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# Establishment of prostate cancer in cynomolgus macaque animal model by orthotropic inoculation of PC-3 cancer cells in situ

## *Induzione di cancro della prostata in modello animale macaco cynomolgus attraverso inoculazione ortotropica di cellule cancerose PC-3 in situ*

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**Summary**

***Background*. Prostate cancer commonly affects men worldwide. Till now, no suitable animal models which could fully embody the characteristics of human prostate cancer due to different deficiencies are still available. This investigation explored the potential application of cynomolgus macaques to establish prostate cancer animal model which is more similar to the characteristics of human disease. *Methods*. Four 4-year-old cynomolgus macaques were used in this study. Cyclosporine conducted as immunosuppressant was subcutaneously injected to cynomolgus macaques once daily. Following 7 days of treatment of cyclosporine, $1 \times 10^7$ PC-3 cancer cells mixed with matrigel were injected into the prostate of two cynomolgus macaque, while the other two cynomolgus macaque were injected by culture medium at the same site as the mock group under anaesthesia state. Cefazolin Sodium acted as antibiotics was administered through intramuscular injection for five days after the operation in protection of bacterium infection. The**

**Riassunto**

***Background*. Il cancro alla prostata colpisce comunemente gli uomini in tutto il mondo. A causa di diverse carenze, ad oggi non sono disponibili modelli animali capaci di incarnare appieno le caratteristiche del cancro umano alla prostata. La presente ricerca ha esplorato la possibilità di impiegare il *cynomolgus* macaco come modello animale per il cancro alla prostata perché maggiormente simile alle caratteristiche della malattia umana. *Metodi.* Per questo studio sono stati usati quattro *cynomolgus* macachi di 4 anni. La ciclosporina usata come immunosoppressore è stata iniettata sottocute ai *cynomolgus* macachi una volta al giorno. Dopo 7 giorni di trattamento di ciclosporina, $1 \times 10^7$ PC-3 di cellule cancerose mescolate con matrigel sono state iniettate all'interno della prostata di due *cynomolgus* macachi mentre agli altri due, considerati come gruppo di controllo, è stato iniettato un medium di coltura sotto anestesia. Cefazolin Sodio, utilizzato come antibiotico, è stato somministrato per via intramuscolare per 5 giorni dopo l'operazione come**

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**serum concentration of prostate-specific antigen (PSA) and alkaline phosphatase (ALP) for each monkey was detected at the beginning of the experiment, on day 30 and 60. On day 60, all the cynomolgus macaques were sacrified and relevant tissues were collected for histopathological examinations and immunohistochemistry of anti-human α-methylacyl coenzyme A racemase (p504s), p63 and basal keratin antibodies (34βE12) to identify the oncogenesis and metastasis of the prostate carcinoma. Prostate tissues of four cynomolgus macaque were also collected for gene chip determination. *Results*. The serum concentration of PSA mildly increased in the two PC-3 cancer cells treated monkey on day 30 and 60 although neither the serum concentration of ALP nor body weight was significantly affected. Prostate cancer has developed in two monkeys following PC-3 cancer cells inoculation. It was shown typical prostate cancer could be observed in histopathological examination. These tissue sections revealed that prostate cancer cells had invaded the nerves, the basal layer was discontinued and the developed tumor lesions had reached the grade II Gleason lesion level. Furthermore, the detection of anti-human α-methylacyl coenzyme A racemase (p504s), p63 and basal keratin antibodies (34βE12) by immunohistochemistry also demonstrated that cancer had developed. However, there was no evidence of prostate carcinoma metastasis occurred in the two monkeys. The gene expression profiling analysis also indicated that most human prostate cancer genes were expressed in the prostate tissue of the monkeys. *Conclusions*. In this study, we have established a cynomolgus macaque model of human prostate carcinoma by an orthotropic injection of PC-3 cancer cell line in situ. These results demonstrated that this model may effectively simulate the biological nature of human prostate carcinomas. Eur. J. Oncol., 17 (4), 189-203, 2012**

***Key words:*** **prostate cancer, animal model, *cynomolgus* macaques**

**protezione da infezione batterica. È stata misurata la concentrazione di siero del PSA e dell'ALP per ciascuna scimmia all'inizio dell'esperimento, dopo 30 e dopo 60 giorni. Al 60° giorno, tutti i *cynomolgus* macachi sono stati sacrificati ed i tessuti più rilevanti sono stati raccolti per effettuare le analisi istopatologiche e l'immunoistochimica utilizzando l'α-metilacil coenzima A racemasi (p504s), il p36 ed anticorpi basali della cheratina (34βE12) per identificare l'oncogenesi e le metastasi del carcinoma prostatico. Sono stati raccolti anche i tessuti della prostata dei quattro *cynomolgus* macachi per la determinazione del frammento gene. *Risultati.* La concentrazione sierica del PSA è cresciuta moderatamente nelle due scimmie trattate con cellule cancerose PC-3 al 30° ed al 60° giorno sebbene né la concentrazione sierica di ALP né il peso corporeo fossero significativamente alterati. Il cancro alla prostata si è sviluppato in due scimmie a seguito dell'inoculazione di cellule cancerose PC-3. È stato dimostrato che un tipico cancro alla prostata può essere osservato all'esame istopatologico. Le sezioni dei tessuti hanno rivelato che le cellule del cancro alla prostata avevano invaso i nervi, lo strato basale era discontinuo e le lesioni tumorali sviluppatesi avevano raggiunto il grado II di Gleason. Inoltre, anche il rilevamento tramite l'α-metilacil coenzima A racemasi (p504s), il p36 e gli anticorpi basali della cheratina (34βE12) attraverso l'immunoistochimica, hanno dimostrato che il cancro si era sviluppato. Tuttavia, non c'era evidenza di metastasi dovute al carcinoma prostatico nelle due scimmie. L'analisi del profilo dell'espressione genetica ha anche dimostrato che la maggior parte dei geni del cancro alla prostata nell'uomo si erano manifestati nel tessuto prostatico delle scimmie. *Conclusioni.* In questo studio, abbiamo istituito un modello *cynomolgus* macaco del cancro prostatico dell'uomo attraverso una iniezione ortotropica di cellule cancerose PC-3 *in situ*. Questi risultati hanno dimostrato che questo modello può effettivamente simulare la natura biologica dei carcinomi prostatici dell'uomo. Eur. J. Oncol., 17 (4), 189-203, 2012**

***Parole chiave:*** **cancro prostatico, modello animale, macaco *cynomolgus***

## Introduction

Prostate cancer is the most common cancer among men, especially for the aged men. One major obstacle for studying the pathogenesis of prostate cancer is due to the lack of appropriate animal models. For example, as dog models, the dog is the only animal known to develop high grade prostatic intraepithelial neoplasia (PIN) and prostate adenocarcinomas spontaneously. Multiple similarities existed between the human and canine cancer models include: late stage onset, metastasis tendency, possible androgen independence of advanced stage, and morphologic and phenotypic heterogeneity of tumor lesions. These similarities support the use of the canine cancer model for the definition of predisposing factors, the assessment of therapeutic agents, and the examination at the cellular and molecular levels of the transitions from prostatic intraepithelial neoplasia to carcinoma and osseous metastasis ultimately. Nevertheless, limitations of this system include the apparently low incidence of spontaneous disease (1), animal handling, and the high expense of maintaining dog colonies. Although the mouse has been applied in modeling other human cancer types, it doesn't work well with the respect to prostate cancer. This may be related to the basic anatomy of the rodent prostate which considerably differs from the corresponding human tissues. Specifically, the rodent prostate is composed of three lobes, namely dorsolateral, ventral and anterior (also called the coagulating gland) that are circumferentially located around the urethra. The human prostate is alobular but can be subdivided into three zones: central, peripheral, and transitional zone, which completely envelops the upper part of the urethra (2). Perhaps because of these species-specific differences in physiology, pathology and morphology of the prostate, there is controversy that the mouse is not an ideal animal for the development of a model for human prostate cancer (3). Nonhuman primates are genetically closer to humans than any other laboratory animal. The cynomolgus macaque is one of the most important and widely used non-human primate animal models in basic and applied biomedical research. Compared to other species, non-human primates have a closer evolutionary relationship to humans and exhibit high physiological similarity well which is suitable to serve as translational models for preclinical drug safety assessment (4).

