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Review

From immune response to cancer: a spot on the low molecular weight protein tyrosine phosphatase

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Abstract. Reversible tyrosine phosphorylation is a key posttranslational regulatory modification of proteins in all eukaryotic cells in normal and pathological processes. Recently a pivotal janus-faced biological role of the low molecular weight protein tyrosine phosphatase (LMWPTP) has become clear. On the one hand this enzyme is important in facilitating appropriate immune responses towards infectious agents, on the other hand it mediates exaggerated inflammatory responses toward innocuous stimuli. The evidence that LMWPTP plays a role in oncological processes has added a promising novel angle. In this review we shall focus on the regulation of LMWPTP enzymatic activity of signaling pathways of different immunological cells, the relation between genetic polymorphism of LMWPTP and predisposition to some type of inflammatory disorders and the contribution of this enzyme to cancer cell onset, growth and migration. Therefore, the LMWPTP is an interesting target for pharmacological intervention, thus modifying both inappropriate cellular immune responses and cancer cell aggressiveness.

Keywords. Low molecular weight protein tyrosine phosphatase, cancer, immune system, inflammation.

Introduction

The widespread nature of protein phosphorylation/dephosphorylation underscores its key role in cell signaling metabolism, growth and differentiation. In fact, cells are able to give a specific response towards diverse changes in their microenvironment through integrated signaling pathways. These pathways occur via sequential phosphorylation or dephosphorylation reactions which are governed by the action of protein kinases and phosphatases, respectively [1, 2]. Protein tyrosine phosphatases (PTPs) in conjunction with protein tyrosine kinases (PTKs) regulate the reversible phosphorylation of tyrosine residues in proteins and thereby control fundamental physiological processes such as cell growth and differentiation, cell cycle, metabolism, cytoskeletal function, and immune response. Accordingly, deregulated activity of PTPs and PTKs is involved in the development of numerous inherited and acquired human diseases such as neurological and cardiovascular disorders, infections, diabetes, cancer, and autoimmunity [3, 4].

Alonso and collaborators [5] demonstrated that the human genome contains 107 genes encoding either experimentally verified PTPs or proteins with a

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Table 1. Classification of protein tyrosine phosphatases based on amino acid sequences of the catalytic domains.

PTP family	Members
Class I cys-based	Receptor PTP CD45, RPTPμ, RPTPκ, RPTPρ, RPTPλ, RPTPσ, RPTPδ, RPTPα, RPTPε, RPTPγ, RPTPξ, RPTPβ, DEP1, SAP1, GLEPP, PTPS31, PCPTP, STEP, IA2 and IA2β Nonreceptor PTP PTP1B, TCPTP, PTP-MEG2, HePTP, STEP, LYP, PTP-PEST, PTP-HSCF, Typ-PTP and HD-PTP MAPKs PAC-1, MKP1, MKP2, MKP3, MKP4, VH3, VH5, PYST2, MKP5, MKP7 and MK-STYX Atypical DSPs VHR, PIR1, BEDP, TMDP, MKP6, DSP20, SKRP, DSP21, MOSP, MGC1136, VHZ, FMDSP, VHX, VHY, HYVH1, VHP, Laforin, RNGTT and STYX PRLs PRL1, PRL2 and PRL3 CDC14 s CDC14A, CDC14B, KAP and PTP9Q22 Slingshots SSH1, SSH2 and SSH3 PTENs PTEN, TPIP, TPTE, tensin and C-1-TEN Myotubularins MTM1, MTMR1, MTMR2, MTMR3, MTMR4, MTMR5, MTMR6, MTMR7, MTMR8, MTMR9, MTMR10, MTMR11, MTMR12, MTMR13 and MTMR14
Class II cys-based	LMWPTP
Class III cys-based	CDC25A, CDC25B and CDC25C
Class IV asp-based	EyA1, EyA2, EyA3 and EyA4

Alonso et al. [5].

domain homologous to the catalytic domain of these PTPs. Among these genes, 81 are predicted to be active protein phosphatases. Based on the primary structure of the catalytic domains and the amino acid used in the catalytic reaction, PTPs are subdivided into four evolutionarily distinct classes: 1, 2 and 3 (cysteine-based PTPs) and 4 (aspartate-based PTPs). Table 1 summarizes PTPs classes and their members [5]. Despite the fact that the three-dimensional structures of the catalytic domains of the cysteine-based PTPs are strikingly similar, they possess different topologies, and their regulatory domains vary significantly [5].

LMWPTP family

Some years ago, an important review on LMWPTP was published by Raugei et al. [6]. Since then, some interesting discoveries regarding this phosphatase have occurred. Therefore, in this review we will provide a general view of this phosphatase and discuss the current state of knowledge regarding its role in the immune response and cancer.

In humans, class II cysteine-based PTPs are represented by the members of LMWPTP family (also known as acid phosphatase locus 1, ACP1), which are widely expressed in humans with no particular tissue-specific expression. Enzymes of this family have molecular weights of about 18 kDa and share no overall sequence homology with the other larger PTPs beyond the signature motif $(H/V)C(X)_5R(S/T)$ in the P-loop common to all PTPs [3].

Four different human LMWPTP messenger RNA isoforms, derived by alternative splicing of a single transcript, have been characterized. Two of them correspond to the classical, active isoforms 1 (IF1, PTP*fast*/isoform F or HCPTPA) and 2 (IF2, PTP*slow*/isoform S or HCPTPB) [7]. Both isoforms are single polypeptide chains of equal length which display a

difference only in a short sequence segment that corresponds to amino acid residues 40–73 in the mature protein. However, these isoforms present divergence in their physical chemistry properties, especially with respect to kinetics and consequently physiological functions [8–11]. In addition, SV3 (splicing variant 3) and LMWPTP-C inactive forms have been described [7, 12].

