EDITORIAL

# NCOA3, a new player in melanoma susceptibility and a therapeutic target



*Cancer Gene Therapy* (2022) 29:399–401; https://doi.org/10.1038/s41417-022-00449-2

The American Cancer Society estimates that ~106,110 new melanoma cases were diagnosed in 2021 in the United States, with 7180 deaths [1]. Melanoma arises from melanocytes, which not only determine hair and skin color but also offer protection to UV radiation (UVR) through production of melanin. However, melanocytes themselves are susceptible to the effects of UVR and its consequent DNA damage, resulting in the development of melanoma, the deadliest skin cancer. While the correlation with sun exposure is well established, the precise mechanism leading from UVR exposure to melanoma development is still unclear, as complex molecular events are required to initiate melanoma and promote its progression [2]. The nuclear receptor coactivator 3 (*NCOA3*) gene (also known as AIB-1 or SRC3), residing on 20q13, a member of the nuclear hormone receptor coactivator family, has recently been shown to play an important role in promoting melanomagenesis through a complex molecular pathway that not only regulates cell-cycle progression but also DNA damage response (DDR), thus regulating UVR sensitivity and ultimately, melanoma susceptibility (Fig. 1) [3]. *NCOA3* was initially identified as differentially expressed in metastatic melanomas by cDNA microarray analysis [4], and validated as a prognostic marker in primary cutaneous melanoma [5, 6]. However, a role for NCOA3 in melanoma susceptibility and as a therapeutic target had not been previously demonstrated.

## NCOA3 ACTIVATION RENDERS IT A THERAPEUTIC TARGET IN MELANOMA

Initial studies demonstrated *NCOA3* copy number elevation (using fluorescence in situ hybridization) in almost 30% of primary melanoma samples versus 0% of a cohort of nevi that included congenital as well as dysplastic nevi. *NCOA3* copy number was elevated in a majority of the melanoma cell lines examined and present regardless of *BRAF* or *NRAS* mutational status. This specific clonal variation, identifiable at the DNA level, can therefore not only be potentially employed as a diagnostic marker but also provides the biologic basis of NCOA3 overexpression being predictive of melanoma-specific survival and metastatic potential to sentinel lymph nodes and distant organs.

While NCOA3 has been identified as a druggable target [7], its role in melanoma progression had not been previously explored. The consequences of modulating NCOA3 expression on melanoma cell growth were evaluated using gene silencing as well as the small molecule bufalin. Bufalin, a toad venom extract used in traditional Chinese herbal medicine, has been shown to target NCOA3 by promoting its degradation at low nanomolar concentrations [3]. Suppression of NCOA3 expression resulted in significantly decreased melanoma cell proliferation and invasion in vitro and was accompanied by suppressed growth and metastatic potential in vivo. Accordingly, bufalin treatment of multiple melanoma cell lines and patient-derived xenografts suppressed melanoma cell viability and in vivo growth. NCOA3 targeting was accompanied by significant suppression of cell-cycle progression, including reduced S phase and G2-M arrest, and increased apoptosis.

## MECHANISTIC ROLE OF NCOA3 IN MELANOMAGENESIS

Cellular responses to DNA damage are essential to the ultimate pathogenic consequences of UVR mutagenesis. These encompass DNA repair, including direct repair, nucleotide and base excision repair, recombinational and crosslink repair [8], and apoptosis [9]. Of considerable interest is the central role for the tumor suppressor p53 in "sensing" DNA damage, and orchestrating DNA repair and apoptotic cell death. While p53's essential role in UVR-mediated cutaneous carcinogenesis is well appreciated [10], *TP53* mutations are significantly less common within melanocytic neoplasms, and melanomas exhibit resistance to typical p53-mediated inducers of apoptosis despite an intact p53, indicating that other molecular events abrogate the response to DNA damage.

Gene expression profiling studies following *NCOA3* gene silencing identified downregulation of multiple cyclins (i.e., *CCNB1*, *CCNB2*, *CCNA1*, and *CCND1*), along with that of *XPC*. The XPC protein plays a key role in regulating sensitivity to UVR by virtue of its recognition of UV-induced DNA damage and its recruitment of repair proteins to complete global genome nucleotide excision repair [11]. An unexpected finding was the upregulation of *CHK2*, which plays a key role in DDR, including following UVR exposure. At the protein level, according to immunofluorescence quantification, the modulation of XPC, CCNB1, and CHK2 in response to *NCOA3* suppression resulted in robust upregulation of p53 and p21. It is not surprising that these molecular events would be accompanied with a distinct cell-cycle profile that is consistent with a G2-M arrest. However, a striking phenotypic change upon *NCOA3* silencing was observed in melanoma cell lines, characterized by multi-nucleation and centrosome amplification, ultimately leading to mitotic catastrophe and cell death. This was accompanied by profound downregulation of proteins that mediate mitotic progression, including CCNB1, CDK1, AURKA, and PLK1. These results indicate why *NCOA3* overexpression is preferentially selected since it promotes the robust and unhindered cell division of melanoma cells.

