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REVIEW ARTICLE

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Phosphatase-independent role of phosphatase of regenerating liver in cancer progression

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Abstract

Phosphatase of regenerating liver (PRL) is a family of protein tyrosine phosphatases (PTPs) that are anchored to the plasma membrane by prenylation. They are frequently overexpressed in various types of malignant cancers and their roles in cancer progression have received considerable attention. Mutational analyses of PRLs have shown that their intrinsic phosphatase activity is dispensable for tumor formation induced by PRL overexpression in a lung metastasis model using melanoma cells. Instead, PRLs directly bind to cyclin M (CNNM) Mg^{2+} exporters in the plasma membrane and potently inhibit their Mg^{2+} export activity, resulting in an increase in intracellular Mg^{2+} levels. Experiments using mammalian culture cells, mice, and *C. elegans* have collectively revealed that dysregulation of Mg^{2+} levels severely affects ATP and reactive oxygen species (ROS) levels as well as the function of Ca^{2+}-permeable channels. Moreover, PRL overexpression altered the optimal pH for cell proliferation from normal 7.5 to acidic 6.5, which is typically observed in malignant tumors. Here, we review the phosphatase-independent biological functions of PRLs, focusing on their interactions with CNNM Mg^{2+} exporters in cancer progression.

KEYWORDS
acid addiction, cancer progression, cyclin M, Mg^{2+}, phosphatase of regenerating liver

1 | CANCER PROGRESSION BY PRL AND ITS PHOSPHATASE-INDEPENDENT ROLE

1.1 | PRL, an enigmatic phosphatase

PRL-1 was discovered as a unique protein tyrosine phosphatase (PTP) highly expressed in mitogen-stimulated cells and regenerating liver.[1] Mammals have two additional similar proteins, PRL-2 and PRL-3; collectively, these three PRL family proteins share 79–88% sequence identity. Structurally, they possess a single phosphatase domain followed by a C-terminal tail with a CAAX motif (Figure 1A). Through farnesylation at the motif, PRLs are anchored to the plasma membrane,[2] which is a unique feature among all PTPs. Sequence alignment shows the conservation of the active center Cys residue (Figure 1B), which plays a catalytically essential role by removing phosphates from the substrates and forming covalent Cys-phosphate reaction intermediates. In most PTPs, this Cys-phosphate conjugate is rapidly hydrolyzed to release

Abbreviations: AMPK, AMP-activated protein kinase; CatSper, cation channels of sperm; CBS, cystathionine-β-synthase; CNNM, cyclin M; CRISPR, clustered regularly interspaced short palindromic repeats; DUF21, domain of unknown function 21; EGFR, epidermal growth factor receptor; mTOR, mammalian target of rapamycin; NASH, non-alcoholic steatohepatitis; PRL, phosphatase of regenerating liver; PTP, protein tyrosine phosphatase; ROS, reactive oxygen species; TRPML, transient receptor potential mucolipin; TRPV1, transient receptor potential vanilloid 1.

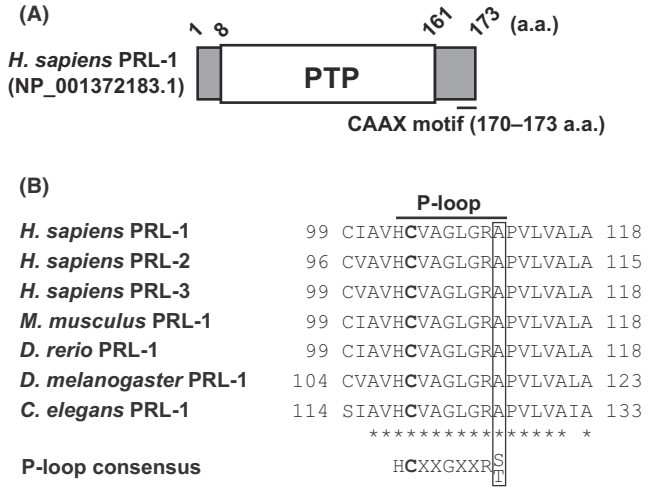

FIGURE 1 Domain structure and sequence alignment of PRL. (A) Domain structure of human PRL-1 protein. The regions for PTP domain and CAAX motif are indicated by the box and black bar, respectively. The amino acid residue numbers are also indicated. (B) Sequence alignment of the amino acid region surrounding the catalytic center Cys residue of various PRL proteins. The accession numbers of each protein are: *H. sapiens* PRL-1: NP_001372183.1, *H. sapiens* PRL-2: NP_001356788.1, *H. sapiens* PRL-3: NP_116000.1, *M. musculus* PRL-1: NP_035330.1, *D. rerio* PRL-1: NP_001007776.2, *D. melanogaster* PRL-1: NP_723956.1, and *C. elegans* PRL-1: NP_001379679.1. The region of phosphate-binding (P) loop, which directly associates with the phosphate moiety in substrates, is indicated by the black bar, with the consensus sequence shown at the bottom. Catalytic Cys residues are shown in bold letters. Consensus Ser/Thr residues, replaced with Ala in PRL proteins, are boxed. The residues conserved among all listed proteins are indicated with asterisks.

