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Impaired Hypoxic Response
Increased in Ischemic Myoblasts as a Response to Impaired Hypoxic Response
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Background: Angiotsentin-converting enzyme 2 (ACE2), the pulmonary epithelial receptor for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), plays a significant role in attenuating muscle fibrosis in dystrophic muscle models. Evidence suggests ACE2 is downregulated in prolonged hypoxia by hypoxia-inducible factor 1-alpha (HIF1-α). We hypothesize that myoblasts affected by chronic ischemia would have attenuated ACE2 expression, potentially modifying the detection of and response to SARS-CoV-2 infection in muscle tissue. This holds an important impact for peripheral arterial disease (PAD) patients who are also at risk for severe coronavirus disease 2019 (COVID) infection based on age.

Methods: Cells were harvested from ischemic and perfused muscle during lower extremity amputations and bypasses in PAD patients. Myoblasts (Pax7- /MyoD+) were isolated using preplating technique and cell sorting. Commercially available healthy donors myoblasts (PAD−) were purchased (Cook MyoSite). All experiments were performed in normoxic (20% O2) and hypoxic (1% O2) conditions. ACE2 expression was quantified via immunofluorescence staining after five days differentiation. HIF-1α ELISAs (Elabscience) were performed on cell lysates after 24 hours of proliferation. Cell lysis in response to exposure to COVID spike protein (RayBiotech) was assessed using lactate dehydrogenase assays (Invitrogen). Analysis of variance with post hoc analysis confirmed statistical significance (α = 0.05).

Results: Hypoxia exposure induced significant increase in HIF1-α expression in perfused myoblasts (P < 0.05). Hypoxia also increased ACE2 expression in ischemic (n = 6) compared to perfused (PAD−; n = 2; perfused PAD; n = 5) [P < 0.05] myoblasts (Figure A). Lactate dehydrogenase concentration suggestive of cell lysis and cytotoxicity was higher in perfused than ischemic myoblasts. Myoblasts from perfused muscle also had higher cell lysis from COVID spike protein exposure while ischemic cells exposed to COVID spike proteins seemed to survive (Figure B).

Conclusions: Chronically ischemic myoblasts from PAD muscle increased ACE2 expression in response to additional hypoxia. This may suggest greater susceptibility of ischemic muscle to SARS-CoV-2 effects in the setting of additional hypoxic insults like pneumonia. While cytotoxicity was not a feature of spike protein exposure in ischemic PAD cells, this might suggest cell survival in the setting of viral infection, which is unfavorable. Further research is needed to understand whether cell survival mechanisms exist in PAD myoblasts exposed to COVID infection.

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