Comparative Study of Prostate Tissues in Non-human Primate (Cynomolgus Monkey) and Human on the Histomorphology and Immunohistochemistry

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Abstract

AIM: To compare the differences of prostate between cynomolgus monkey and human on the histomorphology and immunohistochemistry and lay a foundation for the establishment of animal experimental model of prostate disease. METHODS: The intact prostates of 9 naturally dead cynomolgus monkeys, 9 electrosurgical tissues of human benign prostatic hyperplasia and 36 prostate tumors (9 in each of the low, medium and high risk and metastatic groups) were collected. The samples were fixed by neutral formalin, embedded by paraffin, sliced up, stained by HE and IHC and observed with microscope in histomorphology and immunohistochemistry. 54 tissue samples which immunohistochemical staining (P504s, P63, 34βE12, PSA, AR, P53, PTEN, RB1) were used for qualitative evaluation by positive staining cell score. RESULTS：(1)HE staining revealed that there was a basal cell layer in the prostatic tissues of cynomolgus monkey, with enlarged nucleoli of few glandular epithelial cells, which was similar to the morphological characteristics of human prostatic hyperplasia, but significantly different from that of human prostate tumors of different grades, with basal cell loss and enlarged nucleoli. (2)Immunohistochemical staining results showed that the expression of P504s, P53, PTEN and RB1 in the prostatic tissues of cynomolgus monkey and human prostatic hyperplasia tissues were all negative (1 point), with high expression of P63, 34βE12, AR (3.78-4 points) .A small amount of PSA was expressed in human prostatic hyperplasia tissues(1.78 points) while the expression level in cynomolgus monkeys was negative (1.11 points).In human prostate tumor tissues, the expression of P504s, PSA and AR increased significantly (3.67-4 points), while the low expression of P63 and 34βE12, P53, PTEN and RB1 (1-1.33 points).The expression of related markers in the prostatic tissues of cynomolgus monkey showed no statistically difference with human prostatic hyperplasia (P >0.05), but statistically difference with human prostatic tumor tissues (P<0.05). CONCLUSION: The prostatic tissue of cynomolgus monkey was similar to that of human benign prostatic hyperplasia， and different from that of prostate tumors of different grades. The expression of P504s, PSA, P53, PTEN and RB1 in immunohistochemistry were low, while the expression of P63, 34βE12 and AR were high. The expression of related markers in benign prostatic hyperplasia tissues of cynomolgus monkeys was similar to that of human, but significantly different from tumor in P504s, P63, and 34βE12. Cynomolgus monkeys could be used as one of the best model for the study of prostate cancer.

Keywords

Prostate, Non-human primate, Cynomolgus monkey, Immunohistochemistry.

1. Introduction

The prostate is a male reproductive organ, and prostate cancer is highly prevalent in male reproductive tumors[1, 2]. Experimental models of prostate animals such as mice, rabbits, and dogs differ greatly from human prostate tissue in morphology, immune environment, and pathological types [3, 4]. Observed from the molecular time scale of chemistry, mice and human ancestors are separated by about 40 million years, while the common ancestor of non-human primate (NHP) and humans is about 25 million years[5]. The DNA sequence similarity between NHP and humans is as high as 98.77%, which makes NHP's performance in the immune system and drug sensitivity and other related research closer to humans. It may become the best human tumor research model[6]. Cynomolgus Monkey (Macacafascicularis) is one of the most widely used NHP models, mainly used in the research of hematopoietic system, parasitology, embryonic stem cells and nervous system, etc. But it was rarely reported in prostate-related diseases[7-10]. We compared the morphology and expression of tumor markers related to the immunohistochemistry of the prostate tissues of cynomolgus monkeys and humans by microscopy, and confirmed that the morphology and immunohistochemistry-related markers of prostate tissues of cynomolgus monkeys are very similar to humans, which is an ideal animal model for simulating human prostate-related diseases.

