MINI-REVIEW

Tumor-Suppression Mechanisms of Protein Tyrosine Phosphatase O and Clinical Applications

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Abstract

Tyrosine phosphorylation plays an important role in regulating human physiological and pathological processes. Functional stabilization of tyrosine phosphorylation largely contributes to the balanced, coordinated regulation of protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs). Research has revealed PTPs play an important suppressive role in carcinogenesis and progression by reversing oncoprotein functions. Receptor-type protein tyrosine phosphatase O (PTPRO) as one member of the PTPs family has also been identified to have some roles in tumor development. Some reports have shown PTPRO over-expression in tumors can not only inhibit the frequency of tumor cell division and induce tumor cell death, but also suppress migration. However, the tumor-suppression mechanisms are very complex and understanding is incomplete, which in some degree blocks the further development of PTPRO. Hence, in order to resolve this problem, we here have summarized research findings to draw meaningful conclusions. We found tumor-suppression mechanisms of PTPRO to be diverse, such as controlling G0/G1 of the tumor cell proliferation cycle, inhibiting substrate phosphorylation, down-regulating transcription activators and other activities. In clinical anticancer efforts, expression level of PTPRO in tumors can not only serve as a biomarker to monitor the prognosis of patients, but act as an epigenetic biomarker for noninvasive diagnosis. In addition, the re-activation of PTPRO in tumor tissues, not only can induce tumor volume reduction, but also enhance the susceptibility to chemotherapy drugs. So, we can propose that these research findings of PTPRO will not only support new study ideas and directions for other tumor-suppressors, importantly, but also supply a theoretical basis for researching new molecular targeting agents in the future.

Keywords: PTPRO - tumor suppressor - mechanism prognostic factor - anticancer therapy

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Introduction

Tyrosine phosphorylation plays an important regulation role in sustaining the human physiological activities, such as keeping the cell shape and motility, deciding the cell proliferation, regulating gene transcription, transporting molecules in or out cell and so on (Andres et al., 2004). Therefore, by coordination these physiological processes, tyrosine phosphorylation could sustain internal environment homeostasis of the human. So, if life activities are controlled by abnormal tyrosine phosphorylation, diverse diseases will be induced, for example immune deficiencies and cancers (Andres et al., 2004). Generally, the functional stabilization of tyrosine phosphorylation mainly owes to the balanced, coordinated regulation of protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs) (Andres et al., 2004; Izabela L et al., 2011). To our knowledge, although most researches mainly focused on PTKs, recently, more and more experiments have revealed PTPs may play much more important roles in many physiological and pathological processes regulated by tyrosine phosphorylation (Andres et al., 2004), especially regulating carcinogenesis and progression by reversing oncoprotein functions (Izabela et al., 2011). In human, although, about 107 PTP genes have been published, only 81 PTPs are active protein phosphatases with the ability to dephosphorylate phosphotyrosine (Andres et al., 2004). Because of difference in architectures and functions of catalytic domains, these PTPs are divided into four separate families, of the largest family is the class I which is also classified into transmembrane, receptor-like enzymes (RPTPs) and intracellular, non-receptor PTPs (NRPTPs) relying on their catalytic domain architectures (Andres et al., 2004; Andersen et al., 2004). Therefore, all RPTPs share similar architecture, which was comprised by an N-terminal extracellular domain, a single transmembrane domain and one or two highly conserved intracellular

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catalytic domains (Johnson et al., 2003; Andres et al., 2004; Wei et al., 2013). PTPRO which belongs to a member of R3 subtype RPTP families (Andersen et al., 2001; Yoji et al., 2010) is also called glomerular epithelial protein 1 (GLEPP1), because of first identified in renal glomerular epithelial cell (Thomas et al., 1994; Yang et al., 1996; Yoji et al., 2010). In renal glomerular epithelial cell, PTPRO is essential for sustaining the structure and function of the foot processes though regulating tyrosine phosphorylation of podocyte proteins (Yoji et al., 2010). In addition, some animal trials also showed PTPRO can regulate development of nervous system. In zebrafish cerebellar development, PTPRO plays a crucial role in modulating Fgf signaling by dephosphorylating Fgfr1a (Wei et al., 2013). Besides, PTPRO also can regulate the axon outgrowth and guidance in embryonic chick lumbar spinal cord and retinotectal projection system (Laurie et al., 2005).

In the tumor, most researches have shown the PTPRO can play important suppression roles. For example, in the hepatocellular carcinoma, Hou (Jia et al., 2013) found the PTPRO can inhibit the frequency of cell division and induce greater tumor cells death. Meanwhile, the GFP-expressing Py8119 mouse breast cancer cells were separately implanted into female wide-type mice and PTPRO-/- C57B1/6 mice. After 4 weeks of implantation, the result showed the tumor volumes and metastasis formed of PTPRO-/- mice were both significant high compared with the mice over-expression PTPRO (Zhao et al., 2015). However, tumor-suppression mechanisms of PTPRO are so complicated and dispersive that we can't understand it well, which in some degree block the further development of PTPRO. Hence, in order to resolve this problem, we summarized lots of researches and put these results concluded together. In this review, we will detailedly describe these suppression mechanisms and briefly discuss some important applications of PTPRO in clinical anticancer. On the one hand, it is good for us to further research PTPRO, on the other hand, it also supply theoretical basis and study directions for researching other tumor-suppressors and searching for a new anticancer target.

GST pull-down assay

Although, PTPRO as a receptor-type PTPs has the same ability with specially binding some substrate molecules like some intracellular enzymes, signal pathways and other some small molecules as other inactive receptors, apparently different with inactive receptors, it has the catalytic function of dephosphorylating substrate molecules. Therefore, normally, PTPRO indirectly play suppression function other than directly through inactivating substrate molecules. In order to find out the tumor-suppression mechanisms, we should discover these substrates combining with PTPRO. So, some experts take advantage of the GST pull-down assay to search for substrates of PTPRO. Firstly, the catalytic domain of PTPRO-wild type (WT) was used a template to generate 2 substrate-trapping mutants (PTPRO-CS and PTPRO-DA) which have the ability to bind substrates but lack catalytic activity. Subsequently, by a series of experimental processes, the produced GST-fusion proteins with the ability to encode PTPRO-WT, CS and DA are separately eluted with vanadate-containing buffer which can connect with catalytic cysteinyl residue of PTPs to form a covalent bond. Thereafter, in this way, the substrates combining with the two PTPRO mutants are easily eluted but not the WT. Finally, analyzing these substrates by immunoblotting (Chen et al., 2006; Takafumi et al., 2006; Hsu et al., 2013; Jia et al., 2013).

