ORIGINAL ARTICLE

Study of Validity of PC-3 Derived Xenograft Tumor Model in Athymic Nude Mice for Targeting Therapy of Androgen Receptors in Preclinical Studies of Prostate Cancer

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ABSTRACT

Background: Androgen receptors (AR) play an important role in proliferation of cancerous prostatic cells and prevention of apoptotic signaling. Current drugs cannot inhibit the activity of these receptors very well. Establishment of appropriate animal models is essential for discovery of new drugs of target therapy in hormone dependent prostate cancer.

Materials and Methods: Standard prostatic cancer cell line (PC-3) was injected heterotopically into 9 nude mice. After tumor formation they were excised and sent for histopathologic and AR expression studies by immunohistochemistry.

Results: Histological characteristics of prostate cancer were confirmed. Androgen receptors had been expressed in 65 ± 25% of the xenograft tumor cells.

Conclusion: This xenograft model is suitable for pharmacological and molecular studies of androgen dependent prostate cancer.

Keywords: Androgen receptor, prostate cancer, xenograft model, Athymic nude mice

زمینه و هدف: گیرنده های آندروژن (AR) نقش مهمی در پرولیفراسیون سلول های سرطان پروستات ایفا میکنند و از ایجاد سیگنالهای آپوپتوز ممانعت می نمایند. داروهای موجود به خوبی نمی توانند فعالیت این گیرنده وابسته به هورمون را مهار نمایند. ایجاد مدل های حیوانی مناسب به منظور مطالعه و کشف داروهای جدیدی که در درمان هدفدار سرطان پروستات وابسته به هورمون به کار روند، ضروری به نظر می رسد.

مواد و روش ها: رده سلولی استاندارد PC-۳ به ۹ سر موش بی موی فاقد تیموس نر تلقیح شد. سپس تومورهای تشکیل شده از نظر هیستوپاتولوژیک و بیان AR با ایمونوهیستوشیمی مورد مطالعه قرار گرفتند.

یافته ها: ویژگی های هیستوپاتولوژیک سرطان پروستات مورد تأیید قرار گرفت. میزان بروز AR در تومورهای زنوگرافت ۲۵ ± ٦۵ درصد گزارش گردید.

نتیجه گیری: این مدل به منظور مطالعات فارماکولوژیک و و مولکولی سرطان پروستات وابسته به آندروژن مناسب می باشد.

واژه های کلیدی: گیرنده آندروژن؛ سرطان پروستات؛ مدل زنوگرافت؛ موش بی موی فاقد تیموس

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Introduction

Prostate cancer is one of the most widespread tumors among men above 50. In 2010, according to national cancer institute (NCI) report 217,730 new cases of prostate cancer and 32,050 deaths from prostate cancer in the United States has happened.[1] The most important risk factors of prostate cancer are age; race, diet and family history.[1, 2] Assessment of prostate specific antigen (PSA) in blood and digital rectal exam are the most common screening test for prostate cancer. Treatment strategy is based on Gleason's score, PSA level, histopathological criteria and other factors such as age.[3] Androgen receptors (AR) play an important role in proliferation of cancerous prostatic cells and prevention of apoptosis of tumor cells. AR is an intra-nuclear steroid receptor which is expressed more in luminal epithelial cells than Basal epithelial cells. Animal studies showed castration before puberty resulted in prevention of malignant changes in prostate cells.[4, 5] In the absence of the ligands, AR receptors become inactive and get attached to chaperon molecules. Bond between ligands and these receptors activates genes related to transcriptional factors and proliferation of prostate cancer cells. The specific gene for androgen receptor was found on the Xq12 chromosome.[6] Androgen receptors have high activity during puberty that decreases after the development of prostate gland. In the most cases of prostate cancer a mutation occurs that ultimately stimulates AR activity. This mutation leads to disturb CAG sequence in N terminal that increases prostate cancer risk.[7] Increase in AR activities have also found in ovary and breast cancer in females.[8] Mechanisms can decrease AR activity are; decrease the attachment of testosterone to AR or complete testosterone removal. A variety of drugs such as luteinizing hormone, estrogens, anti-androgens or receptor agonists are used for decreasing activity of AR. Although these drugs successfully control the progress of prostate cancer at first but most hormone dependent cases become refractory to this treatment in 1-3 years and considered as failure of therapy.[9, 10] For this reason other strategies such as targeting therapy of AR related genes are necessary. Such studies depend on appropriate in-vitro and in-vivo models of prostate cancer. A few prostate cancer cell lines have been established to investigate the mechanism involved in prostate cancer. LNCaP and PC-3 are the most commonly used prostate cancer cell lines. National Cancer Research (NCI) of USA uses these cell lines in its preclinical studies. The LNCaP cancer cell line expresses androgen receptor (AR) and it was derived from a human lymph node metastatic lesion in prostatic adenocarcinoma. PC-3 cells were established from human prostatic adenocarcinoma metastasis to bone. Both cell lines are tumorigenic after injection to nude mice. We established an xenograft animal model using LNCaP cell line previously. In this article the results of experiment with PC-3 is presented.