2. Materials and Methods

2.1 Materials and Sample

The 9 naturally dead cynomolgus monkeys came from the monkey breeding base of Guangdong Landau Biotechnology Co. Ltd. (2017.01-2019.12). The process was approved by the Animal Care and Use Committee. Prostate tissue was obtained by dissection and made into paraffin sections for this study. 9 cases of benign prostate tissue and 36 cases of prostate tumor tissue were from the Department of Urology and Pathology of the First Affiliated Hospital of Jinan University and the Third Affiliated Hospital of SunYat-sen University (2018.01-2019.12). The above samples were confirmed by pathology, microscopic examination of HE and immunohistochemical staining. The pathological results were interpreted by 2 senior doctors.

2.2 HE and immunohistochemical staining

Table 1. Purchase information of immunohistochemical primary antibody

Antibody	concentration	Brand	Code
PTEN	1:100	ABclonal	A11189
P53	1:100	Novocastra	P53-DO7-L-CE
PSA	RTU	Zsbio	ZM-0218
RB	RTU	Zsbio	ZM-0223
AR	1:50	MXB	RMA-0807
P504s	1:50	MXB	KIT-8802
P63	1:100	MXB	KIT-8802
34βE12	1:100	MXB	KIT-8802

HE staining: Fixed the prostate tissue in 4% paraformaldehyde, embedded in paraffin, and serially sliced at a thickness of 4 μm. After dewaxing by xylene, it was stained with hematoxylin and eosin. The cell morphology was observed under a microscope after neutral gum sealing. Immunohistochemical staining: Place paraffin sections of prostate tissue in a 65℃ incubator and bake for 120 min. Xylene and alcohol were used to dewax and hydrate the sections. Place in citrate buffer (PH=6.0) and heat for antigen repair. Incubated at room temperature for 10 min by 3% H2O2 endogenous peroxidase blocking solution. Blocked at room temperature for 15 min by goat serum working solution. P504s, P63, 34βE12, PSA, AR, P53, PTEN, RB1 primary antibody (50ul) were

added (Table 1 for antibody information). Place in an incubator at 4℃ overnight. After rewarming, we add 50ul of biotin-labeled goat anti-mouse / rabbit secondary working solution to each slice. Incubated at 37 ℃ for 30 min. Add HRP-labeled streptomyces Avidin at 37℃ for 10 min.Then freshly prepared DAB developer was added. After counterstained by hematoxylin, and air-dried by gradient alcohol dehydration, the slice was put into xylene for 5 min. Finally, it was sealed with neutral resin and observed under OLYMPUS FV1000 laser confocal microscope.

2.3 Analysis of immunohistochemistry results

P504s, PSA, and PTEN exist in the prostate cytoplasm. The presence of brown particles in the prostate cytoplasm is positive. The ratio of the number of cells expressed by the brown particles to the total number of cells is used as the judgment standard. The results are recorded as: cytoplasm is not staining (negative, 1 point), cytoplasm appears weak brown particles <25% (weak positive, 2 points), 25% - 50% cytoplasm appears brown particles (positive, 3 points), > 50% cytoplasm diffuse brownish yellow or brown particles (strong positive, 4 points).

P63 and 34βE12 respectively label the nucleus and cytoplasm of prostate basal cells. The coloring is orange-red. The grading standard is: basal cell nuclei and / or cytoplasm is negative without staining (1 point); the basal cell layer has less discontinuous cytoplasm and/or nuclei staining, and the missing cell region ≥25% is weakly positive (2 points); the basal cell layer is colored and interrupted, and the missing cell area <25% is moderately positive (3 points); the basal cell layer colored continuously and obviously is strongly positive (4 points).

AR, P53, and RB1 are positive for the appearance of brown particles in the nucleus. The judgment standard is the same as above.

2.4 Statistical analysis

Statistical analysis was performed using SPSS 22.0 software. The measurement data were expressed as mean ± SD, and the rank sum test was used to compare the data between multiple groups. $P<0.05$ indicated that the differences were statistically significant.