Tumor-suppression mechanisms of the PTPRO

Arresting G0/G1 of the tumor cell proliferation cycle

The proliferation is a vital characteristic in the cell life activity, which is regulated strictly and precisely. The carcinogenesis and development result from diverse in or out cell oncogenic factors inducing cell disordered proliferation in the level of genes, which will result in the tumor-cell unlimited growth, called immortality. Normally, the cell proliferation cycle is divided four phases including G1 phase, S phase, G2 phase and M phase. G1 phase also called the prophase of DNA synthesis which mainly prepares enough proteins, enzymes and different kinds of composition-factors for DNA synthesis opens the door of the cells limited proliferation. Naturally, normal cells go to three directions after the G1 phase: continuing mature differentiation, restricting proliferation and stopping proliferation, the latter is also called G0 phase or quiescent phase. When a normal cell turns to be the tumor-cell induced by some oncogenic factors, although, few cells in G1 phase will still enter the G0 phase, most of cells will enter the proliferation cycles to produce un-restrictedly tumor-cells. Normally, the tumor-cells in G0 phase may not enter the proliferation cycle under the circumstance of without any stimulating factors, which to a certain degree can stop the tumor-cells going to proliferation. However, the fact is some dead tumor-cells resulting from the process of tumor therapy will stimulate the tumor-cells in G0 phase reenter the proliferation cycle, which will result in recurrence and migration of the tumor and make the tumor difficult to control and treat. So in order to improve the tumor therapy effects as well as enhance disease free survival (DFS) and overall survival (OS) of patients, we could inhibit the tumor growth, recurrence and migration through arresting G0/G1.

Recently, someone have identified the PTPRO could play the suppression function in the tumor by arresting the G0/G1. Ricardo (Ricardo et al., 1999) used immunoblotting to analyze the cell lysates from DHL-4 cells transfected with vector only, pcDNA3-PTPROt sense and pcDNA-PTPROt antisense in B-lymphoid cells, the result showed the PTPROt protein which has the function of tyrosine dephosphorylation was only expressed in PTPROt sense cDNAs and the level of PTPROt expression was most abundant in quiescent naive B cells compared with germinal center B cells and memory B cells. As we all known, although the germinal center B cells and memory B cells both have the potential ability to proliferate, the quiescent naive B cell doesn't have. Besides, in order to

further demonstrate the catalytic function of PTPROt in arresting G0/G1, they planed the PTPROt sense, antisense and vector-only DHL-4 transfectants into 2% or 10% serum including a nocodazole which synchronizes the cells in G2-M in order to enhance the sensitivity of the assay. Because nocodazole-treated cells arrest in G2-M and do not exit mitosis, the cells in G0/G1 phase are more obvious. Finally, the result showed the group encoding PTPROt was over 28% of the cells remained in G0/G1, but the control group was only 6% to 12%.

From above identifications, we can implicate the PTPRO could block the cell in G0 phase into the G1 phase, in other words, it can stop tumor-cells in quiescent or G0 phase to reenter the cell proliferation cycle. So PTPRO could not only suppress the tumor-cell growth, recurrence and migration, but improve clinical tumor therapy by inhibiting the tumor-cell in G0 phase into unlimited proliferation cycle.

Inhibiting the substrate-phosphorylation

The process of phosphorylation and dephosphorylation catalyzed by numerous PTKs and PTPs is one of the key mechanisms to keep the cell homeostasis (Izabela L et al., 2011). PTKs catalyze the transfer of a phosphate group from the coenzyme adenosine-5'-triphosphate (ATP) to specific proteins or lipids. PTPs catalyze a reverse process, that is, it can remove the phosphate group from a substrate (Arena et al., 2005; Tabernero et al., 2008 Izabela et al., 2011). Phosphorylation/ dephosphorylation of a protein can result in a change between active and inactive form, which is connected with conformational changes (Arena et al., 2005; Izabela et al., 2011). In other words, when the conformation of protein complexes are affected by phosphorylation and dephosphorylation, the function of the protein is also altered.

The PTPRO as a receptor-type tyrosine phosphatase can specifically bind the ligand corresponding, which can activate the catalytic function by changing conformation of PTPRO itself. Ultimately, the activation of a substrate protein specifically combining with PTPRO is also inhibited, because of dephosphorylation catalyzed by PTPRO. In other words, the PTPRO can play the role of the tumor suppressor by catalyzing substrate dephosphorylation.

Suppressing the phosphorylation of the protein kinases: PTKs as the oncoprotein play vital roles in different kinds of signal transportation pathways including the cell differentiation, proliferation and migration by catalyzing the hyperphosphorylation of substrate-proteins (Fabbro et al., 2002; Bhise et al., 2004). SYK as a protein tyrosine kinase had been identified that is a major substrate of tissues-specific and developmentally regulated PTP, PTP receptor-type O truncated (PTPROt) (Chen et al., 2006). B cell receptor(BCR)-dependent activation of the SYK PTK initiates downstream signaling events and amplifies the original BCR signal (Gauld et al., 2002; Rolli et al., 2002; Chen et al., 2006). The downstream signaling events activated by the SYK can regulate various signal transportation pathways particularly immune receptors signaling including proliferation, differentiation and phagocytosis (Coopman et al., 2000; Paolo et al., 2009), in which the activation of mitogen-activated protein kinase/ extracellular regulated protein (MAPK/ERK) which is a signal transportation pathway (Campbell KS., 1999) and SHC BLNK that are target proteins of the SYK (Panchamoorthy et al., 1996; Fu et al., 1998) are major events. MAPK/ERK is a vital pathway in transporting extracellular signals into nucleus. In this pathway, the ERK1/2 activated by phosphorylation which can transfer from cytoplasm to nucleus can regulate transportation of various oncoproteins including c-Myc, c-fos and CREB though phosphorylation (Gammarota et al., 2001; Johnson et al., 2002; Liu et al., 2012; D'Arcy et al., 2014; Shi et al., 2014). Besides, it also takes part in regulating several kinds of biological responses including proliferation, differentiation of the cell, sustaining the pattern of the cell, constructing the framework of the cell, regulating apoptosis (Roskoski et al., 2012; Miller et al., 2014). SHC and BLNK are all adaptor proteins (Chen LF et al., 2006). SHC activated by phosphorylation can activate the RAS by specifically combining with GRB2, which can trigger the proliferation of the cell (Gaughn et al., 2000). BLNK activated which can connect the protein kinase of the SYK with several signal transportation pathways can provide some combination locations for BTK, GRB2, VAV and NCK (Fu et al., 1998 ; Hashimoto et al., 1999).

