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REVIEW

The p53 family reaches the final frontier: the variegated regulation of the dark matter of the genome by the p53 family in cancer

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ABSTRACT

The tumour suppressor p53 and its paralogues, p63 and p73, are essential to maintain cellular homoeostasis and the integrity of the cell's genetic material, thus meriting the title of 'guardians of the genome'. The p53 family members are transcription factors and fulfill their activities by controlling the expression of protein-coding and non-coding genes. Here, we review how the latter group transcended from the 'dark matter' of the transcriptome, providing unexpected and intriguing anti-cancer therapeutic strategies.

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Introduction

Almost twenty years ago, the sequencing of the human genome was officially completed [1]. This Herculean effort lasted for almost two decades and had already produced its preliminary results in 2001 [2,3], when it became irrefutable that the protein coding portion of the genome – i.e. that behaving accordingly to the central dogma of molecular biology – was not as large as expected. Indeed, despite the fact that more than 85% of the human genome is actively transcribed [4], only ~2% of this transcriptome corresponds to messenger RNAs (mRNAs). Most of the remaining 98% comprises a large amount of RNA species with poorly characterized or completely unknown functions, which were initially dubbed as the 'dark matter' of the genome [5] and have since then represented an intensive field of research. This research has contributed to the expansion of the ever-growing classification of RNA species. In addition to the long-known yet still surprising groups of ribosomal RNAs (rRNAs), transfer RNAs (tRNAs), and small nuclear and nucleolar RNAs (snRNAs and snoRNAs), there are 4 other main classes of RNA molecules [6]. The largest of these classes comprises the PIWI-interacting RNAs (piRNAs), whose expression is limited to specific developmental stages of germ cells [7]. The remaining categories are more widely expressed and include the ~22 nucleotides long microRNAs (miRNAs) [8], the longer than 200 nucleotides long non-coding RNAs (lncRNAs) [9], and the back-splicing generated circular RNAs (circRNAs) [10]. Notably, many members of these RNA species are dysregulated in human cancers [11–15] underscoring the relevance of these RNA molecules for tumour initiation and progression. This dysregulation is intertwined with the genetic alterations affecting one of the most important tumour suppressive pathways, the p53 pathway. Indeed, the tumour suppressor p53 is the main cellular hub responsible for maintaining cellular homoeostasis and genome integrity, and it fulfils its roles by controlling the expression of protein-coding and non-coding genes alike [16–18]. Given its centrality in counteracting stimuli that can perturb the physiological conditions of cells [19,20], the loss of p53 functions is a common feature of human cancers [21], and numerous animal models have shown that either lack [22,23] of or mutations [24,25] in the *TP53* gene determines the onset of a variety of tumours and can recapitulate the human tumour-predisposing syndrome, known as Li-Fraumeni.

Despite being considered unique to the point of deserving the title of the 'guardian of the genome'[26], it is now clear that p53 is supported in its activities by the other members of its family, p63 and p73 [27–29]. These three transcription factors share a similar DNA binding domain, that allows them to regulate the expression of a common pool of genes crucial to prevent tumorigenesis, including genes involved in cell-cycle arrest [30], DNA repair [31], apoptosis [32], autophagy [19], and cellular metabolism [20]. Beyond these commonalities, however, different biological functions are associated with each of the p53 family members, as highlighted by the phenotypes of the respective knockout mouse models. Indeed, p63 emerged as crucial for the proper formation and differentiation of pluri-stratified epithelia [33,34], while p73 is essential for the development of the central nervous system [35]. Further diversification in their biological roles is provided by the complex structure of the p53 family genes, all of which encode numerous isoforms due both to the usage of alternative promoters and to the splicing events involving the respective 3′ UTRs [36–38]. The various isoforms are grouped in two sets based either on the presence of a transcriptional activation domain resembling that present in p53 (TA isoforms) or on the absence thereof (ΔN isoforms). The TA isoforms have tumour suppressive properties and can

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be bound and inhibited by the ΔN isoforms, which can conversely exert oncogenic activities [39–41]. In line with this, human cancers generally show an imbalance among these isoforms in favour of the ΔN isoforms [42]. The distinct roles of the various isoforms are corroborated by the unique phenotypes of the isoform-specific knockout mouse models. Indeed, even though both the *TAp63*$^{-/-}$ and the *TAp73*$^{-/-}$ mice are tumour prone [43,44], the former is characterized by premature ageing[45], stem cell defects [46] and tendency to diabetes and obesity [47], while the latter cannot properly differentiate multiciliated cells, thereby having impaired functionality of several organs, such as ear, ependyma, fallopian tube, and trachea [48,49]. In the case of the ΔN isoforms, instead, loss of ΔNp63 prevents the terminal differentiation of the epidermis and causes craniofacial abnormalities and limb defects [50–52], while lack of ΔNp73 is associated with severe neurodegeneration [53,54].

The specific roles of the different isoforms of the p53 family members are ultimately achieved via the regulation of unique transcriptional programmes, which comprise both protein-coding and non-coding target genes. Here, we focus on the numerous connections between the p53 family members and the different classes of non-coding RNAs in physiological conditions as well as in cancers, and we discuss the possible therapeutic approaches targeting such connections.

