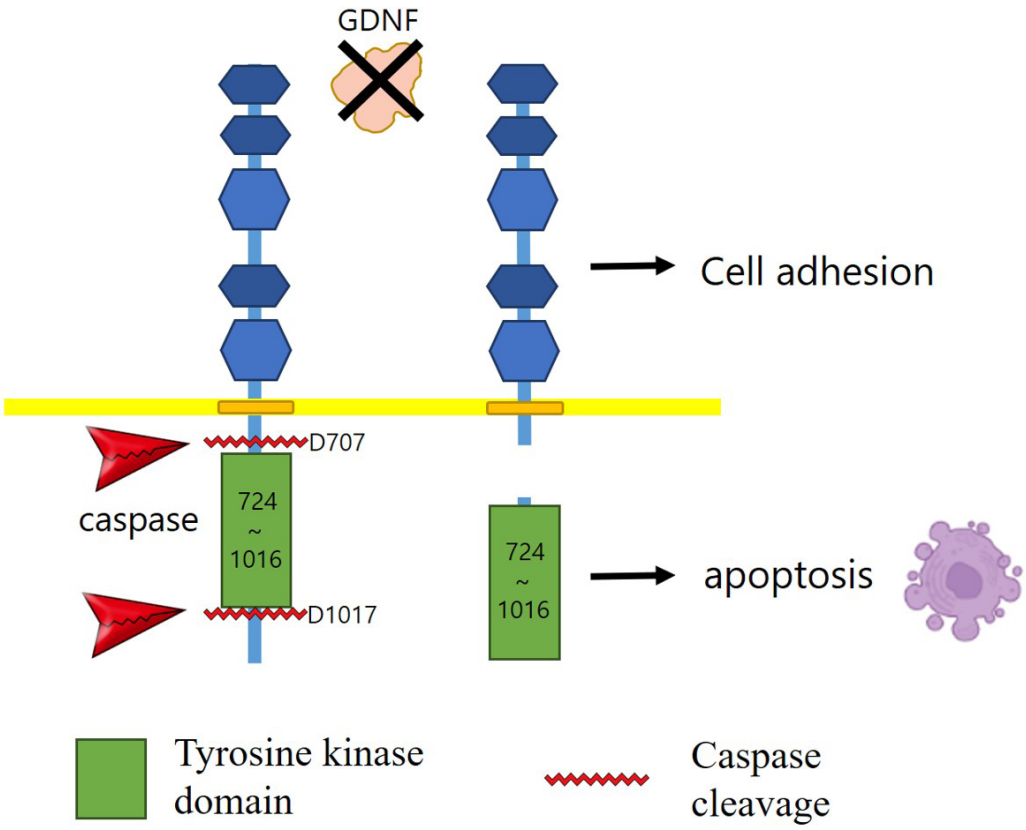

Fig. 3. Cleavage of RET by caspases. In the absence of the RET ligand GDNF, RET is cleaved at the Asp-707 and Asp-1017 sites by caspase-3. The intracellular cleavage product induces apoptosis, and the membrane-anchored truncated ectodomain contributes to cell adhesion.

discussed in detail, and the fate of the cleaved products will be described.

RET CLEAVAGE

Rearranged during transfection (RET) is a receptor tyrosine kinase encoded by *ret* proto-oncogene and activated by glial cell line-derived neurotrophic factor (GDNF) family ligands (Arighi *et al*., 2005). RET is involved in the development process during embryogenesis as well as the proliferation and survival of cells in several neural and neuroendocrine lineages (Mulligan, 2014). RET has become a therapeutic target for several cancer types based on the discovery of RET mutations. Two types of oncogenic alterations of RET (point mutations resulting in the constitutive activation of kinase activity and chromosomal rearrangements resulting in RET fusion proteins) have been identified in various cancer types (Drilon *et al*., 2018). These studies were successfully translated to the development of RET kinase inhibitors such as selpercatinib (LOXO-292) and pralsetinib (BLU-667) for RET-driven cancers (Thein *et al*., 2021).

The cleavage of RET was first reported while investigating the RET as a dependence receptor (Bordeaux *et al*., 2000). In the absence of a ligand, the RET receptor triggers apoptotic cell death, while a different signal is transduced by the RET activation in the presence of its ligand GDNF (Gibert and Mehlen, 2015). The overexpression of RET protein induced apoptosis in the absence of GDNF and the addition of GDNF can block this cell death (Bordeaux *et al*., 2000). The analyses of RET sequence and mutagenesis experiments revealed two caspase-3 cleavage sites: Asp-707 and Asp-1017. These two sites are in the intracellular domain; therefore, caspase cleavage can result in a fragment comprised of amino acids 708 to 1017 (Fig. 3). The authors postulated that this intracellular fragment may trigger a pro-apoptotic signal. The authors also investigated the relationship between RET-induced apoptosis and Hirschsprung disease (HSCR), a congenital disorder associated with loss-of-function mutations of RET (Amiel *et al*., 2008). The results implied that HSCR might arise from apoptosis induced by RET mutations.

As a result of cleavage, another RET fragment at the N-terminal, membrane-anchored truncated ectodomain, is generated as well as an intracellular fragment (Fig. 3). While the intracellular fragment appears to contribute to apoptosis as a dependence receptor (Bordeaux *et al*., 2000), the N-terminal domain contributes to cell adhesion (Cabrera *et al*., 2011). RET belongs to the cadherin superfamily with four cadherin-like domains (Arighi *et al*., 2005). Cadherins are cell adhesion proteins with Ca^{2+}-dependent properties. The presence of cadherin-like domains in the extracellular region of RET protein indicates that RET may have adhesive properties. This hypothesis was tested in sympathetic neurons and the N-terminal domain function as a cadherin accessory protein

