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The ARF tumor suppressor: structure, functions, and status in cancer

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The ARF tumor suppressor: structure, functions, and status in cancer

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Key words : ARF, tumor suppressor gene, p53, DNA damage, human tumor.

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Abstract

The INK4b-ARF-INK4a locus encodes two members of the INK4 family of cyclin-dependent kinase inhibitors, p15^{INK4b} and p16^{INK4a}, and a completely unrelated protein called ARF. ARF is a nucleolar protein with unusual structure that exhibits tumor suppressive functions. There is growing evidence that ARF signaling is complex, and involves p53-dependent or p53-independent pathways aiming mainly at restrain abnormal cell growth and at maintain genomic stability. As such, ARF is a critical component of tumor surveillance, and its expression is decreased in human tumors. In this review, we present the current knowledge on ARF regulation and major functions. The ARF status in human tumors is also briefly summarized.

1
2
3 ARF (known as p14^{ARF} in human and p19^{ARF} in mouse) was originally identified as an
4 alternative transcript of the INK4b-ARF-INK4a locus located on human chromosome 9p21¹.
5
6 This locus encodes two members of the INK4 family of cyclin-dependent kinase inhibitors,
7
8 p15^{INK4b} and p16^{INK4a}, that regulate progression through the G1 phase of the cell cycle. The
9
10 intercalation of an additional exon (called exon 1 beta) between INK4b and INK4a renders
11
12 the simple tandem arrangement more complex, as its transcription by a distinct promoter
13
14 produces a transcript that also incorporates exons 2 and 3 of INK4a. However, because exon 2
15
16 of ARF is translated in an alternative reading frame (ARF) to that used for INK4a, the ARF
17
18 product is unrelated to the INK4a protein. As a consequence, p16^{INK4a} and ARF are not
19
20 isoforms, do not share any amino acid homology and have distinct functions in the cells.
21
22 Nevertheless, like p16^{INK4a}, ARF exhibits tumor suppressive functions as demonstrated by the
23
24 tumor susceptibility phenotype of ARF/INK4a-deficient mice (Table 1). Indeed, mice that are
25
26 defective for any one of the genes have increased susceptibility to spontaneous or carcinogen-
27
28 induced tumors, albeit to different degrees^{2, 3}. Arf-null mice develop tumors early in life
29
30 whereas p16^{INK4a}-null mice do not show predisposition to spontaneous tumor within 17
31
32 months⁴. Furthermore, the phenotype resulting from disruption of both p16^{INK4a} and p19^{ARF} is
33
34 comparable to that produced by disruption of p19^{ARF} alone^{2, 5}. Although these results suggest
35
36 a predominant role of p19^{ARF} over p16^{INK4a}, each product acts in a non-redundant manner to
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38 significantly contribute to tumorigenesis « *in vivo* » and the effects are exacerbated in animals
39
40 that lack both genes. Studies of mouse embryo fibroblasts (MEFs) derived from the knockout
41
42 strains also emphasize the independent role of ARF. In wild type MEFs, both p19^{ARF} and
43
44 p16^{INK4a} accumulate significantly after passaging. However, spontaneous escape from
45
46 senescence occurs through loss of the ARF-p53 axis rather than INK4a-Rb. In addition, ARF-
47
48 null MEFs expressing functional p16^{INK4a} fail to undergo crisis after multiple passages « *in*
49
50 *vitro* » and are efficiently transformed by oncogenic Ha-ras². In contrast, p16^{INK4a}-null MEFs

1
2
3 display a phenotype similar to wild-type MEFs and are not sensitive to Ha-ras transformation
4
5 3,6. Although these observations in MEFs do not apply to all cell types, they highlight ARF as
6
7 a bona fide tumor suppressor « *per se* », independent from p16^{INK4a}.
8
9