Ribosomal biogenesis, nucleolar stress, and the p53 family

Ribosomal RNAs (rRNAs) represent ~85% of the RNA mass in eukaryotic cells [6] and their biogenesis is finely regulated to guarantee a constant balance between their expression and that of the 80 ribosomal proteins (RPs) that ultimately constitute the ribosomes [55]. To facilitate such regulation, ribosomal biogenesis is temporally and spatially confined in the nucleolus, where one the two rRNA precursors, 47S, is transcribed by the RNA polymerase I and processed into the mature 18S of the 40S ribosomal subunit, and the 28S and 5.8S rRNAs of the 60S subunit [56]. The fourth rRNA, 5S, is instead transcribed by the RNA polymerase III in the nucleus, but it is then actively transported in the nucleolus, where it undergoes through the coordinated assembly with the remaining rRNAs and RPs [56]. This multistep process, which concludes with the export of the 40S and 60S into the cytoplasm, requires numerous accessory factors comprising both non-ribosomal proteins and small nucleolar RNAs (snoRNAs) [57] and is coordinated with cell growth and division so that ribosomal biogenesis may occur only during the interphase [58].

Dysregulated ribosomal biosynthesis is a trait of both solid [59,60] and liquid [61] tumours and entails the disruption of the nucleolar organization, an event known as 'nucleolar stress'[62]. An ever-growing body of evidence unequivocally demonstrates the link between nucleolar stress and p53 activation (Fig. 1). The presence of free RPs, which is indicative of a disequilibrium in the ratio between rRNAs and RPs, can be detected by MDM2, an E3 ubiquitin ligase acting as negative regulator of p53 [63]. RPL5 and RPL11 were the first two RPs shown to interact with MDM2 and to block its function, thus leading to p53 accumulation in the disrupted nucleoli [64–66]. There, p53 interacts with the promyelocytic leukaemia (PML) tumour suppressor, which enhances p53 acetylation by p300 thus further preventing the MDM2-dependent ubiquitination of p53 [67]. An additional stabilization of p53 is provided by another RP, RPL26, which interacts with both the 5′ and 3′ UTRs of the p53 mRNA and promotes its translation [68]. In unstressed conditions, this is prevented

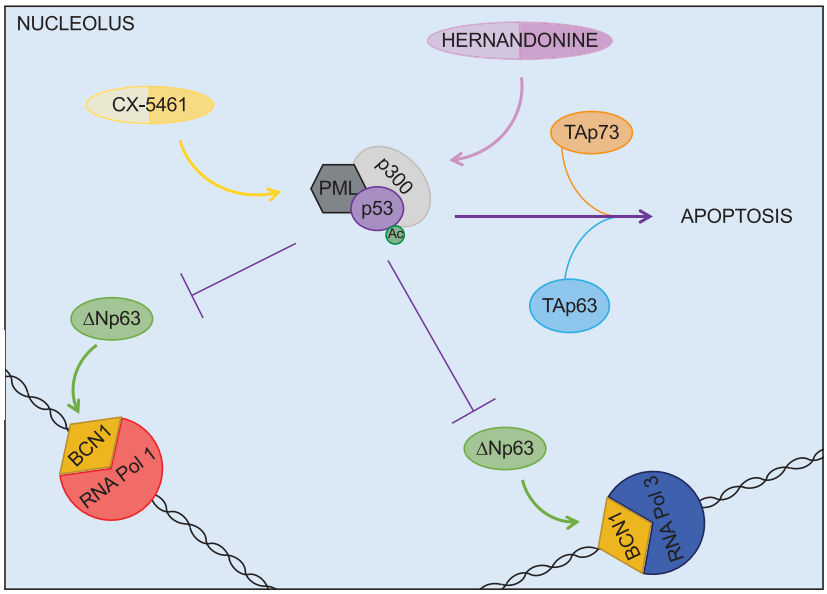

Figure 1. The p53 family modulates the nucleolar stress response. Nucleolar structure and function are impaired by multiple stressors, including inhibitors of ribosomal DNA transcription such as CX-5461 and hernandonine. These compounds induce p53 stabilization, which relies on its interaction with PML and subsequent p300-mediated acetylation. Once activated, p53 counteracts the ΔNp63-induced transcription of ribosomal genes and prompts cell death in concert with the other pro-apoptotic members of the family, TAp63 and TAp73.

by MDM2, which blocks the interaction between RPL26 and the p53 mRNA, thereby realizing an additional feedback loop linking the ribosomal biogenesis with the p53 pathway [69]. The ultimate goal of this activation of p53 following nucleolar stress is to halt ribosomal biosynthesis. This is achieved by p53 at multiple levels including: i) the regulation of the transcriptional activity of RNA polymerase I and III, either directly [70] or by counteracting the cMyc-dependent induction of the rRNA genes [71]; ii) preventing the nuclear import of the RPs that are translated in the cytoplasm [72]; and iii) blocking the nuclear export of the 40S and 60S ribosomal subunits [72]. All these events contribute to suppress ribosomal biogenesis until the unbalance between rRNAs and RPs that led to the nucleolar stress is resolved [73].

The pivotal role of p53 in controlling the homoeostasis of the ribosomal biogenesis is corroborated by the investigation of several RP-deficient mice. Although these mice display different phenotypes, such as embryonic (*Rps6*$^{-/-}$) [74] or perinatal (*Rpl24*$^{-/-}$) [75] lethality, impaired T cell development (*Rpl22*$^{-/-}$) [76], and low body weight and anaemia (*Rpl27a*$^{-/-}$) [77], all these defects are due to the hyperactivation of p53 and can be rescued by the concomitant deletion of the *Trp53* gene [74–77].