At the early stage of a new human antibody drug development, because no corresponding immune response in mice and dog model is present, such antibody drugs could not show anti-cancer effect while they may act in human. Rodent model and dog model may induce such drugs failed due to improper animal models. With the development of pathogenesis of cancer, more and more antibody drugs have been developed. Therefore, it is urgent to explore the more suitable animal models for human prostate cancer. However, nonhuman primates have not been studied in such fields mainly because of the widely held view that nonhuman primates do not spontaneously develop prostate cancer. Furthermore, the relationship between the immune system and cancer is very complicated. Normally the inoculation of foreign prostate cancer cells or tumor tissues into monkeys is very hard to succeed, because their immune systems may attack foreign tissues (5). Following the method of organ transplantation applied in human, we applied the cyclosporine to suppress the immune system of cynomolgus macaque and found cancer lesion in the prostate of cynomolgus macaque. Thus, cyclosporine at the dose of 25 mg/day was subcutaneously injected into the monkeys for 7 days, prior to PC-3 cancer cells inoculation to prostate of monkeys, in order to inhibit the immune reaction. After 60 days of operation, the animals were euthanized and the prostate including other related tissues were harvested for histopathological examination, immunohistochemistry and microarray analysis. The histopathological changes to the prostates of the macaques were remarkable and indicated that cancer lesions had developed, the basal layers were discontinued, and the cancerous cells had invaded the nerves. Concurrent immunohistochemistry staining further demonstrated tumor formation (6).

The cyclosporine was discovered on 31 January 1972 by employees of Sandoz (now Novartis) in Basel, Switzerland. The success of cyclosporine in preventing organ rejection was shown in kidney transplants by Calne and colleagues at the University of Cambridge (7). Cyclosporine was subsequently approved for use in 1983. Since then, it has been used to prevent and treat graft-versus-host reactions in bone-marrow transplantation and to prevent rejection of kidney, heart, and liver transplants (8). Many

articles reported that cyclosporin could induce cancer, e.g. Bouwes *et al*. who found cyclosporin A causes skin cancer in 40% of the transplant patients within 5 years after transplantation, including squamous cell carcinoma, basal cell carcinoma and cutaneous melanoma (9). Furthermore, Cyclosporine is listed as IARC Group 1 carcinogens (sufficient evidence of carcinogenicity in humans).

But at the same time, many researchers demonstrated cyclosporine has broad-spectrum anti-tumor action in vitro and in vivo. For example, Miguel Munoz et al. found cyclosporine exerts anti-tumor action against gastric (AGS cancer cell), colon (HT-29 cancer cell), and other seven kinds of carcinoma cell lines (10). A Phase I/II clinical trial were completed at the University of California, at Irvine and at the Cancer Research Center of Hawaii between July 1989 and June 1994. The results showed that the 2-year survival of the test group was 25% compared to 4% for the control group (11). All information made us confused. We try to explore the action mechanism of cyclosporine and find out the relationship between the immune system and cancer. The prostate cancer cynomolgus macaque animal model is a suitable tool to investigate above problems.

In addition, gene expression profiling indicated that more than 41,000 human genes were expressed in the PC-3 cancer cells treated prostate tissues. Approximately 56 of 72 genes were related to prostate cancer according to the OMIM database while these genes were expressed in both samples. We concluded that this study may work as an exploring animal model for future human prostate cancer research.

## Materials and methods

### *Animals*

Four male cynomolgus macaques were purchased from Suzhou Xi'shan Zhongke Laboratory Animal Co., Ltd. located at Suzhou in the Jiangsu Province of China. All animal care and use procedures conformed to the Chinese regulations for the Human Care and Use of Laboratory Animals. They were housed individually in a temperature and light-controlled room at 18-26°C, on a 12 light:12 dark (on 7 a.m., off 7 p.m.) light cycle and fed with monkey specific food and water ad libitum in the large animal center of Shanghai Institute of Planned Parenthood Research (SIPPR). The 4 monkeys in this study were 4 years old and weighed between 3.6 and 4.0 kg.

### *PC-3 cancer cells culture, implantation and tissue collection*

PC-3 cancer cells were purchased from Cell Bank of Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences and were cultured in RPMI 1640 supplemented with 10% fetal serum in a 5% $CO_2$ atmosphere at the temperature of 37°C. For the animals, monkeys were subcutaneously injected with 25 mg cyclosporine once daily for 7 days after one month of acclimation period. Prior to the operation, monkeys were all labeled and anaesthetized by intravenous injection with 15 mg/kg ketamine. Then, a catheter was placed through the urethra to help drain the urine and peritoneum was incised from the umbilicus to the pubic symphysis along the ventral line at the base of the bladder. $1x10^7$ PC-3 cancer cells or the culture medium which both were mixed with matrigel were inoculated into prostate at the base of the bladder. The wound was then closely stitched. Following the operation, cefazolin sodium at the dose of 20 mg/day was intramuscularly injected for 5 days. On day 0, 30, and 60, serum was collected from the animals for the determination of prostate-specific antigen (PSA) and alkaline phosphatase (ALP).

On day 60, after the monkeys were euthanized, the prostates and inguinal lymph nodes were excised. Tissue samples were either frozen at −80°C or were fixed in neutral buffered formalin for paraffin embedding and sectioning at a thickness of 5 μm. These sections were then stained with hematoxylin and eosin for routine histological examination and immunohistochemistry for anti-human α-methylacyl coenzyme A racemase (p504s), p63 and basal keratin antibodies (34βE12).

### *Serum PSA and ALP evaluation*

PSA levels were evaluated using the Magimuzyme III Shengzhen, CHINA. The ALP levels were assayed using the HITACHI automatic analyzer 7020.

*Histopathological examination and immunohistochemistry*

Tissue samples were fixed in neutral buffered formalin, embedded in paraffin and sectioned at a thickness of 5 μm. One part of sections was stained with hematoxylin and eosin for routine histopathological examination. The other sections were then determined for anti-human α-methylacyl coenzyme A racemase (p504s), p63 and basal keratin antibodies (34βE12) by immunohistochemistry kit (Pacific biotech Co. Fujian, China).

*Microarray assay*

Total RNA from each sample was quantified using the NanoDrop 1000, and RNA integrity was assessed by standard denaturing agarose gel electrophoresis. Approximately 5 μg of total RNA from each sample was used for labeling and array hybridization. Firstly, reverse transcription was performed with the Invitrogen Superscript ds-cDNA synthesis kit, and then ds-cDNA labeling was done using the NimbleGen one-color DNA labeling kit. Next, array hybridization was accomplished using the NimbleGen Hybridization System, which was followed by washes with the NimbleGen wash buffer kit. Lastly, array scanning was performed using the Axon GenePix 4000B microarray scanner (Molecular Devices Corporation).

Scanned images (TIFF format) were then imported into the NimbleScan software (version 2.5) for grid alignment and expression data analysis. The expression data were normalized using quantile normalization and the Robust Multichip Average (RMA) algorithm, which was included in the NimbleScan software. The probe level (*_norm_RMA.pair) files and gene level (*_RMA.calls) files were generated after normalization. The two gene level files were imported into the Agilent GeneSpring GX software (version 11.5) for further analysis. Genes that had values greater than or equal to cutoff of 50.0 in all samples ("all targets value") were chosen for data analysis. Differentially expressed genes were identified through fold change filtering. Pathway analysis and GO analysis were performed to determine the roles of these differentially expressed genes in various biological pathways or GO terms. Finally, hierarchical clustering was performed to determine distinguishable gene expression profiling among samples.

