

## PDRG1 at the interface between intermediary metabolism and oncogenesis

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### Abstract

PDRG1 is a small oncogenic protein of 133 residues. In normal human tissues, the p53 and DNA damage-regulated gene 1 (*PDRG1*) gene exhibits maximal expression in the testis and minimal levels in the liver. Increased expression has been detected in several tumor cells and in response to genotoxic stress. High-throughput studies identified the PDRG1 protein in a variety of macromolecular complexes involved in processes that are altered in cancer cells. For example, this oncogene has been found as part of the RNA polymerase II complex, the splicing machinery and nutrient sensing machinery, although its role in these complexes remains unclear. More recently, the PDRG1 protein was found as an interaction target for the catalytic subunits of methionine adenosyltransferases. These enzymes synthesize S-adenosylmethionine, the methyl donor for, among others, epigenetic methylations that occur on the DNA and histones. In fact, downregulation of S-adenosylmethionine synthesis is the first functional effect directly ascribed to PDRG1. The existence of global DNA hypomethylation, together with increased PDRG1 expression, in many tumor cells highlights the importance of this interaction as one of the putative underlying causes for cell transformation. Here, we will review the accumulated knowledge on this oncogene, emphasizing the numerous aspects that remain to be explored.

**Key words:** Epigenetic modifications; Glutathione; Methylation; Oncogenes; Intermediary metabolism; p53 and DNA damage-regulated gene 1; Protein complexes; R2TP/prefoldin complex; S-adenosylmethionine synthesis; Redox stress

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**Core tip:** PDRG1 is an understudied protein of the R2TP/prefoldin-like complex that has been found as part of different multiprotein complexes. Increased

PDRG1 expression levels have been associated with several types of tumors that concomitantly show global DNA hypomethylation. More recently, this protein has been uncovered as an interaction target for methionine adenosyltransferase catalytic subunits MAT $\alpha$ 1 and MAT $\alpha$ 2. Through this interaction, PDRG1 downregulates nuclear S-adenosylmethionine synthesis, hence impacting epigenetic methylations.

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## INTRODUCTION

Late in the 20<sup>th</sup> century, global DNA hypomethylation was described as a main characteristic of cancer cells and as the underlying cause of their altered expression pattern<sup>[1,2]</sup>. Moreover, tumor cell growth was found to be totally dependent on glutamine and methionine, and the latter amino acid could not be replaced by homocysteine<sup>[3]</sup>. Methionine is one of the substrates required for the synthesis of the main cellular methyl donor, S-adenosylmethionine (AdoMet), which is used for epigenetic methylations occurring at both the DNA and its associated proteins for the regulation of gene expression. These modifications are among the dozens of methylation reactions that occur in a cell using S-adenosylmethionine (AdoMet) as a methyl donor<sup>[4,5]</sup>. However, the methyltransferases catalyzing these modifications are not the main consumers of AdoMet, despite their importance in regulating cell function. This fact is especially clear in the liver, where three methyltransferases involved in the synthesis of small compounds use the majority of the methyl donor synthesized<sup>[6,7]</sup>. Those are glycine N-methyltransferase (GNMT), guanidinoacetate N-methyltransferase (GAMT) and phosphatidylethanolamine N-methyltransferase (PEMT), which produce sarcosine, creatine and phosphatidylcholine, respectively<sup>[8-11]</sup>. Nevertheless, these estimations were made on the basis that AdoMet synthesis occurs exclusively in the cytoplasm and that the methyl donor would be transported to other subcellular localizations as required. This assumption has been challenged lately by reports describing the existence of nuclear pools of several enzymes of the methionine cycle, the pathway where AdoMet is produced, in turn suggesting the existence of two independent AdoMet stocks in the cytoplasm and the nucleus<sup>[12-16]</sup>. The existence of a nuclear branch of this pathway is supported by the importance of epigenetic methylations and the need to guarantee their AdoMet supply.

To date, information regarding the cytoplasmic and nuclear branches of the methionine cycle has been mainly obtained in liver, and the accumulated knowledge can be summarized as follows. The cytoplasmic

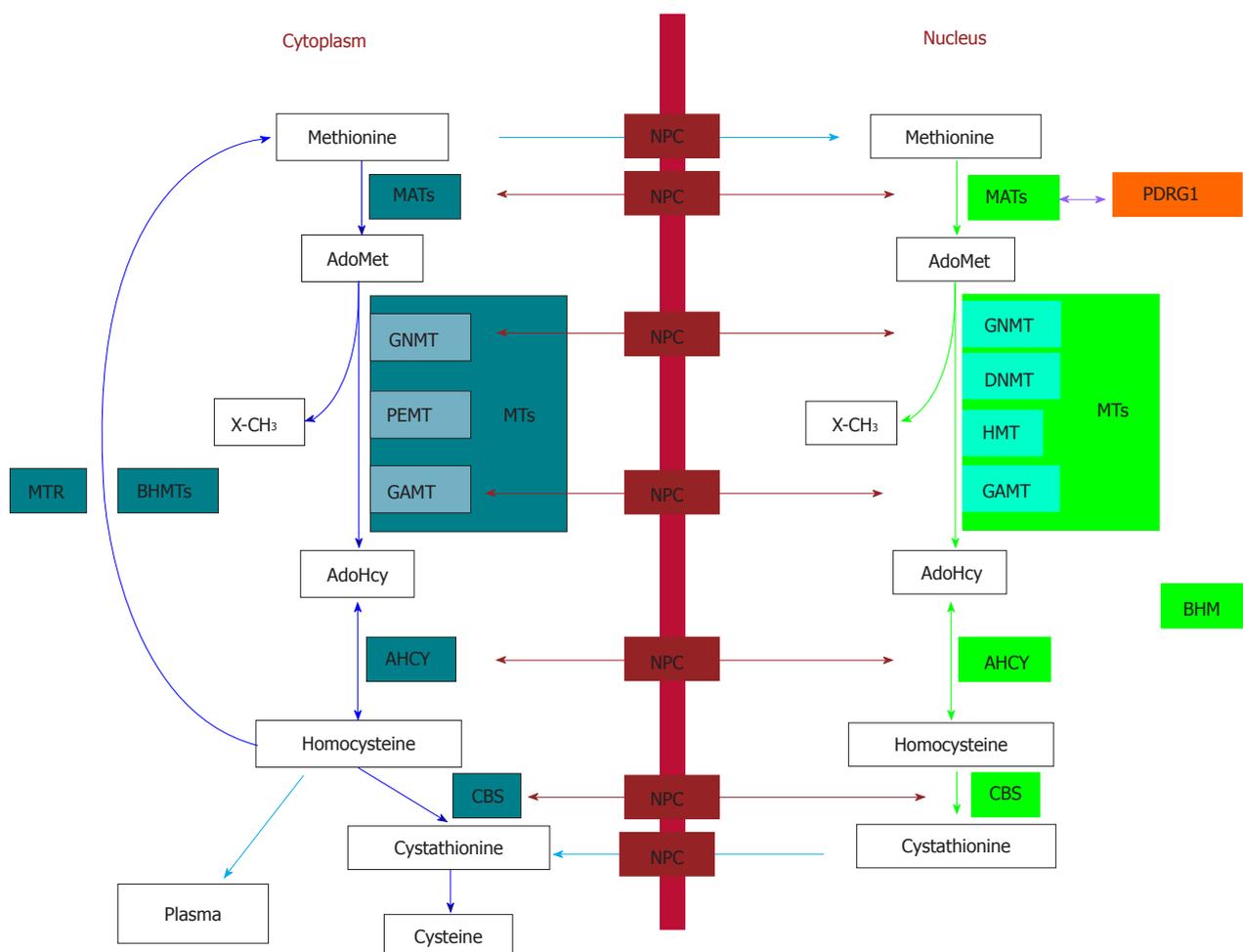
methionine cycle involves the following (Figure 1): (1) methionine adenosyltransferases (MATs) that synthesize AdoMet; (2) a large number of methyltransferases, which methylate many types of substrates, also rendering S-adenosylhomocysteine (AdoHcy); (3) AdoHcy hydrolase (AHCY), which eliminates AdoHcy leading to adenosine and homocysteine (Hcy); and (4) betaine homocysteine S-methyltransferases (BHMT and BHMT2) and methionine synthase (MTR), which methylate Hcy to obtain methionine using betaine or S-methylmethionine and 5-methyltetrahydrofolate, respectively<sup>[17,18]</sup>. In contrast, the nuclear branch of the pathway involves a more reduced set of enzymes as follows (Figure 1): (1) MATs; (2) methyltransferases such as those involved in DNA and histone methylations, GNMT and GAMT; (3) AHCY<sup>[12,13,15,16]</sup>; and (4) BHMT<sup>[19]</sup>. Of note, levels of MATs, GNMT and AHCY are markedly higher in the cytoplasm than in the nucleus, but increases in the latter compartment can be observed as a consequence of pathological changes in glutathione concentrations and, precisely, decreases in the GSH/GSSG ratio<sup>[14]</sup>. More recently, PDRG1 [Consensus nomenclature for the human (*PDRG1*) and mouse genes (*Pdrg1*) is used throughout the text. The protein is in all the cases referred to as PDRG1] was added to this nuclear branch of the cycle as an interaction target and regulator of MATs, and hence, of AdoMet synthesis<sup>[20,21]</sup>.