phosphates, with contributions from several conserved residues in the phosphatase domain. However, the Ser/Thr residue, which is involved in phosphate release from Cys-phosphate, is replaced with Ala in all PRL family proteins from worms (*Caenorhabditis elegans*) to humans (Figure 1B). Therefore, the Cys-phosphate reaction intermediate is unusually stable in PRLs, perturbing the catalytic cycle progression.[3] Details of this inefficient catalysis of PRLs and the functional importance of Cys-phosphate have recently been extensively reviewed by Gehring et al.[4] Here, we focus on the phosphatase-independent biological functions, with an emphasis on tumorigenic function of PRLs in the following sections.

1.2 | Promotion of cancer malignancy by PRL overexpression

The relationship between PRLs and cancers came to attention with the report of specific overexpression of PRL-3 in colorectal cancer metastases.[5] PRL-3 overexpression was subsequently found to occur in many types of cancers and to intimately correlate with their malignancy and poor prognosis.[6] Overexpression of PRL-1 and PRL-2 in malignant cancers has also been reported, although less frequently.[7,8] Consistent with these studies on clinical samples, experimental analyses using various cultured cancer cells, such as CHO cells, B16 cells, and LoVo cells, showed that ectopic overexpression of PRL-1 and PRL-3 made these cells more tumorigenic when inoculated into mice.[9–12] Furthermore, genetic disruption of PRL-3 in mice suppressed tumor formation by ~50% in a colitis-associated colon cancer model.[13] These studies using cultured cancer cells and mice collectively established the concept that PRL overexpression actively promotes cancer progression. The molecular mechanisms underlying PRL overexpression are complex and cannot be explained in a unified manner. The first report of PRL-3 overexpression in colorectal cancer metastases showed the occurrence of gene amplification,[5] but it was later found to be a minor cause; rather, overexpression is considered to be driven at multiple levels via regulation of the synthesis and stability of PRL mRNAs and proteins.[14]

On the contrary, PRL-3 overexpression in mouse embryonic fibroblasts was reported to result in cell cycle arrest at the G1 phase.[15] Moreover, experiments using MDCK epithelial cells showed that PRL-3 overexpression can stimulate cell death when cells are placed under moderately alkaline conditions[16] or reached confluency.[17] Therefore, the oncogenic function of PRLs seems to be dependent on cell type or the conditions surrounding them.

1.3 | Direct interaction with cyclin M (CNNM) proteins

A crucial discovery for understanding the molecular action of PRLs was the identification of CNNM proteins as direct and major binding partners for PRLs.[18,19] CNNMs are a family of membrane proteins that mediate Mg^{2+} export across the plasma membrane,[20,21] and are characterized by the presence of a tandemly aligned set of a membrane-spanning domain of unknown function 21 (DUF21) and a cytosolic cystathionine-β-synthase (CBS) domain (Figure 2A). There are four family proteins having the DUF21-CBS domain set structure in mammals, namely CNNM1, CNNM2, CNNM3, and CNNM4, and orthologous proteins are found even in bacteria and archaea. It should be noted that, whether CNNMs are direct Mg^{2+} transporters or are indirectly involved in Mg^{2+} level regulation, was under debate.[22,23] Recently, crystal structures containing the membrane-spanning DUF21 domain have been solved for two bacterial CNNM orthologs, CorB[24] and CorC.[25] These proteins formed a homodimer, and in each protomer, three membrane-spanning α-helices collectively formed a cavity for recognizing a single Mg^{2+} (Figure 2B). In addition, reconstitution experiments using recombinant CorB proteins embedded in liposomes have shown Mg^{2+} transport activity. Considering that the amino acid residues involved in Mg^{2+} recognition are conserved in mammalian CNNMs (Figure 2C), they should also directly mediate Mg^{2+} transport across the plasma membrane.

All PRLs directly bind to the cytoplasmic CBS domains in all CNNMs.[18] Crystal structures for the PRL-CNNM complex have

