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Mini-review

The role of the NORE1A tumor suppressor in Oncogene-Induced Senescence

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ABSTRACT

The Ras genes are the most frequently mutated oncogenes in human cancer. However, Ras biology is quite complex. While Ras promotes tumorigenesis by regulating numerous growth promoting pathways, activated Ras can paradoxically also lead to cell cycle arrest, death, and Oncogene-Induced Senescence (OIS). OIS is thought to be a critical pathway that serves to protect cells against aberrant Ras signaling. Multiple reports have highlighted the importance of the p53 and Rb tumor suppressors in Ras mediated OIS. However, until recently, the molecular mechanisms connecting Ras to these proteins remained unknown. The RASSF family of tumor suppressors has recently been identified as direct effectors of Ras. One of these members, NORE1A (RASSF5), may be the missing link between Ras-induced senescence and the regulation of p53 and Rb. This occurs both quantitatively, by promoting protein stability, as well as qualitatively via promoting critical pro-senescent post-translational modifications. Here we review the mechanisms by which NORE1A can activate OIS as a barrier against Ras-mediated transformation, and how this could lead to improved therapeutic strategies against cancers having lost NORE1A expression.

Introduction

Ras proteins make up a small family of three closely related proteins. They are small GTPases that act as molecular switches to regulate multiple pathways involved in proliferation and cell survival [1–3]. Gain of function mutations in K-Ras, H-Ras, or N-Ras were the first oncogenic alterations identified in human cancer over 30 years ago [4], and the available data suggest that Ras may be distinguished as the most frequently mutated oncogene in human cancer [5]. Ras is a key driver of transformation due to its ability to regulate multiple signaling pathways including growth, cell-cycle progression, cytoskeletal changes, and migration [4]. Though Ras is mutated in approximately 30% of all human tumors [6], additional cancers contain genetic alterations in Ras regulatory components, such as the Epidermal Growth Factor Receptor (EGFR), Raf, and negative regulators of Ras, such as NF1 (Neurofibromin 1) and DAB2IP (DAB2 Interacting Protein) [7–9]. Thus, tumors can be commonly driven by aberrant Ras activation even in the absence of *ras* gene mutations. While Ras proteins remain one of the cornerstones of cancer biology, Ras biology remains extremely complex and is not fully understood.

Although Ras acts as a powerful driver of growth and transformation, it has the ability to regulate several growth inhibitory pathways [10–12]. Reports in the 1980s showed that in primary cells, mutant (activated) Ras inhibits cell growth rather than promotes transformation [13]. Multiple groups have since shown that mutation or over-expression of Ras leads to cell death and cell cycle arrest. The ability of oncogenes like Ras to promote a state of irreversible cell-cycle arrest has been coined Oncogene-Induced Senescence (OIS) [14], and multiple reports have now implicated H-Ras, N-Ras, and even K-Ras in OIS [15–18]. OIS is believed to be a critical barrier against the development of cancer, which must be overcome in order for oncogenes like Ras to promote tumorigenesis [16,19] (Fig. 1). Interestingly, as Ras-driven tumors progress toward a more malignant phenotype, they lose multiple markers

Abbreviations: OIS, Oncogene-Induced Senescence; NORE1A, Novel Ras Effector 1A; Rb, Retinoblastoma Protein; PTM, Post-Translational Modification; EGFR, Epidermal Growth Factor Receptor; NF1, Neurofibromin 1; DAB2IP, DAB2 Interacting Protein; RA, Ras Association; RASSF1A, Ras Association Domain Family 1 Isoform A; PP1A, Protein Phosphatase 1A; HIPK2, Homeodomain Interacting Protein Kinase 2; MDM2, Mouse Double Minute 2 Homolog; SASP, Senescence-Associated Secretory Phenotype; IL-6, Interleukin-6.

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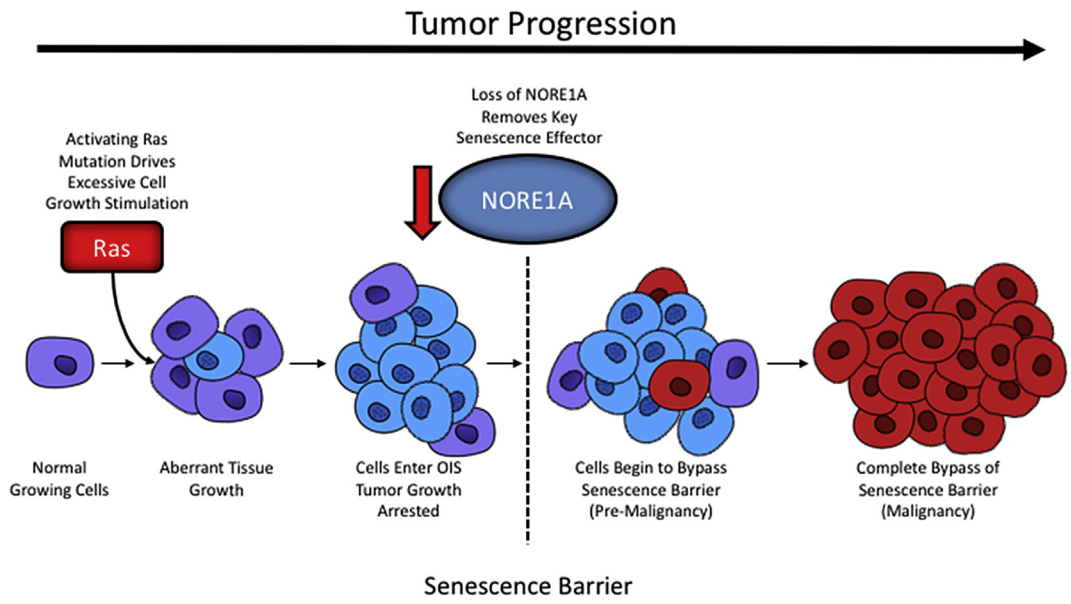

