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Modulation of PDGF receptors function by posttranslational modifications

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Abstract
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The platelet-derived growth factor receptors (PDGFRs) play important roles in multiple cellular processes including cell survival, cell growth and cell migration. Dysregulation of PDGFRs causes aberrant PDGF signaling, which leads to various diseases. Insights from the research about PDGF/PDGFR signaling have enlightened new opportunities to understand the molecular mechanisms of cancer and other diseases. The goal of this thesis is to further investigate the mechanisms of the modulation of PDGF/PDGFR signaling to identify new ways of controlling aberrant signaling of these RTKs in various diseases, including cancer.

Ubiquitination is an important post-translational modification related to protein degradation, receptor internalization, intracellular trafficking, cell proliferation, and other cellular processes. It can be reversed by DUBs, and the overexpression of DUBs is involved in various diseases including cancers. In paper I, we identified two main DUBs working on PDGFRβ, USP17 and USP4. They affected the timing of STAT3 activation and trafficking via different mechanisms, thus fine-tuning its transcriptional activity, which further regulated the proliferative response induced by PDGF-BB.

SUMOylation is another post-translational modification that is important for the regulation of protein subcellular localization, protein stability, protein-DNA interactions, protein-protein interactions, genome organization, DNA repair and transcriptional regulation. In paper II, we have identified PDGFRα as a SUMOylation substrate and performed a characterization of the functional role of SUMOylation in PDGFRα signaling and cell proliferation.

Proteolytic cleavage of RTKs regulates their downstream signaling pathways by affecting their structure, stability, subcellular localization and interaction with other proteins. In paper III, we have identified that PDGFRβ is cleaved in the region Y579-Y857 upon ligand stimulation by a Ca2+-dependent protease, which is dependent on its internalization. The proteasomal inhibitor bortezomib blocked the internalization, as well as the cleavage of PDGFRβ, and also affected its downstream signaling.

Apart from the classic degradation in lysosomes and proteasomes, several RTKs have also been found to undergo autophagy and to be targeted in autophagosomes which further fuse with lysosomes. In liver hepatocytes (LX2) cells, it has been reported that PDGFRα, but not PDGFRβ, undergo selective autophagy. In paper IV, we identified that in certain types of cells, PDGFRβ may be involved in the autophagy pathway, which may affect the synthesis of new PDGFRβ.

To conclude, in this study, we investigated how the turnover and signaling of the RTKs PDGFR isoforms α and β are modulated by ubiquitination, SUMOylation, proteolytic cleavage and degradation.

Keywords: PDGF signaling, receptor tyrosine kinase, ubiquitination, DUB, STAT3, SUMO, SUMOylation, cleavage, autophagy, SQSTM1/p62, LC3, GABARAP, bafilomycin A1

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Dedicated to my family

List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I.   Sarri N, **Wang K**, Tsioumpekou M, Castillejo-López C, Lennartsson J, Heldin CH, Papadopoulos N. (2022) Deubiquitinating enzymes USP4 and USP17 finetune the trafficking of PDGFRβ and affect PDGF-BB-induced STAT3 signalling. *Cell Mol Life Sci.* 2022 Jan 21;79(2):85.

II. **Wang K**, Papadopoulos N, Hamidi A, Lennartsson J, Heldin CH. SUMOylation of PDGF receptor α affects signaling via PLCγ and STAT3 and promotes cell proliferation (2023). *Submitted*

III. Rubin Sander M, **Wang K**, Papadopoulos N, Rorsman C, Heldin J, Tsiatsiou K, Söderberg O, Heldin CH, Lennartsson J. PDGF-induced internalization promotes proteolytic processing of PDGFRβ which can be inhibited by bortezomib (2023). *Manuscript*

IV. **Wang K**, Lennartsson J, Heldin CH, Papadopoulos N. Involvement of autophagy pathways in the degradation of PDGFRβ in normal and cancer cells (2023). *Manuscript*

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Contents

Abbreviations

ADAM	A disintegrin and metalloprotease
AP2	Adaptor protein complex 2
AD	Alzheimer's disease
ALS	Amyotrophic lateral sclerosis
AEP	Asparagine endopeptidase
ATG8	Autophagy-related protein 8
BAD	Bcl2 associated agonist of cell death
BUB1	Benzimidazoles 1
BioID	Biotinylation
BTZ	Bortezomib
CAFs	Cancer-associated fibroblasts
CDC25A	Cell division cycle 25A
CNS	Central nervous system
CMA	Chaperone-mediated autophagy
CQ	Chloroquine
CHC	Clathrin heavy chain
CCPs	Clathrin-coated pits
CCVs	Clathrin-coated vesicles
CIE	Clathrin-independent endocytosis
CME	Clathrin-mediated endocytosis
CSF1	Colony stimulating factor 1
CSF1R	Colony stimulating factor 1 receptor
CNAs	Copy number aberrations
CP	Core particle
CDKs	Cyclin-dependent kinases
DUBs	Deubiquitinating enzymes
DAG	Diacylglycerol
EEs	Early endosomes
ESCRT	Endosomal sorting complexes required for the transport
EGFR	Epidermal growth factor receptor
Eps15	Epidermal growth factor receptor substrate 15
Eps15R	Eps15-related protein
ETV6	ETS variant transcription factor 6

ECD	Extracellular domain
ERK	Extracellular signal-regulated kinase
FGFR1	Fibroblast growth factor receptor 1
FLT-3	Fms like tyrosine kinase 3
FOXO	Forkhead box class O
GABARAP	Gamma-aminobutyric acid receptor-associated protein
GISTs	Gastrointestinal stromal tumors
GA	Ginkgolic acid
GBM	Glioblastoma multiforme
GG	Glycine-glycine
GSK-3	Glycogen synthase kinase-3
GrB	Granzyme B
Grb2	Growth factor receptor-bound protein 2
GAP	GTPase activating protein
HGFR	Hepatocyte growth factor receptor
HIF-1α	Hypoxia-inducible factor-1α
IBGC	Idiopathic basal ganglia calcification
IM	Infantile myofibromatosis
IP3	Inositol 1,4,5-triphosphate
IGF-1R	Insulin-like growth factor 1 receptor
IRF8	Interferon regulatory factor 8
ISG15	Interferon stimulated gene 15
IFNs	Interferons
IL-1α	Interleukin 1α
IARC	International Agency for Research on Cancer
ICDs	Intracellular domains
ILVs	Intra-lumenal vesicles
ITH	Intratumour genetic heterogeneity
IKK	IκB kinase
JAMMs	JAB1/MPN/Mov34 metalloproteases
JAKs	Janus kinases
LEs	Late endosomes
LRP	Lipoprotein receptor-related protein
LX2	Liver hepatic stellate cells
MJDs	Machado-Josephin domain proteases
mTOR	Mammalian target of rapamycin
MAP2K	MAPK kinase
MAP3K	MAPK kinase kinase
MMPs	Matrix metalloproteinases
MSCs	Mesenchymal stem cells

LC3	Microtubule-associated protein 1A/1B-light chain 3
MEK	Mitogen-activated ERK kinase
MAPK	Mitogen-activated protein kinase
mESCs	Mouse embryonic stem cells
MVBs	Multivesicular bodies
MDM2	Murine double minute 2
NPCs	Neural progenitor cells
NSCLC	Non-small cell lung cancer
NPC	Nuclear pore complex
OTUs	Ovarian tumor domain-containing proteases
PD	Parkinson's disease
PES1	Pescadillo ribosomal biogenesis factor 1
PG	Phagophore
PTEN	Phosphatase and tensin homolog
PE	Phosphatidylethanolamine
PIP3	Phosphatidylinositol 3,4,5-triphosphate
PIP2	Phosphatidylinositol 4,5-bisphosphate
PI3K	Phosphoinositide 3-kinase
PLCγ	Phospholipase Cγ
PDGF	Platelet-derived growth factor
PDGFR	Platelet-derived growth factor receptor
PH	Pleckstrin homology
PAE	Porcine aortic endothelial
PTMs	Post-translational modifications
PMA	Progressive muscular atrophy
PIAS	Protein inhibitor of activated STAT
PKB	Protein kinase B
RME	Raft/caveolin-mediated endocytosis
RanBP2	RAN binding protein 2
Raf	Rapidly accelerated fibrosarcoma
RHEB	Ras homolog enriched in brain
Rab	Ras-associated binding
Ras	Rat sarcoma virus
ROS	Reactive oxygen species
RTKs	Receptor tyrosine kinases
REs	Recycling endosomes
RPE1	Retinal peripheral epithelial 1
Rpn1	Ribophorin1
SENP	Sentrin-specific protease
SQSTM1	Sequestosome 1

SHP-2	SH2 domain-containing protein tyrosine phosphatase-2
STAT	Signal transducer and activator of transcription
STAM1/2	Signal transducing adaptor molecule 1 and 2
SUMO	Small ubiquitin-like modifier
SOS1	Son of sevenless homolog 1
SH2	Src homology 2
SAE	SUMO-activating enzyme subunit
SIM	SUMO-interacting motif
Th1	T helper 1
TβRI	TGF-β type I receptor
tPA	Tissue plasminogen activator
tTG	Tissue transglutaminase
TRAF2	TNF receptor associated factor 2
ETS	Transcription factor family
TGF-β	Transforming growth factor-β
TM	Transmembrane
TRIM21	Tripartite motif-containing protein
TSC1/2	Tuberous sclerosis complex 1/2
TNF	Tumor necrosis factor
Ub	Ubiquitin
UCHs	Ubiquitin carboxyl-terminal hydrolases
UIMs	Ubiquitin-interacting motifs
Ubl	Ubiquitin-like protein
UPS	Ubiquitin-proteasome system
USPs	Ubiquitin-specific proteases
UNP	Ubiquitous nuclear protein
ULK1	Unc-51-like autophagy activating kinase 1 complex
uPA	Urokinase-type plasminogen activator
Vps4	Vacuolar protein sorting 4
VEGF	Vascular endothelial growth factor
VSMCs	Vascular smooth muscle cells
WT	Wild type
ZUP1	Zinc finger containing ubiquitin peptidase 1

Introduction

Cells rely on a complex but highly ordered signaling network to communicate with each other and the environment, thus regulating cellular events, such as proliferation, differentiation, migration, and apoptosis. A cell receives signals through the binding of various ligands to specific receptors on the cell surface. Receptor tyrosine kinases (RTKs) are an essential family among these receptors.

The RTK family comprises 55 members belonging to 19 subfamilies (reviewed in Trenker & Jura, 2020). All the members share a similar domain organization: an N-terminal extracellular ligand-binding domain, a single transmembrane (TM) domain with an α-helical propensity, and an intracellular part with a juxtamembrane region, a catalytic tyrosine kinase domain, and a C-terminal tail (reviewed in Hubbard, 1999). RTKs play important roles in cell growth, differentiation and migration. Dysregulation of RTKs can lead to various diseases, notably cancers.

Cancer is a group of diseases involving uncontrollable cell growth, survival and invasion. It is usually the consequence of the accumulation of genetic changes and can originate from any tissue or organ. Cancer is one of the leading causes of death worldwide. According to the data from the International Agency for Research on Cancer (IARC), around 18.1 million new cancer cases and around 9.5 million deaths related to cancer were reported in 2018 worldwide. By 2040, the expected new cancer cases and cancer-related deaths will rise to 29.5 million and 16.4 million, respectively (https://gco.iarc.fr/). Therefore, improved strategies for cancer therapy is of great importance.

Among the targets for cancer treatment are RTKs and signal transduction molecules activated by them. Emerging evidence has shown that multiple regulatory mechanisms, including post-translational modifications (PTMs), internalization, proteolytic cleavage, cellular trafficking and degradation, significantly affect RTK signaling and cellular responses.

In this study, we investigated how the turnover and signaling of the RTKs platelet-derived growth factor receptor (PDGFR) isoforms α and β are modulated by ubiquitination, SUMOylation, proteolytic cleavage and degradation.

PDGF and PDGFR

PDGF

Platelet-derived growth factor (PDGF) is a potent mitogen for certain cell types, including , smooth muscle cells (Ross et al., 1974), fibroblasts (Kohler & Lipton, 1974) and glial cells (Westermark & Wasteson, 1976), and has important roles in embryonic development (Soriano, 1994), normal tissue homeostasis (Rodt et al., 1996), wound healing (Pierce et al., 1995) and tumorigenesis (Buchdunger et al., 1996). It was first discovered in the 1970s in the whole blood serum and then isolated from platelets (Antoniades et al., 1979; Heldin et al., 1979; Kohler & Lipton, 1974; Ross et al., 1974; Westermark & Wasteson, 1976). PDGF isoforms are mainly secreted by platelets (Antoniades, 1981), monocytes (Pencev & Grotendorst, 1988), epithelial cells (Campochiaro et al., 1989), endothelial cells (Hermansson et al., 1988) and glial cells (Pringle et al., 1992) and primarily act as paracrine growth factors.

There are five dimeric isoforms of PDGF consisting of four PDGF polypeptide chains, i.e. PDGF-A, -B, -C and -D chains, that form four homodimers (PDGF-AA, -BB, -CC and -DD) and one heterodimer (PDGF-AB) (Bergsten et al., 2001; Hart et al., 1990; Johnsson et al., 1982; Li et al., 2000) (Figure 1). PDGFs are synthesized as inactive precursors that need to undergo proteolytic processing before they become active. PDGF-A and -B are known as classical PDGFs with short N-terminal extensions that are cleaved and activated intracellularly in the exocytic pathway (Ostman et al., 1992). In contrast, the novel PDGF isoforms, PDGF-C and -D, are secreted as latent factors containing distinct CUB (complement subcomponents Clr/Cls, Urchin EGF-like protein and bone morphogenic protein 1) domain in the N-terminals (Bergsten et al., 2001; Li et al., 2000). For PDGF-CC, tissue plasminogen activator (tPA) (Fredriksson et al., 2004) or plasmin (Lei et al., 2008) catalyzes the removal of the CUB domain, while urokinase-type plasminogen activator (uPA) (Ustach & Kim, 2005) or matriptase (Huang et al., 2007) cleaves and activates PDGF-D. In the C-terminus, both PDGF-A and -B have mainly basic polypeptides contributing to binding to the extracellular matrix, while C-terminal sequence extensions are essentially absent in both PDGF-C and -D (LaRochelle et al., 1991; Ostman et al., 1991).
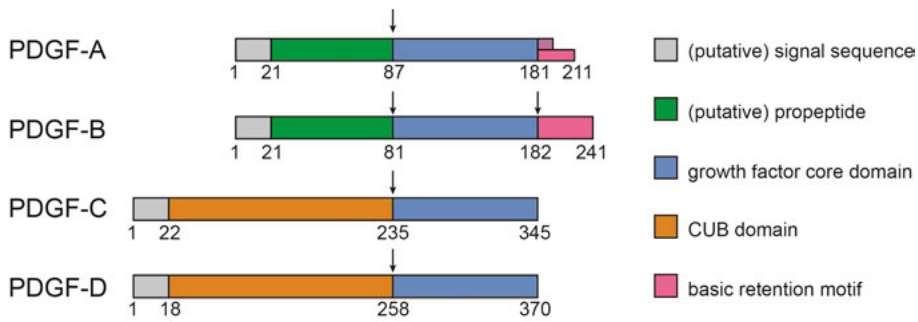
Figure 1. The family of human PDGF ligands and their domain compositions. The starting and ending sites of each domain are marked under the boundaries. The arrows indicate the cleavage sites. Two isoforms of PDGF-AA are shown with different basic retention motifs. See detailed explanation in the main text.

