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The reemergence of nitric oxide and cancer

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Biological effects of nitrogen oxides have been associated with mechanisms that are life giving as well as toxic. Nitrogen oxide chemistry is critical in the nitrogen cycle, converting nitrate and nitrite to ammonia, which is an essential component of protein synthesis. In contrast, the chemistry of nitrogen oxides has also been associated with the deleterious effects of air pollution, leading to damage in plants and animals. In human health, nitrite and nitrate have been used for millennia as antibacterial agents in the preservation of food. However, in the 1970s, it was found that nitrite/nitrate in food can lead to formation of carcinogenic nitrosamines [1,2]. More recently, these dietary nitrogen oxides have demonstrated significant beneficial effects by protecting the cardiovascular system, which may in part explain the reduction in risk factor of cardiovascular disease associated with vegetable consumption [3–6].

In the 1980s, nitric oxide was identified as an integral component of both the cardiovascular system and the immune response to pathogens. These seminal discoveries prompted an explosion in the study of nitrogen oxide chemistry in biological systems, leading to one of the largest and fastest growing areas in biomedical science. Like nitrogen oxides, this small diatomic radical has been shown to have both beneficial and deleterious effects on different biological systems. Understanding the mechanism of these biological effects was further complicated by the complex chemistry of nitrogen oxides. The mechanistic determinant for the biological properties of NO and nitrogen oxide is its chemistry and how it interacts with different molecular targets. This complex chemistry as well as diverse biology has challenged NO research, requiring discussions from fundamental chemistry and biochemistry as they relate to normal physiology and disease processes. Though daunting, the study of this simple molecule offers immense opportunities for new mechanisms and therapeutic outcomes.

With regard to cancer, various studies have demonstrated roles for NO in the induction of genotoxic lesions as well as its participation in tumor promotion and progression by mediating critical processes, including angiogenesis, tumor cell growth, and invasion [7–10]. Yet, nitric oxide is an important component of the immune response of some types of tumors. For example, iNOS is protective against colon cancer in mice [11]. A better mechanistic understanding of these conflicting properties may require elucidation of the role of NO within a specific tumor microenvironment as it relates to the development of wound response vs. immune response to an aberrant cell type.

Nitric oxide tumoricidal activity of macrophages was one of the major findings that led to the discovery that this diatomic radical could be generated in vivo. Hibbs and co-workers described

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an arginine-dependent substance that decreased mito-chondrial respiration and interfered with iron metabolism, resulting in the killing of tumors and pathogens [12,13]. During the same time, Tannenbaum and co-workers showed that infection led to endogenous increases in nitrite and nitrate levels, suggesting a potential risk for generation of carcinogenic nitrosamines [14, 15]. Later, Steuhr and Marletta showed that nitrite, nitrate, and nitrosamine formation in macrophage was arginine dependent [16,17] and was later found in hepatocytes [18,19]. The substance mediating tumoricidal activity was later identified as NO [20]. In addition to demonstrating that NO was generated in vivo, this research brought attention to the dualistic nature of NO in cancer.

The unifying factor in this paradox involves the requirement of high levels of NO (sustained micromolar steady state NO) to kill tumor cells. Similarly, high NO levels are also genotoxic through formation of carcinogenic nitrosamines or by directly modifying DNA or DNA repair proteins. It was found that aerobic solutions of NO, NO_2, and N_2O_3, which were identified as critical intermediates of smog and air pollutants, led to deamination of nucleic acids [21,22]. Unlike oxidation by peroxynitrite or ROS that preferentially results in transversions [23], nitrosative mixtures of NO_2/ N_2O_3 mediate transitions [24–26]. In colon cancer, many p53 mutations are located in CpG-rich regions that have a significant proportion of transitions [27,28]. Cancerous lungs of smokers have revealed both transversions and transitions in p53 [29], which may in part be a result of the oxidants generated in tar and the RNS in smoke. In contrast, in the cancerous lungs of non-smokers transitions are more prominent, suggesting that inflammation favors transitions. In addition to these direct chemical modifications of DNA, nitrogen oxide inhibits DNA repair proteins [30]. Of particular interest were those that contained zinc finger motifs [31–33]. Despite these mechanisms of genotoxicity, NO also reduces oxidation of DNA by reactive oxygen species, suggesting protective mechanisms of NO that are dependent upon the specific microenvironment [34].

