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COMMENTARY

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ZNF768: controlling cellular senescence and proliferation with ten fingers

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ABSTRACT

We recently identified Zinc-finger protein 768 (ZNF768) as a novel transcription factor controlling cell fate decision downstream of Rat sarcoma virus (RAS). We showed that ZNF768 depletion impairs cell cycle progression and triggers cellular senescence, while its overexpression allows cells to bypass oncogene-induced senescence. Elevated ZNF768 levels is common in tumors, suggesting that ZNF768 may help to escape cellular senescence, sustain proliferation and promote malignant transformation. Here, we discuss these recent findings and highlight key questions emerging from our work.

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Cellular senescence is a multifaceted response that halts cells that are at risk of neoplastic transformation. This response plays roles in suppressing tumor development *in vivo*.[1] The aberrant activation of many oncogenes, such as Rat sarcoma virus (RAS), induces cellular senescence in primary cells.[2] However, the mechanisms linking oncogene activation to cellular senescence remain poorly understood.

In our recent study, we report the identification of Zinc finger protein 768 (ZNF768) as a novel player controlling cellular senescence and proliferation downstream of the RAS pathway.[3] ZNF768 is a poorly characterized transcription factor with unique features. In addition to its 10 canonical C_2H_2 zinc finger motifs, this conserved protein contains several heptad repeats which have a high similarity with the C-terminal domain (CTD) of the DNA-directed RNA polymerase II subunit B1 (RPB1). Phosphorylation of the CTD of RPB1 on multiple sites, which yield specific patterns often refered to as the 'CTD code', plays important roles in regulating transcription.[4] Regarding ZNF768, we found that RAS activation leads to the phosphorylation of PXS*P motifs present in this domain and promotes ZNF768 degradation (Figure 1a). Although we did not identify the exact kinase(s) that directly phosphorylate(s) ZNF768, we found that the mitogen-activated protein kinase (MAPK) and mechanistic target of rapamycin complex 2 (mTORC2)/protein kinase B/Akt (Akt) axis act in concert to control ZNF768 phosphorylation and stability downstream of RAS. Our findings suggest that the heptapeptide repeats in ZNF768 may serve as a hub allowing cells to integrate RAS signaling and adapt to its activation state. Whether a precise CTD code exists to control ZNF768 stability and function is an interesting possibility that warrants further investigation.

In a first attempt to characterize ZNF768 functions, knockdown studies were performed in various cell lines. Following ZNF768 depletion, a striking loss of proliferation was observed, which was associated with severe mitotic catastrophes and apoptosis. Interestingly, we showed that partial depletion of ZNF768 did not induce cell death, but rather promoted cellular senescence. These results indicate that ZNF768 levels are tightly linked to the proliferation-senescence-apoptosis cell fate decision machinery. In a previous study, Rohrmoser et al. showed that ZNF768 is recruited to genomic regions called Mammalian-wide interspersed repeats (MIRs) to control the expression of various genes in a cell-specific manner, including many other transcription factors.[5] Our work further extends this by showing that ZNF768 regulates proliferation through two distinct mechanisms, that are i) the transcriptional regulation of a "core" set of cell cycle genes and ii) the repression of *bona fide* targets of cellular tumor protein 53 (TP53, also known as p53). These results suggest that ZNF768 is an important transcription factor controlling cell fate decision downstream of growth factor signaling.

To better understand the relation between ZNF768 levels and the proliferation-to-senescence decision, ZNF768 levels were measured following the induction of replicative and premature senescence (including oncogene- and stress-induced senescence). A strong decrease in ZNF768 was observed in both types of senescence. Supporting a functional impact of ZNF768 depletion on the activation of cellular senescence, we

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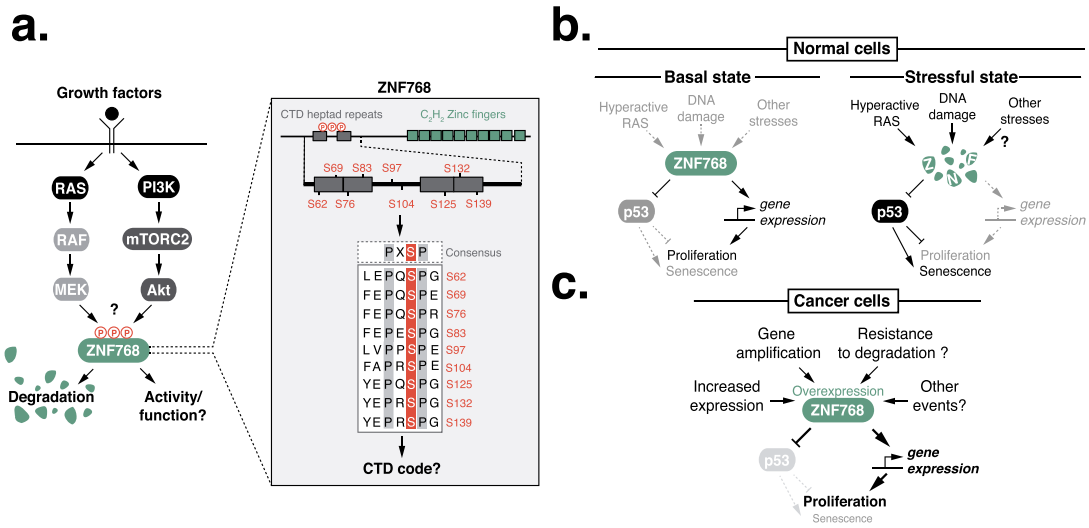

