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Redox-Redux and NADPH Oxidase (NOX): Even More Complicated than We Thought it Might Be

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The NOX (nicotinamide adenine dinucleotide phosphate oxidase) family includes seven unique members that are involved in a multitude of physiological functions, including extensive interaction with UVR and the skin. NOX1 is uniquely present and activated by UVB radiation with biphasic expression of the enzyme immediately and then after a several-hour delay. Specific inhibition of both early and late NOX1 activation leads to evidence of decreased photocarcinogenesis in vitro keratinocytes and in well-characterized mouse models in which antitumor efficacy has been shown; inhibiting only late NOX activation does not exhibit such effects. These results suggest a crucial function of early NOX activation in transducing a signal for cellular protection after UVB carcinogenesis provocation. We term this an intrinsic cellular ROS priming function for quenching DNA damage and promoting survival. Evolutionally, this type of priming function may be essential for addressing various types of stimuli from adverse environments.


Introduction

Cellular growth and its control is an exceedingly complex affair, and the pathways and regulators involved are increasingly recognized. Although the importance of redox in regulating or modifying physiologic processes related to cardiovascular health, energy metabolism (e.g., diabetes), and neurocognition has been recognized for some time (Jones and Sies, 2015), understanding its importance in carcinogenesis has been slow in coming, despite the unfortunate demonstration in a large randomized trial over 20 years ago that supplementation with β-carotene leads to a marked increase in lung cancer in tobacco smokers (Omenn et al., 1996). As summarized elsewhere, “Redox signaling is a long-overlooked form of signal transduction that proceeds through the reversible oxidation of the amino acid cysteine in proteins and that use hydrogen peroxide as a second messenger” (Hornsved and Dansen, 2016, pp 300). Raad et al. (2017) explore the role of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX1) in photocarcinogenesis and its importance in the DNA damage repair (DDR) network. Evidence from this article suggests that a transient surge of reactive oxygen species (ROS) may serve as a priming signal to trigger a series of redox reactions, which, in fact, may stimulate cellular defense mechanisms, including the DNA damage response.

The established paradigm of carcinogenesis

For nearly 50 years, the classical concepts of carcinogenesis have been divided into initiation, promotion, and progression, with each phase being regarded as discrete. In the last decade, this simple schema has been expanded and modified to include roles for epigenetic influences, mitochondrial energy regulation, and, most recently, microbiome effects. We now recognize from recent studies that carcinogenesis is much more complex and that it is accompanied by a series of subtle mutational changes that suggest preventive and therapeutic interventions. These may affect early but not late stages or may even produce adverse acceleration of the malignant process, including, most strikingly, effects in colon and nonmelanoma skin carcinogenesis (Meyskens et al., 2016). Another prominent complexity that has emerged is that the effects of agents that may be used for both treatment and prevention are dose, schedule, and...
Clinical Implications

- Blockage of both the early and late phases of NOX1 activation by UVB with a specific inhibitor (InhNOX1) may be a new approach to the prevention and treatment of cutaneous squamous cell carcinoma.
- Because InhNOX1 inhibits NOX1 activation in xeroderma pigmentosum-1 cells, in which nucleotide excision repair is markedly diminished, clinical trials should be initiated in patients with this aggressive genetic condition.
- Because NOX1 is also a key NOX for melanoma carcinogenesis, exploration of the effectiveness of the newly synthesized InhNOX1 or similar compounds should be explored in individuals at risk.

The work undertaken by Raad et al. (2017) takes us to a new level of complexity and, at the same time, a better understanding of the basic mechanisms at work (Figure 1). The induction of redox changes by a carcinogen (UVB) results in biphasic activation of NOX1. Parallel responses in human keratinocytes and in SKH-1 hairless mice are similar, suggesting that NOX1 plays an important role in photocarcinogenesis. Of course, the next step of our understanding of this set of observations is to ask, Why? What underlies these responses?

We will explore this phenomenon briefly from two vantage points.

What other major factors of carcinogenesis are affected by oxidation that may affect the impact of inhibitors?

Three major factors come into play:

**Protein oxidation.**
- The proteome is a significant target for damage by UVR-induced ROS (Karran and Brem, 2016).
- Nucleotide excision repair (NER) oxidation replication protein (RPA), a DNA-binding protein, is an essential component of NER damaged by oxidation, thereby compromising NER function (Guven et al., 2015).

