ELUCIDATING THE ROLE OF BRCA1 IN SKELETAL MUSCLE MYOGENESIS AND FUNCTION

by

Ananya Koripella

A Senior Honors Project Presented to the

Honors College

East Carolina University

In Partial Fulfillment of the

Requirements for

Graduation with Honors

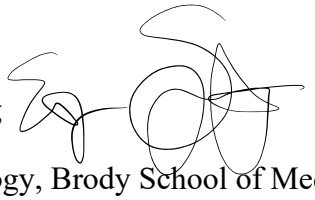
by

Ananya Koripella

Greenville, NC

May 8, 2020

Approved by:



Dr. Espen Spangenburg

Department of Physiology, Brody School of Medicine

East Carolina Diabetes & Obesity Institute

Abstract

The breast cancer 1 early onset gene (BRCA1) is a tumor suppressor gene susceptible to mutations associated with cancer development. We have identified that the BRCA1 gene is expressed in skeletal muscle and plays a critical role in regulating muscle function, in part through maintaining DNA integrity. In this project, we hypothesized that BRCA1 content is necessary for myogenesis, an essential process for skeletal muscle development and repair following injury. Development and repair of skeletal muscle is mediated by stem cells committed to the myogenic lineage (i.e. satellite cells) undergoing myogenesis. While it is understood that DNA damage is an unavoidable consequence of myogenesis, the mechanisms of satellite cell DNA repair are unknown. Here, we hypothesized that BRCA1 expression is necessary in satellite cells to repair DNA to ensure appropriate development and repair of skeletal muscle.

To test this hypothesis, we employed three novel mouse models, where BRCA1 content was either overexpressed or ablated in the skeletal muscle or satellite cells. To induce myogenesis, the anterior compartment of a lower limb was injured with an injection of 1.2% $BaCl_2$. The contralateral limb was injected with the same volume of sterile PBS. One week after injections, the skeletal muscles were removed, and the muscle quality was assessed. Myofiber size (i.e. cross-sectional area) was measured in the tibialis anterior (TA) muscles to determine the ability of the muscle to recover from injury when the satellite cells either overexpress or lack BRCA1. In addition, the number of cells with central nucleation was determined as a marker of pathology within the muscle. The data shows that the overexpression of BRCA1 in the skeletal muscle provides no improvement in skeletal muscle quality after an acute injury. However, the overexpression of BRCA1 in the satellite cells leads to larger muscle cells after the same insult and smaller muscle cells when BRCA1 expression is lost from satellite cells. In conclusion, the

results suggest that a known DNA repair protein, BRCA1, is critical for satellite cell-induced repair of skeletal muscle after an acute injury.

Introduction

The breast cancer 1 early onset gene (BRCA1) is a tumor suppressor gene that is responsible for DNA repair. The BRCA1 gene is susceptible to mutations which are associated with an increased risk of cancer development. We have identified that the BRCA1 gene is expressed in skeletal muscle and plays a critical role in regulating muscle function by preventing the accumulation of DNA damage in nuclei and mitochondria. We also previously determined that the loss of BRCA1, specifically in skeletal muscle, results in mice developing weakened skeletal muscles and a decreased mitochondrial efficiency. The development and repair of skeletal muscle requires stem cells committed to the myogenic lineage (i.e. satellite cells) to undergo myogenesis (Bentzinger *et al.* 2012). DNA damage is unavoidable in myogenesis (Puri *et al.* 2002), yet the mechanism for DNA repair in satellite cells is unknown. In addition, BRCA1 expression increases in satellite cells as they enter myogenesis, suggesting that BRCA1 signaling may be critical for DNA repair.

Skeletal muscle stem cells, i.e. satellite cells, make up approximately 2-10% of myonuclei in skeletal muscle fibers (Dumont *et al.* 2015). In normal adult activity, they usually remain dormant. However, following an injury or a growth stimulus, satellite cells will become activated in order to contribute to a necessary and critical part of the repair process. Once activated, satellite cells commit to the myogenic lineage, becoming and differentiating to either form new myofibers or repair existing myofibers. Satellite cells are crucial for the continual growth, maintenance and repair of skeletal muscles throughout the life span (Dumont *et al.* 2015). Reduction in satellite cell function early in life leads to poorly developed skeletal muscle

(Bachman *et al.* 2018), thus optimal satellite cell dynamics is critical to the establishment of optimally functioning skeletal muscle.