10 11 12 **ARF is a peculiar protein with unusual primary structure** 13

14
15 The primary structure of ARF is puzzling. The mouse protein is predicted to be 169
16
17 amino acids and the human protein only 132 amino acids. Both proteins share only limited
18
19 sequence homology (50%) that could explain some of their functional differences.
20
21 Nevertheless, they are both composed of more than 20% arginine residues conferring them
22
23 highly basic and hydrophobic properties. Interestingly, there are no recognizable structural
24
25 motifs in ARF proteins and the protein probably needs to form complexes with other
26
27 molecules, both to be folded and for its charge to be neutralized at physiological pH. This
28
29 probably explain the increasing number of yet identified ARF partners ⁷. Mouse p19^{ARF}
30
31 contains only one lysine and human p14^{ARF} has none. Mouse p19^{ARF} and human p14^{ARF}
32
33 contain only single internal methionine residues (Met45 and Met48 respectively) which is
34
35 absent in other species (rat, opossum, pig and chicken). Translational initiation from these
36
37 methionine residues produces both in mouse and human a short form of the protein that, when
38
39 overexpressed, localizes to mitochondria (smARF) ⁸. Nevertheless, full-length ARF
40
41 preferentially localizes in the nucleoli thanks to nucleolar localization signals (NoLS). p19^{ARF}
42
43 contains a unique NoLS in its exon1 β (aa 26-37) which deletion induces the nuclear
44
45 translocation of the protein ⁹. The situation is more complex for p14^{ARF} as two NoLS have
46
47 been identified in the protein. The first one localized in exon1 β plays a key role in the
48
49 antiproliferative function of p14^{ARF} as its deletion inhibits the ability of p14^{ARF} to stop the cell
50
51 cycle and to bind Mdm2 ¹⁰. The second one stands in exon 2 (aa83-101) and is involved in the
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53 ability of p14^{ARF} to promote the sumoylation of its binding partners ¹¹.
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ARF expression and turnover

P19^{ARF} expression is very low during embryogenesis except in a subset of perivascular cells in the eyes of developing mouse embryos, in which upregulation of p19^{ARF} blocks proliferation driven by PDGF β to promote hyaloid vascular regression¹². Induction of p19^{ARF} expression has been recently ascribed to TGF β 2 control in that way¹³. Expression of p19^{ARF} is also increased in senescent mouse fibroblasts¹⁴. The situation is less clear for p14^{ARF} as its expression level remains low as cells near senescence¹⁴ and p14^{ARF} depleted cells still undergo a senescence-like arrest when challenged with Ras. However, p14^{ARF} seems to be an important mediator of the senescent phenotype induced by E2F1 suggesting that p14^{ARF} could play a role in some types of oncogene-induced senescence¹⁵. Ectopic expression of a variety of oncogenes such as Ras, c-myc, E1A, and E2F1 upregulates p19^{ARF} expression as part of a checkpoint response that limits cell cycle progression in response to hyperproliferative signals¹⁶. The regulation of p14^{ARF} is less known and its responsiveness is distinct from that of p19^{ARF} as E2F1 induces the transcription of p14^{ARF} whereas Ras or c-myc do not. P14^{ARF} expression is also increased after exposure to some radiations and genotoxic drugs, and contributes to the DNA damage response that eliminates damaged cells from the proliferative pool⁷. It has also been shown that ARF expression is induced by viral infection and acts to reduce viral infectivity¹⁷. Together, these data place ARF as a general sensor of different types of cellular stress. The mechanisms involved in ARF regulation are largely unknown but recent data give some clues as to how ARF expression might be regulated in response to specific stresses. As an example, it has been demonstrated that in a context of oncogene- and stress-induced senescence, the histone H3 Lysine 27 demethylase JMJD3 can overcome INK4a/ARF silencing imposed by the polycomb group (PcG), by removal of H3K27me3 from the INK4a/ARF promoters¹⁸. **In addition, transcription-independent mechanisms of**

