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Dead but not gone: The case for PRL as a pseudophosphatase

Abstract

Background: Protein phosphorylation and dephosphorylation is an integral component of many cellular signaling pathways and regulatory mechanisms. Phosphatases are enzymes that catalyze the removal of phosphate groups from proteins. The phosphatases of regenerating liver (PRLs) are a family of phosphatases which have been correlated with cancer development and metastasis. However, they appear to have weak phosphatase activity and little is known about their physiological substrates. This review discusses PRL from a structural and functional perspective, including recent findings on its interaction with another family of proteins, cyclin M (CNNM).

Methods: Articles were obtained from the scientific literature using databases like PubMed and McGill University's open access institutional repository. This paper specifically focuses on those articles that provided an overview of phosphatases, PRLs, CNNMs, and structural and functional studies of PRLs and CNNMs. In total, 40 articles were selected for the purpose of this review.

Summary: Although PRLs retain many of the structural features of other protein tyrosine phosphatases (PTPs) including the phosphatase catalytic motif and regulation via oxidation, other structural features such as mutation of a conserved serine/threonine residue to alanine in the active site disfavor catalytic activity. Moreover, PRL interaction with CNNM appears to be responsible for its oncogenic potential, yet this interaction does not appear to require PRL phosphatase activity. Thus, PRL may be best classified as a pseudophosphatase, which are phosphatase-like proteins that are structurally similar to phosphatases but have acquired a dominant function that does not require phosphatase activity.

Introduction

Protein phosphorylation is a key post-translational modification that is involved in the regulation of protein structure and function. Two classes of protein kinases, serine/threonine kinases and tyrosine kinases, catalyze the majority of phosphorylation reactions. (1) However, there are also two classes of protein phosphatases: protein serine/threonine phosphatases and protein tyrosine phosphatases (PTPs) (Fig. 1), which both mediate the dephosphorylation of proteins. (2) Both phosphorylation and dephosphorylation play critical roles in cellular signaling pathways, enzyme regulation, and protein diversity. (3)

Protein phosphatases are grouped into several classes, the largest of which are the PTPs. This class consists of the classical PTPs and the dual-specificity phosphatases (DUSPs), which can dephosphorylate both tyrosine and serine/threonine residues. (4) Examples of classical phosphatases include tyrosine-protein phosphatase non-receptor type 1 (PTP1B), which can dephosphorylate phospho-tyrosine sites on the insulin receptor kinase (5), and receptor-like protein-tyrosine phosphatase (RPTPα), which has been implicated in the activation of the protein-tyrosine kinase c-Src. (6) Members of the DUSPs include phosphatase and tensin homolog (PTEN), which dephosphorylates both polypeptide and phosphatidylinositol substrates (7), and VH1-related (VHR), which dephosphorylates phospho-tyrosine and phospho-threonine residues on c-Jun N-terminal kinase (JNK) to downregulate apoptosis-associated signaling. (8) The three phosphatases of regenerating liver (PRL1-3) are also classified as DUSPs, and although investigations have shown that PRL expression is correlated with various cancers (9, 10), there is no consensus on the physiological substrates of the PRLs. Detailed reviews of the PRLs in cancer and studies of PRL substrates can be found in Bessette et al. (11) and Rios et al. (12), respectively. Although PRLs share many similarities with the other DUSPs, there are key differences in their structure and function which suggest that PRLs may not function in vivo as phosphatases. This is supported by recent results that show another protein, cyclin M (CNNM), can bind to PRLs (13, 14) while structural studies of this interaction suggest that this interaction may not be dependent on PRL phosphatase activity. (15) In this review, I use structural and functional considerations to suggest that PRLs may be best classified as pseudophosphatases.

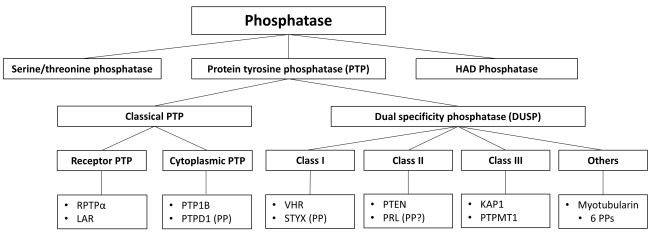

Fig. 1: Classification of protein phosphatases. Major families and subfamilies are shown with examples of phosphatases from each PTP subfamily. Those phosphatases which have been identified as pseudophosphatases are labelled in brackets with "PP". Although PRLs are not generally classified as pseudophosphatases, they share characteristics of pseudophosphatases: weak catalytic activity and a role in mediating protein-protein interactions.

