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Review Article

Multi-talented DEAD-box proteins and potential tumor promoters: p68 RNA helicase (DDX5) and its paralog, p72 RNA helicase (DDX17)

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Abstract: P68 (DDX5) and p72 (DDX17) are members of the DEAD-box RNA helicase family. They can unwind double-stranded RNA and also contribute to the remodeling of ribonucleoprotein complexes. These activities of p68/p72 are required for efficient RNA splicing and microRNA processing. In addition, p68/p72 perform functions that are independent of their enzymatic activity. This is especially common to their role in gene regulation, where p68/p72 coactivate various transcription factors, including the tumor suppressor p53, estrogen receptor α and β-catenin. P68/p72 are posttranslationally modified by SUMO attachment and phosphorylation that regulate their coactivation potential, binding to known interactants or protein stability. Knock-out mouse models revealed that both DDX5 and DDX17 are essential genes during development. Furthermore, together with their ability to stimulate cell proliferation and prevent apoptosis, the reported overexpression of p68/p72 in three of the major human cancers (colon, breast, prostate) strongly suggests that p68/p72 promote tumorigenesis and might even represent proto-oncoproteins. If so, their inhibition holds promise as a novel way to contain or cure various carcinomas.

Keywords: Cancer, DDX5, DDX17, DEAD-box, p68 RNA helicase, p72 RNA helicase

Introduction

RNA, for instance in various viruses, can exist in double-stranded form, but single-stranded RNA is also able to form double-stranded regions by engaging in intra- and intermolecular interactions. Examples are the secondary structures of tRNAs or rRNAs within ribosomes. Also, RNA base-pairing is important during transcript splicing and RNA interference, indicating that unwinding as well as forming of double-stranded RNA is involved in numerous cellular processes. RNA helicases support, or are even indispensable for unwinding RNA [1]. Moreover, RNA helicases are capable of disrupting RNA-protein interactions and are thus crucial for the remodeling of many ribonucleoproteins [2, 3].

The largest family of RNA helicases, comprising 38 members in humans, are the DEAD-box (DDX) proteins, which are named after a conserved signature amino acid sequence (Asp-Glu-Ala-Asp, or D-E-A-D). DDX proteins hydrolyze ATP, which is often stimulated by the presence of double- or single-stranded RNA. However, only in a minority of cases has it been demonstrated that a DDX protein is a true RNA helicase [4]. Furthermore, the yeast Dbp9p DEAD-box protein was reported to exhibit DNA helicase activity, suggesting that DDX proteins may not always exclusively act on RNA [5]. This review will focus on two paralogous DEAD-box RNA helicases, p68 (DDX5) and p72 (DDX17), highlighting their function in normal cells and their potential role as tumor promoters.

Identification, structure and expression of p68/p72 RNA helicases

Three decades ago, an antibody directed against the simian virus 40 large T oncoprotein was shown to cross-react with a 68 kDa cellular

protein that resides within the nucleus [6]. This antibody was then utilized to screen a cDNA expression library and the respective DDX5 gene cloned [7]. Sequence analysis revealed homology to the eukaryotic translation initiation factor eIF4A, the first protein identified to unwind RNA in an ATP-dependent manner. Thus, it was no surprise that the p68 protein was subsequently shown to be an RNA-dependent ATPase and a helicase capable of unwinding RNA [8, 9]. Thereafter, the DDX17 gene was cloned and shown to encode for two proteins, p72 and p82 RNA helicase. The larger isoform is due to alternative translation initiating at a non-AUG start codon preceding the AUG codon that defines the translation start site for p72 RNA helicase in the DDX17 mRNA [10, 11]. Western blotting of breast and colon cancer cell lines indicated that p72 and p82 are generally expressed at similar levels [12-14]. Biochemical studies suggest that p72 and p82 have nearly identical properties [11], but this does not exclude that their physiological functions could differ. Moreover, p72/p82 and p68 RNA helicase can form both homo- as well as heterodimers [15], implicating that these paralogous proteins have overlapping functions.

Amino acid alignment exposes that 439 (71.5%) out of the 614 amino acids within p68 RNA helicase are identical and 478 (77.9%) similar to those in p72/p82 (**Figure 1**). This homology is even higher within the helicase domain consisting of p68 amino acids 96-436: 298 (87.4%) amino acids are identical, and 313 (91.8%) are similar to the DDX17 gene products. In contrast, the N-terminus (amino acids 1-95 of p68) and C-terminus (amino acids 437-614 of p68) are only 71.6% and 54.5% similar to the respective domains in p72/p82, suggesting that DDX5 and DDX17 gene products may perform distinct functions.