Recently, some studies have found the activations of SHC, BLNK and MAPK/ERK which are all downstream events of the SYK are all blocked, because the function of the SYK as a direct PTPROt substrate is inhibited by PTPROt. Chen et al. (2006) use centrifugation and immunoblotting with antiphosphotyrosine antibody to analyze substrate-trapping mutants: PTPROt-CS, PTPROt-DA which lack catalytic activity but retain the ability to bind substrate and wild type PTPRO with catalytic function in lymphoma cell lines, the result showed tyrosyl-phosphorylated SYK is significantly more abundant in PTPROt-CS than DA or wild type *in vivo* or *in vitro*. Besides, for further demonstrating the relationship between SYK tyrosyl phosphorylation and expression of WT or mutant PTPROt, he also tests the immunoprecipitation which is over-expression PTPROt induced by DOx and anti-human Ig by immunoblotting. The result revealed the tyrosyl phosphorylation of SYK is significantly inhibited in PTPROt wild type comparing with the DA or CS-mutant PTPROt. In addition, they also found because the catalytic function of the SYK is inhibited by PTPROt, the phosphorylations activated by the SYK of SHC and BLNK are all blocked in the wild type group compared with CS or DA group. Meanwhile, the functions of ERK1/2 which is a key protein in MAPK/ERK regulating cellular proliferation and apoptosis are also suppressed accompanying with phosphorylation decrease.

From above results, we can postulate that the over-expression of PTPROt inhibits the BCR-triggered SYK tyrosyl phosphorylation and downstream signaling events such as SHC, BLNK and MAPK/ERK. In this way, PTPROt can suppress the occurrence, growth and proliferation of the tumor by inhibiting the activation of PTKs and downstream signaling events.

Down-regulating the activation of ATPase: Valosin

Containing Protein (VCP) is one member of ATPases which are super family relating with various cell activities (Sauer et al., 2004; Jentsch et al., 2007; Stolz et al., 2011; Hsu et al., 2013). Besides, VCP as an ATPase is a abounded expression enzyme which has a wide variety of cellular functions (Wang et al., 2004; Frohlich et al., 1991; Hsu et al., 2013). To our knowledge, NF-kB which is a nuclear transcription factor can regulate diverse oncogenic activation in the human body (Bradford et al., 2014), and it relates with different kinds of biological responses including the tumor infiltration, migration, immune response and apoptosis (Vaiopoulos et al., 2013; Gasparini et al., 2014). VCP which play a central role in ubiquitin degradation of misfolded proteins (Bursavich et al., 2010) involves in tumor cell invasion, migration and anti-apoptosis by activating the signaling transportation pathway of the NF-kB (Bursavich et al., 2010; Long et al., 2013). According some studies reported over-expression VCP is closely associated with the tumor size, the invasion depth, the histological type, the histological grade, lymph node involvement and prognosis of patient in hepatocellular carcinoma, esophageal carcinoma, gastric carcinoma, thyroid carcinoma and breast cancer (Rao et al., 1999; Yamamoto et al., 2003; Yamamoto et al., 2003; Yamamoto et al., 2004; Yamamoto et al., 2005). These findings strength the notion VCP is closely associated with tumor formation and development. Hsu (Hsu et al., 2013) used the mass spectrometry of the peptides pulled down by the substrate-trapping mutant of PTPRO to identify the VCP is a PTPRO novel substrate in hepatocellular carcinoma (HCC). Besides, the phosphorylation of VCP following over-expression of wild-type PTPRO in H293T and HepG2 cells are also inhibited. In addition, he also found the cell growth of the tumor is also inhibited, when the tyrosyl dephosphorylation of VCP is identified in HepG2 cell with over-expressing PTPRO.

From above results, we can suppose PTPRO as a tumor suppressor can use the way of blocking the tyrosyl phosphorylation of ATPase, such as VCP, to regulate the tumor-cell growth.

Regulating negatively Eph receptors: The Eph receptors as a new class of receptor-type tyrosine kinases are first identified in a human cDNA library screen for homologous sequences to the viral oncogenes vfips (Hirai et al., 1987; Nikki et al., 2002; Dana et al., 2004). Generally, the Eph receptors could be classified into two types, class A and class B on the basis of sequence similarity and affinity to relevant ligands (; Gale et al., 1996; Nikki et al., 2002; Dana et al., 2004). Normally, Eph receptors and their ligands, ephrins utilize way of the cell-cell contract to communicate with each other in order to activate receptors and downstream signaling events (Himanen et al., 2003; Takafumi et al., 2006). When Eph receptors are activated by binding their ligands ephrins, the phosphorylation of Eph receptors juxtamembrane region, and a sterile-a-motif (SAM) domain located in intracellular region which contain several tyrosine residues, could supply a number of cytoplasmic signaling proteins with binding locations, such as Ras-GTPase-activating protein (RasGAP), src and Abl family of non-receptor tyrosine kinases, low molecular weight phosphotyrosine phosphatase (LMW-PTP), phospholipase C γ, phosphatidylinositol 3-kinase, and adaptor proteins SLAP, Grb2, Grb10, and Nck (Flanagan et al., 1998; Brukner et al., 1998; Kalo et al., 1999; Nikki et al., 2002; Dana et al., 2004). Recently, some reporters have revealed, although, Eph receptors don't transport the proliferation signal in the tumor cell, they can enhance tumor cell motility, invasion and metastasis by regulating angiogenesis of tumor microenvironment, cell-cell and cell-matrix attachment (Nikki et al., 2002; Dana et al., 2004; Surawska et al., 2004). In melanoma cells, someone identified the ephrin-A1 as a cell survival factor or a promoter can increase the tumor cell growth (Easty et al., 1999; Dana et al., 2004). In addition, in transfected NIH3T3 cells, the over-expression of ephrinA8 induced by EphA5-Fc can enhance cell-matrix adhesion by activating the Fyn kinase as well as Erk pathways and increase focal adhesions (Davy et al., 2000; Nikki et al., 2002). The elevated ephrinB1 and EphB1 of endothelial cells can regulate integrin-dependent cell attachment, migration (Nikki et al., 2002). Besides, in mutant mice experiment, the EphB/ephrin can stimulate the vasculature development of the tumor microenvironment by several steps (Adams et al., 1999; Dana et al., 2004). In breast cancer, some reports shown, on the one hand, elevated EphA2 and EphB4 mainly located in undifferentiated and invasive tumor cells of transgenic mice expressing the Ha-ras oncogene (Andres et al., 1994; Dana et al., 2004), on the other hand, the over-expression of EphB4 in breast cells can accelerate tumor onset in MMTV-Neu animals (David et al., 2008). In lung cancer, patients developing brain metastases have a significant high expression of EphA2 compared with those who don't relapse (Kinch et al., 2003).

