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Reactive oxygen species: The good, the bad, and the enigma

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Work carried out primarily in the laboratory of Fabrizio d'Adda di Fagagna unveils the mitogenic properties of Ras-induced reactive oxygen species (ROS) and their relationship with the DNA damage response. Combined data from studies of cultured cells, zebrafish models, and clinical material consistently support a role of the RAS-RAC1-NOX4 axis in ROS induction, hyperproliferation, and senescence.

Oncogene-Induced Reactive Oxygen Species and Genome Instability: Hijacking the Good for Hyperproliferation

Oncogenes are mutated forms of cellular genes that are responsible for driving rampant cellular proliferation. However, the expression of an activated oncogene in normal human cells does not lead to their transformation, but to a permanent cell cycle arrest known as cellular senescence.[1,2] Hence, oncogene-induced senescence (OIS) serves as a tumor suppressor mechanism that restricts the progression of pre-neoplastic lesions.

The role of oncogene-induced reactive oxygen species (ROS) production in cancer development has been a controversial subject. ROS is a collective term describing a number of highly reactive molecules and free radicals that are formed upon incomplete reduction of oxygen. ROS can either directly damage or modulate redox-sensitive signaling pathways, depending on the molecular concentrations at which they are present.[3] In particular, the role of oncogenic RAS-regulated ROS production in cellular transformation has been the subject of several studies.[4] Nonetheless, it remains unclear whether oncogenic ROS have a direct role in genome instability and, if so, which molecular mechanisms are involved.

We approached this issue by taking a closer look at the mode of action of oncogene-induced ROS in the model system of H-RAS oncogenic activation in normal human fibroblasts. We observed that removal of ROS by the broad specificity scavenger N-acetyl cysteine (NAC) inhibits oncogene-induced hyperproliferation, prevents activation of the DNA damage response (DDR), and reduces the establishment of senescence. This experiment led us to propose the novel concept that oncogene-induced ROS are modulators of cell hyperproliferation that engage the DDR by exposing cells to DNA replication stress.[5]

We then focused on the origins of the oncogene-induced ROS, which are ubiquitously generated through diverse enzymatic pathways. In particular, we turned our attention to the NOX family of oxidases because initial studies suggested them as a source of ROS in cancer.[4] Our experiments identified the RAS-RAC1-NOX4 axis as the pathway responsible for ROS generation by oncogenic RAS in human cells. Therefore, we propose that RAS-induced ROS are mitogenic signaling molecules for the initial hyperproliferative phase, which is causally associated with the altered DNA replication that precedes and prompts DDR activation and ultimately leads to senescence.

Oncogene-Induced ROS in Proliferation and Survival: Addiction to the Bad

Cell transformation is associated with bypass of OIS and ongoing rampant proliferation. Since oncogene activation hijacks the ROS-modulated proliferative cues for the initial hyperproliferative phase, we investigated the effect of ROS scavengers and NOX4 inhibitors on proliferating cells. These cells either harbor mutations to bypass OIS or are fully transformed, and either express oncogenic RAS or do not. We observed that the expression of oncogenic RAS in a cell is sufficient to confer sensitivity to ROS manipulations. Thus, oncogene dependency translates into ROS, and hence NOX4, dependency.

We also treated cells derived from human pancreatic cancer with gemcitabine, the standard of care chemotherapy treatment for pancreatic cancer, and NOX4 inhibitors. Although we observed a modest effect of NOX4 inhibitors alone,

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we discovered an exciting synergic effect of NOX4 inhibitors and gemcitabine that reduced the half-maximal inhibitory concentration (IC50) of this nucleoside analog by up to 6-fold. Deregulation of survival pathways by NOX-derived ROS through JAK, STAT, AKT, and NFκb pathways has been previously implicated[4]; however, the mechanism by which combined use of NOX4 inhibitors and gemcitabine enhances apoptosis in cancer cells remains unclear.

Oncogene-Induced ROS Manipulations in Cancer: The Enigma

Using a well-established mouse model of Ras-induced pancreatic cancer, we reported increased induction of Nox4 during progression from the earliest neoplastic lesions to more invasive ones. In addition, we observed robust NOX4 expression in a set of human pancreatic tumors, with signal intensity correlating with neoplastic stage. Importantly, and concordant with our results in cultured cells, NOX4 levels in these human lesions correlated with markers of DDR activation.

Cancer cells produce elevated levels of ROS and exhibit altered metabolic pathways and regulatory mechanisms in order to maintain redox balance. Therefore, high levels of ROS production are counterbalanced by an equally high antioxidant activity. Studies of the same murine model showed that an antioxidant system mediated by Nrf2 and Nqo1 is activated upon oncogenic Ras activation.[6] However, on the basis of our experimental results it appears that this compensatory response to Ras-induced ROS production is not sufficient to fully buffer Ras-mediated ROS accumulation during the process of tumorigenesis. Another example of altered redox homeostasis resulting from an impaired Nox4-Nrf2 balance has recently been reported in the context of lung fibrosis.[7]

A recent article proposed a novel axis involving activation of p38 through Nox1 for ROS generation and consequent malignant cellular transformation upon oncogenic K-Ras activation in rodent fibroblasts.[8] Another study reported that oncogenic H-Ras cooperates with Nox1 in rodent fibroblasts or with NOX4 in human fibroblasts for establishment of OIS.[9] Therefore, it is worth exploring whether the oncogenes that transform both human and murine cells toward tumorigenicity exhibit different preferences for ROS source between the two species. NOXzymes are differentially expressed not only among distinct tissues, but also among several different types of cancer (see Fig. S6J in the article by Ogrunc et al.).[4,5] In certain cancers one member of the family is activated as the primary source, whereas the enzymes collaborate in other cancers, for example upregulation of both NOX1 and NOX4 in colorectal cancer. Understanding which oncogenic stimulus activates each Nox enzyme as an initial source for transformation and later proliferation, and how this is influenced by the *in vivo* microenvironment during tumorigenesis, will undoubtedly help the design of better therapeutic strategies based on ROS manipulations.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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