Role of the Aryl Hydrocarbon Receptor (Ahr) in Skeletal Muscle

National Institute on Aging

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Introduction & Hypothesis

- Aging is associated with loss of muscle mass and strength which contributes to falls, fractures, and disability.
- The aryl hydrocarbon receptor seems to have a profound negative impact on healthy aging as well as on the cardiovascular system (Eckers, 2016).
- Recently this receptor was shown to be downregulated with resistance exercise in both young and old individuals, suggesting that it may play a role in muscle adaptation to physical activity (Phillips, 2013).

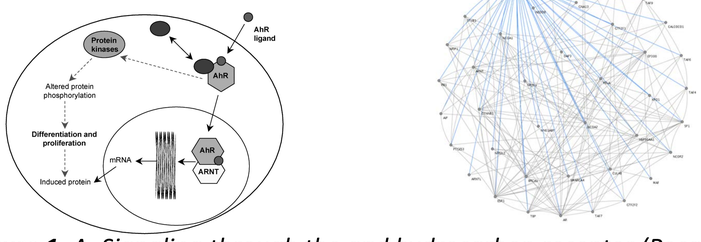


***Figure 1**. A. Signaling through the aryl hydrocarbon receptor (Pocar et al. Reproduction April 1, 2005 129 379-389) B. Gene networks that interact with the AhR (toppgene)*

Materials & Methods

Real-time PCR analysis of gene expression

- Frozen muscle sections were homogenized either mechanically with a sonicator and trizol on ice or with liquid nitrogen and a mortar and pestle
- mRNA was collected using a Qiagen RNeasy Mini Kit
- cDNA was collected using a Qiagen reverse transcription Kit
- Real time PCR was performed using a QuantiTect SYBR Green PCR Kit with primers (SABioscences) specific for the AhR

ELISA assay

- Protein was isolated from muscle homogenates
- Elisa was performed using a LifeSpan BioSciences, Inc. Mouse AHR ELSIA Kit (Sandwich Elisa)

Immunostaining

- Antibody staining was performed on frozen muscle (extensor digitorum longus) sections stained with rabbit anti-human (Novus NB100-2289) Ahr primary antibody and goat anti rabbit secondary antibody labeled with Texas Red.
- Sections were imaged using Zeiss upright confocal microscope in the Medical College of Georgia Cell Imaging Core Facility

Results

- **Immunostaining of Extensor Digitorum Longus (EDL) of 12 month vs. 24 month old mice shows elevated AhR with age**

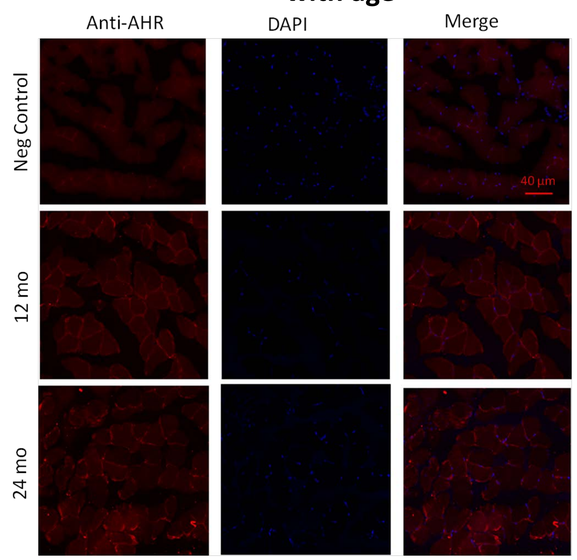


***Figure 2**. Confocal images of AhR antibody (abcam, rabbit antihuman polyclonal antibody) stained with goat anti rabbit secondary antibody labeled with Texas Red. Control received no primary antibody. Image is using Zeiss upright confocal microscope in cell imaging core facility.*

Results

- **PCR of AhR relative to the housekeeping genes 18s RNA and GAP using the ΔΔCT method in young and old mice**

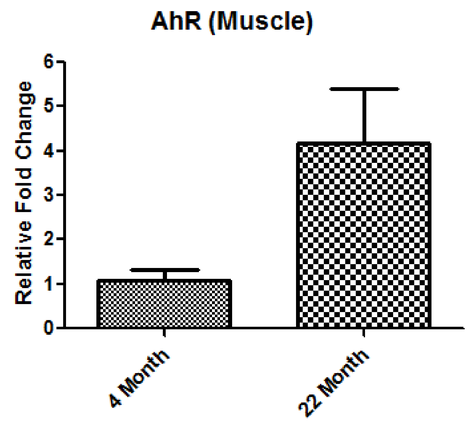
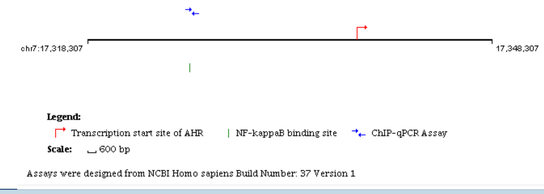


***Figure 3**. PCR results for difference in AhR expression between young and old mice*

Discussion

- The immunostaining of the EDL from the old and young mice showed a significant increase in the concentration of AhR in the older mice when compared to the young mice and negative control. This was further confirmed using PCR to analyze gene expression.
- These findings then raise the question of why AhR would increase with age? The inflammatory factor NFkB is known to increase with age (see below), and it has a transcription factor binding site in the AhR promoter region.



Conclusions

- Together these data indicate that the Ahr is present in skeletal muscle and becomes more sensitive with age. It appears to play a role in the age related decline in muscle function.
- The next step in our research is to determine the impact of AhR on anabolic and catabolic factors in knockout mice lacking AhR. Muscle samples from knockout mice will be analyzed using immunostaining and PCR.

Literature Cited

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Acknowledgments

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