Therefore, it is well known the Eph activated by their ligands, the ephrins, is required for activating a series of downstream signaling events. However, Takafumi and his colleagues (Takafumi et al., 2006) identified the activation of Eph receptors can be inhibited by PTPRO in the chick retinotectal projection system. They took advantage of substrate-trapping mutants of PTPRO to show not only the Eph receptors including EphAs and EphBs are the physiological substrates of PTPRO, but the tyrosl phosphorylation level of Eph receptors are also inhibited because of catalytic activity of PTPRO. *In vitro*, in order to further demonstrate the Eph receptors are the substrates of PTPRO, they identified the intracellular region (ICR) of DA mutation of PTPRO has a close interaction with the ICR of Eph receptors, but not the PTPRO-WT. Absolutely, the similar result was also identified *in vivo*. Besides, *in vitro* and vivo, they found the tyrosyl phosphorylation level of Eph receptors in NIH3T3 cells without expressing PTPRO is significant high compared with the PTPRO-WT.

From above researches, we can see, although Eph receptors activated by ligands, the ephrins, play an important regulation role in tumor formation and development, the research also demonstrated the activation of Eph receptors can be suppressed by PTPRO. Therefore, we can speculate the PTPRO can negatively regulate the function of Eph receptors to indirectly inhibit the tumor, although now there are not any obvious researches to prove it.

down-regulation transcription activator: Signal transducer and activator of transcription 3 (STAT3) as a potentially carcinogenic factor is one important member of the signaling transportation pathway of JAK-STAT (Kreis et al., 2007). Normally, when tumor cells undergo sustained stimulation from a variety of cytokines and growth factors, such as IL-6, IFN-r (interferon-gamma), EGF (epidermal growth factor), FGF (fibroblast growth factor), HGF (hepatocyte growth factor), the JAK2 located in the downstream of these factors is also activated in a tyrosine-phosphorylation dependent manner and the activated JAK2 also potentially leads to the activation of its substrate, STAT3 by phosphorylation of both serine 727 (S727) and tyrosine 705 (Y705) (Boccaccio et al., 1998; Yokogami et al., 2000; Rane et al., 2000; Song et al., 2003; Laurie et al., 2005; Alvarez et al., 2006; Dudka et al., 2010). Then the activated STAT3 will access the nucleus to regulate the signal factor transcription relating with the tumor-cell differentiation, proliferation, apoptosis, angiogenesis and metastasis (Bournazou E et al., 2013). Besides, the activated STAT3 also can affect the tumor-cell by different transportation pathways, such as, inhibiting the tumor-cell apoptosis by up-regulating BCL-2 and BCL-X (ValdeZ et al., 2008); inducing the tumor-cell proliferation by regulating the expression of cyclinD1 and c-Myc (Saha et al., 2014); inducing the angiogenesis by up-regulating the expression of VEGF (Chen Z et al., 2008) and the STAT3 activated by FGF-1 can promote the tumor-cell migration and invasion through over-expressing MMP-7 (Udayakumar et al., 2002). From above identifications, we can conclude the STAT3 can play important regulation roles in diverse transportation pathways relating with the tumor-cell proliferation, apoptosis, angiogenesis, migration and immune response.

Hou (Jia et al., 2013) identified the over-expression PTPRO in the HCC can lead the tyrosyl dephosphorylation of STAT3 by inhibiting the phosphorylation of both serine 727 (S727) and tyrosine 705 (Y705). To demonstrate the PTPRO potential suppression mechanism, they measured the tyrosyl phosphorylation state of STAT3 regulated by PTPRO in HCC and adjacent tissues by western blotting and immunohistochemistry (IHC), the result showed PTPRO over-expression HCC cells can inhibit the STAT3 Y705, S727 phosphorylation through inactivating corresponding JAK2 and PI3K. Besides, they also found the relationship between phosphorylation STAT3 and PTPRO levels in HCC is opposite.

Taken together, these findings indicated STAT3 as a transducer and activator of oncogenes can influence the tumor formation and development by regulating diverse tumor factors. But PTPRO can down-regulate the activation of STAT3 as a substrate of PTPRO by dephosphorylation, which is important reason for the tumor-suppression of PTPRO.

Other tumor-suppression mechanisms of PTPRO

To our knowledge, cell and tissue homeostasis results from the dynamic balance of cell-cell and cell-extracellular component cross-talk that regulates such cell activities as proliferation, differentiation, and apoptosis (Sung et al., 2002; Sung et al., 2007). The cell-extracellular component including numerous cells from different tissue-types and functions, endothelium, fibroblasts and extracellular matrix interactions provide the microenvironment for epithelial cells (Sung et al., 2002; Sung et al., 2007). Only under microenvironment regulating, the organ-specific human epithelial cells can maintain their polarity, grow, survive, and express tissue-specific proteins (Sung et al., 2007). So, when the disruption of the homeostatic interaction between epithelial cells and microenvironment, it will initiate and promote carcinogenesis (Sung et al., 2007). After carcinogenesis, the microenvironment destroyed can confer reciprocal signal cascades in the tumor-cell to promote further carcinogenesis processes (Sung et al., 2007). Finally, these changes can produce different factors that enhance the proliferation and invasion of the tumor and confer the ability to metastasize to different organs (Sung et al., 2007). An experiment mainly focusing on preferential invasion and growth of tumor cell metastases in specific organs in a mouse model of melanoma had identified the sites of metastasis were determined not solely by the characteristics of the tumor cells but also by the microenvironment of the host tissue (Hart., et al., 1980 ; Sung et al., 2007). As Paget published the 'seed and soil' hypothesis, he thought that metastases formed only when the seed and soil were compatible (Sung et al., 2007).

Liu (Zhao et al., 2015) identified the over-expression PTPRO can inhibit the tumor-metastasis by disturbing the appropriate host microenvironment which is essential for the formation of metastasis. For demonstrating the finding, the luciferase-tagged Py8119 cells from breast cancer in the mice were injected intracardiacally into ptpro+/+ and ptpro-/- C57B1/6 mice. Then 4 weeks after the intracardic injection, they identified the ptpro-/- group formed more metastases in the mice than the control group by monitoring the pre-metastatic niche which was direct effected by PTPRO in the way of fluorescence and bioluminescence imaging. So, from above results, we can conclude the PTPRO can inhibit the metastasis formation in the pre-metastatic niche by disturbing the microenvironment of the host tissue.

Besides, they also found the microvessel density was significantly high in the tumor tissues of the ptpro-/- group compared with the wild-type group expressing higher PTPRO by immunohistochemical staining performed with the CD31 and CD34 (Zhao et al., 2015). The result indicated PTPRO over-expression in the niche is essential for the inhibition of the tumor angiogenesis which is regarded as a hallmark of cancer (Douglas et al., 2011). As we all known, the microvessel density reflecting angiogenesis is the net result of cumulative phases of angiogenesis(De Raeve et al., 2004). Like normal cells, angiogenesis is also important for the tumor-cell. In order to sustained proliferation, growth, development and migration, the tumor cell requires enough nutrients and oxygen as well as an ability to evacuate metabolic wastes and carbon dioxide which all can be addressed by angiogenesis (Douglas et al., 2011). Recently, most experiment data indicated angiogenesis not only is importance for a rapidly growing tumor which had formed, also contributes to the microscopic premalignant phase of tumor progression (Hart et al., 1980; Raica et al., 2009).

