Apoptosis in the prostate

J. M. Fitzpatrick, MD,[1] R. W. G. Watson, PhD[2]

[1]Professor and Chairman, Department of Surgery, University College Dublin, Dublin, Ireland
[2]Director of Surgical Research Laboratory, University College Dublin, Conway Institute of Biomolecular and Biomedical Research, Dublin, Ireland

FITZPATRICK JM, WATSON RWG. Apoptosis in the prostate. The Canadian Journal of Urology. 2003;10(2):1796-1798.

The prostate requires androgens for development and glandular maintenance, dying by the process of apoptosis following their removal. Anti-androgen therapy is targeted to induce this process but eventually fails with the emergence of an androgen independent cancer. These cells have development mechanisms to survive with out androgen impart due to the expression of anti-apoptotic factors.

Key Words: prostate, cancer, apoptosis, androgen receptor

Remembrance

I am delighted and proud to remember Ernest Ramsey, particularly so as he was an Irishman who found great success. I am also happy to mention that he was as appreciated in Ireland as he was in Canada. It was always a pleasure to meet him. My fondest memory of Ernest was when I was his guest in Winnipeg. The outside temperature was -25˚C with the snow heaped like mounds of caster sugar; the warmth of Ernest's welcome quickly made one forget the inhospitable temperatures. It is a pleasure to contribute to this special issue of the Canadian Journal of Urology in Ernest's memory.

John M. Fitzpatrick

Accepted for publication February 2003

Address correspondence to Professor John Fitzpatrick, Consultant Urologist & Professor of Surgery, University College Dublin, 47 Eccles Street, Dublin 7, Ireland

Introduction

The prostate is an androgen dependent gland. Androgens maintain the differentiated phenotype and drive proliferation of the epithelium within the prostate. While organ confined androgen dependent prostate cancer can be treated with anti-androgens such as cytoproterone acetate, hydroxyflutamide and bicalutamide, treatment for metastatic androgen independent prostate cancer remains elusive. This has lead to a variety of well-established and novel therapeutic approaches that have one underlying principle, to target and induce apoptosis or programmed cell death. While apoptosis ultimately results in cell death it is quite clear that the activation of this mechanism is dependent upon the phenotype of the cell and the therapy used.

Androgens, metabolism and action

The prostate requires androgens for development and glandular maintenance. Within the gland testosterone

is metabolized to 5α-dihydrotestosterone (5α-DHT) by 5α-reductase (3-oxo-5α-steroid-reductase). 5α-DHT preferentially binds to the androgen receptor and is most probably the only active androgen in the prostate.[1,2] The binding of 5α-DHT to the nuclear androgen receptor initiates the androgenic effects of the steroid by binding to the androgen response element (ARE) as a homodimer.[3]

Hormonal dependence of the prostate

The rat prostate represents a good model for the study of apoptosis, since the secretory epithelial cells are critically dependent on androgens for survival and die by apoptosis after their withdrawal, induced by castration or anti-androgen treatment. The reduction in prostate size is due to the selective loss of the secretory luminal epithelial cells in the distal and intermediate regions of the ducts, resulting in the complete obliteration of many of the ducts while maintaining the proximal segments of the ducts. The luminal epithelial cells in the intermediate and distal regions of the ducts are highly differentiated and die extensively due to their critical dependence on androgens. The loss of the androgen dependent tall columnar epithelial cells occurs via apoptosis.

There are a number of prostate-specific secretory and non-secretory proteins that are androgen dependent and downregulated after castration. One of these proteins is prostate steroid binding protein, a marker of androgenic control that consists of three different subunits C1, C2, and C3. The synthesis of each subunit is completely dependent on androgens.[4] Secretory prostatic acid phosphatase activity is androgen regulated and downregulated after castration in the prostate and has been used as an indicator of the androgenic status of the rat ventral prostate.[5] Human prostatic acid phosphatase and rat prostatic acid phosphatase are glycoproteins synthesized by the epithelial cells of the prostate gland and released into the lumen as part of the prostatic secretion.[6] Androgens also regulate the synthesis and secretion of human prostate specific antigen (PSA or hK1) and human prostate specific glandular kallikrein (hK2).[7] The expression of these genes diminishes very substantially after castration.