PDGF-A and PDGF-C are mainly expressed in epithelial cells, muscle cells, and neuronal progenitor cells (Eitner et al., 2002; Li et al., 2000; Reneker & Overbeek, 1996; Tian et al., 2021). PDGF-B is commonly found in vascular endothelial cells, neurons, and megakaryocytes (Enge et al., 2003; Gladwin et al., 1990; Lindahl et al., 1998). The expression of PDGF-D has been observed in fibroblasts and smooth muscle cells (Buhl et al., 2016; Chaabane et al., 2014).

PDGFR

PDGF ligands mediate their cellular functions by binding to and activating two receptors, i.e. PDGFRα and PDGFRβ, which are members of the class III RTK subfamily together with KIT, colony stimulating factor 1 receptor (CSF1R) and Fms like tyrosine kinase 3 (FLT-3) (Rosnet et al., 1991; Yarden et al., 1986). PDGF receptors contain an extracellular part consisting of five immunoglobulin-like domains (D1-5) which recognize and bind the ligands (Lokker et al., 1997), a transmembrane helix passing information into the cells and an intracellular part, including a juxtamembrane domain that auto-inhibits the kinase domain before ligands binding (Chan et al., 2003), a protein-tyrosine kinase domain as the effector domain, and a carboxy-terminal tail which before ligand binding helps to keep the kinase inactive (Chiara et al., 2004).

Upon ligand binding, inactive monomeric PDGFRs combine to form three types of receptor dimers PDGFRαα, PDGFRαβ or PDGFRββ; the specificity of dimerization is determined by the PDGF isoforms (reviewed in Heldin & Lennartsson, 2013) (Figure 2). Receptor dimerization is followed by auto-phosphorylation on specific tyrosine residues, which causes a conformational change in the intracellular domain and promotes activation of the kinase.
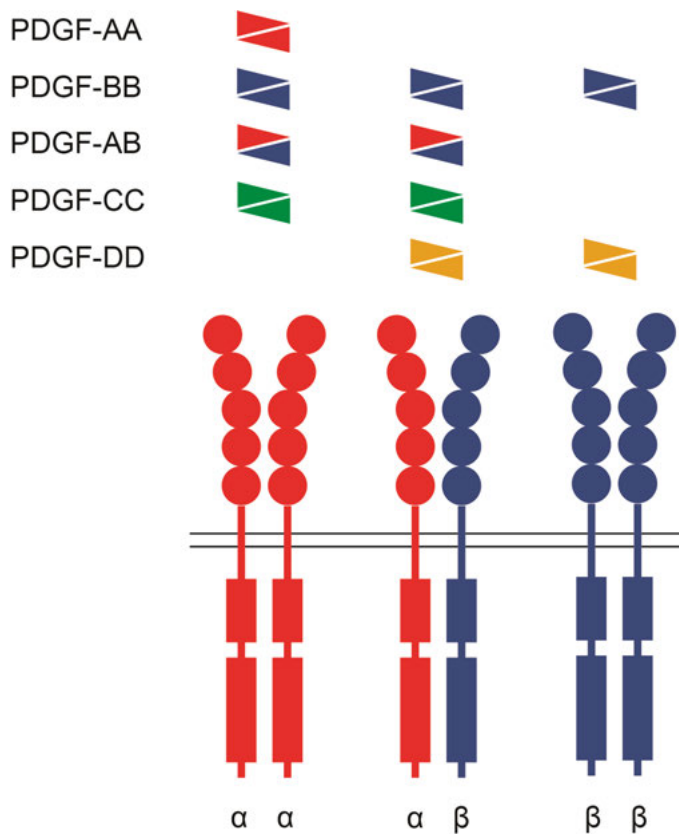
Figure 2. Specific binding of different PDGF ligands to PDGFRs. Four PDGF poly-peptides form five PDGF dimers: PDGF-AA, -BB, -AB, -CC and -DD, which specif-ically induce different PDGFR dimers.

PDGFRs are mainly expressed in cells of mesenchymal origin. PDGFRα has a particularly strong expression in lung mesenchymal progenitor cells (Bos-tröm et al., 1996), intestinal mesenchymal progenitor cells (Roulis et al., 2020) and mesenchymal progenitors in the skin (Orr-Urtreger & Lonai, 1992). PDG-FRβ is mainly expressed in mesenchymal tissues, especially in fibroblasts (Gao et al., 2005), vascular smooth muscle cells (VSMCs) (Kiyan et al., 2005) and pericytes (Winkler et al., 2010).

Under basal conditions *in vivo*, PDGFRs are expressed at a low level, while during inflammation their expression is induced dramatically by several cyto-kines, including transforming growth factor-β (TGF-β) and interleukin 1α (IL-1α) (Lindroos et al., 1995). Overexpression of PDGFRs has been found in certain tumor types, including glioblastoma multiforme (GBM) (Cenciarelli et al., 2014), synovial sarcomas (Ho et al., 2012), and cholangiocarcinoma (Boonjaraspinyo et al., 2012). Mutations in the PDGF or PDGFR coding genes have also been observed in certain tumors. For example, a study based on 1105

cases of gastrointestinal stromal tumors (GISTs) revealed that PDGFRα is mutated in 7.2% of GISTs and the most common mutant, D842V, which is resistant to the receptor kinase inhibitor imatinib, occurred in the activation loop (Corless et al., 2005). A systematic survey in glioblastomas identified a 2-bp deletion in the C-terminal of PDGFRα which results in a truncated mutant PDGFRα (Rand et al., 2005), and PDGFRα has also been found to be amplified in this tumor type (McLendon et al., 2008). In certain leukemias, fusion PDGFRs have been reported, such as FIP1L1-PDGFRα in the acute eosinophilic leukemia (Griffin et al., 2003) and transcription factor family (ETS) variant transcription factor 6 (ETV6)-PDGFRβ in the chronic myelomonocytic leukemia (Golub et al., 1994).

PDGF/PDGFR biological functions

PDGF ligands and their receptors play important roles *in vivo* in both normal physiological and pathological conditions. PDGFRα and PDGFRβ are expressed in different cell types, therefore have different functions. The knockout of PDGFRβ is e.g. associated with vascular defects, while the knockout of PDGFRα is related to various deficiencies in early embryogenesis (Hellstrom et al., 1999; Soriano, 1997).

One important role of PDGF signaling is to induce angiogenesis. PDGF ligands and their receptors have been reported to promote vascular endothelial growth factor (VEGF) production and recruit perivascular cells to newly formed blood vessels (Laschke et al., 2006). Different studies have shown that knockout of PDGF-B or PDGFRβ in mice results in significant deficiency in vessel stability in many organs due to the low pericyte coverage of microvessels (Hellstrom et al., 1999; Lindahl et al., 1997; Soriano, 1994). Endothelial-specific knockout of PDGF-B further demonstrated the importance of endothelial derived PDGF-B as the depletion leads to loss of pericyte and persistent pathological changes, including glomerular, cardiac and placental abnormalities (Bjarnegård et al., 2004).

PDGFs and PDGFRs are also expressed in the neural system and exert their functions during the embryonic development, normal physiology, and pathology (reviewed in Sil et al., 2018). They have been reported to play vital roles in the differentiation of neural progenitor cells (NPCs) into neurons (Erlandsson et al., 2001; Williams et al., 1997). Knockout of PDGF-AA in mice causes severe central nervous system (CNS) demyelination leading to tremors in the surviving pups (Fruttiger et al., 1999). Mutations in *Pdgfb* gene in mice and humans lead to brain calcifications, which is related to the degree of pericyte and the deficiency of blood-brain barrier (Keller et al., 2013). Similarly, mutations of the gene encoding its receptors, PDGFRβ, result in idiopathic basal ganglia calcification (IBGC) (Nicolas et al., 2013). PDGFRα was reported to be essential in both mesoderm cells and neural crest cells during calvarial de-

velopment (Umar et al., 2023). In the murine neural crest cells, PDGFRα primarily contributes to cell migration, while PDGFRβ mainly promotes the proliferation of the facial mesenchyme after mid-gestation (Mo et al., 2020). PDGF-C appears to mediate neurovascular crosstalk due to its dual role in both angiogenesis and neuronal survival (Lee et al., 2013). The alterations in PDGF-CC levels as well as its signaling is related to multiple neurodegenerative diseases, such as amyotrophic lateral sclerosis (ALS) (Lewandowski et al., 2016), Parkinson's disease (PD) (Tang et al., 2010), and stroke (Su et al., 2008). Although PDGF-D is hardly detectable in the embryonic spinal cord, it is highly expressed in the adult motoneurons (Hamada et al., 2002).

Knockout of PDGFRα can lead to severe consequence, i.e. embryonic lethality of the homozygotes (Qian et al., 2017). PDGFRα deficient embryos exhibit incomplete cephalic closure, thin epidermal layer and loss of cornified layer (Schatteman et al., 1992; Soriano, 1997). In Xenopus embryos, the expression of a dominant negative PDGFRα mutant gene results in abnormal gastrulation (Ataliotis et al., 1995).

A Y740/751F mutant PDGFRβ, that lacks binding sites for phosphoinositide 3-kinase (PI3K), mediates abnormal spreading of the mesodermal cells, while other mutants lacking binding sites for other signaling transduction molecules do not have the same effect (Symes & Mercola, 1996). Knock-in in mice of a Y739/750F (corresponding to Y740/751F in humans) mutant PDGFRβ lacking PI3K binding sites lose the ability to normalize the decreased interstitial fluid pressure resulting from the injection of the mast cell degranulating agent C48/80 (Heuchel et al., 1999).

PDGFR activation and signaling

PDGFRs are activated by the binding of PDGF ligands, followed by the dimerization of two receptor monomers. The ligand binding sites are located in Ig-like domains D2 and D3 (Heidaran et al., 1990; Lokker et al., 1997; Miyazawa et al., 1998), whereas direct receptor-receptor interactions involving Ig-like domains D4 and D5 help to stabilize the dimerization (Omura et al., 1997; Yang et al., 2008). Both hydrophobic interactions and salt bridges are involved in ligand-receptor interactions (Shim et al., 2010). The dimerization of the receptors promotes activation of the receptor kinase and auto-phosphorylation of 10 tyrosine residues in PDGFRα and 11 tyrosine residues in PDGFRβ (Figure 3), leading to the recruitment of signal transduction molecules containing Src homology 2 (SH2) domains, such as Src, PI3K, phospholipase Cγ (PLCγ), and growth factor receptor-bound protein 2 (Grb2)/ son of sevenless homolog 1 (SOS1) which activates Ras (Tallquist & Kazlauskas, 2004). The signals are transient and decline as the activated PDGFRs are internalized and degraded.
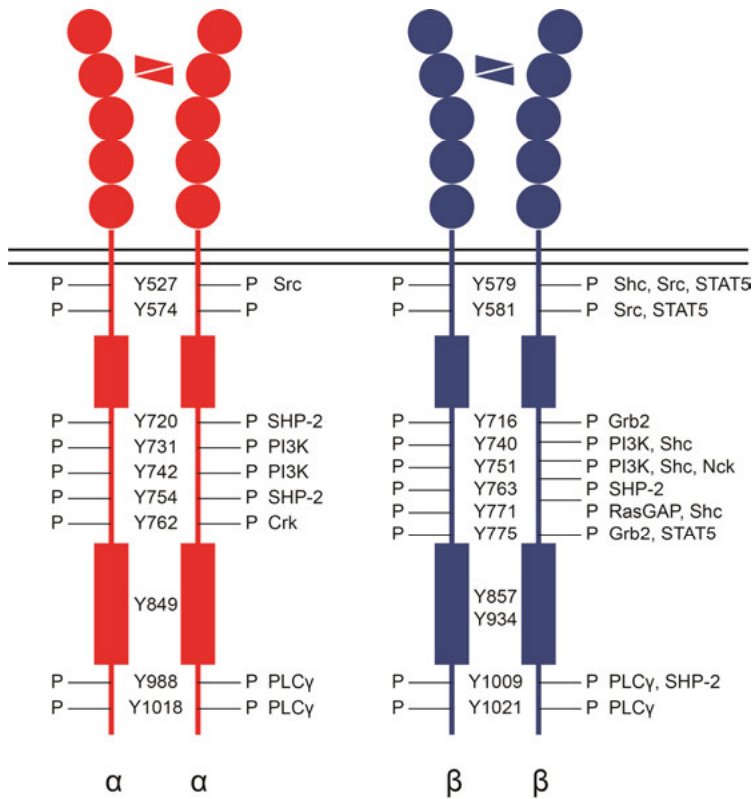
Figure 3. Phosphorylated sites in PDGFRα and PDGFRβ and their binding to signaling molecules containing SH2 domains. Y849 in PDGFRα and Y857 in PDGFRβ are localized in the activation loops of their kinase domains which are not known to bind to any signaling molecules. Y934 in PDGFRβ is phosphorylated by Src, but not autophosphorylated.

In some cases, the activation of PDGFRs is not caused by PDGF ligands but by members of other growth factor families, such as VEGFs, which was found in pathological settings (Pennock et al., 2014). PDGFRα, but not PDGFRβ, can be activated continuously by downstream signaling which is triggered by the binding of certain non-PDGF growth factors and their receptors. The activation of Ras is involved in this event (Lei et al., 2015). VEGF-A can also bind directly to and trigger tyrosine phosphorylation of both PDGFRα and PDGFRβ in mesenchymal stem cells (MSCs) isolated from bone marrow which do not express VEGFR receptors (Ball et al., 2007).