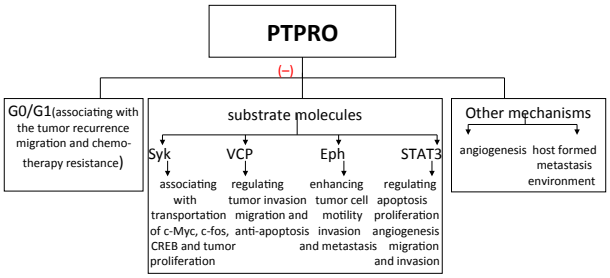

Figure 1. Overview of the PTPRO Tumor-suppression Mechanisms

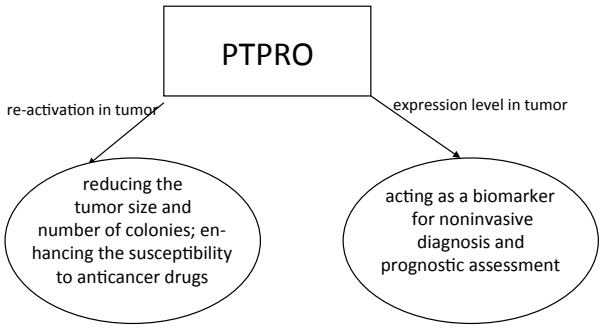

Figure 2. Applications of PTPRO in Anticancer

These findings showed PTPRO can inhibit the tumor-cell growth, proliferation and migration by suppressing tumor angiogenesis.

The implication of the PTPRO in clinical antitumor therapy

Recently, more and more researches have revealed the expression and function of PTPRO as a tumor-suppressor are both inhibited, because of the promoter hypermethylation in various cancer cell lines (Tasneem et al., 2003; Tasneem et al., 2004; Yuriko et al., 2004; Motiwala et al., 2007; You et al., 2012; Shao et al., 2014). Besides, further studies implicated the methylation of PTPRO could represent a biomarker for noninvasive diagnosis and a prognostic factor in tumors (Yu et al., 2015). Meanwhile, the consequent reactivation of PTPRO not only diminishes the tumor-size, also can enhance the susceptibility to anticancer drug.

You (You et al., 2012) explored the methylation level of PTPRO as a biomarker in the peripheral blood of esophageal squamous cell carcinoma. The result showed PTPRO methylation was 36.1% (13/36) in peripheral blood of carcinoma, while no PTPRO methylation was observed in normal peripheral blood. Therefore, this finding showed the PTPRO methylation is an epigenetic biomarker for noninvasive diagnosis of esophageal squamous cell carcinoma. Meanwhile, in breast cancer, a study identified PTPRO was positively associated with lymph node involvement (P=0.014), poorly differentiated histology (P=0.037), depth of invasion (P=0.004), and HER2 amplification (P=0.001) (Shao YL et al., 2014). In addition, someone also found the prognosis of patients with over-expressing PTPRO and erbB2(-) is much more better than those with aberrant expression of PTPRO especially erbB2(+) patients (Yi et al., 2013). So, we can implicate the aberrant PTPRO expression in the tumor could serve as a poorly prognostic factor for breast cancer patients, especially for patients with HER2-positive. In esophageal squamous cell carcinoma, the ratio of PTPRO methylation for patients in the elderly phase (T3/T4) is significantly high compared with the early phase (T1/T2) (P=0.013) (You et al., 2012). In conclusion, the expression level of PTPRO in various solid tumors can serve as a biomarker to monitor the prognosis of patients.

From above researches, we have understood the PTPRO how to inhibit the tumor-cell growth, proliferation and migration by different suppression mechanisms. However, when the promoter of PTPRO is methylated, the function of tumor-suppression is also inhibited accompanying the down-regulation of PTPRO expression. So, in order to control and treat tumors in clinical, we suppose the PTPRO can replay the function of suppression tumors by reactivating the expression.

Many preclinical studies had identified the 5-AzaC, a DNA hypomethylating agent, can alleviate the promoter methylation by removing the methyl groups from the DNA, which can reactivate the expression of PTPRO (Samson, 2005). Motiwala (Tasneem et al., 2004) identified the PTPRO was silenced by methylation in some human lung cancer cells, but the PTPRO in the corresponding normal adjacent tissues was relatively methylation-free. However, when the PTPRO was reactivated after treatment with the 5-AzadC, inhibition of DNA methyltransferase, the tumor with re-expressing PTPRO demonstrated obvious reduction in the size and number of colonies. This observation suggested the over-expression PTPRO reactivated also can play the function to suppress the tumor. In addition, in some animal experiments also found the over-expression PTPRO can not only suppress the tumor-cell as a tumor-suppressor, but enhance the susceptibility of the tumor-cell to potent anticancer drugs. Hsu (Hsu et al., 2013) treated the HepG2 cells, respectively expressing wild type PTPRO and mutant of PTPRO, with the Doxorubicin, a DNA damaging drug commonly used in primary HCC, the result showed the tumor cell expressing PTPRO exhibited highly sensitivity to the anticancer drug compared with the mutant after 72 hours of treatment. Similarly, Motiwala (Motiwala et al., 2007) also identified the over-expression PTPRO can enhance the susceptibility of chronic lymphocytic leukemia cells to fludarabine. In addition some studies also found the over-expression PTPRO can not only significantly enhance the susceptibility to estrogen-mediated tamoxifen therapy in breast cancer (Ramaswamy et al., 2009), but negatively regulate the resistance of anti-EGFR therapy in colon cancer through activation of SRC-mediated EGFR signaling (Layka et al., 2014).

Discussion

PTPRO as a receptor-type PTPs plays an important suppression function in the tumor-cell growth, proliferation and migration through some suppression mechanisms, such as, arresting G0/G1 of the tumor cell proliferation cycle, inhibiting substrates-phosphorylation, down-

regulating transcription activator and so on. Furthermore, clinical studies identified the aberrant expression PTPRO, because of hypermethylation in cancer, can represent a biomarker for noninvasive diagnosis and a prognostic factor evaluating therapeutic effect of patients, which is important for making the tumor-therapy project. In addition, when the methylated PTPRO is reactivated, the over-expression PTPRO can not only reduce the tumor size by inhibiting the tumor-cell growth and proliferation, but improve the DFS and OS of patients by enhancing the susceptibility of the tumor-cell to anticancer drugs. In my opinion, the research of PTPRO function in suppressing the tumor-cell as well as influencing the tumor-therapy is just beginning. So, we can suppose the suppression mechanisms of PTPRO, when they are discovered more comprehensively, not only will support new study ideals and directions for other studies of tumor-suppressors, importantly, but will supply theoretical basis for researching new molecular targeting agents in the near future.