LMWPTP structure and activity regulation

PTPs were initially defined as household enzymes with constitutive activity, able to dephosphorylate any substrate. However, recently it has become clear that cellular PTP activity is under much more dynamic control than previously thought. PTP activity is controlled at different levels, including expression, alternative splicing and limited proteolysis, subcellular localization, oxidation, phosphorylation, dimerization and interaction with ligands [13]. Regarding LMWPTP, the modulation of its activity is based on phosphorylation/dephosphorylation and reversible oxidation of cysteine residues: Phosphorylation/dephosphorylation – LMWPTP contains two conserved adjacent tyrosines, Tyr131 and Tyr132, which are preferential sites for phosphorylation by PTKs [14, 15]. The phosphorylation of Tyr131/or Tyr132 by Src, Lck and Fyn kinases leads to different effects on enzyme behavior and are important in regulating its activity [14, 15]. Although the tyrosine kinase Src strongly and specifically phosphorylates both Tyr resisdues, Tyr131 phosphorylation by Src induces an approximate 25-fold increase in LMWPTP specific activity, while phosphorylation at Tyr132 does not affect the enzyme activity. Additionally, Lck and Fyn tyrosine kinases also phosphorylate LMWPTPs at Tyr131 and, to a lesser extent at Tyr132 in T lymphocytes, causing twofold enzymatic activation. Despite not having an obvious effect on LMWPTP enzymatic activity, phosphorylation of Tyr132 by Src seems to be crucial for this phosphatase to interact with other proteins via SH2 domains. Bucciantini and co-workers demonstrated that the SH2 domain-containing protein Grb2 (growth factor receptor bound protein 2) can bind LMWPTP only when this phosphatase is phosphorylated at Tyr132 [14]. LMWPTP activity is directly influenced by the interaction of this enzyme with the SH2-containing proteins. Since the Tyr132 is located close to the active site, the interaction of Grb2-like proteins with phosphorylated LMWPTP could limit access of the ligand binding surface and active site entrance, leading to enzyme inactivation or selection of substrates by size (Fig. 1A) [14]. Thus, the phosphorylation state of the different tyrosine residues in LMWPTP defines the specificity of this enzyme.

Redox modulation – It has been shown that reactive oxygen species (ROS) such as O_2^- or H_2O_2 are transiently generated intracellularly in various physiological and pathological conditions, such as the lymphocytic and macrophage oxidative burst, cell stimulation with cytokines and growth factors and reperfusion. Under these conditions, the generation of reactive molecules induces an increase in tyrosine phosphorylated proteins, which can be achieved by the activation of PTKs and/or inactivation of PTPs. In fact, recent evidence shows that both mechanisms are triggered by oxidants, which can not only reversibly modulate PTP activity [13, 16–18] but also some PTKs [18–20]. In both situations cysteine oxidation may occur; however, only some PTKs can be activated by oxidation: Src, FAK, ERK, Akt, Fer, insulin receptor, EGFR, PDGFR and Ret [18–22]. In the case of PTPs, such as LMWPTP, the oxidation of catalytic site cysteine leads to the opposite effect through transformation of the sulphydrylic residue in sulphenic acid. The consequent inactivation of the enzyme occurs due to its inability to form a cysteinyl-phosphate intermediate during the first step of the catalysis (Fig. 1B). Additionally, the oxidation/inhibition of LMWPTP can be considered a critical event in the modulation of its activity since the mechanism constitutes a way to assure the stability of Src-mediated phosphorylation of LMWPTP by preventing the enzyme from dephosphorylating itself (Fig. 1C).

It has been demonstrated that activation of growth factors, e.g. PDGFR, induces a transient increase in ROS due to induced activation of membrane NADPH oxidase complex [23]. LMWPTP is oxidized *in vivo* after PDGFR activation and becomes unable to dephosphorylate its substrates, such as p190RhoGAP and PDGFR (Fig. 2A). LMWPTP oxidation can be reversed by the glutaredoxin/glutathione/glutathione reductase/NADPH system [24], leading to the rescue of the availability of the reduced catalytic cysteines. The same redox mechanism of LMWPTP regulation can also be observed during the activation of Eph receptors [25], reinforcing the concept that oxidation/reduction cycles of the enzyme during growth factor signaling is a crucial regulatory mechanism of its activity (Fig. 2B).

In addition, LMWPTP shuttles between different subcellular compartments, providing levels of control. Indeed, although LMWPTP is constitutively retrieved from both cytosolic and cytoskeleton subcellular fractions, Src-phosphorylated-LMWPTP has been found exclusively in the cytoskeleton fraction, whereas only the cytosolic fraction is able to interact with activated PDGF receptor [13, 26]. LMWPTP mem-