*PDRG1* stands for p53 and DNA damage-regulated gene 1 as it was discovered during studies devoted to the identification of components of the cellular response to DNA damage, or genotoxic stress, and it is regulated by p53<sup>[22]</sup>. However, what do we really know about PDRG1? This is the question that will be addressed in the next sections of the present mini-review.

## *PDRG1* GENE STRUCTURE AND REGULATION

Studies devoted to the identification of components of the cellular response to DNA damage have been carried out for a number of years, and many of them used UV-irradiation as a stressor. Some of these investigations identified several novel UV-induced genes using a UV-treated low-ratio hybridization subtraction-enriched cDNA hamster library, and they were followed by screenings of partial-length expressed sequence tag (EST) clones regulated by this type of stress<sup>[22-24]</sup>. This latter procedure rendered a UV-induced EST clone that hybridized with a 1.4 kb transcript, which was named *PDRG1*<sup>[22]</sup>. Sequencing of the corresponding human and mouse EST clones from different tissues showed a single open reading frame coding for a protein of 133 amino acids.

The *PDRG1* gene is organized among five exons and four introns in both humans and mice (Figure 2)<sup>[22]</sup>. The start codon is located at position 120 in exon 1 of the human gene, whereas the stop codon occurs at position 85 on exon 5. The mouse gene is located on chromosome 2, whereas the human gene appears on the



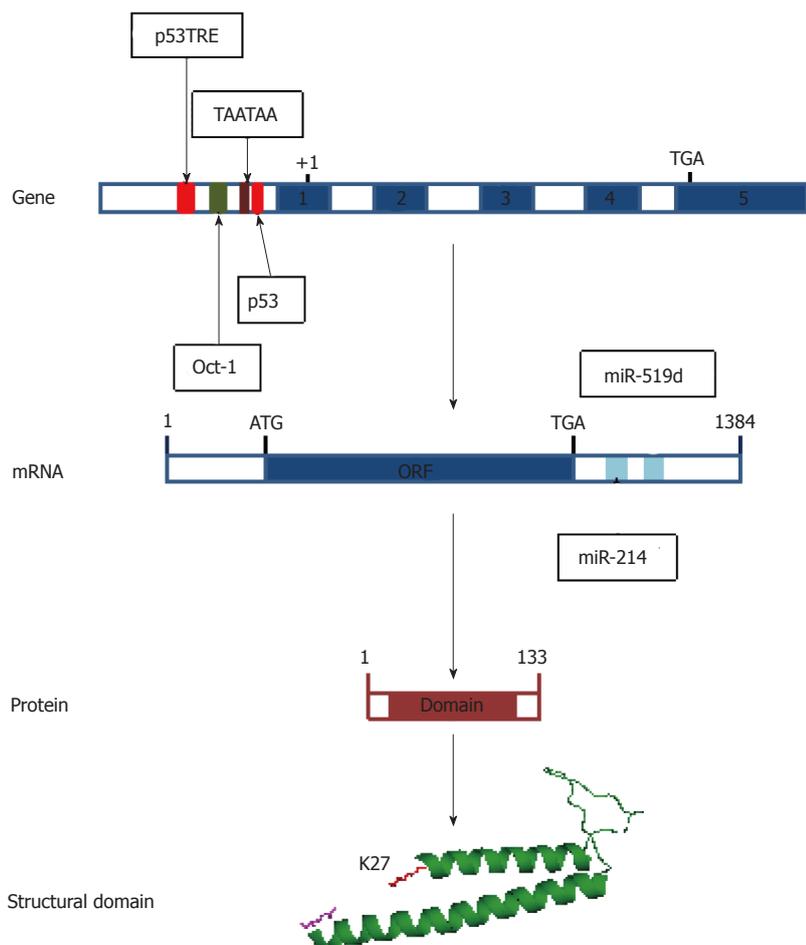
**Figure 1** The cytoplasmic and nuclear branches of the hepatic methionine cycle and transsulfuration. Essential components (metabolites and enzymes) of the pathways are shown in the figure. Cytoplasmic enzymes appear in aquamarine boxes, their nuclear equivalents are shown in green boxes, and protein interaction targets are depicted in orange. Only key methyltransferases (MTs) mentioned in the text are illustrated in both subcellular compartments. The nucleocytoplasmic transport of metabolites (blue arrows) and enzymes (maroon arrows) is shown, whereas protein interactions are indicated by violet arrows. AdoHcy: S-adenosylhomocysteine; AdoMet: S-adenosylmethionine; AHCY: S-adenosylhomocysteine hydrolase; BHMT: Betaine homocysteine S-methyltransferase; CBS: Cystathionine β-synthase; DNMT: DNA methyltransferase; GANT: Guanidinoacetate N-methyltransferase; GNMT: Glycine N-methyltransferase; HMT: Histone methyltransferase; MATs: Methionine adenosyltransferases; MTR: Methionine synthase; NPC: Nuclear pore complex; *PDRG1*: p53 and DNA damage-regulated gene 1; PEMT: Phosphatidylethanolamine N-methyltransferase; X-CH<sub>3</sub>: Methylated acceptors (metabolites, proteins, DNA, etc).

long arm of chromosome 20 at the 20q11.2 region<sup>[22]</sup>. Interestingly, the human gene localization coincides with a position in which gains of DNA are detected in hepatocellular carcinoma (HCC) and dysplastic nodules, and where alterations in cirrhotic processes are also found<sup>[25]</sup>. Moreover, these are pathologies in which impairment of the methionine cycle, especially of AdoMet production, have been described<sup>[17]</sup>.