Figure 1. Schematic overview of ZNF768 and its role in controling cellular senescence and proliferation. a. ZNF768 is a protein phosphorylated in response to growth factor signaling activation. b. Schematic overview of the conditions controling ZNF768 levels and the impact of ZNF768 on the control of cellular senescence and proliferation in basal state (left) and in response to stress (right). c. Overview of the model linking ZNF768 to cancer cell proliferation. Abreviations: Protein kinase B/Akt (Akt), C-terminal domain (CTD), Dual specificity mitogen-activated protein kinase kinase (MEK), Phosphoinositide 3-kinase (PI3K), tumor protein 53 (p53), Rat sarcoma virus (RAS), Rapidly accelerated fibrosarcoma (RAF), Zinc Finger Protein 768 (ZNF768).

found that ZNF768 overexpression was sufficient to bypass RAS-induced senescence in primary cells. Mechanistically, we showed that ZNF768 regulates this process at least in part by repressing p53 function through direct interaction (Figure 1b). As extensively discussed elsewhere, identifying markers of cellular senescence is needed to facilitate the study of this process both *in vitro* and *in vivo*.[6] Our findings indicate that measuring ZNF768 degradation could serve as a new tool to identify and characterize senescent cells.

Although the relation between ZNF768 and cellular senescence was confirmed in various experimental contexts, many important questions remain. For instance, we still do not know whether the MAPK and mTORC2/Akt pathways contribute to the degradation of ZNF768 during replicative senescence. Also, it is still unknown whether ZNF768 levels gradually decrease after each cell doubling or simply fall when cells are exposed to a certain threshold of cellular stress or damages. Interestingly, we observed that induction of DNA damage with genotoxic compounds induced a rapid decrease in ZNF768, an effect that occurred before senescence entry. The rapid decrease in ZNF768 levels in this context indicates that ZNF768 degradation is an early signaling event that likely preceeds the apparition of end-stage senescence markers. Taking into account the established link between activation of the DNA damage response (DDR) and induction of cellular senescence,[7,8] it is tempting to speculate that ZNF768 downregulation during cellular senescence might be linked to the activation of the DDR. Whether ZNF768 plays functional role in the control of the DDR is an intriguing hypothesis that should be tested in the future.

Because variations in ZNF768 levels greatly impacts cell proliferation, we next sought to determine whether *ZNF768* gene was mutated or its expression altered in human cancers. Analyses of the TCGA PanCancer Atlas Studies revealed that gene amplification is the most frequent alteration found in *ZNF768* gene. Accordingly, we found that *ZNF768* expression and ZNF768 protein levels were also increased in different types of cancer. Interestingly, our studies revealed that many tumors showed very high ZNF768 protein levels despite small changes in transcript expression, indicating that post-translational mechanisms likely take place to increase ZNF768 protein expression in tumors. These processes might explain why tumors often overexpress ZNF768 protein, even in the presence of oncogenic RAS mutations. Taking all these observations into account, we propose that ZNF768 overexpression in tumors might offer a proliferative advantage by promoting the expression of key cell cycle regulators and by repressing cellular senescence (Figure 1c). Supporting this model, we observed in a follow up study that ZNF768 protein expression correlates with proliferative clinicopathological features in non-small cell lung cancer.[9] Overall, our data suggest that ZNF768 could serve as a novel biomarker for proliferation and senescence in tumors *in vivo*. Since senescence affects many facets of tumor development and treatment response,[10] it will be interesting to test whether changes in ZNF768 levels can directly impact these processes. Studies with ZNF768 knockout and overexpressing animal models are needed to define the exact contribution of ZNF768 to tumorigenesis *in vivo*.

Overall, the discovery of ZNF768 opens a new chapter in understanding the molecular mechanisms regulating cell proliferation and senescence. Knowing that exciting new research and discoveries lie ahead, time will tell if ZNF768's fingers will

succeed in disentangling the complexity of tumors to improve the treatment of cancer.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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