During the past decade, a few reports have shed light on the connections between the ribosomal biogenesis and other members of the p53 family. For example, two papers have recently unveiled a role for TAp73 in controlling the translation of mRNAs encoding nucleolar proteins, thus in turn affecting rRNA processing and global protein synthesis [78,79] (Fig. 2). Acute downregulation or chemical inhibition of TAp73 impairs the translation of nucleolar protein, which reduces the rRNA processing and the polysomal/subpolysomal ratio, ultimately leading to an impaired global protein synthesis [78]. In *TAp73*$^{-/-}$ mice, a compensatory mechanism occurs that allows the maintenance of global protein synthesis by bypassing the checkpoint set up by the translational elongation factor eEF2K [79].

The other p53 family member reported to affect ribosomal biogenesis is ΔNp63, which directly induces the levels of Basonuclin1 (BCN1) [80], a transcription factor controlling the expression of a subset of rRNA genes via RNA polymerase I and III [81,82] (see Fig. 1). The ΔNp63-BCN1-rRNAs axis is particularly relevant in basal cell carcinomas and in head and neck squamous cell carcinomas, where the levels of both ΔNp63 [42] and BCN1 [80,83] are upregulated in comparison with normal matched tissue and could increase the ribosomal biogenesis to sustain the higher demand for protein production by proliferating cells.

Given that upregulated ribosomal biogenesis is a feature of numerous human cancers, extensive efforts have been made to design inhibitors of this process and some of these small molecules are in advanced pre-clinical investigations or in early clinical trial phases [84]. A promising compound, called CX-5461, is a non-genotoxic drug inhibiting the recruitment of the ribosomal DNA transcription factor Selectivity factor 1 (SL1) on the promoters of the rRNA genes [85]. Notably, when tested in a Eμ-MYC mouse model of Burkitt lymphoma, CX-5461 triggered the p53-dependent cell death of malignant B cells without affecting normal cells and extended the survival of these mice [86]. P53-dependent apoptosis is also induced by another inhibitor of rDNA transcription, hernandonine, which binds to RPA194, the large catalytic protein of the RNA polymerase I, and promotes its degradation via the proteasome [87]. It will be interesting to evaluate in the future whether CX-5461 and hernandonine can stimulate the activities of the other pro-apoptotic members of the p53 family, namely TAp63 and TAp73, thus providing an alternative therapeutic strategy to treat tumours characterized by the functional loss of p53.

The p53 family and tRNA deregulation in cancer

In 1958, 'soluble ribonucleic acid intermediates in protein synthesis' were discovered [88] making these molecules, later named transfer RNAs (tRNAs), the first class of non-coding RNAs to be identified. Given their essential role in translation together with rRNAs, upregulation of tRNAs in tumours has for too long been considered just a consequence of the high metabolic rate and increased demand in protein synthesis

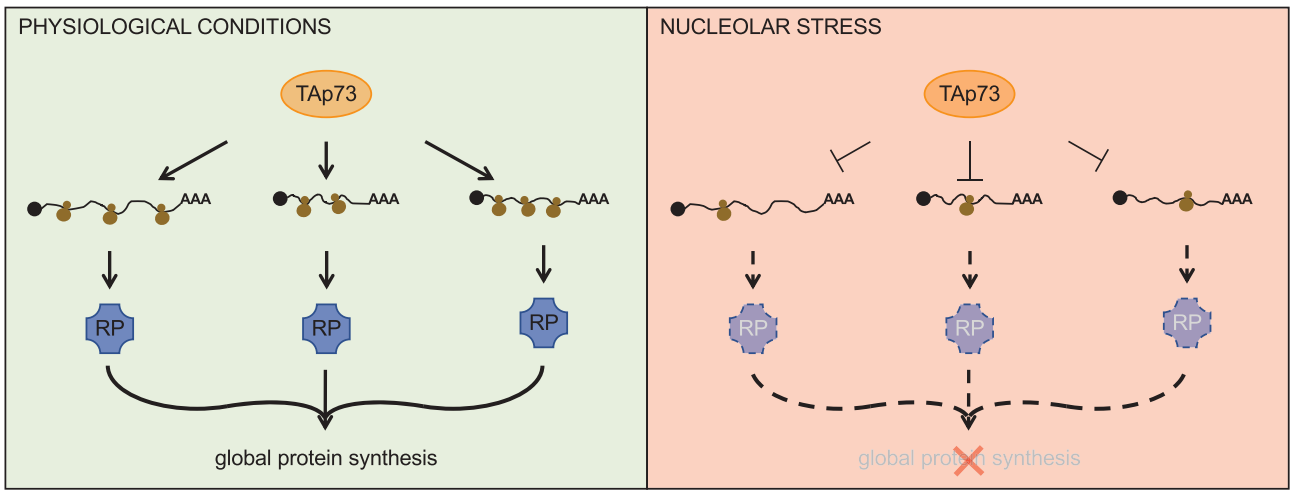

Figure 2. TAp73 controls global protein synthesis via the translation of ribosomal proteins. In physiological conditions, TAp73 promotes the translation of mRNAs encoding ribosomal proteins (RPs), which in turn are required to sustain global protein synthesis. When nucleolar stress occurs, instead, TAp73 forestalls the production of RPs thus ultimately halting global protein synthesis.

typical of cancer cells [89]. However, the regulation of tRNA levels is more complex than previously envisioned. Indeed, not all of the 506 human tRNA genes are simultaneously overexpressed in a given tumour [90]. Instead, the expression of tRNA isoacceptors (i.e. different tRNA isoforms loaded with the same amino acid) is tissue-specific and finely tuned to the expression of the mRNAs needed for the tissue's optimal functions [91]. Accordingly, unbalance in the coordinated transcription of tRNAs and mRNAs may lead to tissue degeneration and even death [92].