References

Andres A, Joanna S, Nunzio B, et al (2004). Protein tyrosine phosphatases in the human genome. *Cell*, **117**, 699-711.

Andersen JN, Jansen PG., Echwald, S.M., et al (2004). Agenomic perspective on protein tyrosine phosphatases: gene structure, pseudogenes, and genetic disease linkage. *FASEB J*, **18**, 8-13.

Andersen JN, Mortensen, OH, Peters GH, et al (2001). Structural and evolutionary relationships among protein tyrosine phosphatase domains. *Mol Cell Biol*, **21**, 7117-36.

Arena S, Benvenuti S, Bardelli (2005). Genetic analysis of the kinome and phosphatome in cancer. *Cell Mol Life Sci*, **62**, 2092-9.

Adams RH, Wilkinson GA, Weiss C, Diella F, et al (1999). Roles of ephrinB ligands and EphB receptors in cardiovascular development: demarcation of arterial/venous domains, vascular morphogenesis, and sprouting angiogenesis. *Genes Dev*, **3**, 295-306.

Andres A-C, Reid HH, Zurcher G, Blaschke RJ, et al (1994). Expression of two novel eph-related receptor protein tyrosine kinases in mammary gland development and carcinogenesis. *Oncogene*, **9**, 1461-7.

Alvarez JV, Greulich H, Sellers WR, et al (2006). Signal transducer and activator of transcription 3 is required for the oncogenic effects of non-small-cell lung cancer-associated mutations of the epidermal growth factor receptor. *Cancer Res*, **66**, 3162-8.

Bhise SB, Nalawade AD, Wadhawa H (2004). Role of potein tyrosine kinase inhibitors in cancer therapeutics. *Indian J Biochem Biophys*, **41**, 273-80.

Bradford JW, Baldwin AS (2014). Ikk/nuclear factor-kappaB and oncogensis: roles in tumor-initiating cells and in the tumor microenvironment. *Adv Cancer Res*, **121**, 125-45.

Bursavich MG, Parker DP, Willardsen JA, et al (2010). 2-Anilino-4aryl-1,3-thiazole inhibitors of valosin-containing protein(VCP or p97). *Bioorg Med Chem Lett*, **20**, 1677-9.

Brukner KKR (1998). Signaling by Eph receptors and their ephrin ligands. *Curr Opin Neurobiol*, **8**, 375-82.

Boccaccio C, Ando M, Tamagnone L, et al (1998). Induction of epithelial tubules by growth factor HGF depends on the STAT pathway. *Nature*, **391**, 285-8.

Bournazou E, Bromberg J (2013). Targeting the tumor microenvironment JAK-STAT3 signaling. *JAKSTAT*, **2**, 23828.

Chen LF, Juszcynski P, Takeyama K, et al (2006). Protein tyrosine phosphatase receptor-type O truncated (PTPROt) regulates SYK phosphorylation proximal B-cell-receptor signaling, and cellular proliferation. *Blood*, **108**, 3428-33.

Coopman PJ, Do MT, Barth M, et al (2000). The Syk tyrosine kinase suppresses malignant growth of human breast cancer cells. *Nature*, **406**, 742-7.

Campbell KS (1999). Signal transduction from the B cell antigen-receptor. *Curr Opin Immunol*, **11**, 256-64.

Chen Z, Han ZC (2008). STAT3: a critical transcription activator in angiogenesis. *Med Res Rev*, **28**, 185-200.

D'Arcy P, Maruwge W, Wolahan B, et al (2014). Oncogenic functions of the cancertestis antigen SSX on the proliferation, survival, and singaling pathways of cancer cells. *PloS One*, **9**, 95136.

Dana BS, Sonja S, Monica P, et al (2004). Eph Receptor Tyrosine Kinases in Tumor and Tumor Microenvironment. *Current Pharmaceutical Design*, **10**, 3431-42.

Davy A, Robbins SM (2000). EphrinA5 modultes cell adhesion and morphology in an integrin-dependent manner. *EMBO J*, **19**, 5346-406.

David V, Dana M BS, Jin C (2008). Eph receptors in breast cancer: roles in tumor promotion and tumor suppression. *Breast Cancer Res*, **10**, 217-24.

Dudka AA, Sweet SM, Heath JK (2010). Signal transducers and activators of transcription-3 binding to the fibroblast growth factor receptor is activated by receptor amplification. *Cancer Res*, **70**, 3391-401.

Douglas Hanahan, Robert A (2011). Weinberg. hallmarks of cancer: the next generation. *Cell*, **144**, 646-674.

De Raeve H, Van Marck E, Van Camp B, et al (2004). Angiogenesis and therole of bone marrow endothelial cells in haematological malignancies. *Histol Histopathol*, **19**, 935-50.

Easty D, Hill S, Hsu M, et al (1999). Up-regulation of ephrin-A1 during melanoma progression. *Int J Cancer*, **84**, 494-501.

Fabbro D, Ruetz S, Buchdunger E, et al (2002). Protein kinases as a targets for anticancer agents: from inhibitors to useful drugs, *Pharmacol Ther*, **93**, 79-98.

Fu C, Turck CW, Kurosaki T, et al (1998). BLNK: a central linker protein in B cell activation. *Immunity*, **9**, 93-103.

Frohlich KU, Fries HW, Rudiger M, et al (1991). Yeast cell cycle protein CDC48p shows full-length homology to the mammalian protein VCP and is a member of a protein family involved in secretion, peroxisome formation, and gene expression. *JCell Biol*, **114**, 443-53.

Flanagan JG, Vanderhaeghen P (1998). The eprhins and Eph receptors in neural development. *Annu Rev Neurosci*, **21**, 309-45.

Gauld SB, Dal Porto JM, Cambier JC (2002). B cell antigen receptor signaling: roles in cell development and disease. Sci, **296**, 1641-2.

Gammarota M, Bevilaqua LR, Dunkley PR, et al (2001). Angiotensin II promotes the phosphorylation of cyclin AMP-responsive element binding protein (CREB) at Ser 133 through an ERK1/2 dependent mechanism. *J Neurochem*, **79**, 1122-8.

Gaughn C, Liu WQ, Vidal M, et al (2000). Inhibitors of Ras signal transductio as antitumor agents. *Biochem Pharmacol*, **60**, 1165-9.

Gasparini C, Celeghini C, Monasta L, et al (2014). NF-kB pathways in hematological malignancies. *Cell Mol Life Sci*, **71**, 2083-102.