Both of these influences will affect the outcome.

**Figure 1. Time frame and effects on DNA damage response network.**

(a) Time frame. UVB activates NOX1 (NADPH oxidase-1) in keratinocytes with a biphasic response: immediate strong and rapid ($\leq 1$ hour) burst (ROS priming) and later sustained, but lower, activation (full response) over several hours (hours 8–12). (b) Effects on DDR network. Using a chemically engineered peptide (InhNOX1) to block both early and delayed responses, NER is increased and apoptosis is decreased, and overall the DDR is increased. Consequently, in an in vivo mouse model, tumor formation (cutaneous SCC) is decreased markedly as well. In contrast, when only the second peak is blocked, NER and apoptosis are both decreased, the DDR response is decreased in keratinocytes, and tumor formation is increased. CPD, cyclopyrimidine; DDR, DNA damage response; hr, hour; InhNOX1, inhibitor of NOX1; NADPH, nicotinamide adenine dinucleotide phosphate; NER, nucleotide excision repair; ROS, reactive oxygen species; SCC, squamous cell carcinoma.
**Single-nucleotide polymorphisms (SNPs).** Many studies of the effect of SNPs involving various components of the DDR network, as well as cell cycle parameters, have been published. Two of the larger and hence more informative studies include the following:

- A large meta-analysis of SNPs in pigmentation genes and non-melanoma skin cancer have shown strong associations with the pigment-related genes (Asgari et al., 2016) MCIR and ASIP (Binstock et al., 2014).
- A large genome-wide association study of cutaneous squamous cell carcinoma (SCC) has shown a strong association with six loci-containing genes in the pigmentation pathway. Surprisingly, SNPs at these loci appear to modulate SCC risk independently of the pigmentation phenotype (Asgari et al., 2016).

The observation that DNA photoproducts are induced long after UV exposure by chemixcitization of melanin derivatives is a new and unique observation (Premi et al., 2015). As in vivo keratinocytes receive melanosomes donated by melanocytes, these results provide an important recognition that oxidation events affecting melanocytes inevitably will be modified or amplified in keratinocytes. As noted by Denat et al. (2014), melanocytes are instigators and victims of oxidative stress.

**Biphasic response and inhibition results.** The intriguing biphasic response and the inhibition results in the work by Raad et al. (2017) strongly suggest an intrinsic ROS-priming process that may have evolved as a self-defense mechanism to ensure cell survival under carcinogenic challenge or under other adverse environmental challenges. It has been noted that a small dose of pro-oxidant pretreatment is able to provoke a defense response in many cell types, either against ROS or against other stimuli (Brewer et al., 2011; Khan et al., 2011). However, these previous studies were based on an additional purposeful pretreatment, whereas the study by Raad et al. suggests an intrinsic mechanism. By separately inhibiting either the early and delayed response together or the delayed response alone, Raad et al. have shown that the early response (ROS priming) may have played an essential role in signaling the DNA repair response. The caveat, however, is that it is not possible to inhibit only the early event without affecting the delayed response. For this reason, the suggested ROS priming warrants further investigation.

**Does the effect of InhNOX1 on UVB on NOX1 expression tell us enough or too much to develop a safe chemical intervention?** Notwithstanding the multiple general effects on carcinogenesis described, the major limitation of the study by Raad et al. (2017) is that the UVR used was confined to the UVB spectrum. A large amount of data regarding the effects of UVA indicate that many other oxidative-related events may be involved as well. Hence, a study of solar UVR should be considered before launching this approach into clinical trials. The interactions of NOX1 in the redox milieu are extremely complex, as recently reviewed (Liu-Smith et al., 2014). At a minimum, the measurements of NOX1 activation and the effects of InhNOX1 when solar energy is used as the carcinogen should be determined. Recently, similar compounds have entered clinical trials for the treatment of a range of inflammatory and fibrotic diseases, and they have shown excellent tolerability and promising effects (Teixeira et al., 2016). This information should facilitate entry of InhNOX into clinical trials for the prevention and treatment of SCC and possibly melanoma. We look forward to these results and to the development of InhNOX1 as a powerful inhibitor of photocarcinogenesis.

**CONFLICT OF INTEREST**

The authors state no conflict of interest.

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