Two Faces of SIVA

Lois Resnick-Silverman and James J. Manfredi

Summary: In non-small cell lung cancer cells that contain a mutated KRAS gene, SIVA, a p53 target gene that is critical for apoptosis, is overexpressed in a p53-independent manner and promotes tumorigenesis through the stimulation of mTOR signaling. The ablation of Siva in conditional knockout mice results in an inhibition of tumor development that makes SIVA an interesting new candidate therapeutic target for the treatment of a carcinoma with few therapeutic options. Cancer Discov; 5(6); 581–3. ©2015 AACR.

See related article by Van Nostrand and colleagues, p. 622 (5).

The contribution of p53 as a sensor of many kinds of stresses that provoke cell-cycle arrest and repair, senescence, and apoptosis is undeniable. Diverse conditions that include DNA damage, oncogenic signaling, ribonucleotide and nutrient depletion, or oxidative stress result in the upregulation of a plethora of p53 targets that act either to maintain genomic stability or lead to cell elimination (1). What remains unsatisfying is the mechanism by which p53 can act as a tumor suppressor (2, 3). The classic view that p53 mediates tumor suppression through target genes that include CDKN1A (p21), BBC3 (PUMA), and PMAIP1 (NOXA) has been challenged by recent studies (4). It seems like it is time to dust off these long-held notions and look elsewhere for answers. Perhaps the answer will come from studying context-dependent mechanisms, and there will be no one-size-fits-all solution to this key question of tumor biology. Perhaps the answer will lie in the identification of novel p53 targets. In this issue of Cancer Discovery, Van Nostrand and colleagues (5) focus on a novel p53 target gene, SIVA, that has been shown to play a critical role in apoptosis. Unlike other such target genes, it has a counterintuitive p53-independent role as a tumor promoter in the context of a non-small cell lung carcinoma (NSCLC) driven by the KRASG12D mutation. Seeking to define the role of SIVA in tumorigenesis, conditional knockout of Siva in mice was surprisingly found to inhibit NSCLC development. Of notable importance is the fact that high levels of SIVA in NSCLC patients correlate with a poor prognosis.

Lung cancers present a formidable challenge for cancer therapeutics. NSCLC accounts for approximately 85% of lung cancers. These are mostly adenocarcinomas and squamous cell carcinomas. Treatment options are based on the patients’ medical history and the stage of the disease and include surgery, radiation therapy, and chemotherapy. The identification of specific genetic alterations in key oncogenes has led to a targeted therapy approach (6). Molecular testing has identified mutually exclusive mutations affecting three genes: EGFR, ALK, and KRAS. Patients whose tumors overexpress EGFR or have activating mutations in its catalytic domain have benefited from tyrosine kinase inhibitors, such as erlotinib and gefitinib. However, 15% to 25% of NSCLC patients have mutations in KRAS that lead to constitutively activated protein. Whereas patients who have EGFR mutations are generally nonsmokers and of Asian descent, patients who have KRAS mutations are mostly smokers of non-Asian descent. These tumors are typically not responsive to tyrosine kinase inhibitors, because KRAS activation is downstream of EGFR. They are predictive of poor prognosis and survival (7). Unfortunately, current options for treatment are limited. SIVA was originally identified as a novel protein that bound to the cytoplasmic tail of CD27, a member of the tumor necrosis factor receptor (TNFR) family using a yeast two-hybrid system (8). The protein has a death domain (DD) homology region, a box-B-like ring finger and a zinc ring finger-like domain. Overexpression of SIVA resulted in apoptosis in several different cell lines. A proapoptotic role for this protein has been shown in the immune system, cerebellar granule neurons, and in injury-induced apoptosis in postmitotic neurons. It has since been reported to be a transcriptional target of both p53 and E2F1 (9, 10). Jacobs and colleagues previously used microarray analysis to identify SIVA as an essential p53 target gene selectively induced during apoptosis (9). SIVA is associated with the plasma membrane and, in combination with other extrinsic apoptotic factors, plays a critical role in p53-dependent apoptosis. Jacobs and colleagues used a mouse embryo fibroblast (MEF) system in which DNA damage elicited either growth arrest or apoptosis (9). (Apoptosis was induced in MEFs that were engineered to express adenovirus E1A.) Disimilar to other p53 targets that were induced during both cell-cycle arrest and apoptosis, SIVA was expressed only during the cell death response (Fig. 1, left). Although such evidence raises the possibility of SIVA having tumor suppressor activity, other contradictory findings suggested a role in proliferation. In this issue, the authors shed light on the dual nature of this p53 target (5).

The authors have chosen to examine the effects of SIVA knockdown in oncogenic KRAS-driven NSCLC development using a mouse model (5). NSCLC cells have wild-type p53 that has been shown to suppress malignant progression. The mice have been engineered to be conditional for KRASG12D.
expression and Siva knockout. The introduction of Cre recombinase by intratracheal injection can accomplish the necessary recombination for expression. Unexpectedly, SIVA loss reduces tumor initiation and therefore suggests that SIVA is necessary for efficient oncogenesis in the development of KRAS-driven NSCLC. Similar observations were made in human NSCLC cell lines in that SIVA knockdown slows proliferation and transformation. This suggested the idea that in the context of KRAS-driven lung carcinoma, SIVA is functioning in a p53-independent manner with a role that was yet to be teased out. As the authors note, this might make SIVA a good target for lung cancer therapy due to its overexpression and specificity in tumor cells. The authors’ analysis continued by using two mouse NSCLC cell lines that were derived from the wild-type p53-expressing KRASG12D mice that express SIVA. Using lentiviral transduction of shRNAs to knock down SIVA, a reduction in proliferation was observed that was accompanied by reduced bromodeoxyuridine (BrdUrd) incorporation. Apoptosis did not account for this reduction because no increase was observed in the percentage of Annexin V-positive cells. Hallmarks of transformation, such as low-density plating, anchorage independence, and growth in soft agar, all indicated that knockdown of SIVA reduced the transformational potential of these cells. How, then, was SIVA promoting tumorigenesis? It was not occurring through p53, because knockdown of SIVA did not result in a change of p53 levels or a change in the expression of p53 targets such as Cdkn1a (p21), Bbc3 (Puma), Pmaip1 (NOXA), Bax, and Siva, which are selectively expressed during apoptosis. Right, KRASG12D-mutant NSCLC that also contains wild-type p53 overexpresses SIVA. These cells proliferate and display a transformed phenotype. Upon SIVA knockdown, cells die by autophagy due to a pronounced reduction of mTOR activity.

**Figure 1.** Context-dependent outcomes of SIVA expression. Left, E1A 12S-expressing MEFs that are wild-type for p53 undergo apoptosis after DNA damage due to upregulation of BBC3 (PUMA), PMAIP1 (NOXA), BAX, and SIVA, which are selectively expressed during apoptosis. Right, KRASG12D-mutant NSCLC that also contains wild-type p53 overexpresses SIVA. These cells proliferate and display a transformed phenotype. Upon SIVA knockdown, cells die by autophagy due to a pronounced reduction of mTOR activity.
REFERENCES

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