PDRG1 at the interface between intermediary metabolism and oncogenesis

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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 MATα1 and MATα2. Through this interaction, PDRG1 downregulates nuclear S-adenosylmethionine synthesis, hence impacting epigenetic methylations.

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INTRODUCTION

Late in the 20th century, global DNA hypomethylation was described as a main characteristic of cancer cells and as the underlying cause of their altered expression pattern[1,2]. 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[3]. 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[4,5]. 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[6,7]. Those are glycine N-methyltransferase (GNMT), guanidinoacetate N-methyltransferase (GAMT) and phosphatidylethanolamine N-methyltransferase (PEMT), which produce sarcosine, creatine and phosphatidylcholine, respectively[8-11]. 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 partial-length expressed sequence tag (EST) clones rendered a UV-induced EST clone that hybridized with a 1.4 kb transcript, which was named PDRG1[12]. 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 S-methyltetrahydrofolate, respectively[17,18]. 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[12,13,15,16]; and (4) BHMT[19].

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[20]. 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[22-24]. This latter procedure rendered a UV-induced EST clone that hybridized with a 1.4 kb transcript, which was named PDRG1[22]. 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)[22]. 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
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. Moreover, these are pathologies in which impairment of the methionine cycle, especially of AdoMet production, have been described. 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. The gene contains a TATA-like element (TAATAA) localized at -268 from the start codon, but no canonical TATA box was identified. Promoter activity was eliminated by deletion of the -138/+1 region, in turn suggesting the presence of key regulatory elements in this segment. 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. The second corresponds to a putative p53 transcriptional repressor element (TRE) located at -854/-834. Regulation of the PDRG1 promoter by p53 was further proven when luciferase activity was abolished in the presence of this tumor suppressor. 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; this factor is known to upregulate expression of a number of genes when activated by UV irradiation. Cotransfection of a PDRG1 promoter-luciferase construct and Oct-1 into
MCF-7 cells rendered approximately 8-fold induction of the luciferase activity, thus demonstrating the functionality of this Oct-1 binding element. This increased promoter activity was far greater than that caused exclusively by UV irradiation, a fact that Luo et al. 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. 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. 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).

Regarding the PDRG1 expression profile, this aspect was initially studied using commercial human poly(A) + Northern blots, where a 1.4 kb transcript was identified in brain, heart, liver, lung, spleen, stomach, testis and skeletal muscle. More recently, real-time RTqPCR of rat tissues also demonstrated wide expression of Pdgri, further including the cerebellum, intestine, pancreas, kidney and cava vein. 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.

**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...
3, 52, 54 and mouse serine 120 and ubiquitinations on human lysine residues 27, 108 and 125 have been postulated or found [35-41]. 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
Motif searches identified a helix-turn-helix (LNQDEL KALKVILKG) at the C-terminal end of the human and mouse protein sequences. 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 β-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 MATα1 and MATα2. 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. 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. 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. 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\textsuperscript{[43]}. 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\textsuperscript{[43]}. 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\textsuperscript{[43]}.

Additional studies to analyze the cytoplasmic assembly of RNA polymerase II were later performed by Boulon et al\textsuperscript{[46]}. These MS-proteomics studies showed association of unassembled RPB1, the largest subunit of this transcription machinery, with the R2TP/prefoldin-like complex\textsuperscript{[46]}. 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 RPB5, although this binding seemed indirect through its association with hSpagh\textsuperscript{[46]}. The PDRG1-URI interaction is abundant, according to the number of peptides retrieved\textsuperscript{[46,49]}.

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\textsuperscript{[45,46]}. 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\textsuperscript{[47]}. These results linked PDRG1 with the modulation of apoptosis and the U12-type spliceosome, a process and a complex that involve PDCD7\textsuperscript{[48]}.