The major element determining tRNA expression is the activity of the RNA polymerase III, which is synchronized to the cell cycle and kept under control by the tumour suppressor RB and p53 [70]. On the contrary, numerous oncogenes have been proven to promote RNA polymerase III transcriptional activity, including cMyc [71], Ras/ERK [93], PI3K/AKT/mTOR [94], and TERT [95]. Notably, these oncogenes induce the expression of selective tRNAs in a tissue-specific manner, thus underlining the fact that the conditions in which specific tRNAs are upregulated are crucial for them to exert their pro-oncogenic effects. The best example of this crosstalk between oncogenes and tRNAs is that of TERT upregulating $tRNA^{Leu}$ and $tRNA^{Tyr}$ in highly aggressive triple-negative breast cancers but inducing different tRNAs in other organs [95].

Additional complexity in the regulation of tRNA levels has recently been provided by a report demonstrating that the p53 induced miR-34a post-transcriptionally regulates the initiator $tRNA^{Met}$ [96] (Fig. 3). Although this is the only case of miRNA-tRNA interaction known so far, these two RNAs create a feedback loop that is highly relevant for human tumours. Indeed, if on one hand miR-34a decreases the levels of $tRNA^{Met}$, on the other hand the overexpression of this tRNA bypasses the S/G2 cell cycle transition controlled by p53 and miR-34a thus promoting tumour initiation [96]. Furthermore, $tRNA^{Met}$ can sustain tumour progression by increasing the migratory and invasive potential of melanoma cells [97], a property shared with $tRNA^{Glu}$ and $tRNA^{Arg}$ in metastatic breast cancer cells [89].

Not only are tRNAs now established as key elements in tumour and metastasis formation, but they are also emerging as potential cancer biomarkers. In renal clear cell carcinomas, the overexpression of $tRNA^{Arg}$ and the downregulation of $tRNA^{Pro}$ and $tRNA^{Thr}$ correlate with poor overall survival [98], and in lung adenocarcinoma the overexpression of $tRNA^{Glu}$ and $tRNA^{Tyr}$ and the downregulation of $tRNA^{Asn}$ and $tRNA^{Thr}$ are associated with increased recurrence risk [99]. It is very likely that the constantly-growing availability of next generation sequencing data will aid in unveiling prognostic markers for tRNAs in other tumour types as well. It has been hypothesised that tRNAs might also be predictive of responsiveness to cancer treatment. This is based on the fact that the elevated protein synthesis rate present in fast proliferating tumours causes a reduction in translational accuracy [100]. This augmented protein synthesis error (PSE) is due to tRNA misreading (i.e. the incorporation of a wrong amino acid in a protein), which leads to an *in vivo* tumour growth similar to what is achieved by a potent oncogene, such as $K\text{-}ras^{G12V}$ [101], and can endow cancer cells with drug resistance and adaptation to tumour suppressive signals, a phenomenon referred to as adaptive mistranslation [102]. The biological consequence of PSE is the induction of the unfolded protein response (UPR) also known as the ER stress response [103]. Although UPR helps cancer cells to cope with translational inaccuracy by upregulating molecular chaperones and increasing the proteasome-dependent degradation of misfolded proteins [103], it can also be an Achilles' heel for the tumour. Indeed, if PSE is not cleared, USP triggers the pro-apoptotic members of the p53 family thus leading to tumour cell death [104–106]. This could be pharmacologically exploited, since tumours relying on tRNA misreading may be more responsive to UPR and/or proteasome inhibitors [107].

The bidirectional crosstalk between the p53 family and miRNAs in cancer

MicroRNAs (miRNAs) are a large group of small RNAs that function by regulating mRNA stability and translation in a sequence-specific manner [108]. Although they are ~22 nucleotides long, they are produced as longer precursors either via splicing of host gene pre-mRNA transcripts (so called mirtrons) [109] or via RNA polymerase II transcription of dedicated genes (primary miRNAs or pri-miRNAs) [110]. In the latter case, the precursors undergo 5′ capping and 3′ polyadenylation similar to mRNAs [111] but are characterized by a unique feature, the presence of a ~ 70 nucleotides long RNA stem-loop. This structure is recognized by the microprocessor, a multiprotein complex including DGCR8, which mediates the association with the stem-loop [112], and Drosha, which processes pri-miRNAs into precursor miRNAs or pre-miRNAs [113]. The resulting pre-miRNAs and the mirtrons, whose synthesis instead does not require the microprocessor, are then exported from the nucleus in an exporting-5 dependent manner [114]. Once in the cytoplasm, they are further processed by the RNAse III enzyme Dicer into mature miRNAs [115], which subsequently interact with the RNA-induced silencing complex (RISC), where the miRNA-mRNA interaction and the Argonaute-dependent cleavage of the mRNA occur [116].

The miRNA biogenesis pathway is regulated by the p53 family members at various levels (Fig. 4). The first of such regulatory events to be unveiled was the interaction between p53 and the microprocessor component DDX5 to promote the maturation of tumour suppressive miRNAs in response to DNA damage [117]. In similar conditions, p53 also affects the RISC complex through its binding to Ago2, hence globally perturbing the miRNA-mRNA interactions [118]. Mutant p53 proteins were demonstrated to retain the capability to associate with both DDX5 and Ago2 and to highjack them as part of the so-called mutant p53 gain of function [117,118].