Analysis of the human *PDRG1* promoter was initially carried out using a 1.7 kb fragment that included the 5'-UTR and part of the non-coding region of exon 1, and it showed high levels of promoter activity in breast cancer MCF-7 cells when fused to the luciferase gene<sup>[22]</sup>. The gene contains a TATA-like element (TAATAA) localized at -268 from the start codon, but no canonical TATA box was identified<sup>[22]</sup>. Promoter activity was eliminated by deletion of the -138/+1 region, in turn suggesting the presence of key regulatory elements in this segment<sup>[22]</sup>. These same authors also detected two

putative downregulatory elements in the promoter. The first, a p53 binding element, was identified at position -240/-214 and includes two RRRCWWGYYY (R = purine, Y = pyrimidine, W = A or T, S = G or C, D = A, G or T) repeats arranged head to tail and separated by a 6 bp sequence<sup>[26-28]</sup>. The second corresponds to a putative p53 transcriptional repressor element (TRE) located at -854/-834<sup>[29]</sup>. Regulation of the *PDRG1* promoter by p53 was further proven when luciferase activity was abolished in the presence of this tumor suppressor<sup>[22]</sup>. Basal *PDRG1* expression in p53 null HCT116 colon cancer cells was in fact higher than that in their wild-type counterparts, results that further supported repression of *PDRG1* by p53.

Additionally, the *PDRG1* promoter includes a canonical Oct-1 binding element located at -689/-683<sup>[22,30]</sup>; this factor is known to upregulate expression of a number of genes when activated by UV irradiation<sup>[31,32]</sup>. Cotransfection of a *PDRG1* promoter-luciferase construct and Oct-1 into



**Figure 2** Regulatory elements on the human *PDRG1* gene and mRNA and structural features of the protein. From top to bottom, the figure shows first a scheme of the human *PDRG1* gene, indicating the regulatory elements on the promoter identified to date as follows: p53 and p53TRE elements (red), an Oct-1 binding element (green) and the TATA-box-like element (crimson). Exons appear as blue boxes. Second, a schematic representation of the mRNA is depicted, including the ORF (blue box) and the miRNA binding sites identified at the 3'-UTR (aquamarine boxes). Third, a scheme of the *PDRG1* protein is shown where the sequence encoding the prefoldin-like domain is indicated as a crimson box. Finally, a cartoon of the modeled prefoldin-like domain of rat *PDRG1* is included with the N-terminal K27 (red) and the C-terminal Q106 (magenta) depicted as sticks.

MCF-7 cells rendered approximately 8-fold induction of the luciferase activity, thus demonstrating the functionality of this Oct-1 binding element<sup>[22]</sup>. This increased promoter activity was far greater than that caused exclusively by UV irradiation, a fact that Luo *et al*<sup>[22]</sup> ascribed putatively to p53 activation in these cells.

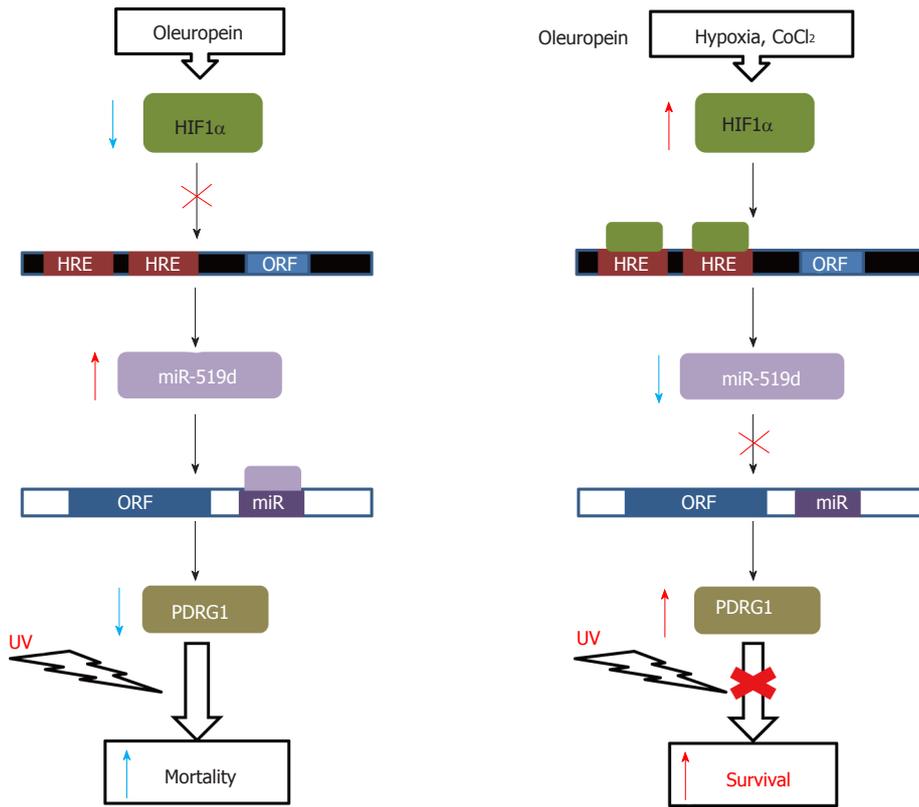
More recently, the presence of complementary sequences for miR-214 (position 842-850) and miR-519d (position 1076-1082) in the 3'-UTR of the *PDRG1* mRNA was reported<sup>[33,34]</sup>. Overexpression of miR-214, a miRNA with dual functionality as tumor suppressor or oncogene, in bladder cancer cell lines reduced *PDRG1* expression and protein levels<sup>[33]</sup>. Similarly, overexpression of miR-519d leads to decreased *PDRG1* mRNA and protein levels in two nasopharyngeal carcinoma cell lines, which in turn correlates with a higher radiation-induced mortality (Figure 3)<sup>[34]</sup>.