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\textsuperscript{[49]}. URI stands for unconventional prefoldin RPBS 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 TIP49ab in HEK293/ FRT and HeLa cells, as well as bound to BC014022, DPCD, FLJ20643, NUFIP, RPB5MP and UXT1\textsuperscript{[49]}. Reciprocal studies using FLAG-tagged PDRG1 identified interactors with moderate (POL3A, PPP1CA, PPP1CC, PFDN2, HKE2, RPB5, PPP1CB, NPM1, UXT1, H2B5, H2B8, RPB5MP, FLJ21908, BC014022, H2AZ, NOP5/ NOP58, FLJ20643) or low probability scores (TUB6, HSP70/1, HSP70/1B, BAF53, TIP49a, TIP49b)\textsuperscript{[49]}. 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\textsuperscript{[49]}. Both studies considered the PDRG1-URI interaction abundant, according to the number of peptides retrieved\textsuperscript{[46,49]}. The PDRG1-URI interaction seems to involve the hook-shaped β-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\textsuperscript{[49]}. 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\textsuperscript{[50]}. 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\textsuperscript{[51,52]}. 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\textsuperscript{[53]}.

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\textsuperscript{[50]}. On the other hand, YTH screening of a rat liver library identified PDRG1 interacting with Mat\textsubscript{α1}, a result that was further confirmed by immunoprecipitation and pull-downs\textsuperscript{[20]}. These results linked PDRG1 with methionine metabolism and AdoMet-dependent methylations\textsuperscript{[20]}, which are altered in tumor cells. Mat\textsubscript{α1} 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\textsuperscript{(12,14)}. Despite the nucleocytoplasmic localization of both MAT\textalpha\textalpha\textalpha and PDRG1, their interaction was restricted to the nuclear compartment, where large association states (approximately 360 kDa) could be detected\textsuperscript{(20,21)}. 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\textalpha\textalpha catalytic subunit of heterotrimeric MAT II, the main isoenzyme found in extrahepatic tissues, fetal liver and hepatic diseases\textsuperscript{(20,21)}. This fact could be expected from the high percentage of identity (84\%) between the MAT\textalpha\textalpha and MAT\textalpha\textalpha subunits\textsuperscript{[54]}. Additionally, the PDRG1-MAT\textalpha\textalpha interaction interface seems to overlap with the binding site for the regulatory MATI subunit that is displaced from the oligomer upon PDRG1 attachment\textsuperscript{[20]}. 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\textsuperscript{[20]}. Analysis of their interaction with MAT\textalpha\textalpha and MAT\textalpha\textalpha 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\textsuperscript{[42]}

Tissue Pdrg1 expression mimics the expression profile of Mat2a (the MAT\textalpha gene), while following the opposite pattern from that exhibited by Mat1a (the MAT\textalpha\textalpha gene)\textsuperscript{[20]}. This fact coincides with the preferential nuclear localization of MAT\textalpha 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\textalpha\textalpha or MAT\textalpha\textalpha 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\textalpha\textalpha\textalpha. 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\textsuperscript{(25,55-59)}. 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\textsuperscript{(33,60-62)}. 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\textsuperscript{[47]}. 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\textsuperscript{[33]}. 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\textsuperscript{[63]}. 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\textsuperscript{[64,65]}. 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\textsuperscript{[61]}. Levels of this repression mark are enhanced in HCC\textsuperscript{[66]}, where nuclear accumulation of MAT\textalpha 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\textalpha/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)\textsuperscript{[47,67]}. 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\textsuperscript{(20,22,67)}. Regarding apoptosis, no signs of this process were detected in NIH 3T3 or HCT116 cells after overexpression\textsuperscript{[21,67]}. 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\textsuperscript{[33]}. PDRG1 knockdown in RKO colon and A549 and H446 lung cancer cells also caused reduction in cell growth\textsuperscript{[47,67]}.
However, stable H35 hepatoma cells did not induce significant changes in growth of any of the clones analyzed or in their morphology. 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 shutting 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 cells. Nevertheless, important changes in the expression profile were found using microarrays, these alterations affecting a large variety of pathways. 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. Lpin1 codes for a bifunctional protein that catalyzes diacylglycerol synthesis and acts as a transcriptional coactivator in hepatocytes to regulate fatty acid oxidation. 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.