## Results

*The determination of serum PSA and ALP concentration*

Serum PSA concentration obviously changed from 0.7 ng/mL to 6.2 ng/mL and 6.8 ng/ml on day 0, day 30 and day 60, respectively in one of PC-3 cancer cells treated cynomolgus macaques, for the other monkey, the data were 0.7 ng/mL, 5.9 ng/mL and 6.6 ng/mL accordingly. At the same time, no change in mock group was observed; whereas the serum concentration of ALP in all monkeys was changed from 537 to 658 U/mL. No statistic difference between the two groups is present. The detailed data were shown in Table 1 and Table 2.

*Histopathological examination and Immunohistochemistry*

From the result of histopathological examination, the cancerous lesions were visible under a light microscope. The structure of the basal cells was abnor-

**Table 1 -** The determination of serum PSA concentration

| Conc / Day | 1# (ng/mL) | 2# (ng/mL) | 3# (ng/mL) | 4# (ng/mL) |
|---|---|---|---|---|
| 0 | 0.7 | 0.7 | 0.4 | 0.4 |
| 30 | 5.9 | 6.2 | 1.3 | 1.2 |
| 60 | 6.6 | 6.8 | 1.5 | 1.4 |

Note: Monkey 1#, 2# belongs to the monkeys treated by PC-3 cancer cells; Monkey 3#, 4# belongs to the mock group

**Table 2 -** The determination of serum ALP concentration

| Conc / Day | 1# (U/mL) | 2# (U/mL) | 3# (U/mL) | 4# (U/mL) |
|---|---|---|---|---|
| 0 | 564 | 537 | 544 | 623 |
| 30 | 599 | 587 | 630 | 597 |
| 60 | 658 | 562 | 615 | 569 |

Note: Monkey 1#, 2# belongs to the monkeys treated by PC-3 cancer cells; Monkey 3#, 4# belongs to the mock group

mal and partially absent. Lesions had infiltrated the nerves which were equivalent to those found in human prostate cancer specimens (Gleason grade II) as shown in Fig. 1, 2 and 3. No change in the prostate of mock group was observed as shown in Fig. 4. The results of immunohistochemistry indicated that strong immunoreactions against p504s were detected in these sections, whereas p63 and 34βE12 staining also significantly showed the same effect, which indicated that the prostate cancer had been developing in the cynomolgus macaques. The results were shown in Fig. 5, 6 and 7.

*Microarray assay*

NimbleGen 12135K microarray containing 45,034 human gene probes was used to detect gene expression in the PC-3 cancer cells treated two monkey samples. More than 39,068 genes expressed in all the samples were present. Retrieved from OMIM database, 67 genes were related with prostate cancer: 26 genes were highly expressed in cancer groups, 26 genes had almost the same expression intensity and 2 genes were highly expressed in the control group. Thirteen genes had no expression in all the samples,

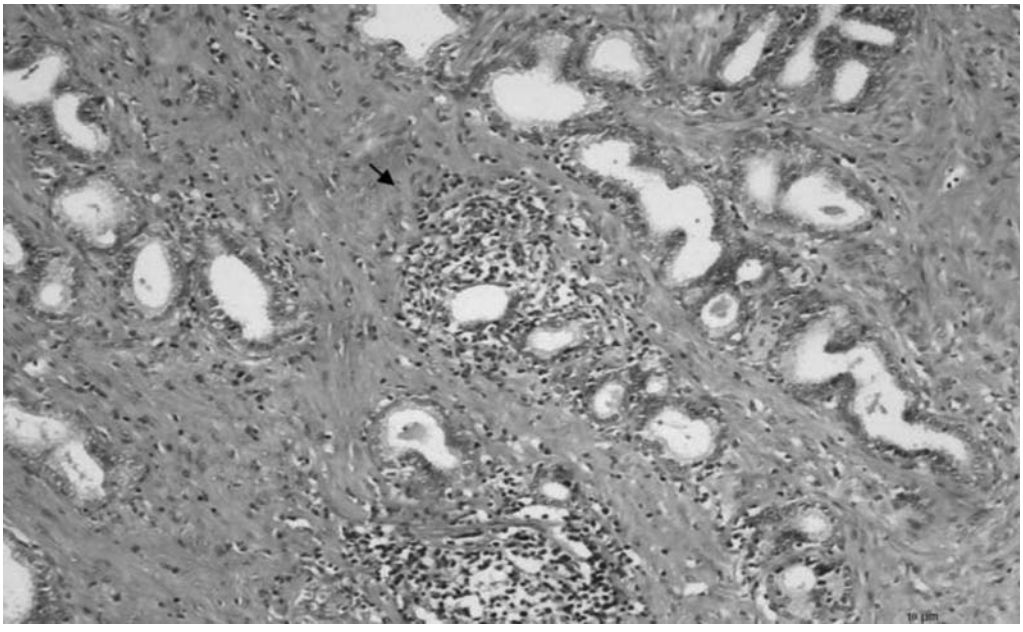

**Fig. 1.** Cancer lesion (H&E staining, 10×). Uniform proliferation of small glands, single cell layer, and prominent nucleoli in the prostate tissue of PC-3 cancer cells treated monkeys