Androgen replacement in castrated rats results in luminal epithelial cell proliferation, without significant proliferation of basal cells or stromal cells. This cycle of androgen withdrawal and replacement can be repeated several times with constant repopulation of the stroma by the epithelial cells and the restoration of a fully functional gland.[8,9] This suggests there is a precursor epithelial stem-like cell in the prostate that is androgen independent that will replace the lost luminal secretory epithelium after androgen replacement.

Aspects of apoptosis in the prostate

Much of the underlying research on apoptosis in the prostate has relied on the use of a variety of animal, cell culture and to a lesser extent primary culture models. The cellular events that occur during apoptosis in the prostatic epithelium have yet to be fully elucidated. However, it is clear that apoptosis in the glandular epithelial cells of the human prostate share many features of apoptosis seen in other cell types.

Cell death, or apoptosis, is not a single phenomenon, but a series of morphologically and biochemically related processes.[10] Cell death of lymphocytes and other cells of reticulo-endothelial origin is dominated by changes in nuclear morphology[11] and mitochondrial biology,[12] while apoptotic death of glandular epithelial cells, such as those of the prostate, also requires profound cytoplasmic changes and alterations in the cell-cell and cell-substratum interactions .[13] The cytoplasmic changes that occur in epithelial cells undergoing apoptosis that lead to the release of the dying cell from the underlying in ECM occurs through the upregulation and action of proteins such as tissue transglutaminase and the activation of caspases.[14] Cell death in the prostate is known to be an active process as the rapid rate of prostatic cell death following castration can be inhibited by actinomycin D or cycloheximide, inhibitors of RNA and protein synthesis, respectively.[15]

The mechanism of anti-androgens

Apoptosis is a fundamental part of the events that occur in the regressing prostate after androgen ablation. How this process is activated following androgen withdrawal remains unknown. A number of anti-androgens have been developed, including: cytoproterone acetate, a steroidal anti-androgen and hydroxyflutamide and bicalutamide, two non-steroidal anti-androgens. These compounds bind to the steroid binding hydrophobic pocket of the androgen receptor blocking its action.

The most widely used anti-androgen in the world is bicalutamide, which inhibits gene expression and cell growth stimulated by androgens. However very little is known about the exact cellular events that

occur during apoptosis in the prostate epithelial cells after bicalutamide administration.[16] Bicalutamide induces regression in the prostate after administration and can limit the growth of the transplantable androgen-responsive Dunning rat tumor. When bound to the androgen receptor, bicalutamide causes the rapid degradation of the receptor in the nucleus of prostate epithelial cells.[17]

Conclusions

Androgens are required to maintain the integrity of the prostate and the survival of androgen dependent epithelial cells within the gland. While tissue remodeling occurs within the prostate, apoptosis is central to the regression process after androgen ablation.

Many of the receptor and mitochondrial mediated apoptotic pathways have been clearly defined in a variety of model systems, however the cell death pathway induced within the epithelial portion of the prostate after hormonal ablation *in vivo* remains to be elucidated. It is clear that the cell death pathways induced by the anti-androgens, currently used in treating androgen dependent prostate cancer are poorly understood and require further investigation.

There has been a focus in prostate cancer research on the use and design of better anti-androgens to induce apoptosis in prostate cancer cells. Most of this research is based on the physiological observation that the majority of prostate epithelial cells are androgen dependent.

However, this therapeutic approach does not work in the long term and leads to androgen independent prostate cancer. One possible reason for this may be that this therapeutic approach may only activate a specific cell death pathway within the prostate epithelial cell. Therefore, through a selection process, the cells may use survival signals like Akt, IGFBP's or Bcl-2 to block the cell death pathway induced by androgen withdrawal ultimately leading to androgen independence and disease progression.