Satellite cell dysfunction leads to poor skeletal muscle function and quality. Loss of muscle quality significantly increases the risk of requiring assisted living, causing an increase in mortality (Brown *et al.* 2016). Thus, it is critical to identify key regulators of cellular function that are necessary for satellite cell maintenance to create interventions that prevent loss of muscle quality in people. This study will focus on determining if BRCA1 expression is necessary in satellite cells during myogenesis induced by acute muscle injury. Overall, we will phenotype the skeletal muscle of mice with varying levels of BRCA1 expression to determine if manipulation of BRCA1 signaling affects muscle recovery from an acute injury.

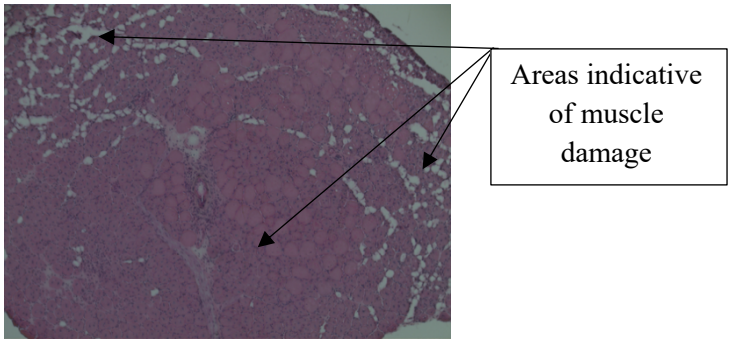
Methods

Using three novel mouse models, we assessed BRCA1's impact on myogenesis. In the first model, BRCA1 was deleted from satellite cells ($BRCA1^{KOSC}$) in skeletal muscle and in the second, BRCA1 was overexpressed ($BRCA1^{R26SC}$) in the satellite cells. The third model over-expressed BRCA1 in the general skeletal muscle ($BRCA1^{R26SM}$). Using age-matched $BRCA1^{KOSC}$, $BRCA1^{R26SC}$, $BRCA1^{R26SM}$ mice and age-matched control mice (wild-type (WT)), we phenotyped skeletal muscle after an acute injury induced by $BaCl_2$ to determine if muscle recovery had been affected by variations in BRCA1 expression. All procedures were approved by the ECU IACUC committee.

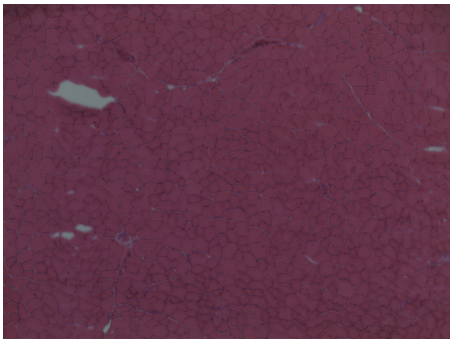
After administering isoflurane-induced anesthesia, the $BaCl_2$ model of mouse muscle injury was performed using 20-µL intramuscular injections of 1.2% $BaCl_2$ solution into the TA muscle of anesthetized mice. An equivalent volume sham injection of 1× PBS was also

administered to the muscles of the contralateral hind limb. The animals were returned to their cages and allowed to recover. The skeletal muscles were then isolated 7 days after the injection to determine the extent of injury using histological analysis.

After the 7 days, the mice were euthanized using an overdose of isoflurane. Once isolated, the TA muscles were flash frozen in optimal cutting temperature (OCT) solution for sectioning and staining. The TA muscle sections were stained using Hematoxylin & Eosin (H&E) and dystrophin to assess nuclei localization and the cross-sectional area (CSA) of muscle cells respectively. Cross-sections of the muscles were taken at random across the belly of the TA muscle. After staining, images of the cross sections were captured using a 20x objective and an EVOS XL core microscope with the accompanying software (Life Technologies, Bothell, WA). The CSA and the number of cells with nuclei localization were then measured using Image J software (Tarpey *et al.* 2019). The number of muscle cells were used as a proxy for assessing the degree of injury, while the CSA was used to gauge the recovery of the muscle from the injury.



H&E stain of a TA muscle section 7 days post $BaCl_2$ injection



H&E stain of a TA muscle section 7 days post 1xPBS injection

Results

Using the H&E staining of the cross-sections, we assessed the impact of $BaCl_2$ induced injury on the WT mice and both of the heterozygote and homozygote forms of the $BRCA1^{R26SM}$ mice. The H&E stained sections showed no significant difference between the percentages of cells with centralized nuclei in the female and male mice of both strains (Fig 1&2), indicating that the animals were equally susceptible to the injury insult.