Activation of PDGFRβ promotes its association with the low-density lipoprotein receptor-related protein (LRP) in WI-38 fibroblasts, indicating that LRP acts as a co-receptor of the PDGFRβ (Newton et al., 2005). In fibroblasts, tissue transglutaminase (tTG) functions as the bridge between PDGFR and integrins, thus enhancing their association and the signal transduction (Zemskov et al., 2009).

PLCγ/PKC pathway

Phospholipases are a group of metabolizing enzymes that mediate the break-down of phospholipids to generate bioactive lipid mediators (Park et al., 2012). PLC isoforms constitute a major familiy of phospholipases which specifically hydrolyze phosphatidylinositol 4,5-bisphosphate (PIP2) to generate two intracellular secondary messengers, i.e. inositol 1,4,5-triphosphate (IP3) and diacylglycerol (DAG). IP3 promotes the release of calcium ions (Ca^{2+}) from the endoplasmic reticulum, which together with DAG, activate protein kinase C (PKC) (Rhee, 2001). These processes contribute to various cellular events related to tumorigenesis, including cell survival (Bai et al., 2002), proliferation (Zhang et al., 2014), autophagy (Sakaki & Kaufman, 2008) and angiogenesis (Yoshiji et al., 1999).

According to the structure, PLCs are divided into 6 sub-families, among which PLCγ contains SH2 domains allowing them to interact with tyrosine phosphorylated RTKs (Owusu Obeng et al., 2020; Rotin et al., 1992). To date, two mammalian PLCγ isoforms, PLCγ1 and PLCγ2, have been identified. PLCγ1 is widely expressed, while PLCγ2 mainly existed in hematopoietic cells (Liao et al., 2002).

After ligand binding, PDGFRs are dimerized and autophosphorylated. Autophosphorylation of PDGFR at its tyrosine residue Y1021 provides a docking site for the SH2 domain of PLCγ1, leading to the enzymatic activation of PLCγ1 and stimulation of mitogenesis (Valius et al., 1993). Our previous results have demonstrated that depletion of c-Cbl and Cbl-b results in enhanced phosphorylation of Src and PLCγ, which might contribute to the observed increased chemotaxis of the cells (Rorsman et al., 2016). It should be noted that Cbls also bind to PDGFRβ at Y1021, so the enhanced activation of PLCγ may be the result of less competition with Cbls for binding to Y1021.

Ras/Raf/MEK/ERK pathway

Mitogen-activated protein kinase (MAPK) is a family of serine/threonine protein kinases, which is essential for the regulation of cell proliferation (Qu et al., 2009), cell differentiation (Ding et al., 2001) and apoptosis (Zhan et al., 2012). MAPK signaling is dependent on a sequential cascade, including GTPase activating protein (GAP), MAPK kinase kinase (MAP3K), MAPK kinase (MAP2K) and MAPK (reviewed in Guo et al., 2020). The extracellular signal-regulated kinase (ERK) is a prototypic subfamily of MAPK, which is translocated from the cytoplasm to the nucleus to regulate downstream transcription factors and gene expression upon activation (Boulton et al., 1991). Different isoforms ERK1-ERK7 have been found in the ERK family, among which ERK1 and ERK2 are the two members most studied (Wang et al., 2007). In the ERK pathway, rat sarcoma virus (Ras) is the GTPase that activates the signaling, rapidly accelerated fibrosarcoma (Raf), mitogen-activated

ERK kinase (MEK) and ERK, act as the MAP3K, the MAP2K and the MAPK, respectively, thus forming the Ras/Raf/MEK/ERK signaling pathway (reviewed in Yang & Liu, 2017).

Activated PDGFRβ can bind to Grb2 directly at Tyr716 or indirectly via SH2 domain-containing protein tyrosine phosphatase-2 (SHP-2) at Tyr763 and Tyr1009 (Arvidsson et al., 1994; Rönnstrand et al., 1999). Grb2 form a complex with SOS1, which is a GTP exchange factor activating Ras (Egan et al., 1993). Besides the function in mediating the interaction with Grb2 and PDGFR, SHP-2 also dephosphorylates the receptors, as well as its substrates, thus modulating PDGFR signaling both positively (Dance et al., 2008; Peng & Cartwright, 1995; Zhao & Zhao, 1999) and negatively (Klinghoffer & Kazlauskas, 1995). The association of RasGAP with activated PDGFRβ downregulates the activation of Ras, while PDGFRα does not engage with RasGAP, which makes the activation of ERK signaling via PDGFRα more durable (Jurek et al., 2011; Lei et al., 2015).

JAK/STAT pathway

Signal transducer and activator of transcription (STAT) family, which shuttles continuously between cytoplasm and nucleus, consists of seven mammalian members: STAT1-4, 5a, 5b and 6 (Kiu & Nicholson, 2012). Each STAT protein is about 750-900 amino acid residues consisting of 6 conserved domains that mediate different functions: an N-terminal domain, a coiled-coil domain, a DNA-binding domain, a linker domain, an SH2 domain and a C-terminal domain (Hanada et al., 2004; Kisseleva et al., 2002). Members of STAT families exert distinct roles in tumor progression: STAT1 and STAT2 usually inhibit tumor survival, whereas STAT3 has been reported to promote various cancers, such as cutaneous squamous cell carcinoma (Suiqing et al., 2005), prostate cancer (Bishop et al., 2014), and breast cancer (Berishaj et al., 2007). The balance of different family members of the STAT family may play an important role in controlling tumor development.

Typically, STAT molecules are activated by the binding of cytokines, such as ILs to their receptors on the cell surface (Demoulin et al., 1996). Upon ligand binding and dimerization, the receptors recruit and activate Janus kinases (JAKs). Activated JAKs then phosphorylate the cytoplasmic tyrosine residues of the receptors to provide a docking site for the SH2 domain of STAT, leading to its phosphorylation at Tyr705 (Kaptein et al., 1996). The phosphorylation of STAT then triggers its homo- or hetero-dimerization, which releases them from the receptor and promotes their translocation into the nucleus via importins (Fagerlund et al., 2002; Liu et al., 2005), where they bind to DNA and modulate the expression of target genes. PKCδ (Jain et al., 1999; Uddin et al., 2002) and MAPKs including ERK (Chung et al., 1997), p38 (Goh et al., 1999), and SEK-1/MKK-4 (Schuringa et al., 2000) mediate the phosphoryla-

tion of STAT1 and STAT3 at Ser727 in the nucleus which enhances its transcriptional activity after DNA binding (Wen & Darnell, 1997). In the nucleus, transcription factor STAT3 induces transcription of multiple genes including *c-myc* (Yamanaka et al., 1996), *junB* (Kojima et al., 1996), *CDKN1A* (Cattaneo et al., 1999), *cyclin D1* (Cressman et al., 1996), *β-catenin* (Hao et al., 2006), *Bcl-xL* (Yamauchi-Takihara, 2002), its own transcription (Yang et al., 2005) and its inhibitor suppressor of cytokine signalling (SOCS) (Krebs & Hilton, 2001).

Knockdown of different STAT members in mice revealed the different function of each STAT. Mice depleted with STAT1 (Meraz et al., 1996) or STAT2 (Park et al., 2000) lack innate response to interferons (IFNs) and are highly sensitive to viral or bacterial infection. Knockdown of STAT3 in mice leads to early embryonic lethality prior to gastrulation (Takeda et al., 1997). Mice depleted in STAT4 and STAT6 exhibit impaired T helper 1 (Th1) or no Th2 cell function, respectively, revealing their roles in IL-12 or IL-4 signaling (Kaplan et al., 1996; Takeda et al., 1996). STAT5a-deficient mice exhibit curtailed breast development or lactation (Liu et al., 1997), while STAT5b-deficient mice exhibit disturbed sexual dimorphism of body growth rates as well as liver gene expression (Udy et al., 1997).

Different RTKs, including PDGRs, have been reported to activate STATs. PDGFRβ have been reported to activate STAT1, STAT3 and STAT5 and interact with STAT5 directly at phosphorylated Tyr579, Tyr581 and Tyr775 (Valgeirsdóttir et al., 1998). STAT1 was also shown to directly interact with PDGFR, while the activation of STAT3 induced by PDGF is dependent on JAKs (Vignais & Gilman, 1999). It was found that the full activation of STAT3 requires the internalization of PDGFRβ, implying a requirement of receptor signaling from endosomes for PDGFRβ-mediated activation of STAT3 (Jastrzębski et al., 2017).

Wild type (WT) RTKs usually activate STAT weakly and the activation has not been suggested to mediate key physiologic functions so far, which is contrary to mutated RTKs. For example, mutant forms of PDGFRs are found to strongly activates STATs. In idiopathic hypereosinophilic syndrome, FIP1L1-PDGFRα fusion protein has been reported to activate STAT5 which can be inhibited by imatinib (Cools et al., 2003). In a transformed IL-3 independent cell line, Ba/F3, the tyrosine kinase fusion protein TEL/PDGFRβ was found to activate STAT1 and STAT3 in a JAK-independent pathway (Wilbanks et al., 2000). Two PDGFRβ mutants found in patients with familial infantile myofibromatosis (IM), R561C and N666K, constitutively activate STAT3 and STAT5 in the absence of PDGF stimulation (Arts et al., 2016). Mutation of PDGFR at some sites also leads to the impairment of STAT3 activation. For example, the R987W mutant PDGFRβ which is mutated in its C-terminal tail promoted receptor degradation, thus causing deficient STAT3 signaling (Arts et al., 2015).

PI3K/AKT pathway

PI3K is a group of lipid kinases that mediate the phosphorylation of phospho-inositides at the 3'-hydroxyl group. Based on their specific structures and substrates, PI3Ks are divided into 3 classes, among which class I PI3Ks can be further grouped into class IA and class IB. To date, class IA PI3K is the only type that has been clearly implicated in human cancers (reviewed in Yang et al., 2019). Members of class IA PI3K are heterodimers of p85 regulatory subunits and p110 catalytic subunits and are activated by RTKs (Engelman et al., 2006; Hiles et al., 1992). Activated PI3KIA catalyzes the phosphorylation of PIP2 to phosphatidylinositol 3,4,5-triphosphate (PIP3), which serves as a binding site for signaling proteins containing pleckstrin homology (PH) domains, such as protein kinase B (PKB, also known as AKT) (Manning & Cantley, 2007). Phosphatase and tensin homolog (PTEN) is a tumor suppressor which negatively regulates PI3K signaling by dephosphorylating PIP3 to generate PIP2 (Maehama & Dixon, 1998).

The serine/threonine protein kinase AKT is the main downstream signaling molecule of the PI3K pathway. It comprises three members, AKT1, AKT2, and AKT3, which share three conserved functional domains: the N-terminal PH domain, the central kinase domain, and the C-terminal regulatory domain containing the hydrophobic motif (Hanada et al., 2004). The full activation of AKT depends on the phosphorylation of Thr308 in its activation loop and Ser473 in the C-terminal hydrophobic motif (Alessi et al., 1996). Activated AKT then phosphorylates its downstream signaling molecules including forkhead box class O (FOXO), Bcl2 associated agonist of cell death (BAD), murine double minute 2 (MDM2), glycogen synthase kinase-3 (GSK-3), IκB kinase (IKK), and mammalian target of rapamycin (mTOR), to regulate the cell cycle (Gao et al., 2004), cell proliferation (Nelson & Chen, 2002), cell survival (Souza et al., 2001), apoptosis (Yao & Cooper, 1995) and tumor growth (Janda et al., 2002).

It has been reported that activated PDGFRβ provides binding sites for the SH2 domain of the p85-like subunit of class IA PI3Ks via its Tyr740 and Tyr751 residues, thus activating the PI3K/AKT pathway (Kim et al., 2011). Similarly, activated PDGFRα binds PI3K via Tyr 731 and Tyr742. By activating the PDGFRα/PI3K/AKT pathway, PDGF-AA inhibits apoptosis of cells induced by H_2O_2 (Vantler et al., 2006) and by activating the PDGFRβ/PI3K/AKT pathway, PDGF-DD has been found to promote Schwannoma cell proliferation (Ammoun et al., 2011).

Ubiquitination and SUMOylation

PTMs of proteins occur after their translation from mRNA and can take place in multiple cellular compartments, including the Golgi apparatus, nucleus, endosomes, and the plasma membrane (Rahimi & Costello, 2015). Numerous PTMs have been identified, among which phosphorylation, ubiquitination, acetylation, methylation and glycosylation are the most studied (Kokkinidis et al., 2020; Prus et al., 2019). PTMs dramatically enrich the diversity of the proteome and provide an efficient way to regulate and fine-tune protein functions, especially related to signal transduction (Liddy et al., 2013).

Among the commonest types of PTMs of PDGFRs is phosphorylation which has been studied extensively (Kelly et al., 1991; Suzuki et al., 2010; Szöőr et al., 2016), as well as ubiquitination and SUMOylation which are the subject of the present study. The phosphorylation of PDGFRs has been discussed previously in part 2. In this part, I will focus on ubiquitination and SUMOylation.

Ubiquitination

Ubiquitination is a key PTM regulating degradation, subcellular localization and activity of many proteins including PDGFRs. It involves the covalent attachment of a small protein, ubiquitin (Ub), to target proteins (Hershko, 1983). Ubiquitin is a highly conserved 8 kDa protein, which consists of 76 amino acid residues including 7 lysine residues (K6, K11, K27, K29, K33, K48 and K63). As the name suggests, ubiquitin is expressed ubiquitously. It can be attached to target proteins in different ways including monoubiquitination (mono-Ub), multiubiquitination (multi-Ub) and polyubiquitination (poly-Ub) (Foot et al., 2016).

Protein ubiquitination is mediated in a sequential ATP-dependent manner by three enzymes, E1 activating enzyme, E2 conjugating enzyme and E3 ligase (Wilkinson, 1987). The process starts with the activation of ubiquitin by the E1 enzyme and is followed by the passing of activated ubiquitin to an E2 enzyme to form an E2-Ub complex. Finally, this complex and the target protein are simultaneously conjugated to an E3 ligase; then, the ubiquitin, through its C-terminal glycine residue, forms an isopeptide bond with lysine residue(s) in the substrate (Mansour, 2018) (Figure 4).

