Effects of Moderate Versus Severe Hypoxic Exposure on Myoblast Autophagy

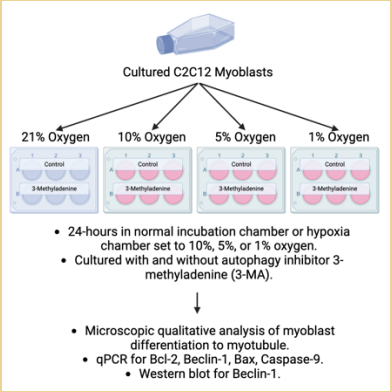
Joseph Tomecki, Megan Moore, Sheridan Hayes, Yong Li, MD, PhD

Department of Surgical Sciences, Western Michigan University Homer Stryker MD School of Medicine, Kalamazoo, MI

INTRODUCTION

- Moderate oxygen deprivation, defined as 5% to 20% oxygen, generally enhances muscle cell survival and differentiation. In contrast, severe oxygen deprivation of <3% oxygen can induce muscle atrophy and reduced protein production in some conditions, especially in cells exposed to hypoxic stress for extended periods of time. The mechanisms by which varying levels of oxygen deprivation influence intracellular pathways to alter cell growth remain poorly characterized.[1,2]
- Autophagy, a cellular process of engulfing cellular contents to recycle proteins and organelles, offers a mechanistic explanation for muscle cell survival under moderate hypoxia by facilitating the degradation of damaged proteins and organelles that would otherwise trigger apoptotic cell death.
- We hypothesize that 24-hour exposure to 5-10% oxygen upregulates autophagy, supporting cell survival and differentiation, while extended exposure to 1% oxygen begins to overwhelm autophagic flux, leading to increased apoptosis.

METHODS



RESULTS

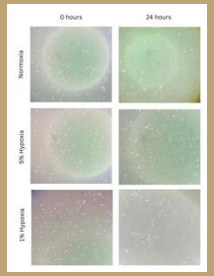
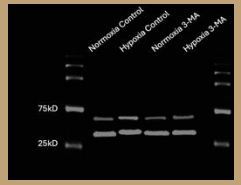


Figure 1: C2C12 cultured for 24 hours in 21% oxygen (normoxia), 5%, and 1% oxygen.



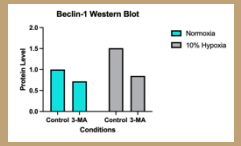
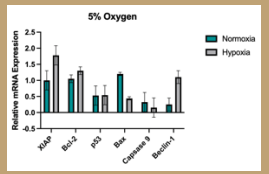


Figure 2: Western blot (above) and quantification (below) for Beclin-1 in C2C12 myoblasts. Increased Beclin-1 protein is seen in samples cultured in 10% hypoxia and decreased Beclin-1 is found in samples cultured with 3-methyladenine for both normoxia and hypoxia conditions.



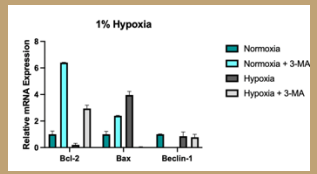
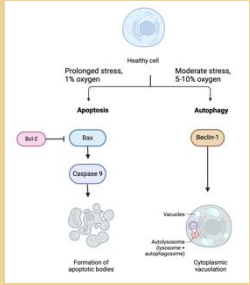


Figure : C2C12 cells cultured for 24 hours in 5% or 1% oxygen (Hypoxia) and 21% oxygen (Normoxia). Bcl-2 expression is increased in cells treated with 3-MA both in normoxia and hypoxia. Beclin-1 is increased in 5% oxygen but not 1% oxygen.

CONCLUSION

- C2C12 cells cultured under 5% oxygen exhibited increased Beclin-1 expression compared to those grown in 21% oxygen.
- In contrast, cells cultured in 1% oxygen showed reduced Beclin-1 protein by Western blot and reduced Beclin-1 mRNA by qPCR.
- Cells treated with 3-MA under moderate or severe hypoxia expressed similar Beclin-1 levels to normoxia, suggesting that autophagy may be involved in changes seen with hypoxia.



- Moderate hypoxia stimulates increased markers of autophagy and decreased markers of apoptosis.
- These findings may explain the beneficial effect of moderate hypoxia in myoblast survival. In contrast to moderate hypoxia, severe hypoxia at 24 hours may surpass the protective capacity of autophagy, leading to apoptosis and muscle atrophy, as seen by the increase in apoptotic marker Bax and lack of increase in autophagy marker Beclin-1.

ACKNOWLEDGEMENTS

Dr. Yong Li lab materials, support, and funding.

REFERENCES

1. Chaillou T, Lanner JT (2016) Regulation of myogenesis and skeletal muscle regeneration: Effects of oxygen levels on satellite cell activity. FASEB Journal 30:3929–3941. https://doi.org/10.1096/FJ.201600757R
2. Sakushima K, Yoshikawa M, Osaki T, et al (2020) Moderate hypoxia promotes skeletal muscle cell growth and hypertrophy in C2C12 cells. Biochem Biophys Res Commun 525:921–927. https://doi.org/10.1016/J.BBRC.2020.02.152