Regarding the *PDRG1* expression profile, this aspect was initially studied using commercial human poly(A)<sup>+</sup> Northern blots, where a 1.4 kb transcript was identified in brain, heart, liver, lung, spleen, stomach, testis and

skeletal muscle<sup>[22]</sup>. More recently, real-time RTqPCR of rat tissues also demonstrated wide expression of *PdrG1*, further including the cerebellum, intestine, pancreas, kidney and cava vein<sup>[20]</sup>. In human tissues the highest levels were found in testis, whereas in the rat similar expression levels were detected in brain, cerebellum and testis. Nevertheless, both species showed very low *PDRG1* expression in the liver<sup>[20,22]</sup>.

## PDRG1 PROTEIN

Mammalian *PDRG1* proteins share a high level of conservation, and most of them are over 90% identical (Figure 4). The sequences usually comprise 133 residues (15.5 kDa for the human protein), although larger proteins have been predicted in several marine mammals. In those cases, *PDRG1* presents with N-terminal extensions of variable length. The protein's isoelectric point is 5.81, and several post-translational modifications have been predicted and identified in high-throughput studies. Among them, phosphorylations at human serine residues



**Figure 3 Regulation of cell survival.** A schematic representation of the effects on cell survival exerted by *PDRG1* regulation through *miR-519d* expression is shown. Binding sites for HIF1a (HRE) in the promoter of the *miR-519d* gene (black box) and for this miRNA on the *PDRG1* mRNA (white box) are indicated. Agents used for induction or blockade of UV-irradiation effects are indicated on the top.

Rattus norvegicus	<sup>1</sup> MVSP EADRVLRYLVEVEELAEAVLSDK RQIVDLDTKRNQ NREGLRALQKDPSVSEDVMVC
Mus musculus	<sup>1</sup> ML SPEAERVLRYLVEVEELAEAVLSDK RQIVDLDTKRNQ NREGLRALQKDL SVSEDVMVC
Homo sapiens	<sup>1</sup> ML SPEAERVLRYLVEVEELAEAVLADK RQIVDLDTKRNQ NREGLRALQKDL S SEDVMVC
Bos taurus	<sup>1</sup> ML SPEAERVLRYLVEVEELAEAVLADK RQIVDLDTKRNQ NREGLRALQKDL S L TEDVMVC
Cricetulus griseus*	<sup>1</sup> ML SPEAERVM RYLVEVEELAEAVLSDK RQIVDLDTKRNQ NREGLRALQKDL SVSEDVMVC
Microtus ochrogaster*	<sup>1</sup> ML SPEAERVLRYLVEVEELAEAVLSDK RQIVDLDTKRNQ NREGLRALQKDL SVSEDVMVC
Pteropus vampyrus*	<sup>1</sup> ML SPEAERVLRYLVEVEELAEAVLADK RQIVDLDTKRNQ NREGLRALQKDL S SEDVMVC
Canis lupus*	<sup>1</sup> ML SPEAERVLRYLVEVEELAEAVLADK RQIVDLDTKRNQ NREGLRALQKDL S SEDVMVC
Jaculus jaculus*	<sup>1</sup> ML SPEAERVLRYLVEVEELAEAVLADK RQIVDLDTKRNQ NREGLRALQKDL S SEDVMVC
Ictidomys tridecemlineatus*	<sup>1</sup> ML SPEAERVLRYLVEVEELAEAVLADK RQIVDLDTKRNQ NREGLRALQKDL S SEDVMVC
Erinaceus europaeus*	<sup>1</sup> ML SPEAERVLRYLVEVEELAEAVLADK RQIVDLDTKRNQ NREGLRALQKDL S SEDVMVC
Ursus maritimus*	<sup>1</sup> ML SPEAERVLRYLVEVEELAEAVLADK RQIVDLDTKRNQ NREGLRALQKDL S SEDVMVC
Physeter catodon*	<sup>1</sup> MDRGRTELEQVNTML SPEAERVLRYLVEVEELAEAVLADK RQIVDLDTKRNQ NREGLRALQKDL S SEDVMVC

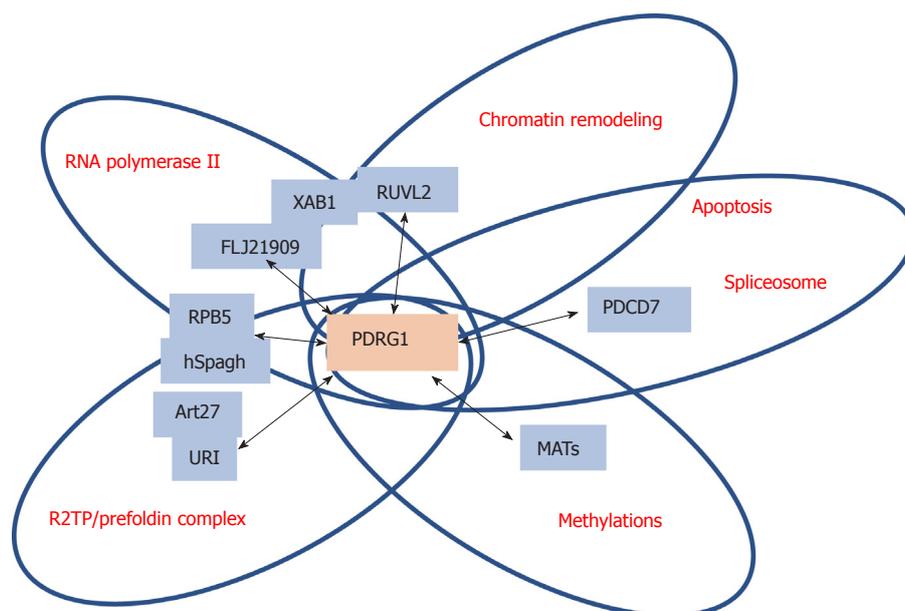
  

Rattus norvegicus	<sup>61</sup> FGNMF IKMPH LKTKEMI QKDQEHLDKE IERLRSQ LKVKVNRLLEA QGKPELKG FNLP LNQDELKALQVILKG
Mus musculus	<sup>61</sup> FGNMF IKMPH PTKEMI QKDQEHLDKE IERLRSQ LKVKVNRLFEA QGKPELKG FNLP LNQDELKALQVILKG
Homo sapiens	<sup>61</sup> FGNMF IKMPH PTKEMI QKDQEHLDKE IERLRSQ LKVKVNRLFEA QGKPELKG FNLP LNQDELKALQVILKG
Bos taurus	<sup>61</sup> FGNMF IRMPH PTKEMI QKDQEHLDKE IERLRK QLKVKVNRLFEA QGKPELKG FNLP LNQDELKALQVILKG
Cricetulus griseus*	<sup>61</sup> FGNMF IKMPH PTKEMI QKDQEHLDKE IERLRN QLKVKVNRLFEA QGKPELKG FNLP LNQDELKALQVILKG
Microtus ochrogaster*	<sup>61</sup> FGNMF IKMPH PTKEMI QKDQEHLDKE IERLRN QLKVKVNRLFEA QGKPELKG FNLP LNQDELKALQVILKG
Pteropus vampyrus*	<sup>61</sup> FGNMF IKMPH PTKEMI QKDQEHLDKE IERLRN QLKVKVNRLFEA QGKPELKG FNLP LNQDELKALQVILKG
Canis lupus*	<sup>61</sup> FGNMF IKMPH PTKEMI QKDQEHLDKE IERLRN QLKVKVNRLFEA QGKPELKG FNLP LNQDELKALQVILKG
Jaculus jaculus*	<sup>61</sup> FGSMF IRMPH PTKEMI QKDQEHLDKE IERLRN QLKVKVNRLFEA QGKPELKG FNLP LNQDELKALQVILKG
Ictidomys tridecemlineatus*	<sup>61</sup> FGSMF IKM SHP TKEMI QKDQEHLDKE IERLRN QLKVKVNRLFEA QGKPELKG FNLP LNQDELKALQVILKG
Erinaceus europaeus*	<sup>61</sup> FGDMF IKMPH PTKEMI QKDQEHLDKE IERLRN QLKVKVNRLFEA QGKPELKG FNLP LNQDELKALQVILKG
Ursus maritimus*	<sup>61</sup> FGNMF IRMPH PTKEMI QKDQEHLDKE IERLRN QLKVKVNRLFEA QGKPELKG FNLP LNQDELKALQVILKG
Physeter catodon*	<sup>74</sup> FGNMF IRMPH RPTKEMI QKDQEHLDKE IERLRN QLKVKVNRLFEA QGKPELKG FNLP LNQDELKALQVILKG