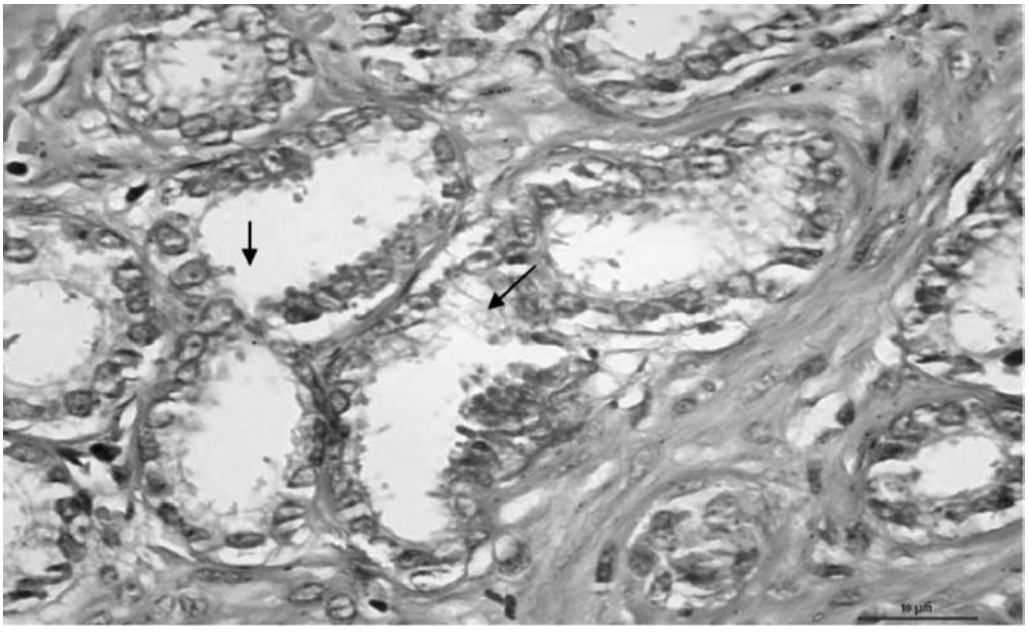

**Fig. 2.** Basal cell staining is partially absent in PC-3 cancer cells treated monkeys. (H&E staining, 40×)

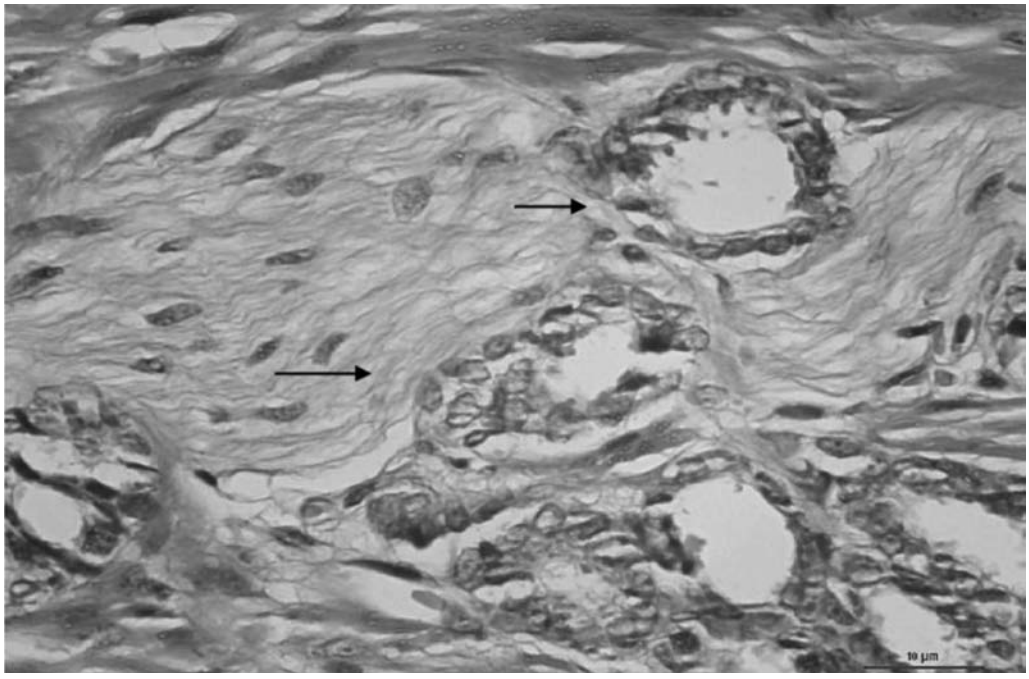

**Fig. 3.** Lesion infiltrates into the nerve fiber in the prostate tissue of PC-3 cancer cells treated monkeys. (H&E staining, 40×)

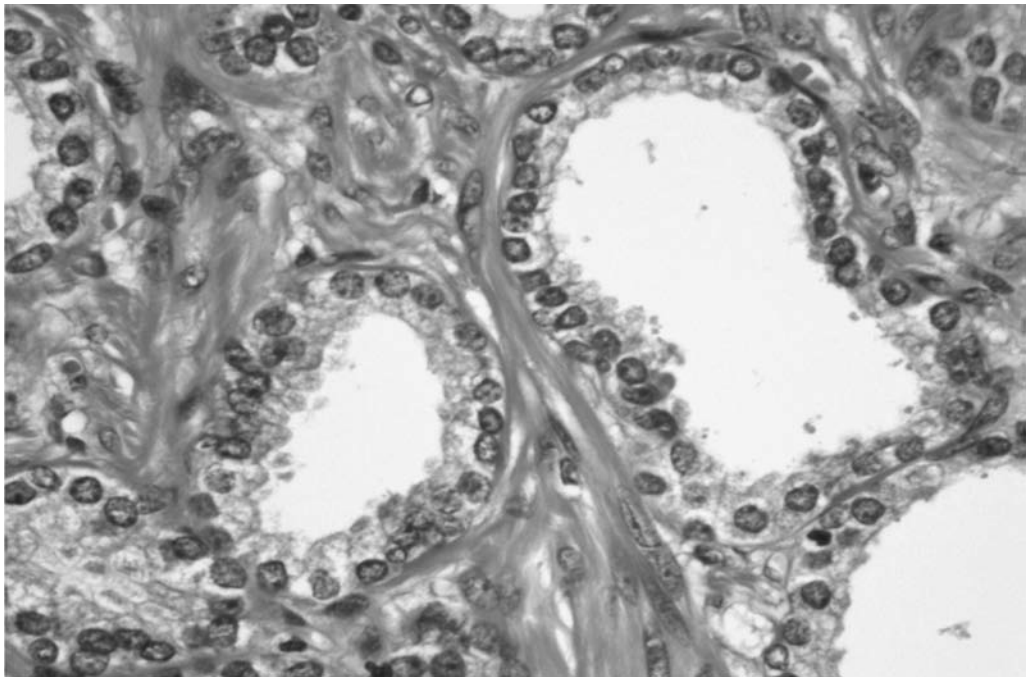

**Fig. 4.** The normal prostate tissue of the monkeys (H&E staining, 40×). Normal tissues have an integrated ring of double-layer basal cells in the prostate tissue

those genes are hereditary in prostate cancer as shown in Table 3.

*Pathway analysis and GO analysis*

Pathway analysis is a functional analysis mapping genes to KEGG pathways. The p-value (Fisher-P value) denotes the significance of the pathway correlated to the conditions. Lower is the p-value, more significant is in the pathway. From the result, it showed several signal pathways which were significantly changed in the samples. In up regulatory gene list, the most notably changed signal pathway was focal adhesions. Focal adhesions are large, dynamic protein complexes through which the cytoskeleton of a cell connects to the extracellular matrix (ECM). Up regulation of focal adhesions means that cell establishes new contacts at the lead-

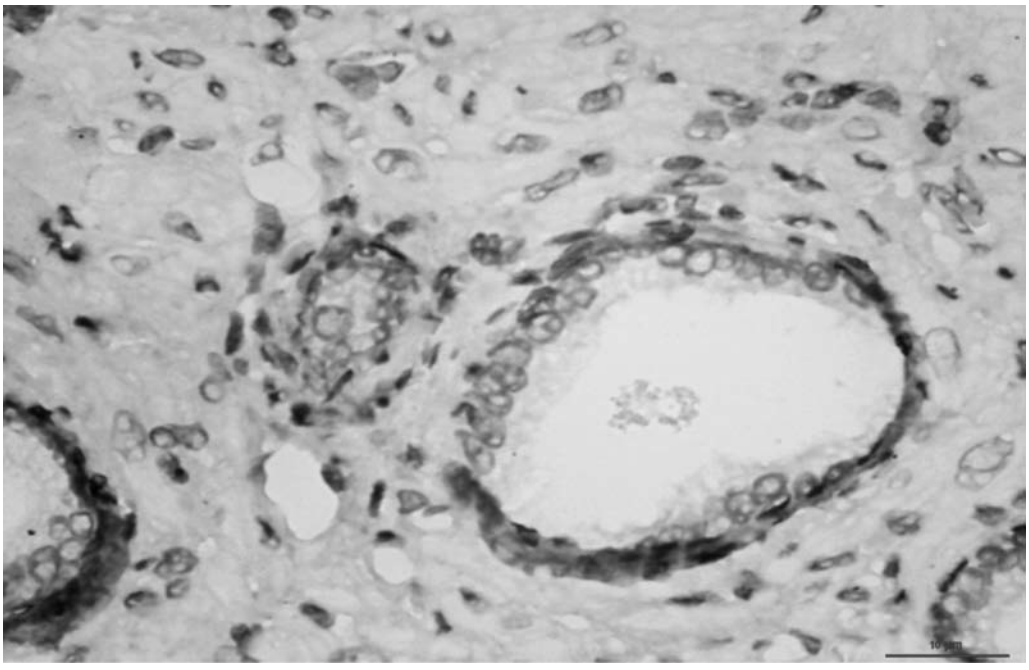

**Fig. 5.** P504s immunohistochemistry staining showed positive in the prostate tissue of PC-3 cancer cells treated monkeys