Like other RNA helicases, p68 and p72/p82 possess eight conserved motifs (see **Figure 1**) within their catalytic core [1]. In addition, a Q-motif that is specific for DEAD-box RNA helicases is present at the N-terminus of the catalytic core and is preceded by an aromatic amino acid located 17 residues upstream of the Q-motif. This conserved aromatic amino acid and the Q-motif are regarded as an adenine recognition motif, which is important for binding of RNA substrates, ATP hydrolysis and helicase activity [16, 17]. Motifs I and II (also called Walker A and B motif, respectively) are found in many NTPases and bind ATP as well as associated Mg^{2+}. These domains are indispensable for ATP hydrolysis and thus also helicase activity. Further, motifs III and VI are thought to interact with the γ-phosphate of ATP and couple ATP hydrolysis with helicase activity, whereas motifs Ia, Ib, IV and V are engaged in binding of the RNA substrate [18].

Originally, both p68 and p72 RNA helicase were described as nuclear proteins [6, 9, 10]. However, more detailed biochemical and immunohistochemical analyses revealed that significant amounts of p68 RNA helicase are also present in the cytoplasm of various cell lines, whereas little, if any, p72 is localized within the cytoplasm [13, 19, 20]. Recently, it was found that p68 is indeed shuttling between the cell nucleus and the cytoplasm, which involves two nuclear localization and two nuclear export signals; however, it remains unknown how this shuttling is regulated [21]. Moreover, the intranuclear distribution of p68 is cell cycle-dependent: whereas p68 is excluded from nucleoli during interphase, it appears in nascent nucleoli during telophase [22, 23]. Again, it is unknown how this is regulated and if this bears any physiological relevance. Furthermore, mass spectrometry of purified HeLa nucleoli suggests that p72 is a nucleolar protein similar to p68 RNA helicase, but it was not studied if this is limited to nucleoli in telophase [24].

Expression of DDX5 or DDX17 mRNA appears to be ubiquitous, but expression levels vary significantly between different organs [10, 25] (see also **Figure 2**). Northern blotting reveals two major species of DDX5 mRNA (~2.4 and ~4.2 kb), with the longer transcript most likely representing an incompletely spliced mRNA, whose translation is predicted to result in a truncated DDX5 gene product [25]. As such a truncated DDX5 protein has not been observed by Western blotting, it is likely that either this truncated protein is very labile or that the 4.2 kb DDX5 mRNA is inefficiently translated. Similarly, at least two major mRNAs (5.3 and 9.3 kb) are derived from the DDX17 gene [10], indicating that differentially spliced DDX17 mRNAs exist that may give rise to several protein isoforms.

Essential roles in development

The yeast ortholog of p68/p72 is Dbp2p.