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2
3 ARF regulation that involve ubiquitination and degradation of ARF have been reported during
4
5 responses to oncogenic stress such as c-myc ¹⁹.
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8 The mechanisms that regulate ARF turnover is still not yet completely clear although
9
10 two residues in exon1 β were recently found to be essential for p14^{ARF} stability ²⁰. Both mouse
11
12 and human ARF are relatively stable proteins with estimated half-life ranging from
13
14 approximately 1-8h. Some studies have shown that ARF degradation depends, at least in part,
15
16 on the proteasome and that, although it lacks lysine, ARF can undergo N-Terminal
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18 ubiquitination independently of p53 and MDM2 ²¹. Recently, a specific ubiquitin ligase for
19
20 ARF called ULF was identified ¹⁹. Interestingly, oncogenic stress such as c-Myc abrogates
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22 ULF-mediated ARF ubiquitylation and promotes ARF-dependent, p53-mediated growth
23
24 arrest. On the other hand, an « *in vitro* » degradation of ARF by the 20S proteasome in the
25
26 absence of ubiquitination has also been reported, a process counteracted by TBP1, a
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28 component of the regulatory subunit of the proteasome ²¹. While it remains unclear how ARF
29
30 could be addressed to the proteasome in the absence of ubiquitination, recent data have reveal
31
32 that the REG- γ proteasome could be involved in the ubiquitin-independent regulation of ARF
33
34 turnover ²¹. Studies on subcellular localization of ARF also give some clues about its stability.
35
36 ARF is stable when expressed within the nucleolus, but turns over more rapidly in the
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38 nucleoplasm. In the nucleolus, the ARF protein assumes a stable structure thanks to its
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40 sequestration by nucleophosmin (NPM/B23) that prevents its nucleoplasmic degradation ^{22,23}.
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42 Therefore, NPM is responsible not only for protecting ARF from degradation, but also for its
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44 nucleolar compartmentalization.
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55 **Biological functions of ARF**

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57 The ARF response is quite complex. Although it was first assessed that the chief
58
59 function of ARF was to suppress aberrant cell growth by inducing the p53 pathway which
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3 mediates tumor suppression, there is now ample evidences that ARF also displays p53-
4
5 independent activities. These p53-independent functions are heterogeneous and although
6
7 several potential regulators have been identified, the molecular basis of p53-independent ARF
8
9 signaling remains largely unelucidated. Here, we briefly review the major functions of ARF
10
11
12 (Table 2).

15 The role of ARF in tumor suppression

17 *ARF is a key activator of the p53 pathway*

20 One of the most well-defined function of ARF is to suppress aberrant cell growth in
21
22 response to oncogene activation, by activating the transcription factor p53 that triggers the
23
24 expression of many apoptosis inducers and cell cycle inhibitory genes ¹⁶. ARF is thought to
25
26 stabilize and stimulate p53 activity by neutralizing the inhibitory effects of two ubiquitin
27
28 ligases, Mdm2 and ARF-BP1/Mule (ARF-binding protein1/Mcl1-ubiquitin ligase E3) (Fig 1).
29
30 Both proteins are specific ubiquitin ligase for p53 and can inhibit its tumor suppressor
31
32 functions. Overexpressed ARF interacts directly with Mdm2 and blocks Mdm2-mediated
33
34 ubiquitination, nuclear export and degradation of p53 by the proteasome ⁷. ARF can also
35
36 relocalizes MDM2 to nucleoli thus blocking ubiquitination and nuclear export of p53. Other
37
38 studies show that ARF promotes p53 stabilization and cell cycle arrest without relocalization
39
40 of endogenous Mdm2 to nucleoli ^{24 25}. Beside, ARF can also stimulate the p53 pathway by
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42 targeting the ARF-BP1 ubiquitin ligase. Following aberrant oncogene activation, ARF is
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44 induced and inhibits the proteasomal degradation of nuclear p53 by ARF-BP1, thereby
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46 promoting p53-dependent cell cycle arrest or apoptosis ²⁶.
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55 *ARF displays inhibitory cell growth control independently of p53*

57 If ARF is undoubtedly a critical component of the p53 pathway, there is now evidence
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59 that ARF has also the ability to restrain cell growth independently of p53. Overexpression of
60