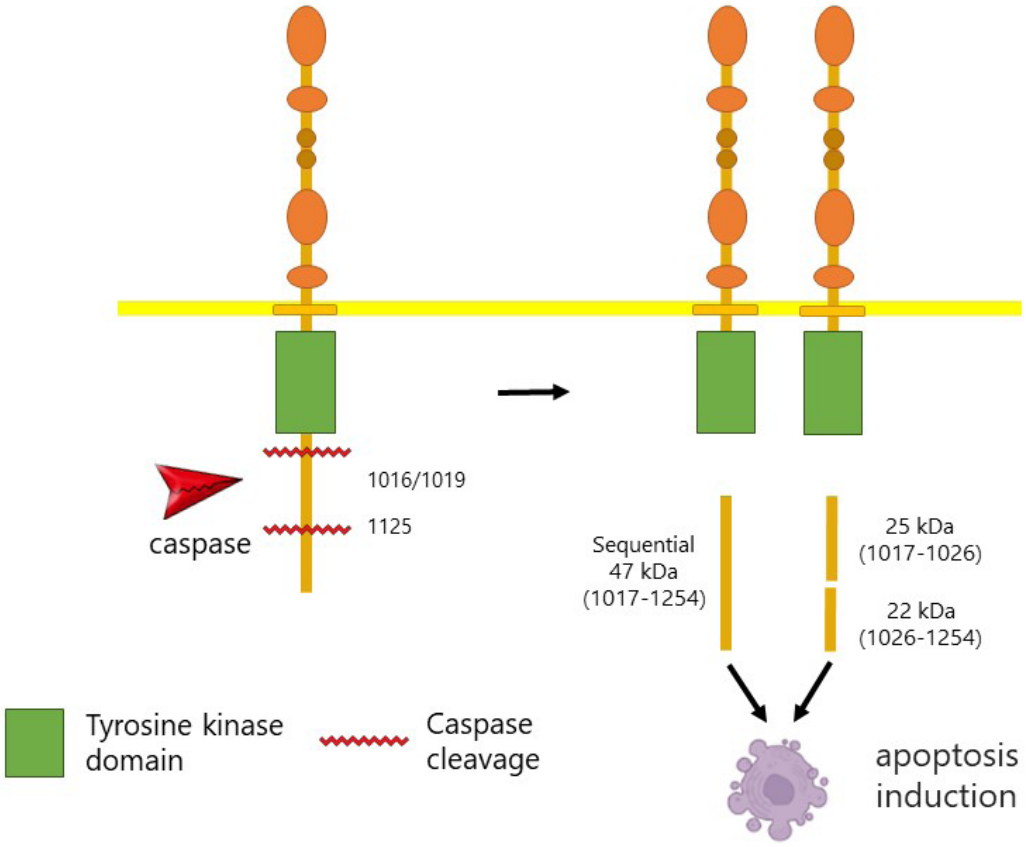

Fig. 4. Cleavage of HER2 by caspases. HER2 is cleaved at the Asp-1016/1019 and Asp-1125 sites by caspases.

inducing cell aggregation (Cabrera *et al*., 2011).

The primary function of RET is signal transduction via ligand-induced dimerization and phosphorylation. In addition to its function as a receptor tyrosine kinase, RET can modulate cellular function via caspase-induced cleavage. Further investigations are required to understand the role of individual cleavage products clearly.

HER2 CLEAVAGE

Human epidermal growth factor receptor-2 (HER2/ErbB2/*neu*) is a receptor tyrosine kinase that belongs to the EGFR family with no cognate ligand. Instead of activation by ligands, HER2 functions as a partner for the heterodimerization of other receptors in the EGFR family (Roskoski, 2019). Based on the overexpression of HER2 in breast and ovarian cancers, drugs interfering with the HER2 function have been developed and used in clinics. Trastuzumab is an antibody drug for HER2, and lapatinib is a kinase inhibitor targeting EGFR and HER2 (DiPiro, 2023).

The cleavage of HER2 in response to apoptotic stimulation such as geldanamycin, staurosporine, and tumor necrosis factor α has been reported (Tikhomirov and Carpenter, 2001; Benoit *et al*., 2004). The caspase cleavage at Asp-1016/1019 generates a 47 kDa fragment at the C-terminal (Fig. 4). One more cleavage at Asp-1125 results in a 25 kDa fragment from Asp-1016/1019 to Asp-1125 (Strohecker *et al*., 2008). These two fragments contain a Bcl-2 homology domain 3 (BH3)-like domain; therefore, these fragments induce apoptosis through cytochrome c release (Strohecker *et al*., 2008).

MET CLEAVAGE

MET is a receptor tyrosine kinase activated by its ligand hepatocyte growth factor (HGF), thereby mediating cell proliferation, migration, differentiation, and morphogenesis. MET has been investigated as a cancer drug target for a long time based on the overexpression and activated mutations found in cancer cells. The kinase activity inhibitors against MET approved by the US Food and Drug Administration (FDA) are capmatinib and tepotinib for non-small cell lung cancer (NSCLC) patients (Mathieu *et al*., 2022).

The caspase cleavage of MET was reported in 2004, and this research classified MET as a dependence receptor. Two cleavage sites are Asp-1002 near the juxtamembrane site and Asp-1374 within the C-terminal (Fig. 5) (Tulasne *et al*., 2004; Foveau *et al*., 2007). The two cleavages are sequential, with the cleavage at Asp-1374 preceding Asp-1002 cleavage (Foveau *et al*., 2007). This caspase-dependent cleavage at both sites generates a 40-kDa fragment containing kinase domain (p40 MET). The ectopic expression of this p40 MET causes apoptosis concomitantly with caspase-3 activation. In apoptotic cells, p40 MET localizes in mitochondria and induces mitochondrial permeabilization (Lefebvre *et al*., 2013). Detailed

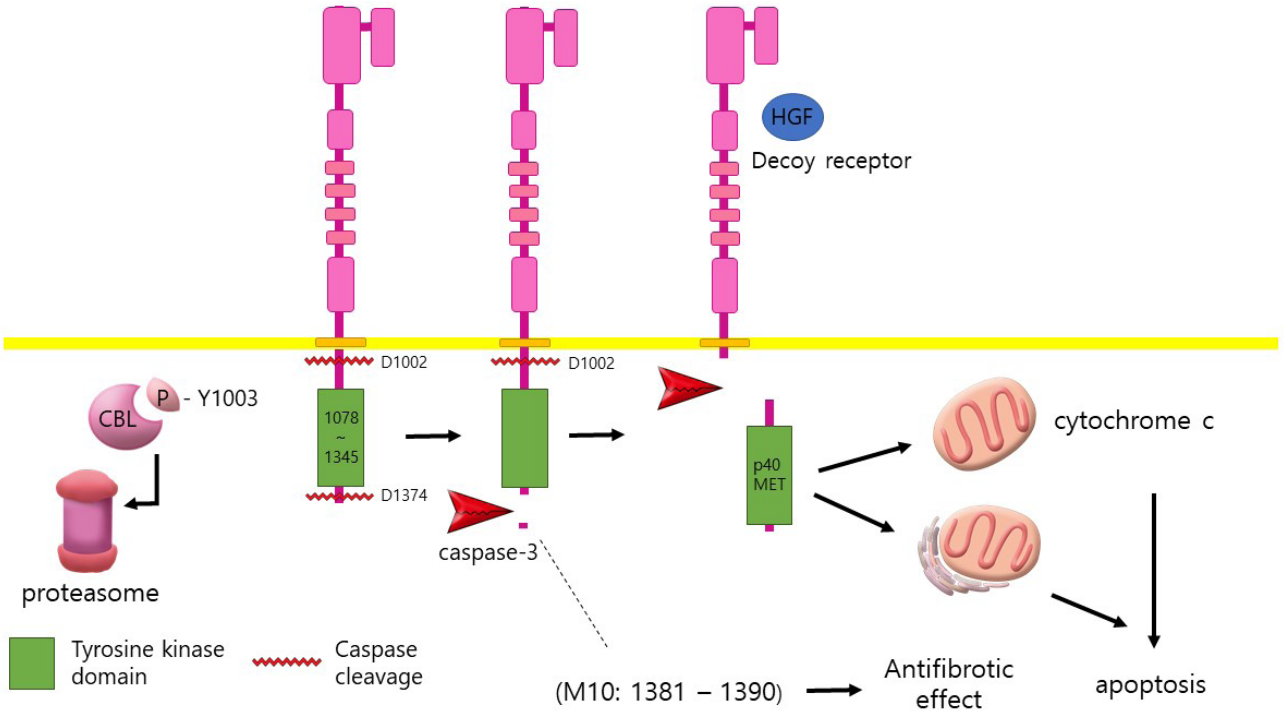

Fig. 5. Cleavage of MET by caspases. In the absence of the MET ligand HGF, the MET is cleaved at Asp-1374 and Asp-1002 sites by caspase-3. The intracellular cleavage product induces apoptosis. Detailed information is explained in the main text.

investigation using GFP-p40 MET revealed that this fragment localized to the site is called the mitochondria-associated ER membrane (MAM) (Duplaquet *et al*., 2020). This fragment localized to the zone between ER and mitochondria triggers calcium exchange between ER and mitochondria. It is unclear if kinase activity is required for the proapoptotic function of the p40 MET fragment. Some of the studies have shown that the kinase-dead mutant of p40 MET reduced apoptosis (Tulasne *et al*., 2004). However, other studies have reported that the p40 MET fragment-induced apoptosis is independent of tyrosine kinase activity (Lefebvre *et al*., 2013).