**Figure 4 Alignment of mammalian *PDRG1* proteins.** The figure shows the BLASTP alignment of thirteen representative mammalian *PDRG1* sequences using that of *Rattus norvegicus* as a reference. Most of the data correspond to predicted sequences (\*). Conservative changes are highlighted in green, non-conservative substitutions appear in red, and N-terminal extensions are indicated in yellow. The C-terminal helix-turn-helix motif and the prefoldin-like domain are indicated in blue and crimson boxes, respectively.

3, 52, 54 and mouse serine 120 and ubiquitinations on human lysine residues 27, 108 and 125 have been postulated or found<sup>[35-41]</sup>. Initial studies suggested

association of the protein into homo-oligomers, a fact that was later confirmed by detection of HA-*PDRG1* hexamers in embryonic kidney HEK293T cells using analytical gel



**Figure 5 Schematic representation of the main PDRG1 interaction targets.** The figure highlights the main targets of interaction with PDRG1 identified using either affinity purification/mass spectrometry or yeast two-hybrid screening. The proteins involved are shown in blue boxes within circles, according to the function or multiprotein complex in which they are involved. PDRG1 appears at the intersection of these circles.

filtration chromatography<sup>[20-22]</sup>.

Motif searches identified a helix-turn-helix (LNQDEL KALKVILKG) at the C-terminal end of the human and mouse protein sequences<sup>[22]</sup>. These motifs are normally involved in interactions with either the DNA or other proteins. Moreover, human and mouse PDRG1 are characterized by the presence of a  $\beta$ -prefoldin-like domain that is also recognized by the Protein Homology/analogy Recognition Engine (PHYRE) in the rat counterpart. PHYRE2 identified prefoldin as the closest homologue to rat PDRG1 during the preparation of a structural model. This model included residues K27-Q106 (Figure 2), thus excluding approximately 25 residues from each protein end. Elimination of the C-terminal end has divergent effects on the interaction of PDRG1 with MAT catalytic subunits MAT $\alpha$ 1 and MAT $\alpha$ 2<sup>[20,21]</sup>. Additionally, motif searches carried out with the rat PDRG1 sequence predicted nucleocytoplasmic localization (PSORT II) and a nuclear export signal (NES) involving serine 93; the NetNES score increases between residues E89-L95.

Subcellular localization was initially examined by fluorescence microscopy using PDRG1-GFP and GFP-PDRG1 tagged forms in fixed NIH 3T3 fibroblasts and HCT116 colon cancer cells. In both cases, cytoplasmic aggregates of the protein were described, whereas no localization within subcellular compartments such as the mitochondria, endoplasmic reticulum and nucleus was observed using specific dyes for these organelles<sup>[22]</sup>. Moreover, these authors indicated that this distribution was not affected by UV irradiation, and hence, a putative PDRG1-DNA interaction was excluded. In contrast, PDRG1 was found in the nuclear fractions used to isolate protein complexes from LNCaP prostate cancer cells, hence suggesting that the protein may be distributed

between subcellular compartments<sup>[42]</sup>. This aspect was reexamined using confocal microscopy and several cell lines in which HA-PDRG1 or PDRG1-EGFP was overexpressed. The results of direct fluorescence and immunofluorescence experiments demonstrated that, in fact, PDRG1 is a nucleocytoplasmic protein in ovary (CHO), kidney (COS-7), neuroblastoma (N2a) and hepatoma (H35) cell lines<sup>[20,21]</sup>. Moreover, quantification of the fluorescence signals indicated a stronger protein level in the nuclear compartment, where PDRG1 also colocalized with nuclear speckles containing SC-35.

## PROTEIN-PROTEIN INTERACTIONS INVOLVING PDRG1

Lately, several high-throughput studies have been carried out with the goal of identifying components of several large protein complexes (Figure 5). For this purpose, the following two main techniques were used: (1) affinity purification of subunits including single (AP) or tandem tags (TAP) followed by mass spectrometry (MS); and (2) yeast two-hybrid (YTH). During some of these studies PDRG1 was identified either as a subunit or interaction target of several complexes, and interestingly, methylation plays a direct or indirect role in many of the processes involving these multimers (e.g., nutrient signaling and mRNA capping).

### *PDRG1 is indirectly linked to the RNA polymerase II machinery*

MS, together with a computer method to minimize the contribution of false positives, was used to identify the interaction network of human RNA polymerase II

using tagged subunits that allowed TAP purification from kidney HEK293 cells<sup>[43]</sup>. Some key proteins were selected for additional TAP-MS experiments, and among them are XAB1, which appears placed at the interface with members of chaperone complexes (C19orf2/URI, PFDN6, PFDN2 and UXT), and the AAA+ chaperone-like ATPases RUVBL1/TIP49a and RUVBL2/TIP49b. These experiments showed TAP-XAB1 copurification with FLJ21909 and subsequent TAP-FLJ21909 copurification with PDRG1<sup>[43]</sup>. Moreover, this same work also demonstrated copurification of PDRG1 with TAP-RUVBL2, C1orf82/RPAP2 and FLJ21908/RPAP3, although no further validation of the interactions using additional techniques was carried out<sup>[43]</sup>.

Additional studies to analyze the cytoplasmic assembly of RNA polymerase II were later performed by Boulon *et al.*<sup>[44]</sup>. These MS-proteomics studies showed association of unassembled RPB1, the largest subunit of this transcription machinery, with the R2TP/prefoldin-like complex<sup>[44]</sup>. Moreover, in this same study the interactions of a central component of the R2TP/prefoldin-like complex, hSpagh that binds HSP90 as a cofactor, were further explored. Independent interactions of hSpagh with RPB1 and RPB5 subcomplexes were then identified, which also involved other members of the R2TP/prefoldin-like complex, such as PDRG1. However, YTH only confirmed the implication of PDRG1 in the interaction with RBP5, although this binding seemed indirect through its association with hSpagh<sup>[44]</sup>.

