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Nitric Oxide's Role in Mechanically-Stimulated Sclerostin Degradation

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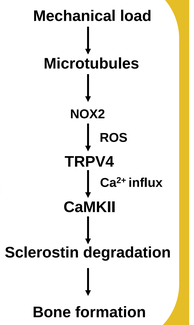
INTRODUCTION

- Nitric oxide (NO) is a secondary messenger produced by osteocytes in response to mechanical stimulation.[1]
- The three isoforms of NOS (nitric oxide synthase) are distributed across many cell types and work under varying conditions and cues to produce NO from L-Arginine.[2]
- NO's impact and position in the mechano-response pathway controlling sclerostin has not been definitively established.



AIM

- Determine the position and role of NO in the mechano-pathway controlling sclerostin protein.
- Determine which NOS isoform(s) are most relevant to sclerostin control in response to mechanical strain.

RESULTS

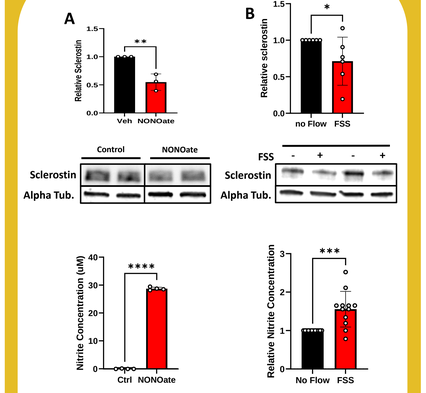


Fig 1. NO donation and FSS (fluid-shear stress) are each sufficient to induce sclerostin degradation and nitrite production. (A) Nitric oxide donation (NONOate, 10μM, 5min) or **(B)** FSS (4 dynes/cm^2, 5min) were applied to mycSOST transfected OCY454s.

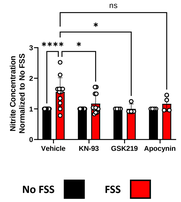


Fig 2. Mechano-activated NO production is downstream of several mechano-pathway elements. FSS (4 dynes/ cm^2, 5min) was sufficient to increase nitrite production by OCY454 cells. Inhibition of CamKII (KN-93, 10μM, 1hr), TRPV4 channels (GSK219, 15μM, 10min), or NOX2 (Apocynin, 500μM, 1hr) prevented the production of nitrite with FSS.

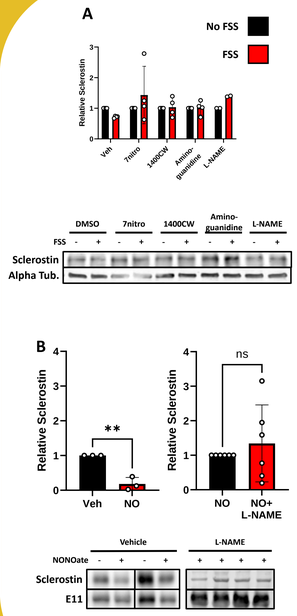


Fig 3. Pharmacological inhibition of NOS isoforms prevents the degradation of sclerostin following FSS. (A) Sclerostin abundance is decreased with FSS (4 dynes/ cm^2, 5min). Inhibition of nNOS (7nitro, 5μM, min), iNOS (1400CW, 1μM, 30min) (Aminoguanidine, 250μM, 30min), or non-specific NOS inhibition (L-NAME, 1mM, 30min) each prevent the loss of sclerostin protein in OCY454 cells. **(B)** NO donation (NONOate, 10μM, 3min) drives the degradation of sclerostin in C56BL/6 murine long bones, while non-specific NOS inhibition (L-NAME, 1μM, 30min) blunts the degradation of sclerostin in the presence of NONOate (10μM, 15min).

Fig 4. Knockdown of specific NOS isoforms prevents the degradation of sclerotin. mycSOST and scramble or NOS isoform-specific siRNA co-transfected OCY454 cells were subjected to FSS (4 dynes/cm^2, 5min). iNOS and eNOS siRNAs prevented the degradation of sclerostin. In contrast, nNOS did not affect the rapid loss of sclerostin protein.

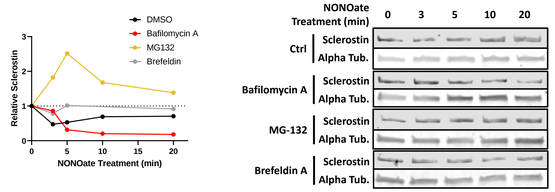


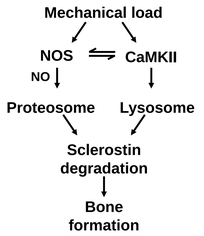
Fig 5. The NO-driven degradation of sclerostin may be mediated by the proteasome. OCY454 cells were treated with a protein synthesis inhibitor (cycloheximide, 150μg/ml, 4hr) and with inhibitors of the lysosome (bafilomycin A, 100nM, 1hr), proteasome (MG-132, 10μM, 1hr) or vesicle formation (brefeldin A, 2μM, 1hr) prior to NO donation (NONOate, 10μM, 5min).

METHODS

- In vitro: OCY454 osteocyte-like cells
- Ex vivo: C57BL/6 WT mice long bones (soft tissues removed and marrow flushed)
- Fluid-shear stress (FSS) applied via custom peristaltic pump system[3,4]
- Nitrite concentration calculated via Griess assay
- Statistics: data analyzed using t-test (two-tailed) or two-way ANOVA (Holm-Sidak post-hoc). Values represented as mean ± SD. *=p<0.05, **=p<0.01,***=p<0.001, ****=p<0.0001

CONCLUSIONS

- Exogenous nitric oxide (NO) is sufficient to stimulate the degradation of sclerostin protein. Non-specific pharmacologic inhibition of nitric oxide synthases prevents the FSS induced degradation of sclerostin protein.
- eNOS and iNOS appear to be most biologically relevant effectors of NO's-impact on sclerostin in osteocyte-like cells. Further investigation using primary murine osteocytes, ex vivo and in vivo models with selective NOS isoform deletion or knockdown will be necessary for confirmation.
- Nitric oxide production appears downstream of NOX2-TRPV4 and CaMKII activation
- Nitric oxide may drive the degradation of sclerostin via the proteasome in a pathway parallel to the calcium-mediated, lysosome-driven crinophagy pathway.
- The temporal relationship between FSS degradation of sclerostin via the lysosome[5] and NO's apparent effect on sclerostin degradation via the proteasome remains to be clarified.



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