The caspase cleavage at Asp-1002 also results in another fragment at the N-terminal, which contains an extracellular/transmembrane domain and juxtamembrane domain with 100 kDa size (Fig. 5). This N-terminal fragment appears to act as a decoy receptor by binding HGF, thereby preventing HGF from activating a fully functional MET (Deheuninck *et al*., 2008).

The third fragment generated by caspase cleavage at Asp-1374 is the C-terminal cytoplasmic tail (Fig. 5). To examine the function of this small fragment, the 10-amino acid peptide was synthesized and designated as M10 (Atanelishvili *et al*., 2016). When the M10 synthetic peptide is treated in the cells, the peptide moves into the cells and exhibits an antifibrotic effect. The M10 interacts with Smad2 and inhibits the phosphorylation of Smad2, and this observation can be a putative mechanism for the antifibrotic properties of M10.

The Caspase cleavage site Asp-1374 at C-terminal consists of a DNAD-DEVD$_{1374}$-T amino acid sequence with dual caspase consensus site in humans. An attractive hypothesis is presented for the function of this domain. Caspase-3 recognizes and binds to this domain, resulting in the sequestration of caspase-3 and the inhibition of apoptosis (Ma *et al*., 2014). Contrary to the previous reports that suggested cleavage products can amplify the apoptotic process (Tulasne *et al*., 2004), this study suggests that cleavage sites can function as an inhibitory regulation. Tulasne's team proposed the model for the MET regulation in survival/apoptosis balance, thus integrating these discrepancies (Furlan and Tulasne, 2014). Caspase 3 is bound to the C-terminal at the DNAD-DEVD-T at an early stage of apoptosis, thus protecting cells from apoptosis. At a later stage of apoptosis, caspase activation becomes strong enough to overcome the protection of the C-terminal region. Cleavage at the juxtamembrane site occurs, thus generating a proapoptotic p40 MET fragment. However, this model cannot explain the caspase cleavage at the C-terminal (Asp-1374), which is required for the cleavage of Asp-1002.

The Tyrosine residue at 1003, which is right next to the Asp-1002, is essential for the negative regulation of MET by an E3 ubiquitin ligase Casitas B-lineage lymphoma (CBL). MET is activated by HGF and phosphorylated in several tyrosine residues, including Y1003. CBL ubiquitinates MET protein with phosphorylated Y1003, leading to degradation by the ubiquitin-proteasome system (Peschard *et al*., 2004). CBL recognizes the DYR site (ESVD$_{1002}$Y$_{1003}$R). and phosphorylation at Y1003 prevents the cleavage of D1002 by caspase and generation of p40 MET (Fig. 5) (Deheuninck *et al*., 2009). These data indicate that the ESVD$_{1002}$Y$_{1003}$R motif is crucial in determining the cell fate of apoptosis and ligand-activated survival.

Approximately 3% of NSCLC patients have a mutation of exon 14 deletion caused by splice site mutations, resulting in the constitutive activation of MET (Cortot *et al*., 2017). Exon 14 contains the juxtamembrane domain. Therefore, cells with exon 14 skipping mutation lack regulation mechanisms by caspase and CBL. Along with this indirect clue for the tumori-

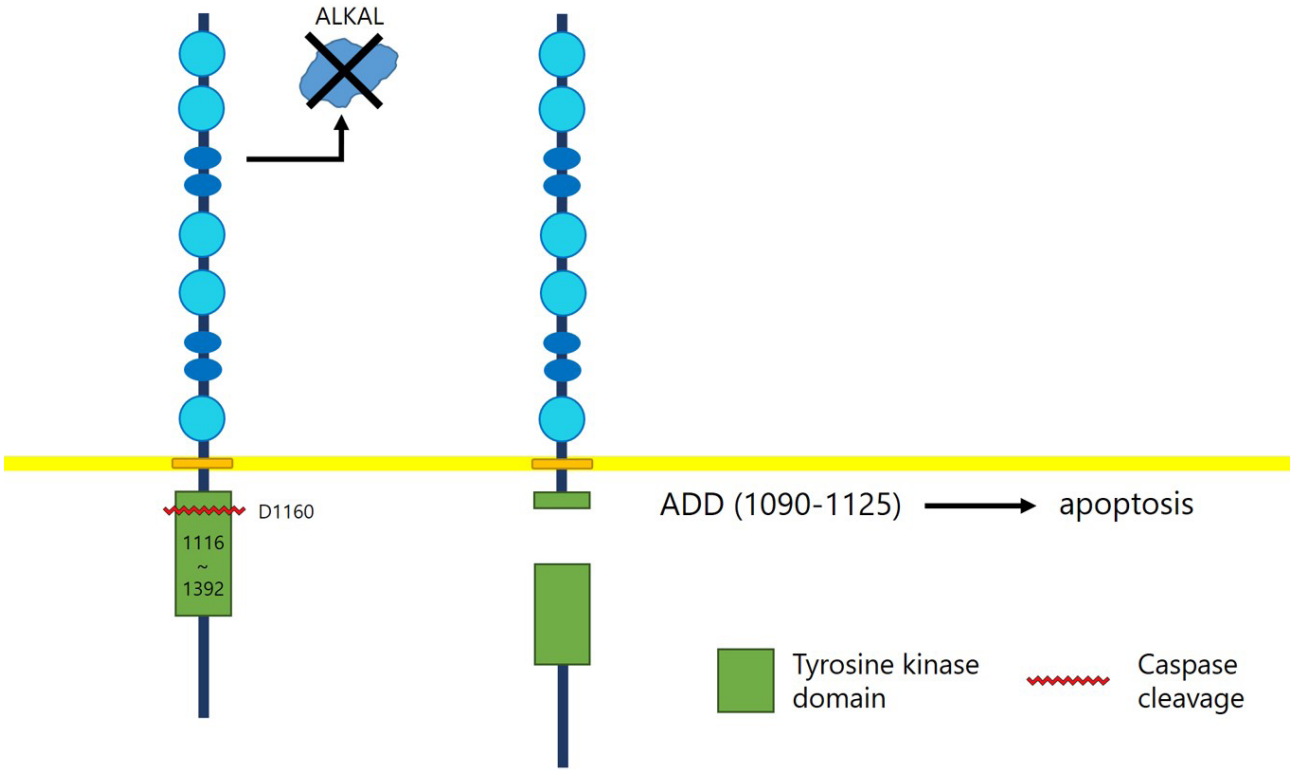

Fig. 6. Cleavage of ALK by caspases. In the absence of the ALK ligand ALKAL, the ALK is cleaved at the Asp-1160 site by caspases. The addiction/dependence domain (ADD) of ALK induces apoptosis. Detailed information is explained in the main text.

genic potential of the D1002 site, knock-in mice with caspase cleavage site mutation can provide direct evidence of tumorigenesis.

