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The ARFul truth about melanoma susceptibility genes

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Abstract

For many years, *ARF* has languished in the shadow of *INK4a*, a famous and well-characterized tumor suppressor gene. The *INK4a/ ARF* locus at chromo-some 9p21 is a frequent target for loss-of-function alterations in human cancers, including melanoma. Despite sharing largely overlapping DNA sequences, the *INK4a/ARF* locus encodes two completely distinct proteins [p16*INK4a*, which regulates Rb, and p14ARF (or p19Arf in mice), which regulates p53]. This is made possible through the use of two separate first exons (1α for *INK4a* and 1β for *ARF*) that splice into common second and third exons in different reading frames. Not surprisingly, mutation/deletion at the *INK4a/ARF* locus often affects both proteins. Numerous studies have also reported germline and somatic deletions in melanoma that inactivate only *INK4a*. This had not been the case for ARF, leading many to assume that *INK4a* is the critical melanoma susceptibility gene at the *INK4a/ ARF* locus, and to question the role of ARF. However, recently a few patients from melanoma prone families have been discovered that harbor *ARF*-specific germline mutations consisting of deletions, insertions or missense mutations within exon 1β, thereby resurrecting arguments concerning the possible importance of *ARF* in human melanomagenesis (reviewed by Saporita et al., 2007). In a new study Polsky and colleagues (Freedberg et al., 2008) have employed a systematic evaluation of a wide array of *ARF* alterations in metastatic melanoma to elevate *ARF* to its proper status as a bone fide melanoma susceptibility gene.

Unlike many previous studies, Freed-berg et al. (2008) performed a systematic analysis of both genetic and epigenetic alterations in both *ARF* and *INK4a*, using melanoma cell lines initially as proof-of-principle, and then focusing on metastatic melanoma tissue. To cast a broad net a variety of techniques were employed, each having distinct strengths. Deletions were detected by high resolution multiplex ligation-dependent probe amplification (MLPA) and multiplex PCR, promoter methylation by methylation-specific PCR, and point mutations by direct sequencing. Surprisingly, *ARF* was not only found to be frequently inactivated in the context of wildtype *INK4a* (30% of metastases), but also more frequently inactivated than *INK4a* (43% versus 22% of metastases). The *ARF* promoter was methylated, an epigenetic event commonly associated with inhibition of gene expression, in a percentage of metastatic melanomas (57%) that is higher than that reported for a variety of other tumor types, and higher than the *INK4a* promoter (27%), corroborating the importance of *ARF* in melanoma. In fact, the most common mechanism by which ARF expression was lost in metastatic melanomas was hemizygous deletion combined with promoter methylation at the other allele, placing ARF in the class of candidate tumor suppressor genes likely to be affected by treatment with demethylating agents.

Much has been gleaned about the tumor suppressor role(s) of the *INK4a/ Arf* locus from analysis of genetically engineered mice. By combining INK4a/Arf-deficiency with expression of either mutant RAS, or a deregulated receptor tyrosine kinase that can constitutively activate RAS, mice can be induced to develop melanoma, resulting in an excellent model system to dissect the functions of this important locus (reviewed by Larue and Beermann, 2007). These models have shown that simultaneous loss of both *INK4a* and *Arf* has a dramatic effect on melanoma incidence and latency, testament to the significant consequence of losing the function of both gene products. However, loss of either *INK4a* or *Arf* also facilitates melanomagenesis, albeit to a degree that is less impressive than the loss of both. Importantly, loss of one *Arf* allele can enhance the development of melanoma and other tumors in INK4a-deficient mice. Taken together, mouse models have strongly supported a role for ARF in melanomagenesis that is independent of INK4a.

So what then are the critical INK4a-independent roles of ARF in melanomagenesis? ARF is a highly basic, structurally nondescript, mostly nucleolar protein containing two alpha helices at its amino terminus. It is of course best known for its ability to regulate p53 pathway activities, including apoptosis and senescence. ARF can stabilize p53 by interacting with and sequestering MDM2, an E3 ubiquitin ligase able to degrade p53, thereby facilitating p53 tumor suppressor function. This is a scenario often triggered by arising oncogenic stimulation. However, because p53 mutations are relatively infrequent in melanoma relative to other cancers, its importance in suppressing melanomagenesis has been a point of some contention. Then what is known about ARF's p53-independent functions? The very basic ARF has been shown to interact with a wide variety of key proteins such as MYC, NFκB, E2F1 and CtBP, interactions that can determine cellular survival, whether through apoptosis or auto-phagy (reviewed by Saporita et al., 2007). Recent evidence has suggested that senescence also represents a significant impediment to the progression of tumors in vivo, including melanoma, and it has been reported that ARF can induce senescence in melanocytes and melanoma cells independently of p53 (reviewed by Ha et al., 2008). Unfortunately, this situation is complicated by the fact that *INK4a* is also a potent melanocyte senescence gene. A crucial independent regulatory function of nucleolar ARF is thought to occur through its interaction with nucleophosmin (NPM, or B23), which promotes the biogenesis and nuclear exportation of ribosomes, the very heart of the translational machinery. Further analysis of *ARF*-NPM interaction is warranted, and should prove to be most illuminating.

Positioned at the nexus of pathways responsible for preventing sustained and unfettered cellular growth and proliferation, as well as survival, ARF would constitute a formidable barrier to melanoma progression. It should therefore be appreciated that ARF may represent a valuable therapeutic target against melanoma. Notably, the first 14 amino acids at the amino terminus of ARF appear to be sufficient for all of its biological functions, or at least those currently known. This raises the possibility that a small amino terminal ARF peptide, if it could be effectively delivered, may possess considerable p53-independent as well as p53-dependent therapeutic value. Ironically, by stepping back to reset our sights on a protein target whose expression was once thought to be lost as a by-product of *INK4a* deletion at chromosome 9p21 in human melanoma, we may in fact be taking a most significant step forward in our quest to combat this deadly disease.

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