In addition to p53, other members of the family are involved in miRNA processing. For example, TAp63 was shown to induce Dicer expression [43]. This is particularly relevant for tumorigenesis, since the tumour and metastatic suppressive functions of TAp63 rely on this mechanism [43] and deletion of either *TAp63* [43] or *Dicer* [119] predisposes mice to the onset of metastatic tumours. This crucial link between TAp63 and Dicer is further supported by the finding that the loss of both factors is a common feature of different types of aggressive human tumours, including breast cancers, head and neck

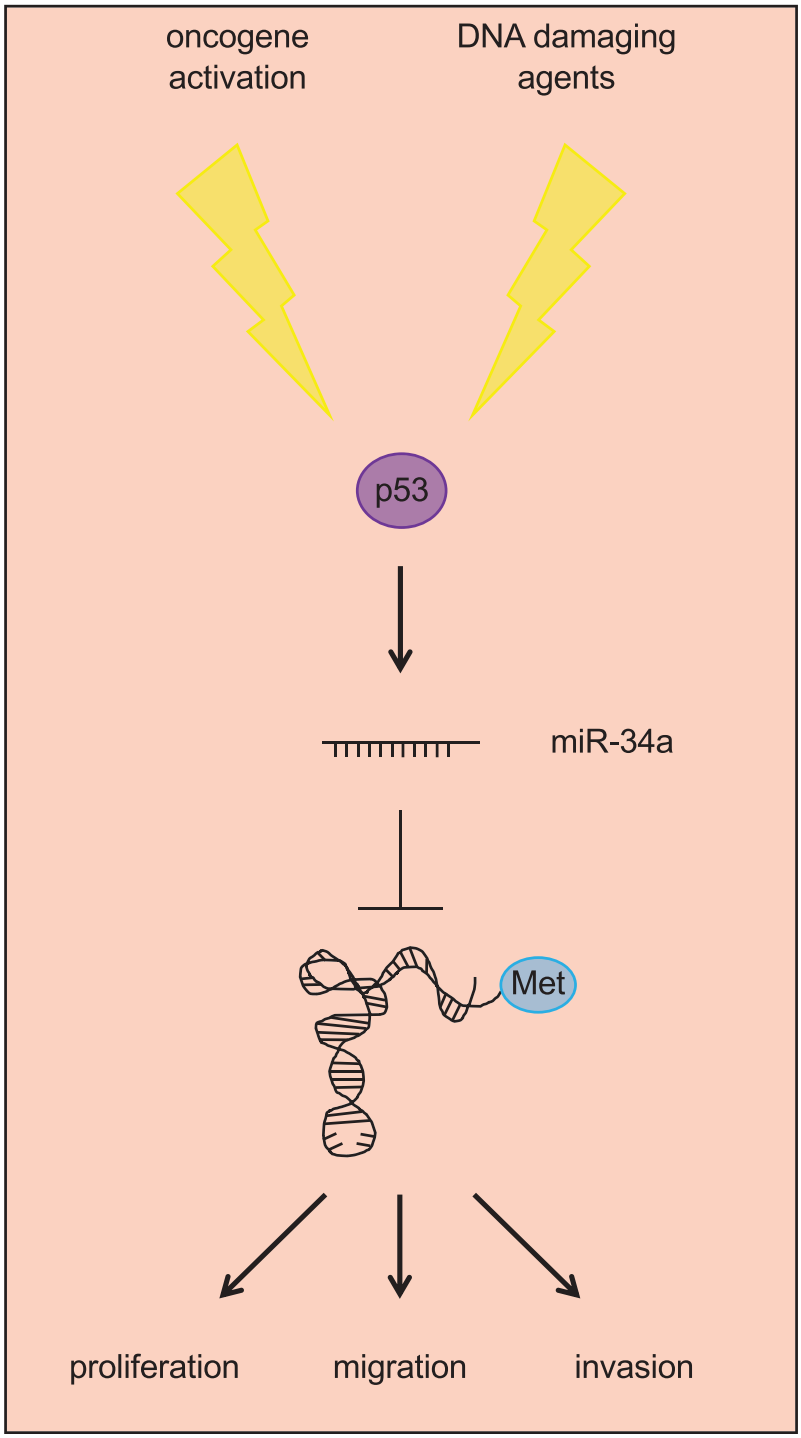

Figure 3. miR-34a inhibits the tRNA initiator $tRNA^{Met}$. Following either endogenous (e.g. oncogene activation) or exogenous (e.g. DNA damaging agents) stimuli, p53 is activated and promotes the expression of miR-34a, which is the only miRNA known to target tRNAs. Specifically, miR-34a binds to $tRNA^{Met}$ and prevents it from promoting biological processes supporting tumour formation and progression.

squamous cell carcinomas, and lung adenocarcinomas [43]. Importantly, in addition to having a global effect on miRNA biogenesis through Dicer, TAp63 achieves its tumour suppressive functions by directly inducing the expression of specific miRNAs, including miR-130b that abolishes the migratory and invasive potential of cancer cells [43].