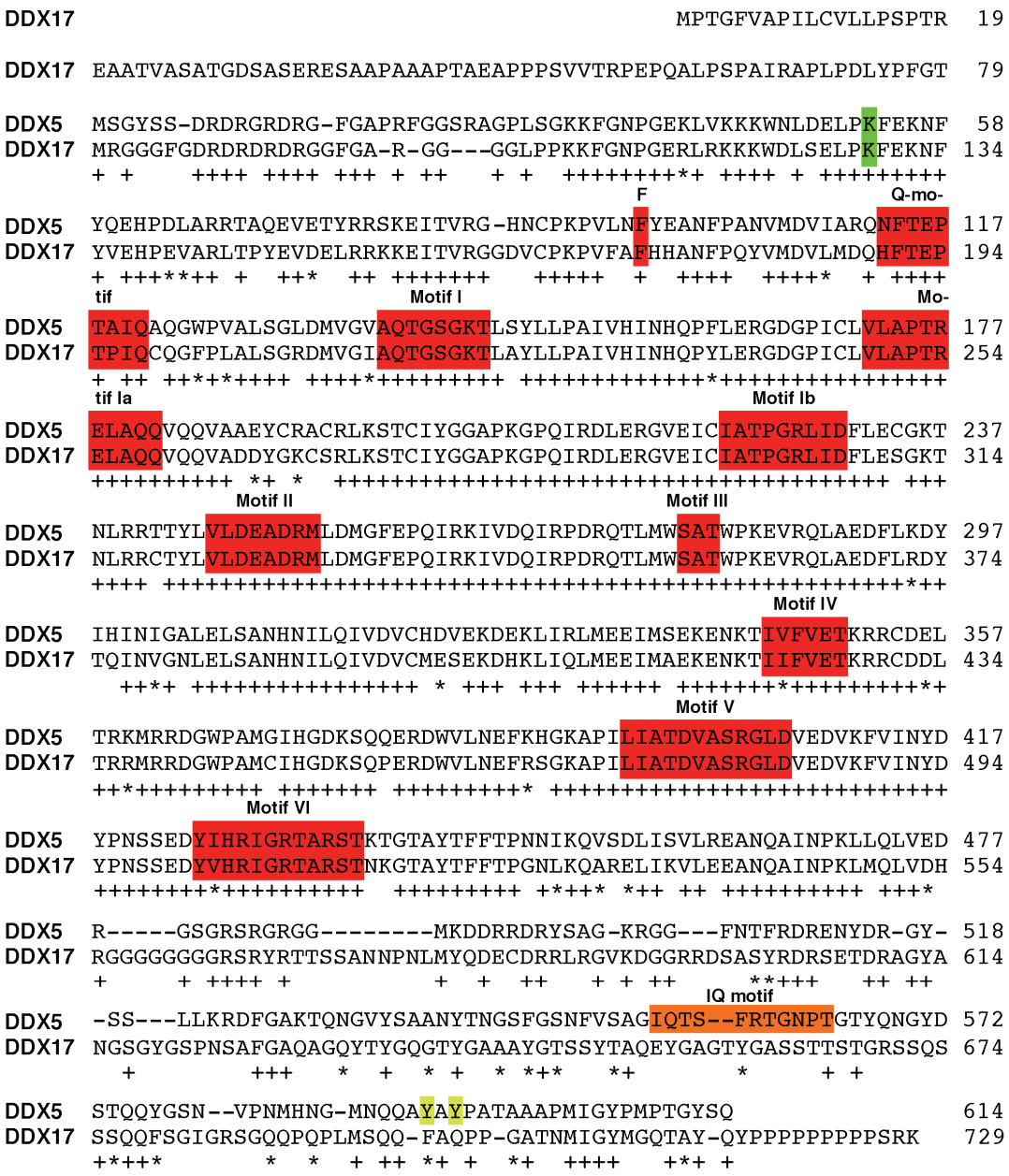

Figure 1. Protein sequence alignment of p68 RNA helicase (DDX5) with DDX17. The 729 amino acid long DDX17 protein corresponds to p82 RNA helicase, whereas DDX17 amino acids 80-729 represent p72 RNA helicase. DDX5 amino acids were derived from NCBI reference sequence NP_004387.1 and DDX17 amino acids from NP_006377.2. Identical amino acids are highlighted by "+" and similar ones by "*". Nine motifs characteristic for DEAD-box RNA helicases are boxed in red, as is the conserved phenylalanine preceding the Q-motif. Motifs I and II are also known as Walker A and B motifs, respectively. The center of motif II consists of the amino acid sequence Asp-Glu -Ala-Asp, after which the DEAD-box family of RNA helicases is named. Sumoylation and tyrosine phosphorylation sites are highlighted in green and yellow, respectively, and the IQ motif in orange.

Although originally reported to be essential for viability, a later study demonstrated that inactivation of the DBP2 gene was not lethal, but rather vastly reduced cell growth and resulted in a cold-sensitive phenotype [22, 26]. Regardless, this was the first hint that p68/p72 RNA helicases perform crucial functions. Moreover, mutations within the Drosophila p68 RNA helicase

