ARF Tumor Suppressor

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P14ARF (ARF; Alternative Reading Frame) is an extensively characterized tumor suppressor which, in response to oncogenic stimuli, mediates cell cycle arrest and apoptosis via p53-dependent and independent routes. ARF has been shown to be frequently lost through CpG island promoter methylation in a wide spectrum of human malignancies, such as colorectal, prostate, breast, and gastric cancers, while point mutations and deletions in the p14ARF locus have been linked with various forms of melanomas and glioblastomas. Although ARF has been mostly studied in the context of tumorigenesis, it has been also implicated in purely developmental processes, such as spermatogenesis, and mammary gland and ocular development, while it has been additionally involved in the regulation of angiogenesis. Moreover, ARF has been found to hold important roles in stem cell self-renewal and differentiation. As is often the case with tumor suppressors, ARF functions as a pleiotropic protein regulating a number of different mechanisms at the crossroad of development and tumorigenesis.

ARF Tumor Suppressor Stem Cell Biology

1. Introduction

The CDKN2A locus which is found on human chromosome 9p21 encodes two overlapping transcripts that produce two different proteins, p16INK4a and p14ARF (ARF) [1], both of which are established tumor suppressors. P16INK4a and ARF display no sequence identity, and function through distinct pathways. P16INK4a acts through inhibition of the Cyclin D-CDK4/6 complex, which maintains the Rb protein in its active form [2][3]. On the other hand, ARF acts as a sensor of various types of cellular stress, including oncogenic, heat-shock, and oxidative stress [4][5][6], to subsequently trigger either growth arrest or apoptotic mechanisms in a p53-dependent or independent fashion [7]. Interestingly, ARF has been considered to be an intermediate link between the Rb and p53 pathways, as Rb inactivation leads to increased ARF transcription, which in turn activates a p53-dependent checkpoint [5][8][9].

Early studies on the role of ARF in response to DNA damage had originally dismissed its contribution [10][11], mainly on account of the fact that p53 could still become activated in the absence of ARF, which was however elevated in response to oncogenic stimuli. These initial studies considered the DNA damage and oncogenic response as separate processes with different mediators and outcomes. However, it is now known that oncogene activation can trigger ARF signaling, readily contributing to senescence and cell cycle arrest [5][12][13][14][15][16][17][18]. Importantly, a link was established between ARF and the DNA double-strand break sensor Ataxia Telangiectasia Mutated (ATM), demonstrating that, following genotoxic stress in cancer cells, ATM negatively regulates ARF protein levels [15]. Of note, ARF was also shown to play a role in DNA single-strand break repair [19].

Regarding its established role in cancer biology, ARF was identified as a second line of defense against cancer following DNA damage response, with a higher threshold of oncogenic signals being potentially required for its activation [12]. ARF also holds a pivotal role in the Nucleotide Excision Repair (NER) pathway, facilitating the repair of UV-induced DNA lesions [20][21]. Moreover, loss of ARF was also shown to co-operate with BRAF mutations, resulting in increased UV-induced DNA damage and melanoma formation in BRAFV600E mice [22]. Although ARF is well-known for its role in stabilizing p53 levels, including those of mutant p53 [8][23], interestingly, increased ARF expression has been reported in some cases of lung cancer [24], cervical cancer [25], lymphomas [26], and cancer cell lines, such as HeLa and H1299, accompanied by p53 inactivation. Of note, in tumors such as thyroid carcinomas where ARF is upregulated, ARF is unusually found delocalized in the cytoplasm [27]. On the other hand, the role of ARF in the cytoplasm regulating cytoskeleton remodeling and cell adhesion processes has also been postulated [28], thus providing further insight into the pleiotropic roles of ARF in cancer.