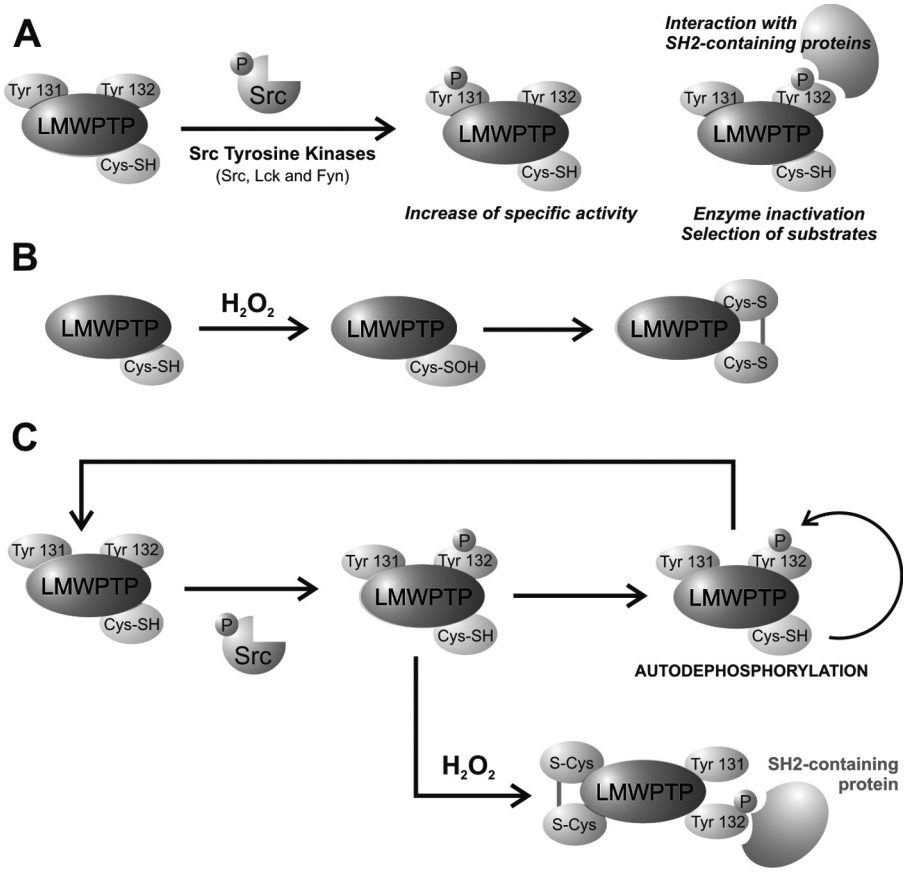

Figure 1. Modulation of LMWPTP activity. (*A*) Phosphorylation of Tyr131 by the Src kinases induces an increase in LMWPTP specific activity, while phosphorylation at Tyr132 enables LMWPTP interaction with SH2-containing proteins which can, in turn, regulate substrate access to the PTP active site, leading to enzyme inactivation or selection of substrates [14, 15]. (*B*) In the presence of ROS or other oxidants, such as H_2O_2, the oxidation of the cysteine residue (Cys-SH) in the catalytic site leads to production of sulphenic acid (Cys-SOH). To avoid the reaction of the Cys-SOH with another reactive molecule and the consequent irreversible inactivation of the LMWPTP, the Cys-SOH is blocked by S-S disulfide formation through its reaction with an intramolecular cysteine [16, 18, 19]. (*C*) LMWPTP oxidation not only inhibits the catalytic activity of the enzyme, but also potentiates the action of PTKs, such as Src. Once phosphorylated by a PTK, oxidized/inhibited LMWPTP cannot dephosphorylate itself and therefore has no ability to counteract the action of PTK.

brane-associated pool binds and dephosphorylates activated PDGFR, while the cytoskeleton-associated pool specifically acts on substrates that become tyrosine phosphorylated upon PDGFR treatment [26]. These findings reveal the importance of these differences in localization on LMWPTP molecular functioning.

A further finding, recently reported by Caselli and collaborators [27], is the influence of caveolin1 on LMWPTP activity. These authors found that IF1 and IF2 isoforms are capable of interacting with defined regions of caveolin1. Importantly, the formation of LMWPTP/caveolin1 complexes is accompanied by strong inhibition of the HCPTPA/IF1 isoform. Additionally, the same research group demonstrated that LMWPTP associated with caveolae structures is also able to bind and dephosphorylate caveolin1 previously phosphorylated by Src Kinases [28].

LMWPTP in the immune system

Although some research groups have contributed to an increase in knowledge of LMWPTP function and its relation to pathological states, at the moment little is known about its exact biological functions. Reports are found in literature showing the implications of deregulated activity and expression of LMWPTPs in common diseases, including allergy, asthma, obesity, myocardial hypertrophy, and Alzheimer's disease [29–31]. The correlation between altered expression and activity of its variants with various human diseases greatly indicates this phosphatase involvement in key processes in cell physiology [32]. In particular, some studies [29, 31] have shown the association between the different genotypes of LMWPTP and the predisposition/resistance to immunological disorders, suggesting the importance of this PTP in the modulation of the immune response.