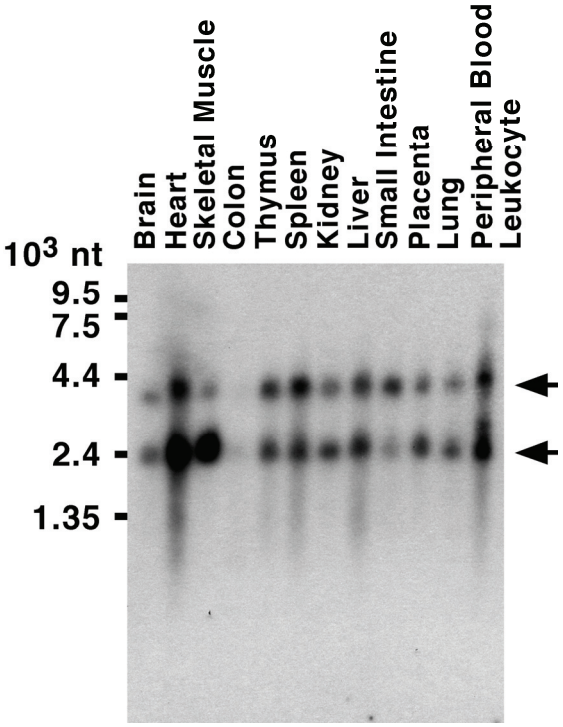

Figure 2. Human multiple tissue Northern blot containing equal amounts of poly(A)$^+$-RNA in each lane was hybridized with a human DDX5 cDNA probe. Arrows point to major DDX5 mRNAs of 4.2 and 2.4 kb size.

gene showed phenotypes ranging from sterility to lethality [27], indicating that p68 RNA helicase is essential during development. Indeed, both DDX5 and DDX17 are expressed in chick, frog, mouse and rat embryos [28-30], and individually knocking out DDX5 or DDX17 resulted in lethality in mice [31]. However, there was a distinction between DDX5 and DDX17: whereas DDX5 knockout embryos died at embryonal day 11.5, DDX17 knockout mice survived for up to two days after birth. No gross changes in organogenesis were observed, but both knockout mice displayed malformation of blood vessels.

Analysis of DDX17$^{-/-}$ mouse embryonic fibroblasts revealed a drastic reduction in cell growth and enhanced apoptosis, and similarly p68 downregulation by RNA interference reduced cell growth and survival [31]. Further, joint downregulation of DDX5 and DDX17 in RKO colon cancer or HeLa cervical carcinoma cells suppressed cell proliferation [13, 32], and likewise knock-down of DDX17, but not DDX5, in MCF-7 breast cancer cells curtailed estrogen-dependent growth [33]. Altogether, these results demonstrate that DDX5 and DDX17 are both essential for development and may have profound effects on cell growth and survival.

Unwinding RNA

P68 and p72 RNA helicase bind to double- and single-stranded RNA, albeit the affinity for the former appears to be much higher. Furthermore, binding to RNA stimulates their ATPase activity, which provides the energy to unwind RNA duplexes in both the 3'→5' and 5'→3' direction [34, 35]. In addition, p68 and p72 possess an RNA annealing activity, which, together with their helicase activity, catalyzes the rearrangement of secondary structures in RNA [36]. It may be via such an action that p68/p72 RNA helicases contribute to ribosome biogenesis that requires extensive rRNA remodeling to form mature ribosomes [32], and consistently the yeast ortholog of p68/p72 has been shown to be associated with pre-ribosomal complexes [37].

RNA splicing involves the association and dissociation of the pre-mRNA with snRNAs, which may be facilitated by RNA helicases. Indeed, mass spectrometric analysis of the spliceosome identified p68 RNA helicase to be an associated protein [38]. Thereafter, it was demonstrated that both p68 and p72 specifically interact with the U1 small nuclear ribonucleoprotein particle that recognizes the 5' splice site [35, 39, 40]. In fact, p68 molecules devoid of ATPase or RNA helicase activity inhibited the dissociation of U1 from the 5' splice site, and accordingly downregulation of DDX5 resulted in the accumulation of unspliced RNA [41]. Moreover, p68 and p72 RNA helicase have been shown to affect alternative splicing [42, 43]. Altogether, these results suggest that p68 and p72 RNA helicase are crucial factors required for efficient RNA splicing.

Another function of p68 and p72/p82 relates to the processing of microRNA (miRNA). The primary miRNA transcript (pri-miRNA) is processed in the cell nucleus by the Drosha complex into pre-miRNA, a hairpin intermediate. Mass spectrometry of the Drosha complex revealed that p68 and p72 are part of this complex [44]. Moreover, knock-down of either DDX5 or DDX17 resulted in impaired processing of selected pri-

miRNAs as well as of the 5.8S rRNA precursor [31]. The fact that ATPase activity is required for p72 to potentiate pri-miRNA processing suggests that p68/p72 unwind RNA to make it accessible for cleavage in the Drosha complex [31]. Another function of p68/p72 in the Drosha complex is based upon their ability to bind Smad proteins and the tumor suppressor p53 [45, 46]. When recruited into the Drosha complex by association with p68 RNA helicase, Smad proteins as well as p53 stimulate the maturation of selected miRNAs [47, 48]. Finally, it has been shown that p68 RNA helicase unwinds the let-7 miRNA duplex in vitro [49]. This suggests that p68 RNA helicase may help loading miRNAs into the silencing complex. In fact, this may not be limited to miRNAs, but also extend to siRNAs [50].