## NCOA3 IN MELANOMA SUSCEPTIBILITY

Even the identification of high- and low-penetrance susceptibility loci (e.g., *CDKN2A*, *CDK4*, *MC1R*, *TYR*, *TYRP1*, and *OCA2*) in melanoma-prone families [12] does not fully account for the distinct mechanisms of UVR-induced melanomagenesis. Thus,

Received: 5 January 2022 Revised: 12 February 2022 Accepted: 24 February 2022
Published online: 23 March 2022

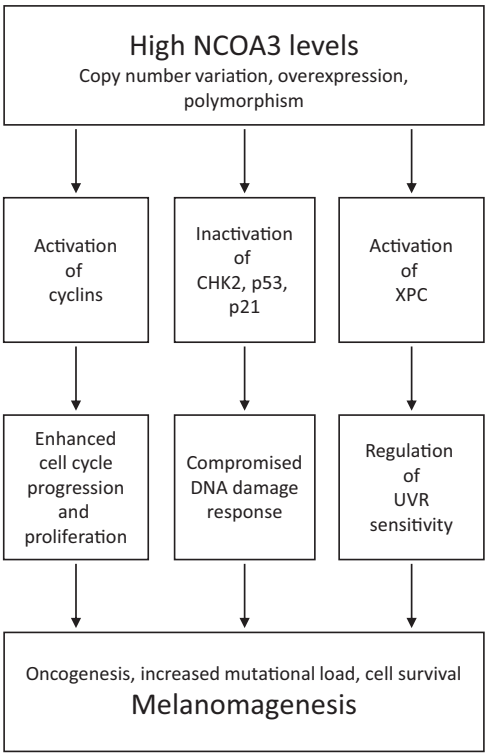


**Fig. 1 Pathways regulated by NCOA3 leading to melanomagenesis.** Increased NCOA3 expression, whether through copy number elevation or polymorphism, regulates multiple distinct pathways leading to oncogenesis, involving cell cycle regulation, circumvention of the DNA damage response, and regulation of susceptiblity to UV exposure.

ongoing studies are necessary to identify other key mediators, including the non-mutational events that regulate UVR sensitivity in melanocytes.

*NCOA3* polymorphisms have been previously linked to breast cancer susceptibility [13]. The *NCOA3 T960T* (2880 A > G or rs2076546) polymorphism, with reduced prevalence in familial breast cancer cases lacking *BRCA1* and *BRCA2* mutations [14], was also found to be significantly less prevalent in a 97-patient familial melanoma cohort without a *CDKN2A* gene mutation when compared to 364 individuals without history of cancer. Thus, the 2880 A > G variant was present in 9.3% of the familial melanoma cohort versus 19.5% of the control cohort, indicating that *NCOA3* polymorphism status was significantly different in a cohort whose susceptibility to melanoma could not be accounted for by *CDKN2A* mutation status. In addition, the 2880 A > G polymorphism was present in 22.6% of a sub-Saharan African population versus 10.6% of Europeans, which have a higher melanoma risk. Finally, the *T960T* polymorphism was present in only two of 23 melanoma cell lines, and none of 53 melanoma specimens examined. These studies firmly implicated a role for NCOA3 in melanoma susceptibility.

Additional studies identified regulation of UVR sensitivity as the mechanism by which NCOA3 modulates melanoma susceptibility. NCOA3 targeting in melanoma cell lines, either by bufalin administration or gene silencing, decreased cell survival and colony formation following exposure to UVR. Conversely, the overexpression of *NCOA3* cDNA in normal melanocytes of both adult and neonatal lineage resulted in reduced sensitivity to UVR and was accompanied by accumulation of the DNA damage marker γH2AX. NCOA3 overexpression resulted in concomitant elevation of XPC and CCNB1 expression, as well as suppression of phospho-CHK2, p53, and p21. It is interesting to note that several of the genes regulated by NCOA3 (*XPC*, *TP53*, and *CHK2*) are themselves established markers of cancer susceptibility.

Finally, normal melanocytes expressing a cDNA construct encoding wild-type *NCOA3* expressed higher levels of NCOA3 protein, with consequently significantly increased resistance to UVR compared to control, while overexpression of the *T960T* construct produced an intermediate level of sensitivity to UVR. Analysis of UV photoproducts further reinforced the susceptibility hypothesis, as it was shown that normal melanocytes overexpressing the *T960T* polymorphism were more successful in removing these photoproducts post-UVR exposure, compared to wild-type *NCOA3*-overexpressing cells. Thus, NCOA3-mediated abrogation of DDR and UVR response is clearly dose dependent, emphasizing the important role played by NCOA3 in orchestrating events that can lead to melanomagenesis.

## SUMMARY

Taken together, these studies identify NCOA3 as a new player in melanomagenesis and as a novel target for therapy. An important aspect of these findings is that elevated expression of *NCOA3* in melanoma, in part through increased copy number, promotes melanoma progression through enhanced survival following UVR exposure and increased accumulation of UV-mediated DNA damage. These data lend themselves to a model in which elevated NCOA3 expression, in the setting of chronic UVR exposure, can promote melanomagenesis by circumventing DDR and facilitating cell-cycle progression, thereby enabling melanocyte survival at the expense of a higher mutational burden (Fig. 1). These effects are attenuated in individuals harboring the *T960T* polymorphism, in which intermediate levels of NCOA3 expression are insufficient to override DDR. In addition, these studies uniquely implicate NCOA3 as a marker of melanoma susceptibility, as a diagnostic and prognostic marker, and as a target for therapy. Clearly, additional studies are required to advance NCOA3 targeting to the clinical arena. While preliminary clinical trials with bufalin demonstrated modest antitumor activity in molecularly unselected cancer patients [15], the observation of elevated *NCOA3* copy number may have ultimate utility as a biomarker in identifying melanoma patient subsets to undergo therapy with NCOA3-targeting agents.

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## DATA AVAILABILITY

Not applicable.

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## AUTHOR CONTRIBUTIONS

Both authors contributed equally.

## FUNDING

This work was supported by NIH R01's CA114337 and CA215755 (to MKS), and the Cancer Avatar Program Fund of the California Pacific Medical Center Foundation.

## COMPETING INTERESTS

The authors declare no competing interests.

## ADDITIONAL INFORMATION

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