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ARF sees Pdgfrβ through the miR

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ARF, the alternative reading frame protein encoded by the *Ink4a–ARF* (*Cdkn2a*) locus, is best known for suppressing cancer through p53-dependent and p53-independent pathways.[1] Although less widely appreciated, ARF is also important in development. Loss of ARF causes blindness in mice, mimicking persistent hyperplastic primary vitreous (PHPV) eye disease in humans.[2] Blindness in ARF-null animals results from increased expression of platelet-derived growth factor receptor β (Pdgfrβ) in perivascular cells within the vitreous of the eye, which stimulates their inappropriate proliferation and prevents the vascular regression needed for sight. Pdgfrβ loss rescues the *ARF*$^{-/-}$ eye phenotype and restores vision, establishing the physiological significance of the ARF–Pdgfrβ pathway.[3,4] For years, ARF was thought to inhibit Pdgfrβ independently of p53, but new evidence reveals it occurs through combined p53-dependent and p53-independent mechanisms.[4,5] Specifically, ARF blocks Pdgfrβ mRNA expression by p53-mediated repression and can inhibit Pdgfrβ translation independently of p53 via induction of miR-34a (**Fig. 1**).

microRNAs (miRNAs) control gene expression through post-transcriptional mechanisms, leading Iqbal et al. to hypothesize that miRNAs could mediate the p53-independent suppression of Pdgfrβ translation by ARF.[5] Consequently, they compiled a list of miRNAs expressed in the developing eye that might target the 3′ untranslated region (UTR) of Pdgfrβ, and of those found that miR-34a was the most highly induced by ARF in p53-null cells. This is the first reported p53-independent connection between ARF and miR34a. The authors then showed that miR-34a directly targets the 3′ UTR and represses translation of Pdgfrβ mRNA, and that miR34a is required for ARF's ability to inhibit Pdgf-B ligand-induced cell proliferation in MEFs lacking p53. That this mechanism may function in vivo is suggested by reduced levels of miR-34a in the vitreous of ARF-null embryos. Together, these findings establish miR-34a as an essential p53-independent mediator of ARF–Pdgfrβ regulation, suggesting a critical role for miR34a in vascular remodeling during eye development. While provocative, that prediction remains to be tested in vivo.

Work by the Skapek group has also clarified the involvement of p53 in ARF–Pdgfrβ regulation and eye development. Early studies showed ARF is required for eyesight regardless of p53 status, and most p53-null mice have normal eyes, indicating little if any role for p53.[2] However, p53 must play some role, because *p53*$^{-/-}$ mice on pure C57BL/6 or BALB/c backgrounds can develop primary vitreous hyperplasia and vision defects. The discovery that ARF represses Pdgfrβ transcription via p53[4] and blocks Pdgfrβ protein synthesis via miR-34a[5] helps resolve this puzzle by showing that ARF regulates Pdgfrβ through complementary p53-dependent and p53-independent mechanisms (**Fig. 1**). Intriguingly, while ARF can induce miR-34a independently of p53, miR-34a is a well-recognized p53 transcriptional target, and both ARF and p53 are required for maximal miR-34a levels.[5] These findings led Iqbal et al. to conclude that ARF may promote sufficient expression of miR-34a to compensate for p53 loss and prevent eye defects on most genetic backgrounds, an effect that would be enhanced by the upregulation of ARF resulting from p53 deficiency.[1,4] However, it remains unclear why the ARF-miR-34a-Pdgfrβ pathway cannot compensate for p53 loss in some inbred strains. Is the pathway defective in those animals, or are there other regulators still to be identified?

Interestingly, ARF controls the expression of other miRNAs besides miR-34a in the eye and in p53-null cells, including the miR-34a relatives miR-34b/c.[5] What is the contribution of those miRNAs to p53-independent ARF activities? Also, how does ARF regulate miR-34a and the other miRNAs identified in this study? Repression of Drosha, the catalytic core of miRNA-processing complexes, seems most likely, since ARF was recently found to block its translation and thereby influence the expression of numerous miRNAs.[6] Finally, ARF, miR-34a, and Pdgfr signaling each have demonstrated or predicted roles in other important biological processes besides development, including cancer, aging, and diabetes (**Fig. 1**). This prompts many new questions. For

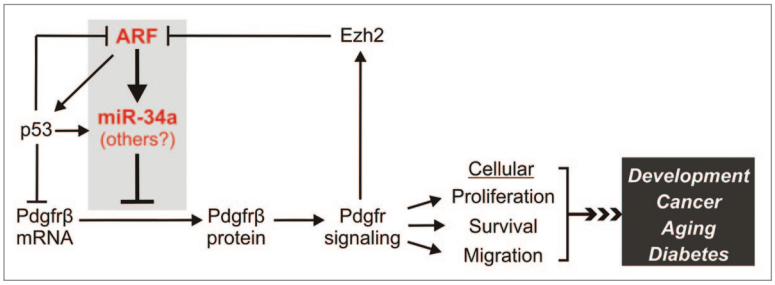

Figure 1. ARF employs complementary mechanisms to suppress Pdgfrβ expression and inhibit cell proliferation. ARF inhibits Pdgfrβ mRNA expression via p53-mediated repression and blocks Pdgfrβ protein synthesis by upregulating miR-34a (and possibly other miRNAs). The novel ARF-miR-34a link to Pdgfrβ (highlighted in red) may contribute to other significant biological processes in addition to development, including cancer, aging, and diabetes.

example, is miR-34a critical for ARF-mediated tumor suppression in the absence of p53? Does Pdgfr signaling, which upregulates Ezh2 (a repressor of *INK4a-ARF*), form an autoregulatory feedback loop with ARF-miR-34a that controls pancreatic β-cell proliferation and diabetes?[7,8] If so, it would expose a currently undefined role for ARF (unlike the established contribution of $p16^{INK4a}$) in that disease. Clearly, the implications of the Iqbal et al. study connecting ARF-miR-34a to Pdgfrβ repression extend beyond eye development and may represent a unique mechanism through which ARF influences other human conditions independent of p53.

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