Addition of hormones to non-metastatic prostate cancer and its effect on myosin IC expression

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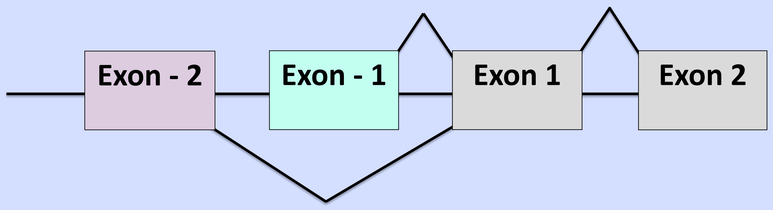
Abstract:

Myosin IC is member of a superfamily of cellular motor proteins that, together with the cytoskeletal element, actin filaments, facilitates many different types of cellular movement and intercellular transport. The myosin IC gene expresses three different isoforms including myosin IC isoform A, B, C. Expression of myosin IC isoform A was found to be elevated specifically in metastatic prostate cancer cells of both mouse and human origin when compared to non-cancer prostate cells and when compared to other (non-prostate cancer) cancer cells from other tissues. The addition of select hormones to a non-metastatic prostate cancer is able to induce an expression of myosin IC isoform A. LNCaP, a non-metastatic prostate cancer cell line, were exposed to the hormone, dihydrotestosterone (DHT). These prostate cells then had their relative expression of myosin IC isoform A and B tested and compared to control cells.

The most common cancer diagnosis for men is prostate cancer (Baade et al., 2009). Often times, prostate cancer will develop into a metastatic or aggressive form that will become lethal. Currently, there is no dependable method or marker known for the early detection of prostate cancer.

The myosin IC gene expresses three different isoforms that are named myosin IC isoforms A, B, and C. Isoforms A and B contain additional unique amino acids when compared to isoform C that allow them to localize to the nucleus (Ihnatovych et al., 2012).

MYOIC Gene structure



Expression analysis of the myosin IC isoforms in mouse tissues showed that of the three isoforms specifically isoform A, is present in only a few tissues while myosin IC isoform B is relatively equally present in all the tissues tested (Sielski et al., 2014). Importantly, expression of myosin IC isoform A was found to be elevated specifically in metastatic prostate cancer cells of both mouse and human origin when compared to non-cancer prostate cells and when compared to other (non-prostate cancer) cancer cells from other tissues. This suggests that this specific protein may be used as a biomarker that would allow for testing for prostate cancer (Ihnatovych et al., 2014). However, the reason for elevation of expression is not known.

References and Acknowledgements:

References:

1.Baade, P.D., D.R. Youlden, and L.J. Krnjacki, *International epidemiology of prostate cancer: Geographical distribution and secular trends.* Molecular Nutrition & Food Research, 2009. **53**(2): p. 171-184.

2.Ihnatovych, I., et al., *Identification and characterization of a novel myosin Ic isoform that localizes to the nucleus.* Cytoskeleton, 2012. **69**(8): p. 555-565.

3.Ihnatovych, I., mN.L. Sielski, and W.A. Hofmann, *Selective expression of myosin IC isoform A in mouse and human cell lines and mouse prostate cancer tissues.* Plos One, 2014. **9**(9): p. 8.

4.Sielski, N.L., et al., *Tissue specific expression of myosin IC isoforms.* BMC Cell Biology, 2014. **15**: p. 6.

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Methods:

1) Flow Chart of general methods used

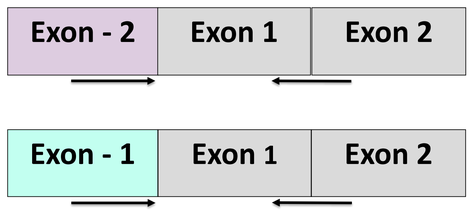
Cell Cultures
- LNCaP – a human non-metastatic prostate cancer cell line
- Cultured with RPMI 1640, FBS, and penicillin streptomycin at 5% CO_2 at 37°C
- Plated at $8.0x10^5$ with normal growth medium for 24 hours

Treatment with DHT
- Media was exchanged for serum free media after equilibration
- A final concentration of 10 nM DHT (dissolved in methanol) was added
- Control cultures only received methanol
- Cultured for 48 hours with treatment

Cell Harvest
- Cells were rinsed twice with PBS and harvested
- Total RNA was isolated using TRIzol® Reagent
- cDNA was obtained by reverse transcription

RT-PCR
- PCR was performed to test for expression of PSA, myosin isoforms A and B, and actin
- PCR products were ran on an agarose gel and visualized with ethidium bromide

2) Design and location of myosin IC isoform specific primer



Forward Primer (5'-3')
gcgctaccgggcatcg
Reverse Primer (5'-3')
ggaccgatgtaggtataaatgagg

Results:

