Mechanistic basis of differential regulation of polyamine pathway by testosterone in prostate and androgen-responsive skeletal muscle

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Specific Aims

To determine whether the conversion of ornithine to putrescine by the enzyme ODC1 is obligatory for mediating testosterone's effects on the ventral prostate, but not on the androgen-responsive skeletal muscle groups

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Background

Administration of recombinant follistatin (rFst), a down-stream target of testosterone's promyogenic action (Singh et. al. Endo 2009; Braga et. al. Mol Cell Endo 2012) increases muscle mass in mice but has no effect on prostate mass (Jasuja, Costello, Singh, et. al. Aging Cell 2014)

Combined administration of testosterone plus ornithine decarboxylase (ODC1) inhibitor could offer a therapeutic strategy for developing selective prostate-sparing anabolic therapy (Jasuja, Costello, Singh, et. al. Aging Cell 2014)

Development of selective androgen receptor modulators (SARMs) with preferential anabolic effects on the muscle without unwanted side-effects on prostate mass are of great clinical importance

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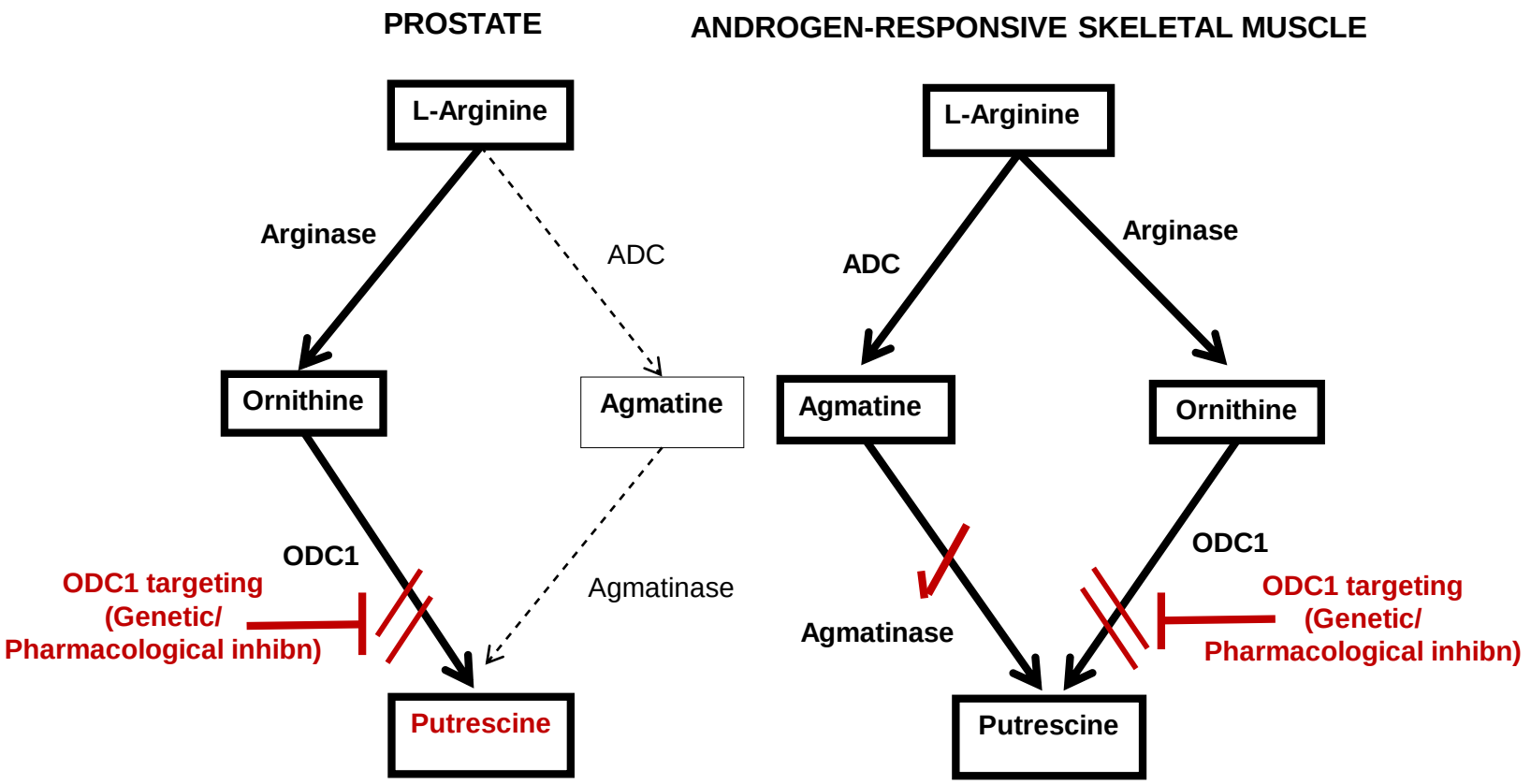
Rationale

