Androgen-Mediated Repression of the Maspin Tumor Suppressor Gene in Prostate Cancer

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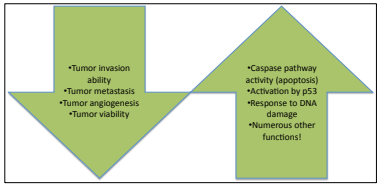
Abstract

This year in the United States, cancer is projected to cause one out of every four deaths. Prostate cancer alone is estimated to take the life of over 30,000 men. One area of intense research as a potential therapy against cancerous growth is in tumor suppressing genes, which function in cell cycle checkpoint responses, detection and repair of damaged DNA, protein ubiquitination and degradation, mitogenic signaling, cell specification, differentiation and migration, and tumor angiogenesis. One particular gene, the mammary serine protease inhibitor (maspin), is critically important in both breast and prostate cancer, which in the latter the gene is repressed. However, the exact mechanism that leads to the repression of the maspin gene is not entirely understood. In my thesis work, I first showed that the maspin tumor suppressor gene was under direct regulation by the androgen receptor protein. By analyzing the quality and quantity of the mRNA produced under various growth conditions, we confirmed that the activation of the androgen receptor was critical for the repression of maspin. To do this, cell cultures were selectively grown with or without androgens and the mRNA was extracted, converted to cDNA, and qPCR was performed to analyze relative levels of maspin expression. I next attempted to verify that the androgen receptor was physically binding the promoter region of maspin by performing chromatin immunoprecipitation (ChIP) assays. After immunoprecipitating any chromatin fragments with the androgen receptor bound, PCR was performed to amplify the maspin promoter. Obtaining a PCR product confirmed that the AR binds the androgen response element (ARE) within the maspin promoter, repressing its expression.

Background

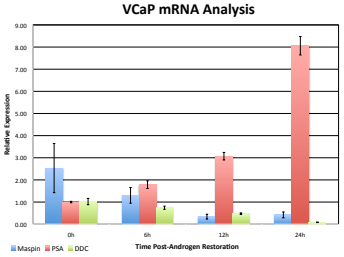
•When functional maspin, a pleiotrophic tumor suppressing gene, is expressed:

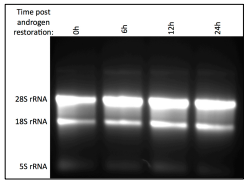


•BUT, in an androgen environment (i.e. the prostate), maspin expression is repressed
•We hypothesize that activated androgen receptor (AR) inhibits transcription of maspin by binding an androgen response element (ARE) in its promoter
•We will perform ChIP assays to investigate this relationship

mRNA Analysis

To investigate maspin expression, androgen-starved VCaP cells were treated with 1nM mibolerone at T=0, and the mRNA was extracted at 0, 6, 12, and 24 hours post treatment. Maspin expression levels were determined using quantitative RT-PCR.

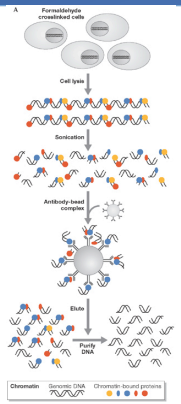




A 1% TAE gel verifying RNA integrity.

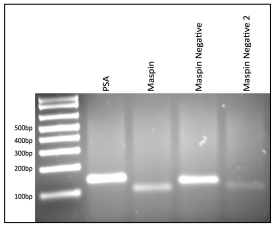
Maspin expression is reduced by 83%. DDC, a gene also repressed by AR, is reduced by 91%. PSA, a gene activated by AR, was increased by over 800%.

ChIP Protocol



Primer Testing

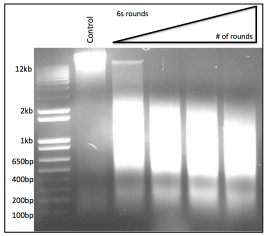
Prior to performing the ChIP assays the primers were tested. The PSA primers are a positive control. The maspin primers amplify the predicted maspin ARE. Both the maspin negative and negative 2 primers are negative controls, as they amplify regions away from the predicted ARE.



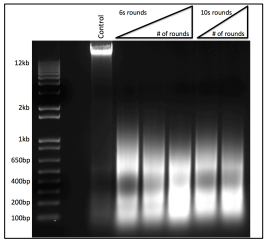
The expected sized fragments were produced: 139, 100, 125, and 105bp, respectively.