3. Results

3.1 Determination of PSA value of cynomolgus monkey prostate

The value of PSA expression in cynomolgus monkeys cannot be measured, which is close to the normal PSA level of humans (0-4ng/ul) [11]. The prostate size of cynomolgus monkeys is also similar to the normal size of human prostate (2*3*4cm) by weight conversion [12] (Table 2).

Table 2. Specimen information of cynomolgus monkey, BPH and prostate tumor (mean±SD, %)

Group（n）		Cynomolgus monkey（9）	BPH（9）	Prostate tumor（36）
Age（y）		19±3.7（14~24）	57.6±3.9（53~67）	66.1±7.0（53~81）
	10~29	9（100）	-	-
	30~49	-	-	-
	50~69	-	9（100）	26（72.2）
	70~89	-	-	10（27.8）
Prostate Size（g）		3.99±1.0 （3.3~6.80）	45.2±20.3 （21.3~76.9）	32.6±18.8 （10.4~123.41）
	0~20	9（100）	-	5（13.9）
	21~40	-	5（55.6）	24（66.7）
	41~60	-	3（33.3）	5（13.9）
	> 60	-	1（11.1）	2（5.6）
tPSA（ng/ml）		0.003±0.006 （0~0.017）	2.7±1.7（0.3~4.5）	32.1±27.9 （3.84~105）
	0~4	9（100）	6（66.7）	1（2.8）

	4~10	-	3（33.3）	6（16.7）
	10~20	-	-	10（27.8）
	> 20	-	-	19（52.8）
Weight（kg）		9.16±2.4 (6.80~14.55)	63.7±8.5 (51.9~76.2)	-
	5~25	9（100）	-	-
	26~45	-	-	-
	46~65	-	5（55.6）	-
	66~85	-	4（44.4）	-
	≥86	-	-	-
Pathological T stage	T1	-	-	-
	T2	-	-	21（58.3）
	T3	-	-	15（41.7）
	T4	-	-	-
Puncture.GS	6（3+3）	-	-	7（19.4）
	7（3+4/4+3）	-	-	9（25.0）
	8（4+4/5+3）	-	-	15（41.7）
	9（4+5/5+4）	-	-	5（13.9）
	10（5+5）	-	-	-
postoperative.GS	6（3+3）	-	-	9（25.0）
	7（3+4/4+3）	-	-	9（25.0）
	8（4+4/5+3）	-	-	9（25.0）
	9（4+5/5+4）	-	-	9（25.0）
	10（5+5）	-	-	-
Pathological lymph node	Positive	-	-	6（16.7）
	Negative	-	-	30（83.3）
Postoperative cut edge	Positive	-	-	14（38.9）
	Negative	-	-	22（61.1）

3.2 Histomorphology of cynomolgus monkey and human prostate tissue with HE staining

The histomorphological results of HE staining showed that there were basal cell layers in the prostate tissue of cynomolgus monkey, and few cases of enlarged nucleoli of glandular epithelial cells. In the human prostatic hyperplasia tissue, part of the glands were dilated, the hyperplasia of glands and smooth muscle was obvious, and the basal cell was continuous. The morphology of the two tissues was similar. The low-risk group of prostate tumors showed that the tumor cells formed a single gland.

The gland was small, irregular in shape, closely arranged, with few stroma and discontinuous basal cells. In the middle-risk group, the glands were small and fused, showing small clusters of cells with irregular boundaries, large and dark nuclei, and irregular nucleoli. The high-risk group showed that the glandular structure was disappeared accompanied by large and pale stained cells ("adrenoid"). In the metastasis group, normal acinar structure and basal cells were basically disappeared, lumen cells were increased, accompanied by increased nuclear chromatin and enlarged nucleoli. The tumor cells were irregular masses composed of solid sieve tumors and grew into pieces or cords. The changes in human prostate tumor tissues were significantly different from cynomolgus monkeys and human prostate hyperplasia tissues (Figure 1).