Hypothetical framework of the experimental approach



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Innovation

- Novelty of the proposed hypothesis (ODC1 pathway for putrescine biosynthesis is critical for mediating testosterone's trophic effects on the prostate but not on muscle)
- Combined application of genetic as well as pharmacological approaches
- Translational value for drug development
- Discovery that alternate pathway for putrescine synthesis through Agmatine in muscle can maintain putrescine levels and preserve the anabolic effects of testosterone on the muscle in spite of ODC1 inhibition

Experimental Approach

A. Inhibition of putrescine synthesis by blocking ODC1 expression/ and or activity –

1. Employ genetically-modified mice with conditional prostate-and muscle-specific disruption of *Odc1* (Odc1 $^{flox/flox}$/ ACTA1^{Cre} mice) to assess the effect of testosterone withdrawal and replacement

2. Employ pharmacological inhibitors of ODC1 (DFMO and or POB) to assess the effect of testosterone withdrawal and replacement

B. Outcome measures-

1. Weights of prostate and androgen-responsive skeletal muscle groups
2. Whole body lean mass (NMR)
3. Tissue concentrations of primary polyamine putrescine as well as– ornithine, agmatine, spermidine and spermine (LC-MS)

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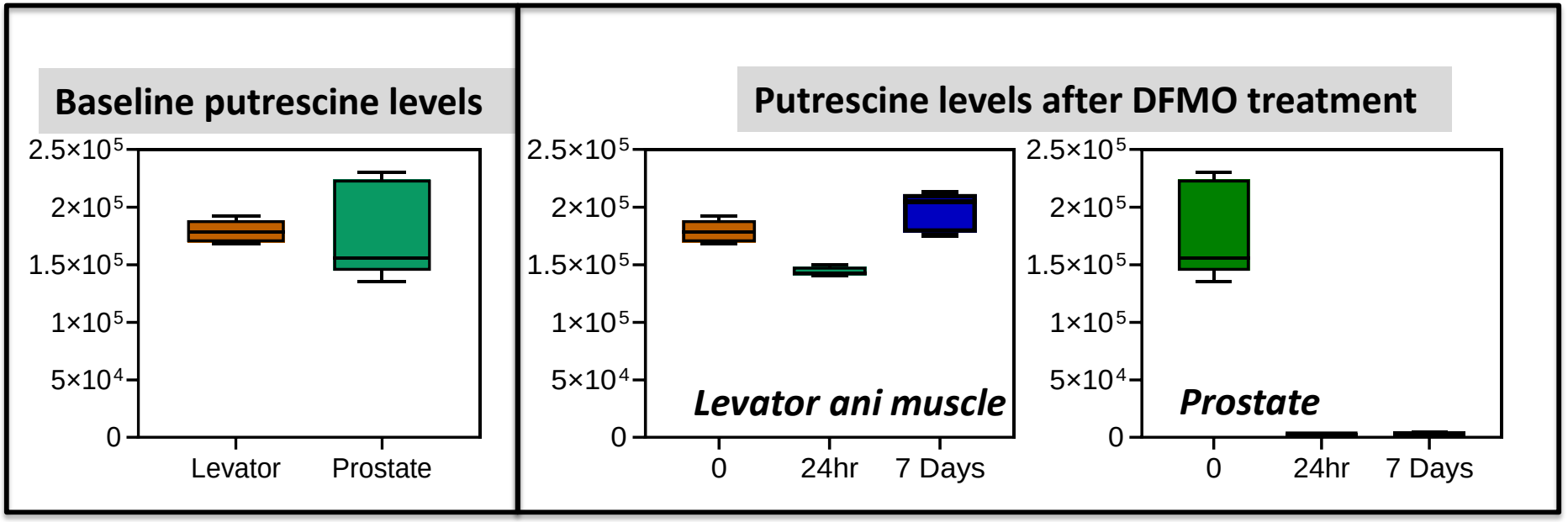
Results