ALK CLEAVAGE

Anaplastic lymphoma kinase (ALK) was discovered as a nucleophosmin (NPM)-ALK fusion in anaplastic large cell lymphoma (ALCL). ALK fusion with partner proteins from chromosomal rearrangements has been found in various cancers, including NSCLC (Lin *et al*., 2017). The constitutive activation of kinase activity from gene fusions is oncogenic; therefore, ALK inhibitors such as crizotinib and alectinib were developed and are currently used in clinics for NSCLC patients (Lin *et al*., 2017). The physiological function of ALK has only begun to be elucidated. ALK was an orphan receptor until 2015 when ALKAL1 (also known as FAM150A or Augmentor-β) and ALKAL2 (also known as FAM150B or Augmentor-α) were identified as ALK ligands (Guan *et al*., 2015; Reshetnyak *et al*., 2015).

The cleavage of ALK was also discovered while investigating the dependence receptor function (Mourali *et al*., 2006). Because the ligands for ALK were not identified at the time of the publication on dependence receptors, activating antibodies specific to ALK were used instead of ligands (Moog-Lutz *et al*., 2005). During apoptosis, the ALK protein is cleaved by the caspase, and this phenomenon is blocked by ALK antibodies (Mourali *et al*., 2006). The caspase cleavage site at Asp-1160 with the DELD motif was identified and located within the kinase domain (Fig. 6), indicating that the cleavage of ALK during apoptosis results in the loss of kinase activity.

A deletion mutagenesis study revealed the segment of ALK responsible for apoptosis enhancement (Mourali *et al*., 2006). This segment is located in the intracellular region close to the membrane (i.e., juxtamembrane) upstream of the tyrosine kinase domain between the amino acids 1090 and 1125 (Fig. 6). This segment is called “addiction/dependence domain (ADD),” with the function of apoptosis amplification (Mehlen and Thibert, 2004). Interestingly, no sequence similarity has been found in the ADD of ALK with any known protein motif associated with apoptosis. The 36 amino acid sequence was made as a synthetic peptide (P36) and tested for therapeutic purposes in ALK-positive tumors. P36 exhibited a proapoptotic effect in cancer cells harboring oncogenic ALK fusion proteins in a caspase-dependent way (Aubry *et al*., 2015). The P36 peptide can be useful for cancers with ALK tyrosine kinase inhibitors or as a combination therapy with ALK inhibitors (Aubry *et al*., 2019).

TRKC CLEAVAGE

The tropomyosin receptor kinase C (TrkC) is one of the neurotrophin receptors with neurotrophin-3 (NT-3) as a ligand (Nakagawara, 2001). Working as a dependence receptor, TrkC can also undergo caspase cleavage at two sites: Asp-496 and Asp-641 (Fig. 7) (Tauszig-Delamasure *et al*., 2007). The intracellular fragment between amino acids 496 and 641 generated by cleavage, termed killer fragment (TrkC-KF), can induce apoptotic cell death (Tauszig-Delamasure *et al*.,

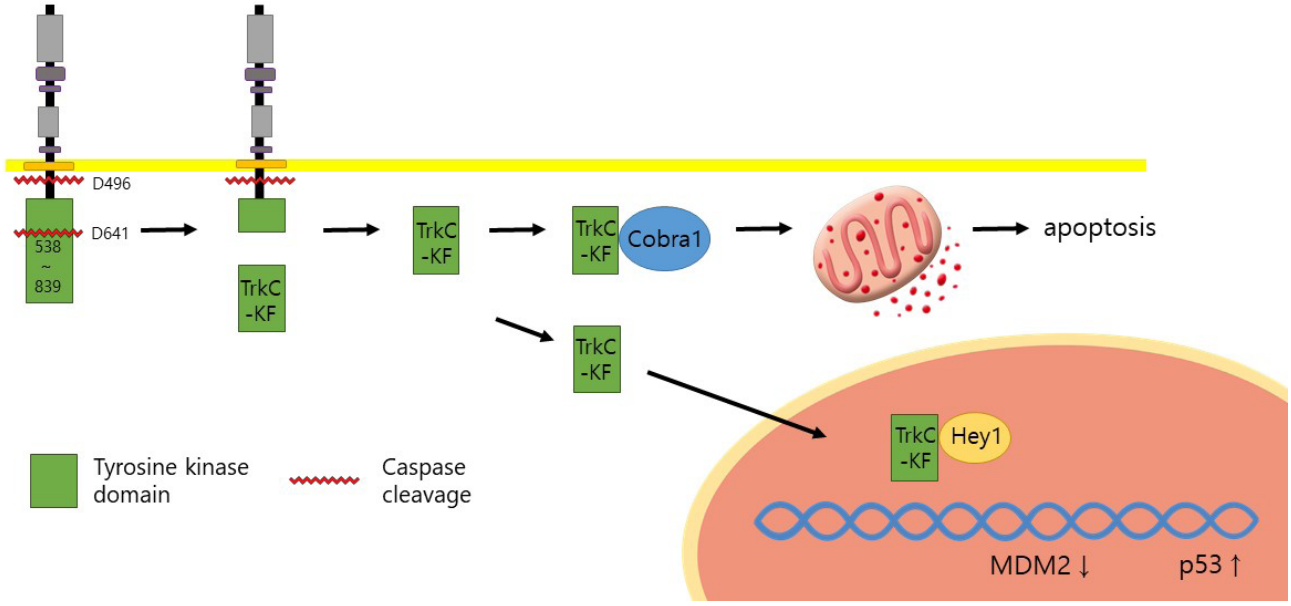

Fig. 7. Cleavage of TrkC by caspases. In the absence of the TrkC ligand neurotrophin-3, the TrkC is cleaved at Asp-496 and Asp-641 sites by caspases. Intracellular cleavage products TrkC-KF contribute to apoptosis. Detailed information is explained in the main text.

2007). To explore the mechanism of TrkC-KF, several interacting partners of TrkC-KF were identified. The cofactor of breast cancer 1 (Cobra1) is one of the interactors localized in the cytosol (Ichim *et al*., 2013). The function of Cobra1 appears to shuttle TrkC-KF to mitochondria, and Trk-KF induces cytochrome c release, resulting in apoptosis. Another interacting protein of TrkC-KF identified is Hey-1, a transcription factor (Menard *et al*., 2018). The Hey-1 protein mediates the function of TrkC-KF in the nucleus as transcriptional control of MDM2. The inhibition of MDM2 transcription results in proapoptotic effects mediated by p53 stabilization.

The prototype dependence receptors such as DCC (Deleted in Colorectal Carcinoma) and UNC5H3 are considered tumor suppressors. In contrast, receptor tyrosine kinases in the dependence receptor group described above (i.e., RET, ALK, and MET) are oncogenic (Brisset *et al*., 2021). Given the proapoptotic activity of dependence receptors without ligands, it is logical to assume that dependence receptors function as tumor suppressors. Indeed, the down-regulation of TrkC in certain types of cancer indicates the tumor suppressor function of TrkC (Genevois *et al*., 2013; Luo *et al*., 2013; Menard *et al*., 2018). Unlike other tyrosine kinases classified as dependence receptors, TrkC follows the paradigm of dependence receptors.