Structure Characteristics of PRLs and the Other PTPs

One feature of cysteine-based PTPs like PRLs is the presence of the phosphatase signature motif, HCXXGXXR, in the active site of the enzyme. (7) The cysteine residue in this motif mediates a nucleophilic attack on the phosphate group attached to the substrate, forming a phospho-enzyme intermediate and releasing the dephosphorylated substrate. (16) A conserved aspartic acid residue in a neighboring WPD loop is involved in protonation of the dephosphorylated substrate and in subsequent activation of a water molecule by deprotonation, leading to regeneration of the

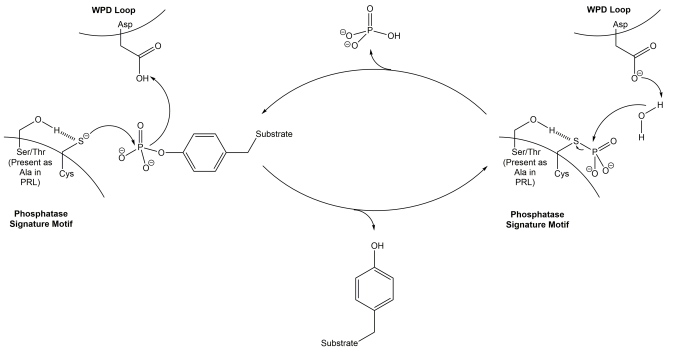

Fig. 2. Mechanism of dephosphorylation by PTPs. A conserved cysteine residue in the signature motif mediates nucleophilic attack and removal of the phosphate group from the substrate. Then, a conserved aspartate residue in the WPD loop activates a water molecule for regeneration of the active enzyme. A conserved serine/threonine residue in the catalytic pocket also stabilizes the catalytic cysteine residue.

active enzyme (Fig. 2). (17)

The size of the catalytic pocket of PTPs is also responsible for conferring substrate specificity. In PTP1B, the catalytic pocket is approximately 9 Å in depth, which corresponds well with the length of a phospho-tyrosine residue. (18) On the other hand, in the case of most DUSPs, the catalytic pocket is relatively shallow, and this feature is thought to confer the ability to dephosphorylate the shorter phospho-serine and phospho-threonine residues. PRLs have particularly shallow catalytic pockets, with the pocket of PRL-3 being the shallowest of the known phosphatases. (19) Conversely, PTEN has a large catalytic pocket even though it is a DUSP, and this is consistent with PTEN's ability to dephosphorylate the larger PI(3,4,5)P3 substrate. (7) Thus, the depth of the catalytic pocket is indicative of the size of the phosphatase substrate, and in regard to PRL, the relatively shallow pocket appears to predict broad substrate specificity.

The physical and chemical characteristics of the residues in and around the active site are also responsible for PTP substrate specificity. For example, PTP1B is known to dephosphorylate the insulin receptor kinase at a region with tandem phospho-tyrosine residues. (20) While the first phospho-tyrosine interacts with the active site of PTP1B, another groove in PTP1B near the active site binds to the second phospho-tyrosine residue, thus conferring greater affinity for the tandemly phosphorylated site in insulin receptor kinase. (20) Interestingly, the G129E mutation of PTEN, observed in Cowden syndrome (18), abolishes lipid phosphatase activity but not protein phosphatase activity. (21) Thus, G129 appears to be responsible for the distinctive ability of PTEN to dephosphorylate PI(3,4,5)P3. The active site of PRLs, unlike the basic active sites of other DUSPs like PTEN and VHR, is unusually hydrophobic. (19) Although this predicts a preference for hydrophobic substrates, there has been no conclusive evidence identifying such substrates of PRLs.

One feature particular to PRLs is their slow enzymatic activity, which is thought to be the result of a mutation of the conserved serine/threonine in the PTP active site to alanine in PRLs (Fig. 2). (19) This eliminates the stabilizing hydrogen bonding interactions that normally occur between the serine/threonine and the catalytic thiolate intermediate (Fig. 2). (22) Therefore, the phosphatase activity of PRLs appears to be either intrinsically low or binding to physiological substrates may be required for sufficient PRL enzymatic activity. Studies with PRL-1 and PRL-3 also show that regeneration of the enzyme is the rate-limiting step, resulting in a relatively long-lived phospho-enzyme intermediate. (19, 23) In fact, due to its stability, this phospho-enzyme intermediate may be better viewed as a phosphorylated variant of PRL, where phosphorylation acts as a post-translational modification rather than as a transient step in the catalytic mechanism.