The other isoform of p63, ΔNp63, transcriptionally activates *DGCR8* [50], affecting in this way the cleavage of pri-miRNAs into pre-miRNAs. Notably, the ΔNp63/DGCR8 axis is necessary for the maturation of a group of miRNAs required for the proper terminal differentiation of the epidermis, thereby explaining the skin defects observed in the $\Delta Np63^{-/-}$ mice [50]. Because of the pleiotropic roles of ΔNp63 in the initiation and progression of multiple human cancer types [120], the ΔNp63/DGCR8 axis is crucial in human tumours as well. Indeed, tumours overexpressing ΔNp63 are generally addicted to this oncogene and can be treated by targeting ΔNp63 either genetically [40] or pharmacologically via histone deacetylase inhibitors (HDACi) based

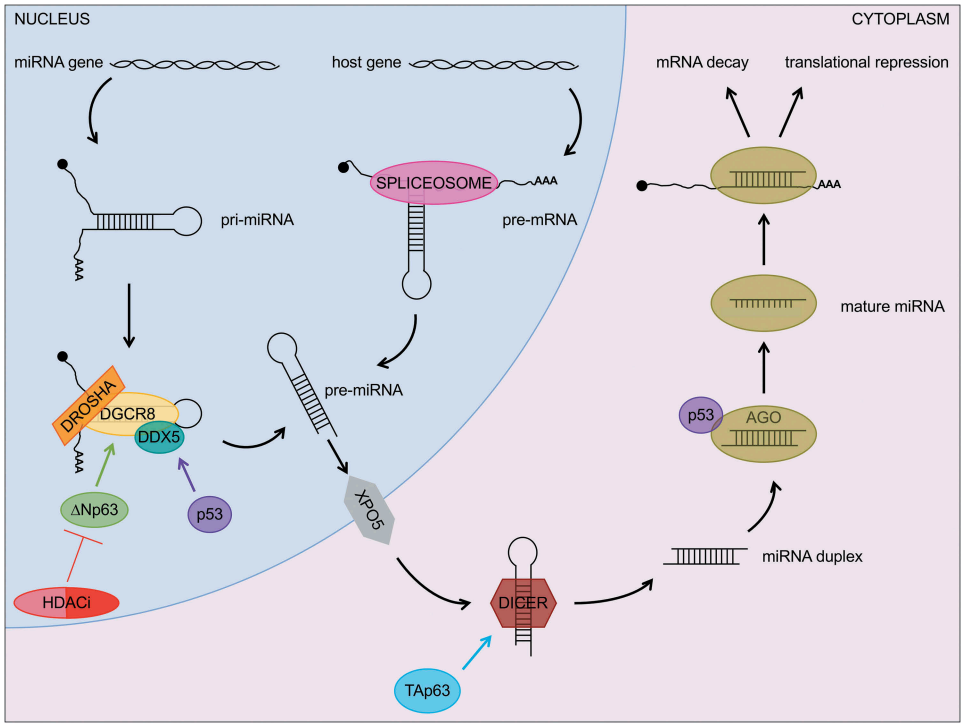

Figure 4. The p53 family regulates multiple layers of the miRNA biogenesis pathway. In addition to directly regulating the expression of miRNAs (see Table 1), p53 and its family members, TAp63 and ΔNp63, affect miRNA processing as well as the activity of miRNAs on their mRNA targets.

therapies [41]. Resistance to HDACi treatment due to low levels of ΔNp63's E3 ubiquitin ligase, Fbxw7, can be bypassed by directly targeting DGCR8 or oncogenic miRNAs processed in a ΔNp63/DGCR8-dependent manner, such as let-7d and miR-128 [41].

In addition to control miRNA biogenesis, the p53 family can directly affect the expression of specific miRNAs (Table 1). One of the best characterized examples is the induction of miR-34 by p53 in response to DNA damaging agents, such as ionizing radiations [121] and chemotherapeutic drugs [117]. This induction is essential for the p53-dependent cell cycle arrest and senescence [121], during which p53 also represses the expression of several miRNAs, including miR-17-5p, miR-106b, and miR-155 [122]. P53-induced senescence is counteracted by ΔNp63 both through: i) the repression of senescence-specific miRNAs, such as miR-181 and miR-130b [123], the latter being instead induced by TAp63 [43]; and ii) the induction of pro-proliferative miRNAs, like miR-630 [124]. Furthermore, ΔNp63 regulates the expression of the miR-200 family and miR-205, through which ΔNp63 promotes the epithelial-mesenchymal transition (EMT) [125]. This is crucial for the development of epithelial tissues such as the mammary gland [126] and for the pro-metastatic activity of ΔNp63 in bladder [127] and prostate [128] cancers.

One intriguing miRNA induced by TAp73 is miR-193b, which in turn binds to the 3′UTR of TP73, thus inducing a negative feedback loop that keeps TAp73 activity under check [129]. This is not the only miRNA directly regulating the stability of the p53 family mRNAs. Additional examples are the oncogenic miR-125b, that inhibits p53 activation by interacting with its 3′ UTR [130], and the tumour suppressive miR-203, which inhibits the ΔNp63-dependent proliferation of cancer cells by binding to its 3′ UTR [131]. These findings demonstrate that the intricate crosstalk between the p53 family and miRNAs acts in both directions and has crucial repercussions for human cancers.