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. 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+/– 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.

Additional studies carried out by Jiang et al. 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. 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. Oleuropein treatment of nasopharyngeal cancer cell lines reduced Pdrg1 and HIF1α expression and protein levels, while inducing miR-519d levels. Further examination of the effects of Pdrg1 was never observed.
of the treatment revealed that oleuropein avoids HIF1α 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[200]. However, acute liver injury induced by D-galactosamine intoxication induced the *Mat* expression switch, nuclear accumulation of MATα1 and the upregulation of *Pdrg1* expression and its nuclear levels[20]. 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[60]. 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 *Pdgr1* 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[69].

**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 MATα1 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 MATα1. Moreover, the interest and impact of this nuclear interaction could be of major importance for most cells, where PDRG1 expression is higher and MATα1 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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**REFERENCES**

1. Díaz ES, Hoffman RM. Hypomethylation of HeLa cell DNA and the absence of 5-methylcytosine in SV40 and adenovirus (type 2) DNA: analysis by HPLC. *Biochem Biophys Res Commun* 1982; 107: 19-26 [PMID: 6289818 DOI: 10.1016/0006-291X(82)91663-1]
2. Laird PW, Jaenisch R. DNA methylation and cancer. *Hum Mol Genet* 1994; 3 Spec No: 1487-1495 [PMID: 7849743 DOI: 10.1093/hmg/3.suppl.1.1487]
3. Mecham JO, Rowitch D, Wallace CD, Stern PH, Hoffman RM. The metabolic defect of methionine dependence occurs frequently in human tumor cell lines. *Biochem Biophys Res Commun* 1983; 117: 429-434 [PMID: 6661235 DOI: 10.1016/0006-291X(83)91218-4]
4. Cantoni GL. Biological methylation: selected aspects. *Annu Rev Biochem* 1975; 44: 435-451 [PMID: 1094914 DOI: 10.1146/annurev.bi.44.070175.002251]
5. Pettrossian TC, Clarke SG. Uncovering the human methyltransferase: *Mol Cell Proteomics* 2011; 10: M110.000976 [PMID: 20936037 DOI: 10.1074/mcp.M110.000976]
6. Stead LM, Brosnan JT, Brosnan ME, Vance DE, Jacobs RL. Is it time to revaluate methyl balance in humans? *Am J Clin Nutr* 2006; 83: 5-10 [PMID: 16400042]
7. Mudd SH, Brosnan JT, Brosnan ME, Jacobs RL, Stabler SP, Allen RH, Vance DE, Wagner C. Methyl balance and transmethylation fluxes in humans. *Am J Clin Nutr* 2007; 85: 19-25 [PMID: 17209172]
8. Luka Z, Mudd SH, Wagner C. Glycine N-methyltransferase and regulation of S-adenosylmethionine levels. *J Biol Chem* 2009; 284: 22507-22511 [PMID: 19480883 DOI: 10.1074/jbc.M109.019273]
9. Brosnan JT, da Silva RP, Brosnan ME. The metabolic burden of creatinine synthesis. *Amino Acids* 2011; 40: 1325-1331 [PMID: 21387089 DOI: 10.1007/s00726-011-0853-y]
10. Vance DE. Phospholipid methylation in mammals: from biochemistry to physiological function. *Biochem Biophys Acta* 2014; 1838: 1477-1487 [PMID: 24184426 DOI: 10.1016/j.bbamem.2013.10.018]
11. Mato JM, Pajares MA, Varela I. How many phospholipid methyltransferases are there in mammalian cells? *Trends Biochem Sci* 1984; 9: 471-472 [DOI: 10.1016/0968-0004(84)90311-6]
12 Reytor E, Pérez-Miguel Sanz J, Alvarez L, Pérez-Sala D, Pajares MA. Conformational signals in the C-terminal domain of methionine adenosyltransferase VIII determine its nucleotycloprotein distribution. *FASEB J* 2009; 23: 3347-3360 [PMID: 19497982 DOI: 10.1096/fj.08-130758]