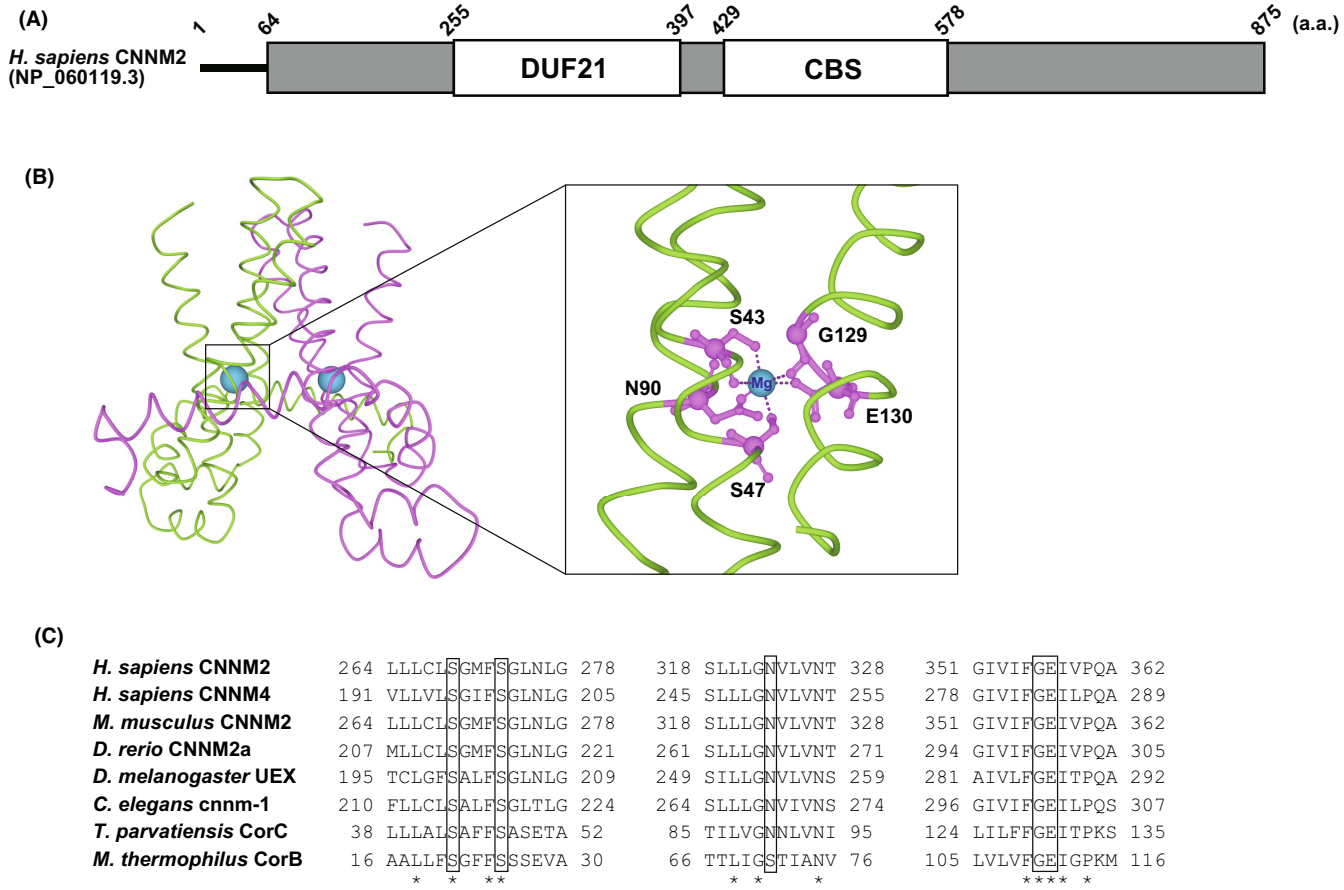

FIGURE 2 Structure and alignment of CNNM and its orthologs. (A) Domain structure of human CNNM2 protein. The region of the signal peptide, which is necessary for co-translational entry into endoplasmic reticulum (ER) and cleaved off in the mature protein at the plasma membrane, is indicated with a black line. DUF21 and CBS domains are indicated with white boxes. The amino acid residue numbers are also indicated. (B) Ribbon diagram of the 3D structure of DUF21 domain of *T. parvatiensis* CorC in the Mg^{2+}-bound dimer form [from the data deposited at protein data bank (PDB), ID: 7CFG]. An enlarged view of the Mg^{2+}-binding site is shown on the right side. Light blue sphere indicates the Mg^{2+} ion, and the electrostatic interactions formed with the surrounding amino acids are shown by dashed lines. (C) Sequence alignment of the amino acid region surrounding the Mg^{2+}-binding sites of CNNM and its orthologs. The accession numbers of each protein are as follows, *H. sapiens* CNNM2: NP_060119.3, *H. sapiens* CNNM4: NP_064569.3, *M. musculus* CNNM2: NP_291047.2, *D. rerio* CNNM2a: NP_001138257.1, *D. melanogaster* UEX: NP_001104391.2, *C. elegans* CNNM-1: NP_503052.1, *T. parvatiensis* CorC: WP_060384576.1, and *M. thermophilus* CorB: SDJ87353.1. The residues involved in Mg^{2+} binding are boxed. The residues conserved among all listed proteins are indicated with asterisks.

also been solved, showing a unique mode of interaction: a short polypeptide loop extending from the CBS domains is captured in the shallow cavity in the PRL catalytic pocket like a pseudo-substrate (Figure 3).[26–28] As described above, overexpressed CNNMs in cultured cells can induce Mg^{2+} efflux from cells, but co-expression of PRLs almost completely suppressed Mg^{2+} efflux.[18] Consistent with the functional inhibition of CNNMs, RNAi knockdown of endogenous *PRL-1* in HEK293 cells decreased intracellular Mg^{2+} levels.[18] Furthermore, when endogenous *PRL-1* and *PRL-2* were simultaneously knocked down in HeLa cells, the cells became vulnerable to Mg^{2+} depletion from the medium.[29] These results implicate the role of PRLs in the regulation of intracellular Mg^{2+} levels, indicating a tight functional connection with CNNM Mg^{2+} transporters.