Table 1. Connections between miRNAs and the p53 family.

miRNA	Connection with the p53 family	Reference
let-7d	oncogenic miRNA processed via the ΔNp63/DGCR8 axis	[41]
miR-17-5p	oncogenic miRNA repressed by p53	[122]
miR-34a	p53 induced miRNA mediating cell cycle arrest and senescence	[117,121]
miR-106b	oncogenic miRNA repressed by p53	[122]
miR-128	oncogenic miRNA processed via the ΔNp63/DGCR8 axis	[41]
miR-130b	tumour suppressive miRNA repressed by ΔNp63 and induced by TAp63	[43,123]
miR-155	oncogenic miRNA repressed by p53	[122]
miR-181	tumour suppressive miRNA suppressed by ΔNp63	[123]
miR-193b	miRNA induced by TAp73 and binding to the 3′ UTR of p73	[129]
miR-200	induced by ΔNp63 to regulate EMT	[125]
miR-203	tumour suppressive miRNA binding the 3′ UTR of p63	[131]
miR-205	induced by ΔNp63 to regulate EMT and mammary gland development	[125,126]
miR-630	pro-proliferative miRNA induced by ΔNp63	[124]

LncRNAs and the activity of the p53 family members in human tumours

The vast majority of the 'dark matter' comprises long non-coding RNAs (lncRNAs), RNA species arbitrarily defined as RNA polymerase II transcripts that are longer than 200 nucleotides and devoid of open reading frames [132]. Due to the ever-growing identification of the lncRNAs thanks to the advances in RNA sequencing techniques, lncRNAs have

been believed to represent transcriptional noise [133]. However, a small but steadily growing list of lncRNAs has been confirmed to have biological roles, including modulation of gene expression (both in cis [134] and in trans [135]), mRNA stability control [136], sequestration of miRNAs [137], regulation of protein localization [138], and organization of scaffolds both for RNA binding proteins [139] and for subnuclear domains [140].

Several lncRNAs have recently been identified as key components of the p53 pathway (Table 2), including known oncogenic lncRNAs that act as upstream modulators of p53, such as ANRIL, MALAT1, PURPL, and PVT1. The former is also known as CDKN2B-AS1, because it is transcribed from the same promoter but on the opposite strand compared to CDKN2B (also known as ARF) [141]. During the last stage of the DNA damage response, i.e. when the DNA repair is completed and p53 levels return to normal, ANRIL directly binds to the nascent ARF transcript and recruits polycomb repressor complex (PRC) 1 and 2 to silence ARF expression [142]. As a consequence of the reduced ARF levels, MDM2 is free to interact with p53 and to drive its degradation [143]. In several human tumours, including breast [142], lung [144], and ovarian [145] cancers, ANRIL is overexpressed thereby leading to the hypoactivation of p53. A similar result is obtained in tumours where cMYC is coamplified with the lncRNA PVT1 [146]. This RNA promotes the loading of EZH2 on the promoter of the large tumour suppressor kinase 2 (LATS2) [147], the kinase at the core of the Hippo pathway responsible for the phosphorylation and subsequent inactivation of YAP/TAZ [148]. Similar to ARF, LATS2 also counteracts MDM2 binding to p53 [149]. Therefore, PVT1 overexpression results in reduced LATS2 levels and increased MDM2-dependent degradation of p53 [147]. In addition to regulating p53 ubiquitination levels via MDM2, there are lncRNAs affecting other p53 posttranslational modifications (PTMs). This is the case for MALAT1, an oncogenic lncRNA enhancing the deacetylation activity of SIRT1 on p53, thus reducing the ability of p53 to be recruited to promoters of its target genes [150]. Besides these effects on p53 PTMs, another lncRNA, named WRAP53, can instead stabilize p53 post-transcriptionally. Indeed, this lncRNA is an antisense transcript of *TP53*, which binds to the 5′ UTR of the p53 mRNA via a perfectly complementary sequence and promotes p53 mRNA translation and the subsequent accumulation of the p53 protein in response to DNA damage [151]. Finally, a special case of upstream modulator of p53 is that of PURPL. Indeed, not only does this lncRNA prevent the interaction between p53 and MYBBP1A thus counteracting p53 stabilization, but PURPL itself is a direct target of p53 [152]. This creates a negative feedback loop between PURPL and p53, which is an important mechanism to avoid the hyperactivation of p53 in unstressed conditions [152]. p53 has also been shown to induce the expression of other lncRNAs that act as downstream tuners of different p53's biological responses, such as apoptosis (PINCR) [153], cell cycle arrest (DINO) [154], DNA repair (GUARDIN) [155], and senescence (PANDA) [156].

In contrast to the large body of evidence linking p53 and lncRNAs, only a handful of reports have clearly demonstrated an interplay between these RNA species and the other members of the p53 family. Notable examples are SNHG1 [157], which inhibits TAp63 via an uncharacterized mechanism leading to metastatic lung squamous cell carcinomas, and BLNCR [158] and XIAP-AS1 [159], two direct targets of ΔNp63 mediating its proliferative and invasive effects, respectively. Given the ever-growing interest in the lncRNA field, it is very likely that additional and surprising connections between lncRNAs and the p53 family will be unveiled in the near future.

Table 2. Connections between lncRNAs and the p53 family.

lncRNA	Connection with the p53 family	Reference
ANRIL	oncogenic lncRNA destabilizing p53 by repressing ARF	[141]
BLNCR	direct ΔNp63 target promoting cancer cell proliferation	[158]
DINO	interacts with p53 and promotes p53-mediated cell cycle arrest	[154]
GUARDIN	direct p53 target involved in DNA repair	[155]
MALAT1	promotes p53 deacetylation via SIRT1	[150]
PANDA	direct p53 target promoting senescence	[156]
PINCR	p53-induced lncRNA counteracting p53-dependent apoptosis	[153]
PURPL	direct p53 target inhibiting p53 stabilization	[152]
PVT1	oncogenic lncRNA destabilizing p53 by repressing LATS2	[147]
SNHG1	oncogenic lncRNA inhibiting TAp63's anti-metastatic activity	[157]
WRAP53	promotes the translation of the p53 mRNA	[151]
XIAP-AS1	direct ΔNp63 target promoting cancer cell invasion	[159]