13 Katoh Y, Ikura T, Hoshikawa Y, Tashiro S, Ino T, Ohta M, Kera Y, Noda T, Igarkashi K. Methionine adenosyltransferase II serves as a transcriptional corepressor of Maf oncoprotein. *Mol Cell* 2011; 41: 554-566 [PMID: 21362551 DOI: 10.1016/j.molcel.2011.02.018]

14 Delgado M, Garrido F, Pérez-Miguel Sanz J, Pacheco M, Partearroyo T, Pérez-Sala D, Pajares MA. Acute liver injury induces nucleotycloprotein redistribution of hepatic methionine metabolism enzymes. *Antioxid Redox Signal* 2014; 20: 2541-2554 [PMID: 24124652 DOI: 10.1089/ars.2013.5342]

15 Krupenko NI, Wagner C. Transport of rat liver glycine N-methyltransferase into rat liver nuclei. *J Biol Chem* 1997; 272: 27140-27146 [PMID: 9341155 DOI: 10.1074/jbc.272.43.27140]

16 Radomski N, Kaufmann C, Dreyer C. Nuclear accumulation of S-adenosylhomocysteine hydrolase in transcriptionally active cells during development of Xenopus laevis. *Mol Biol Cell* 1999; 10: 4283-4298 [PMID: 10586858 DOI: 10.1091/mbc.10.12.4283]

17 Pajares MA, Markham GD. Methionine adenosyltransferase (s-adenosylmethionine synthetase). *Adv Enzymol Relat Areas Mol Biol* 2011; 78: 449-521 [PMID: 22220481 DOI: 10.1002/9781118110571.ch11]

18 Pajares MA, Pérez-Sala D. Betaine homocysteine S-methyltransferase: just a regulator of homocysteine metabolism? *J Biol Chem* 2003; 278: 19679-19686 [DOI: 10.1074/jbc.M203020200]

19 Johnson RA, Ince TA, Scotto KW. Transcriptional repression by p53 through direct binding to a novel DNA element. *J Biol Chem* 2001; 276: 27716-27720 [PMID: 11350951 DOI: 10.1074/jbc.C100121200]

20 Resnick-Silverman L, St Clair S, Maurer M, Zhao K, Manfredi JJ. Identification of a novel class of genomic DNA-binding sites suggests a mechanism for selectivity in target gene activation by the tumor suppressor protein p53. *Genes Dev* 1998; 12: 2102-2107 [PMID: 9679504 DOI: 10.1101/gad.12.14.2102]

21 el-Deiry WS, Kern SE, Pietenpol JA, Kinzler KW, Vogelstein B. Definition of a consensus binding site for p53. *Nat Genet* 1992; 1: 45-49 [PMID: 1301998 DOI: 10.1038/ng0492-45]

22 Wong J, Li PX, Klummt HJ. A novel p53 transcriptional repressor element (p53TRE) and the asymmetrical contribution of two p53 binding sites modulate the response of the placental transforming growth factor-beta promoter to p53. *J Biol Chem* 2002; 277: 26699-26707 [PMID: 12011055 DOI: 10.1074/jbc.M203020200]

23 Latchman DS. POU family transcription factors in the nervous system. *J Cell Physiol* 1999; 179: 126-133 [PMID: 10199551 DOI: 10.1002/(SICI)1097-4652(199905)179:2.CO;2-M]

24 Takahashi S, Saito S, Ohtani N, Sakai T. Involvement of the Oct-1 regulatory element of the gadd45 promoter in the p53-independent response to ultraviolet irradiation. *Cancer Res* 2001; 61: 1187-1195 [PMID: 11221850]

25 Zhao H, Jin S, Fan F, Fan W, Tong T, Zhan Q. Activation of the transcription factor Oct-1 in response to DNA damage. *Cancer Res* 2000; 60: 6276-6280 [PMID: 11103783]