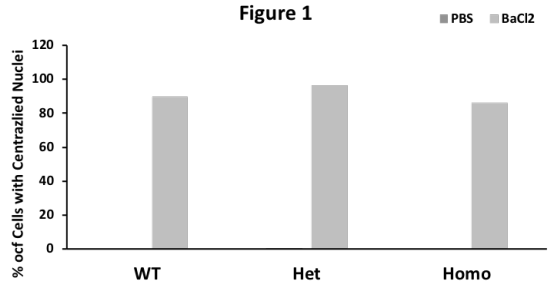


Figure 1. Female $BRCA1^{R26SM}$ mice in homozygote form display a similar percentage of cells with centralized nuclei compared to age-matched WT mice

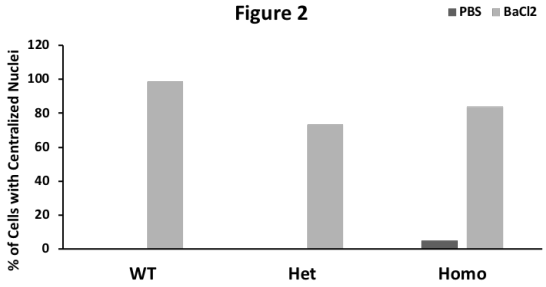


Figure 2. Male $BRCA1^{R26SM}$ mice in homozygote form display a similar percentage of cells with centralized nuclei compared to age-matched WT mice

Next, we assessed the size of the muscle fibers after recovery from injury in the mice using dystrophin staining to outline the sarcolemma of the muscle fibers. The CSA of the individual muscle fibers after the $BaCl_2$ in the homozygote form of both the female and male WT and $BRCA1^{R26SM}$ mice were smaller than those seen in the PBS injected mice, indicative of the regeneration of smaller muscle fibers following injury (Fig 3&4). No differences were seen across genotype after injury suggesting that BRCA1 expression in only skeletal muscle does not affect recovery from injury.

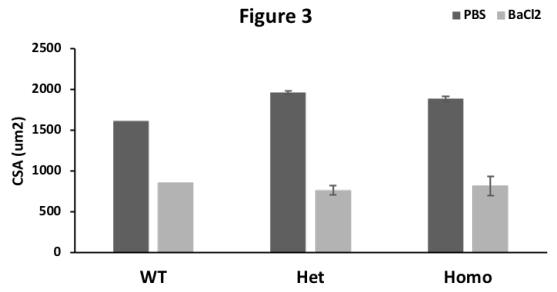


Figure 3. Acute injury reduces muscle size in female BRCA1^{R26SM} mice to the same extent as age-matched WT mice

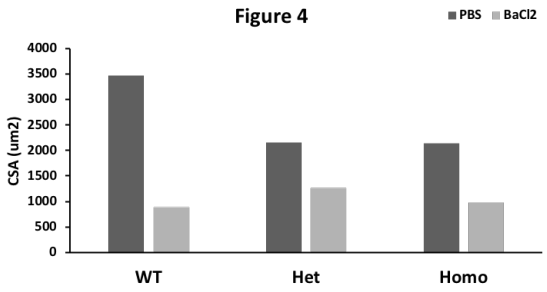


Figure 4. Acute injury reduces muscle size in male BRCA1^{R26SM} mice to the same extent as age-matched WT mice

In a similar manner, we also assessed the size of the muscle fibers of BRCA1^{KOSC} and BRCA1^{R26SC} mice after $BaCl_2$ induced injury. In contrast to the previous results, we now found that the CSA of the muscle sections in the BRCA1^{KOSC} were smaller than those in the WT mice. In addition, the CSA of the recovered muscle fibers after injury in the BRCA1^{R26SC} mice were larger than the fibers in both the BRCA1^{KOSC} and WT control mice (Fig 5) suggesting that BRCA1 expression is critical in the satellite cells and contributes to the recovery process from injury.

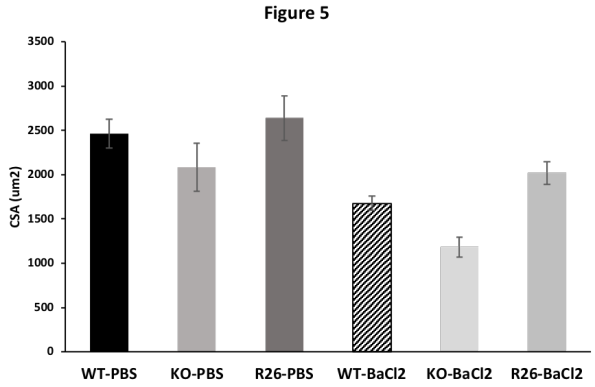


Figure 5. BRCA1^{KOSC} and BRCA1^{R26SC} show altered muscle fiber size following acute injury and repair in comparison to age-matched WT mice