In the 1990s, it was discovered that NOS was associated with several tumors in humans [35–38]. To determine its role in tumor progression, several groups transfected iNOS in different tumor lines [10,39]. As expected, in vitro growth was significantly decreased, indicating a cytostatic effect of NO. However, in vivo, the outcome was tumor specific; while iNOS transfection of some tumors led to less aggressive phenotypes and others became more aggressive. This dichotomy suggested a role for the specific tumor microenvironment in dictating the outcome in response to the high levels of NO generated by iNOS.

One of the critical insights into this dichotomy was that iNOS-induced p53 caused cell cycle arrest. However, in p53 mutants iNOS increased VEGF expression and promoted tumor growth, suggesting that the tumoricidal activity of NO is dependent on p53 status of the tumor [40]. This interplay between p53 and iNOS is an important aspect in determining the role of NO in carcinogenesis [41]. Other studies showed that HIF-1α could be stabilized by nitric oxide, leading to increased VEGF expression [42]. This growth factor phosphorylates eNOS, which generates low-level NO to promote angiogenesis and endothelial function [43]. The fluxes of NO are considerably lower than that generated with iNOS to interact with p53. These findings suggest a balance between NO concentration and specific signal transduction pathways.

While NO has multiple roles in carcinogenesis, NO donors or NOS inhibitors can affect conventional therapy such as radiation and chemotherapy. A limiting factor in radiation treatment of solid tumors is low oxygen in vivo. In 1957 Howard–Flanders demonstrated radiosensitization of Escherichia coli, grown under hypoxic conditions, by O_2 and NO [44]. Several decades later it was found that NO performs nearly as well as O_2 in the radiosensitization of hypoxic mammalian cells [45]. In vivo, local administration of NO donors prior to radiation enhanced tumor blood flow and oxygenation, resulting in modest

radiosensitization of the tumor [46]. Similarly, iNOS gene therapy in combination with an inducible promoter also caused tumor radiosensitization in vivo [47], while eNOS knockout animals showed decreased sensitization [48]. Interestingly, NOS inhibition also enhanced radiation response of animal xenographs when given post irradiation by modulation of the tumor's wound response [49]. These studies further indicate the temporal importance of NO modulation in tumor outcome.

In addition to radiation, NO donors sensitize tumor cells to che-motherapeutic compounds, in particular alkylating agents. This is in part due to nitrosation of critical thiols in DNA repair enzymes such as alkyltransferase, an essential enzyme that repairs alkylation damage caused by BCNU [50]. Other studies have demonstrated NO sensitization to cisplatin and melphalan toxicities, which persisted for several hours after NO treatment [51,52]. These results implied substantial modification of key biological target(s) including DNA repair proteins and transcription factor known to be inhibited by NO.

In 2007 the conference ''Nitric Oxide and Cancer" brought into perspective the widely diverse properties of NO in cancer. In the last 10 years the combination of molecular and redox biology techniques has shed light on these diverse mechanisms. The conference was a long overdue gathering that presented numerous studies demonstrating how NO is integrated into many aspects of cancer. This conference identified molecular mechanisms involving NO chemistry, its interaction with different molecular motifs, and the significance of NO concentration in cancer outcome.

The reviews in this issue represent the range of actions of nitric oxide in cancer and offer insight into the dichotomous nature of NO. As a larger picture emerges and new mechanisms of NO in cancer are discovered, it has become evident that the devil is in the details. Nitric oxide's biological determinant involves the chemistry of the intermediates formed, their molecular targets, and the subsequent influence on cellular, immunological, and physiological functions. Therefore NO location, concentration, and timing placed in context with the tumor microenvironment are imperative in the development of novel strategies for cancer treatment and prevention.

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