LC-MS analysis of putrescine levels in levator ani muscle and prostate in male mice following DFMO treatments



DFMO dose: 15µg/day

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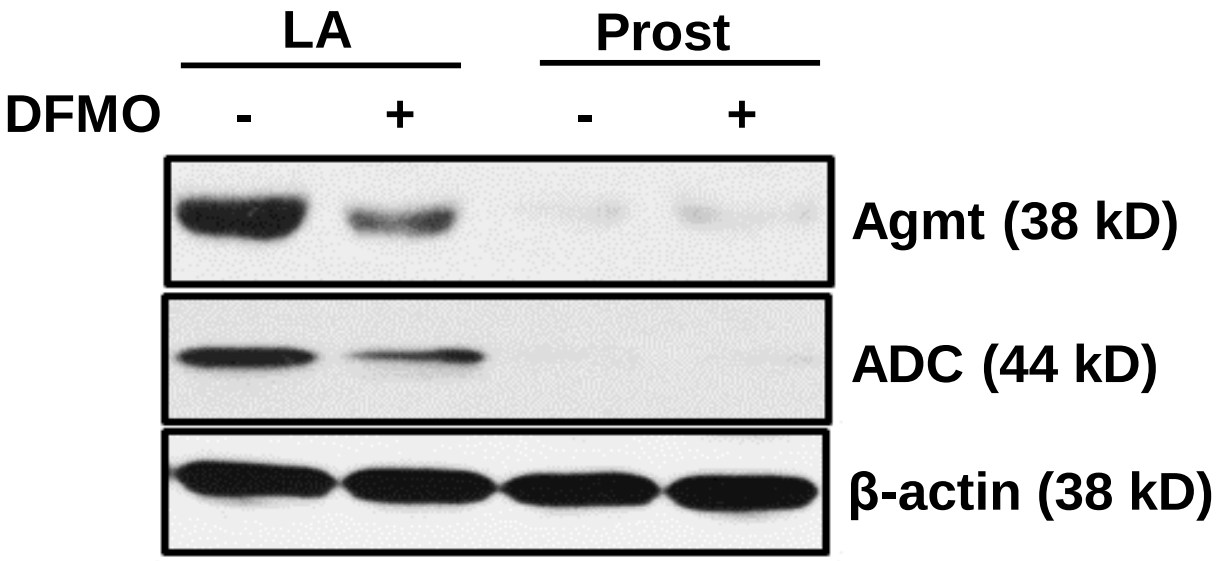
Results

Basal ADC and Agmt expression in prostate tissues is very low compared to the levator ani muscle



Results

Generation of conditional Odc1 KO mice (consultation with Taconic Lab)

1. Generation of targeting vector containing mouse genomic region from exon 2 to 11 flanked by loxP sites (3.6 kb), selection markers flanked by FRT (NeoR) and F3 (PuroR) inserted between intron 1 and 11- ***completed and sequence verified.***

2. ES cell generation using ES cell line Art B6 3.7 transfected with targeting vector. 192 resistant ES clones picked; 3 positive clones (PCR-based screening) were expanded- ***completed and confirmed by Southern blot analysis for correctly targeted Odc1 allele.***