Many other processes involving RNA may require helicase activity and thus p68/p72 RNA helicases. For instance, the yeast and Drosophila orthologs of p68/p72 affect nonsense-mediated mRNA decay and RNA release from chromatin, respectively [27, 51]. Or the zinc-finger antiviral protein, which binds to viral mRNAs and targets them for degradation, interacts with p72 RNA helicase, whose enzymatic activity is thought to restructure viral RNA and thereby makes it more amenable for degradation [52]. On the other hand, cellular p68 is utilized by the hepatitis C virus RNA-dependent RNA polymerase to facilitate the production of negative-strand from positive-strand viral RNA. Of note, the RNA-dependent RNA polymerase binds to p68 RNA helicase and thereby leads to its relocalization from the nucleus into the cytoplasm [53]. Thus, it is likely that also other RNA viruses highjack p68/p72 to promote their replication. And indeed, the influenza virus RNA polymerase complex and the SARS (severe acute respiratory syndrome) coronavirus helicase have been shown to bind to p68 RNA helicase, and DDX5 downregulation led to impaired SARS coronavirus replication [54, 55].

Roles in gene transcription

The transcription of chromosomal genes can be roughly divided into three parts: initiation, elongation and termination. At present, it is unclear whether p68/p72 participate in the latter processes, but ample of evidence suggests that they are involved in the initiation of gene transcription. Consistently, p68/p72 interact with RNA polymerase II and ubiquitous transcriptional cofactors such as CBP, p300 and PCAF [12, 19]. CBP and p300 are two homologous acetyltransferases that acetylate lysine residues in histones and many transcription factors [56, 57], and PCAF is another acetyltransferase that forms complexes with CBP/p300 [58, 59]. Interestingly, p68/p72 also bind to histone deacetylase (HDAC) 1, 2 and 3 [14, 60] that can antagonize the action of CBP/p300 and PCAF. Which of these interactions prevails (acetyltransferases versus HDACs) may determine whether p68/p72 activate or repress gene transcription.

The first time that p68/p72 were shown to function as transcriptional coactivators was in estrogen-dependent transcription. P68/p72 bind directly to the estrogen receptor α (ERα) [61, 62] and are one of the earliest proteins being recruited to the ERα-regulated pS2 gene promoter after estrogen stimulation [63]. Notably, p68/p72 molecules mutated in their helicase domain were as able to stimulate estrogen-dependent transcription as respective wild-type molecules [33, 61], indicating that p68/p72 do not act as RNA helicases in stimulating ERα-dependent transcription. Similarly, p68/p72 appear to bind to the androgen receptor, but only p68 RNA helicase is capable of stimulating androgen-dependent transcription, which again did not require its catalytic activity [64, 65]. Also, when functioning as a coactivator for the p53 tumor suppressor, Runx2 or MyoD, RNA helicase activity of p68/p72 was not essential [12, 46, 66, 67].

In contrast, RNA helicase activity is required for p68/p72 to activate cyclin D1 transcription [68]. In this case, p68/p72 mediate their effect through binding to β-catenin [13, 20]. Interestingly, there seem to be two modes of how these proteins affect β-catenin: (i) p68, upon platelet-derived growth factor (PDGF) stimulated phosphorylation on Y593, facilitates the translocation of β-catenin from the cytoplasm into the nucleus [20], although this finding is controversial [69]. Notably, p72 is not predicted to act in this way, since it lacks a phosphorylation site that is homologous to Y593 (see **Figure 1**). (ii) p68/p72 function as transcriptional coactivators of β-catenin in the cell nucleus, and consistently interact with the gene promoters of the β-catenin targets, cyclin D1 and c-Myc [13, 68].