1
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3 p19^{ARF} can induce a G1 arrest in cells lacking p53^{27,28}. Human p14^{ARF} can stop the cells in S
4
5 phase and/or trigger apoptosis by mechanisms that do not require the expression of a wild
6
7 type p53 protein^{29,30}. We have also demonstrated that p14^{ARF} inhibits the growth of human
8
9 lung tumor cells lacking p53 by inducing a G2 arrest followed by apoptosis both in “*in vitro*”
10
11 and “*in vivo*” models. In this last case, enforced p14^{ARF} expression prevents tumor growth
12
13 and induces the regression of lung tumors established in nude mice³¹⁻³³. Moreover, several
14
15 groups of investigators have shown that ARF interacts with and antagonizes the
16
17 transcriptional function of Myc and E2F1 independently of p53, both proteins being potent
18
19 oncogenes required for cell cycle progression⁷. ARF does not interfere directly with the
20
21 DNA-binding of these transcription factors on their target promoters, but induces their
22
23 nucleolar sequestration and/or prevents the recruitment of coactivators. As both Myc and
24
25 E2F1 stimulate ARF expression, these results highlight p53-independent negative feed-back
26
27 mechanisms. All these studies are consistent with the findings that mice lacking ARF, p53
28
29 and Mdm2 are more tumor prone than those lacking only p53 and Mdm2, and that ARF^{-/-} and
30
31 +/- mice develop a broader spectrum of tumors than p53-null animals^{4,28}. They support a role
32
33 of ARF in mediating p53-independent tumor suppressive functions, and suggest that ARF
34
35 also acts independently of the Mdm2-p53 axis in tumor surveillance.
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46 *ARF attenuates ribosomal RNA transcription and processing*

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48 ARF predominantly resides within the nucleolus where it can bind to NPM^{34,35}. NPM
49
50 is an abundant nucleolar protein which expression level correlates directly with the
51
52 proliferative state of a cell. NPM is involved in diverse cellular processes including ribosome
53
54 biogenesis³⁶. In response to oncogenic stress, ARF enters the nucleolus to form stable
55
56 complexes with NPM. The biological consequences of the ARF-NPM complex is a subject of
57
58 debate⁷. The prevailing view is that ARF exerts its growth inhibitory activities within the
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3 nucleolus. Indeed, ARF retards rRNA transcription and processing, interferes with NPM
4
5 nucleocytoplasmic shuttling, and impedes ribosome export from the nucleus to the cytoplasm.
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7
8 On the other hand, it has been suggested that sequestration by NPM in the nucleolus could
9
10 hold ARF inactive towards growth suppression, and that its nucleoplasmic translocation
11
12 promotes p53 interaction and cell cycle arrest notably through Mdm2 inhibition. Interestingly,
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14 as same domains of ARF mediate nucleolar localization and Mdm2 binding, it has been
15
16 suggested that Mdm2 and NPM could compete for ARF association. Therefore, ARF may use
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18 ribosome function to inhibit cell growth through binding with NPM in the nucleolus, and may
19
20 also regulate the p53 pathway through its interaction with Mdm2 and ARF-BP1 in the
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22 nucleoplasm.
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29 *ARF contributes to the DNA damage response*