Another reason may be that the tumor is only androgen sensitive not androgen dependent and while a proportion of the cells will die after androgen ablation the majority of the cells never required androgen for survival. Making this therapeutic strategy useful for palliative treatment, but ultimately useless as a tool to improve long-term survival.

Either way, alternative treatment modalities should be investigated that may induce apoptosis in the prostatic epithelium using a number of different cell death pathways to induce apoptosis within the same cell type.

Understanding the pro- and anti-apoptotic mechanisms that exist in the prostatic epithelium may ultimately lead to the design and use of therapeutic strategies that will target a variety of apoptotic pathways in prostate cancer. □

References

1. Mainwaring WI. The binding of (1,2-3H) testosterone within nuclei of the rat prostate. *Journal of Endocrinology* 1969;44:323-333.
2. Tenniswood M, Abrahams P, Winterton V, Bird CE, Clark AF. 1982 Binding of testosterone, 5 alpha-dihydrotestosterone and 5 alpha-androstane (3 alpha- and 3 beta-), 17 beta-diols to serum proteins in the rat. *Journal of Steroid Biochemistry* 1982;16:617-620.
3. Wong CI, Zhou ZX, Sar M, Wilson EM. Steroid requirement for androgen receptor dimerization and DNA binding. Modulation by intramolecular interactions between the NH2-terminal and steroid binding domains. *Journal of Biological Chemistry* 1993;268:19004-19012.
4. Parker MG, Scrace GT, Mainwaring WI. Testosterone regulates the synthesis of major proteins in rat ventral prostate. *Biochemical Journal* 1978;170:115-121.
5. Tenniswood M, Bird CE, Clark AF. Acid phosphatases: androgen dependent markers of rat prostate. *Canadian Journal Biochemistry* 1976;54:350-357.
6. Vanha-Perttula T, Niemi R, Helminen HJ. Separate lysosomal and secretory acid phosphatases in the ventral prostate. *Investigative Urology* 1972;9:345-352.
7. Wolf DA, Schulz P, Fittler F. Transcriptional regulation of prostate kallikrein-like genes by androgen. *Molecular Endocrinogy* 1992;6:753-762.
8. Lesser B, Bruchovsky N. The effects of testosterone, 5-dihydrotestosterone and adenosine 3',5' -monophosphate on cell proliferation and differentiation in rat prostate. *Biochimica et Biophysica Acta* 1973;308:426-437.
9. Berry SJ, Isaacs JT. Comparative aspects of prostatic growth and androgen metabolism with aging in the rat versus dog. *Endocrinology* 1984;114:511-520.
10. Clarke PG. Developmental cell death: morphological diversity and multiple mechanisms. *Anatomical Embryology* 1990;181:195-213.
11. Cohen JJ. Apoptosis. *Immunology Today* 1993;14:126-130.
12. Zamzami N, Susin SA, Marchetti P, Hirsch T, Gomez-Monterrey I, Castedo M, Kroemer G. Mitochondrial control of nuclear apoptosis. *Journal of Experimental Medicine* 1996;183:1533-1544.
13. Sandford N, Searle JW, Kerr JFR. Successive waves of apoptosis in the rat prostate after repeated withdrawal of testosterone stimulation. *Pathology* 1984;16:406-410.
14. Cummings M. Apoptosis of epithelial cells in vivo involves tissue transglutaminase upregulation. *Journal of Pathology* 1996;179:288-293.
15. Stanisic T, Sadlowski R, Lee C, Grayhack JT. Partial inhibition of castration induced ventral prostate regression with actinomycin D and cycloheximide. *Investigative Urology* 1978;16:19-22.
16. Furr BJA. The development of CasodexTM (Bicalutamide): Preclinical studies. *European Urology* 1996;29:83-95.
17. Waller AS, Sharrard RM, Berthon P, NMaitland NJ. Androgen receptor localisation and turnover in human prostate epithelium treated with the antiandrogen, Casodex. *Journal of Molecular Endocrinology* 2000;24:339-351.