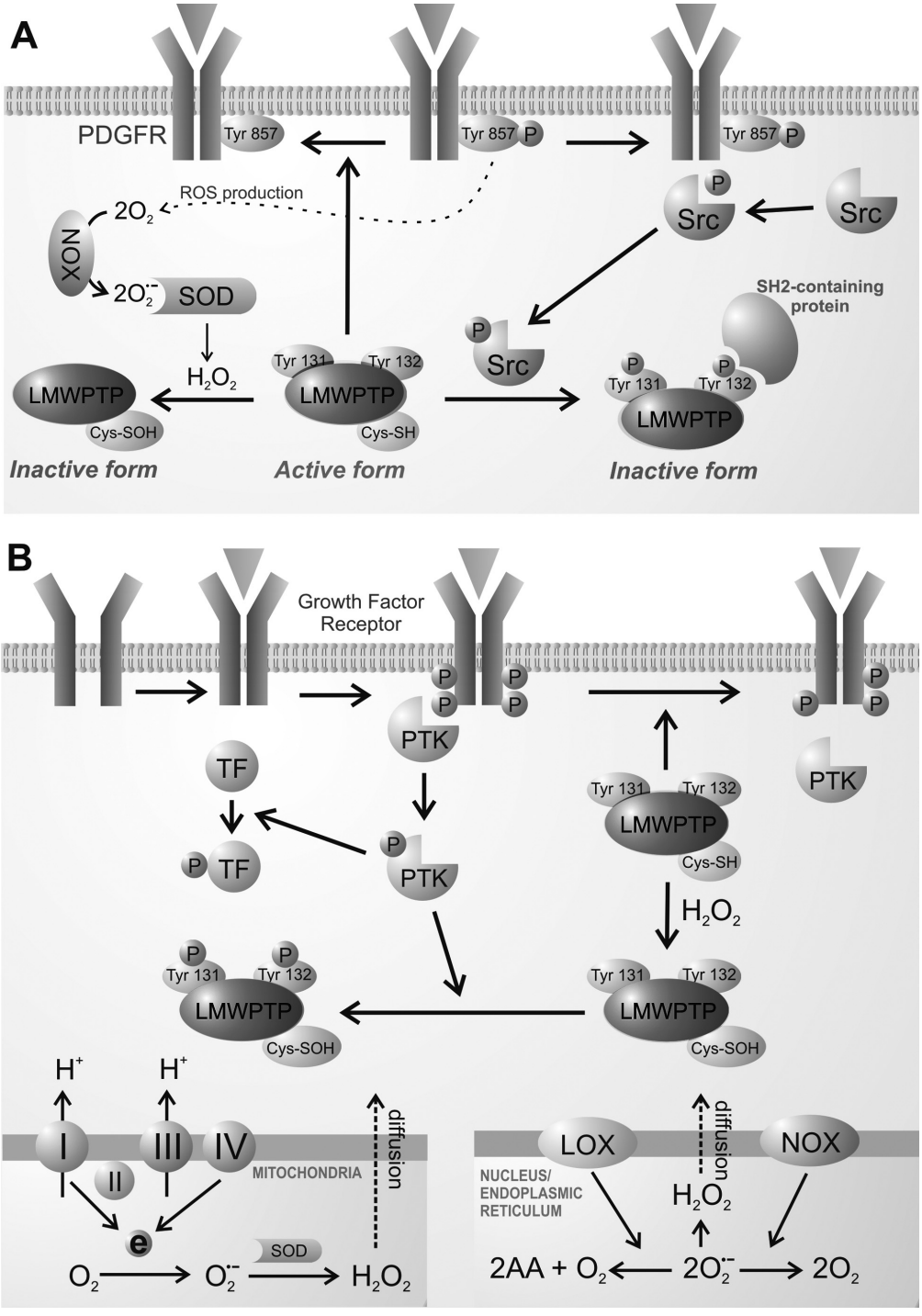

Figure 2. The role of LMWPTP in growth factor receptor signaling. (*A*) The binding of PDGF to its receptor on the cell surface induces dimerization and intrinsic tyrosine kinase activation, leading to its trans-autophosphorylation and binding of the c-Src kinase. Activated Src phosphorylates a broad set of proteins including the LMWPTP. The PDGFR activation process is accompanied by induction of H_2O_2 production through the activity of NADPH oxidase system (NOX) and superoxide dismutase (SOD). LMWPTP inhibition by the ROS produced during the process prevents it from dephosphorylating the receptor and ensures the binding of c-Src. (*B*) The binding of growth factors and cytokines to its receptors induces their dimerization and activation through trans-autophosphorylation. This is the starting point of signaling pathways that lead to an overall increase in tyrosine phosphorylation in intracellular targets. This occurs due to activation of PTKs and inhibition of PTPs, which is the case for LMWPTP. The activated receptor can phosphorylate PTKs and also triggers an increase in intracellular ROS, which seems related to the activation of NOX, 5-Lipoxygenase (LOX) and increased mitochondrial electron chain flux. The increase of intracellular ROS directly affects LMWPTP activity through its oxidation and the inhibited LMWPTP cannot dephosphorylate the receptor. Additionally, the activated PTKs can increase phosphorylation of LMWPTP, contributing to its inhibition. (TF = transcription factors; AA = Arachidonic acid) [6, 16].

The production of ROS in biological systems has been associated with the induction of biochemical alterations in macromolecules and the initiation or aggravation of pathological states. At low concentrations, ROS are able to activate transcription factors and alter mitogenic signals that affect growth, differentiation and a variety of many other cellular processes [33]. In immune cells, production of ROS is generally associated with the antimicrobial arsenal of phagocytes such as neutrophils and monocytes. However, the role of ROS in the immune system has acquired greater attention from researchers. A variety of activation pathways in immune cells induces the generation of ROS in the intracellular milieu through activation of NADPH oxidase (NOX), 5-lipoxygenase (LOX) and an increase in mitochondrial ROS production. The ROS produced during immune cell activation acts through positive modulation of some PTKs and reversible oxidation/inhibition of PTPs [18–22, 33]. In this scenario, LMWPTP has been shown to be a key mediator in the regulation of signaling pathways in receptor-stimulated immune cells.

LMWPTP is able to associate and dephosphorylate many growth factors such as platelet-derived growth factor receptor (PDGFR), fibroblast growth factor receptor (FGFR), macrophage-colony-stimulating factor receptors (MCSFR), insulin receptor (IR), and ephrin receptors (Eph), thus downregulating many of the tyrosine kinase receptor functions that lead to cell division. Additionally, LMWPTP has been shown to be a critical regulator of integrin signaling and, therefore, a key player in cell adhesion. Adhesion is required for proper T cell migration to inflammatory/infectious sites and contact establishment with antigen-presenting cells (APCs) or targets cells. LMWPTPs modulate the signal transduction by T cell receptor (TCR) [34] and contribute to the regulation of cell division, adhesion and spreading through cytoskeleton rearrangement, especially due to their ability to regulate the small GTPase Rho and p125FAK (focal adhesion kinase) [15, 35].

LMWPTP and T lymphocyte function. Proper physiological function of lymphocytes requires that these cells be able to circulate in a non adhesive form through blood and lymph. However, lymphocytes must become adherent to interact with endothelial cells for transmigration into sites of inflammation or infection, to stabilize cell-cell contact with APCs (establishing a proper immune response) or act as effectors cells, killing their targets [36]. The main receptor responsible for the regulation of T cell adhesion is the β_2 integrin LFA-1 (lymphocyte function-associated antigen-1 or CD11a/CD18). LFA-1 mediates adhesive phenomena through interaction with its counter-receptors, intercellular adhesion molecule-1 (ICAM-1 or CD54), ICAM-2 (CD102), or ICAM-3 (CD50) triggering a variety of intracellular signals including phosphorylation of phospholipase Cγ, protein kinase C activation, mobilization of intracellular Ca^{2+}, and activation of tyrosine kinases such as Src and p125FAK [36]. The Tyr397 autophosphorylation of p125FAK during LFA-1 allows the binding of $p60^{src}$ kinase to the p125 FAK molecule, an event that usher in two main consequences: a) induces an increase in p125FAK activity and the simultaneous activation of Src; b) activation of Src results in phosphorylation of p125FAK, thus creating specific docking sites for the binding of signaling and cytoskeleton proteins such as paxillin, tensin, Grb2, PI3K, which are themselves phosphorylated by p125FAK. In this way the phosphorylation of p125FAK seems itself to be a critical event for the functioning of LFA-1, since its activity is a required step for the liberation of LFA-1 from its binding to the actin cytoskeleton network [36]. This allows its clustering and polarization in the cell membrane surface, a condition that increases the LFA-1 avidity for ICAMs. Additionally, the activation of FAK also induces the clustering of antigen-specific T cell receptor (TCR), a primary event involved in the LFA-1 aggregation, and seems intimately associated with the overall clustering and polarization of cell surface receptors required for the formation of the immunological synapses (IS) in T cells. IS is a stable supramolecular structure initiated by the congregation of specific adhesion and signaling molecules at the contact site between T cell and APCs, which facilitates activation and further differentiation of T cells.