26 Wang J, Zhang X, Wang L, Yang Y, Dong Z, Wang D, Lu L, Wang C. MicroRNA-214 suppresses oncogenesis and exerts impact on prognosis by targeting PDRG1 in bladder cancer. *PLoS One* 2015; 10: e0118806 [PMID: 25709619 DOI: 10.1371/journal.pone.0118806]

27 Xu T, Xiao D. Oleuropein enhances radiation sensitivity of nasopharyngeal carcinoma by downregulating PDRG1 through HIF1α-repressed microRNA-519d. *J Exp Clin Cancer Res* 2017; 36: 3 [PMID: 28057028 DOI: 10.1186/s13046-016-0480-2]

28 Sharma K, D’Souza RC, Tyanova S, Schaub C, Wisniewski JR, Cox J, Mann M. Ultra deep human phosphoproteome reveals a distinct regulatory nature of Tyr and Ser/Thr-based signaling. *Cell Rep* 2014; 8: 1583-1594 [PMID: 25159151 DOI: 10.1016/j.celrep.2014.07.036]

29 Mertins P, Yang F, Liu T, Mani DR, Petukh VA, Gillette MA, Clauser KR, Qiao JW, Gritsenko MA, Moore RJ, Levine DA, Smith RD, Paulovich AG, Ellis M, Carr SA. Ischemia in tumors induces early and sustained phosphorylation changes in stress kinase pathways but does not affect global protein levels. *Mol Cell Proteomics* 2014; 13: 1690-1704 [PMID: 24719451 DOI: 10.1074/mcp.M113.036392]

30 Zhou H, Di Palma S, Preisinger C, Peng M, Polat AN, Heck AJ, Mohammed S. Toward a comprehensive characterization of a human cancer cell phosphoproteome. *J Proteome Res* 2013; 12: 260-271 [PMID: 23188613 DOI: 10.1021/pr300630k]

31 Olsen CM, Carroll HJ, Whiteman DC. Estimating the attributable fraction for cancer: A meta-analysis of nevi and melanoma. *Cancer Prev Res (Phila)* 2010; 3: 233-245 [PMID: 20068181 DOI: 10.1158/1940-6207.CAPR-09-0148]

32 Wu X, Tian L, Li J, Zhang Y, Han V, Li Y, Xu X, Li H, Chen X, Chen J, Jin W, Xie Y, Han J, Zhong Q. Investigation of receptor interacting protein (RIP3)-dependent protein phosphorylation by quantitative phosphoproteomics. *Mol Cell Proteomics* 2012; 11: 1640-1651 [PMID: 22942356 DOI: 10.1074/mcp.M111.019091]

33 Wisniewsky JR, Nagaraj N, Zougram A, Gnaid F, Mann M. Brain phosphoproteome obtained by a FASP-based method reveals plasma membrane protein topology. *J Proteome Res* 2010; 9: 3280-3289 [PMID: 20415495 DOI: 10.1021/pr1002214]

34 Kim W, Bennett EJ, Huttlin EL, Guo A, Li J, Possemato A, Sowa ME, Rad R, Rush J, Comb MJ, Harper JW, Gygi SP. Systematic and quantitative assessment of the ubiquitin-modified proteome. *Mol Cell Proteomics* 2011; 10: 1265-1276 [PMID: 21906983 DOI: 10.1074/mcp.M110.102241]

35 Pita S, Savas JN, Pfander J, Lange A, Jancekova V, Hlubokova M. Determination of the subcellular localization of tetraspanin CD63 using a novel anti-CD63 antibody. *Cell Biol Int* 2011; 35: 1424-1432 [PMID: 21684226 DOI: 10.1002/1097-4187.cib.2307]