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32 Several studies have underscored a role of ARF in the DNA damage response. ARF-
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34 null mice exposed to ionizing radiations develop more frequently tumors than do wild-type
35
36 mice ⁴. Furthermore, ARF enhances DNA-damage-induced apoptosis ³⁷ and is required for
37
38 some forms of DNA damage response ³⁸. The p53 pathway is activated during the DNA
39
40 damage response and the induction of p53 is coordinated by the ataxia-telangiectasia mutated
41
42 (ATM) and ataxia-telangiectasia and Rad3-related (ATR) protein kinases which mediate the
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44 rapid destruction of Mdm2. In irradiated ARF^{-/-} MEFs, sustained induction of p53 requires
45
46 the expression of p19^{ARF} ³⁸ and the UV sensitization effect of ectopic ARF is dependent on
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48 p53 coexpression ³⁹. Other studies have demonstrated that ARF is a component of the p53
49
50 response following ionizing radiation, UV exposure and genotoxic treatment ⁴⁰. Therefore,
51
52 there is good evidence that ARF contributes to the p53 response following DNA damage.
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55 Nevertheless, some studies also point to the fact that ARF could influence the activity of
56
57 ATM/ATR kinases by mechanisms that do not involve the p53-Mdm2 axis. Enforced
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3 expression of p14^{ARF} has been reported to trigger ATR/CHK1-dependent NFkappaB
4
5 phosphorylation leading to the inhibition of the transactivating activity of NFkappaB and to
6
7 further TNF α -dependent apoptosis ⁴¹. Moreover, we have demonstrated that p14^{ARF} activates
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9 ATM/ATR/CHK signaling pathways in response to various genotoxic drugs and induces p53-
10
11 independent cell cycle arrest and apoptosis ⁴². Activation of ATM/CHK2 signaling cascade is
12
13 triggered by a p14^{ARF}-mediated stabilization of the Tip60 protein, an histone acetyltransferase
14
15 that activates ATM through acetylation ⁴³. Therefore, these results situate ARF as an upstream
16
17 regulator of the ATM/ATR signaling pathways and suggest that ARF might act as a sensor of
18
19 damaged cells. Consistent with this hypothesis, we and others have demonstrated that UV and
20
21 genotoxic agents stimulate p14^{ARF} expression in cultured cells ⁷. Very recently, a p53-
22
23 independent role of ARF in the regulation of XPC expression after UV irradiation that is
24
25 independent of p53 has been described, implicating ARF in nucleotide excision repair (NER)
26
27 ⁴⁴. Moreover, p14^{ARF} has also been reported to control chromosomal stability in p53-null
28
29 cells, and three key residues that reside outside the established functional domains of p14^{ARF}
30
31 have been identified as critical for maintaining and/or promoting chromosomal stability ²⁰.
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33 Collectively, these studies support the idea that ARF stimulates pathways that are significant
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35 in maintaining genomic integrity and plays a role in genome stability (Fig 2).
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46 Other ARF functions

47 *ARF promotes autophagy*

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50 Translational initiation from internal methionine residue produces a short
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52 mitochondrial form of ARF (smARF) in both mouse and human ⁸. If smARF is rapidly
53
54 degraded by the proteasome under physiological conditions, it accumulates in mitochondria in
55
56 response to abnormal proliferative signals. smARF alters the mitochondrial membrane
57
58 potential and triggers type II caspase-independent autophagic cell death, a process usually
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3 initiated in response to nutrient starvation in which cells digest their own organelles to drive
4 energy. Depending on the cellular context, this cell death is dependent or independent of p53
5 and Bcl-2⁸. More recently, full-length ARF was also reported to trigger autophagy in both
6 p53-dependent and p53-independent manners, suggesting that autophagy is another effector
7 of ARF functions⁴⁵.
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17 *ARF induces the sumoylation of its binding partners*

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20 Enforced expression of ARF promotes the sumoylation of lysine residue of the
21 proteins to which it binds, including Mdm2 and NPM⁴⁶. Sumoylation is a process analogous
22 to ubiquitination in which a SUMO (Small Ubiquitin-like Protein) motif is covalently added
23 to a target protein by using sequentially the activating (E1), conjugating (E2) and ligase (E3)
24 enzyme cascade. The mechanism of ARF-induced sumoylation is unknown. ARF does not
25 seem to control the activity of SUMO proteases required to cleave SUMO proteins before
26 conjugation⁴⁷. However, its ability to associate with UBC9 (E2)⁴⁸ indicates that it might
27 facilitate the transfer of SUMO from the E2 complex to ARF-binding proteins. The
28 biological impact of ARF-mediated sumoylation is unknown. Effects of sumoylation are
29 diverse and can control protein trafficking and stability, ubiquitination, transcription factors
30 activities, DNA repair and centromeric cohesion. Therefore, it has been suggested that
31 sumoylation could contribute to the various p53-independent functions of ARF⁴⁶.
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50 **Deregulation of p14^{ARF} in tumors**