As mentioned above, a prominent role of ubiquitination is to mark its target proteins for degradation. During this process, K48-linked chains target proteins for degradation by the 26S proteasome, while K63-linked chains could be involved in autophagy-lysosomal degradation (Ciechanover, 2005). Apart from this, protein ubiquitination also participates in certain other cellular processes, such as protein-protein interactions, protein trafficking, and activation or inactivation of substrates.
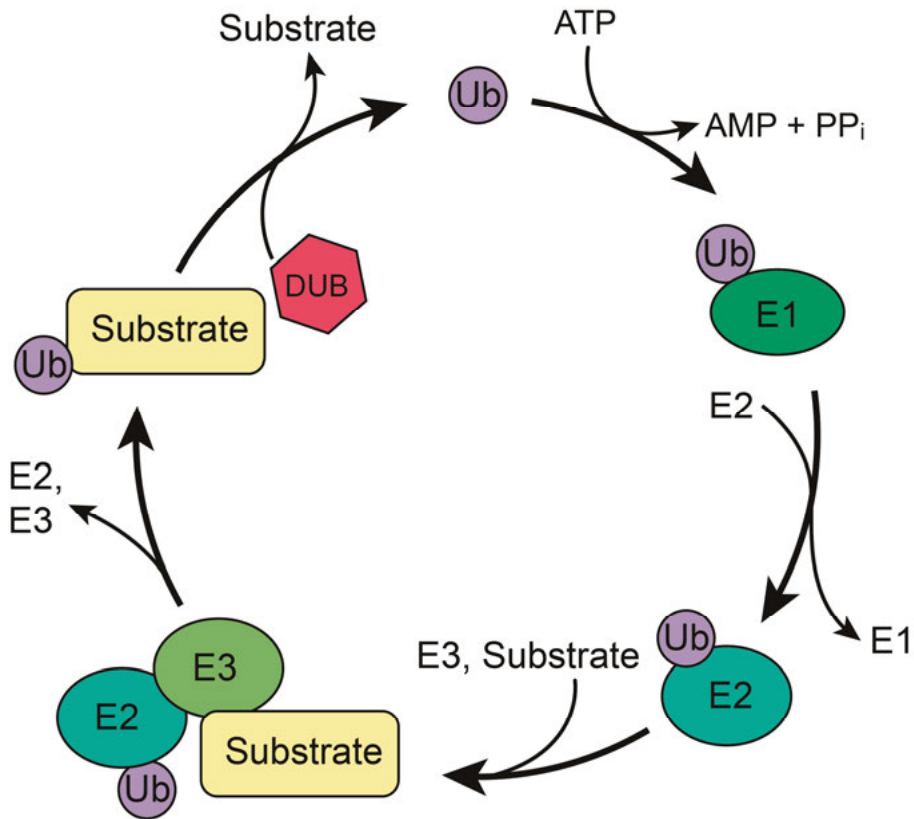
Figure 4. The ubiquitination conjugation mechanism. Ubiquitin is activated by the E1 enzyme in an ATP-dependent manner, and then with the mediation of E2 enzyme and E3 ligase, it is coupled to its substrate. Ubiquitin is removed from its substrate by DUBs to enter into another cycle.

Various RTKs are modified by ubiquitination, in both basal conditions and ligand-stimulated conditions. It has been reported that ubiquitination of non-activated VEGFR2 significantly affects its ligand-induced signaling and cellular responses (Smith et al., 2017). Ubiquitination of RTKs is linked to their trafficking from cell membranes to different intracellular compartments and consecutive degradation in the endosome-lysosome system (Critchley et al., 2018).

Deubiquitinating enzymes (DUBs)
Protein ubiquitination can be reversed by the removal of ubiquitin from ubiquitinated proteins mediated by deubiquitinating enzymes (DUBs), thus giving this modification flexibility. There are 99 DUBs in humans, which can be categorized into seven subfamilies: six cysteine protease families, including ubiquitin-specific proteases (USPs), ovarian tumor domain-containing prote-

ases (OTUs), ubiquitin carboxyl-terminal hydrolases (UCHs), Machado-Josephin domain proteases (MJDs), motif interacting with ubiquitin-containing novel DUB family (MINDYs) and zinc finger containing ubiquitin peptidase 1 (ZUP1), as well as JAB1/MPN/Mov34 metalloproteases (JAMMs) (reviewed in Ruan et al., 2020).

DUBs have several functions, including activating ubiquitin precursors, removing ubiquitin from target proteins, modulating signaling and subcellular localization and regulating proteasomal or lysosomal degradation (reviewed in Schauer et al., 2020). The epidermal growth factor receptor (EGFR), for instance, can be deubiquitinated by USP18 (Duex et al., 2011), by an endosome-associated ubiquitin isopeptidase AMSH (McCullough et al., 2004), and by USP9X indirectly through the endocytic adaptor Eps15 (Savio et al., 2016). USP8, another DUB, is involved in the deubiquitination of several RTKs including EGFR, the EGFR family member ErbB3, hepatocyte growth factor receptor (HGFR, also called c-Met), and VEGFR2 (Niendorf et al., 2007; Reincke et al., 2015). In paper I, we report that PDGFRβ is deubiquitinated by USP4 and USP17.

USP4

USP4, which is also named as ubiquitous nuclear protein (UNP), shuttles between the nucleus and the cytoplasm, and the localization of USP4 varies among distinct cell types (Soboleva et al., 2005). Phosphorylation of USP4 by kinases, such as Akt and cyclin-dependent kinases (CDKs), is essential for its export from the nucleus to the cytoplasm, while dephosphorylation of USP4 causes its nuclear accumulation (Das et al., 2019; Zhang et al., 2012). USP4 can remove both K48- and K63-linked ubiquitin chains (Kwon et al., 2017; Xu et al., 2018). Due to its various substrates, USP4 is involved in several signaling pathways, including Wnt/β-catenin (Zhao et al., 2009), TGF-β (Zhang et al., 2012), nuclear factor kappa B (NF-κB) (Fan et al., 2011), and p53-related signaling pathways (Zhang et al., 2011), thus modulating various physiological and pathological processes.

Loss of USP4 in cells disturbs the accumulation of several correctly spliced cell cycle regulators, including budding uninhibited by benzimidazoles 1 (BUB1) and α-tubulin, resulting in the impairment of cell cycle progression (Song et al., 2010). The study based on the USP4-depleted mouse embryonic stem cells (mESCs) has revealed that USP4 binds to SMAD4 and prevents its monoubiquitination, which enhances both bone morphogenetic protein (BMP) and activin pathways (Zhou et al., 2017). USP4 interacts with and deconjugates K48-linked polyubiquitin chains from an immune system-restricted interferon regulatory factor 8 (IRF8), which is vital for the normal function of regulatory T cells (Lin et al., 2017).

Aberrant USP4 action has been observed in various cancers, either suppressing or promoting tumor progression. In human lung adenocarcinoma ep-

ithelial cells, USP4 downregulates the NF-κB signaling pathway by deubiquitinating and inactivating tumor necrosis factor (TNF) receptor associated factor 2 (TRAF2) and TRAF6, thus inhibiting TNFα-induced cancer cell migration (Xiao et al., 2012). In breast cancer cells, deubiquitination of TGF-β type I receptor (TβRI) mediated by phosphorylated USP4 in the cytoplasm is essential for Akt-induced cell migration (Zhang et al., 2012). A tumor-promoting role of USP4 was also found in liver cancer and glioblastoma, involving deubiquitination of TβRI and activation of SMAD and ERK1/2 pathways (Qiu et al., 2018; Zhou et al., 2019).

USP17

USP17, also known as DUB3, consists of multiple similar proteins encoded by a block of tandemly repeated gene sequences with high copy number variation (Burrows et al., 2010). These genes were originally discovered in mice and named DUB-1, DUB-1A, DUB-2 and DUB-2A (Zhu et al., 1996, 1997). The expression of USP17 can be induced by several cytokines, including several ILs (Baek et al., 2004; Burrows et al., 2004).

USP17 is involved in various cellular processes. It is essential for clathrin-mediated endocytosis (CME) of the RTK EGFR as the depletion of USP17 impairs the recruitment of the key components of CME machinery including clathrin itself and the adaptor protein complex 2 (AP2) (Jaworski et al., 2014). Tightly regulated expression of USP17 is required for proper cell cycle progression as depletion of USP17 blocks the cell cycle transition from G1 to S phase (Burrows et al., 2010). USP17 binds to the phosphatase cell division cycle 25A (CDC25A), a crucial mitotic regulator de-phosphorylating CDKs, and removes the polyubiquitin chains that mark it for ubiquitin-proteasome system (UPS) (Pereg et al., 2010). Deubiquitination of cyclin A by USP17 stabilizes it and promotes G1/S transition (Hu et al., 2019). The transient induction of USP17 in response to the chemokines IL-8 and SDF-1 is necessary for cell motility. Additionally, USP17 changes the cellular localization of Ras, thus modulating the Ras pathway which is crucial for cell proliferation and migration (de la Vega et al., 2011). Induction of USP17 enzymes leads to the attenuation of the Ras/MAPK signaling pathway, and decreases cell viability (Borbely et al., 2015). Ubiquitination of the ETS transcription factor ELK-1 at K35 can be reversed by USP17 and is important for the regulation of nuclear ERK signaling, as well as cell proliferation (Ducker et al., 2019).

USP17 is overexpressed in many types of tumors, such as non-small cell lung cancer (NSCLC) (McCann et al., 2018), human ovarian cancer (Zhou et al., 2015), osteosarcoma (Song et al., 2017) and breast cancer (Wu et al., 2017), and this overexpression is correlated with the formation of metastases and poor prognosis. Given the role that USP17 plays in multiple cancer types, it has been reported as a potential therapeutic target. However, there are also studies indicating a role of USP17 in suppressing tumorigenesis. For example,

in breast cancer, USP17-mediated downregulation of asparagine endopeptidase (AEP) was reported to disturb ERK signaling and inhibit breast cancer tumorgenesis and growth (Chen et al., 2019). Inhibition of USP17 suppresses NF-κB/p65 signal transduction via the promotion of the reactive oxygen species (ROS), thus exerting anti-tumor activities in prostate cancer (Baohai et al., 2019).

SUMOylation

SUMOylation is a PTM that plays an important role in the regulation of protein subcellular localization (Liu et al., 2007), protein-protein interactions (Jakobs et al., 2007), protein-DNA binding (Tateishi et al., 2009), genome organization (den Besten et al., 2006), transcriptional regulation (Oishi et al., 2008) and DNA repair (Dou et al., 2010). Dysregulation of SUMOylation is highly related to various diseases, such as neurodegenerative disease (Mun et al., 2016), cardiac disease (Da Silva-Ferrada et al., 2016), and cancers (Seeler & Dejean, 2017).

SUMOylation involves the covalent attachment of a 97-amino acid residue small ubiquitin-like modifier (SUMO), to lysine residues of its substrates (Filippopoulou et al., 2020). SUMO belongs to the ubiquitin-like protein (Ubl) family, which consists of approximately 20 proteins, such as neuronal precursor cell-expressed developmentally downregulated protein 8 (NEDD8) and interferon stimulated gene 15 (ISG15) (Pirone et al., 2017). Most Ubls contain a glycine-glycine (GG) motif at their C-terminus, which is responsible for their conjugation to substrates. The 20 amino acid residue long N-terminus is one feature that defines SUMO protein from other Ubls (Bayer et al., 1998). Multiple SUMO genes occur in humans, encoding distinct SUMO proteins, i.e. SUMO1-5. SUMO1-3 members are expressed more ubiquitously than SUMO4 and SUMO5. SUMO1 shows 48% similarity with SUMO2 and 46% with SUMO3, while SUMO2 and 3 share 97% sequence similarity and cannot be distinguished by specific antibodies (Saitoh & Hinchey, 2000).

Similar to the enzymatic mechanism of ubiquitination, the conjugation of SUMOs to their target proteins involves three SUMO-specific enzymes: an E1 activating enzyme which consists of the two subunits SUMO-activating enzyme subunit 1 and 2 (SAE1/SAE2), an E2 conjugating enzyme Ubc9, and E3 ligases, such as members of the protein inhibitor of activated STAT (PIAS) family and RAN binding protein 2 (RanBP2). SUMO proteins are expressed as precursors and need to be cleaved by SUMO specific proteases, such as members of the sentrin-specific protease (SENP) family, to reveal their GG motif (Xu & Au, 2005). The mature SUMO molecule is then activated by the E1 enzyme, dependent on ATP hydrolysis, and transferred to the catalytic center of the E2 enzyme Ubc9. Ubc9 recognizes and binds to a specific motif in the target proteins, i.e. ψKxD/E (ψ, hydrophobic amino acid residue; K, the target lysine; x, any amino acid residue; D/E, aspartate or glutamate) (Johnson

& Blobel, 1999). Some of the substrates also contain a SUMO-interacting motif (SIM) to help them recruit SUMO (Engelhardt et al., 2003). The final step of protein SUMOylation involves an E3 ligase which transfers SUMO from Ubc9 to the target protein. Like ubiquitination, SUMOylation is reversible, it can be remove from protein by the same enzyme that processes the SUMO precursor, i.e. SUMO specific proteases (Gong et al., 2000) (Figure 5).
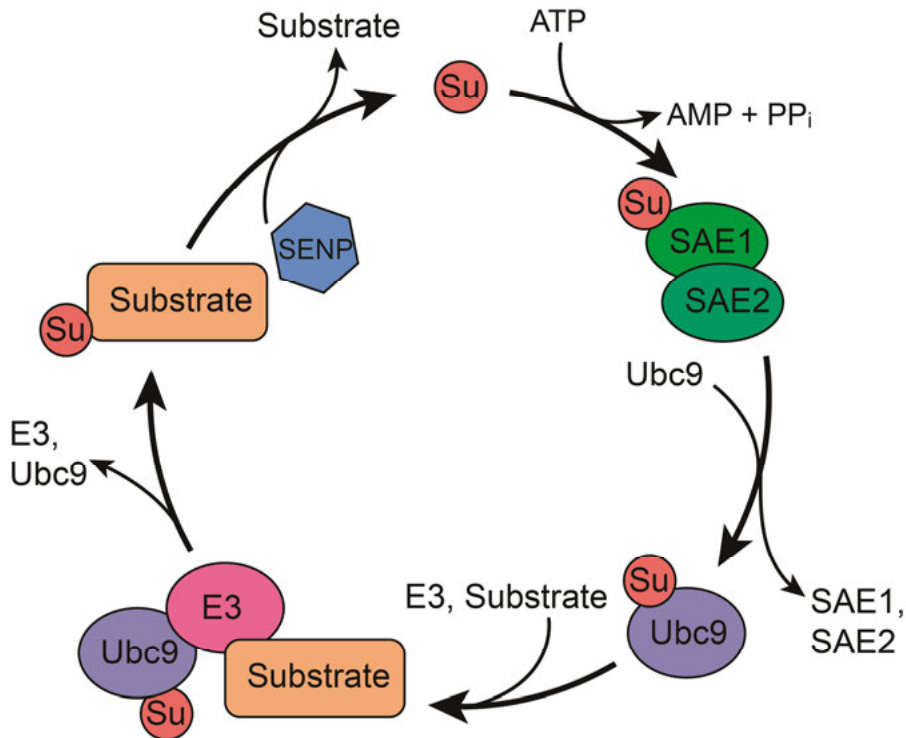

Figure 5. The SUMO conjugation mechanism. SUMO precursors are processed by SENP and then activated by the SUMO E1 enzyme in an ATP-dependent manner and transferred to Ubc9. With the help of a SUMO E3 ligase, it can be conjugated to its target proteins. SENP mediates the removal of SUMO from its substrate.