Like many of the other PTPs and DUSPs, PRL appears to be regulated by redox reactions that occur at its catalytic cysteine residue. Several studies have demonstrated that PRLs can form an intramolecular disulfide bond between its catalytic cysteine residue and a neighboring cysteine residue (C49 in PRL-1 and PRL-3, C46 in PRL-2). (13, 14, 19, 23) Similar disulfide bond formation in other DUSPs like KAP (24) and PTEN (25) is associated with a loss of catalytic activity. One contributing factor to the prevalence of the disulfide bond is the comparatively low pKa of the catalytic cysteine residue in the active site, which leaves the cysteine prone to oxidation. (26) In fact, even in PTPs where disulfide bond formation is not observed, oxidation of the catalytic cysteine still occurs. In PTP1B, the catalytic cysteine reacts with the backbone nitrogen of the adjacent serine residue to form a sulfenyl-amide bond. (27, 28) This sulfenyl-amide is thought to prevent irreversible oxidation and inactivation of the catalytic cysteine to the sulfinic or sulfonic acid species. Thus, oxidation of PTPs, although inactivating catalytic activity, also serves a protective role.

The structural features of PRLs, particularly the mutation of the conserved PTP serine/threonine residue to alanine, the shallow catalytic pocket, and the susceptibility of the catalytic cysteine residue to oxidation, predict weak and indeterminate phosphatase activity. Thus, based on structural considerations, PRL does not appear to be particularly suited to function as a phosphatase. However, as will be seen later, this does not mean that PRL cannot act at all as a phosphatase, and only with consideration of functional properties does PRL's status as a pseudophosphatase become clear.

PRLs as Pseudophosphatases

Pseudophosphatases are broadly defined as phosphatases that lack catalytic activity. In the majority of cases, this is due to the mutation of conserved residues of the phosphatase signature motif. For example, in the pseudophosphatase, STYX, the catalytic cysteine is replaced by a glycine residue. (29) Moreover, the presence of tandem PTP domains with an active domain closer to the membrane (D1) and an inactive pseudophosphatase domain farther from the membrane (D2) is conserved throughout the receptor protein tyrosine phosphatases (RPTPs). Although the function of the D2 domain has not been well-characterized, it is thought to have a role in regulation of the D1 domain. In LAR, the D2 domain can lower the catalytic activity of the D1 domain and can regulate the type of substrates to which the D1 domain can bind. (30) In contrast, in another RPTP found in Drosophila, PTP99A, the presence of the D2 domain augments the catalytic activity of the D1 domain. (30) Moreover, the D2 domain appears to have greater sensitivity to oxidation than the D1 domain, which postulates a redox-sensing role for this domain in the function of RPTPs. (31) Although pseudophosphatases may not have catalytic activity, their structural similarity to active phosphatases makes them susceptible to many of the regulatory modifications, such as oxidation, seen in active phosphatases.

Although pseudophosphatases have been classified as so because of lack of catalytic activity, this criterion is not very robust. Pseudophosphatases that lack catalytic activity in vivo can show catalytic activity in vitro or vice versa. (32) Moreover, some pseudophosphatases such as PTPN14 do not have a mutation at the catalytic cysteine residue, and their classification as pseudophosphatases is primarily due to undetected phosphatase activity. (33) This does not exclude the existence of physiologically relevant substrates, and another possibility is that the protein may have a dominant function that does not involve phosphatase activity. Further studies have found that PTPN14 binds in a phosphatase-independent manner to another oncoprotein, YAP1, in order to inhibit its activity. (34) Therefore, an alternative classification of a pseudophosphatase would not focus solely on lack of catalytic activity, but rather on the dominance of some other function of the phosphatase, such as protein-protein interaction, over the expected phosphatase activity of the enzyme.