Given the relevant function of tyrosine phosphorylation during integrin-mediated T-cell adhesion, PTPs may be good candidates for the downregulation of LFA-1 activation. Figure 3 summarizes the participation of LMWPTP in the signaling pathway activated by LFA-1, which is based on the findings reported by Giannoni et al. [36]. These authors demonstrated that LMWPTP binds and dephosphorylates FAK mainly at the Tyr397, its Src-binding site, which leads to the inactivation of FAK and loss of the binding sites for many signaling and cytoskeleton proteins associated with T cell adhesion and function. As a consequence, FAK inhibition prevents cytoskeleton rearrangement and impairs the LFA-1/ICAM-1 dependent T cell-cell adhesion by avoiding LFA-1 clustering and conversion into its functional state. Also, the authors demonstrated that, upon antigen stimulation, LMWPTP-dependent FAK inhibition is associated with a strong reduction of LFA-1 and TCR co-clustering towards a single region of T cell surface,

thus impairing the activity of the receptor by preventing changes in their avidity state. As co-localization of both TCR and LFA-1 is an essential event during encounters of T cells with APCs and IS formation, LMWPTP seems to play a pivotal role in the regulation of IS formation and stability, and in the consequent modulation of sustaining the immune response. Interestingly, Zeyda and collaborators [37] have demonstrated that teriflunomide, the active metabolite of the anti-inflammatory and immunosuppressant agent leflunomide, impairs integrin avidity and integrin mediated signals, leading to abrogation of successful T cell/APC interaction and inhibition of mature IS formation. As teriflunomide action resembles that elicited by LMWPTP, we can speculate that this enzyme could mediate or contribute to teriflunomide immunosuppressant effects. In fact, the molecular mechanisms responsible for failure of IS maturation induced by clinically used immunosuppressants is still unclear [38], which might suggest that LMWPTP could be a potential candidate for mediator of their actions.

Additionally, Bottini's work [39] demonstrated that LMWPTP has the ability to dephosphorylate a negative regulatory site of the ZAP-70 protein tyrosine kinase, an event that leads to a several-fold increase in the activity of this enzyme. Dephosphorylation of ZAP-70 inhibitory site (Tyr292) by the LMWPTP is important to maintain the functionality of ZAP-70 in TCR signaling. Giannoni [36] suggested a model where LMWPTP may enhance TCR signaling in the early phases by positively regulating the activity of ZAP-70 while, in the later phases, the same enzyme reduces the cytoskeleton rearrangements by acting on FAK. Therefore, it is possible that a broad spectrum of LMWPTP actions in T cell can be orchestrated by the intensity and duration of signal as well as compartmentalization.

As mentioned previously, LMWPTP and caveolin1 can present mutual modulation. Recently caveolin1 has been recognized as an important mediator of signaling pathways in APCs [40, 41]. Therefore, we can speculate that the capacity of the LMWPTP to interact with caveolin1 can significantly modulate the immune response also at the APCs level, an indirect modulatory effect on T cell activity through regulation of caveolin-1 phosphorylation status in the APCs.

Immune cells respiratory burst and LMWPTP. Since reactive oxygen (ROS) and nitrogen species (RNS) production are involved in a variety of activation pathways in both phagocytic and non-phagocytic cells, Cys-based PTPs have been considered targets of both reactive species during activation and establishment of adhesion of immune cells [42]. Activation of mast cells and basophiles is accompanied by the production of ROS and RNS. These regulate diverse signaling pathways leading to the release of inflammatory mediators and production of a variety of cytokines. In mast cells these events are associated with the antigen-mediated aggregation of the high affinity IgE receptor (FcεRI) on the cell surface. IgE aggregation induces the secretion of allergy mediators such as β-hexosaminidase, histamine, serotonin, proteoglycans, proteases and the synthesis of multiple cytokines including interleukin (IL)-2, IL6, IL8 and interferon (INF)-γ. Indeed, PTPs such as LMWPTP are involved directly in FcεRI-mediated activation pathways in mast cells and basophils, suggesting that these cells may undergo ROS- and RNS-mediated regulation of Cys-based PTPs involved in receptor-mediated signaling [42–44].

Apart from being pivotal for the immunological activation of classical immune effector cells, a recent study by Mancini et al. [45] demonstrated expression and function of LMWPTP in circulating platelets and in DAMI megakaryocytic cells. LMWPTP plays a crucial role in the regulation of cell activation downstream immunoreceptor-tyrosine-based activation-motif (ITAM)-containing receptors such as the low affinity receptor for immunoglobulin G (FcγRIIA). Importantly, the work showed that, although LMWPTP is less abundant than other phosphatases in platelets, it can efficiently dephosphorylate FcγRIIA both *in vitro* and *in vivo*. In fact, it is the first described enzyme able to dephosphorylate this immunoreceptor and to negatively regulate cell activation [45].

Genetic approach of LMWPTP role in immune system function. LMWPTP is highly polymorphic and is transcribed from the LMWPTP/ACP1 locus on the short arm of chromosome 2 (p25 band). In Caucasian populations there are common alleles, *A, *B and *C, causing the existence of six different genotypes, *A/*A, *A/*B, *A/*C, *B/*B, *B/*C and *C/*C. The three ACP1 alleles contain single base substitutions located at specific sites: the ACP1*C allele differs from *A and *B alleles at codon 43 (exon 3) while ACP1*A and *B alleles differ by two base substitutions, a silent G-T transition at codon 41 (exon 4) and an A-G transition at codon 105 (exon 6) [46]. The six different genotypes diverge with respect to electrophoretic pattern, enzyme concentration, activity, modulation by purines and pteridines, stability and also show strong variations in total enzymatic activity and in the ratio between the activities of the isoforms. Spencer and co-workers [47] described the following enzymatic activities: (μmol p-nitrophenol produced in 30 min per gram of haemoglobin at 37°C): ACPl*A/