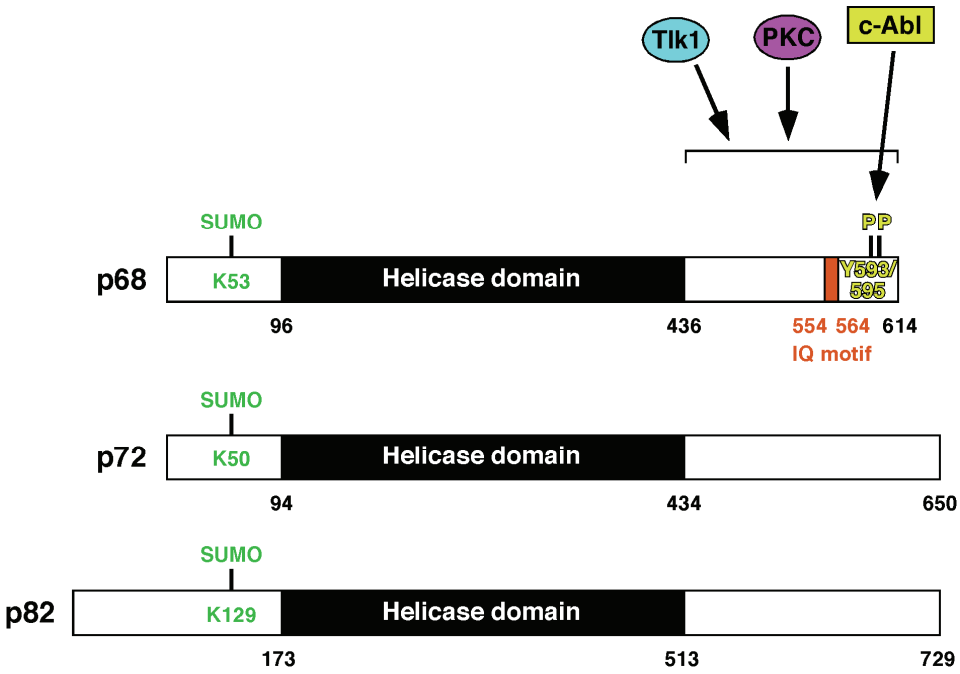

Figure 3. Reported sumoylation and phosphorylation sites in p68, p72 or p82 RNA helicase.

The steroid receptor coactivator (SRA) gene encodes for several alternatively spliced mRNAs, which give rise to two different gene products: a non-coding RNA molecule and the SRA protein, SRAP [70-72]. While it is still unresolved which functions SRAP exerts, it is evident that the SRA RNA is a coactivator of steroid hormone receptors [70]. Moreover, p68/p72 bind to the SRA RNA molecule as well as the p160 family of steroid hormone coactivators, thereby enhancing ERα-dependent gene transcription [62]. In addition, p68/p72 cooperate with the SRA RNA in stimulating the transcription factor MyoD [67]. Since no helicase activity of p68/p72 is required for ERα and MyoD coactivation, p68/p72 are not thought to remodel the exquisite secondary structure of the SRA RNA that is important for its function [73]. Rather, p68/p72 may help to recruit the SRA RNA into transcriptional complexes by virtue of their RNA binding activity. It remains to be determined whether SRA recruitment is a general mechanism of how p68/p72 stimulate gene transcription or if this is limited to ERα- and MyoD-dependent transcription.

Regulation by posttranslational modification

The first demonstration that p68 RNA helicase can be posttranslationally modified was its in vitro phosphorylation by PKC, a protein kinase that stably binds to p68 RNA helicase [74, 75]. Although the phosphorylation site was not mapped, it is likely to occur within the IQ motif (see **Figures 1** and **3**), a sequence that is involved in calmodulin binding, has a (I/L/V)QXXXRXXXX(R/K) consensus and often contains a PKC or PKA site [76]. Notably, the IQ motif found in p68 is absent in p72/p82 (see **Figure 1**). It was shown that both PKC-mediated phosphorlyation and calmodulin binding of p68 RNA helicase inhibit its ATPase activity [74]. Moreover, PKC inhibits the ability of the p68 C-terminal region to bind to single-stranded RNA [77]. A similar phenomenon was observed upon phosphorylation by the serine/threonine kinase Tlk1 [78], suggesting that PKC and Tlk1 may target the same phosphorylation site(s) in p68 RNA helicase.