SUMOylation is a vital modification that regulates various cellular processes, especially nuclear functions (Geiss-Friedlander & Melchior, 2007; Hendriks et al., 2017). The first protein that was discovered to be modified by SUMO was RanGAP1. SUMOylation of RanGAP1 is required for targeting it to the nuclear pore complex (NPC) where it can interact with RanBP2, which is essential for its role in nuclear protein import (Mahajan et al., 1997). SUMOylation has also been found to regulate protein stability. The SUMOylation of pescadillo ribosomal biogenesis factor 1 (PES1) enhances its stability by inhibiting its ubiquitination, promoting the proliferation of breast cancer cells (Li et al., 2016). SUMOylation is able to induce accumulation of α-synuclein,

a presynaptic neuronal protein, directly or by blocking the ubiquitin-dependent degradation of α-synuclein (Rott et al., 2017).

There is evidence that SUMOylation plays a role in the intracellular localization of RTKs. SUMOylation of insulin-like growth factor 1 receptor (IGF-1R) has been found to be related to its nuclear translocation, as the mutation of its SUMOylation sites blocked the accumulation of receptors in the nucleus (Sehat et al., 2010). Similarly, SUMOylation is essential for the nuclear localization and function of the intracellular domain of ErbB4 (Knittle et al., 2017). SUMOylation of VEGFR2 at lysine 1270, promoted its accumulation in the Golgi apparatus and decreased its expression on the cell surface, impairing VEGFR2 signaling (Zhou et al., 2018).

Not much is known about SUMOylation of PDGFRs. A fusion protein, FIP1L1-PDGFRα, which is found in patients with idiopathic hypereosinophilic syndrome, is able to associate with PIAS1, a SUMO E3 ligase. However, it is the FIP1L1 part that contribute to this interaction (Ibata et al., 2017). The advances of mass spectrometry-based proteomics has allowed the exploration of new PTM sites (Hornbeck et al., 2015). Recently, SUMOylation of Lys917 of PDGFRα was detected using this approach (Lumpkin et al., 2017), but the functional role of this modification has not been determined.

PDGFR internalization

One important mechanism of PDGF signaling regulation is the internalization of PDGFRs which happens continuously in a cell. Under normal conditions, there is a basic internalization level of PDGFRs at a rate similar to other membrane proteins, which is matched by the delivery of PDGFRs to the cell membrane (Goh & Sorkin, 2013). Upon ligand binding and dimerization, the internalization rate of PDGFRs is highly elevated, which is usually composed of two major processes: rapid endocytosis of PDGFRs from the plasma membrane and the sorting of internalized PDGFRs through the endosomal system destined for degradation or, under some circumstances, recycling (Hellberg et al., 2009).

PDGFR endocytosis and sorting

The mechanism of RTK endocytosis has been widely studied and the knowledge is mostly based on EGFR (Kreitman et al., 2018; Zhang & Simons, 2014). The dominant endocytosis route of PDGFRs, as well as most RTKs, is the dynamin-dependent CME. The canonical model of the endocytosis of a RTK is shown in Figure 6.
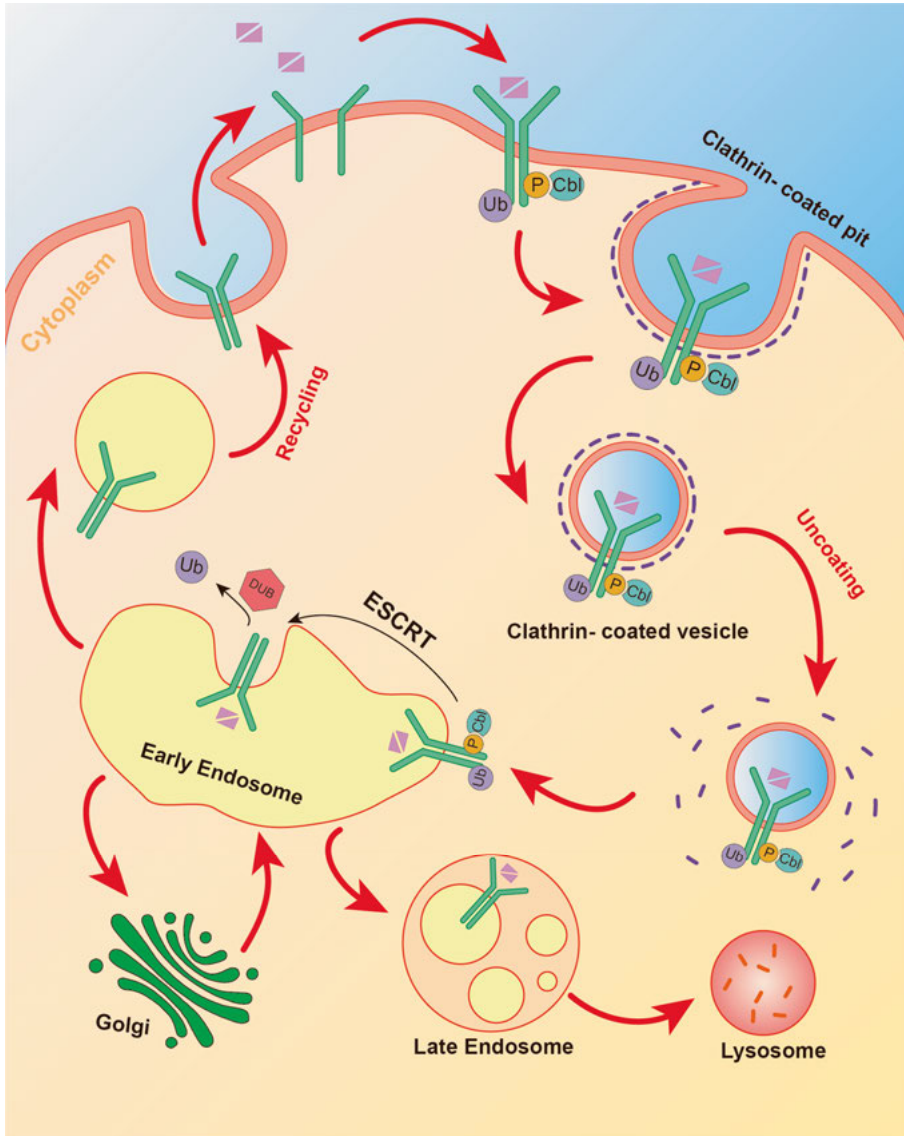
Figure 6. Clathrin-mediated endocytosis of RTKs. Upon ligand binding, RTKs are dimerized, phosphorylated, and ubiquitinated by Cbl E3 ligases that are recruited to the receptors via its SH2 domains. Clathrin-coated pits are formed around internalized receptor to mediate the internalization of RTKs. After uncoating, the vesicles with RTKs fuse to the early endosomes, from where most of them will be delivered to the late endosomes and then lysosomes for degradation. Some portion of RTKs can go back to the cell membrane through recycling endosomes and signal again.

Upon ligand binding, activation and autophosphorylation in their cytoplasmic domains, certain RTKs bind E3 ubiquitin ligases of the Cbl family, mediating their ubiquitination. There are at least two Cbl members which are involved in the ubiquitination of RTKs, i.e. c-Cbl and Cbl-b. Ubiquitination of RTKs helps them recruit ubiquitin-interacting motifs (UIMs)-containing proteins such as epsin, epidermal growth factor receptor substrate 15 (Eps15) and Eps15-related protein (Eps15R) (Chen & De Camilli, 2005; van Bergen en Henegouwen, 2009). UIMs of these proteins could also interact with clathrin directly by binding to the clathrin heavy chain (CHC) or indirectly through AP2 (Jastrzębski et al., 2017; Oldham et al., 2002). Thereafter, the clathrin triskelions recruited to the plasma membrane polymerize and form the lattice-like model, clathrin-coated pits (CCPs), which further bud off from the cell membrane and form clathrin-coated vesicles (CCVs) that contain RTKs and their ligands (McMahon & Boucrot, 2011). Dynamin mediates the fission and budding of the CCVs from the cell membrane (Schmid & Frolov, 2011).

Following the internalization of the CCVs, the clathrin lattice that surrounds the vesicles is disassembled, and the uncoated vesicles are then transferred to the early endosomes (EEs) for sorting (McMahon & Boucrot, 2011). The sorting of RTKs in endosomal compartments is highly dependent on the regulation of different Ras-associated binding (Rab) proteins, which are frequently used as markers to map the localization of proteins in the endosomal system (Xie et al., 2019). Rab5 mediates the trafficking of RTKs to early endosomes, where the fates of the RTKs are decided (Eichmann & Simons, 2012). From early endosomes, most of the RTKs are allocated to late endosomes (LEs) which further mature into multivesicular bodies (MVBs) under the control of Rab7 to be further translocated to the lysosomes for degradation (Guerra & Bucci, 2016). Another portion of RTKs at the early endosomes could be transported to the recycling endosomes (REs) through short-loop recycling or long-loop recycling mediated by Rab4 and Rab11, respectively (Ballmer-Hofer et al., 2011; Barford et al., 2017).

During the sorting of activated and ubiquitinated RTKs, the endosomal sorting complexes required for the transport (ESCRT) system play an important role in sorting them towards lysosomes (Tu et al., 2011). The ESCRT machinery mainly consists of five protein complexes: ESCRT-0, ESCRT-I, ESCRT-II, ESCRT-III, as well as the vacuolar protein sorting 4 (Vps4) complex (Umbaugh & Jaeschke, 2021). Ubiquitinated RTKs on the endosomal membrane are recognized by ESCRT-0 consisting of hepatocyte growth factor regulated tyrosine-kinase substrate (Hrs) and signal transducing adaptor molecule 1 and 2 (STAM1/2), which then recruit ESCRT-I, ESCRT-II, and ESCRT-III sequentially to the endosomal membrane to mediate the intra-lumenal vesicles (ILVs) budding for recycling (Henne et al., 2011). Vps4 drives the final step of this process and facilitates the dissociation and recycling of the ESCRT components from endosomal membranes (Legent et al., 2015).

The timing of RTK deubiquitination is also vital according to the ESCRT sorting model. The ubiquitination of RTKs remains until the formation of ILVs since it is important for them to be recognized by the ESCRT machinery, whereas to save ubiquitin, RTKs are deubiquitinated by DUBs before their incorporation into the ILVs. Deubiquitination is also needed for the recycling of RTKs so that they could engage in another cycle of signaling from the plasma membrane (Eden et al., 2012).

Different signals contribute to the internalization of PDGFRs. Ubiquitination serves as a critical mark to initiate internalization of RTKs. The E3 ubiquitin ligases c-Cbl and Cbl-b mediate the ubiquitination of PDGFRs and were found to be required for both PDGFRα (Miyake et al., 1998) and PDGFRβ (Miyake et al., 1999) internalization. Initially, internalization of PDGFR was thought to occur via CME, while in recent years, clathrin-independent endocytosis (CIE) has been also found to be engaged in it. Both routes generally target the receptors to early endosomes for degradation or recycling (Goh & Sorkin, 2013). CME is the most prominent route through which PDGFRs are internalized. It can be both dynamin-dependent and dynamin-independent (Sadowski et al., 2013). Upon PDGF stimulation, Grb2 serves as an adaptor protein for the interaction of PDGFRβ and dynamin (Kawada et al., 2009). It has been reported that PDGFRβ kinase activity is not necessary for its internalization, while the dimerization via a C-terminal hydrophobic motif 952–965 is essential to drive its endocytosis (Pahara et al., 2010).

Internalization and signaling

Upon ligand binding, RTKs undergo endocytic trafficking rapidly, which modulates their downstream signaling in different ways. First, signaling activated from the plasma membrane is attenuated following the internalization of RTKs because of the separation of receptors and their extracellular ligands and membrane substrates. Second, the sorting of RTKs to recycling or degradation positively or negatively, respectively, regulates the time of signaling. Last, RTK internalization also fine-tune the time and space of the downstream signaling. Many RTKs remain active after internalization, which allows them to initiate signaling continuously in other cellular compartments, such as endosomes (Irannejad et al., 2015; Sorkin & von Zastrow, 2009; Villaseñor et al., 2016).

Activated PDGFR in endosomes is able to recruit various adaptor proteins including Shc and Grb2, as well as signaling molecules including PLCγ1 and PI3K. Endosomal PDGFR signaling has been shown to be sufficient to activate both the Ras/ERK and the PI3K/AKT pathways which are linked to cell survival and cell proliferation (Wang et al., 2002, 2004). The endocytic internalization of PDGFRβ has been reported to contribute to the full activation of STAT3 signaling induced by PDGF and the expression of certain PDGF target genes (Jastrzębski et al., 2017).

In a study performed in NIH3T3 cells, it has been reported that different PDGF concentrations induce different endocytic routes, thus leading to different cellular responses. At low concentrations, PDGF induces CME which mostly triggers signaling pathways linked to cell migration, whereas high doses of PDGF (>5 ng/ml) induce the shift of CME partially to a type of CIM, i.e. raft/caveolin-mediated endocytosis (RME) which activate proliferation-related pathways (De Donatis et al., 2008). The activation of different signaling pathways in ligand concentration-dependent manner has also been reported before in NPCs, where PDGFRα is activated by the colony stimulating factor 1 (CSF1); PI3K is activated at low CSF1 concentrations (5 and 10 ng/ml), promoting proliferation, migration and differentiation, while PLCγ is activated at high CSF1 concentrations (20 ng/ml), promoting only proliferation (McKinnon et al., 2005). Although both dynamin-dependent and dynamin-independent routes mediate PDGFR uptake, it was found that dynamin activity is necessary for full activation of STAT3 and PDGF-induced mitogenesis (Sadowski et al., 2013).