Methods

Nine male BALB/c Athymic nude mice 6-8 weeks of age were used in this study. The animals were kept in IVC (individual ventilated cage) in experimental tumor implantation laboratory which is under supervision of Tehran University of Medical Sciences (TUMS). All phases of this investigation were designed and performed according to the internationally accepted ethical principles of working with laboratory animals.

PC-3 cells were provided by the National Cell bank of Iran (NCBI) affiliated to Pasteur Institute. Cells then were cultured in RPMI 1640 culture medium containing 10% FBS. To develop a xenograft model, 5×106 cells in 200µl of RPMI 1640 culture medium without FBS were injected into the right and left flank region of nude mouse subcutaneously. Tumor growth was measured twice a week. The volume of tumors was calculated by standard formula (Length×Width2×0.52) and growth curves were drawn.[11] Experiments were terminated after 30 days and euthanasia was conducted on the mice in a humane manner. The resulted tumors were removed from the animals and fixed in 10% buffered formalin and sent for histopathological studies. Specimens were stained by H&E and also by IHC (Anti AR antibody -Abcom Company). A pathologist then studied the slides. Numbers of AR were recorded in 4 microscopic fields with a magnification of 400.

Results

Fifteen tumors formed in 9 animals after one month (**Figure 1**) and tumor cells didn't grow in 3 injection sites. Growth curve of tumors is shown in (**Figure 2**). In 12 specimens histopathological characteristics of adenocarcinoma of prostate were confirmed. We excluded remaining 3 samples from the study because histopathological characteristics of adenocarcinoma of prostate were not confirmed. The average number of androgen receptors was determined 65±25% by IHC. In the 8 specimens AR expression was over 60% and it is equal to 66% in entire samples (**Figure 3**).

