Breast Cancer RESEARCH

EDITORIAL

S-nitrosylation of Ras in breast cancer

Harvey E Marshall and Matthew W Foster*

See related research by Switzer *et al.*, http://breast-cancer-research.com/content/14/5/R125

Abstract

Elevated expression of nitric oxide synthase 2 has been recently shown to correlate with poor survival in estrogen receptor-negative breast cancer. In an article in *Breast Cancer Research*, Switzer and colleagues identify the transcription factor Ets-1 as a critical mediator of nitric oxide-dependent oncogenic gene expression in basal-like breast cancer. This pathway is driven by S-nitrosylation of wild-type Ras, which leads to mitogen-activated protein kinase-dependent phosphorylation and activation of Ets-1. These results establish a new role for S-nitrosylation in mediating an aggressive breast cancer phenotype.

Unlike the constitutive nitric oxide (NO) synthase isoforms, the inducible isoform of NO synthase – nitric oxide synthase 2 (NOS2) – is capable of producing sustained intracellular levels of NO, and it is increasingly appreciated that protein S-nitrosylation, the covalent modification of cysteine thiol by NO, is important for NOS2-dependent signal transduction.

In the previous issue of *Breast Cancer Research*, Switzer and coworkers delineate a novel pathway for S-nitrosylation in regulation of estrogen receptor (ER)-negative breast cancer invasion [1]. This work adds to the growing appreciation that S-nitrosylation can regulate myriad pathways important for tumorigenesis, including gene transcription, apoptosis and DNA repair [2-4], and it complements another recent study by these coworkers showing that S-nitrosylation and activation of epidermal growth factor receptor is associated with the induction of epithelial-to-mesenchymal transition as well as chemoresistance in ER-negative breast cancer [5]. More generally, these data begin to explain why expression of NOS2 correlates with aggressive tumor phenotypes and poor clinical outcomes in a variety of malignancies, including breast cancer, lung cancer, colon cancer and prostate cancer [6-9].

The small GTPase Ras was one of the earliest described regulatory targets of S-nitrosylation. Modification of a single cysteine residue that is located in the nucleotide-binding region of wild-type Ras and is conserved among all Ras isoforms (Cys118 in human H-Ras) stimulates guanine nucleotide exchange and downstream pathways, including activation of mitogen-activated protein kinase signaling [10]. S-nitrosylation of wild-type Ras by endothelial NO synthase has been shown to promote pancreatic tumor growth [11]. Switzer and colleagues identify a role for S-nitrosylation of wild-type Ras in ER-negative breast cancer [1]. They find that the only elements common to genes upregulated in high NOS2-expressing breast cancer are binding sites for the Ets-1 transcription factor. Using a model ER-negative breast cancer cell line, they further show that NO induces S-nitrosylation of wild-type Ras, leading to phosphorylation and activation of Ets-1 through the Ras/MEK/ERK pathway. Knockdown of Ets-1 inhibits the NO-dependent expression of basal-like breast cancer markers and attenuates NO-dependent cancer cell invasion. These findings delineate a mechanism by which NOS2 promotes an aggressive tumor phenotype in ER-negative breast cancer.

While NOS2 expression is high in ER-negative tumors [9], it is not constitutively expressed in the triple-negative breast cancer cell line MDA-MB-468 (lacking ER, progesterone receptor and epidermal growth factor receptor 2) that was utilized in this and related studies [1,5]. Indeed, the loss of NOS2 expression in cultured tumor cells *ex vivo* is not uncommon and could reflect a deficiency in cytokine signaling and/or an inability to replicate the hypoxic tumor environment. Regardless of mechanism, this deficit in NOS2 expression can make challenging the study of the (patho)physiological functions of the enzyme in cell culture systems. To model NOS2-based signaling as it occurs in the solid tumor, Switzer and colleagues employ a long-lived NO donor as well as adenoviral expression of NOS2 [1]; in earlier work, they also co-cultured these cells with macrophages stimulated to produce high levels of NOS2-derived NO [5]. While these conditions may adequately recapitulate pathophysiological

*Correspondence: mwfoster@duke.edu
Division of Pulmonary, Allergy and Critical Care Medicine, Duke University Medical Center, Durham, NC 27710, USA

NO levels within the tumor, further work is needed to determine the extent to which these manipulations fully reconstitute NOS2-dependent signal transduction. These questions may in part be addressed by defining the precise mechanisms underlying enhanced expression of NOS2 in breast cancer and other malignancies. In addition to the environmental factors of cytokine secretion and hypoxia, aberrant regulation of NOS2 transcription, mRNA stability, and proteasomal degradation in the tumor cells present potential mechanisms.

There is increasing recognition that denitrosylases – in particular, S-nitrosoglutathione reductase (GSNOR) and thioredoxin (Trx) – are critical modulators of S-nitrosothiol (SNO) homeostasis [12]. As the name implies, GSNOR metabolizes S-nitrosoglutathione, a small molecule intermediate in S-nitrosylation and denitrosylation. This enzyme may have general housekeeping functions, one of which is to protect against indiscriminate protein S-nitrosylation (nitrosative stress) that may occur as a result of excessive NOS2 activity. In this regard, GSNOR deficiency has been tied to inhibition of DNA repair and to hepatocellular carcinoma [3], and the enzyme is also deficient in some lung cancers [13]. Trx reduces protein SNOs directly, and inhibition of thioredoxin reductase (TrxR) can increase protein SNOs by blocking Trx turnover. Trx has another major role as a modulator of oxidative stress, and it is in this capacity that chemotherapeutics targeting TrxR are believed to affect cancer cell killing. Given the potential for protein S-nitrosylation to stimulate tumor growth and invasion, TrxR inhibitors may have unexpected deleterious effects. More generally, the balance of SNO formation/degradation is clearly an important factor in malignant cell transformation and thus warrants further investigation.

Given the potential role of NOS2 in tumor chemoresistance and aggressiveness, it is important to note that NOS2 inhibitors have had some success in inhibiting tumor growth [14,15]. At the same time, interventions that increase SNO levels, either through application of NO donors and low-mass SNOs or through inhibition of SNO-metabolizing enzymes, are being proposed as therapeutics in numerous diseases [16]. Given recent findings in the cancer field, it may be necessary to weigh the risk–benefit of therapies designed to increase protein S-nitrosylation for those individuals at high risk of developing cancer.

Abbreviations

ER, estrogen receptor; GSNO, S-nitrosoglutathione reductase; NO, nitric oxide; NOS2, nitric oxide synthase 2; SNO, S-nitrosothiol; Trx, thioredoxin; TrxR, thioredoxin reductase.

Competing interests

The authors declare that they have no competing interests.

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