Like PTPN14, PRLs also have low in vitro phosphatase activity even though there is no mutation of the catalytic cysteine residue. (15, 19) The substitution of the conserved serine/threonine residue in the phosphatase signature motif of other PTPs to alanine in PRL may confer such low enzymatic activity (15, 19) that PRL phosphatase activity becomes essentially nonexistent. Recent studies are now revealing that PRL binding and inhibition of a class of putative magnesium transporters, CNNMs, may be the predominant PRL activity in cells. (13, 14) Features which were thought to be a consequence of PRL phosphatase activity such as the long-lived phos-

phorylated intermediate and redox regulation now appear to responsible for mediating the binding of PRLs to CNNMs.

PRL and CNNM: The Developing Story

CNNMs were originally termed ancient conserved domain proteins (ACDP). The family has four members (CNNM1-CNNM4), and each member consists of a transmembrane region, a cystathionine β-synthase pair (CBS-pair) domain, a cyclin box motif, and a cNMP-binding domain. (35) The presence of the cyclin box motif initially suggested these proteins were involved in regulation of the cell cycle, hence the name, cyclin M (CNNM). (35) The role of these proteins, however, in the cell cycle has not been fully characterized, and the CNNM proteins have been found to localize primarily to the plasma membrane. (36) Homology to Mg^{2+} transport proteins like CorC and MgtE along with recent functional studies postulate a Mg^{2+} transport role for the CNNM family. (14, 19, 36, 37) There is disagreement, however, about whether CNNM mediates Mg^{2+} efflux (38) or Mg^{2+} influx (14). Nonetheless, experiments show that PRL can bind to CNNM and that this protein-protein interaction causes an increase in intracellular Mg^{2+} levels by either inhibiting Mg^{2+} efflux (13) or by stimulating Mg^{2+} influx (14). Since Mg^{2+} is linked to cellular energy state and to the activation of processes that lead to cancer development (39), PRL binding to CNNM and subsequent increase in intracellular Mg^{2+} affords one explanation for the correlation between PRL and cancer. (9, 10) Notably, this explanation does not require the phosphatase activity of PRL.

Results from structural studies reveal that PRL binds to CNNM via its phosphatase catalytic pocket. (15) Since this interaction effectively blocks off the PRL catalytic pocket, it is unlikely that PRL would be able to function as a phosphatase for other substrates when bound to CNNM. Furthermore, the binding between PRL and CNNM can be modulated by oxidation of the PRL catalytic cysteine residue since a decrease in binding affinity is observed when PRL is placed in oxidizing conditions. (11, 12) These results suggest that structural modifications on PRL traditionally associated with its role as a phosphatase may also influence its interaction with CNNM. Further studies are needed to determine if PRL catalytic activity is dispensable in its biological function.

Conclusion

The term 'pseudophosphatase', while formally denoting those phosphatases that lack catalytic activity, is in reality ambiguous and often open to interpretation in its application. There are examples of 'pseudophosphatases' that show catalytic activity towards small molecule substrates yet not towards larger synthetic peptides. (40) Moreover, it is not entirely accurate to infer catalytic activity using synthetic substrates. The structure of many phosphatases require specific interactions with properly shaped physiological substrates before manifestation of any catalytic activity.

On the other hand, there are phosphatases like PRL for which it appears some other function has taken precedence over phosphatase activity. The term 'pseudophosphatase' would best be applied to these proteins. For one, they may still retain catalytic activity, which in the case of PRL is observable in vitro albeit very slow. More importantly though, this classification would come with a recognition that this group of phosphatases, while similar to traditional protein phosphatases in structure, have a completely different function. For PRL, this function appears to come from its interactions with CNNM, a class of ion transporters. From what is currently known, PRL interaction with CNNM could potentially be a significant player in regulation of cellular energy state through Mg^{2+} levels. Thus, PRLs, once viewed as laggard members of the PTP family, have now been shown to have a completely different side - one which promises to be a source of intrigue in the future.