Gale NW, Holland SJ, Valenzuela DM, et al (1996). Eph receptors and ligands comprise two major specificity subclasses and are reciprocally compartmentalized during

embryogenesis. *Neuron*, **17**, 9-19.
Hsu, Tasneem M, Satavisha R, et al (2013). Methylation of gene encoding the growth suppressor protein tyrosine phosphatase receptor-type O (PTPRO) in human hepatocellular carcinoma and identification of VCP as its bona fide substrate. *J Cellular Biochemistry*, **1002**, 134-55.
Hashimoto S, Iwamatsu A, Ishiai M, Okawa K, et al (1999). Identification of the SH2 domain binding protein of Bruton's tyrosin kinase as BLNK-functional significance of Btk-SH2 domain in B-cell antigen receptor coupled calcium signaling. *Blood*, **94**, 2357-64.
Hirai H, Maru Y, Hagiwara K, et al (1987). A novel putative tyrosine kinase receptor encoded by the Eph gene. *Sci*, **238**, 1717-20.
Himanen JP, Nikolov DB (2003). Eph receptors and ephrins. *Int J Biochem Cell Biol*, **35**, 130-4.
Hart, I. R, Fidler, I. J (1980). Role of organ selectivity in the determination of metastatic patterns of the B16 melanoma. *Cancer Res*, **40**, 2281-7.
Izabela L, Maria M S (2011). Tyrosine phosphatases as a superfamily of tumor suppressors in colorectal cancer. *Acta Biochimica Polonica*, **4**, 467-70.
Johnson KG, Van Vactor D (2003). Receptor protein tyrosine phosphatases in nervous system development. *Physiol Rev*, **83**, 1-24.
Jia JH, Juan X, Run QJ, You JW, et al (2013). Estrogen-sensitive PTPRO expression represses hepatocellular carcinoma progression by control of STAT3. *Hepatol*, **57**, 678-88.
Johnson GL, Lapadat R (2002). Mitogen-activated protein kinase pathways mediated by ERK, JNK, and p38 protein kinases. *Sci*, **298**, 1911-2.
Jentsch S, Rumpf S (2007). Cdc48 (p97): a "molecular gearbox" in the ubiquitin pathway? *Trends Biochem Sci*, **32**, 6-11.
Kalo MS, Pasquale EB (1999). Signal transfer by Eph receptors. *Cell Tissue Res*, **298**, 1-9.
Kinch MS, Moore MB, Harpole DH Jr (2003). Predictive value of the EphA2 receptor tyrosine kinase in lung cancer recurrence and survival. *Clin Cancer Res*, **9**, 613-8.
Kreis S, Munz GA, Haan S, et al (2007). Cell density dependent increase of constitutive signal transducers and activators of transcription 3 activity in melanoma cells is mediated by Janus Kinases. *Mol Cancer Res*, **5**, 1331-41.
Laurie Stepanek, Andrew W. Stoker, Esther Stoeckli, et al (2005). Receptor tyrosine phosphatases guide vertebrate motor axons during development. *Soc Neurosci*, **25**, 3813-23.
Liu B, Kuang A (2012). Genetic alterations in MAPK and PI3K/ Akt signaling pathways and the generation, progression, diagnosis and therapy of thyroid cancer. *Sheng Wu Yi Xue Gong Cheng Xue Za Zhi*, **29**, 1221-5.
Long XH, Zhang ZH, Liu ZL, et al (2013). Inhibiting valosin-containing protein suppresses osteosarcoma cell metastasis via AKT/nuclear factor of kappa B singaling pathway *in vitro*. *Indian J Pathol Microbiol*, **56**, 190-5.
Layka AA, Iria V, Loredana V, et al (2014). The tyrosine phosphatase PTPRO sensitizes colon cancer cells to anti-EGFR therapy through activation of SRC-mediated EGFR signaling. *Oncotarget*, **5**, 10070-83.
Miller CR, Oliver KE, Farley JH (2014). MEK1/2 inhibitors in the treatment of gynecologic maligancies. *Gynecol Oncol*, **133**, 128-37.
Motiwala T, Majumder S, Kutsy H, et al (2007). Methylation and silencing of protein tyrosine phosphatase receptor type O in chronic lymphocytic leukemia. *Clin Cancer Res*, **13**, 3174-81.
Nikki Chenga, Dana M. Brantley, et al (2002). The ephrins and Eph receptors in angiogenesis. *Cytokine Growth Factor Rev*, 13, 75-85.
Paolo R, Barbara B, Andrea C (2009). Therapeutic prospect of Syk inhibitors. *Exper Opin Ther Pat*, **19**, 1361-76.
Panchamoorthy G, Fukazawa T, Miyake S, et al (1996). p120cbl is a major substrate of tyrosine phophorylation upon B cell antigen receptor stimulation and interacts *in vivo* with Fyn and Syk tyrosine kinases, Grb2 and shc adaptors, and the p85 subunit of phosphatidylinositol 3-kinase. *J Biol Chem*, **271**, 3187-94.
Ricardo C.T. A, Yoshihiro Y, Samir K, et al (1999). Freeman, Margaret A. Shipp. PTPROt: An Alternatively Spliced and Developmentally Regulated B-Lymphoid Phosphatase That Promotes G0/G1 Arrest. *Blood*, **94**, 2403-13.
Rolli V, Gallwitz M, Wossning T, et al (2002). Amplification of B cell antigen receptor signaling by a Syk/ ITAM positive feedback loop. *Mol Cell*, **10**, 1057-69.
Roskoski R Jr (2012). ERK1/2 MAP kinases: structure, function, and regulation. *Pharmacol Res*, **66**, 105-143.
Rao PN, Levine E, Myers MO, et al (1999). Elevation of serum riboflavin carrier protein in breast cancer. *Cancer Epidemiol Biomarkers Prev*, **8**, 985-90.
Rane SG, Reddy EP (2000). Janus kinases: components of multiple signaling pathways. *Oncogene*, **19**, 5662-79.
Raica M, Cimpean AM, Ribatti D (2009). Angiogenesis in pre-malignant conditions. Eur J Cancer, 45, 1924-1934.
Ramaswamy B, Majumder S, Roy S, et al (2009). Estrogen-mediated suppression of the gene encoding protein tyrosine phosphatase PTPRO in human breast cancer: mechanism and role in tamoxifen sensitivity[J]. *Mol Endocrinol*, **23**, 176-87.
Shi W, Hou X, Peng H, et al (2014). MEK/ERK signaling pathway is required for enterovirus 71 replication in immature dendritic cells. *Virol J*, **11**, 227.
Sauer RT, Bolon DN, Burton BM, et al (2004). Sculpting the proteome with AAA(+) proteases and disassembly machines. *Cell*, **119**, 9-18.
Stolz A, Hilt W, Buchberger A, et al (2011). Cdc48: a power machine in protein degradation. *Trends Biochemical Sci*, **36**, 515-23.
Surawska H, Ma PC, Salgia R (2004). The role of ephrins and Eph receptors in cancer. *Cytokine Growth Factor Rev*, **15**, 419-33.
Song L, Turkson J, Karras JG, et al (2003). Activation of Stat3 by receptor tyrosine kinases and cytokines regulates survival in human non-small cell carcinoma cells. *Oncogene*, **22**, 4150-65.
Saha A, Blando J, Silver E, et al (2014). 6-Shogaol from dried ginger inhibits growth of prostate cancer cells both *in vitro* and *in vivo* through inhibition of STAT3 and NF-kB signaling. *Cancer Prev Res*, **7**, 627-38.
Sung SY, Chung LW (2002). Prostate tumor-stroma interaction: molecular mechanisms and opportunities for therapeutic targeting. *Differentiat*, **70**, 506-21.
Sung SY, Hsieh CL, Wu D, et al (2007). Tumor microenvironment promotes cancer progression, metastasis, and therapeutic resistance. *Curr Probl Cancer*, **31**, 36-100.
Shao YL, Rong L, Yu LC, et al (2014). Aberrant PTPRO methylation in tumor tissues as a potential biomarker that predicts clinical outcomes in breast cancer patients. *Biol Med Central Genet*, **15**, 67.
Samson T J, Tasneem M (2005). Epigenetic regulation of protein tyrosine phosphatases: potential molecular targets for cancer therapy. *Cancer Gene Therapy*, **12**, 665-72.
Thomas P.E, Wharram B.L., Goyal M, et al (1994). GLEPP1, a renal glomerular epithelial cell (podocyte) membrane protein tyrosine phosphatase. Identification, molecular cloning, and characterization in rabbit. *J Biol Chem*, **269**, 19953-62.
Takafumi S, Masaru I, Hiraki S, et al (2006). Eph receptors are negatively controlled by protein tyrosine phosphatase