Discussion

In these experiments, we demonstrate the necessity of BRCA1 expression, specifically in satellite cells, during myogenesis for skeletal muscle repair. Injection of $BaCl_2$ into

BRCA1^{R26SM} and WT mice resulted in a similar injury and no difference in the recovery of the muscle post-injury. This suggests that BRCA1 overexpression in the general skeletal muscle prior to the injury insult does not prevent or improve muscle repair. In contrast, when BRCA1 expression was manipulated in the satellite cells, we found differences in the recovery from the induced muscle injury. Specifically, $BaCl_2$ injury to the BRCA1^{KOSC} mice resulted in smaller muscle fibers compared to the WT animals, while BRCA1^{R26SC} mice exhibited larger muscle fibers post-injury compared to the WT control mice. There was also a significant overall improvement in the muscle quality when BRCA1 was overexpressed in the satellite cells.

We and others had previously identified that BRCA1 expression increases during the myogenesis process (Jackson *et al.* 2014; Kubista *et al.* 2002). However, it was unclear if BRCA1 is important to the myogenic process. In this study, our findings now indicate that BRCA1 is not only crucial to myogenesis but also that BRCA1 expression specifically in the satellite cells is necessary for proper muscle repair and recovery. While the overexpression in the general skeletal muscle prior to injury is not beneficial and does not affect muscle quality, our data shows that proper functioning satellite cells are key to recovery post injury. In order to ensure proper regeneration of muscle fibers, as a key regulator of DNA repair, BRCA1 needs to be present in the satellite cells prior to myogenesis occurring.

So far, BRCA1 has been mostly associated with its function in the mammary glands and little has been known about its critical role in skeletal muscle and other tissues. Our research provides critical data suggesting that BRCA1 expression is specifically needed in satellite cells for myogenesis following acute injury and its absence impedes proper muscle regeneration. This is similar to other studies demonstrating the importance of satellite cells for muscle growth by specifically targeting the satellite cell as opposed to the actual muscle cell (Petrany *et al.* 2020).

Further investigations should address the mechanisms for how BRCA1 overexpression is affecting satellite cell dynamics and the myogenic process. By observing differences in skeletal muscle function and metabolism of various levels of BRCA1 overexpression, we can identify any underlying pathologies and find the proper level of BRCA1 expression that can improve muscle quality without posing significant side effects. With recent studies demonstrating how satellite cell dysfunction can lead to complications in human health, this study and further investigation can help tailor future medical treatments to prevent the loss of skeletal muscle quality.

Acknowledgements

Thanks for the help from Mr. Adam Amorese with this project. Funding for this project came from an ECU URCA award and NIH RO1AR066660.

References

Bachman JF, Klose A, Liu W, Paris ND, Blanc RS, Schmalz M, et al. (2018). Prepubertal skeletal muscle growth requires Pax7-expressing satellite cell-derived myonuclear contribution. *Development* **145**

Bentzinger CF, Wang YX, Rudnicki MA (2012). Building muscle: molecular regulation of myogenesis. *Cold Spring Harb Perspect Biol* **4**, a008342.

Brown JC, Harhay MO, Harhay MN (2016). The muscle quality index and mortality among males and females. *Ann Epidemiol* **26**, 648–653.

Dumont NA, Wang YX, Rudnicki MA (2015). Intrinsic and extrinsic mechanisms regulating satellite cell function. *Development.* **142**, 1572-1581.

Jackson KC, Gidlund EK, Norrbom J, Valencia AP, Thomson DM, Schuh RA, et al. (2014). BRCA1 is a novel regulator of metabolic function in skeletal muscle. *J Lipid Res.* **55,** 668-680

Kubista M, Rosner M, Kubista E, Bernaschek G, Hengstschlager M (2002). BRCA1 regulates *in vivo* differentiation of mammary epithelial cells. *Oncogene.* **21,** 4747-4756.

Petrany MJ, Song T, Sadayappan S, Milay DP (2020). Myocyte-derived myomaker expression is required for regenerative fusion but exacerbates membrane instability in dystrophic myofibers. *JCI Insight.* doi: 10.1172/jci.insight.136095

Puri PL, Bhakta K, Wood LD, Costanzo A, Zhu J, Wang JY (2002). A myogenic differentiation checkpoint activated by genotoxic stress. *Nat Genet.* **32**, 585-93.

Tarpey MD, Valencia AP, Jackson KC, Amorese AJ, Balestrieri NP, Renegar RH, et al. (2019). Induced *in vivo* knockdown of the BRCA1 gene in skeletal muscle results in skeletal muscle weakness. *J Physio.* **597,** 869-887,