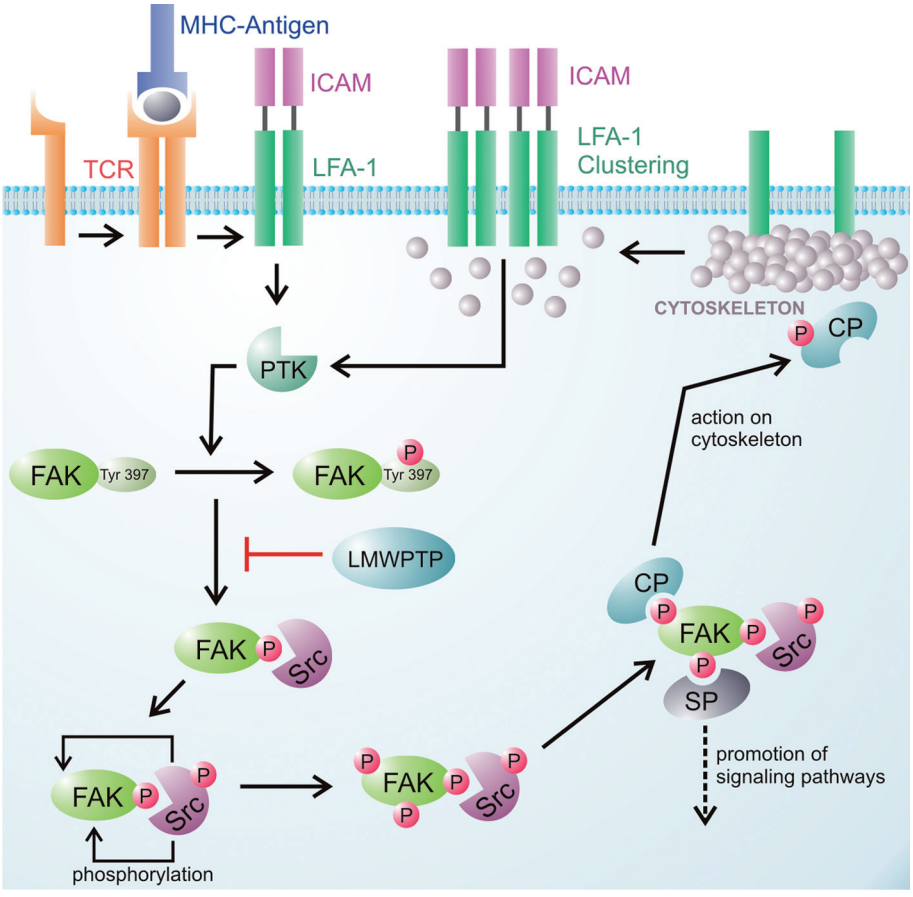

Figure 3. LMWPTP as a negative modulator of T cell activation and adhesion. Engagement of TCR and CD2, or cytokine and chemokine stimuli lead to the increase in LFA-1 activity through the rearrangement of the actin cytoskeleton network mediated by the p125FAK activation. LMWPTP acts as an inhibitor of FAK activation, thus preventing LFA-1 clustering and activation of the receptor when not necessary. (CP = Cytoskeleton Protein, PTK = Protein Tyrosine Kinase, SP = Signaling Protein) [36].

*A, 122.4; ACPl*A/*B, 153.9; ACPl*B/*B, 188.3; ACPl*A/*C, 183.8; and ACPl*B/*C, 212.3. These differences in activity might have profound influence on human physiology in general and in the immune system in particular.

Comparison of LMWPTP haplotype distribution in mothers and their newborns and analysis of the joint wife-husband LMWPTP phenotype distribution in couples with repeated spontaneous abortion suggest a negative effect of the ACP1*C allele on early life viability [48]. Furthermore, Bottini et al. [49] investigated the possible effect of LMWPTP on the relationship between maternal age at delivery and risk of type 1 diabetes in the offspring, since it has been demonstrated that the incidence of this Th1-mediated disease in children from older mothers is higher [50], an intriguing finding apparently due to later conception of the first child. The results obtained showed that the proportion of low activity ACP1 genotypes is much higher among children born from older mothers than among children born from relatively young mothers. This indicates that the risk and clinical course of type I diabetes seem to be dependent on both environment during intrauterine development and fetal genetic factors. The different levels of LMWPTP activity seem to be a crucial factor in the development of the disease. The mechanism of such association is unknown at the present but appears to be strongly associated with the role of LMWPTP in the modulation of T cell receptor mediated-signaling pathways involved in the genesis of the disease [51, 52]. Interestingly, besides the effects of LMWPTP in the genesis of type I diabetes, the same enzyme is also associated with glucose metabolism and insulin action in type II diabetes and has been pointed by recent studies as a new key negative regulator of insulin signaling *in vivo* and a novel drug target for the treatment of type II diabetes [46, 51].

In addition, an association between ACP1 polymorphism and allergic disorders such as asthma, rhinitis and allergic atopic eczema/dermatitis syndrome (AEDS) has been demonstrated in various reports, suggesting LMWPTP as a key player in the predisposition or resistance to allergic disorders [29, 34]. In general, carriers of *A allele who show low enzymatic activity have a greater susceptibility to allergy, whereas subjects carrying the genotypes with the highest activity (CB genotype) show lower total serum IgE levels [29, 34, 52]. Accordingly, Bottini's work [34]

showed that the proportion of allergic subjects is much higher among carriers of *A allele than other ACP1 phenotypes. Also, the presence of allergic disorders seems significantly associated with a lower F isoform concentration, while no association with S isoform concentration could be detected.

Among the signal transduction pathways involved in allergic disorders, the IL-4 receptor pathway might be a good candidate for the ACP1 regulatory role. Several tyrosine phosphorylation phenomena are activated following IL-4 stimulation, and many pathways activated by the IL-4 receptor are similar to those activated by insulin, whose signal transduction is modulated by ACP1. Indeed a significant correlation between ACP1 and the intracellular Glu/Arg polymorphism of IL4 has been detected, indicating that allergy can be associated with the levels of LMWPTP action on the IL4-activated signaling pathways [15, 34, 53].

In view of the current interest in gender differences in autoimmune diseases, Bottini and collaborators noted a significant interaction among LMWPTP, sex and class of inflammatory bowel disease. These authors noted a positive association of Crohn′s disease with one isoform of LMWPTP. More recently, Gloria-Bottini [54] carried out a study with subjects presenting allergic disorders (Crohn's disease and type I diabetes). This group found an association between ACP1 and allergy (Th2 class) that depends on gender showing that the presence of ACP1 allele seems to make females more susceptible to allergic manifestations as compared to males. In the Th1 class of immunological disease the ACP1 and gender also share a relationship, but in a pattern different from that in the Th2 class. In subjects with Crohn's disease and type I diabetes, the presence of *A allele, which is associated with low LMWPTP activity, seems to make females less susceptible to this class of diseases as compared to males. The results obtained by Gloria-Bottini and co-workers demonstrated that low levels of LMWPTP activity, determined by the ACP1 allele, appear responsible for a complex relationship involving gender, ACP1 and Th1/Th2 orientation. They found that low LMWPTP activity influences ZAP-70 activity and, in turn, T cell activation seems to have opposite effects on Th1/Th2 orientation, depending on gender [54].