receptor type O. *Nature Neuroscience*, **9**, 761-9.

Tabernero L, Aricescu AR, Jones EY, et al (2008). Protein tyrosine phosphatases: structure-function relationships. *FEBS J*, **275**, 867-82.

Takafumi S, Masaru I, Hiraki S, et al (2006). Eph receptors are negatively controlled by protein tyrosine phosphatase receptor type O. *Nature Neurosci*, **9**, 761-9.

Tasneem M, Kutay H, Ghoshal K, et al (2004). Protein tyrosine phosphatase receptor-type O(PTPRO) exhibits characteristics of a candidate tumor suppressor in human lung cancer. *Med Sci*, **38**, 13844-9.

Tasneem M, Kalpana G, Das A, et al (2003). Suppression of the protein tyrosine phosphatase receptor type O gene (PTPRO) by methylation in hepatocellular carcinomas. *Oncogene*, **22**, 6319-31.

Udayakumar TS, Stratton MS, Nagle RB, et al (2002). Fibroblast growth factor-1 induced promatrilysin expression through the activation of extracellular-regulated kinases and STAT3. *Neoplasia*, **4**, 60-7.

Vaiopoulos AG, Athanasoula KCh, Papavassiliou AG (2013). NF-kB in colorectal cancer. *J mol Med*, **91**, 1029-37.

ValdeZ BC, Murray D, Ramdas L, et al (2008). Altered gene expression in busulfan-resistant human myeloid leukemia. *LeuK Res*, **32**, 1684-97.

Wei HL, Chia HC, Kuo SH, et al (2013). Protein tyrosine phosphatase receptor type O (Ptpro) regulates cerebellar formation during zebrafish development through modulating Fgf signaling. *Cell Mol Life Sci*, **70**, 2367-81.

Wang Q, Song C, Li CC (2004). Molecular perspectives on p97-VCP: progress in understanding its structure and diverse biological functions. *J Structural Biol*, **146**, 44-57.

Yoji M, Munemasa M, Takenori K, et al (2010). Tyrosine phosphorylation of R3 subtype receptor-type protein tyrosine phosphatases and their complex formations with Grb2 or Fyn. *Genes to Cells*, **15**, 513-24.

Yang DH, Goyal M, Sharif K, et al (1996). Glomerular epithelial protein1 and podocalyxin-like protein 1 in inflammatory glomerular disease (crescentic nephritis) in rabbit and man. Lab. *Invest*, **74**, 571-84.

Yamamoto S, Tomita Y, Nakamori S, et al (2003). Elevated expression of valosin-containing protein(p97) in hepatocellular carcinoma is correlated with increased of tumor recurrence. *J Clin Oncol*, **21**, 447-52.

Yamamoto S, Tomita Y, Nakamori S, et al (2004). Expression level of valosin-containing protein(p97) is associated with prognosis of esophageal carcinoma. *Clin Cancer Res*, **10**, 5558-65.

Yamamoto S, Tomita Y, Nakamori S, et al (2003). Expression level of valosin-containing protein(p97) is strongly associated with prognosis of gastric carcinoma. *J Clin Oncol*, **21**, 2537-44.

Yamamoto S, Tomita Y, Nakamori S, et al (2005). Increased expression of valosin-containing protein(p97) is correlated with disease recurrence in follicular thyroid cancer. *Ann Surg Oncol*, **12**, 925-34.

Yokogami K, Wakisaka S, Avruch J, et al (2000). Serine phosphorylation and maximal activation of STAT3 during CNTF signaling is mediated by the rapamycin target mTOR. *Curr Biol*, **10**, 47-50.

You YJ, Chen YP, Zheng XX, et al (2012). Aberrant methylation of the PTPRO gene in periheral blood biomarker in esophageal cell carcinoma patients. *Cancer Letters*, **315**, 138-44.

Yuriko M, Jing Y, Fumiaki S, et al (2004). Identification of Genes Uniquely Involved in Frequent Microstaellite instability Colon Cancinogenesis by Expression Profiling Combined with Epigenetic Scanning. *American Associat Cancer*, **64**, 2434-8.

Yu Du1, Jennifer R. Grandis (2015). Receptor-type protein tyrosine phosphatases in cancer. *Chinese J Cancer*, **34**, 61-9.

Yi TH, Fei FL, Chen K, et al (2013). PTPRO promoter methylation is predictive of poorer outcome for HER2-positive breast cancer: indication for personalized therapy. *J Translational Med*, **11**, 245-52.

Zhao L, JIA JIE H, Li DONG R, el at (2015). Protein tyrosine phosphatase receptor type O expression in the tumor niche correlates with reduced tumor growth, angiogenesis, circulating tumor cells and metastasis of breast cancer. *Oncol Reports*, **3862**, 1-7.