EPHA4 CLEAVAGE

The ephrin type-A receptor 4 (EphA4) receptor binds the ephrins family as ligands, thus making them belong to the A class of the Eph receptor family. Since both receptors and ligands are membrane-bound proteins, the interaction of Eph and ephrin occurs in the context of cell-cell contact (Wilkinson, 2001). EphA4 pairs with ephrinB3 as a ligand and regulates cell migration and adhesion such as neuronal guidance.

EphA4 is also a dependence receptor triggering apoptotic cell death in the absence of ephrinB3. The cleavage of EphA4 occurs at D773/774 by caspase 3 (Fig. 8), and this cleavage is required for the pro-apoptotic function of EphA4 (Furne *et al*., 2009). The cleavage results in approximately 19-23 kDa fragments at the C-terminal. Unlike RET and MET, this intracellular fragment does not induce apoptosis. Instead, the expression of the N-terminal fragment (i.e., amino acid 1-774) induced cell death, implying that the addiction/dependence domain is located at the N-terminus rather than the C-terminus.

The research on the EphA4 receptor following the dependence receptor paradigm has been reported (Royet *et al*., 2017). EphA4 triggers apoptosis acting as a putative tumor suppressor. The overexpression of the ephrinB3 ligand together with EphA4 has been observed in human glioblastoma (Royet *et al*., 2017). With the expression of both ligand and dependence receptor pairs, glioblastoma cells appear to bypass the pro-apoptotic effect of EphA4.

EPHB3 CLEAVAGE

The ephrin type-B receptor 3 (EphB3) also belongs to the Eph receptor family and displays effects similar to EphA4 as a dependence receptor. EphB3 mediates cell death in the nervous system as a dependence receptor, and these effects are blocked by its ligand Ephrin B3 (Theus *et al*., 2014; Tsenkina *et al*., 2015). EphB3 is cleaved by the caspase at Asp-849, resulting in the 20 kDa fragment at the C-terminal (Fig. 9).

c-KIT CLEAVAGE

Proto-oncogene c-KIT (also known as CD117) is activated by its ligand, the stem cell factor (SCF). c-KIT transmits signals related to cell proliferation, differentiation, and migration (Ali and Ali, 2007). The function of c-KIT is highlighted in the

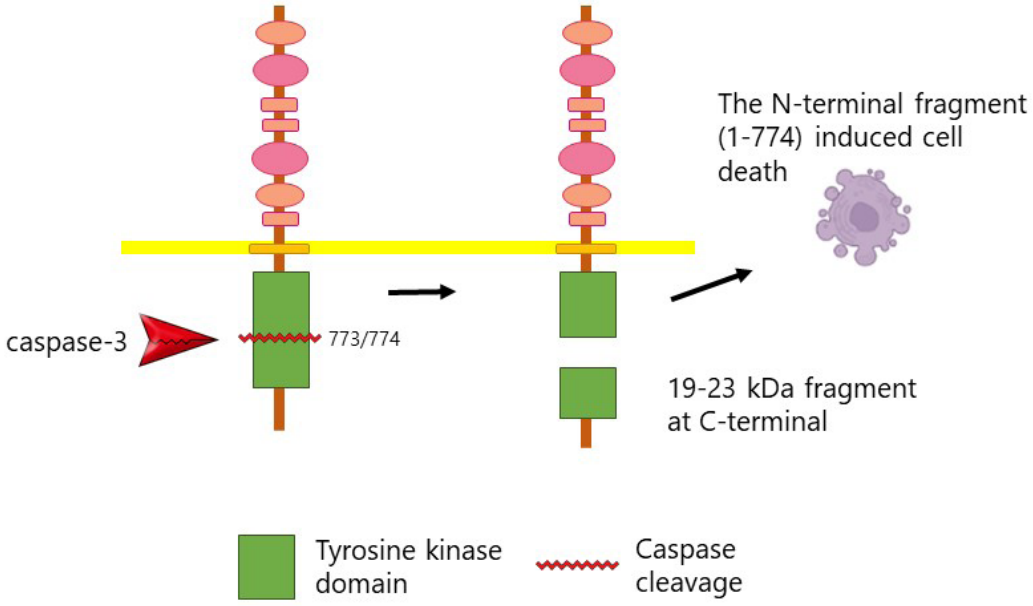

Fig. 8. Cleavage of EphA4 by caspases. In the absence of the EphA4 ligand EphrinB3, the EphA4 is cleaved at the Asp-773/774 site by caspases.

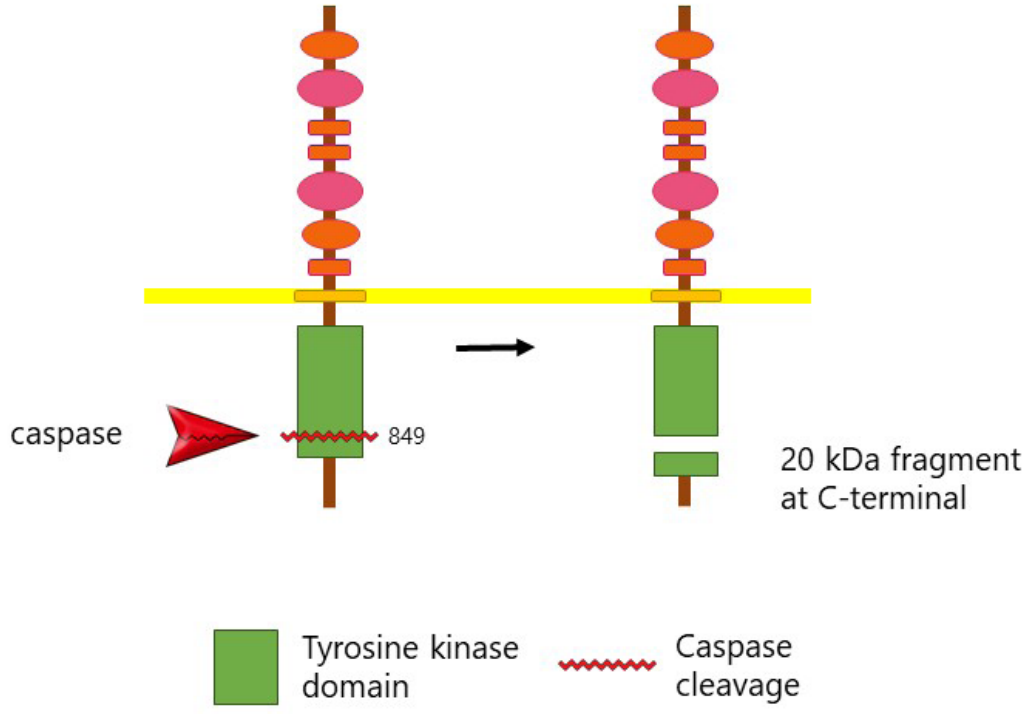

Fig. 9. Cleavage of EphB3 by caspases. In the absence of the EphB3 ligand EphrinB3, the EphB3 is cleaved at the Asp-849 site by caspases.