36 Jeronimo C, Forget D, Bouchard A, Li Q, Chua G, Poitras C, Thérien C, Bergeron D, Bourassa S, Greenblatt J, Chabot B, Poirier GG, Hughes TR, Blanchette M, Price DH, Coulombe B. Systematic analysis of the protein interaction network for the human transcription machinery reveals the identity of the 7SK capping enzyme. *Mol Cell* 2007; 27: 262-274 [PMID: 17643375 DOI: 10.1016/
Boulin S, Pradet-Balade B, Verheggen C, Mollé D, Boireau S, Georgiev A, Azzag K, Robert MC, Almad N, Neel H, Lamond AI, Bertrand E. HSFP90 and its R2TP/ prelin-like co-chaperone are involved in the cytoplasmic assembly of RNA polymerase II. Mol Cell 2010; 39: 912-924 [PMID: 20864308 DOI: 10.1016/j.molcel.2010.08.023]

Ruhl DD, Jin J, Cai Y, Swanson S, Flores L, Washburn MP, Conaway RC, Conaway JW, Chrivia JC. Purification of a human SRCAP complex that remodels chromatin by incorporating the histone variant H2A.Z into nucleosomes. Biochemistry 2006; 45: 5671-5677 [PMID: 16634648 DOI: 10.1021/bi0604343]

Jin J, Cai Y, Yao T, Gottschalk AJ, Flores L, Swanson SK, Gutiérrez JL, Coleman MK, Workman JL, Mushegian A, Washburn MP, Conaway RC, Conaway JW. A mammalian chromatin remodeling complex with similarities to the yeast INO80 complex. J Biol Chem 2005; 280: 41207-41212 [PMID: 16230350 DOI: 10.1074/jbc.M509128200]

Jiang L, Luo X, Shi J, Sun H, Sun Q, Sheikh MS, Huang Y. PDRG1, a novel tumor marker for multiple malignancies that is selectively regulated by genotoxic stress. Cancer Biol Ther 2011; 11: 567-573 [PMID: 21938342 DOI: 10.4161/cbt.11.6.14414]

Will CL, Schneider C, Hosbach M, Urlaub H, Rauther R, Elbashir S, Tuschi T, Lührmann R. The human 18S U1/U12 snRNP contains a set of novel proteins not found in the U2-dependent spliceosome. RNA 2004; 10: 929-941 [PMID: 15146077 DOI: 10.1261/ma.7320064]

Sardiu ME, Cai Y, Jin J, Swanson SK, Conaway RC, Conaway JW, Flores L, Washburn MP. Probabilistic assembly of human protein interaction networks from label-free quantitative proteomics. Proc Natl Acad Sci USA 2008; 105: 1454-1459 [PMID: 18218781 DOI: 10.1073/pnas.0706983105]

Gstaiger M, Luke B, Hess D, Oakeley EJ, Wirbelauer C, Bloddel M, Vigneron M, Peter M, Krek W. Control of nutrient-sensitive gene expression by targeting UDP-N-acetyl-α-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 7. J Biol Chem 2012; 287: 14301-14309 [PMID: 22539294 DOI: 10.1074/jbc.M111.337642]

Verbeek W, Schultz HJ, Sperling M, Tiesmeier J, Stoop H, Dinjens W, Looijenga L, Wörnmann B, Fuzesi L, Donhuijzen K. Rectal adenocarcinoma with choriocarcinomatous differentiation: clinical and genetic aspects. Hum Pathol 2004; 35: 1427-1430 [PMID: 15668990 DOI: 10.1016/j.humpath.2004.06.005]

Peng RQ, Wan HY, Li HF, Liu M, Li X, Tang H. MicroRNA-214 suppresses growth and invasiveness of cervical cancer cells by targeting UDP-N-acetyl-a-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 7. J Biol Chem 2012; 287: 14301-14309 [PMID: 22539294 DOI: 10.1074/jbc.M111.337642]

Sengupta S, Tanaka K, Toiyama Y, Matsushita K, Kawamura M, Okugawa Y, Hiro J, Inoue Y, Uchida K, Mohri Y, Kusunoki M. Gene expression profiles of tumor regression grade in locally advanced rectal cancer after neoadjuvant chemoradiotherapy. Oncol Rep 2012; 28: 855-861 [PMID: 22711167 DOI: 10.3892/or.2012.1863]

