COMMENTARY

NO and COX2: Dual targeting for aggressive cancers

Daniel Davila-Gonzalez*, Jenny C. Chang*, and Timothy R. Billiar*,1

A number of tumor-specific characteristics are known to associate with a cancer’s growth, metastatic potential, and response to therapy. Among these are mutational load and gene-expression patterns in the tumor cells and the interaction of the tumor cells with the many cellular and noncellular components that comprise the microenvironment of the tumor. One molecule that has received considerable attention in tumor biology is nitric oxide (NO), a short-lived locally acting but highly diffusible intra- and intercellular signaling molecule (1, 2). This attention is well warranted because NO can regulate almost every process important to carcinogenesis, including DNA damage/repair, cell proliferation, blood flow/angiogenesis, cell migration and invasion, cell death and survival, inflammation, and immune responses (3). However, nearly three decades of intense mechanism-based research has yielded a large number of conflicting results on the even basic question of whether NO is pro- or antineoplastic (1). This has led many to conclude that the role of NO in tumor biology is highly context-dependent and determined by the sources of NO, the level and duration of NO production, and the background phenotype of the tumor. However, an emerging theme with some consistency in human cancers is that the expression of the high-output inducible NO synthase (iNOS or NOS2) associates with poor outcome in many solid and hematological malignancies. For example, NOS2 can act as a poor prognostic indicator for survival in many aggressive cancers, including glioblastoma, melanoma, pancreatic, liver, esophageal, gastric, cervical, ovarian, prostate, and lung adenocarcinoma, with hazard ratios (HR) greater than threefold in most of these cancers (4–9). Similarly, NOS2 has been shown to be a predictor of poor survival in estrogen receptor-negative (ER−) breast cancer patients (10), especially in the subset of highly aggressive chemoresistant metaplastic breast cancers (11). Although expression of NOS2 in human macrophages is limited compared with rodents, the expression of NOS2 in human epithelial cells can be robust, which lead to its initial cloning and characterization in human hepatocytes (12). This may explain, in part, its prevalence in aggressive human epithelial malignancies.

*Houston Methodist Cancer Center, Houston Methodist Hospital, Houston, TX 77030; and †Department of Surgery, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261


The authors declare no conflict of interest.

Published under the PNAS license.

See companion article on page 13030 in issue 49 of volume 114.

1To whom correspondence should be addressed. Email: billiartr@upmc.edu.

www.pnas.org/cgi/doi/10.1073/pnas.1717440114

PNAS | December 26, 2017 | vol. 114 | no. 52 | 13591–13593
However, no trials have been reported to establish if NOS2 inhibition has therapeutic benefit.

In PNAS, Basudhar et al. (13) provide additional evidence that tumor-specific factors—and specifically the coexpression of the eicosanoid-producing enzyme, cyclooxygenase-2 (COX2)—in ER− and triple-negative breast cancers (TNBC) associate with poor prognosis. The authors studied 248 human ER− breast cancers and found NOS2 to be the strongest predictor of poor outcome in a multivariable analysis, adjusted for age, tumor grade, TNM (tumor, node, and metastasis) stage, chemotherapy, and tumor p53 mutation status. Whether COX2 alone predicted poor survival could not be established because so few patients had tumors that were NOS2-low and COX2-high, and this raised the possibility that the expression of COX2 is mechanistically linked to NOS2 expression in aggressive breast cancers. In fact, interaction analysis identified a highly significant impact of NOS2 and COX2 interaction on patient survival. Patients whose tumors expressed high levels of both NOS2 and COX2 had dismal outcomes (HR >21), with only one-third of patients alive at 5 y compared with over 95% survival in patients with low NOS2 and COX2 expression.