LMWPTP in cancer

Protein tyrosine phosphorylation is the most important signal necessary to promote cell growth, proliferation, invasion and migration of normal and cancer cells [46], and thus it is not surprising that LMWPTP is important in the cancerous process. LMWPTP expression is upregulated in various human cancers [32, 55] and its biological roles may be of interest to understand cancer biology and the establishment of new cancer therapy strategies. The list of cancer relevant molecules apparently regulated by LMWPTP is growing and now includes the PDGFR [56], janus kinase 2 (JAK2) [57], signal transducer and activator of transcription 5 (STAT5) [58, 59], Focal adhesion kinase (FAK) [35], Ephrin A2 receptor (Eph A2) [60], insulin receptor (IR) [61, 51] and β-Catenin [62]. Often, this phosphatase is regarded as a negative regulator of such proteins but LMWPTP has been also shown to act as a positive signal for cell growth and proliferation [61]. For instance, in v-Ha-Ras-transformed cells, LMWPTP increases cell proliferation [62, 63]. The *Xenopus laevis* LMWPTP is a functional component of the fibroblast growth factor receptor-1 complex and acts as a positive regulator of the Ras [64]. Most likely, this diversity in functionality is a representation of its ubiquitous function in cellular physiology, as it is employed by the cellular machinery for many different processes. In our view, it is important to consider the different and sometimes opposite functions displayed by the LMWPTP depending on its activation status. On the one hand the catalytically active form of LMWPTP is able to dephosphorylate receptors and signaling molecules, resulting in the inhibition of cell proliferation. On the other hand, the catalytically inactive form of LMWPTP can also act as a scaffold protein for SH2-containing molecules, contributing to the positive regulation of survival and proliferative signaling pathways. It is therefore fair to say that the real contribution of LMWPTP to the development and progression of a specific cancer type needs to be analyzed considering the catalytic activity status of the enzyme in addition to its expression levels. Given the potential pro-mitogenic activities of the non-catalytically active enzyme, increased expression of LMWPTP in tumoral cells could not always be accompanied by a cellular increase in the total enzyme activity.

LMWPTP oncogenic potential is closely related to the receptor tyrosine kinase EphA2 (Figs. 4A and B). EphA2 is dephosphorylated in a wide variety of cancer cells and this phenomenon seems to correlate with malignancy. Activated EphA2 negatively regulates tumor cell growth, survival, migration, and invasion [65]. Kikawa and collaborators [60] showed that LMWPTP-mediated dephosphorylation of EphA2 promotes transformation of normal epithelial cells. Eph2A was also shown to be the main substrate for LMWPTP in engrafted tumor cells [66]. In fact, LMWPTP overexpression alone is sufficient to pro-

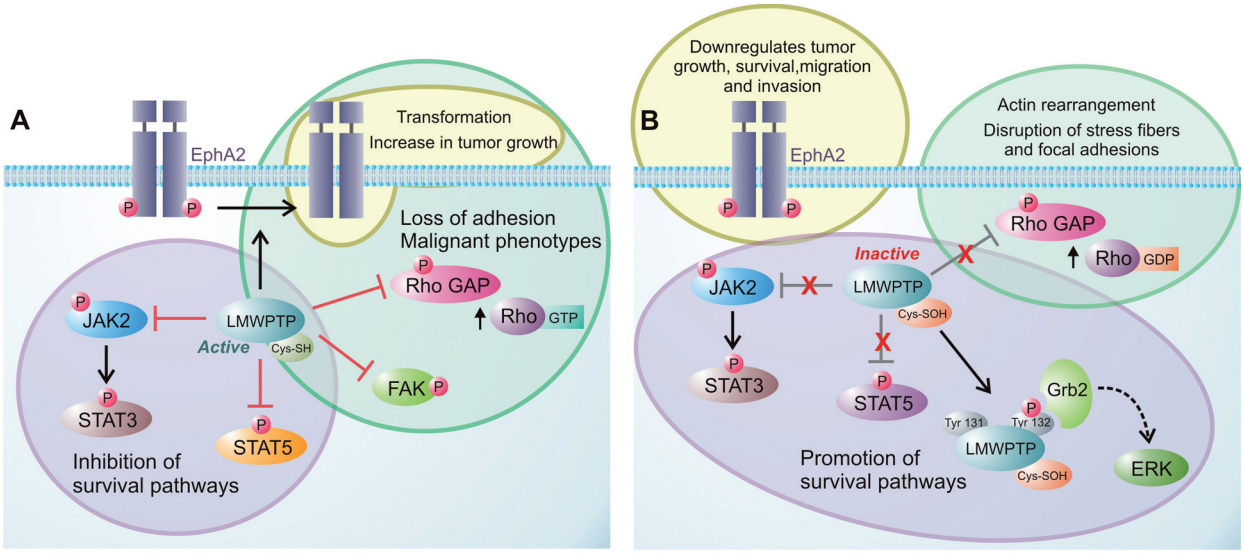

Figure 4. Effects of LMWPTP redox regulation on cancer cells. Since both active and oxidized forms of LMWPTP play essential and somewhat controversial roles in cancer cell signaling, we propose that these cells can use and benefit from the redox regulation and perhaps compartmentalization of LMWPTP functions. (*A*) Active LMWPTP dephosphorylates FAK, inhibiting integrin mediated adhesion. Inactivation of p190 RhoGAP increases Rho-GTP levels, leading to destabilization of adherens junctions. Dephosphorylated EphA2 promotes transformation and accelerates tumor growth and implantation *in vivo*. In contrast, dephosphorylation of JAK2 and STAT5 prevents activation of pro survival pathways. (*B*) Oxidized LMWPTP is unable to dephosphorylate and inactivate JAK2 and STAT5, increasing pro-survival signaling. Active p190 RhoGAP increases Rho-GDP levels, leading to actin rearrangement and disruption of stress fibers and focal adhesions. Oxidation of LMWPTP also prevents autodephosphorylation of Tyr132, which is essential for its interaction with Grb2 and consequent increase in ERK survival signaling. However, activated EphA2 dowregulates major tumoral processes.