#### ***PDRG1 may have a role in chromatin remodeling, splicing and apoptosis***

AP-MS data also suggested that PDRG1 might be linked to the SWR-C and INO80 chromatin remodeling complexes. Such a relationship could be established by the identification of its interaction targets, RUVBL1 and RUVBL2, in both complexes in experiments carried out in nuclear fractions of human cells<sup>[45,46]</sup>. Additionally, YTH screens identified PDRG1 interacting with programmed cell death 7 (PDCD7), Cip1 zinc interacting finger (CIZ1) and microtubule-associated protein 1S (MAP1S) in human testis, although immunoprecipitation only confirmed the PDRG1-PDCD7 interaction<sup>[47]</sup>. These results linked PDRG1 with the modulation of apoptosis and the U12-type spliceosome, a process and a complex that involve PDCD7<sup>[48]</sup>.

#### ***PDRG1 is a member of the URI/prefoldin complex involved in nutrient signaling***

PDRG1 was identified, together with HKE2, BC014022, POL3A, FLJ21908 and FLJ20643, as a new member of the URI/prefoldin complex with abundance similar to that exhibited by the consensus subunits known at that time<sup>[49]</sup>. URI stands for unconventional prefoldin RPB5 interactor, also known as RMP, and some of the interaction studies described in this section showed this interaction. Additional data from this AP-MS study identified PDRG1 interacting with TIP49a/b in HEK293/FRT and HeLa cells, as well as bound to BC014022,

DPCD, FLJ20643, NUFIP, RPB5MP and UXT1<sup>[49]</sup>. Reciprocal studies using FLAG-tagged PDRG1 identified interactors with moderate (POL3A, PPP1CA, PPP1CC, PFDN2, HKE2, RPB5, PPP1CB, NPM1, UXT1, H2BS, H2BR, RPB5MP, FLJ21908, BC014022, H2AZ, NOP5/NOP58, FLJ20643) or low probability scores (TUB6, HSP70/1, HSP70/1B, BAF53, TIP49a, TIP49b)<sup>[49]</sup>. However, none of these PDRG1 interactions were confirmed by immunoprecipitation. More recently, MS analysis of immunoprecipitates from nuclear fractions of LNCaP prostate cells stably transfected with FLAG-URI also showed the presence of PDRG1 in the R2TP/prefoldin-like complex, and the interaction was confirmed by detection of URI and PDRG1 in anti-Art27 immunoprecipitates<sup>[42]</sup>. Both studies considered the PDRG1-URI interaction abundant, according to the number of peptides retrieved<sup>[42,49]</sup>.

PDRG1 overexpression did not modify URI or Art27 levels, whereas overexpression of URI upregulates PDRG1 protein levels, an effect that was ascribed to protein stabilization<sup>[42]</sup>. The PDRG1-URI interaction seems to involve the hook-shaped  $\beta$ -strands of the URI N-terminal prefoldin-like domain, through which interaction with Art27 is also established. In fact, these authors suggested that all prefoldin-like proteins of the R2TP/prefoldin-like complex (URI, Art27, PDRG1, PFD2, PFD6) use the hook to interact with each other<sup>[42]</sup>.

AP-MS experiments carried out in HeLa cells identified URI, TIP49/RUVL, RMP and RPB5 in association with STAP-1, and hence potentially linking PDRG1 with signal transduction and the TOR complex<sup>[50]</sup>. These interactions were further confirmed by immunoprecipitation, where anti-URI binds RPB5 and STAP-1 and anti-TIP48 rendered TIP49, URI, STAP1 and SKP2. Similarly, PDRG1 could be indirectly implicated in the roles of URI as a transcriptional repressor and as a regulator of apoptosis that involve interactions with the RPB5/POLR2E subunit of the three RNA polymerases and PPI $\gamma$ <sup>[51,52]</sup>. In the latter case, phosphorylation of mitochondrial URI by S6K1 allows dissociation of the complex. Moreover, the URI complex is targeted by nutrient signaling and, in turn, participates in the control of gene expression downstream of the TOR kinase, a process that is regulated by methylation of the protein phosphatase PP2A<sup>[53]</sup>.

#### ***PDRG1 controls production of S-adenosylmethionine***

In yeast, URI deletion alters expression of genes involved mainly in amino acid metabolism, although none of the key genes in the methionine cycle seems affected<sup>[50]</sup>. On the other hand, YTH screening of a rat liver library identified PDRG1 interacting with MAT $\alpha$ 1, a result that was further confirmed by immunoprecipitation and pull-down<sup>[20]</sup>. These results linked PDRG1 with methionine metabolism and AdoMet-dependent methylations<sup>[20]</sup>, which are altered in tumor cells.

MAT $\alpha$ 1 is the catalytic subunit of homotetrameric MAT I and homodimeric MAT III, the isoenzymes synthesizing AdoMet in normal liver, and accumulates

in the cell nucleus in liver disease and extrahepatic cells<sup>[12,14]</sup>. Despite the nucleocytoplasmic localization of both MAT $\alpha$ 1 and PDRG1, their interaction was restricted to the nuclear compartment, where large association states (approximately 360 kDa) could be detected<sup>[20,21]</sup>. Taking into account the size of each protein and their individual oligomeric states, this means binding of two PDRG1 hexamers per nuclear MAT I homotetramer. Moreover, pull-down assays also showed the ability of PDRG1 to interact with the MAT $\alpha$ 2 catalytic subunit of heterotrimeric MAT II, the main isoenzyme found in extrahepatic tissues, fetal liver and hepatic diseases<sup>[20,21]</sup>. This fact could be expected from the high percentage of identity (84%) between the MAT $\alpha$ 1 and MAT $\alpha$ 2 subunits<sup>[54]</sup>. Additionally, the PDRG1-MAT $\alpha$ 2 interaction interface seems to overlap with the binding site for the regulatory MAT $\beta$  subunit that is displaced from the oligomer upon PDRG1 attachment<sup>[20]</sup>. Further insights into the putative areas of interaction were obtained through the use of truncated PDRG1 forms. These protein variants were designed according to information obtained from the available structural model and lack the N-terminus, the C-terminus or both protein ends<sup>[20]</sup>. Analysis of their interaction with MAT $\alpha$ 1 and MAT $\alpha$ 2 indicated a role for the C-terminal end of PDRG1 in the interaction, an area that is not involved in the interaction with other members of the R2TP/prefoldin-like complex, which seem to use the hook for this purpose<sup>[42]</sup>.