1) Addition of DHT induces PSA expression in LNCaP cells

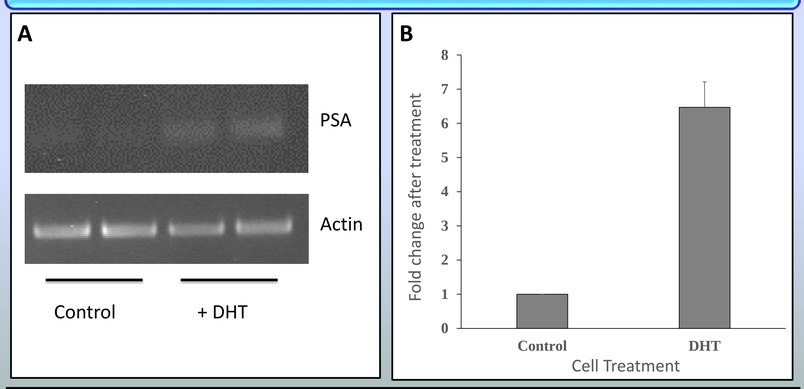


Figure 1: Semi-quantitative end-point PCR analysis of PSA expression.
A) Representative image of PCR products analyzed by electrophoresis on 1.5% agarose gels. **B)** Quantification of PCR signal using densitometry. PSA data were normalized to actin control. Error bars = SD. Dev; n = 4.

Results Continued:

2) Addition of DHT induces myosin IC isoform A expression in LNCaP cells

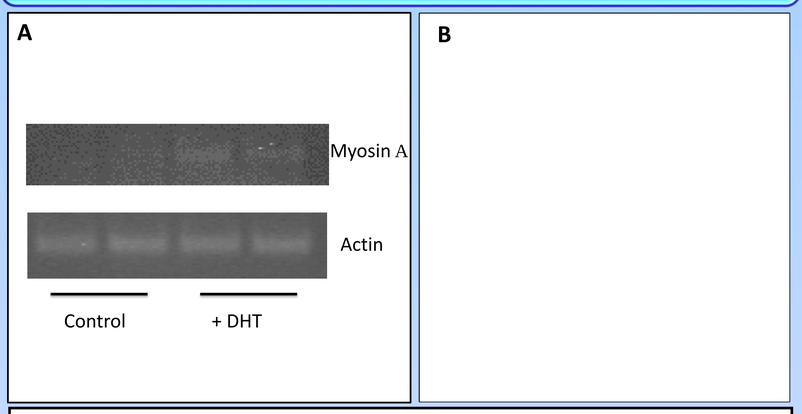


Figure 2: Semi-quantitative end-point PCR analysis of Myosin IC isoform A expression.
A) Representative image of PCR products analyzed by electrophoresis on 1.5% agarose gels. B) Quantification of PCR signal using densitometry. Myosin IC isoform A data were normalized to actin control. Error bars = SD. Dev; n = 2.

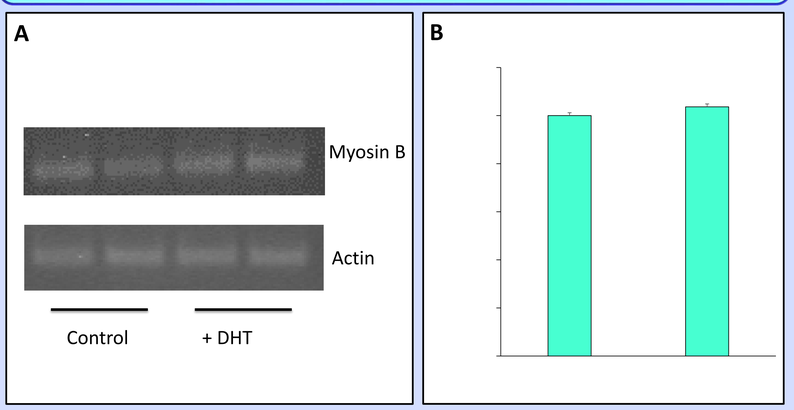


Figure 3: Semi-quantitative end-point PCR analysis of Myosin IC isoform B expression.
A) Representative image of PCR products analyzed by electrophoresis on 1.5% agarose gels. **B)** Quantification of PCR signal using densitometry. Myosin IC isoform B data were normalized to actin control. Error bars = SD. Dev; n = 2.

Conclusion and Future Studies:

Conclusions:
- The addition of the hormone DHT to a non-metastatic prostate cancer cell line causes an increase expression of PSA
- Furthermore addition of DHT induces expression of myosin isoform A from a non-metastatic prostate cancer cell line
- Supports previous findings that myosin isoform A expression is a potential indicator or marker for the diagnosis of metastatic prostate cancer

Future Studies:
- Why does DHT induce expression of myosin isoform A? Look for androgen receptor binding sites within the sequence of the MYOIC gene.
- Measure the expression of myosin isoforms in various other non metastatic cancer cell lines/tissues after addition of DHT, comparing them to prostate cancer cell lines