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References

1. Hanks S, Quinn A, Hunter T. The protein kinase family: conserved features and deduced phylogeny of the catalytic domains. Science. 1988;241(4861):42-52.
2. Tonks NK. Protein tyrosine phosphatases–from housekeeping enzymes to master regulators of signal transduction. Febs Journal. 2013;280(2):346-78.
3. Hunter T. Protein kinases and phosphatases: The Yin and Yang of protein phosphorylation and signaling. Cell. 1995;80(2):225-36.
4. Kharitidi D, Manteghi S, Pause A. Pseudophosphatases: methods of analysis and physiological functions. Methods. 2014;65(2):207-18.
5. Seely BL, Staubs PA, Reichart DR, Berhanu P, Milarski KL, Saltiel AR, et al. Protein tyrosine phosphatase 1B interacts with the activated insulin receptor. Diabetes. 1996;45(10):1379-85.
6. Tracy S, van der Geer P, Hunter T. The Receptor-like Protein-tyrosine Phosphatase, RPTP, Is Phosphorylated by Protein Kinase C on Two Serines Close to the Inner Face of the Plasma Membrane. Journal of Biological Chemistry. 1995;270(18):10587-94.
7. Lee JO, Yang H, Georgescu MM, Di Cristofano A, Maehama T, Shi Y, et al. Crystal structure of the PTEN tumor suppressor: implications for its phosphoinositide phosphatase activity and membrane association. Cell. 1999;99(3):323-34.
8. Todd JL, Rigas JD, Rafty LA, Denu JM. Dual-specificity protein tyrosine phosphatase VHR down-regulates c-Jun N-terminal kinase (JNK). Oncogene. 2002;21(16):2573-83.
9. Bardelli A, Saha S, Sager JA, Romans KE, Xin B, Markowitz SD, et al. PRL-3 expression in metastatic cancers. Clinical Cancer Research. 2003;9(15):5607-15.
10. Wang Q, Holmes DI, Powell SM, Lu QL, Waxman J. Analysis of stromal-epithelial interactions in prostate cancer identifies PTPCAAX2 as a potential oncogene. Cancer Letters. 2002;175(1):63-9.
11. Bessette D, Qiu D, Pallen C. PRL PTPs: mediators and markers of cancer progression. Cancer Metastasis Rev. 2008;27(2):231-52.
12. Rios P, Li X, Köhn M. Molecular mechanisms of the PRL phosphatases. FEBS Journal. 2013;280(2):505-24.
13. Funato Y, Yamazaki D, Mizukami S, Du L, Kikuchi K, Miki H. Membrane protein CNNM4–dependent Mg^{2+} efflux suppresses tumor progression. The Journal of Clinical Investigation. 2014;124(12):5398-410.
14. Hardy S, Uetani N, Wong N, Kostantin E, Labbe DP, Begin LR, et al. The protein tyrosine phosphatase PRL-2 interacts with the magnesium transporter CNNM3 to promote oncogenesis. Oncogene. 2015;34(8):986-95.
15. Gulerez IE. Structural Characterization of the Complex between the Oncogenic Phosphatase PRL2 and the CBS pair domain of the Magnesium Transporter CNNM3. McGill University; 2015.
16. Gulerez IE, Gehring K. X-ray crystallography and NMR as tools for the study of protein tyrosine phosphatases. Methods. 2014;65(2):175-83.
17. Andersen JN, Mortensen OH, Peters GH, Drake PG, Iversen LF, Olsen OH, et al. Structural and Evolutionary Relationships among Protein Tyrosine Phosphatase Domains. Molecular and Cellular Biology. 2001;21(21):7117-36.
18. Jia Z, Barford D, Flint AJ, Tonks NK. Structural basis for phosphotyrosine peptide recognition by protein tyrosine phosphatase 1B. Science. 1995;268(5218):1754-8.
19. Kozlov G, Cheng J, Ziomek E, Banville D, Gehring K, Ekiel I. Structural insights into molecular function of the metastasis-associated phosphatase PRL-3. The Journal of Biological Chemistry. 2004;279(12):11882-9.
20. Salmeen A, Andersen JN, Myers MP, Tonks NK, Barford D. Molecular basis for the dephosphorylation of the activation segment of the insulin receptor by protein tyrosine phosphatase 1B. Molecular Cell. 2000;6(6):1401-12.
21. Myers MP, Pass I, Batty IH, Van der Kaay J, Stolarov JP, Hemmings BA, et al. The lipid phosphatase activity of PTEN is critical for its tumor supressor function. Proceedings of the National Academy of Sciences of the United States of America. 1998;95(23):13513-8.
22. Denu JM, Dixon JE. A catalytic mechanism for the dual-specific phosphatases. Proceedings of the National Academy of Sciences of the United States of America. 1995;92(13):5910-4.
23. Sun JP, Wang WQ, Yang H, Liu S, Liang F, Fedorov AA, et al. Structure and biochemical properties of PRL-1, a phosphatase implicated in cell growth, differentiation, and tumor invasion. Biochemistry. 2005;44(36):12009-21.
24. Song H, Hanlon N, Brown NR, Noble MEM, Johnson LN, Barford D. Phosphoprotein–Protein Interactions Revealed by the Crystal Structure of Kinase-Associated Phosphatase in Complex with PhosphoCDK2. Molecular

