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EDITORIAL FOCUS

S-nitrosylation/denitrosylation regulate myoblast proliferation. Focus on "Balance between S-nitrosylation and denitrosylation modulates myoblast proliferation independently of soluble guanylyl cyclase activation"

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MYOGENESIS INVOLVES A COMPLEX SERIES of signaling events that will result in the formation of muscle fibers (3). The participation of nitric oxide (NO) in myogenesis has been evidenced over the years. Most of the endogenous production of NO in skeletal muscle comes from the neuronal isoform of the NO synthase (nNOS) (5). Sub cellular compartmentalization of nNOS is essential to enable site specific generation of NO and signaling through cGMP production and s-nitrosylation of proteins in skeletal muscle (8). It was earlier suggested that in skeletal muscle, nNOS interacts with two domains of caveolin-3 which is part of caveolae an invagination of the sarcolemma. At the caveolae nNOS and caveolin-3 forms a signaling complex through additional interactions with Src kinase and p21Ras (8).

Specific NO targets in skeletal muscle development include the ryanodine receptor calcium release channel (Ryr) and soluble guanylyl cyclase (sGC). Xu et al. (9) showed that Ryr purified from canine hearts was endogenously *S*-nitrosylated. In myoblasts, the mechanisms by which NO acts during the transition between myoblast proliferation and fusion have been reported to be regulated by a transient generation of cGMP (4). Most importantly, sGC activity potentially is down regulated by s-nitrosylation (6). S-nitrosylation which is characterized by the covalent attachment of a NO group to a Cys thiol side chain, regulates the cellular responses mediated by ligand-gated ion channels, G protein coupled receptors, cytokine receptors and receptor tyrosine kinases. In the context of signal transduction, reversibility of S-nitrosylation is achieved through denitrosylation of GSNO and s-nitrosylated proteins which is carried out by two specific enzymatic systems: the Thioredoxin/Thioredoxin reductase system and the GSH/GSNO reductase (GSNOR) system (1).

GSNO is the most physiologically relevant s-nitrosothiol (SNO) initially isolated in human airways. GSNO is formed in cells by the reaction between NO (or another SNO) with

GSH, or through GSH-mediated denitrosylation of S-nitrosylated proteins and other cellular SNOs. The metabolism of GSNO is carried out by the GSNOR, an evolutionary conserved and widely expressed enzyme in prokaryotic and eukaryotic organisms. GSNOR specifically catalyses the denitrosylation of GSNO. Nevertheless, studies using GSNOR knockouts in yeast and in mammalian cells clearly indicated that GSNO is in equilibrium with S-nitrosylated proteins (1).

In this issue of the American Journal of Physiology-Cell Physiology, Yamashita and coworkers convincingly demonstrate that in primary cultures of chick embryonic skeletal muscle cells, the effects of NO on myogenesis are importantly mediated by s-nitrosylation/denitrosylation (10). Another important conclusion drawn from the studies was the role played by nNOS as the solely NO source in myogenesis. Together with GSNOR, nNOS activities were shown to determine the intracellular SNO levels. Indeed, pharmacological inhibition or knocking-down of GSNOR on myoblasts promoted an increase on the SNO levels during myogenesis. On the other hand, inhibition of nNOS strongly inhibited the generation of SNO in myoblasts. Increasing intracellular SNO levels during myogenesis stimulates cell proliferation in both myoblasts and fibroblasts, and diminishes myoblast fusion. Importantly, authors also demonstrate that myoblast fusion only occurred after increase on GSNOR activity. In addition, by stimulating myoblasts with CysNO, Yamashita et al (10) were able to recapitulate the pro-proliferative effects derived from the enhanced intracellular SNO levels. Most important, Yamashita et al (10) showed for the first time that NO-stimulated myoblast proliferation occurs without the participation of cGMP. However, fibroblasts behaved like other cells and cGMP levels have to rise for SNO-induced proliferation to proceed. Yamashita et al (10) also demonstrated that an increase on the intracellular SNO pool drives myoblast proliferation but not fusion. Their findings clearly

indicate that optimal intracellular SNO levels have to be maintained to regulate proliferation of myoblasts and their differentiation to myotubes.

The use of primary cultures of skeletal muscle progenitor cells from chick embryos by Yamashita et al (10) provides an environmental condition that is similar to the *in vivo* condition. These primary cultures contain both myoblasts and fibroblasts which proliferate in different phases of myogenesis. Myoblasts proliferate before they withdraw from the cell cycle to fuse, forming myotubes, whereas fibroblasts will continuously proliferate (3). Although the data presented support a major role for NO and s-nitrosylation as key regulators of myoblast proliferation, some questions related to the signaling pathway associated with the NO/SNO-driven proliferation of myoblasts, remain unanswered. The first question was asked by the authors when they suggested that p21Ras could be trans-nitrosylated and activated by GSNO, triggering cell cycle progression and proliferation (7). Second, could s-nitrosylation and activation of Src kinase be important to the overall mechanism of NO/SNO-induced proliferation of myoblast? S-nitrosylation of Src kinase was earlier shown to be important for the assembling of the focal adhesion complex in mouse fibroblasts, stimulating their proliferation (2). S-nitrosylated Src and p21Ras, nNOS, and caveolin-3 (2,7,8), could be organized as a signaling complex that is associated with myoblast proliferation. A general scheme of this putative signaling complex is shown in Fig. 1.