PDGFR degradation

To maintain the homeostasis of cells, the regulated removal of cellular components including proteins and organelles is as important as their synthesis. Degradation of PDGFRs is also an important way to modulate PDGFR signaling pathways.

Proteolytic cleavage

Multiple RTKs have been frequently found to be proteolytically cleaved by various proteases via the hydrolysis of peptide bonds. Proteolytic cleavage of RTKs modulates their downstream signaling by affecting their structure, stability, subcellular localization and interaction with other proteins (reviewed in Huang, 2021).

There are several families of proteases which conduct the cleavage of RTKs. A disintegrin and metalloprotease (ADAM) family members and matrix metalloproteinases (MMPs) cleaves RTKs, such as fibroblast growth factor receptor 1 (FGFR1) and Met in the extracellular domain (ECD) which releases ECD fragments into extracellular space (Levi et al., 1996; Schelter et al., 2010). Caspases and calpains cleave RTKs in their intracellular parts, which may induce apoptosis, as has been reported for Met (Montagne et al., 2015; Tulasne et al., 2004). γ-secretase mediates cleavage of certain RTKs in the transmembrane region and releases their intracellular domains (ICDs), as shown for VEGFR1 (Cai et al., 2011).

The cleaved fragments have different fates in the cells. Similar with full length RTKs, truncated RTKs that are bound to the plasma membrane undergo

endocytosis and sorting for degradation. Free ECD fragments could either be degraded or function as ligand traps in the extracellular space (Barisione et al., 2015; Liu et al., 2006). Free ICD fragments could be released in the cytoplasm to be degraded in proteasome (Montagne et al., 2017), or they can be translocated to different organelles or nucleus to mediate functional roles. For example, ErbB4 is cleaved by γ-secretase, releasing an ICD fragment which is translocated into the nucleus and acts as a coregulatory factor with certain transcriptional regulators, such as the hypoxia-inducible factor-1α (HIF-1α) (Paatero et al., 2012). Cleavage of FGFR1 by the protease Granzyme B (GrB) releases a fragment which can traffic to the nucleus and mediate the migration of breast cancer cells (Chioni & Grose, 2012).

Several studies have been done on the proteolytic cleavage of PDGFRs. A previous study suggests that a Ca^{2+}-dependent cysteine protease cleaves PDGFRβ (Ek & Heldin, 1986). It has been reported that pre-treatment of elastase completely degrades PDGFRα and cleaves PDGFRβ into several fragments, which significantly impairs the activation of ERK1/2 induced by both PDGF-AA and PDGF-BB (Nemoto et al., 2005). Matriptase and hepsin were found to shed the ECD of both PDGFRα and PDGFRβ in a co-transfection system where prostasin cleaved only the ECD of PDGFRα (Chen & Chai, 2017).

Lysosomal degradation

The lysosome is an organelle originating from the Golgi apparatus surrounded by a single membrane, which was first described by Christian de Duve in 1955. It has an acid lumen containing around 60 types of hydrolytic enzymes, such as proteases, phosphatases, lipases, nucleases and sulfatases, which makes it play an important role in protein degradation (Zhang et al., 2021).

Lysosomal degradation of RTKs is the major way of terminating the signaling generated from the cell surface (Kholodenko et al., 2010). In Figure 5, the canonical route of RTK internalization from the plasma membrane to the endosomal-lysosomal system is shown. Ubiquitinated RTKs internalize and traffic through the endosomes. Either AMSH or USP8/UBPy mediates the removal of ubiquitin from RTKs to preserve ubiquitin levels in the cells before RTKs are translocated into the lumen of the MVBs (Urbé, 2005). Deubiquitinated RTKs will finally enter into the lysosomes to be completely degraded. In addition, upon activation by their ligands, RTKs activate the PI3K/AKT pathway, which inhibit the phosphorylation of the tuberous sclerosis complex 1/2 (TSC1/2) and further inhibit the activity of the GTP-binding protein Ras homolog enriched in brain (RHEB), thus promoting the activation of mTORC1 (Huang et al., 2008; Yang et al., 2017). Activated lysosomal mTORC1 signaling promote anabolic processes like synthesis of proteins, lipids and nucleic acids (Howell et al., 2013), while inhibit catabolic processes such as macroautophagy and lysosomal biogenesis (Asrani et al., 2019; Sung et al., 2015).

As with most RTKs, upon ligand binding PDGFRα and PDGFRβ are internalized and translocated into lysosomes for degradation (Pahara et al., 2010; Rogers & Fantauzzo, 2021). In 293T and porcine aortic endothelial (PAE) cell lines, PDGFRβ has been reported to be multi-monoubiquitinated, possibly at several sites, but not poly-ubiquitinated upon PDGF-BB stimulation which further leads the receptor to lysosomes for degradation (Haglund et al., 2003). However, it needs to be noted that other studies have shown the poly-Ub of PDGFRβ since then (Rorsman et al., 2016). The mutation of Tyr1021, which serves as the binding site for both Cbls and PLCγ, leads to blockage of the sorting of PDGFRβ to the lysosomes upon ligand binding. Depletion of Cbls has the same effect on PDGFRβ sorting and also enhanced the association of PDGFRβ with PLCγ, as well as PLCγ-mediated cell migration (Reddi et al., 2007).

Proteasomal degradation

UPS regulates almost all major cellular processes, such as signal transduction (Voutsadakis, 2012), protein quality control (de Vrij et al., 2004), DNA repair (Krogan et al., 2004), cell cycle (Fasanaro et al., 2010), cell proliferation (Benanti, 2012) and cell death (Bader & Steller, 2009).The proteasome is a highly organized, multi-catalytic ATP-dependent complex of proteases that processes the degradation of selected proteins. It is widely established that polyubiquitination is the modification that serves as the signal for targeting proteins to be proteolytically degraded in the proteasomes (Tanaka, 2009).

The ATP-dependent 26S proteasome complex is composed of two subcomplexes: a 20S catalytic core particle (CP) and one or two 19S regulatory particles (RP) which are also known as PA700, as it has a molecular mass of about 700 kDa (Tanaka, 2009). The 20S CP consists of 14 different subunits which can be divided into two groups: α1-α7 and β1-β7. These two types of subunits are arranged into four axially stacked heptameric rings, which form an α1-7, β1-7, β1-7, α1-7 barrel-shaped structure (DeMartino & Gillette, 2007). The outer α-rings act as the gate of the 20S CP, while the inner β-rings contain six active sites with three different types of proteolytic specificities (Dick et al., 1998; Groll et al., 2000). The 19S RP includes six hexameric AAA ATPases, RP triphosphatase protein 1-6 (Rpt1-Rpt6), and three non-ATPases, ribophorin1 (Rpn1), Rpn2 and Rpn13, forming the base of the 19S RP, while the other Rpns form the lid. The 19S RP, which serves as the gatekeeper, binds to one or both sides of the 20S cylinder, deubiquitinates and unfolds the proteins before they enter into the 20S cylinder for degradation (DeMartino & Gillette, 2007; Livneh et al., 2016; Smith et al., 2006).

In PAE cells stably transfected with PDGFRβ, it was reported that different proteasome inhibitors, including Cbz-Leu-Leu-norvalinal (MG115), Cbz-Ile-Glu(O-t-Bu)-Ala-leucinal (PSI), and substrate-related peptidyl aldehydes dramatically block the ligand induced degradation of WT PDGFRβ, but not the

truncated CT98 mutant PDGFRβ which lack the ability to be ubiquitinated (Mori et al., 1995). In NIH3T3 fibroblasts, upon stimulation with high PDGF concentrations (>5 ng/ml), PDGFRs undergo proteasomal degradation (De Donatis et al., 2008). Our previous study in AG01523 cells and human BJ hTERT fibroblasts, has also reported that stimulation with 20 ng/ml PDGF-BB induces both Lys48 and Lys63 poly-ubiquitination of PDGFRβ mediated with c-Cbl and Cbl-b, and the poly-ubiquitinated PDGFRβ is dominantly degraded in the proteasomes (Rorsman et al., 2016). Another research in cardiomyocytes has shown that 50 ng/ml PDGF-BB induced PDGFRβ downregulation is dependent on proteasome at early time points (within 60 minutes), but not late time points (from 4 hours to 24 hours) (Zhang et al., 2011).

Autophagy

Autophagy is another major pathway involved in the regulation of cellular degradation. The term "autophagy" originated from an ancient Greek word meaning "self-eating", was first termed in 1963 by Christian de Duve (De Duve & Wattiaux, 1966). It is an evolutionary conserved intracellular self-degradation process delivering unwanted cytoplasmic components into lysosomes to recycle useless materials or to terminate harmful materials.

There are three types of autophagy: chaperone-mediated autophagy (CMA), microautophagy, and macroautophagy. CMA mediates degradation of individual proteins across the membrane of the lysosomes with the mediation of the Hsp70 family of chaperones (Hsc70) (Chiang et al., 1989). Micro-autophagy is the direct invagination of the lysosomal membranes that engulf the cytoplasmic portions including cytosol (Chiang et al., 1996), mitochondria (Campbell & Thorsness, 1998), ER (Schuck et al., 2014) and certain cytosolic enzymes (Liu et al., 2015). Macroautophagy (hereafter termed autophagy), which we mainly focused on in the present study, relies on the formation of autophagosomes, the double-membrane vesicles, and their fusion with the lysosomes. In physiological conditions, autophagy is maintained at low level, whereas under cellular and environmental stresses, including hypoxia (Mazure & Pouysségur, 2010), oxidation (Yun et al., 2020), starvation (Shang et al., 2011), or growth factor withdrawal (Lum et al., 2005), it is highly induced.

The process of autophagy consists of a series of steps: initiation, nucleation, autophagosome maturation, fusion of autophagosome and lysosome, and degradation (Hansen et al., 2018) (Figure 7). First, different cellular stresses activate AMPK, which further activates the unc-51-like autophagy activating kinase 1 complex (ULK1 initiation complex). After that, PI3K III nucleation complex is formed, resulting in the recruitment of PI(3)P at the omegasome, where phagophore (PG) originates and further forms the double-membrane autophagosome, engulfing cytoplasmic substrates to be degraded. The au-

tophagic membranes are marked by the ubiquitin-like autophagy-related protein 8 (ATG8) family proteins through the formation of covalent bond with phosphatidylethanolamine (PE) on the membranes.

The ATG8 families consist of six orthologues that belongs to two subfamilies: microtubule-associated protein 1A/1B-light chain 3 (LC3), including LC3A, LC3B, and LC3C; Gamma-aminobutyric acid receptor-associated protein (GABARAP), including GABARAP, GABARAPL1, and GABARAPL2 (Schaaf et al., 2016). Among the six ATG8 family members, LC3B is the most studied protein and is widely used as a marker to measure autophagic flux with the benefit of the conversion of unlipidated LC3 I and lipidated LC3 II (Loos et al., 2014).
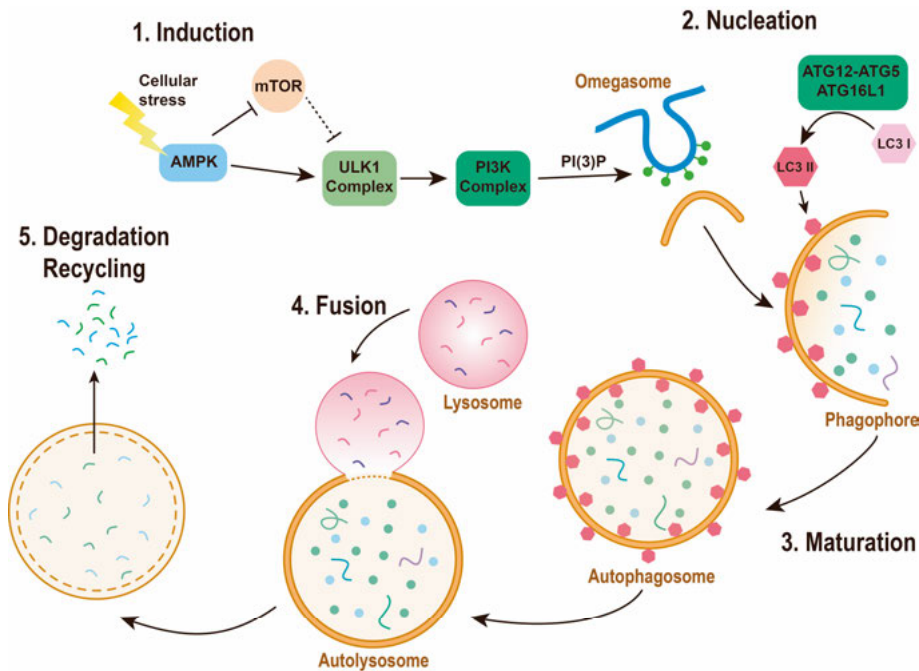

Figure 7. The autophagy program. Autophagy involves a series of steps: induction, nucleation, maturation, fusion and degradation. Autophagy is positively controlled by AMPK and negatively regulated by mTOR. Upon cellular stress, autophagy is induced, ULK1 initiation complex and the PI3K nucleation complex are formed, which recruits PI(3)P at the omegasome, a cup-shaped organelle where the phagophore originates. The expansion of phagophore and maturation of autophagosome is dependent on the attachment of ATG12 to ATG5, and then ATG16L1. The ATG12-ATG5-ATG16L1 complex serves as a ubiquitin E3-like enzyme that facilitates the lipidation of LC3 I to form LC3 II. The matured autophagosome is then fused with the lysosome and the components are degraded and recycled.

38

The conjugation of ATG8 with the PE on the membrane of autophagosomes is en enzymatic process similar to conjugation of ubiquitin to proteins. It is mediated with the E1-type enzyme ATG7, while ATG3 and the ATG12-ATG5-ATG16 complex serve as E2-type and E3-type enzymes (Runwal et al., 2019). ATG12 is conjugated to ATG5, while ATG7 and ATG10 act as E1 and E2 enzymes, respectively. ATG12-ATG5 is then associated with ATG16 and the ATG12-ATG5-ATG16 complex is recruited to the phagophore membrane (Walczak & Martens, 2013).