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53 Considering that the INK4a/ARF locus encodes tumor suppressor genes that play a
54 pivotal role in the control of the RB/p53 network, genetic abnormalities that target this locus
55 are common events in human cancers. However, because of the unusual structure of the locus,
56 inactivation of the *ARF* gene is often accompanied by inactivation of the *INK4a* gene.
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3 Indeed, homozygous deletions of the INK4a/ARF locus is a frequent event in tumors and
4 mutations in exon 2 that affect both p16^{INK4a} and p14^{ARF} proteins are also observed although
5 to a lesser extent¹⁶. Because the incidence of genetic alterations that specifically inactivate
6 *p14^{ARF}* but not *p16^{INK4a}* is still a subject of debate, the pathogenic and biological significance
7 of *p14^{ARF}* gene alterations in human cancers is still unclear. Furthermore, although ARF null-
8 mice develop spontaneous tumors at an early age, germ-line mutations affecting specifically
9 exon 1 beta have not been yet identified, and mutations that specifically target exon1beta are
10 rare in human tumors.
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22 Because the physiological level of p14^{ARF} is very low and the commercial p14^{ARF}
23 antibodies hard to work by immunohistochemistry, expression of *p14^{ARF}* has been mainly
24 studied at the mRNA level in human tumors (Table 3). The promoter region of *p14^{ARF}*
25 possesses CpG islands that can be methylated and control specifically *p14^{ARF}* expression
26 without affecting *p16^{INK4a}* expression⁴⁹. Methylation of the *p14^{ARF}* promoter has been
27 reported, although at various frequencies, in different tumor samples such as colorectal⁵⁰,
28 gastric⁵¹ and prostate carcinomas⁵². In breast cancer, homozygous deletion, loss of
29 heterozygosity and promoter hypermethylation could explain most of the decrease of *p14^{ARF}*
30 mRNA⁵³. Moreover, one of the most frequent chromosomal translocation t(8;21) of acute
31 leukaemia creates a fusion protein named AML1-ETO that specifically represses the
32 transcription of p14^{ARF}⁵⁴. We previously performed a comprehensive analysis of *p14^{ARF}*
33 status in lung tumors of all histological types and observed a loss of p14^{ARF} protein, more
34 frequently in small cell lung cancer (SCLC) than in non small cell lung cancer (NSCLC)⁵⁵.
35 No deleterious mutation was found in exon 1beta and 2, and a beta transcript was still
36 expressed in the majority of NSCLC having lost the protein suggesting a yet non identified
37 mechanism of inactivation. Further studies have confirmed the aberrant pattern of *p14^{ARF}*
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3 expression in NSCLC ^{56, 57}. However, although hypermethylation of *p14^{ARF}* promoter was
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5 reported in some studies ^{56, 58}, this was not found in other ones ⁵⁹.

6
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8 Deregulation of proteins that affect *p14^{ARF}* expression might be an alternative way to
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10 explain its aberrant status in tumors. For example, overexpression of the Bmi-1 repressor
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12 correlates with inhibition of both *p16^{INK4a}* and *p14^{ARF}* proteins in colon cancer ⁶⁰ and
13
14 Pokemon and *p14^{ARF}* expression are negatively associated in lung tumors and cell lines ⁶¹.
15
16 Moreover, an aberrant expression pattern of other *p14^{ARF}* regulators such as TBX3, TBX2 and
17
18 DMP1 has been reported in human tumors. However, further studies are required to analyze
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20 whether these proteins associate or not with deregulated *p14^{ARF}* expression. More
21
22 interestingly, recent data demonstrate that the H3K27me3 demethylase JMJD3 contributes to
23
24 the activation of the INK4a-ARF locus ¹⁸. JMJD3 is located on chromosome 17 in close
25
26 vicinity to the p53 gene. Therefore, allele loss at chromosome 17, which is a frequent event in
27
28 tumors such as lung, might be an alternative way to inactivate *p14^{ARF}*.

29
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31 Aberrant methylation of both *p16^{INK4a}* and *p14^{ARF}* promoters correlates with lymph node
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33 metastasis and higher tumor grade in colon cancer ⁶² and with a poor prognosis in breast,
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35 colon and bladder carcinomas ⁶³. *P14^{ARF}* methylation is associated with lower recurrence rate
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37 in oral cancer patients with a good clinical outcome ⁶⁴. Expression of *p14^{ARF}* is an
38
39 independent predictor of both relapse and survival in squamous cell carcinoma of the anterior
40
41 tongue ⁶⁵. In hepatocellular carcinoma, increased expression of *p14^{ARF}* mRNA is associated
42
43 with a poorly differentiated phenotype ⁶⁶. We did not find correlation between *p14^{ARF}* protein
44
45 expression and tumor grade or prognosis in NSCLC. However, we observed that expression
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47 of *p14^{ARF}* and activated phospho-CHK2(Thr68), a critical determinant of the DNA damage
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49 response, are directly associated ⁴². In keeping with our demonstration on cell lines that
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51 *p14^{ARF}* is a key component for the initiation of the ATM/CHK2 DNA damage signaling
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53 cascade, our results indicate that *p14^{ARF}* is a critical determinant of CHK2 activation in
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3 human lung tumors and suggest that its loss in lung tumors could allow tumor cells to dodge
4 the CHK2 checkpoint control, thus favoring genetic instability and lung tumorigenesis.
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10 **Concluding remarks**