process of melanogenesis, gametogenesis, hematopoiesis, and brain angiogenesis (Lennartsson *et al*., 2005). The mutation causing the constitutive activation of c-KIT has been found in tumors, particularly gastrointestinal stromal tumors (GISTs), acute myeloid leukemia, and mastocytosis (Lennartsson *et al*., 2005). Imatinib, a bcr-abl inhibitor used in chronic myeloid leukemia treatments, has also been used as a c-KIT activity inhibitor for GIST treatments.

c-KIT became a member of the dependence receptor in 2018 by demonstrating a pro-death effect of the kinase-dead

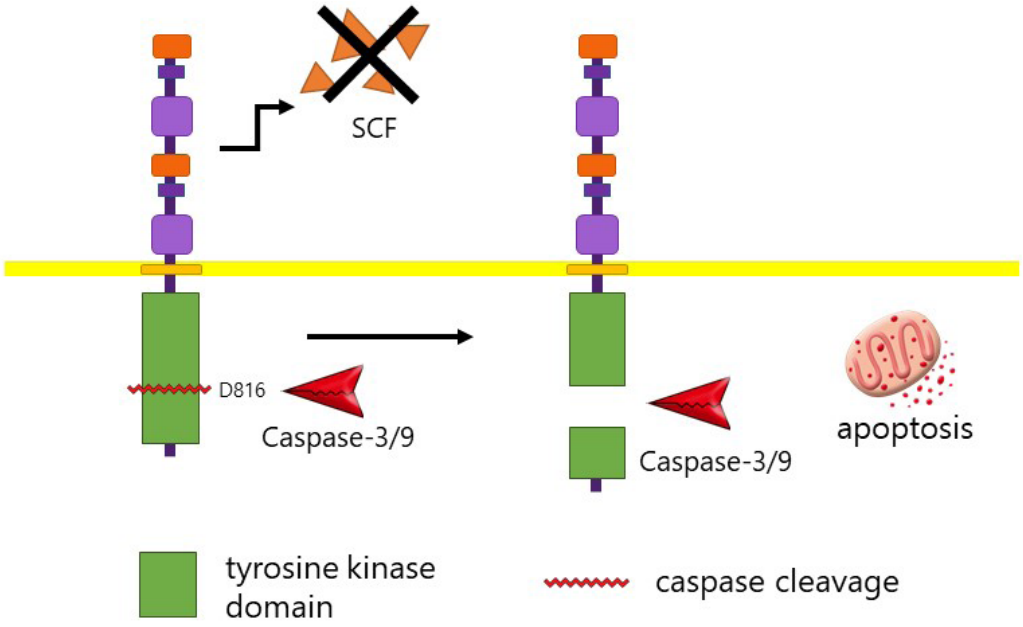

Fig. 10. Cleavage of c-KIT by caspases. In the absence of the c-KIT ligand stem cell factor, the c-KIT is cleaved at the Asp-816 site by caspases.

mutant (Wang *et al*., 2018). The cleavage of c-KIT also occurs at the Asp-816 site by caspase (Fig. 10) (Wang *et al*., 2018). The tumor suppressor function of c-KIT has been investigated as a dependence receptor, although c-KIT is known as tumorigenic. The overexpression of c-KIT induces tumor regression in mice xenograft (Wang *et al*., 2018). The function of the intracellular fragment generated by cleavage has not been reported yet.

CONCLUSION

Eight RTKs that belong to the dependence receptor family with the characteristic of caspase cleavage were discussed in this review (Table 1). The intuitive result of RTK cleavage in the intracellular domain can be a loss of tyrosine kinase function. In addition to the inability to activate downstream signaling, fragments of receptors generated from caspase cleavage have appeared to regulate cellular processes. The amplification of apoptosis by cleavage products was observed in several RTKs such as RET, MET, ALK, and TrkC. Another cellular function other than apoptosis may be regulated by cleavage products, as shown by the antifibrotic effect of the MET cleavage product (Fig. 5). Further research will reveal the regulatory function of the cleavage products of other RTKs.

RTK downregulators are currently being developed extensively for anticancer agents, as represented by PROTAC technology (Khan *et al*., 2020; Dale *et al*., 2021). Along with RTK proteasomal degradation, RTK cleavage induction is another way to downregulate RTK. As with the case of RTK degradation, small molecules inducing cleavage can be developed and utilized for cancer therapy. Anticancer drugs with novel mechanisms are expected, as they follow in the footsteps of kinase activity inhibitors and kinase degraders.

Table 1. Caspase cleavage sites of receptor tyrosine kinases

Receptor tyrosine kinase	Ligand	Cleavage site	Kinase domain
RET	GDNF	707, 1017	636-657
HER2	-	1016/1019, 1125	653-675
MET	HGF	1002, 1374/1380	933-955
ALK	ALKL1, ALKL2	1160	1039-1059
TrkC	Neurotrophin-3	496, 641	439-453
EphA4	EphrinB3	773/774	548-569
EphB3	EprhinB3	849	633-896
c-KIT	Stem cell factor	816	525-545

GDNF, glial cell line-derived neurotrophic factor; HGF, hepatocyte growth factor; ALKL1, ALK ligand 1; ALKL2, ALK ligand 2.
Columns of cleavage site and kinase domain indicate amino acid numbers of receptor tyrosine kinases.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ACKNOWLEDGMENTS

This research was funded by the National Research Foundation, Government of Korea, grant number 2021R1A2C1007790 (S-Y.H.). The authors thank Ms. Jeong Min Lee for her illustrative work with the Figures.

REFERENCES

Ali, S. and Ali, S. (2007) Role of c-kit/SCF in cause and treatment of gastrointestinal stromal tumors (GIST). *Gene* **401**, 38-45.

Amiel, J., Sproat-Emison, E., Garcia-Barcelo, M., Lantieri, F., Burzynski, G., Borrego, S., Pelet, A., Arnold, S., Miao, X., Griseri, P., Brooks, A. S., Antinolo, G., de Pontual, L., Clement-Ziza, M., Munnich, A., Kashuk, C., West, K., Wong, K. K., Lyonnet, S., Chakravarti, A., Tam, P. K., Ceccherini, I., Hofstra, R. M. and Fernandez, R.; Hirschsprung Disease Consortium (2008) Hirschsprung disease, associated syndromes and genetics: a review. *J Med Genet.* **45**, 1-14.

Arighi, E., Borrello, M. G. and Sariola, H. (2005) RET tyrosine kinase signaling in development and cancer. *Cytokine Growth Factor Rev.* **16**, 441-467.

Atanelishvili, I., Shirai, Y., Akter, T., Buckner, T., Noguchi, A., Silver, R. M. and Bogatkevich, G. S. (2016) M10, a caspase cleavage product of the hepatocyte growth factor receptor, interacts with Smad2 and demonstrates antifibrotic properties *in vitro* and *in vivo*. *Transl. Res.* **170**, 99-111.

Aubry, A., Galiacy, S. and Allouche, M. (2019) Targeting ALK in cancer: therapeutic potential of proapoptotic peptides. *Cancers (Basel)* **11**, 275.