PDGF as well as tumor necrosis factor-α induce the phosphorylation of p68 on tyrosine [79, 80]. Furthermore, tyrosine phosphorylation of p68 appears to be prominent in cancer cell lines, but not in non-tumorigenic ones, suggesting that tyrosine phosphorylation of p68 may be a marker for tumor cells [80]. However, a systematic analysis of human normal and tumor tissue

specimens is needed to validate this assumption. Subsequently, it was shown that PDGF stimulation of colon cancer cells leads to the activation of c-Abl, a non-receptor tyrosine kinase and proto-oncogene [81], which then phosphorylates p68 RNA helicase on Y593. This phosphorylation was reported to be required for efficient interaction with β-catenin, its translocation from the cytoplasm into the cell nucleus and the induction of epithelial-mesenchymal transition [20]. Interestingly, Y593 phosphorylation is also needed for p68 RNA helicase to coactivate androgen-dependent transcription in LNCaP prostate cancer cells [64]. In T98G glioblastoma cells, an autocrine PDGF-loop results into additional phosphorylation of p68 on Y595, and phosphorylation on both Y593 and Y595 confers resistance to TRAIL-induced apoptosis [82]. **Figure 1** shows that p72/p82 do not possess any tyrosine residues that are homologous to Y593 and Y595 in p68 RNA helicase, implicating that p68, but not p72/p82, is a downstream effector of PDGF or tumor necrosis factor-α. Finally, p38 MAP kinase phosphorylates p68 RNA helicase, but neither the site(s) of phosphorylation nor the biological significance of this phosphorylation event is known [79].

A second type of posttranslational modification is ubiquitylation. Both p68 and p72 are ubiquitylated in vivo, but whereas p68 shows a high degree of poly-ubiquitylation, p72 is predominantly mono-ubiquitylated [14, 83]. Possibly because of this difference in poly-ubiquitylation that normally targets proteins for degradation, the half-life of p72 is much longer compared to p68 RNA helicase (68 h versus 20 h in 293T cells; ref. [14]). However, up to now the lysine residue(s) becoming ubiquitylated have not been uncovered.

In contrast, one conserved lysine residue in the N-terminus of p68 and p72 RNA helicase (K53 and K50, respectively; see **Figures 1** and **3**) has been identified to be a target for sumoylation [14, 84]. This conserved lysine residue conforms to the consensus sequence for sumoylation ΨKXE, where Ψ is a large aliphatic amino acid [85]. Sumoylation of p68/p72 has a variety of consequences: (i) It doubles protein stability of p68, whereas the protein half-life of p72 is only slightly increased upon sumoylation. (ii) It enhances the interaction of p68 and p72 with HDAC1, but has no effect on their interaction with HDAC2, HDAC3, p300 or ERα. (iii) It affects the coactivation potential of p68/p72 [14, 84]. However, the impact of sumoylation on the coactivation potential differs dependent on the promoter studied for p68: whereas its K53R mutant was slightly more potent to activate an artificial p53-responsive promoter, it was as potent as wild-type p68 in stimulating p53-dependent MDM2 transcription and drastically less able to stimulate ERα-dependent transcription. In contrast, mutation of the p72 sumoylation site resulted in enhanced ERα-dependent transcription as well as p53-mediated MDM2 upregulation, clearly showing that sumoylation affects p68 and p72 differently. Increased recruitment of HDAC1 to gene promoters normally leads to a reduction of gene transcription. Accordingly, this could explain why p72-K50R, which binds less efficiently to HDAC1 than wild-type p72, is transcriptionally more potent than wild-type p72. And similarly, this provides a plausible explanation why p68-K53R activates an artificial p53-responsive promoter more than wild-type p68, but it does not rationalize why p68-K53R coactivates ERα less efficiently than wild-type p68. However, it has not been resolved if changed HDAC1-interaction is indeed an underlying cause for the differences in the transactivation potential of sumoylated versus non-sumoylated p68/p72.

The cancer connection

It is now well established that p68/p72 promote cell proliferation and survival [13, 31-33, 68, 82, 86]. In addition, DDX5 overexpression protects lung carcinoma cells from the topoisomerase-1 poison, camptothecin, which is often employed in cancer therapy [87], and p68 RNA helicase facilitates epithelial-mesenchymal transition [20], a process that is not only important during embryogenesis but also for invasion and metastasis of tumors [88]. Moreover, the DDX5 gene passed three litmus tests for a proto-oncogene: its ectopic expression in NIH3T3 and NC3H10 fibroblasts resulted in the formation of foci on top of a monolayer of cells, caused anchorage-independent growth in soft agar, and induced tumor formation in nude mice [89]. Provided that these latter experiments can be independently confirmed, these data altogether point strongly at a tumor promoting activity of p68 and p72.

