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Targeting phosphatases of regenerating liver (PRLs) in cancer

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ABSTRACT

The phosphatase of regenerating liver (PRL) family, also known as protein tyrosine phosphatase 4A (PTP4A), are dual-specificity phosphatases with largely unknown cellular functions. However, accumulating evidence indicates that PRLs are oncogenic across a broad variety of human cancers. PRLs are highly expressed in advanced tumors and metastases compared to early stage cancers or matched healthy tissue, and high expression of PRLs often correlates with poor patient prognosis. Consequentially, PRLs have been considered potential therapeutic targets in cancer. Persistent efforts have been made to define their role and mechanism in cancer progression and to create specific PRL inhibitors for basic research and drug development. However, targeting PRLs with small molecules remains challenging due to the highly conserved active site of protein tyrosine phosphatases and a high degree of sequence similarity between the PRL protein families. Here, we review the current PRL inhibitors, including the strategies used for their identification, their biological efficacy, potency, and selectivity, with a special focus on how PRL structure can inform future efforts to develop specific PRL inhibitors.

Contents

1. Introduction

Protein tyrosine phosphatases (PTPs) are a large family of enzymes that catalyze the removal of phosphate groups that are attached to tyrosine residues on their substrates. PTPs, together with protein tyrosine kinases (PTKs), precisely maintain the appropriate phosphorylation level of proteins, which is critical for normal cellular functions.

The aberrant phosphorylation of proteins is implicated in many human diseases, including cancer, inflammatory diseases, and diabetes/obesity (Z. Y. Zhang, 2017), suggesting both PTPs and PTKs are potential therapeutic targets. PTK inhibitors have achieved clinical success and become the standard of care in several types of cancer, including Afatinib for non-small-cell lung cancer (Y.-L. Wu et al., 2014) and Imatinib for chronic myeloid leukemia

Abbreviations: CBS, cystathionine-beta-synthase; CHO, Chinese hamster ovary; CNNM, magnesium transporters of cyclin M; EMT, epithelial–mesenchymal transition; HMVEC, human microvascular endothelial cells; mAb, monoclonal antibody; NMR, nuclear magnetic resonance; PDB, protein data bank; PRL, phosphatase of regenerating liver; PTK, protein tyrosine kinase; PTP, protein tyrosine phosphatase; PTEN, phosphatase and tensin homolog; SAR, structure–activity relationship; SHP2, protein tyrosine phosphatase N11; SSG, sodium stibogluconate; TCPTP, T-cell protein tyrosine phosphatase; VHR, vaccinia H1-related phosphatase.

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(Comert, Baran, & Saydam, 2013). Conversely, PTPs have not received attention as therapeutic targets until the past decade, due to misconceptions that phosphatases are only tumor suppressors or that they lack regulatory roles in disease (Lazo & Sharlow, 2016). However, accumulating evidence has shown that phosphatases are suitable therapeutic targets in cancer. For example, protein tyrosine phosphatase 1B (PTP1B) is reported to play a tumor-promoting role in prostate and colorectal cancer (Lessard et al., 2012), and high PTPB1 expression is associated with poor prognosis in colorectal cancer patients (Hoekstra et al., 2016; Lessard et al., 2012). Additionally, protein tyrosine phosphatase SHP2 increases tumor progression and maintains tumor-initiating cells in breast cancer (Aceto et al., 2012; Hu, Li, Gao, Wei, & Yang, 2017). Consequently, the interest in exploring phosphatases as drug targets to treat cancer has risen sharply in the last decade.

2. PRLs as oncogenic phosphatases

The protein tyrosine phosphatase 4A (PTP4A) family, commonly known as phosphatase of regenerating liver (PRLs) are dual-specificity phosphatases, which can act on both tyrosine residues and serine/threonine residues (Bessette, Qiu, & Pallen, 2008). PRLs are largely considered oncogenic phosphatases that play critical roles in tumor progression and metastasis across a variety of human cancers. PRL-3 is the most well-studied of the PRLs, and is highly-expressed in many types of solid tumors and leukemia, reviewed in detail elsewhere (Bollu, Mazumdar, Savage, & Brown, 2017; Campbell & Zhang, 2014; Stephens, Han, Gokhale, & Von Hoff, 2005). Importantly, metastatic lesions in many of these solid cancers expressed PRL-3 at much higher levels than the primary tumor, and high PRL-3 expression was often correlated with poor patient prognosis (Beekman et al., 2011; Dai, Lu, Shou, & Li, 2009; Mayinuer et al., 2013; Qu et al., 2014; Radke et al., 2006; Ren et al., 2009; Saha et al., 2001), suggesting a causative role for PRL-3 in cancer progression. A direct contributing role for PRL-3 in cancer has been demonstrated by over-expression and knock-down of PRL-3 in normal or cancer cell lines. For example, human cell lines transfected with PRL-3, including human melanoma, breast, lung and colorectal cancer, exhibited increased oncogenic properties compared to control, including increased motility, migration, invasion and proliferation *in vitro*. PRL-3 expression significantly enhanced tumor progression and metastasis after transplantation of the transfected cells in mice (Guo et al., 2004; Hardy, Wong, Muller, Park, & Tremblay, 2010; X. Wu et al., 2004). Conversely, PRL-3 knock-down led to decreased cell proliferation, migration, and invasion of melanoma, gastric, ovarian, lung cancer cell lines *in vitro* and inhibited primary tumor proliferation and metastasis in mouse cancers or xenograft models (Achiwa & Lazo, 2007; Hardy et al., 2010; Kato et al., 2004; Li et al., 2006; Polato et al., 2005; Qian et al., 2007; Y. Wang & Lazo, 2012).