Others have linked COX2 to aggressive cancers (14, 15). ER− breast cancer cells that express high levels of COX2 show increased levels of the eicosanoid, prostaglandin E2 (PGE2), and this has been positively correlated with tumor aggressiveness and an increased risk of metastasis (16). The relationship between NOS2 and COX2 has also been previously observed in other tumor types, including nonsmall cell lung cancer (14) and hepatocellular carcinoma (17); however, the mechanistic bases for the NOS2/COX2 synergy in cancer has not been established. Basudhar et al. (13) describe the mechanisms responsible for the NOS2/COX2 cross-talk that underlie the aggressive phenotype in subtype-specific ER− breast cancer. Tumor subtype-specific (mesenchymal/claudin-low Basal B MDA MB-231 or epithelioid-like Basal A MDA MB-468) interaction involving TNF−α and endoplasmic reticulum (EnR) stress were described as key players in an autocrine loop (Fig. 1). In vitro, NO stimulation increased PGE2 levels, an effect that was blocked by pharmacological inhibition of COX2; furthermore, PGE2 induced NOS2 expression, suggesting cross-talk between both inflammatory pathways. NOS2 has been associated with the production of IL-6 and IL-8 (18, 19), which have been linked to the maintenance and survival of cancer stem cells via activation of the STAT3 pathway. In both cell lines, NO stimulation activated membrane TNF−α and increased intracellular pTRAF2. In contrast, the NO-mediated increase in TRAF2 activation, via the EnR stress pathway through IRE1α, was observed only in the Basal B MDA MB-231 cell line.

An important aspect of NO signaling is that it forms a network of feed-forward loops, promoting multiple oncogenic pathways that involve cancer, immune, and stromal cells. NOS2 and COX2 modulate the tumor immune microenvironment: NOS inhibition has been shown to improve response to anti-PD1 therapy in ER− breast cancer models (20), while COX2 inhibition decreases myeloid-derived suppressor cells (21), and reverses IDO1-mediated immunosuppression in multiple cancers (22, 23). One of the most encouraging findings of Basudhar et al. (13) is the discovery that simultaneous inhibition of NOS2 and COX2 using aminoguanidine and aspirin significantly reduced human breast cancer growth in a xenograft murine model, suggesting that dual targeting of NOS2/COX2 may be therapeutically beneficial. However, it would be important to show in future studies that a more selective NOS2 inhibitor (especially one of those already safely tested in humans) and selective COX2 inhibitors are as effective before concluding that the benefits in vivo were due solely to NOS2 and COX2 inhibition. This point is also emphasized by the authors’ own observations that a second nonselective COX2 antagonist (indomethacin) did not add to the increased survival observed with NOS2 inhibition alone in the xenograft model. However, taken together these results are particularly timely, as nonspecific NOS inhibitors are currently being evaluated in clinical trials as a first-in-class cancer therapeutic in metastatic TNBC (24).

It is also worth noting that NO can be derived from a number of sources in the tumor microenvironment other than the tumor cells (Fig. 1). In addition to NOS2, the constitutive NO synthases, including NOS1 (also known as neuronal NOS) and NOS3 (also known as endothelial NOS) have been linked to cancer progression (2). NO can also be formed from reduction of the stable end product of NO metabolism, nitrite, under acidic conditions. Nontumor cell types within the tumor microenvironment can also express NOS, including NOS2, and these include stromal cells and immune cells. These sources of NO formation would also be targeted with systemic delivery of a NOS2 inhibitor, as used in the Basudhar et al. (13) study. The use of nonselective NOS inhibitors as described in the current first-in-class trials (20) may have distinct advantages by targeting all three NOS enzymes in both tumor and stromal cells. However, it is also possible that suppression of endothelial NOS in some tumors could induce abnormalities in tumor vessels, leading to hypoxia and aggressive tumor growth.

However, what Basudhar et al. (13) suggest to us is that in the context of a specific tumor genotype (ER− breast cancer), the identification of high NOS2 and COX2 in the tumor might be used to personalize the testing of a combination of already available targeted therapies for a subset of aggressive breast cancers. This study also points to the importance of understanding the interactions between NOS2 and COX2, with respect to conditions in the tumor microenvironment, and provides a foundation to better understand these niche interactions, thus exposing the vulnerabilities of feed-forward loops.