1.4 | Phosphatase-independent oncogenic function

The role of CNNMs in oncogenesis has been demonstrated in mice by gene disruption analyses of *CNNM4*, which is highly expressed in intestinal epithelial cells.[20] *CNNM4* disruption by itself did not cause tumor formation, but studies using two different mouse models for intestinal tumors, genetic disruption of one *APC* allele, and oral administration of mutagenic and inflammatory chemicals showed that *CNNM4* disruption promoted malignant progression of cancers.[18,30] CNNM4 expression level was inversely correlated with malignancy in human colon cancers. These results suggest that CNNMs suppress cancer malignancy; however, whether PRL overexpression promotes oncogenesis by inhibiting the functions of CNNMs remains to be determined.

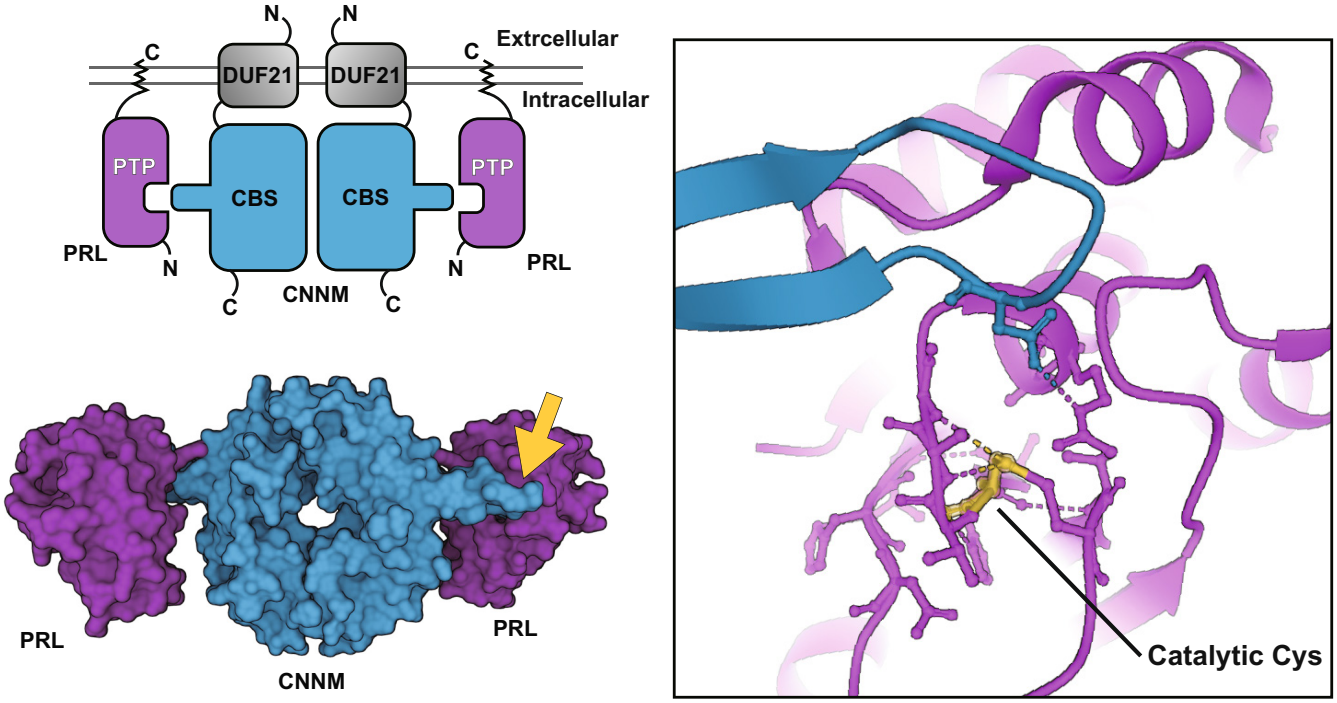

FIGURE 3 3D structure of PRL-CNNM binding site. The cartoon model (top left) and surface model (bottom left, from the data deposited at PDB ID: 5MMZ) of the complex between PRL1 and CNNM2. The CNNM CBS and PRL PTP domains are colored in blue and purple, respectively. The yellow arrow indicates the binding site between the short polypeptide loop extending from the CNNM CBS domain and cavity of the PRL catalytic pocket. An enlarged view of the binding site is also shown (right). The catalytic Cys residue in yellow is located in close proximity to the protein–protein interface. The dashed lines indicate electrostatic interactions.