Aubry, A., Galiacy, S., Ceccato, L., Marchand, C., Tricoire, C., Lopez, F., Bremner, R., Racaud-Sultan, C., Monsarrat, B., Malecaze, F. and Allouche, M. (2015) Peptides derived from the dependence receptor ALK are proapoptotic for ALK-positive tumors. *Cell Death Dis.* **6**, e1736.

Benoit, V., Chariot, A., Delacroix, L., Deregowski, V., Jacobs, N., Merville, M. P. and Bours, V. (2004) Caspase-8-dependent HER-2 cleavage in response to tumor necrosis factor alpha stimulation is counteracted by nuclear factor kappaB through c-FLIP-L expression. *Cancer Res.* **64**, 2684-2691.

Bordeaux, M. C., Forcet, C., Granger, L., Corset, V., Bidaud, C., Billaud, M., Bredesen, D. E., Edery, P. and Mehlen, P. (2000) The RET proto-oncogene induces apoptosis: a novel mechanism for Hirschsprung disease. *EMBO J.* **19**, 4056-4063.

Brisset, M., Grandin, M., Bernet, A., Mehlen, P. and Hollande, F. (2021) Dependence receptors: new targets for cancer therapy. *EMBO Mol. Med.* **13**, e14495.

Cabrera, J. R., Bouzas-Rodriguez, J., Tauszig-Delamasure, S. and Mehlen, P. (2011) RET modulates cell adhesion via its cleavage by caspase in sympathetic neurons. *J. Biol. Chem.* **286**, 14628-14638.

Cortot, A. B., Kherrouche, Z., Descarpentries, C., Wislez, M., Baldacci, S., Furlan, A. and Tulasne, D. (2017) Exon 14 deleted MET receptor as a new biomarker and target in cancers. *J. Natl. Cancer Inst.* **109**, djw262.

Dale, B., Cheng, M., Park, K. S., Kaniskan, H. U., Xiong, Y. and Jin, J. (2021) Advancing targeted protein degradation for cancer therapy. *Nat. Rev. Cancer* **21**, 638-654.

Deheuninck, J., Foveau, B., Goormachtigh, G., Leroy, C., Ji, Z., Tulasne, D. and Fafeur, V. (2008) Caspase cleavage of the MET receptor generates an HGF interfering fragment. *Biochem. Biophys. Res. Commun.* **367**, 573-577.

Deheuninck, J., Goormachtigh, G., Foveau, B., Ji, Z., Leroy, C., Ancot, F., Villeret, V., Tulasne, D. and Fafeur, V. (2009) Phosphorylation of the MET receptor on juxtamembrane tyrosine residue 1001 inhibits its caspase-dependent cleavage. *Cell Signal.* **21**, 1455-1463.

DiPiro, J. T. (2023) Pharmacotherapy: a Pathophysiologic Approach, 12th ed. McGraw-Hill Medical, New York.

Drilon, A., Hu, Z. I., Lai, G. G. Y. and Tan, D. S. W. (2018) Targeting RET-driven cancers: lessons from evolving preclinical and clinical landscapes. *Nat. Rev. Clin. Oncol.* **15**, 151-167.

Duplaquet, L., Leroy, C., Vinchent, A., Paget, S., Lefebvre, J., Vanden Abeele, F., Lancel, S., Giffard, F., Paumelle, R., Bidaux, G., Heliot, L., Poulain, L., Furlan, A. and Tulasne, D. (2020) Control of cell death/survival balance by the MET dependence receptor. *Elife* **9**, e50041.

Foveau, B., Leroy, C., Ancot, F., Deheuninck, J., Ji, Z., Fafeur, V. and Tulasne, D. (2007) Amplification of apoptosis through sequential caspase cleavage of the MET tyrosine kinase receptor. *Cell Death Differ.* **14**, 752-764.

Furlan, A. and Tulasne, D. (2014) How does met regulate the survival/apoptosis balance? *Hepatology* **60**, 1108-1109.

Furne, C., Ricard, J., Cabrera, J. R., Pays, L., Bethea, J. R., Mehlen, P. and Liebl, D. J. (2009) EphrinB3 is an anti-apoptotic ligand that inhibits the dependence receptor functions of EphA4 receptors during adult neurogenesis. *Biochim. Biophys. Acta* **1793**, 231-238.

Genevois, A. L., Ichim, G., Coissieux, M. M., Lambert, M. P., Lavial, F., Goldschneider, D., Jarrosson-Wuilleme, L., Lepinasse, F., Gouysse, G., Herceg, Z., Scoazec, J. Y., Tauszig-Delamasure, S. and Mehlen, P. (2013) Dependence receptor TrkC is a putative colon cancer tumor suppressor. *Proc. Natl. Acad. Sci. U. S. A.* **110**, 3017-3022.

Gibert, B. and Mehlen, P. (2015) Dependence receptors and cancer: addiction to trophic ligands. *Cancer Res.* **75**, 5171-5175.

Guan, J., Umapathy, G., Yamazaki, Y., Wolfstetter, G., Mendoza, P., Pfeifer, K., Mohammed, A., Hugosson, F., Zhang, H., Hsu, A. W., Halenbeck, R., Hallberg, B. and Palmer, R. H. (2015) FAM150A and FAM150B are activating ligands for anaplastic lymphoma kinase. *Elife* **4**, e09811.

Huang, H. (2021) Proteolytic cleavage of receptor tyrosine kinases. *Biomolecules* **11**, 660.

Ichim, G., Genevois, A. L., Menard, M., Yu, L. Y., Coelho-Aguiar, J. M., Llambi, F., Jarrosson-Wuilleme, L., Lefebvre, J., Tulasne, D., Dupin, E., Le Douarin, N., Arumae, U., Tauszig-Delamasure, S. and Mehlen, P. (2013) The dependence receptor TrkC triggers mitochondria-dependent apoptosis upon Cobra-1 recruitment. *Mol. Cell* **51**, 632-646.

Khan, S., He, Y., Zhang, X., Yuan, Y., Pu, S., Kong, Q., Zheng, G. and Zhou, D. (2020) PROteolysis TArgeting Chimeras (PROTACs) as emerging anticancer therapeutics. *Oncogene* **39**, 4909-4924.

Lefebvre, J., Muharram, G., Leroy, C., Kherrouche, Z., Montagne, R., Ichim, G., Tauszig-Delamasure, S., Chotteau-Lelievre, A., Brenner, C., Mehlen, P. and Tulasne, D. (2013) Caspase-generated fragment of the Met receptor favors apoptosis via the intrinsic pathway independently of its tyrosine kinase activity. *Cell Death Dis.* **4**, e871.

Lennartsson, J., Jelacic, T., Linnekin, D. and Shivakrupa, R. (2005) Normal and oncogenic forms of the receptor tyrosine kinase kit. *Stem Cells* **23**, 16-43.

Lin, J. J., Riely, G. J. and Shaw, A. T. (2017) Targeting ALK: precision medicine takes on drug resistance. *Cancer Discov.* **7**, 137-155.

Luo, Y., Kaz, A. M., Kanngurn, S., Welsch, P., Morris, S. M., Wang, J., Lutterbaugh, J. D., Markowitz, S. D. and Grady, W. M. (2013) NTRK3 is a potential tumor suppressor gene commonly inactivated by epigenetic mechanisms in colorectal cancer. *PLoS Genet.* **9**, e1003552.