Autophagy was initially considered as a non-selective bulk degradation pathway while increasing evidence suggests that specific unwanted proteins or damaged organelles undergo selective autophagy, during which multiple autophagy cargo receptors are involved (Gatica et al., 2018). One of the classical and key selective autophagy receptors is p62/SQSTM1 (sequestosome 1), which interacts with ubiquitinated proteins and targets them to the autophagosomes for autophagic degradation, where it directly interacts with ATG8 proteins of the membranes (Pankiv et al., 2007). Bafilomycin A1 is a widely used autophagy inhibitor, which inhibits the autophagic flux in two independent ways. It could either inhibit the V-ATPase, which leads to the blockage of lysosomal acidification, or inhibit the ER-calcium ATPase Ca-P60A/dSERCA, thus blocking the fusion of autophagosome and lysosome (Mauvezin & Neufeld, 2015).

Autophagy is pivotal for the maintenance of metabolic substrates including amino acids, which are needed for crucial protein synthesis (Onodera & Ohsumi, 2005). Nutrient deprivation leads to the inhibition of mTORC1 signaling, which acts as a negative regulator of the autophagy pathway, thus activating autophagy and providing nutrients (Saxton & Sabatini, 2017). Autophagy has been also reported to suppress cancer in its early stages, while helping maintain the tumorigenesis in established cancers via its role in dealing with stresses, such as nutrient starvation, hypoxia or therapeutic stress (reviewed in Amaravadi et al., 2019).

It has been reported that different RTKs undergo autophagy. For example, following stimulation by HGF, its receptor Met interacts with LC3C and is targeted to autophagosomes for degradation, which downregulates its signaling and recycling (Bell et al., 2019). Another RTK, EGFR, is selectively regulated to the autophagasomes via Ack1 upon stimulation (Jones et al., 2014). PDGFRα has been reported to undergo selective autophagy during which the ubiquitination of its Lys971 is essential (Drinane et al., 2017). The same study also showed that PDGFRβ neither colocalize with LC3B nor p62 under serum starvation and autophagic flux blockage in liver hepatic stellate cells (LX2) cells.

Targeting PDGF/PDGFR signaling in cancer

PDGFs and PDGFRs play important roles in multiple cellular processes including cell survival, cell growth and cell migration. Dysregulation of PDGFRs causes aberrant PDGF signaling, which leads to various diseases. Abnormal regulation of PDGFRs could be the result of several different mechanisms: mutation, genomic amplification, abnormal expression and aberrant activation. Aberrant PDGFR signaling is often related to various diseases. Decreased level of PDGF-BB in plasma has been reported to correlate with mild impairments of cognitivity observed in patients with Alzheimer's disease (AD) (Björkqvist et al., 2012). Elevated PDGF-BB level was detected in patients with progressive muscular atrophy (PMA) and ALS (Furukawa et al., 2015). In renal arteriosclerosis, an upregulation of PDGFRα has been noticed in smooth muscle cells (Floege et al., 1998). Compared with non-diabetic patients, patients with Type I diabetes mellitus showed increased PDGF-BB release (Guillausseau et al., 1989).

Abnormal PDGF/PDGFR signaling has been noticed in various human cancers including glioma (Hermanson et al., 1992), gastrointestinal stromal tumors (Heinrich et al., 2003), prostate carcinoma (Sitaras et al., 1988), pancreatic cancer (McCarty et al., 2007), and atypical myeloid neoplasms (Toffalini et al., 2010). Their expression and activation levels in cancer cells are positively related to tumor growth, metastasis, homeostasis, drug resistance, and poor prognosis. By targeting malignant and non-malignant cells, including vascular cells and stromal fibroblasts, PDGFs modulate tumor development, invasiveness, and tumor microenvironment (Cao, 2013; Hanahan & Weinberg, 2011; Li et al., 2021). Therefore, PDGF/PDGFR signaling has become an important target for cancer therapy.

Gene defects of PDGFs or PDGFRs appears in patients who suffer from different cancer types. In melanoma, the incidence of defects can be as many as 30%. The defect could be mutations, deletions, or copy number aberrations (CNAs) (Farooqi & Siddik, 2015). Tumor-derived PDGF ligands act either in an autocrine manner or a paracrine manner. The autocrine loop is commonly observed in certain types of sarcomas (Smits et al., 1992), gliomas (Hermanson et al., 1992), and leukemia (Yang et al., 2010), whereas the paracrine activation of PDGF signaling is usually involved in epithelial cancers, where cancer cells recruit stromal cells, thus promoting angiogenesis, tumor growth, invasiveness and metastasis (Krenzlin et al., 2019; Li et al., 2021; Shao et al., 2000). A study in a genetic model of cervix cancer has shown that PDGF-dependent cancer-associated fibroblasts (CAFs) produced FGFs, which contribute to tumor angiogenesis significantly (Pietras et al., 2008). Intratumour genetic heterogeneity (ITH) contributes to multidrug resistance. A study using PDGF-D-deficient mice indicates that, by providing growth-stimulatory cues, PDGF-DD promotes functional tumor heterogeneity (Cortez et al., 2016).

Regarding the role of PDGF ligands and receptors as promoters of cancer, the general strategy is to inhibit their signaling to impair tumor growth. The use of different PDGF/PDGFR signaling antagonists in preclinical research and clinical treatments has been reviewed previously (Heldin et al., 2018; Zou et al., 2022). These antagonists target either PDGFs or PDGFRs. The ones targeting PDGFs can be monoclonal antibodies (Li et al., 2018), DNA adaptors (Falcon et al., 2011), and soluble receptor extracellular domains (Duan et al., 1991). The antagonists that work on PDGFRs include monoclonal antibodies (Lowery et al., 2018), RNA adaptors (Camorani et al., 2018), PDGFR-specific inhibitors (Yang et al., 2018), and selective tyrosine kinase inhibitors, such as imatinib (Casali et al., 2021). The specific antagonists, including monoclonal antibodies and RNA adaptors, have relatively modest side effects but are expensive. The less specific inhibitors usually cause unwanted side effects, and in some cases, the combination of more than one inhibitor is needed (Heldin et al., 2018).

While antagonists of PDGF or PDGFR is an important strategy for cancer therapy, it is in most cases not enough on its own. Studying the modulation of PDGFR signaling in cancer is also necessary which may develop more targeted and effective therapies. As we mentioned before, cancer is a complex and heterogeneous disease. The same treatment to different patients may lead to distinct responses. Better understanding of mechanisms of PDGFR signaling in cancer could bring more personalized therapies for individual patients. Apart from this, in cancer cells, PDGFR signaling could interact with other signaling pathways, therefore, combination treatments targeting multiple signaling pathways may increase treatment efficiency.

Present investigation

As discussed above, the interconnection of various proteins involved in complex cell signaling networks together modulates the cancer cell itself or its microenvironment, thus impairing apoptotic pathways of cancer cells or promoting survival pathways. RTKs are important components of this network. Among different RTK family members, we focus on PDGFR, which is known to promote tumor progression. Insights from the research about PDGF/PDGFR signaling have enlightened new opportunities to understand the molecular mechanisms of cancer and other diseases, and have provided strategies for cancer therapy.

The goal of this thesis is to further investigate the mechanisms of the modulation of PDGF/PDGFR signaling to identify new ways of controlling aberrant signaling of these RTKs in various diseases, including cancer. Therefore, we explored the mechanisms of PDGF/PDGFR signaling regulation from different aspects to answer the following questions:

I. PDGFRβ is ubiquitinated upon ligand stimulation. Which deubiquitinases catalyze the removal of ubiquitins from PDGFRβ? How do deubiquitinases affect PDGFRβ function and its downstream signaling?

II. Can PDGFRs be modified by SUMO? If so, how does SUMOylation regulate their stability and trafficking as well as the downstream signaling and cellular responses?

III. Is PDGFRβ cleaved upon ligand stimulation? If so, what are the mechanisms?

IV. Is autophagy involved in the degradation of PDGFRα and PDGFRβ?

Paper I

Deubiquitinating enzymes USP4 and USP17 finetune the trafficking of PDGFRβ and affect PDGF-BB-induced STAT3 signalling

Ubiquitination is an important post-translational modification related to protein degradation, receptor internalization, intracellular trafficking, cell proliferation, and other cellular processes (Popovic et al., 2014). Upon ligand binding and activation, ubiquitination of RTKs controls their endocytic trafficking

and their interaction with sorting machinery at both the cell surface and endosomes (Goh & Sorkin, 2013). Ubiquitination can be reversed by DUBs, and the overexpression of DUBs is involved in various diseases including cancers (McCann et al., 2018). Several RTKs have been shown to be substrates of DUBs, while the DUBs that act on PDGFRβ has remained unknown.

In order to identify DUBs working on PDGFRβ, we screened a cDNA library of 64 Flag-HA-DUBs. From the screening results, we noticed that the DUBs that most efficiently deubiquitinated PDGFRβ were USP17, which removed ubiquitin from PDGFRβ almost completely, and USP4, which partially deubiquitinated PDGFRβ. Both USP17 and USP 4 were able to remove both K48- and K63-linked ubiquitin chains on PDGFRβ. The USP17 plasmid that we used was later found to be a truncated version, which was most similar to the USP17L22 isoform. We then tested the full-length flag-tagged USP17L22, L11, L20 and L2 isoforms, and found that they all deubiquitinated PDGFRβ. USP17L22 (hereafter referred to as USP17) was selected for further experiments. To better study and understand the role of USP17 and USP4 on PDGFRβ, we established USP17 and USP4 tet-inducible BJhTERT cell lines and U2OS cell lines.

First, we investigated the effect of the two DUBs on the stability of PDGFRβ by treating the cells with cycloheximide to inhibit the synthesis of new proteins. No effect was found on PDGF-BB-induced PDGFRβ degradation, neither in transient expression nor in tet-inducible cell lines. Deletion of USP4 in BJhTERT fibroblasts using the CRISPR-Cas9 technique also did not affect the stability of PDGFRβ upon PDGF-BB stimulation. We then explored the possibility that they regulate the timing of PDGFRβ subcellular trafficking. The induction of USP17 using the tet-one system in both U2OS cells and BJhTERT cells impaired the internalization of PDGFRβ from the cell surface, while either induction or depletion of USP4 did not have any significant effect. However, upon USP4-CRISPR-Cas9 knockout of Usp4 in BJhTERT and U2OS cells, we noticed faster internalization of PDGFRβ and co-localization with EEA1, an early endosomal marker, but not with Rab7, a late endosomal marker. We further investigated the impact of USP17 and USP4 on downstream signaling. STAT3 was found to be the main affected pathway by overexpression of either USP17 or USP4, while there were no consistent changes in the activation of PLCγ, Akt1/2/3 or Erk1/2. This correlated with the impaired activation of STAT3 upon PDGF-BB stimulation in USP4-CRISPR-Cas9 knockout BJhTERT fibroblasts. No ubiquitination of STAT3, nor any effect of USP17 and USP4 on total STAT3 levels, were detected, suggesting that these two DUBs do not regulate STAT3 directly, but rather affect its activation via modulation of the subcellular trafficking of PDGFRβ. Based on these results, we suggested that USP17 and USP4 affected the timing of activation of STAT3 via different mechanisms. USP17 retained PDGFRβ at the cell surface for a longer time, while USP4 modulated the timing of PDGFRβ delivery to early endosomes.

We then investigated whether the effect on STAT3 activation further led to changes in STAT3 transcriptional activity and found that the induction of USP17 and USP4 both increased the affinity of activated STAT3 binding to its consensus binding element. The short-term expression of STAT3 target genes including *CSF1, myc, junB*, and *SOCS3* was increased and prolonged when USP17 was induced. The long-term expression of *myc* and *CDKN1a*, which are known to positively and negatively regulate cell proliferation, were found to be elevated when DUBs were induced. Finally, in order to verify whether the modulation of the gene expression leads to any functional consequence, we conducted proliferation, contraction and migration assay upon induction or depletion of DUBs in the cells. No significant effects were noticed on ligand-induced cell contraction or cell migration, while there were some effects on cell proliferation. Although the induction of DUBs did not alter the proliferative response to PDGF-BB, the deletion of USP4 decreased PDGF-BB induced proliferation significantly, while deletion of USP17 was lethal for the cells.

In conclusion, we identified two main DUBs working on PDGFRβ, USP17 and USP4. They affected the timing of STAT3 activation and trafficking via different mechanisms, thus fine-tuning its transcriptional activity, which further regulated the proliferative response induced by PDGF-BB.

Paper II

SUMOylation of PDGF receptor α affects signaling via PLCγ and STAT3 and cell proliferation

SUMOylation is another post-translational modification that is important for the regulation of protein subcellular localization, protein stability, protein-DNA interactions, protein-protein interactions, genome organization, DNA repair and transcriptional regulation (Hickey et al., 2012). Aberrant SUMOylation has been observed in certain diseases, including cancers (Seeler & Dejean, 2017). In order to determine whether PDGFRs can be SUMOylated, we validated the finding of SUMOylation sites on PDGFRs detected by mass spectrometry technology on PhosphoSite, a web-based resource dedicated to mammalian PTMs (https://www.phosphosite.org/). We found that Lys917 in PDGFRα can be SUMOylated (Lumpkin et al., 2017), while no SUMOylation was found of PDGFRβ.