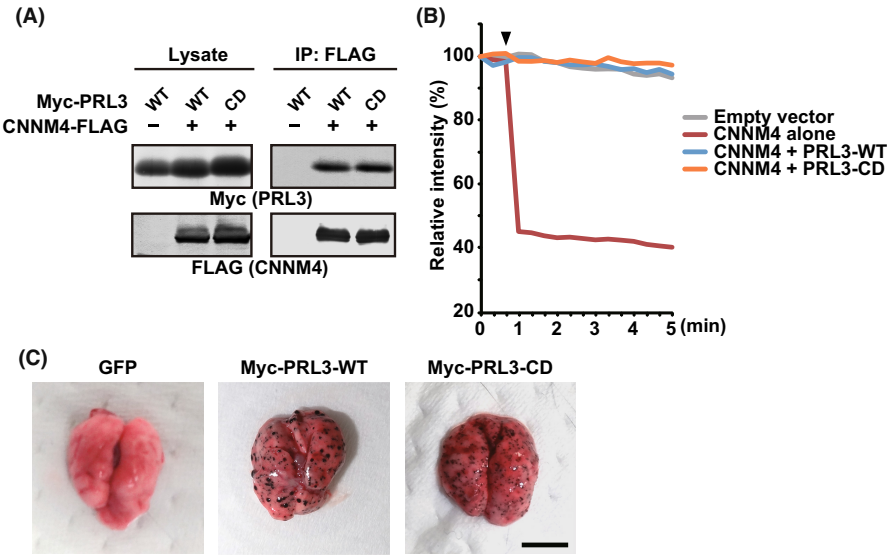

FIGURE 4 Phosphatase-independent function of PRL. (A) Co-immunoprecipitation (IP) analyses of Myc-PRL3 [wild-type (WT) and C104D mutant (CD)] and CNNM4-FLAG. Replacement of catalytic Cys104 to Asp (CD mutation) in PRL3 did not affect the interaction with CNNM4. (B) Mg^{2+} efflux assay using fluorescence Mg^{2+} indicator, Magnesium Green. Decrease in fluorescence after Mg^{2+} depletion (arrowhead) was evaluated. Co-transfection of CD mutant form of PRL3 suppressed the Mg^{2+} efflux function of CNNM4 as effectively as the WT PRL3. (C) Mouse melanoma B16 cells stably expressing Myc-PRL3 (WT and CD mutant) were injected into the tail vein of syngenic mice and metastatic tumor nodules formed on the lungs were evaluated. Stable expression of CD mutant PRL3 augmented the tumor nodule formation the same as WT PRL3. Bar, 5 mm. This research was originally published in the Journal of Biological Chemistry.[31] © Kozlov et al.

PRLs possess a Cys-based phosphatase domain, and in vitro assays using purified recombinant PRL proteins and artificial chemical substrates showed a very weak but significant phosphatase activity.[3] Generally, mutants for the catalytic Cys have been used to investigate the functional importance of phosphatase activity, but the replacement of Cys with Ser in PRLs also abolished the interaction

with CNNMs.[18,19] The crystal structures of the PRL-CNNM complex showed that the catalytic Cys is located in close proximity to the protein–protein interface (Figure 3),[26] and thus, the perturbation introduced by the replacement with Ser was thought to interfere with the protein–protein interaction. To discriminate which of the two functions, the phosphatase activity or the CNNM-binding activity, is important for promoting oncogenesis, the identification of mutations that can selectively kill phosphatase activity is desirable. This was achieved by Kozlov et al.,[31] who found that replacement of Cys with Asp (CD mutation) in PRL-3 did not affect the PRL-CNNM interaction (Figure 4A). The CD mutant form of PRL-3 effectively suppressed the Mg^{2+} transport function of CNNM4 in cells (Figure 4B) and, more importantly, promoted tumor formation when stably expressed in B16 melanoma cells, as did wild-type PRL-3 (Figure 4C). These results from experiments using the CD mutant unambiguously demonstrate that phosphatase activity is dispensable for PRL-3 to promote oncogenesis, thus rather placing the CNNMs as the functional targets of PRLs.

It should be noted that there are reports showing some effects at the cellular level by PRL-3, such as changes in metabolic status and sphere-forming ability, can be also triggered by its Cys to Ser mutant, lacking both phosphatase and CNNM-binding activities.[32,33] Although their importance on cancer progression is unknown and thus will not be further discussed in this review, these reports suggest the existence of other phosphatase-independent functions of PRL besides CNNM inhibition.

2 | MOLECULAR MECHANISM OF CANCER PROGRESSION BY PRL

2.1 | Concomitant elevation of intracellular ATP and ROS levels with Mg^{2+}

To clarify the mechanism underlying the cancer progression triggered by CNNM inhibition, a series of experiments using mammalian culture cells, mice and mouse-derived cells, and *C. elegans* were conducted, which led to the identification of several key molecules affected by CNNM inhibition (Figure 5). In particular, multiple studies have shown the importance of elevated intracellular ATP levels.