Tissue *Pdrg1* expression mimics the expression profile of *Mat2a* (the MAT $\alpha$ 2 gene), while following the opposite pattern from that exhibited by *Mat1a* (the MAT $\alpha$ 1 gene)<sup>[20]</sup>. This fact coincides with the preferential nuclear localization of MAT $\alpha$ 1 in those cell types/tissues where *Mat1a* expression is low, thus increasing the probability of interaction with PDRG1 in those settings. This interaction impairs AdoMet production by approximately 50%, as demonstrated *in vitro* using purified MAT $\alpha$ 1 or MAT $\alpha$ 2 homo-oligomers or MAT II hetero-oligomers and PDRG1. Similarly, decreased global DNA methylation levels were detected in cultured cells upon overexpression of PDRG1 or coexpression of PDRG1 and MAT $\alpha$ 1<sup>[20,21]</sup>. Altogether, these results provide the first evidence for PDRG1 function as a modulator of methyl donor production.

## ROLE OF PDRG1 IN DISEASE

To date, there is no disease directly linked to alterations in *PDRG1*, although the OMIM 610789 code has been associated with this gene. In fact, the NCBI database already includes descriptions of more than 700 SNPs in this gene, including point mutations in the ORF, frameshifts and early stop codons (Table 1). However, the impact of these alterations remains to be studied.

### *PDRG1* and cancer

The chromosomal location of *PDRG1* coincides with a region with high frequency of sequence gains in several types of human cancers, including breast, liver, lung, colon and rectum tumors<sup>[25,55-59]</sup>. Moreover,

p53 is downregulated in several human cancers, and hence, its regulation of the *PDRG1* gene is expected to be impaired. The same is true for miR-214, which is also downregulated in cervical, breast, HCC and bladder tumors<sup>[33,60-62]</sup>. Using commercial cancer profiling arrays, 60%-85% upregulation of *PDRG1* was detected in a variety of tumor types with impaired p53 expression, confirming this hypothesis<sup>[47]</sup>. This same study also showed that the percentage of patients with increased *PDRG1* levels was dependent on the type of tumor analyzed. In bladder cancer, miR-214 levels were associated with the tumor stage and recurrence, whereas an inverse correlation with *PDRG1* expression was detected<sup>[33]</sup>. Moreover, this tumor suppressor role was found to be exerted through miR-214 binding to the 3'-UTR of the *PDRG1* mRNA, in turn leading to a reduction in its levels.

Correlation of elevated *PDRG1* and *GLUT1* expression with poor pathological response has been reported in residual rectal cancer cells after pre-operative chemotherapy<sup>[63]</sup>. However, *PDRG1* expression levels were not associated with the overall survival of the patients despite its proposed roles in DNA damage regulation and tumor growth. Furthermore, a role for PDRG1 in cancer can also be inferred from the reports linking its interaction target URI to the development of ovarian cancer and HCC<sup>[64,65]</sup>. In this same line, the reduced miR-214 levels found in breast cancer cells lead to the accumulation of the enhancer of Zeste homologue 2 (EZH2) methyltransferase. EZH2 is a component of the polycomb repressive complex 2 (PRC2) and catalyzes the inclusion of the repressive epigenetic mark H3K27me3<sup>[61]</sup>. Levels of this repression mark are enhanced in HCC<sup>[66]</sup>, where nuclear accumulation of MAT $\alpha$ 1 is induced, putatively favoring its interaction with PDRG1. This apparent contradictory effect could be explained by the high affinity of EZH2 for AdoMet, which putatively makes this particular methyltransferase insensitive to the decreased methyl donor concentrations resulting from the nuclear MAT $\alpha$ 1/PDRG1 interaction.

The upregulated expression of *PDRG1* correlates with enhanced PDRG1 protein levels, for example in colon cancer samples (mainly in epithelial cells) and in some lung cancer cell lines (A549 and H446)<sup>[47,67]</sup>. Tumor cell lines have been used for stable or transient overexpression and downregulation of PDRG1 to study effects on proliferation, apoptosis or the response to UV irradiation. PDRG1 overexpression in A549 and H446 lung cancer cells enhanced proliferation, whereas no morphological changes in NIH 3T3 fibroblasts, HCT116 colon cancer or H35 hepatoma cells were observed<sup>[20,22,67]</sup>. Regarding apoptosis, no signs of this process were detected in NIH 3T3 or HCT116 cells after overexpression<sup>[22]</sup>. In contrast, downregulation of *PDRG1* in T24 and 5637 bladder cancer cell lines by overexpression of miR-214 led to decreased proliferation, enhanced apoptosis and reduced invasiveness<sup>[33]</sup>. *PDRG1* knockdown in RKO colon and A549 and H446 lung cancer cells also caused reduction in cell growth<sup>[47,67]</sup>.

**Table 1 SNPs for the human *PDRG1* gene listed in the NCBI database leading to mutated protein forms**

SNP code <sup>1</sup>	Base change	Mutated residue	Type of mutation
rs777897331	1A>C	M1L	Missense
rs748585638	19G>A	E7K	Missense
rs867545839	23G>T	R8L	Missense
rs779425406	29T>C	L10P	Missense
rs755286962	32G>A	R11Q	Missense
rs150958567	34T>C	Y12H	Missense
rs142496377	35A>G	Y12C	Missense
rs375829085	46G>A	V16M	Missense
rs760411451	59C>T	A20V	Missense
rs763777690	57_68delCGCCG- AGGAGGT	A20_V23del	Indel
rs765247648	67G>T	V23L	Missense
rs759432900	83G>A	R28Q	Missense
rs772260316	91G>A	V31M	Missense
rs139473332	92T>C	V31A	Missense
rs147359616	110G>T	R37M	Missense
rs201481003	113A>C	N38T	Missense
rs749744129	119A>C	N40T	Missense
rs142890852	121C>G, 121C>T	R41G, R41stop	Missense, stop gained
rs539323269	122G>A	R41Q	Missense
rs746198655	134G>A	R45K	Missense
rs370045763	155G>C	S52T	Missense
rs758120112	157C>G	L53V	Missense
rs748723095	175G>T	V59F	Missense
rs775880591	184G>A	G62R	Missense
rs200644098	188A>G	N63S	Missense
rs767238840	190A>G	M64V	Missense
rs764202244	208C>T	H70Y	Missense
rs777531663	210_211insAA	P71Nfs	Frameshift
rs566583428	211C>T	P71S	Missense
rs768828941	223_224ins <sup>2</sup>	E75Vfs	Frameshift
rs750997250	251_252delTG	L84Rfs	Frameshift
rs745401995	264A>G	I88M	Missense
rs780722451	265G>C	E89Q	Missense
rs201754951	274C>T	R92W	Missense
rs186921219	275G>A	R92Q	Missense
rs140650422	295G>A	V99I	Missense
rs200378363	299A>T	N100I	Missense
rs565384763	301C>T	R101C	Missense
rs779220725	302G>A	R101H	Missense
rs753937019	317A>G	Q106R	Missense
rs143995288	319G>A	G107S	Missense
rs768088765	323A>G	K108R	Missense
rs200900452	326C>T	P109L	Missense
rs759134978	328G>A	E110K	Missense
rs776274160	332T>C	L111P	Missense
rs370586022	340T>C	F114L	Missense
rs764544630	344_345insA	N115Kfs	Frameshift
rs760255766	359A>G	N120S	Missense
rs772012403	361C>T	Q121stop	Stop gained
rs149510387	365A>G	D122G	Missense
rs774273098	366T>G	D122E	Missense
rs548757558	377C>T	A126V	Missense

<sup>1</sup>Information collected in January 2017; <sup>2</sup>Inserted sequence TGAGCT TTTCTTTTTTACATGCCCTGCAATAGAGACTGGGTGA.