Theurillat JP, Metzler SC, Henzi N, Djouner N, Helbling M, Zimmermann AK, Jacob F, Soltermann A, Caduff R, Henzelmann-Schwarz V, Moch H, Krek W. URI is an oncogene amplified in ovarian cancer cells. J Pathol 2012; 57: 584-591 [PMID: 22613005 DOI: 10.1016/j.jpath.2012.04.031]

Saigusa S, Tanaka K, Toiyama Y, Matsushita K, Kawamura M, Okugawa Y, Hiro J, Inoue Y, Uchida K, Mohri Y, Kusunoki M. Gene expression profiles of tumor regression grade in locally advanced rectal cancer after neoadjuvant chemoradiotherapy. Oncol Rep 2012; 28: 855-861 [PMID: 22711167 DOI: 10.3892/or.2012.1863]

Sánchez-Pérez GF, Bautista JM, Pajares MA. Methionine adenosyltransferase as a useful molecular systematics tool revealed by phylogenetic and structural analyses. J Mol Biol 2004; 345: 693-706 [PMID: 14687567 DOI: 10.1016/j.jmb.2003.11.022]

Hodgson JG, Chin K, Collins C, Gray JW. Genome amplification of chromosome 20 in breast cancer. Breast Cancer Res Treat 2003; 78: 337-345 [PMID: 12755492 DOI: 10.1023/A:1020385825042]

Wong MP, Fung LF, Wang E, Chow WS, Chiu SW, Lam WK, Ho KK, Ma ES, Wang WS, Chung LP. Chromosomal aberrations of primary lung adenocarcinomas in nonsmokers. Cancer 2003; 97: 1263-1270 [PMID: 12599234 DOI: 10.1002/cncr.11183]

Schlegel J, Sturmm G, Schertham H, Bocker T, Zimghl H, Rüschhoff J, Hofstädter F. Comparative genomic in situ hybridization of colon carcinomas with replication error. Cancer Res 1995; 55: 6002-6005 [PMID: 8521381]

Lukášová E, Zuzek B, Falk M, Zouzubek M, Zaloudik J, Vagunda V, Pavlovský Z. Topography of genetic loci in the nuclei of cells of colorectal carcinoma and adjacent tissue of colorectal epithelium. Chromosoma 2004; 112: 221-230 [PMID: 14722771 DOI: 10.1007/s00403-002-0623-3]

Verbeek W, Schultz HJ, Sperling M, Tiesmeier J, Stoop H, Dinjens W, Looijenga L, Wörnmann B, Fuzesi L, Donhuijzen K. Rectal adenocarcinoma with choriocarcinomatous differentiation: clinical and genetic aspects. Hum Pathol 2004; 35: 1427-1430 [PMID: 15668990 DOI: 10.1016/j.humpath.2004.06.005]

Peng RQ, Wan HY, Li HF, Liu M, Li X, Tang H. MicroRNA-214 suppresses growth and invasiveness of cervical cancer cells by targeting UDP-N-acetyl-a-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 7. J Biol Chem 2012; 287: 14301-14309 [PMID: 22539294 DOI: 10.1074/jbc.M111.337642]

Verbeek W, Schultz HJ, Sperling M, Tiesmeier J, Stoop H, Dinjens W, Looijenga L, Wörnmann B, Fuzesi L, Donhuijzen K. Rectal adenocarcinoma with choriocarcinomatous differentiation: clinical and genetic aspects. Hum Pathol 2004; 35: 1427-1430 [PMID: 15668990 DOI: 10.1016/j.humpath.2004.06.005]

Peng RQ, Wan HY, Li HF, Liu M, Li X, Tang H. MicroRNA-214 suppresses growth and invasiveness of cervical cancer cells by targeting UDP-N-acetyl-a-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 7. J Biol Chem 2012; 287: 14301-14309 [PMID: 22539294 DOI: 10.1074/jbc.M111.337642]