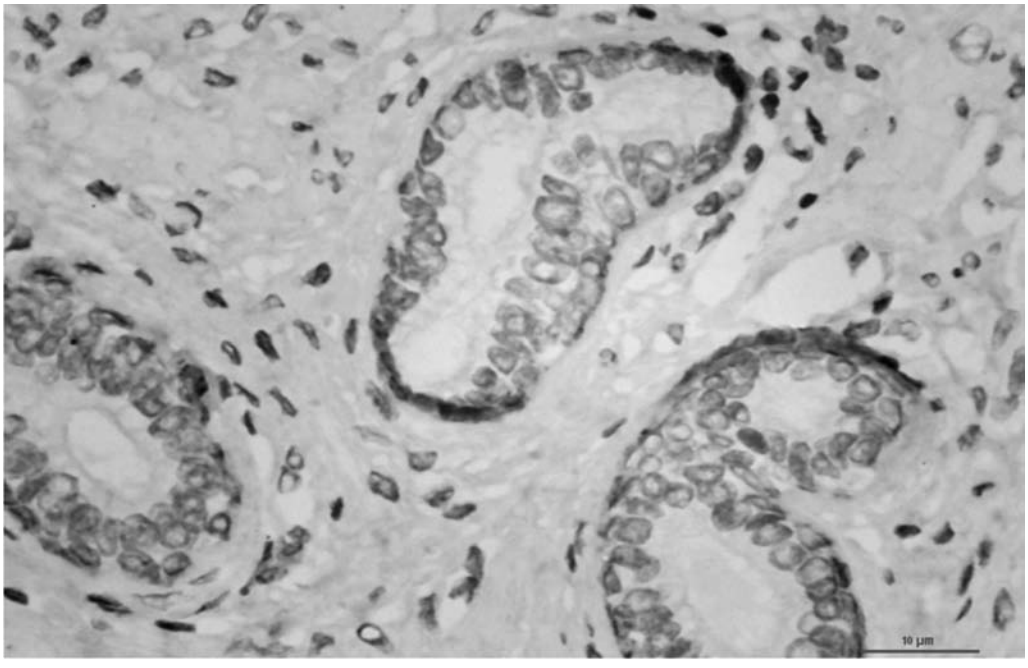


**Fig. 6.** Basal cell staining is partially absent (34βE12 staining) in the prostate tissue of PC-3 cancer cells treated monkeys. Normal tissues should have integrated, double-layer basal cells with positive staining

ing edge, and breaks old contacts at the trailing edge of the cell (12). The second up regulated signal pathway was cell-cell adherens junctions (AJs). Cell-cell adherens junctions, the most common type of intercellular adhesions, are important for maintaining tissue architecture and cell polarity (13). The third up regulated signal pathway was pathway in cancer, the other up regulated signal pathways related with cancer and metastasis too. These results demonstrated that cancer were growing and have invaded normal prostate tissues. Here, we also presented the down regulated signal pathway.

The Gene Ontology project provides a controlled vocabulary to describe gene and gene product attributes in prostate. The oncology covers three domains: biological process, cellular component and molecular function. Fisher's exact test is used to find if there is more overlap between the differentiated expressed genes list and the GO annotation list that would be expected by chance. The results were displayed in figures 8-12.

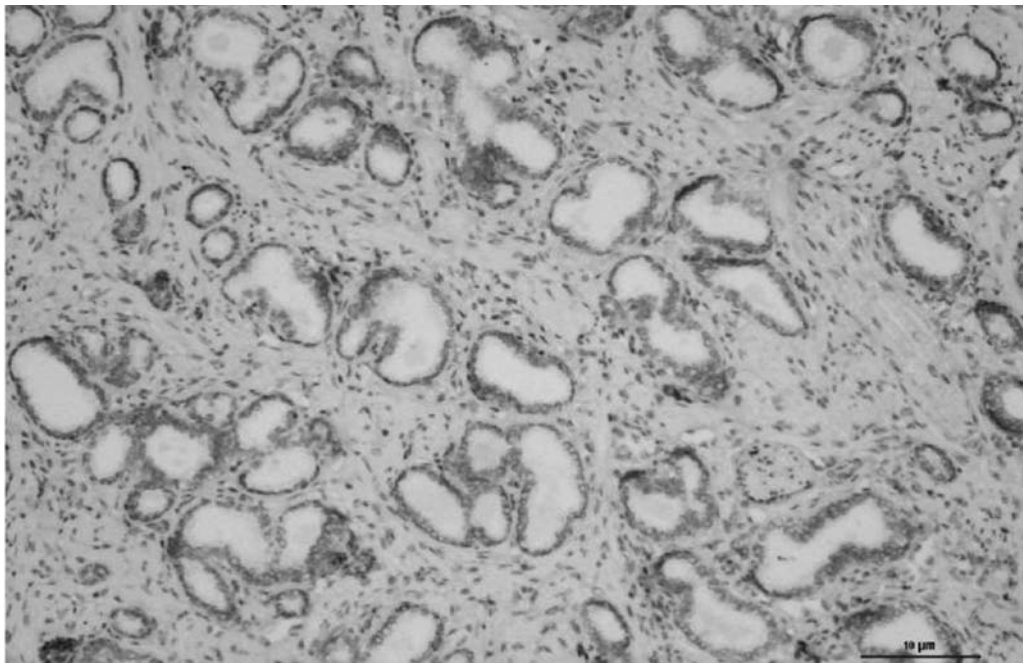

**Fig. 7.** P63 was poorly expressed in the prostate tissue of PC-3 cancer cells treated monkeys

**Table 3 -** The results of the prostate cancer gene expression studies

| | Gene name | OMIM ID | NCBI Gene id | No. 1 expression intensity | No. 2 expression intensity | No. 3 expression intensity | No. 4 expression intensity |
|---|---|---|---|---|---|---|---|
| 1 | LMTK2 | 610989 | 22853 | 121.48059 | 150.2575 | — | — |
| 2 | RANKL | 602642 | 8600 | 69.592094 | 84.36788 | — | — |
| 3 | SLC45A3 | 605097 | 85414 | 61.627987 | 98.2096 | — | — |
| 4 | WAF/CIP1 | 116899 | 56996 | 1737.507 | 172.85983 | — | — |
| 5 | SMOOTHENED | 601500 | 6608 | 1060.9148 | 902.8898 | — | — |
| 6 | CHEK2 | 604373 | 11200 | 810.28723 | 450.8422 | — | — |
| 7 | LFS2 | 609265 | 11200 | 696.5546 | 982.6284 | — | — |
| 8 | EHBP1 | 609922 | 23301 | 1793.9758 | 2359.1165 | — | — |
| 9 | STAT3 | 102582 | 6774 | 15922.543 | 17672.816 | 1983.9445 | 1335.1206 |
| 10 | HNF1B | 189907 | 6928 | 7335.158 | 1206.0488 | 528.43555 | 619.29694 |
| 11 | HNF1B | 189907 | 6928 | 7335.158 | 1206.0488 | 528.43555 | 619.29694 |
| 12 | SRD5A2 | 607306 | 6716 | 1873.0391 | 214.94095 | 169.64464 | 139.2128 |
| 13 | ETV1 | 600541 | 2115 | 558.1835 | 1162.6713 | 231.73816 | 125.612854 |
| 14 | JAZF1 | 606246 | 221895 | 1186.9906 | 574.8173 | 142.37383 | 231.82152 |
| 15 | IKK-β | 603258 | 3551 | 2130.8887 | 689.12427 | 282.0492 | 319.8382 |
| 16 | C15ORF21 | 611314 | 283651 | 22481.943 | 22285.21 | 3755.7407 | 7182.1343 |
| 17 | CHUK | 600664 | 1147 | 764.18286 | 612.1543 | 191.9847 | 144.3884 |
| 18 | ATBF1 | 104155 | 463 | 19745.113 | 6142.2876 | 4404.0444 | 3428.3882 |
| 19 | PLXNB1 | 601053 | 5364 | 3592.962 | 668.8797 | 325.26834 | 1009.72577 |
| 20 | KLF6 | 602053 | 1316 | 958.2522 | 3218.1858 | 855.0163 | 458.56622 |
| 21 | TMRSS2 | 602060 | 7113 | 798.72327 | 118.047295 | 189.37193 | 107.9271 |
| 22 | AKT1 | 164730 | 207 | 2812.9043 | 3336.4336 | 836.3693 | 1162.3569 |
| 23 | PIM1 | 164960 | 5292 | 3967.7131 | 3315.856 | 860.14325 | 1552.2163 |
| 24 | MXI1 | 600020 | 4601 | 1296.7134 | 844.1968 | 276.09998 | 434.21225 |