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12 Because of its peculiar structure and localization within the INK4b-ARF-INK4a locus,
13 dissecting the role of ARF as a “*bona fide*” tumor suppressor has been the focus of intensive
14 studies. It is now clear that both human and mouse proteins function as important sensors of
15 hyperproliferative stimuli, acting to restrict cell growth and tumor progression through both
16 p53-dependent and –independent pathways. However, the ARF response is quite complex and
17 the growing number of yet identified ARF partners warrants further investigations to consider
18 their biological relevance in ARF signaling. As a critical component of tumor surveillance,
19 ARF accumulates in response to oncogenic and genotoxic stresses and activates DNA damage
20 pathways to halt cell cycle division and/or eliminate cells that have sustained irreparable
21 damage. Emerging data now enlarge this point of view and suggest that ARF may counteract
22 DNA damage in a way much more complex than initially thought, making this protein a
23 critical sensor of genomic instability.
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Figures legends

Figure 1: Control of the p53 pathway by ARF

Hyperproliferative signals generated by oncogene activation induce the accumulation of ARF. In turn, ARF stabilizes p53 and stimulates its transcriptional activity by inhibiting the negative regulation imposed by the ubiquitin ligases MDM2 and ARF-BP1/Mule, thereby inducing cell cycle arrest or apoptosis. The mechanism by which ARF controls MDM2 activity toward p53 is multifaceted, depending on the activated signals and on tissue type.

Figure 2: A model for the role of ARF in tumor suppression. Abnormal oncogenic signals, which nature depends on murin or human background, induce transcription-dependent or transcription-independent upregulation of ARF. Elevated expression of ARF counteracts the negative control of MDM2 on p53, leading to stabilization of p53 and activation of a p53-dependent transcriptional programme that potentiates apoptosis or induces cell cycle arrest according to tissue type and activating signals. ARF also functions independently of p53 to

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3 inhibit cell growth, notably by attenuating the transactivating activity of growth promoting
4 genes such as E2F1 and c-myc. DNA damage caused by various cellular stresses stimulates
5 the p53 pathway through activation of the ATM and/or ATR kinases depending of the nature
6 of the inducing signal. These enzymes increase the transcriptional activity of p53 by
7 promoting its phosphorylation by CHK kinases. The ability of ARF to inhibit MDM2 can
8 modify the p53-dependent DNA damage response. Increased expression of oncogenes is
9 thought to activate protective DNA damage responses during the early stages of tumor
10 progression and to induce, independently of ARF, the p53 pathway. Some forms of DNA
11 damage such as UV and cytotoxic drugs can directly stimulate expression of ARF which
12 impinges on ATM and/or ATR signaling by mechanisms that do not involve the p53
13 pathways but modify the activity of the ATM/ATR enzymes either directly or indirectly.
14 Besides, there are also some evidences that ARF contributes to some DNA repair pathways
15 and to chromosomal stability independently of p53.
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Table 1. Phenotype of INK4a/ARF-deficient mice

Genetic background	Tumor susceptibility	MEFs properties	ref
ARF/INK4a -/ disruption of exons2/3	Spontaneous tumors within 8.5 months (sarcomas, lymphomas) High sensitivity to carcinogens	High proliferation rate Immortal Transformed by oncogenic Ras	5
INK4a -/ disruption of exon1 α	Spontaneous tumors within 17 months (sarcomas, lymphomas) High sensitivity to carcinogens	Normal proliferation rate Undergo senescence Resist transformation by Ras	3,6
ARF -/ disruption of exon1 β	Spontaneous tumors within 9,5 months (sarcomas, lymphomas, carcinomas) High sensitivity to carcinogens	High proliferation rate Immortal Transformed by oncogenic Ras	2,4