Sonication Optimization

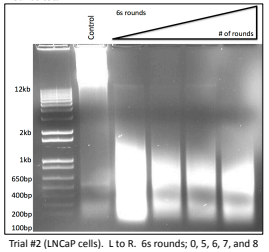
In order for the ChIP assays to be accurate, the sonication step must produce chromatin fragments ranging from 100 to 1000bp in size. Therefore, we optimized sonciation conditions using a Branson 450 Sonicator. One round of sonication consisted of a .5s pulse, followed by a 2s rest, repeated until a total sonication time of 6, 10, or 15s was achieved. The sonicated samples were separated by gel electrophoresis on a 1% agarose TAE gel.



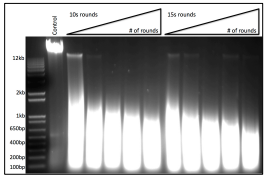
Trial #1 (LNCaP cells). L to R. 6s rounds; 0, 2, 3, and 4 rounds total.



Trial #3 (LNCaP cells). L to R. 6s rounds; 0, 9, 12, and 15 rounds total. 10s rounds; 6 and 9 rounds total.



Trial #2 (LNCaP cells). L to R. 6s rounds; 0, 5, 6, 7, and 8 rounds total.

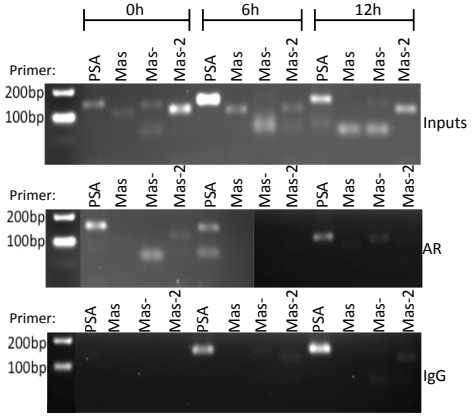


Trial #4 (VCaP cells). L to R. 10s rounds; 0, 2, 4, 5, 6, and 8 rounds total. 15s rounds; 2, 3, 4, 5, and 6 rounds total.

It was decided that 10s rounds of 60s total sonication produced the desired chromatin fragment range, while also limiting the number of rounds and time spent sonicating. This condition also ensured that all of the chromatin was fully sonicated (no full length DNA left in sample).

ChIP Assay of the Maspin Promoter

Androgen starved VCaP cells were treated with 1nM mibolerone at T=0. At 0, 6, and 12 hours post treatment cells were treated with formaldehyde to fix DNA bound protein. After sonicating with the chosen conditions, the samples were immunoprecipitated with AR N-20 to enrich AR bound chromatin, or non-specific rabbit IgG antibody, a negative control. DNA was purified and traditional PCR was performed to amplify the target sequences.



The PCR products visualized on a 1% SB agarose gel.

The input positive control lanes are sonicated chromatin that has not been immunoprecipitated with antibody. Each of these lanes produced the correct sized PCR product, except 6h Mas- and 12h Mas. The IgG lanes were immunoprecipitated with non-specific rabbit IgG. These samples should contain no DNA and serve as a negative control. As seen in the gel, many of the IgG lanes did produce a product. The AR lanes were immunoprecipitated with AR N-20 antibody. In these lanes the positive control PSA primers produced the correct sized product. The negative control primers produced products in some of samples. The experimental Mas primer lanes did not produce products at 0 or 6h, but did at 12h, suggesting the AR interacts with the maspin ARE sometime after 6h post-treatment. However, since many of the controls failed, no definitive conclusions can be made.

Conclusions

•Maspin gene expression is repressed by androgens
•A sonication using 10s rounds with 60s of total sonication produces the desired chromatin fragment size range while limiting the number of rounds and time spent sonicating
•The AR is probably binding to the ARE within the maspin promoter, repressing its expression
•Further trials need to be performed to confirm this

Future Studies

•Optimize ChIP conditions for positive (PSA) and negative (maspin neg./neg. 2) controls
•Continue ChIP experiments investigating the AR-ARE interaction in the maspin promoter
•Perform a Western Blot to determine whether androgens repress maspin protein expression

Acknowledgements/References

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ChIP Protocol Image: http://kenickbiochem09.wikispaces.com/file/view/chip2.jpg/72261523/980x945/chip2.jpg