Most part of the intracellular Mg^{2+} is bound to other intracellular molecules, primarily ATP.[34] Increase in intracellular Mg^{2+} levels by stable expression of PRL-3 or *CNNM4* knockdown in mammalian culture cells or *C. elegans* carrying mutations in both *cnnm-1* and *cnnm-3*, the two major isoforms expressed in their intestine, led to an increase in intracellular ATP levels to an extent similar to those of Mg^{2+}.[18,35] Such elevation in ATP levels was shown to be beneficial for mouse melanoma B16 cells stably expressing PRL-3 to proliferate continuously in low glucose conditions which are commonly found within malignant tumors.[36,37] The increase in intracellular ATP levels also affected the intracellular signaling pathways. In PRL-3-expressing and *CNNM4* knockdown cells, the function of AMP-activated protein kinase (AMPK), an energy sensor molecule activated under low ATP conditions, was suppressed, and the activity of the mammalian target of rapamycin (mTOR) pathway, which is known to be inhibited by AMPK, was maintained at high levels. Consistently, the administration of the mTOR inhibitor rapamycin in mice reversed the increased tumorigenicity of PRL-3-expressing B16 cells when transplanted into mice.[18]

CNNM inhibition was also shown to accelerate mitochondrial electron transfer and overproduction of ATP, which may account for the concomitant increase in intracellular ATP levels with Mg^{2+} levels.[35] Furthermore, the accelerated mitochondrial electron transfer led to an increase in the amount of its byproduct, reactive oxygen species (ROS), which was observed not only in cultured cells with PRL-3-expression or *CNNM4* knockdown, but also in the intestines of *cnnm-1*; *cnnm-3* mutant *C. elegans* and *CNNM4* knockout mice. This increase in mitochondrial ROS led to a shortened lifespan in *C. elegans* and to the expansion of the transient amplifying cell layer in the mouse intestine. More interestingly, analyses with cultured mammalian cells revealed that overproduction of mitochondrial ROS by PRL/CNNM dysregulation caused cells to adapt to the acidic environment found in tumors, which will be described in detail in the following section.

2.2 | PRL-induced adaptation to acidic environment

In the human body, the pH of the extracellular fluid is usually maintained at approximately 7.4, and cells are optimized to proliferate most efficiently under this condition. However, the extracellular environment in tumors (often referred to as "tumor microenvironment") is known to be acidic with pH as low as 6.5.[38] This acidic condition is caused by a metabolic shift in cancer cells, typically the Warburg effect. However, how cancer cells can adapt and proliferate continuously in acidic environments, which are toxic to normal cells, remains largely unknown.

Analyses using mammalian culture cells inducibly expressing PRL-3 shed light on this issue.[16] Induced expression of PRL-3 in MDCK epithelial cells shifted their optimal pH for growth from physiological 7.5 to 6.5, the pH observed in malignant cancer tissues. A series of experiments including clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9-mediated genome-wide screening of genes functionally related to this shift of optimal pH for growth revealed the importance of lysosomal exocytosis, fusion of lysosomes with the plasma membrane, and subsequent extrusion of their contents, including condensed H^+ ions. Indeed, knockout of transient receptor potential mucolipin (TRPML), the lysosomal channel protein essential for lysosomal exocytosis,[39] reversed not only PRL-3-induced lysosomal exocytosis but also the shift in optimal pH and increased tumorigenicity of mouse melanoma B16 cells. Importantly, the induction of lysosomal exocytosis was shown to be caused through Mg^{2+} and ROS, which is known to activate TRPML (Figure 6).[40] Genetic ablation of *CNNM* family genes also promoted lysosomal exocytosis, and PRL-3-induced lysosomal exocytosis was suppressed by the addition of an antioxidant, N-acetyl L-cysteine.[16]