Similarly, both PRL-1 and PRL-2 are reported to have oncogenic roles in cancer, but these are not well-defined. High PRL-1 expression was observed in cervical (Dong, Sui, Wang, Chen, & Sun, 2014) and gastric cancers (Dumaual et al., 2012) and intrahepatic cholangiocarcinoma (Liu et al., 2016). PRL-1 expression was correlated with poor patient prognosis in hepatocellular carcinoma (Jin et al., 2014) and prostate cancer (Shinmei et al., 2014). PRL-2 expression was significantly increased in breast cancer (Hardy et al., 2015) and hepatocellular carcinomas (Dumaual et al., 2012). Inconsistently, *in situ* hybridization and immunohistochemistry showed that PRL-1 expression was lower in ovarian, breast, and lung cancers and PRL-2 was significantly down-regulated in kidney carcinomas compared to normal tissue (Dumaual et al., 2012). However, the number of cases examined in this study was limited, and further research needed to validate the expression level of PRL-1 and PRL-2 in these cancer types.

Studies of PRL-1 or PRL-2 over-expression or knock-down in cell lines show that these PRLs may have similar functions as PRL-3. For example, PRL-1 overexpression in chinese hamster ovary (CHO) cells led to increased cell motility and invasiveness *in vitro*. The injection of those cells in nude mice induced lung tumor and liver metastasis, similar to the effects of PRL-3 overexpression in CHO cells (Zeng et al., 2003). The D27 hamster pancreatic ductal epithelial cells that ectopically overexpress PRL-1 or PRL-2 showed loss of contact inhibition *in vitro* and induced tumor growth in nude mice (Cates et al., 1996). Different mouse mammary tumor–derived cell lines that overexpress PRL-2 showed increased anchorage-independent growth and cell migration. In addition, injection of DB-7 mammary cancer cells with PRL-2 overexpression into the mouse mammary fat pad increased tumor growth (Serge Hardy et al., 2010). Finally, PRL-2 knock-down reduced the anchorage-independent growth and cell migration of human metastatic MDA-MB-231 breast cancer cells and reduced the cell migration and invasion of human A549 lung cancer cells, which can be rescued by co-transfecting an siRNA resistant PRL-2 (Y. Wang & Lazo, 2012).

While the experimental evidence above clearly establishes the oncogenic role for the PRL phosphatase family in cancer cells, PRLs may also play an important role in the tumor angiogenesis. For example, PRL-3 mRNA was detected in endothelial cells within a colon cancer metastasis (Bardelli et al., 2003) and was increased 6-fold in breast tumor endothelium compared to surrounding epithelial cells (Parker et al., 2004). Overexpression of PRL-3 in human microvascular endothelial cells (HMVEC) *in vitro* enhanced endothelial tube formation (Rouleau et al., 2006) and endothelial cell migration (Parker et al., 2004). Additionally, PRL-3 knock-out in mice led to decreased microvessel density in colon tumor tissues compared with wild type controls. In addition, vascular cells isolated from PRL-3-null mice were less invasive and migratory *in vitro*, compared with wild type cells (Zimmerman et al., 2014). Further studies are needed to definitively link PRL-3 to angiogenesis in the cancer setting, and the role of PRL-3 in other migratory cells within the tumor microenvironment, such as fibroblasts and immune cells, remains to be defined.

3. PRL substrates

Despite the relatively well-established functional role of PRLs in cancer progression, the molecular mechanisms through which PRLs promote cancer cell proliferation, invasion and metastasis are largely undefined. Mechanistically, PRLs have been shown to be involved in several major signaling pathways, including regulation of p53, PTEN/PI3K/Akt, Src/ERK1/2, Rho family GTPases and adhesion proteins including integrin, E-Cadherin and matrix metalloproteases (Campbell & Zhang, 2014; Rios, Li, & Kohn, 2013).

Identification of the substrates of phosphatases is highly challenging due to the complicated substrate profiles that may include proteins, lipids, and carbohydrates, as well as the transient interaction between most phosphatases and their substrates (Fahs, Lujan, & Kohn, 2016). This difficulty in identifying substrates is best reflected by the fact that there are only 305 protein substrates and 89 non-protein substrates identified for 194 human phosphatases according to the DEPOD database (http://depod.bioss.uni-freiburg.de/br_s.php) as of April 2018. In contrast, there are 5092 protein substrates for 518 protein kinases according to the RegPhos (http://140.138.144.141/~RegPhos/index.php). It may be even more challenging to identify PRL substrates, as the catalytic pocket of PRLs are more shallow and wider compared to other PTPs (Kozlov et al., 2004), making substrate trapping difficult. Consequently, only a few direct substrates have been suggested for PRLs, including phosphatydilinositol (4,5) bisphosphate [PI(4,5)P2] (McParland et al., 2011), Ezrin (Forte et al., 2008), Stathmin (Zheng et al., 2010), Keratin 8 (Mizuuchi, Semba, Kodama, & Yokozaki, 2009), Integrin α1 (Peng et al., 2006), Elongation factor 2 (Orsatti et al., 2009) and Nucleolin (Semba, Mizuuchi, & Yokozaki, 2010). Different strategies have been used to identify these substrates, including proteomics (Zheng et al., 2010), a yeast two-hybrid system (Peng et al., 2006), immunoprecipitation using wild-type and catalytically inactive PRL (Semba et al., 2010), *in vitro* dephosphorylation assays

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