However, stable silencing in H35 hepatoma cells did not induce significant changes in growth of any of the clones analyzed or in their morphology<sup>[20]</sup>. This downregulation was never above 70%, suggesting that further suppression of *PdrG1* expression in hepatoma cells could be lethal. Additionally, the differences observed may arise

not only from the downregulation extent achieved in each tumor cell but also from the diverse procedures used for silencing and the distinct basal levels in the corresponding normal cells. Altogether, these results suggest a putative role for *PDRG1* in apoptosis and proliferation, which might be exerted through its interaction with different targets. Among those identified to date, *PDCD7* contributes to the modulation of apoptosis, *MAP1S* participates in cell cycle progression through its interaction with *RASSF1A*, and *CIZ* interacts with *p21* for their shuttling from the nucleus to the cytoplasm.

Focusing our attention on stably silenced H35 hepatoma cells, the maximum downregulation achieved is still well above the levels detected in normal liver<sup>[20,21]</sup>. Nevertheless, important changes in the expression profile were found using microarrays, these alterations affecting a large variety of pathways<sup>[20,21]</sup>. A total of 114 genes consistently changed their pattern in different clones, of which only 80% could be ascribed to GO pathways. Among upregulated genes, *Lpin1* was found. This is an essential gene in fat metabolism that is regulated by *p53* and induced by DNA damage and glucose deprivation<sup>[68]</sup>. *Lpin1* codes for a bifunctional protein that catalyzes diacylglycerol synthesis and acts as a transcriptional coactivator in hepatocytes to regulate fatty acid oxidation<sup>[68]</sup>. In contrast, expression of neither the *TP53* nor the *Mat* genes were significantly altered in the silenced cells, but many of the routes affected are implicated in pathological processes in which the pattern of *Mat* gene expression is modified<sup>[20,21]</sup>.

As mentioned in the initial sections of this mini-review, *PDRG1* was identified when the effects of UV irradiation were analyzed. Therefore, it was not surprising that stable HCT116 clones overexpressing HA-*PDRG1* displayed increased sensitivity to UV irradiation or that this agent upregulated *PDRG1* in several human cell lines, including some lacking *p53* or carrying mutant forms of this protein<sup>[22]</sup>. In fact, higher *PDRG1* mRNA levels were detected after irradiation upon *TP53* repression by doxycycline in Tet-inducible DLD1 colon cancer cells as well as in *TP53*<sup>-/-</sup> cells. These data suggested an inverse correlation of *PDRG1* and *TP53* expression but also with that of *Cip1* (the *p21* gene, also named *CDKN1*), in turn indicating involvement of a *p53*-independent mechanism<sup>[22]</sup>. Additional studies carried out by Jiang *et al*<sup>[47]</sup> demonstrated that only agents inducing genotoxic stress (adriamycin, etoposide, camptothecin and UV) upregulated *PDRG1* expression.

The link between *PDRG1* and radioresistance was further supported when an inverse correlation between *PDRG1* and *ATM* levels was detected in lung cancer cells after irradiation<sup>[67]</sup>. In fact, *PDRG1*-silenced cells exhibited high *ATM* levels together with increased *p53* phosphorylation on serine 15, and tumors generated in nude mice using these silenced clones were more susceptible to irradiation<sup>[67]</sup>. Oleuropein treatment of nasopharyngeal cancer cell lines reduced *PDRG1* and *HIF1α* expression and protein levels, while inducing miR-519d levels<sup>[34]</sup>. Further examination of the effects

of the treatment revealed that oleuropein avoids HIF1 $\alpha$  downregulation of miR-519, in turn decreasing PDRG1 levels in the cells and favoring sensitivity to irradiation both in the cells and in tumor xenografts.

### **PDRG1 in liver disease**

*Pdrg1* expression has also been analyzed in models of liver disease, where the *Mat1a* to *Mat2a* expression switch was detected together with oxidative stress. The results reported to date indicate no significant alteration of hepatic *Pdrg1* levels in early stages of a model of Wilson disease, the Long Evans Cinnamon rats, despite the evident accumulation of copper<sup>[20]</sup>. However, acute liver injury induced by D-galactosamine intoxication induced the *Mat* expression switch, nuclear accumulation of MAT $\alpha$ 1 and the upregulation of *Pdrg1* expression and its nuclear levels<sup>[20]</sup>. Of note, the upregulation of *Pdrg1* reported in this model was more modest than that measured in hepatoma cells.

### **PDRG1 in T cell selection**

Autoimmune diseases present altered T cell selection mechanisms that have been explored using microarrays and animal models such as the non-obese diabetic (NOD) mice<sup>[69]</sup>. Thymocytes obtained from this model of human autoimmune diabetes have a reduced number of genes allowing distinction between positive and negative T cell selection compared to cells from a control mouse strain. This fact was especially important in the negative selection gene set, which included *Pdgr1*. Basal *Pdrg1* expression was reduced in NOD thymocytes, and this gene was also identified among the high-quality candidates for *D2mit490*-linked defective thymic deletion, *D2mit490* being a marker for the loci contributing to the defective negative selection detected in NOD mice<sup>[69]</sup>.

## **CONCLUSION**

PDRG1 is a member of the R2TP/prefoldin-like complex with nucleocytoplasmic distribution. In acute liver injury and a variety of tumors, high levels of *PDRG1* expression have been detected. Several protein-protein interactions involving PDRG1 have been found while exploring the components of multiprotein complexes, putatively linking this protein to processes such as splicing, apoptosis and nutrient signaling. However, the role of these interactions and how and when do they occur remains unclear. Increased *PDRG1* expression correlates with decreased levels of AdoMet, the main cellular methyl donor. Moreover, this increased expression also correlates with nuclear accumulation of MAT $\alpha$ 1 and with changes in epigenetic methylations that may be of a different sign depending on the AdoMet affinity of each specific methyltransferase. Such behavior can be explained by the decreased capacity for AdoMet synthesis exhibited by the MAT isoenzymes upon their interaction with PDRG1. This modulatory role of AdoMet levels is the first function ascribed to PDRG1. The fact that this interaction is

restricted to the nucleus may exert a counteracting effect on liver pathology, precluding the objectives pursued by the nuclear accumulation of MAT $\alpha$ 1. Moreover, the interest and impact of this nuclear interaction could be of major importance for most cells, where PDRG1 expression is higher and MAT $\alpha$ 1 localization is restricted to this compartment. Altogether, the aspects that deserve further attention include the identification of the interaction surfaces in complexes involving PDRG1, how the interactions are regulated, and the structure of the oncogene or its regulation. Knowledge of structural features may be of use for the design of new therapeutic options that, for example, interfere with pathological protein-protein interactions involving PDRG1.

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