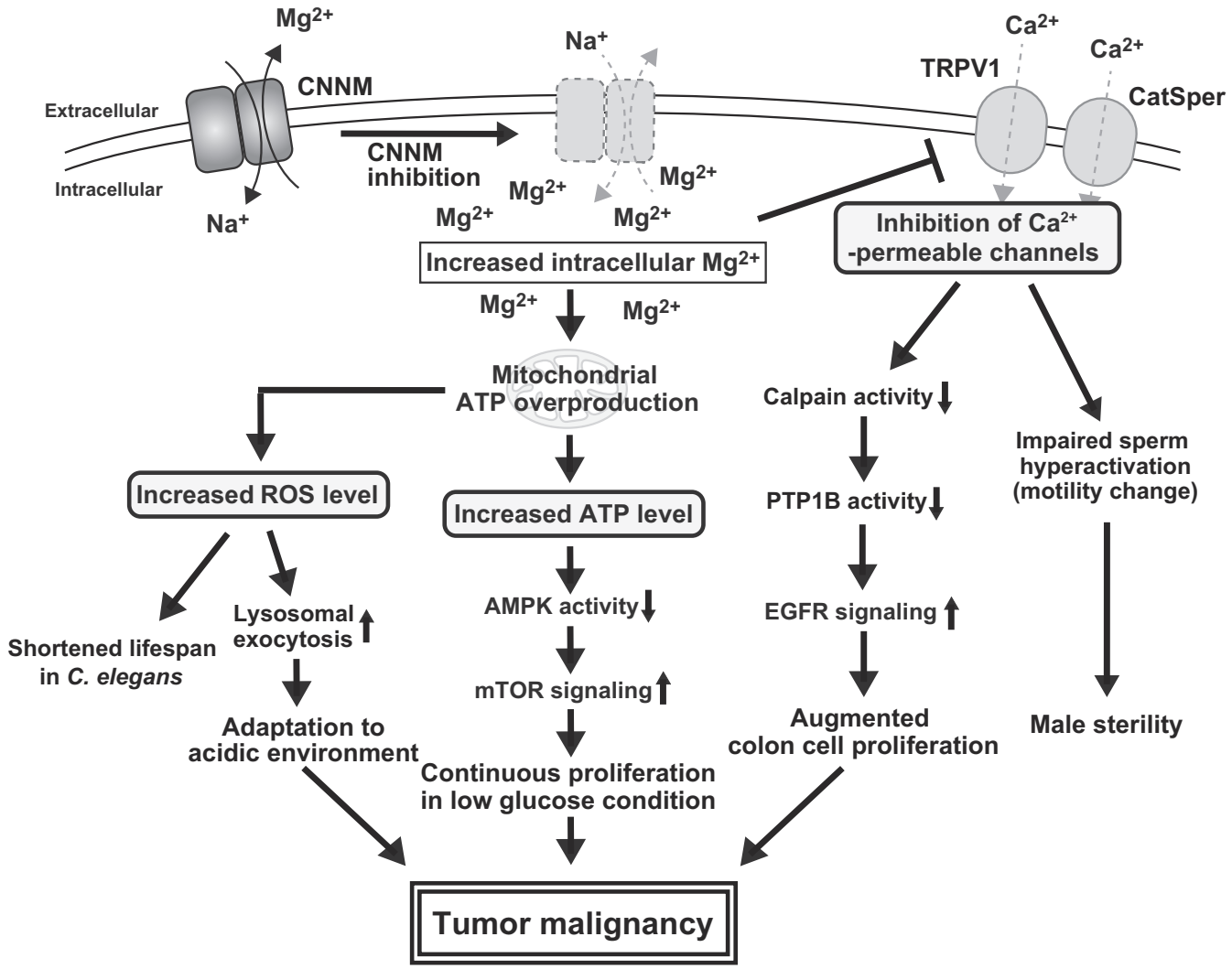

FIGURE 5 Schematic model of the effects caused by the CNNM inhibition, Increased intracellular Mg^{2+} by CNNM inhibition leads to a concomitant increase in the ATP level, which augments mTOR signaling activity and thus enables cells to continuously proliferate under low-glucose conditions, which can be found within malignant tumors. Such an increase in intracellular ATP is presumably caused by accelerated mitochondrial ATP production, which accompanies ROS overproduction. While elevation of mitochondrial ROS shortens the lifespan of *C. elegans*, it enhances lysosomal exocytosis in mammalian cells and induces their adaptation to the acidic environment observed in malignant tumors (see Figure 6 for details). Increased intracellular Mg^{2+} also inhibits Ca^{2+}-permeable channels such as TRPV1 and CatSper. TRPV1 inhibition impairs the processing/activation of PTP1B by calpain protease, which leads to upregulation of EGFR signaling and subsequent augmentation of cell proliferation. CatSper inhibition leads to male sterility by inhibiting sperm hyperactivation.

Studies have suggested that enhanced lysosomal exocytosis can be utilized by a wide range of cancer cells to adapt to acidic environments. Damahgi et al. found that continuous culture of a breast cancer cell line, MCF-7, at pH 6.7 resulted in the accumulation of LAMP, a lysosomal membrane protein, at the cell surface.[41] In addition, immunohistochemical studies of various human cancer tissues revealed elevated levels of V-ATPase, the H^+ pumps for lysosomal acidification, at the plasma membrane of cancer cells.[42] These lysosome-localized proteins are presumably transported to the plasma membrane by lysosomal exocytosis. Further analyses may reveal the general importance of lysosomal exocytosis and potentially PRL and Mg^{2+} in the acid adaptation of cancer cells.

It should also be noted that the activation of lysosomal exocytosis by PRL/CNNM may facilitate not only the adaptation of cancer cells to the acidic environment but also the invasion of cancer cells. Enzymes expelled by lysosomal exocytosis include cathepsin proteases, which are known to digest the extracellular matrix directly or indirectly by activating other matrix metalloproteases.[43] Indeed, *CNNM4* knockout in *APC* hetero-deficient mice, which spontaneously form benign intestinal tumors, led to the emergence of malignant cancer cells invading the muscular layer.[18]

2.3 | Inhibition of Ca^{2+}-permeable channels

Analyses using *CNNM4*-deficient mice and cells derived from these mice revealed Ca^{2+}-permeable channels as additional targets of increased intracellular Mg^{2+} levels (Figure 5). *CNNM4*-deficient mice