*(continued)*

**Table 3 -** The results of the prostate cancer gene expression studies

| | Gene name | OMIM ID | NCBI Gene id | No. 1 expression intensity | No. 2 expression intensity | No. 3 expression intensity | No. 4 expression intensity |
|---|---|---|---|---|---|---|---|
| 25 | HNRNPA2B1 | 600124 | 3181 | 27862.186 | 10644.573 | 7904.1196 | 6569.5405 |
| 26 | CAPB | 603688 | 832 | 3949.584 | 7014.743 | 2120.4316 | 2749.6943 |
| 27 | HPC3 | 608656 | 57332 | 301.89532 | 359.45703 | 170.29254 | 228.14503 |
| 28 | CTBP2 | 602619 | 1488 | 2221.0767 | 756.74756 | 692.9266 | 1145.1272 |
| 29 | CDH13 | 601364 | 1012 | 226.07037 | 531.79614 | 297.31412 | 184.25725 |
| 30 | HPC1 | 601518 | 6041 | 254.90636 | 202.56084 | 126.41432 | 184.82089 |
| 31 | NF-K-B | 164011 | 4790 | 1406.5033 | 1419.2499 | 1008.4077 | 971.944 |
| 32 | MAD1L1 | 602686 | 8379 | 1101.828 | 1154.2561 | 1311.9111 | 294.23456 |
| 33 | H4 | 602824 | 8030 | 1205.6135 | 2285.8167 | 1258.8983 | 1366.2802 |
| 34 | MYC | 190080 | 4609 | 2813.62 | 1986.7385 | 2580.4626 | 1279.7106 |
| 35 | CDKN1B | 600778 | 1027 | 205.03937 | 280.13477 | 177.44113 | 256.89847 |
| 36 | SUZ12 | 606245 | 23512 | 569.1282 | 353.34283 | 695.879 | 150.74788 |
| 37 | HPC2/ELAC2 | 605367 | 60528 | 1425.4224 | 754.29584 | 1637.0469 | 695.5171 |
| 38 | EPHB2 | 600997 | 2048 | 555.4931 | 258.13806 | 348.94122 | 532.2502 |
| 39 | CPNE3 | 604207 | 8895 | 276.47766 | 257.05966 | 253.444 | 425.55478 |
| 40 | AMACR | 604489 | 23600 | 340.41797 | 273.66058 | 590.3244 | 225.28502 |
| 41 | GNMT | 606628 | 27232 | 334.40823 | 181.51498 | 368.687 | 339.2348 |
| 42 | IL16 | 603035 | 3603 | 391.3804 | 470.95364 | 580.9791 | 760.5081 |
| 43 | CYP3A4 | 124010 | 1576 | 122.8809 | 112.44357 | 204.86684 | 200.38142 |
| 44 | HPN | 142440 | 3249 | 701.55597 | 204.54227 | 532.5208 | 1081.7612 |
| 45 | ERG | 165080 | 3757 | 610.9698 | 185.72835 | 446.1777 | 296.14017 |
| 46 | PTEN | 601728 | 65018 | 1314.9012 | 1999.4432 | 563.8486 | 1413.0957 |
| 47 | MASPIN | 154790 | 5268 | 142.26503 | 275.00143 | 371.0795 | 1026.8625 |
| 48 | SARDH | 604455 | 1757 | 303.9309 | 429.61615 | 561.0152 | 168.0939 |
| 49 | CDH1 | 192090 | 999 | 1266.7225 | 395.22977 | 637.7579 | 194.4512 |
| 50 | MUC1 | 158430 | 4582 | 206.14589 | 207.70338 | 150.14297 | 198.7649 |
| 51 | MSMB | 157145 | 4477 | 135.41359 | 135.72034 | 8174.657 | 4264.8237 |
| 52 | SHH | 600725 | 6469 | 476.999 | 793.8585 | 1431.2148 | 1304.3707 |
| 53 | AZGP1 | 194460 | 563 | 141.12143 | 159.28871 | 352.10056 | 336.78436 |
| 54 | H3/HIST2H3C | 142780 | 126961 | 510.0495 | 1081.8435 | 1242.1638 | 2699.7698 |
| 55 | MSR1 | 153622 | 4481 | — | — | — | — |
| 56 | HPCX2 | 300074 | 100188769 | — | — | — | — |
| 57 | HPC11 | 611955 | 100240741 | — | — | — | — |
| 58 | HPC8 | 602759 | 7834 | — | — | — | — |
| 59 | HPCX1 | 300147 | 9566 | — | — | — | — |
| 60 | HPCQTL19 | 607592 | 347747 | — | — | — | — |
| 61 | HPC14 | 611958 | 100188867 | — | — | — | — |
| 62 | HPC15 | 611959 | 100188868 | — | — | — | — |
| 63 | HPC4 | 608658 | 408260 | — | — | — | — |
| 64 | HPC5 | 609299 | 619402 | — | — | — | — |
| 65 | HPC6 | 609558 | 100188789 | — | — | — | — |
| 66 | HPC7 | 610321 | 100188809 | — | — | — | — |
| 67 | HPC9 | 610997 | 100188826 | — | — | — | — |

Note: Monkey 1#, 2# belongs to the monkeys treated by PC-3 cancer cells; Monkey 3#, 4# belongs to the mock group.