3. Heterozygous G1 mice by microinjection of validated ES cell clones into blastocysts and transfer of injected blastocyst into pseudo-pregnant females to generate chimeric G0 mice- ongoing

4. Final G1 heterozygous mice will be generated by in vitro fertilization (IVF) using sperm from Go chimeric mice and C57BL/6 N Tac ***oocytes-ongoing expected to be completed within 2-3 months.***

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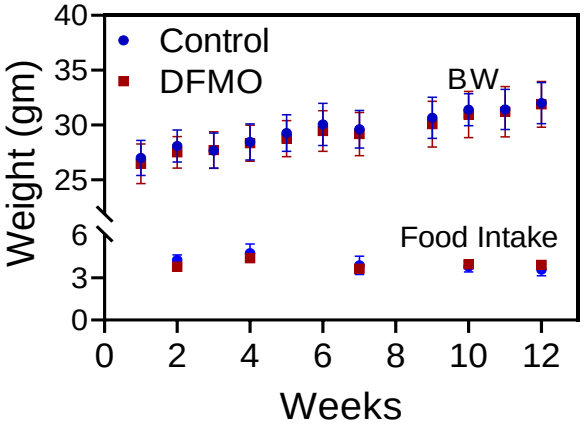
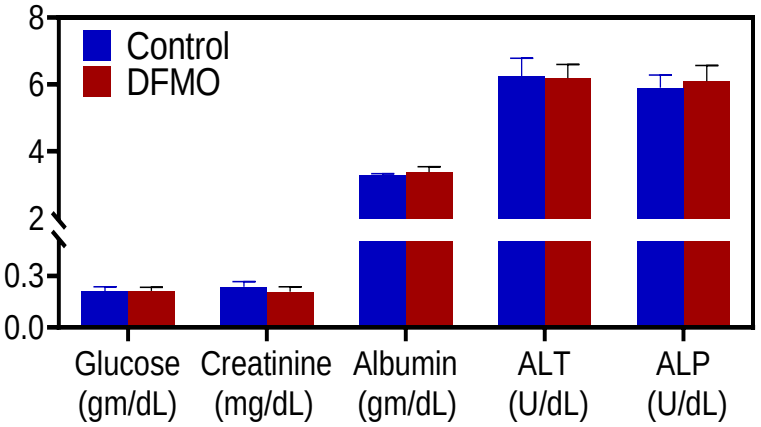
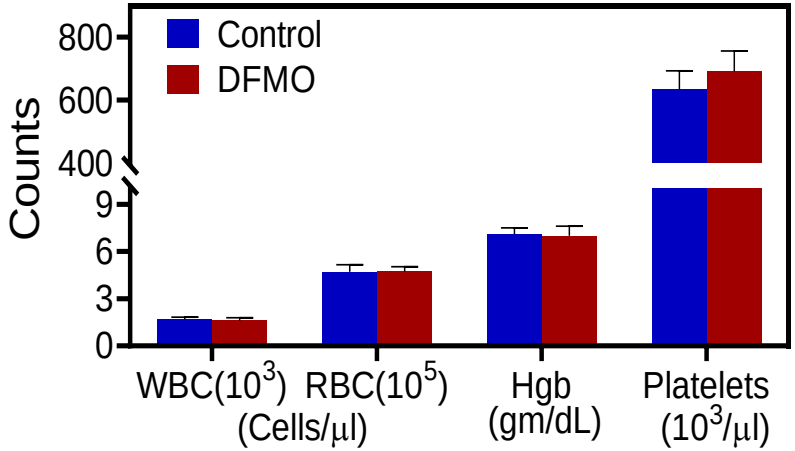
Results

Safety data of DFMO daily injection in male mice







DFMO dose: *75 μg daily (5 times higher)*
Duration: *3-months*

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Next steps/Career trajectory

a) *Establish preclinical models to test the efficacy of combination therapy for safe use of testosterone*

b) *Submission of a highly competitive R01 application/s in collaboration with Pepper Center Faculty member/s*

c) *Full-time transition to Men's Health, Aging and Metabolism Department at BWH*

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