Cell. 2001;7(3):615-26.
25. Lee SR, Yang KS, Kwon J, Lee C, Jeong W, Rhee SG. Reversible inactivation of the tumor suppressor PTEN by H2O2. The Journal of Biological Chemistry. 2002;277(23):20336-42.
26. Tonks NK. Protein tyrosine phosphatases: from genes, to function, to disease. Nat Rev Mol Cell Biol. 2006;7(11):833-46.
27. Salmeen A, Andersen JN, Myers MP, Meng T-C, Hinks JA, Tonks NK, et al. Redox regulation of protein tyrosine phosphatase 1B involves a sulphenyl-amide intermediate. Nature. 2003;423(6941):769-73.
28. van Montfort RLM, Congreve M, Tisi D, Carr R, Jhoti H. Oxidation state of the active-site cysteine in protein tyrosine phosphatase 1B. Nature. 2003;423(6941):773-7.
29. Wishart MJ, Denu JM, Williams JA, Dixon JE. A Single Mutation Converts a Novel Phosphotyrosine Binding Domain into a Dual-specificity Phosphatase. Journal of Biological Chemistry. 1995;270(45):26782-5.
30. Madan LL, Veeranna S, Shameer K, Reddy CCS, Sowdhamini R, Gopal B. Modulation of Catalytic Activity in Multi-Domain Protein Tyrosine Phosphatases. PLoS ONE. 2011;6(9):e24766.
31. Persson C, Sjöblom T, Groen A, Kappert K, Engström U, Hellman U, et al. Preferential oxidation of the second phosphatase domain of receptor-like PTP-α revealed by an antibody against oxidized protein tyrosine phosphatases. Proceedings of the National Academy of Sciences of the United States of America. 2004;101(7):1886-91.
32. Reiterer V, Eyers PA, Farhan H. Day of the dead: pseudokinases and pseudophosphatases in physiology and disease. Trends in Cell Biology. 2014;24(9):489-505.
33. Zhu JH, Chen R, Yi W, Cantin GT, Fearns C, Yang Y, et al. Protein tyrosine phosphatase PTPN13 negatively regulates Her2/ErbB2 malignant signaling. Oncogene. 2007;27(18):2525-31.
34. Wang W, Huang J, Wang X, Yuan J, Li X, Feng L, et al. PTPN14 is required for the density-dependent control of YAP1. Genes & Development. 2012;26(17):1959-71.
35. Wang C-Y, Shi J-D, Yang P, Kumar PG, Li Q-Z, Run Q-G, et al. Molecular cloning and characterization of a novel gene family of four ancient conserved domain proteins (ACDP). Gene. 2003;306:37-44.
36. de Baaij JHF, Stuiver M, Meij IC, Lainez S, Kopplin K, Venselaar H, et al. Membrane Topology and Intracellular Processing of Cyclin M2 (CNNM2). Journal of Biological Chemistry. 2012;287(17):13644-55.
37. Corral-Rodriguez MA, Stuiver M, Abascal-Palacios G, Diercks T, Oyenarte I, Ereno-Orbea J, et al. Nucleotide binding triggers a conformational change of the CBS module of the magnesium transporter CNNM2 from a twisted towards a flat structure. The Biochemical Journal. 2014;464(1):23-34.
38. Yamazaki D, Funato Y, Miura J, Sato S, Toyosawa S, Furutani K, et al. Basolateral Mg^{2+} Extrusion via CNNM4 Mediates Transcellular Mg^{2+} Transport across Epithelia: A Mouse Model. PLoS Genet. 2013;9(12):e1003983.
39. Castiglioni S, Maier JA. Magnesium and cancer: a dangerous liason. Magnesium Research. 2011;24(3):92-100.
40. Barr AJ, Ugochukwu E, Lee WH, King ON, Filippakopoulos P, Alfano I, et al. Large-scale structural analysis of the classical human protein tyrosine phosphatome. Cell. 2009;136(2):352-63.