In most cases of human cancers, both ARF and p16INK4a are lost, rendering it challenging to define their individual contribution to tumor suppression [9]. Alterations of the complete CDKN2A locus are identified in approximately 30% of human tumors, including glioblastoma, pancreatic cancer, adenocarcinoma, and melanoma [9][29]. The identification of chromatin remodeling events in the CDKN2A locus has recently started to delineate the genetic mechanisms governing expression of ARF and p16INK4a. A cis-element located beside the ARF promoter was recently found to down-regulate p16INK4a via long range interaction, thus providing a clear example of transcriptional regulation facilitated by chromatin folding [30].

Apart from the widely characterized canonical functions of ARF as a barrier to tumor progression, ARF was additionally implicated in other fundamental biological processes, including early development and morphogenesis.

2. ARF in Stem Cell Biology

CDKN2A expression increases with age, followed by a decline in the tissue regenerative potential. It has been suggested that during the transition from stemness to differentiation, the CDKN2A locus is remodeled to become responsive to stress and mitogenic signals emerging in the differentiation process [9]. The regenerative potential of tissues depends on the balance between stem cell quiescence and self-renewal, both of which are critical processes implicated in tissue homeostasis. Hence, stem cell exhaustion has been considered to be a major hallmark of aging [31][32]. Aging is characterized by accumulation of chronic stress-induced cellular damage, accompanied by higher incidence of tumorigenesis. The ARF/p53 pathway, which is dormant in several tissues throughout development and postnatal life, is progressively activated from adulthood to old age in a wide spectrum of tissues and species [31]. By introducing regulatory and coding sequences of human ARF into the zebrafish genome, it was shown that ARF increases during epimorphic fin regeneration after amputation, contributing to inhibition of the regeneration process [33]. However, inhibition of ARF alone was insufficient to allow regeneration [34].

A major mechanism through which ARF contributes to stem cell regulation is through p53 stabilization. While reduced p53 activity is linked to increased stem cell self-renewal, p53 hyperactivation in mouse models has

resulted in limited regeneration potential attributed to premature exhaustion of stem cell niches [31]. Thus, depletion of hematopoietic stem cells (HSCs) accompanied by impaired hematopoiesis, disruption of mammary gland morphogenesis, reduction of the neural stem cell pools, and disrupted olfactory functions have been reported in p53 mutant mouse models with higher p53 activity than wild-type counterparts [35][36][37]. In line with those phenotypes, p53 induction in the mouse epidermis through Mdm2 ablation has resulted in compromised stem cell activity and premature skin aging [38]. In contrast, mice with an extra copy of Ink4a/Arf/p53 exhibit extended lifespan and delayed aging, linked to extended preservation of stem cell populations [31][39]. Hence, it has been proposed that moderate and regulated activation of the ARF/p53 pathway during aging yields slower proliferation capacities, likely contributing to stem cell quiescence and ameliorating stem cell aging, by simultaneously preventing the exhaustion of stem cell populations. In support of this notion, p53 or p21 loss in mouse models has resulted in exit from quiescence and long-term depletion of stem cell reservoirs at advanced ages [40][41][42].

The polycomb group gene BMI1, which is required for adult stem cell maintenance in many organs [43][44], was found to regulate cell proliferation and senescence through the CDKN2A locus [45]. Jacobs et al. (1999) demonstrated that in Bmi1-deficient mouse embryonic fibroblasts and lymphocytes undergoing premature senescence, the expression of both p16INK4a and p19ARF was markedly increased, while Bmi1 overexpression led to fibroblast immortalization and a decrease in p16INK4A and p19ARF levels [45]. Depletion of the Cdkn2a locus dramatically rescued the phenotypes observed in Bmi1-deficient mice, rendering Cdkn2a critical in vivo Bmi1 target [45]. In line with this observation, it was additionally shown that Bmi1 repressed Ink4a/Arf and Hox genes to allow stem cell self-renewal in rodents [46]. A recent study confirmed the previously reported links between Bmi1 and the Cdkn2a locus, as it demonstrated that the diminished self-renewal capacity of Bmi1-deficient innate-like B lymphocytes was rescued by additional deletion of the Cdkn2a locus [47].

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