Fig. 1. The role of Oncogene-Induced Senescence as a critical barrier against transformation and tumor development. Normal growing cells can undergo mutations leading to activation of oncogenes, such as Ras. Aberrant Ras signaling can activate a fail-safe mechanism known as Oncogene-Induced Senescence (OIS), and as long as the senescence machinery remains intact, it will suppress tumor progression. However, mutations or loss of tumor suppressor genes involved in OIS, such as NORE1A, p53, and $p16^{INK4a}$, allows Ras to bypass the senescence program and drive tumor progression towards malignancy.

associated with senescence [20]. The detection of Ras-mediated senescence in cell culture systems, primary human tumors, and animal models confirms OIS as a physiologically relevant tumor suppression process [16,20–24]. However, the signaling pathways mediated by Ras that promote senescence are not fully defined.

Pro-mitogenic pathways controlled by Ras are regulated by numerous effector proteins, such as the Raf kinases and PI3-kinase [25,26]. However, not all Ras effectors are mediators of survival signaling [10]. Members of the family of proteins known as the RASSF family of proteins can serve as key effectors of Ras-mediated growth inhibition [27–29]. The six core members have Ras Association (RA) domains and several have been shown to bind or complex with activated Ras [30]. These proteins lack any apparent intrinsic enzymatic activity, and are thought to function as scaffolding and localization molecules, in turn regulating the function of downstream tumor suppressor proteins [29,31]. Notably, most RASSF family members are down-regulated in human cancers, in part by epigenetic mechanisms [32]. The best characterized member, RASSF1A, is now thought to be the most frequently silenced tumor suppressor in human cancers, highlighting the significance of this family in tumor suppression [31,33,34].

The second best characterized member of the RASSF family is NORE1A (RASSF5). NORE1A binds directly to Ras, acting as a *bona fide* Ras death effector/tumor suppressor [35–40]. Initial evidence clearly pointed to NORE1A as a Ras effector and tumor suppressor. For example, over-expression of NORE1A inhibited growth in human cancer cells harboring mutant K-Ras (A549 lung adenocarcinoma) or mutant N-Ras (H1299 non-small cell lung carcinoma), and inhibition of NORE1A expression enhanced cell proliferation [38,41]. However, the underlying mechanisms of Ras/NORE1A action were not clear.

NORE1A shares about 50% homology with RASSF1A [35], but the proteins diverge significantly in their N-termini, suggesting overlapping, but distinct functions. As with RASSF1A, NORE1A is frequently inactivated in many human cancers by promoter methylation. It is also subject to post-transcriptional inactivation via calpain-mediated proteolysis [42]. In addition, inactivation of NORE1A through a translocation event is linked to the development of clear cell renal cell carcinoma, a rare form of familial cancer [43]. Thus, "knockout humans" are predisposed to cancer. Moreover, in primary Ras-driven tumors, loss of NORE1A is associated with a more malignant phenotype, suggesting an important link between Ras and NORE1A *in vivo* [44,45].

NORE1A was first thought to play a role in regulation of the HIPPO pathway due to its ability to bind to MST kinases [37]. However, NORE1A does not seem to activate MST kinases, and the interaction between NORE1A and MST kinases is not required for NORE1A to inhibit growth [46,47]. Collectively, the data suggest that NORE1A acts as a tumor suppressor independently of canonical HIPPO signaling. Additional work in this area, using more physiological systems, suggested that NORE1A had an impact on cell-cycle arrest rather than apoptosis [41]. Several reports have now shown that NORE1A is a critical driver of Oncogene-Induced Senescence [48–52]. In support of this premise, loss of NORE1A impairs the ability of Ras to induce senescence, providing strong evidence that NORE1A is an important component of Ras-induced senescence [48].

Ras-induced senescence appears to act as a critical barrier against the development of cancer, and OIS must be overcome in order for Ras to promote transformation [16,53,54]. For over 25 years, both the p53 and Rb pathways have been implicated in OIS; however, the exact mechanisms by which Ras regulates these proteins was not defined. NORE1A has now been identified as the missing link between Ras and these critical tumor suppressors. Specifically, NORE1A can regulate both the p53 and Rb pathways quantitatively and qualitatively [48,49]. This review describes our current understanding of how NORE1A regulates critical tumor suppressor pathways to promote OIS in order to protect against Ras-mediated transformation.

NORE1A interacts with PP1A to dephosphorylate and activate Rb, inducing OIS

The *Rb* gene was the first tumor suppressor gene identified and was initially linked to the formation of rare cases of pediatric tumors of the retina called retinoblastoma [55–57]. Further studies