In summary, Yamashita et al. presented compelling evidence that manipulation of GSNOR activity with consequent modulation of the intracellular s-nitrosylation levels in myoblasts is important for myoblast proliferation and fusion. These findings may have consequences on the promotion of muscle repair and prevention of skeletal muscle wasting.

AUTHOR CONTRIBUTIONS

HPM and FTO drafted and revised manuscript; HPM and FTO prepared figure.

DISCLOSURES

No conflict of interest, financial or otherwise, are declared by the authors.

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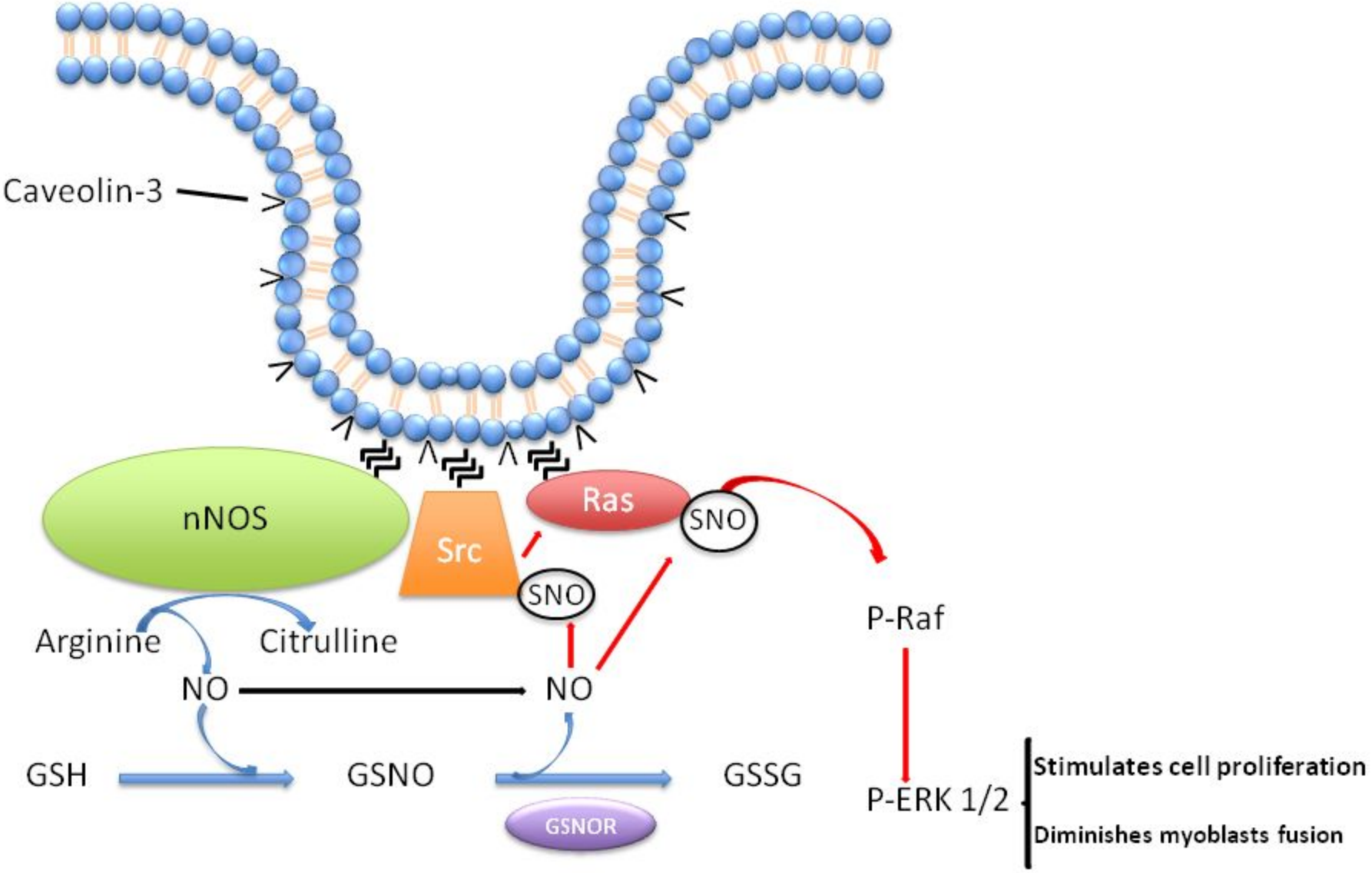
Figure Legend

Fig.1: A schematic overview of a proposed signaling pathway associated with myoblast proliferation. In caveolae, caveolin-3, nNOS, Src kinase, and p21ras form a signaling complex. Neuronal NOS (nNOS) generates NO and NO nytrosilates GSH, and the signaling proteins Src kinase e p21Ras. S-nitrosylation of Src kinase and p21Ras may also occur through trans-nitrosylation mediated by GSNO and modulated by GSNO reductase. S-nitrosylation of Src kinase and p21Ras activate them and promote their engagement in a signaling pathway that results in stimulation of myoblast proliferation and diminishing myoblast fusion.


Caveolin-3
nNOS
Src
Ras
SNO
SNO
Arginine
Citrulline
NO
NO
P-Raf
GSH
GSNO
GSSG
GSNOR
P-ERK 1/2
Stimulates cell proliferation
Diminishes myoblasts fusion