Ma, J., Zou, C., Guo, L., Seneviratne, D. S., Tan, X., Kwon, Y. K., An, J., Bowser, R., DeFrances, M. C. and Zarnegar, R. (2014) Novel Death Defying Domain in Met entraps the active site of caspase-3 and blocks apoptosis in hepatocytes. *Hepatology* **59**, 2010-2021.

Mathieu, L. N., Larkins, E., Akinboro, O., Roy, P., Amatya, A. K., Fiero, M. H., Mishra-Kalyani, P. S., Helms, W. S., Myers, C. E., Skinner, A. M., Aungst, S., Jin, R., Zhao, H., Xia, H., Zirkelbach, J. F., Bi, Y., Li, Y., Liu, J., Grimstein, M., Zhang, X., Woods, S., Reece, K., Abukhdeir, A. M., Ghosh, S., Philip, R., Tang, S., Goldberg, K. B., Pazdur, R., Beaver, J. A. and Singh, H. (2022) FDA approval summary: capmatinib and tepotinib for the treatment of metastatic NSCLC harboring MET exon 14 skipping mutations or alterations. *Clin. Cancer Res.* **28**, 249-254.

Mehlen, P. and Thibert, C. (2004) Dependence receptors: between life and death. *Cell. Mol. Life Sci.* **61**, 1854-1866.

Menard, M., Costechareyre, C., Ichim, G., Blachier, J., Neves, D., Jarrosson-Wuilleme, L., Depping, R., Koster, J., Saintigny, P., Mehlen, P. and Tauszig-Delamasure, S. (2018) Hey1- and p53-dependent TrkC proapoptotic activity controls neuroblastoma growth. *PLoS Biol.* **16**, e2002912.

Moog-Lutz, C., Degoutin, J., Gouzi, J. Y., Frobert, Y., Brunet-de Carvalho, N., Bureau, J., Creminon, C. and Vigny, M. (2005) Activation and inhibition of anaplastic lymphoma kinase receptor tyrosine kinase by monoclonal antibodies and absence of agonist activity of pleiotrophin. *J. Biol. Chem.* **280**, 26039-26048.

Mourali, J., Benard, A., Lourenco, F. C., Monnet, C., Greenland, C., Moog-Lutz, C., Racaud-Sultan, C., Gonzalez-Dunia, D., Vigny, M., Mehlen, P., Delsol, G. and Allouche, M. (2006) Anaplastic lymphoma kinase is a dependence receptor whose proapoptotic functions are activated by caspase cleavage. *Mol. Cell. Biol.* **26**, 6209-6222.

Mulligan, L. M. (2014) RET revisited: expanding the oncogenic portfolio. *Nat. Rev. Cancer* **14**, 173-186.

Nakagawara, A. (2001) Trk receptor tyrosine kinases: a bridge between cancer and neural development. *Cancer Lett.* **169**, 107-114.

Negulescu, A. M. and Mehlen, P. (2018) Dependence receptors - the dark side awakens. *FEBS J.* **285**, 3909-3924.

Peschard, P., Ishiyama, N., Lin, T., Lipkowitz, S. and Park, M. (2004) A conserved DpYR motif in the juxtamembrane domain of the Met receptor family forms an atypical c-Cbl/Cbl-b tyrosine kinase binding domain binding site required for suppression of oncogenic activation. *J. Biol. Chem.* **279**, 29565-29571.

Rabizadeh, S., Oh, J., Zhong, L. T., Yang, J., Bitler, C. M., Butcher, L. L. and Bredesen, D. E. (1993) Induction of apoptosis by the low-affinity NGF receptor. *Science* **261**, 345-348.

Reshetnyak, A. V., Murray, P. B., Shi, X., Mo, E. S., Mohanty, J., Tome, F., Bai, H., Gunel, M., Lax, I. and Schlessinger, J. (2015) Augmentor alpha and beta (FAM150) are ligands of the receptor tyrosine kinases ALK and LTK: hierarchy and specificity of ligand-receptor interactions. *Proc. Natl. Acad. Sci. U. S. A.* **112**, 15862-15867.

Roskoski, R., Jr. (2019) Small molecule inhibitors targeting the EGFR/ErbB family of protein-tyrosine kinases in human cancers. *Pharmacol. Res.* **139**, 395-411.

Royet, A., Broutier, L., Coissieux, M. M., Malleval, C., Gadot, N., Maillet, D., Gratadou-Hupon, L., Bernet, A., Nony, P., Treilleux, I., Honnorat, J., Liebl, D., Pelletier, L., Berger, F., Meyronet, D., Castets, M. and Mehlen, P. (2017) Ephrin-B3 supports glioblastoma growth by inhibiting apoptosis induced by the dependence receptor EphA4. *Oncotarget* **8**, 23750-23759.

Strohecker, A. M., Yehiely, F., Chen, F. and Cryns, V. L. (2008) Caspase cleavage of HER-2 releases a Bad-like cell death effector. *J. Biol. Chem.* **283**, 18269-18282.

Tauszig-Delamasure, S., Yu, L. Y., Cabrera, J. R., Bouzas-Rodriguez, J., Mermet-Bouvier, C., Guix, C., Bordeaux, M. C., Arumae, U. and Mehlen, P. (2007) The TrkC receptor induces apoptosis when the dependence receptor notion meets the neurotrophin paradigm. *Proc. Natl. Acad. Sci. U. S. A.* **104**, 13361-13366.

Thein, K. Z., Velcheti, V., Mooers, B. H. M., Wu, J. and Subbiah, V. (2021) Precision therapy for RET-altered cancers with RET inhibitors. *Trends Cancer* **7**, 1074-1088.

Theus, M. H., Ricard, J., Glass, S. J., Travieso, L. G. and Liebl, D. J. (2014) EphrinB3 blocks EphB3 dependence receptor functions to prevent cell death following traumatic brain injury. *Cell Death Dis.* **5**, e1207.

Tikhomirov, O. and Carpenter, G. (2001) Caspase-dependent cleavage of ErbB-2 by geldanamycin and staurosporin. *J. Biol. Chem.* **276**, 33675-33680.

Tsenkina, Y., Ricard, J., Runko, E., Quiala-Acosta, M. M., Mier, J. and Liebl, D. J. (2015) EphB3 receptors function as dependence receptors to mediate oligodendrocyte cell death following contusive spinal cord injury. *Cell Death Dis.* **6**, e1922.

Tulasne, D., Deheuninck, J., Lourenco, F. C., Lamballe, F., Ji, Z., Leroy, C., Puchois, E., Moumen, A., Maina, F., Mehlen, P. and Fafeur, V. (2004) Proapoptotic function of the MET tyrosine kinase receptor through caspase cleavage. *Mol. Cell. Biol.* **24**, 10328-10339.

Wang, H., Boussouar, A., Mazelin, L., Tauszig-Delamasure, S., Sun, Y., Goldschneider, D., Paradisi, A. and Mehlen, P. (2018) The proto-oncogene c-Kit inhibits tumor growth by behaving as a dependence receptor. *Mol. Cell* **72**, 413-425.e5.

Wilkinson, D. G. (2001) Multiple roles of EPH receptors and ephrins in neural development. *Nat. Rev. Neurosci.* **2**, 155-164.