mote cellular transformation [60] and accelerates both tumor growth as well as required implantation time *in vivo* [66]. EphA2 dephosphorylation is frequently related to loss of cellular adhesion [67] and the connection of this receptor with LMWPTP might be responsible for destabilizing adherens junctions. Fang and co-workers [68] suggested a possible mechanism in which LMWPTP, recruited by EphA2, prevents the phosphorylation of p190 RhoGAP, upregulating Rho-GTP levels, ultimately leading to destabilization of cell-cell adhesion. This and an earlier report [69] suggest that upregulation of LMWPTP leads to impairment of adhesion and more aggressive migratory cell phenotypes. Such cellular behavior is a well-known hallmark of invasive carcinoma. Therefore, high levels of LMWPTP are considered tumoral markers for aggressiveness and unfavorable prognosis [32, 55].

In apparent contrast, in oncogenic Ras transformed cells, oxidation of LMWPTP was required for disruption of stress fiber and focal adhesions. LMWPTP oxidation inhibits its action on p190 RhoGAP and consequently downregulates Rho [70–72]. Therefore, it is clear that the key role of LMWPTP in cell signaling is intricately associated with its ability to be reversibly regulated by oxidation. In fact, LMWPTP oxidation promotes pancreatic cancer (PaCa) cell survival via a JAK-STAT pathway. Lee et al. [57] have found that administration of insulin-like growth factor-1 (IGF-I) to PaCa cells increases generation of ROS by the NADPH oxidase Nox4, thus inhibiting LMWPTP. This, in turn, seems required to enhance and maintain JAK2 phosphorylation and consequently the activation of the JAK2-STAT1/3 pathway, an event essential for the antiapoptotic effects of IGF-I and serum. Another important observation is that LMWPTP colocalizes with Nox4 both in PaCa cells and in human pancreatic adenocarcinoma. Importantly, blocking constitutively activated STAT3 or STAT5 signaling leads to apoptosis of tumor cells [73] and STAT5 is a *bona fide* substrate for LMWPTP [58]. Given that the PTP oxidized state is catalytically inactive, increased ROS generation may contribute to increased STAT5 signaling via an inhibitory action on LMWPTP.

Another example of how oxidative inhibition of LMWPTP may promote cell survival is the Grb2-ERK pathway. As already described, LMWPTP p-Tyr132 serves as a scaffold for the SH2 domain of growth factor receptor-bound protein 2 (Grb2) which, in turn, promotes ERK activation. Oxidative inactivation of the PTP catalytic site prevents LMWPTP from auto-dephosphorylation, which causes an increase of Tyr132 phosphorylation and may induce pro-survival response [74].

Thus there is multiple evidence that LMWPTP is involved in carcinogenesis, although its influence can not be unequivocally classified as pro- or anti-

oncogenic. For various tumors, such as: neuroblastoma, breast and colon cancer – but not lung cancer – increased expression levels have been reported. Moreover, analysis of patient survival shows that higher LMWPTP levels are predictive of an unfavorable outcome [32]. At a molecular level, on the one hand its catalytic activity is important for dephosphorylating EphA2, p190 RhoGAP and FAK, supporting cellular transformation and possibly metastasis. On the other hand, LMWPTP oxidation and thus inactivation might be of importance in maintaining the activation of pro-survival pathways. Interestingly, cancer cells generally are under persistent oxidative stress [75, 76] and recent work has demonstrated that tumoral transformants show an increase in oxidized PTPs [77]. This may indicate that cancer cells could use the environmental redox status to their favor and benefit from LMWPTP redox regulation, since both activated and oxidized states are key players in the regulation of cell survival and adhesion pathways.

Inhibitors targeting LMWPTP

PTPs have recently been implicated in an increasing number of human diseases [3, 4, 78]. Not surprisingly, these enzymes attract widespread interest from both industry and academia as drug targets. To this end, potent and selective PTP inhibitors have been developed [3, 79, 80]. However, establishing how selective small-molecule inhibitors may be the main point to address in this field. Surface topological characterization by crystallization of different PTPs has added a new angle to the development of PTP inhibitors. Tautz et al. [79] demonstrated that the surface topology surrounding the catalytic site of each PTP has numerous unique features and therefore, this can be used for the rational design of selective inhibitors. Regarding LMWPTP, some inhibitors were designed based on the binding of adenine in the active site of the *S. cerevisiae* LTP1 and the inhibitory effect of this nucleotide on the human isoform A. However, these compounds did not present low inhibition constants [81]. We have shown that the flavonoid morin acts as a competitor inhibitor of LMWPTP, displaying an inhibitory constant at microM range [82]. Another natural compound, ferruginol, was able to inhibit the human recombinant LMWPTP and also inhibited this enzyme in prostate cancer treated with ferruginol [83]. Maccari et al., [84] synthesized different 4-(5-Arylidene-2,4-dioxothiazolidin-3-yl)methylbenzoic acids and demonstrated that two of them displayed preferentially inhibitory effect on LMWPTP isoform 1. However, from our point of view, none of the mentioned compounds can be considered specific LMWPTP inhibitors. Therefore, due to the wide-ranging functions of this phosphatase in diseases such as immune disorders and cancer, the development of specific inhibitors may prove useful. Additionally, potent and selective LMWPTP inhibitors will serve as powerful tools to delineate the function of this enzyme in normal and pathological conditions.

Conclusion

The combination of genetic data revealing the spectacular relation between genotype and LMWPTP activities levels, together with the increasing insight into its effects in immune and cancer cell signaling have led to an understanding of the fundamental role that PTP has in controlling appropriate immune responses and cell malignant transformation. In this context, pharmacological intervention of specific signal transduction mediators could prove effective for dealing with these diseases. A fascinating picture depicted in the regulation of LMWPTP, involving oxidation of cysteine residues and/or phosphorylation of tyrosine residues, has emerged, and this should allow rational design of activity-modulating compounds. These might prove highly useful to open novel avenues to treat immune system- and proliferation-based disease.

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