Table 2. Major cellular functions of ARF

Cellular functions	Mechanisms	ref
Tumor suppression		
<u>Cell growth control</u>		
-	Activation of p53 leading to cell cycle arrest or apoptosis	23, 24
-	p53-independent cell cycle arrest or apoptosis	26-28, 31, 32
<u>Ribosome biogenesis</u>		
	Decreased rRNA transcription and processing	33,34
<u>DNA damage response</u>		
	Activation of p53 pathways	40, 41,42, 43,
	Activation of ATM/ATR/CHK pathways	44, 45
	Activation of DNA repair pathways	47
	Maintenance of chromosomal stability	18
Other functions		
<u>Autophagy</u>		
	Alteration of the mitochondrial membrane potential	7, 36
<u>Sumoylation</u>		
	Association with UBC9 (E2). Biological impact unknown	37, 38, 39

Table 3. ARF status in human tumors

Tissue type	ref	Mutation type/ expression change	Frequency	Association with clinical parameters
Colorectal carcinoma	49	promoter methylation	28%	
Colon cancer	61	promoter methylation	50.8%	lymph node metastasis high tumor grade
	62	promoter methylation	33%	tumor stage
Gastric cancer	50	promoter methylation	24%	early stage of intestinal type advanced stage of diffuse type
Prostate carcinoma	51	HD	6%	
		promoter methylation	6%	
		loss of protein exp	12.5%	
Breast cancer	52	HD	4%	
		LOH	21%	
		low/undetectable mRNA	26%	
		High level mRNA	17%	
		promoter methylation	24%	
	62	promoter methylation	24%	vascular invasion
Bladder carcinomas	62	promoter methylation	56%	multicentric foci, muscle invasion tumor size and tumor stage
Oral cancer	63	HD	12%	
		promoter methylation	18%	lower recurrence rate
Squamous carcinoma of the tongue	64	low/undetectable prot	20%	independent predictor of bad prognosis
Hepatocellular carcinoma	65	HD	2%	
		mutation	4%	
		increased mRNA	93%	poor differentiation
Lung carcinoma				
NSCLC	54	low/undetectable prot	25%	
		low level mRNA	18%	
		mutation	4%	
	55	low/undetectable prot	34%	more frequent in ADK than in SQ
		decreased mRNA	31%	
		promoter methylation	30%	
		HD/LOH	9%/26%	
		mutation	2%	
	56	low/undetectable prot	41%	
	57	promoter methylation	8%	
SCLC	54	low/undetectable prot	73%	

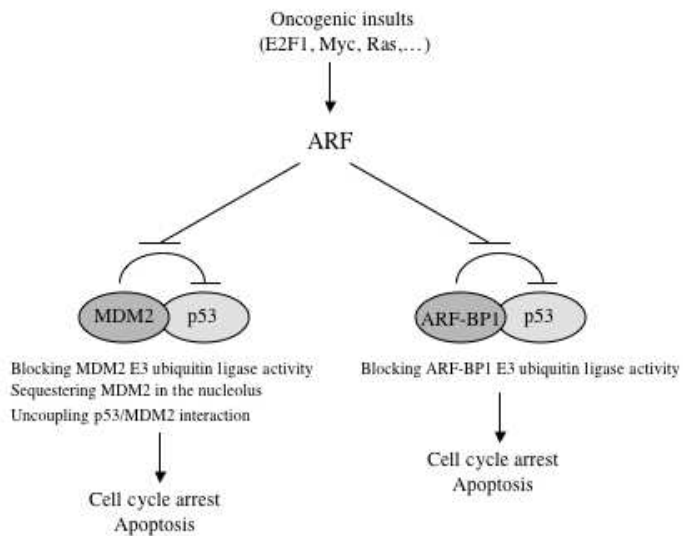
HD, Homozygous deletion; LOH, Loss of heterozygosity; ADK, adenocarcinoma; SQ, squamous carcinoma

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Figure 1



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Figure 2