Consistent with a possible role as proto-

oncoproteins, both p68 and p72 were found to be overexpressed in colorectal tumors [13, 83]. Immunohistochemical staining clearly showed that p68/p72 expression increased with the progression of the disease from hyperplastic polyps to adenomas and adenocarcinomas, the latter ones overexpressing p68/p72 in ≥90% of all cases. How could this overexpression contribute to colorectal tumor formation? A key event during colon carcinogenesis is the aberrant activation of β-catenin [90, 91], and both p68 and p72 RNA helicase bind to β-catenin and stimulate β-catenin-dependent transcription of target genes such as c-Myc, cyclin D1 and c-jun. Furthermore, downregulation of DDX5/DDX17 in colorectal cancer cells reduced proliferation and tumor formation in nude mice [13, 20, 68]. Collectively, these data suggest that p68/p72 overexpression might be an underlying cause of colon cancer formation by augmenting β-catenin.

Similarly, p68 and p72 were reported to be overexpressed in 30-58% or 72-76% of breast tumors, respectively [14, 33]. Approximately 70% of human breast tumors are ERα-positive and pharmacological inhibition of ERα is one mainstay of therapy [92, 93]. Since both p68 and p72 are coactivators of ERα, their overexpression may enhance the oncogenic activities of ERα, providing a plausible mechanism how p68/p72 contribute to breast tumorigenesis. Similarly, p68 was found to be overexpressed in prostate tumors and is capable of coactivating androgen receptor [64], the key villain in prostate tumorigenesis [94], and could thereby facilitate prostate tumorigenesis.

One open question is how do p68/p72 become overexpressed in tumors? Recent analyses suggest that DDX5 mRNA is overexpressed in ovarian cancer or multiple myelomas [95, 96], but this seems not to be true for colon carcinomas [83]. Thus, DDX5 and DDX17 gene expression may be upregulated at the transcriptional level in some tumors, but at the posttranscriptional level in others. Notably, sumoylation of p68 or p72 strongly or moderately, respectively, stabilizes these proteins [14]. Further, SUMO1, the SUMO conjugating enzyme Ubc9 and the SUMO ligase PIAS3 appear to be upregulated in breast cancer cells, whereas the SUMO protease SENP6 is downregulated [14, 97], indicating that posttranslational modification by SUMO is generally enhanced in breast tumors. This could, at least in part, explain why p68 and p72 become overexpressed in these tumors. However, more research is needed to elucidate the mechanisms of p68/p72 overexpression in various tumors. As a caveat, not all tumors will show enhanced p68/p72 levels, one example being the apparent downregulation of DDX17 mRNA in meibomian cell carcinoma [98]. And finally, genetic studies are required to prove that p68/p72 are indeed tumor promoters or even true oncoproteins. To this end, transgenic or conditional knock-out mouse models should be established and analyzed whether overexpression enhances and lack of p68/p72 suppresses spontaneous as well as chemically or genetically induced tumorigenesis.

Conclusion

The RNA helicases p68 and p72 perform a plethora of cellular functions and are essential for development. In most cases, these two proteins require enzymatic activity to perform their functions and thus behave as true helicases. But their role in transcription is often independent of their RNA helicase activity, suggesting that p68/p72 function similar to other transcriptional cofactors that recruit further proteins to gene promoters or establish the contact with the RNA polymerase II holoenzyme. Many lines of evidence strongly suggest, but do not yet prove, that p68/p72 overexpression promotes tumorigenesis. Notably, p68/p72 may also be involved in other diseases, including obesity [99-101], development of brain defects in Down syndrome [102] or hepatic fibrosis [103, 104].

The facts that p68/p72 are overexpressed in major carcinomas (breast, prostate, colon) and are enzymes makes them attractive drug targets. Small molecules could be developed that fit into their catalytic center and block ATPase and/or helicase activity. If the structures of p68/p72 would be known, rational drug design could be of great help to develop such inhibitors. Unfortunately, the structures of p68/p72 can currently only be modeled from known structures of other RNA helicases [18], although attempts are underway to elucidate the structure of p68 RNA helicase [105]. Finally, if the main oncogenic functions of p68/p72 are independent of their enzymatic activity, other means than blocking the catalytic center of p68/p72 must be found to suppress their harmful action in tumors.

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