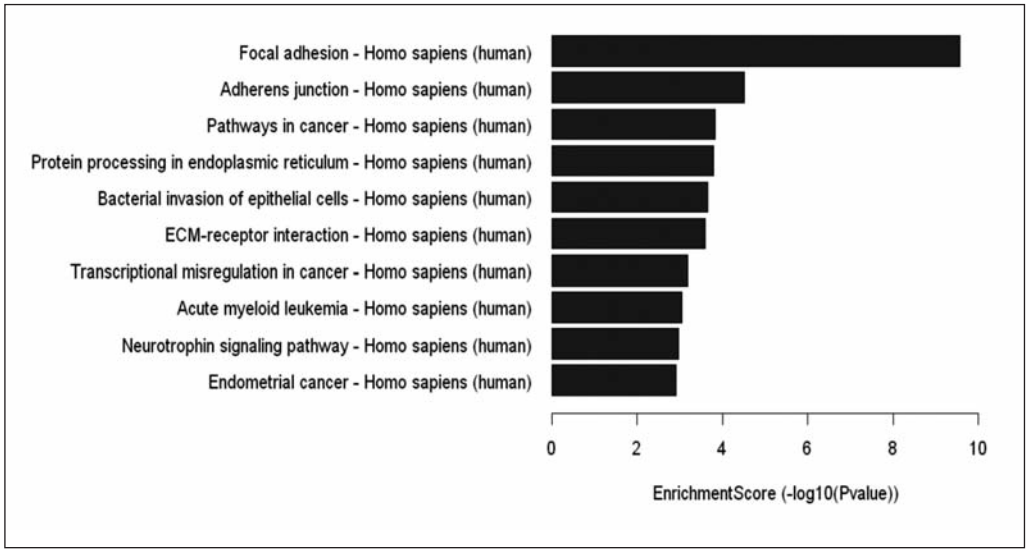


**Fig. 8.** The signal pathway of up regulatory genes

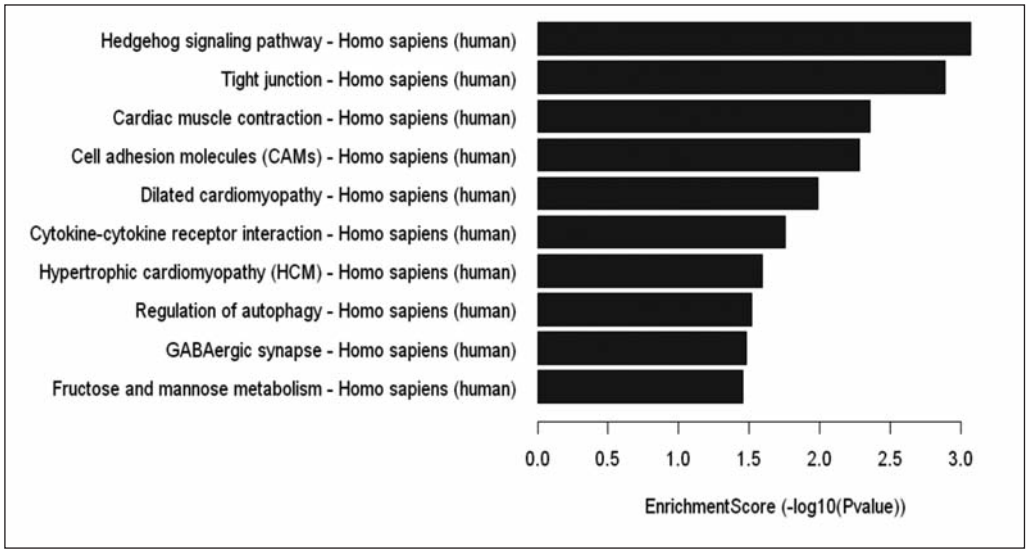


**Fig. 9.** The signal pathway of down regulatory genes

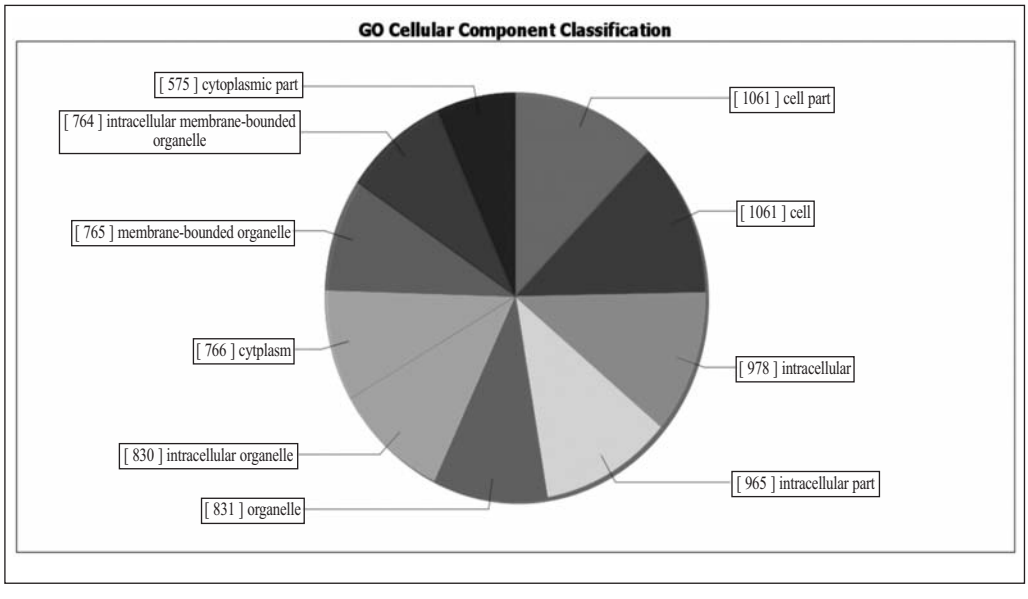


**Fig. 10.** The GO analysis of cellular component

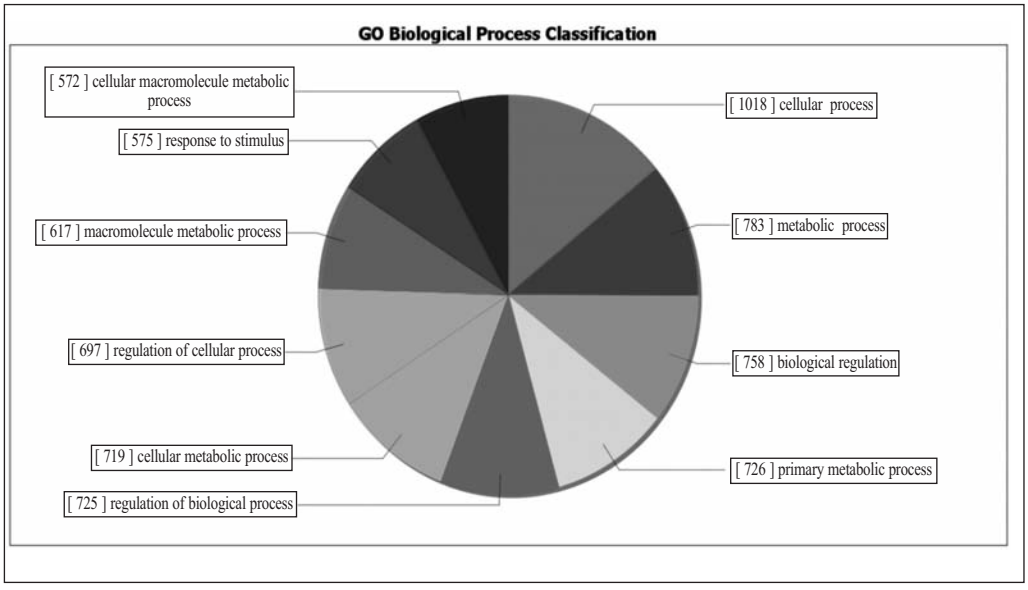


**Fig. 11.** The GO analysis of biological process

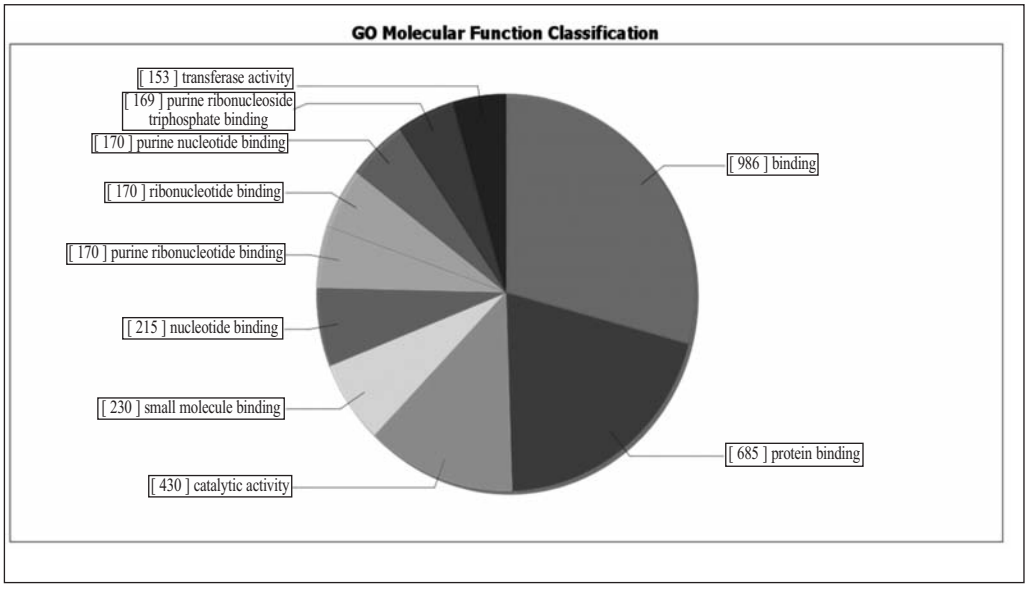


**Fig. 12.** The GO analysis of molecular function

## Discussion

Prostate cancer continues to be a major cause of morbidity and mortality in men around the world. The field of prostate cancer research continues to be hindered by the lack of relevant preclinical models for the study of tumorigenesis and the development of effective preventative and therapeutic strategies. The most widely used models include transgenic mice, variations on SCID mice, and traditional orthotropic and xenotransplantation models. The anatomical, histological, physiological, and biochemical differences between humans and rodents are immense. The currently used prostate cancer preclinical models have hindered the translation of research findings from animal models to human clinical trials. The bottlenecks that have hindered research include the following: the insufficient number of models that appropriately reflect the molecular and biological diversity of human cancer; the lack of understanding regarding the molecular events that define tumorigenesis; the lack of tools for studying the tumor-host interaction; the difficulty of assessing model systems across institutions; and the disconnection between preclinical studies and human clinical trials. Therefore, it is necessary to develop more effective models that mimic progressive and fatal prostate cancer for improving intervention treatment strategies.