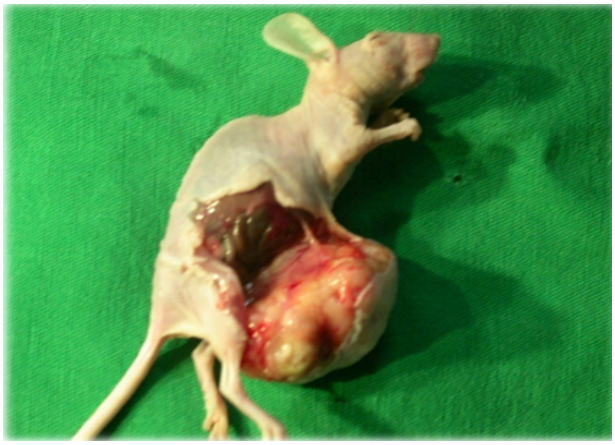

Figure 1: PC-3 xenograft prostate cancer

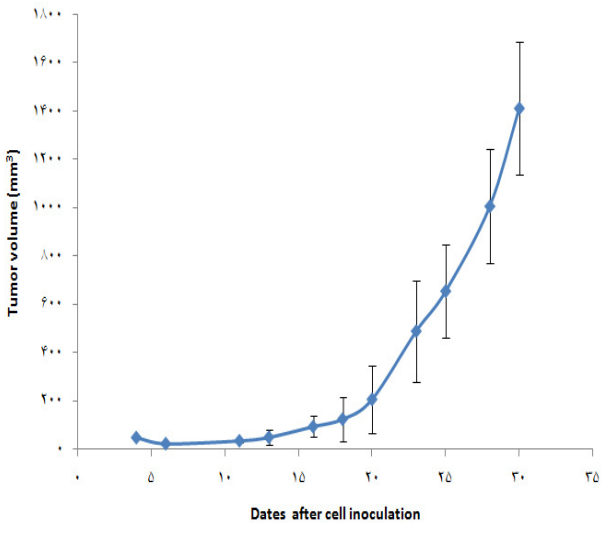

Figure 2: Growth curve of prostate tumors during 30 days after injection of PC-3 cells

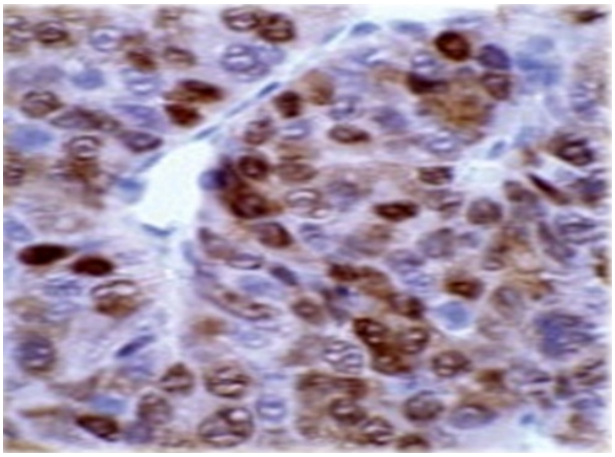

Figure 3: Micrograph × 400 (IHC with AR -Ab) of PC-3 tumor.

Discussion

Androgen receptors belong to the steroidal nuclear receptors and activate androgen dependent transcriptional factors. Androgen signaling mediated by AR plays an important role in the normal development of prostate organ and also during the prostate cancer development. Nowadays in end stage prostate cancer, anti-androgens are used as adjuvant or neoadjuvant therapy.[12] Although orchiectomy is a golden standard but it does not appeal to patients and on the other hand around 10% of androgen is produced by adrenal gland.[13, 14]

Diethylstilbestrol use was limited due to cardiovascular side effects. Gonadotropin-releasing hormone (GnRH) analogues brought a rise in testosterone blood level in the early stage of treatment that might result in clinical failure. Non estradiol anti androgen is usually effective by itself. Meta-analysis of various Clinical trial shows that there is no tangible advantage of using anti androgens along with orchiectomy. However using anti androgens along with GnRH analogue slightly increases the survival rate.[15] Drugs decreasing blood testosterone level cause androgen deprivation syndrome which ultimately leads to bone density drop. On the other hand it causes gynecomastia induction.[16,17] On the whole it could be claimed that the drugs available for AR activity decrease are not remarkably efficient. So targeting therapy is considered inevitable. The first xenograft model of human prostate cancer was established by PC-3 cell line in 1979.[18] PC-3 cell line was derived from stage IV adenocarcinoma pros-

tate and gene activity related to AR was confirmed.[19, 20]

In this study, PC-3 Derived cell line tumor was AR positive. Similar results were reported by using PC-3 cells in other studies.[21,22] Following the possibility of using nude mice with T cell immunodeficiency, this study was carried out for the first time in Iran for developing hormone dependent prostate cancer model so that researchers could pursue therapeutic objectives of inhibition of gene activities related to AR.

Conclusion

Xenograft prostate cancer model established by PC-3 cell line has characteristics of therapeutic and preclinical studies related to AR and researches would be able to utilize this model in anti prostate cancer drug studies.

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