Circular RNAs and their effects on the p53 pathway

In 1976, curious 'single-stranded and covalently closed circular RNA molecules' were described in pathogenic viroids [160]. Since then, circular RNAs (circRNAs) have been discovered in most organisms, including Archaea, plants, and metazoans [10]. Although initially disregarded as artefacts of splicing errors [161], RNA-sequencing methods including the depletion of polyadenylated RNAs and the degradation of linear RNA molecules via RNAse R, revealed that ~10% of all the expressed human genes produce circRNA splice variants [162]. Notably, in several instances, the levels of the circRNA isoforms exceed those of the respective linear transcripts [163], hence confuting the linear RNA-centric view that circRNAs are just 'scrambled exons'[161]. The first functional circRNA to be reported was CDR1as [164], also known as ciRS-7 [165], which is the archetype for miRNA-sponging circRNAs. It indeed contains more than 70 binding sites for miR-7 and suppresses the activity of this miRNA. In addition to the inactivation of miRNAs, circRNAs can exert a variety of mechanisms of function, such as affecting protein localization [166], regulating the transcription of their parental gene [167], and acting as scaffolds for the formation of protein complexes [168].

Intriguingly, a few circRNAs were demonstrated to encode small peptides, thus indicting that not every circRNA is an actual non-coding RNA. One of the peptide-encoding circRNAs is the circular form of the p53-induced transcript PINT, whose peptide acts as a tumour suppressor in glioblastomas by binding to PAF1c, an RNA polymerase II associated factor, and inhibiting the transcriptional elongation of multiple oncogenic transcripts [169]. Another interesting circRNA

is the circular form of the lncRNA ANRIL. While, as described above, linear ANRIL affects p53 stability via ARF [141], circANRIL expression impairs rRNA processing and maturation, ultimately leading to nucleolar stress and p53 activation [170]. The activation of p53 is also affected by other circRNAs, including: i) circ_0000263, which sponges miR-150-5p, in turn causing the up-regulation of the p53 inhibitor MDM4 [171]; ii) circ_0055538, whose loss in oral squamous cell carcinoma attenuates p53's pro-apoptotic response [172]; and iii) circAMOTL1L, whose downregulation promotes prostate cancer progression by impairing the p53-dependent regulation of EMT [173].

Despite the numerous reports demonstrating the functional interactions between circRNAs and the p53 pathway (Table 3), further efforts are still needed to show whether circRNAs can also affect the stability or the activity of the remaining members of the p53 family and what consequences this interplay may have in human cancers and other biological processes.

Conclusions and future perspectives

The world of ncRNAs is ever changing and leading to new venues of research and holds the promise of novel therapies for multiple diseases including cancer. Though initially shadowed by the interest captured by the protein-coding RNAs, the 'dark matter' has undeniably proven to comprise molecules with biological functions crucial for both cell physiology and several human diseases. These findings have provided fuel for the investigation in the ncRNA field to the point that it is now clear how intertwined the ncRNAs are with the components of one of the most important pathways in human cancers, the p53 pathway. Indeed, not only are ncRNAs at the centre of the phenotypes shown by the mouse models lacking specific isoforms of the p53 family members, as in the case of the skin defects of the *ΔNp63*$^{-/-}$ mice [50] or the tumour predisposition of the *TAp63*$^{-/-}$ mice [43], but small molecules targeting ncRNA biogenesis (as CX-5461 [85] and hernandonine [87]) and UPR and/or proteasome inhibitors [107] exert their anti-tumour effects via the activation of the pro-apoptotic members of the p53 family.

In addition to these pharmacological approaches affecting global ncRNA biogenesis, several strategies have been designed to target specific ncRNAs via complementary base-pairing recognition, as provided by antisense oligonucleotides (ASOs) [174], miRNA inhibitors [175], and siRNAs [176]. To guarantee their stability and delivery, these molecules are chemically modified (e.g. cholesterol [177] and N-acetylgalactosamine [178]) and/or loaded on either liposomal, polymeric, or inorganic nanoparticles [179]. Many of these ncRNA-based therapeutics showed significant anti-cancer effects in *in vivo* models and are currently being tested in early clinical trial phases for the treatment of both solid tumours and haematological malignancies [180].

Despite the constant progress made in the ncRNA field, there are still some uncharted territory and unanswered questions worthy of further investigation. For example, are there any lncRNAs or circRNAs whose levels or functions are affected by either TAp73 or ΔNp73? How successful will therapeutic strategies aiming to stabilize p53 by blocking lncRNAs, like ANRIL [142], MALAT1 [150], and PVT1 [147] be? Furthermore, given that the inactivation of p53 and its downstream pathway as well as the overexpression of the oncogenic p53 family members, ΔNp63 and ΔNp73, are among the most common alterations in human cancers [21,42], are these ncRNA inhibitors as well as those currently under investigation effective in treating these tumours? We are certain that the paths leading to the answers of these questions are filled with novel and exciting ncRNA functions and will cast an everlasting light shining upon the world of the 'dark matter'.

Table 3. Connections between circRNAs and the p53 family.

circRNA	Connection with the p53 family	Reference
circ_0000263	destabilizes p53 via miR-150-5p/MDM4	[171]
circ_0055538	tumour suppressor required for p53-dependent apoptosis	[172]
circAMOTL1L	tumour suppressor mediating p53 suppression of EMT	[173]
circANRIL	causes p53 activation via nucleolar stress	[170]
PINT	p53 target encoding a small tumour suppressive peptide	[169]

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