In this study, we used PC-3 cancer cells and monkeys to establish an animal model of prostate cancer that had molecular and histological features similar to those of human prostate cancer. Because it was an exploratory experiment, we still have much research work to verify the results.

Prostate-specific antigen (PSA) is prostate cancer biomarker that is present in the serum, which is secreted by both normal and cancerous cells within the prostate gland (14). Although PSA is mostly found in semen, a small amount is also found in the blood. Most healthy men have PSA levels under 4 nanograms per milliliter (ng/mL) of blood. The likelihood of having prostate cancer increases as the PSA level rises. Increasing PSA levels over time are associated with both localized and metastatic prostate cancer (CaP). Alkaline phosphatase (ALP) is a protein that can be found in all body tissues. However, tissues such as the liver, bile duct, and bone have particularly high levels of ALP, when prostate cancer invades the bone, ALP concentrations increase. Thus, PSA and ALP related to prostate carcinoma were selected here for this investigation. Rapidly increased concentrations of PSA are indicative of prostate cancer. In our study, the two PC-3 cancer cells treated monkeys showed significant PSA level changes similar to that which occurs in human prostate cancer. However, ALP levels were not apparently changed in serum, which was partially attributable to the short time span of the study and the cancer did not invade into liver, bile duct and bone. It should be pointed out that ALP is not early index values for the diagnosis of prostate cancer.

Five days after the operation, the wound had healed and was not inflamed. The monkeys were healthy as before and their weights had slightly increased. When the peritoneum was excised following the operation, we found that the surface of the prostate was smooth and without nodes. The diagnostic criteria for prostate cancer include the following: the uniform proliferation of the small glands; the infiltration of nerves or fat and prominent nucleoli. In this study, the histopathological evaluation of prostate in microscope had provided valuable and critical information for the diagnosis of prostate cancer and tissue sections from both monkeys showed typical features of prostate cancer and samples from both monkeys met the diagnostic criteria for prostate cancer and these have been scored as prostate cancer Gleason grades II or III.

Several studies have suggested that α-Methylacyl Coenzyme A Racemase (AMACR) can also be used as a prostate cancer biomarker (15). By evaluating AMACR protein expression in 94 prostate needle biopsy specimens and by classifying strong and intense staining as positive (score = 3 or 4), Rubin et al demonstrated that AMACR has a 97% sensitivity level and a 100% specificity level for detecting prostate cancer. P504s is the antibody for AMACR which can validate the gene expression of AMACR occurred in prostate cancer. 34βE12, which is often written as β34 E12 and is also known as CK34βE12 (7) and keratin 903 (CK903), is an antibody specific for high molecular weight cytokeratins that is used to stain basal cells in prostatic glands. Loss of basal cells often occurs in prostate adenocarcinomas, which are the most common form of prostate cancer. Tumor protein p63, which is also known as transformation-related protein 63, is a protein that is encoded by the TP63 gene in humans discovered 20 years after the discovery of the p53 tumor suppressor gene (16). Based on structural similarities, TP63 and p73 constitute the p53 gene family. Despite being discovered significantly later than p53, phylogenetic analyses of p53, p63, and p73 suggest that p63 was the original member of the family from which p53 and p73 evolved. Immunostaining for p63 may be useful for differentiating between prostate adenocarcinomas and benign prostate tissue, as normal prostatic glands maybe stained with p63 because they contain basal cells, whereas malignant glands in prostatic adenocarcinomas do not stain for p63 because they lack basal cells. Immunohistochemistry for p504s, 34βE12, and p63 was carried out using monoclonal mouse anti-human antibodies. The absence of basal cells and specific immunohistochemical reactions is labeled as 34βE12 and p63 were negative and p504s was positive, which indicated AMACR gene had expressed in the treated prostate tissue and the lost of 34βE12 and p63 also verified such effect.

Cancer lesions cannot originate from healthy cynomolgus macaques. Mubiru *et al.* reported that a total of 114 baboons, 66 cynomolgus macaques, 12 rhesus macaques, and 5 marmosets were examined across their entire lifespans, and none was found to develop prostate cancer lesions (17). Additionally,

prostate cancer often affects older men. However, in our study, the monkeys were 4 years old and just reached sexual maturity, which is the age equivalent to an adolescent human. In this study, the young monkeys were not likely to develop spontaneously prostate cancer unless artificially inoculation of PC-3 cancer cells under immune system inhibition.

To further confirm the reliability of establishment of a prostate cancer animal model using cynomolgus macaques, the NimbleGen 12x135K microarray containing 45,034 human gene probes was used to detect gene expression in the four monkey samples and demonstrate similarities between monkeys and humans. The microarray analysis indicated that there were more than 41,000 of these genes that were expressed in the monkey samples. Additionally, 54 of 67 prostate cancer-related genes were expressed in these two treated monkeys, whereas the remaining expressed genes were related to familial inheritance. PC-3 cancer cells were originally derived from a male patient who had prostate cancer with metastases of the bone (Gleason grade IV) and have high metastatic potentiality. However, in our study, we found no metastases to the lymph nodes or bone, which may be due to short span of our study.

So, we can have the primary conclusion that the cancerous lesions in the monkeys' prostates were derived from the inoculation of the PC-3 cells. The exhibition of prostate cancer in these monkeys was very similar to that of humans in terms of its histology, biochemistry, immunology, and gene expression profile. The monkey prostate cancer animal model could be useful for disease research and drug efficacy validation. Traditional rodent animal models have apparent deficiency in drug efficacy study, for example, some candidate compounds had demonstrated activity in rodent animals but had no effect in clinic research. Alternatively, some compounds had minor effects in rodents but had shown strong effects in humans (i.e., taxol). As a result, these major differences between rodents and humans in their response to anti-tumor drug have limited the development of various anti-tumor drugs. The use of cynomolgus macaques cancer animal model could improve and accelerate the process of anti-tumor drug discovery and research.

The investigation disclosed the fact that cyclosporine could help cancer cell grow in normal monkey. The mechanism of cyclosporine is still unclear and the relationship between the immune system and cancer is complicated (18). The capacity of immunity to control and transfer to form cancer, is the result of three processes that function either in a dependent manner or in a developing sequence: elimination (cancer immunity surveillance, in which immunity functions as an extrinsic tumor suppression or in the naive hosts); equilibrium (expansion of transformed cells is held in check by immunity); and escape (tumor cell variants with dampened immunogenicity or the capacity to attenuate immune responses grow into clinically apparent cancers). The equilibrium process was largely inferred from clinical observations, including reports of transplantation of undetected or occulted cancer from organ donor into immunosuppressed recipients (19). Herein monkey model could be used to demonstrate that equilibrium has occurred and mechanistically disclosed the details of elimination and escape in vivo.

To our knowledge, this is the exploratory report describing the availability of cyclosporine for the establishment of cancer animal model. The present study also provided the potentiality to establish other types of animal models by this method. However, there were still some limitations in this study. For example, the whole span of the cancer growth was only 60 days which was quite short for the detection of cancer metastasis. Approximately 180 to 720 days may represent a more adequate amount of time for observing metastases to the bone and lungs. A further study has been designed to verify the hypothesis of the metastasis process and will be done based on the current study.

## Conclusions

In this study, we established the cynomolgus macaque model of human prostate carcinoma through the orthotopical injection of the PC-3 cell line in situ. This model may effectively simulate the biological characteristics of human prostate carcinoma. This is the first exploratory report to establish prostate cancer animal model in cynomolgus monkeys. Due to the advantages in our animal model, it could promote the prostate cancer research and exploration of new chemotherapy agents for prostate cancer.

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