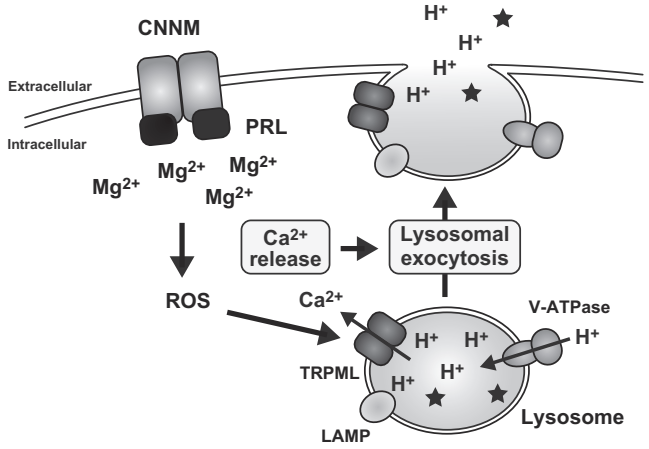

FIGURE 6 Model of PRL-induced lysosomal exocytosis and adaptation to the acidic environment. Increased intracellular Mg^{2+} due to PRL-mediated CNNM inhibition accelerates mitochondrial ATP production and generates ROS (Figure 5), which activates TRPML, a Ca^{2+}-permeable channel on the lysosomal membrane. Ca^{2+} released from lysosomes causes lysosomal exocytosis, fusion of lysosomes with the plasma membrane, and the resulting secretion of lysosomal ingredients, such as condensed H^+ and cathepsin proteases [indicated with a star (★)]. Such extrusion of condensed H^+ from lysosomes suppresses intracellular acidification in acidic environments. Alternatively, V-ATPase, which is located in the lysosomal membrane and transports H^+ into the lysosomal lumen using ATP energy, is translocated to the plasma membrane by lysosomal exocytosis and can extrude H^+ in situ, which may also account for the prevention of intracellular acidification.

showed male sterility, and sperm analysis revealed impaired Ca^{2+} incorporation via cation channels of sperm (CatSper) and subsequent motility change (hyperactivation) during capacitation, the maturation step of sperm.[44] A similar dysfunction of Ca^{2+}-permeable channels by *CNNM4*-deficiency was also observed in colon cells. In organoids established from isolated *CNNM4*-deficient mouse colon crypts, Ca^{2+} influx via transient receptor potential vanilloid 1 (TRPV1) was suppressed. Impaired Ca^{2+} influx led to augmented epidermal growth factor receptor (EGFR) signaling and proliferation, presumably through inactivation of protein tyrosine phosphatase 1B (PTP1B) (Figure 5),[30] and such augmented proliferation may explain the increased colorectal tumor formation in *CNNM4*-deficient mice during the chemically induced tumorigenesis experiment. Whether increased intracellular Mg^{2+} directly inhibits these Ca^{2+}-permeable channels or through other Mg^{2+}-affected molecules, such as ATP and ROS, remains unclear. Detailed functional analyses of these channels, including patch-clamp recordings, might resolve this issue.

3 | CURRENT STATUS OF ANTICANCER DRUG DEVELOPMENT TARGETING PRL AND CNNM

Multiple types of candidate anticancer reagents have been developed to target PRL; they include anti-PRL monoclonal antibodies[45] and chemical compounds inhibiting PRL oligomerization,[46] but the majority are the compounds inhibiting the weak phosphatase activity of PRL.[47] Although at present no compound has reached clinical trials, numerous phosphatase inhibitors such as pentamidine, thienopyridone, and rhodanine (including their derivatives) have been developed.[48–50] Among them thienopyridone is a promising lead,[50] which was eventually shown to inhibit both phosphatase activity and CNNM binding.[51] It should be mentioned that there is a report showing that this compound generates ROS,[52] which inhibits the interaction between PRL and CNNM,[18,19] and thus, it is unclear whether this compound competitively inhibits protein–protein interactions. A direct search for compounds that inhibit the PRL/CNNM interaction may lead to the development of compounds that can inhibit PRL-induced malignancy more efficiently. To date, there has been no report on the search for compounds targeting the PRL/CNNM interaction, but a fluorescence resonance energy transfer-based evaluation method for PRL/CNNM interaction, which can be applied to high-throughput screening, has been reported.[53]

4 | CONCLUDING REMARKS

That PRL induces cancer malignancy independently of its phosphatase activity, by inhibiting CNNM and subsequently increasing intracellular Mg^{2+} levels is clear from the evidence discussed in this review. Furthermore, analyses at the cellular level have revealed the importance of ATP, ROS, and Ca^{2+}-permeable channels as key molecules linking increased intracellular Mg^{2+} to pleiotropic phenotypes related to cancer malignancy. Mg^{2+} is also associated with many other diseases,[54] and particularly in hypertension and nonalcoholic steatohepatitis (NASH), the importance of CNNM has also been demonstrated.[55–57] Thus, identification of molecules that sense the change in Mg^{2+} levels and function downstream of Mg^{2+} will not only lead to the clarification of the Mg^{2+}-driven, unique mechanism of malignant progression, but will also facilitate the understanding of these other Mg^{2+} and CNNM-related diseases.

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CONFLICT OF INTEREST

The authors have no conflict of interest.

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