In this paper, we have focused on the SUMOylation of PDGFRα. First, we validated the SUMOylation of PDGFRα by immunoprecipitation. In the co-transfection system using COS-7 cells, we detected the SUMOylation of PDGFRα under starvation conditions, peaking at 45 minutes of 20 ng/ml

PDGF-AA stimulation, which was later than phosphorylation and ubiquitination of PDGFRα. SUMOylation of PDGFRα was also observed in PAE cells stably transfected with PDGFRα, as well as retinal peripheral epithelial 1 (RPE1) cells which express endogenous PDGFRα. Stimulation with either PDGF-AA or PDGF-BB induced SUMOylation of PDGFRα. To test the possibility that the SUMOylation we observed was caused by some other proteins, interacting with PDGFRα during immunoprecipitation, we boiled the lysates before immunoprecipitation and determined the SUMOylation of PDGFRs under denaturing conditions; the results confirmed that SUMO1 was directly added to PDGFRα, but not to PDGFRβ. Surprisingly, upon PDGF stimulation, the SUMOylation of PDGFRα decreased, which was opposite to analysis under non-denaturing conditions. Thus, it is possible that SUMOylation of some proteins bound to PDGFRα was induced in response to PDGF. When introducing an E1 activating enzyme inhibitor, ginkgolic acid (GA), we observed a decrease in SUMOylation of PDGFRα, which was expected since the activity of E1 enzymes is vital for protein SUMOylation. To investigate the function of PDGFRα SUMOylation, we mutated the only known SUMOylation site in PDGFRα, Lys917, to an arginine residue, to obtain the PDGFRα mutant K917R. The mutation of K917 decreased the SUMOylation of PDGFRα. We then established the WT and K917R mutant PDGFRα tet-inducible PAE cell lines for further experiments. Since SUMOylation has been reported to influence the stability of its substrates, we determined the effects of the K917R mutation on the stability of PDGFR, but found that neither steady state level nor degradation induced by PDGF-AA, were affected. The lysosomal inhibitor chloroquine (CQ) inhibited the degradation of both WT and K917R mutant PDGFRα to the same extent; the proteasomal inhibitor bortezomib (BTZ) also inhibited the degradation of both WT and K917R mutant PDGFRα, but to a lesser extent. Ubiquitination and SUMOylation of proteins are often connected to each other, therefore we analyzed ubiquitination of PDGFRα and observed a decrease of the ubiquitination of the K917R mutant PDGFRα in response to PDGF-AA stimulation, compared to the WT PDGFRα. Since ubiquitination is a key signal for PDGFR internalization, we then conducted a cell surface biotinylation assay to determine the internalization of WT and K917R mutant PDGFRα; we did not notice any differences between WT and mutant receptor. Mutation of K917 did not affect the localization of PDGFRα to different organelles, including Golgi apparatus and endosomes. When determining the downstream signaling induced by PDGFRα, we noticed a delay in the activation of PLCγ and an increase of activation of STAT3 in cells expressing the mutant receptor. Finally, we proceeded to investigate the effect of K917 mutation on cell migration and cell proliferation. The proliferation assay revealed that the proliferative response to ligand stimulation of cells expressing K917R mutant PDGFRα was diminished compared to cells expressing the WT receptor, especially the proliferative response to PDGF-BB.

In summary, in paper II, we have identified PDGFRα as a SUMOylation substrate and performed a characterization of the functional role of SUMOylation in PDGFRα signaling and cell proliferation.

Paper III

PDGF-induced internalization promotes proteolytic processing of PDGFRβ which can be inhibited by bortezomib

The degradation of PDGFRs occurs in both lysosomes and proteasomes. Proteolytic cleavage of RTKs regulates their downstream signaling pathways by affecting their structure, stability, subcellular localization and interaction with other proteins (Huang, 2021). In this study, we focused on the ligand-induced proteolytic cleavage of PDGFRβ in primary human fibroblasts AG01523 and immortalized human fibroblasts BJhTERT.

First, by using two antibodies that recognize either the ECD or ICD, respectively, of PDGFRβ, we observed that upon ligand induction, an ECD fragment of 130 kDa and an ICD fragment of 70 kDa occurred in conjunction with the decrease of the full-length receptor. Treatment of cells with cycloheximide to block new protein synthesis did not abolish the suspected cleavage, which excluded the possibility that 130 kDa band was a newly synthesized receptor. Many proteases are Ca^{2+}-dependent, so to investigate whether the cleavage is Ca^{2+}-dependent, and to check the region from where PDGFRβ was cleaved, we used EDTA to chelate Ca^{2+} outside the cells or BAPTA AM to chelate Ca^{2+} both inside and outside the cells. BAPTA AM, but not EDTA, prevented the formation of the cleaved fragment which suggests that the cleavage occurred in the intracellular part of the receptor by a Ca^{2+}-dependent protease. Furthermore, by immunoblotting with site specific phosphoantibodies recognizing PDGFRβ phosphorylated Y579/581 and Y857, respectively, we narrowed down the cleaved region to between Y579 and Y857. Our previous study showed that PDGFRβ was degraded via the proteasome, so to investigate the role of proteasomes in PDGFRβ cleavage, we inhibited the proteasome with bortezomib or MG132, and found that they both blocked the formation of the cleaved fragment. Our previous results showed that PDGFRβ is internalized after 5 minutes of PDGF-BB stimulation, which is earlier than the appearance of the cleaved fragment at 30 minutes. Therefore, we investigated the relationship between PDGFRβ internalization and cleavage by blocking the internalization with either low temperature or dynamin inhibitors. Interestingly, the blockage of internalization dramatically prevented the cleavage of PDGFRβ. Since the proteasomal inhibitor bortezomib and the inhibition of internalization both blocked the cleavage, we then wondered whether bortezomib could

inhibit the internalization of PDGFRβ. By performing the cell surface biotinylation assay, we found that the proteasomal inhibitor bortezomib restrained PDGFRβ at the cell membrane upon ligand stimulation, while the lysosomal inhibitor chloroquine did not have the same effect. We also determined the effect of bortezomib on the ligand-induced activation of PDGFRβ and its downstream signaling. We found that bortezomib enhanced the phosphorylation of PDGFRβ, as well as PLCγ and STAT3, whereas phosphorylation of AKT was not affected, and ERK1/2 phosphorylation was reduced. Finally, we tried to determine which protease is responsible for this cleavage, by treating the cells with different protease inhibitors, targeting calpain, cathepsin, γ-secretase and β-secretase; none of these inhibitors showed any effect on the cleavage of PDGFRβ.

To conclude, in paper III, we have identified that PDGFRβ is cleaved in the region Y579-Y857 upon ligand stimulation by a Ca^{2+}-dependent protease, which is dependent on its internalization. The proteasomal inhibitor bortezomib blocked the internalization, as well as the cleavage of PDGFRβ, and also affected its downstream signaling.

Paper IV

Involvement of autophagy pathways in the degradation of PDGFRβ in normal and cancer cells

Apart from the classic degradation in lysosomes and proteasomes, several RTKs have also been found to undergo autophagy and to be targeted in autophagosomes which further fuse with lysosomes (Bell et al., 2019; Jones et al., 2014). In LX2 cells, it has been reported that PDGFRα, but not PDGFRβ, undergo selective autophagy (Drinane et al., 2017). In this study, we investigated the possibility of PDGFRβ undergoes autophagy in primary fibroblasts and osteosarcoma cancer cells.

Bafilomycin A1 is a specific V-ATPase pump inhibitor, which inhibits the fusion of autophagosomes and lysosomes. Therefore, it is frequently used by researchers in order to inhibit autophagy (van Weert et al., 1995). In our study, we first determined the level of PDGFRβ after PDGF-BB stimulation upon treatment with bafilomycin A1 under different starvation conditions, including serum starvation, glucose starvation, and nutrient starvation. The results using BJhTERT fibroblasts, AG01523 fibroblasts and human osteosarcoma U2OS cells, indicated that treatment with bafilomycin A1 prevented PDGF-BB-induced PDGFRβ degradation regardless of starvation conditions. By conducting a co-immunoprecipitation assay, we identified an interaction between PDGFRβ and the autophagy receptor p62, but not with the autophagosomal marker protein LC3B. In order to investigate whether bafilomycin A1

affected the accumulation of mature or newly synthesized PDGFRβ, we inhibited the protein synthesis with cycloheximide or actinomycin D. We observed that treatment with either of the inhibitors relieved the accumulation of PDGFRβ caused by bafilomycin and that accumulation of the receptor was located primarily over the Golgi apparatus. To investigate the possibility that the accumulation of PDGFRβ upon bafilomycin A1 was due to its effect on the Golgi apparatus instead of inhibition of autophagy, we knocked-down Atg7, which is an E1-like enzyme that is necessary for conjugation of LC3-like proteins to the membranes. Depletion of Atg7 led to accumulation of PDGFRβ to some extent in AG01523 fibroblasts, stimulated or not with PDGF, but not in U2OS cells, suggesting different involvement of autophagy in PDGFRβ degradation in different cell lines. We then analyzed the possibility of co-localization of PDGFRβ with LC3B and p62 under different starvation conditions, with or without PDGF-BB stimulation, by immunostaining. We could not observe any triple co-localization. Since LC3B is a member of the Atg8 family which includes six proteins, we further explored the possibility that degradation of PDGFRβ could be mediated by some other Atg8 family members, by performing co-immunoprecipitation assays with overexpressed proteins; we observed some weak interactions between PDGFRβ and LC3A, GABARAPL1 and GABARAPL2 proteins. The interaction between PDGFRβ and GABARAPs (using the antibody that recognizes all three GABARAP proteins) was further confirmed by co-immunoprecipitation in bafilomycin-treated AG01523 fibroblasts.

In summary, in paper IV, we identified that in certain types of cells, PDGFRβ may be involved in the autophagy pathway, which may affect the synthesis of new PDGFRβ.

Future perspectives

Do USP17 and USP4 protect newly synthesized PDGFRβ during maturation at ER?

In paper I, we identified USP17 and USP4 as DUBs for PDGFRβ that act during ligand-mediated internalization of PDGFR at the early endosomes and plasma membrane, correspondingly. Since many DUBs have been reported to act at multiple subcellular locations and since both USP17 and USP4 have been reported to localize to the ER, it would be interesting to investigate if USP17 or USP4 function at the ER and are able to prevent the degradation of newly synthesized PDGFRβ during maturation. To investigate this possibility, it would be useful to accumulate the receptors at the ER. Brefeldin A could be used to inhibit the trafficking of newly synthesized PDGFRβ to the Golgi apparatus.

Do USP17 and USP4 have functions in autophagy of PDGFRβ?

In paper IV, we obtained indications for involvement of the autophagy pathway in the degradation of PDGFRβ. As reported before, USP4 interact with an E3 ligase, the tripartite motif-containing protein (TRIM21) (Di Donato et al., 2001), and TRIM21 was reported to interact with key components of autophagosome assembly and initiate autophagy (Kimura et al., 2017). We have identified TRIM21 as an E3 ligase, acting on PDGFRβ (Sarri et al., 2022). Considering the interaction of USP4 and TRIM21 with each other, it would be worth to investigate if USP4 or USP17 have functions in the autophagy of PDGFRβ.

Are there other sites in PDGFRα that are SUMOylated?

In paper II, we determined the SUMOylation of PDGFRα at Lys917. However, after the mutation of this residue, there was still some residual SUMOylation of PDGFRα detected. Therefore, we assume that apart from its main SUMOylation site, K917, there are still other sites that may be SUMOylated. Many SUMOylation sites follow a consensus motif ψ–K–X–E/D (ψ is a hydrophobic amino acid residue, K is the target Lys, X is any amino acid residue and D/E is aspartic acid or glutamic acid) (Wilkinson & Henley, 2010). Based on this, we searched for potential SUMOylation sites in PDGFRα using an online tool, GPS-SUMO 2.0 Online Service (http://sumosp.biocuckoo.org/online.php), and found seven predicted possible SUMOylation

sites in PDGFRα: K385, K492, K702, K917, K971, K994, and K1061, i.e. six other candidates in addition to K917. By mutating these sites separately or together, we should be able to determine if there are additional SUMOylation sites and investigate the functional consequences of complete loss of SUMOylation of PDGFR.

Are there any other PTMs modifying PDGFRs?

So far, the most studied PTMs of PDGFRs are phosphorylation and ubiquitination. Our study validated the SUMOylation of PDGFRα, which affected its signaling and the cellular proliferative response. It would also be interesting to look for other PTMs of PDGFRs that have not been validated. On the phosphosite website (https://www.phosphosite.org), we searched for PTMs for both PDGFRα and PDGFRβ. In addition to phosphorylation, ubiquitination and N-glycosylation, which have been studied, we found one putative mono-methylation site in PDGFRα. This finding is based on the results from mass spectrometry and need to be investigated. It would also be of interest to perform an un-biased mass spectrometry analysis to identify any other unknown PTMs of PDGFRs.

Which protease mediates the cleavage of PDGFRβ? What is the exact cleavage site of PDGFRβ? Do the fragments have any functional properties?

In paper III, we observed a cleavage of PDGFRβ induced by ligand binding mediated by a Ca^{2+}-dependent protease, and located the cleavage region to between Y579 and Y857. Next, we would like to know the identity of the protease that mediates the cleavage and the exact site where the cleavage occurs. According to the appproximate sizes of cleaved bands 130 kDa and 70 kDa, it is likely that the cleaved size is in the juxtamembrane domain just downstream of Y579. Cleavage of RTKs in their intracellular domain has been reported to involve caspases and calpains (Chen & Chai, 2017). Caspases cleave proteins at specific aspartic acid residues. There are several aspartic acid residues in the juxtamembrane domain next to Y579, i.e. D583, D590, and D598, which could be the possible cleavage sites. However, caspases are known to cleave non-activated RTKs, but after ligand stimulation. In our case, we observed the cleaved fragments after stimulation with PDGF-BB. Therefore, it is more likely that calpain protease is involved in the cleavage. Calpains do not recognize a specific cleavage motif, but they prefer a large hydrophobic residue at the P1 position, and another hydrophobic residue at the P2 position. When we searched for two or more consecutive hydrophobic residues after Y579 in PDGFRβ, we found two possible motifs: I580-Y581-V582 and L601-V602-L603 that could be potential cleavage sites. Thus, we could mutate potential cleavage site and test whether cleavage is prevented in the mutant receptors.

To investigate which protease is involved in the cleavage of PDGFRβ, we could try two different methods. First, we could inhibit individual proteases

with more specific inhibitors and check if the cleavage is abolished. Second, we could use proximity dependent biotinylation (BioID) to perform affinity purification or proteins that interact with PDGFR in order to detect and identify the protease that interacts with PDGFRβ.

An important question is if the ICD fragment has any role in signaling. It would be of interest to investigate if the ICD is translocated to the nucleus or to other cell organelles. Moreover, it would be interesting to compare the signaling properties of the WT receptor with a receptor mutant that cannot be cleaved.

Is misfolded PDGFRβ targeted to autophagy?

In paper IV, we observed the involvement of the autophagy pathway in the degradation of PDGFRβ. Autophagy is known to degrade misfolded and aggregated proteins. Therefore, we would like to know whether misfolded and aggregated PDGFRβ is targeted to the autophagy pathway. To investigate this possibility, we will treat the cells with proteasome inhibitors, such as bortezomib and MG132, or the antibiotic tunicamycin, which will cause the accumulation of misfolded proteins for long periods of time, thus initiating ER stress. We can then determine whether this provokes targeting of PDGFRβ via the autophagic degradation route. We also plan to validate the interaction of PDGFRβ with GABARAP proteins and investigate functional consequences of this interaction.

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