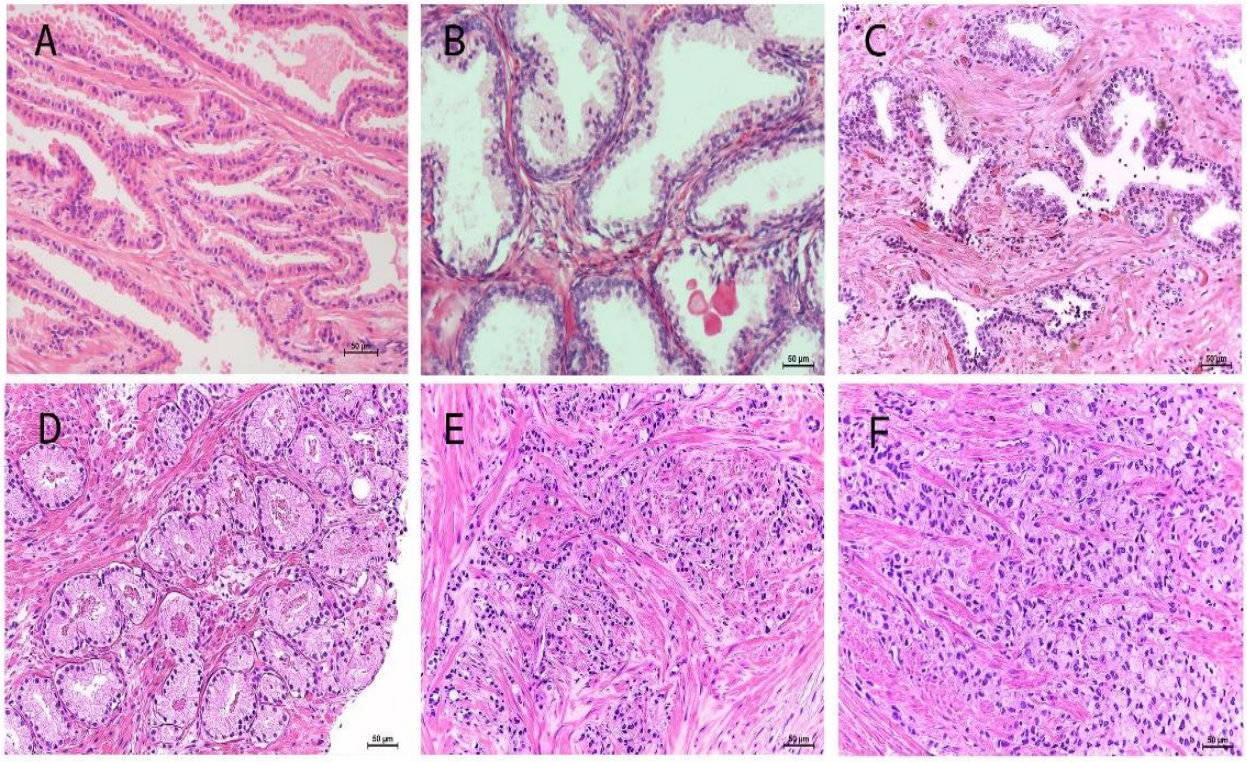

Figure1: Comparison of histomorphology between cynomolgus monkey and human prostate (HE staining, ×200). A: Cynomolgus monkey group; B: BPH group; C: PCa Low-risk group; D: PCa Medium-risk group; E: PCa High-risk group; F: PCa Metastatic groups.

3.3 Immunohistochemical comparison of cynomolgus monkey and human prostate tissue

The expression levels of P504s, P53, PTEN and RB1 in the prostate tissues of cynomolgus monkey and human prostate hyperplasia were all low, but the expression levels of P63, 34βE12 and AR were high. In the human prostatic hyperplasia tissues, a small amount of PSA was expressed, while the expression levels of cynomolgus monkeys were negative. In the human prostate tumor tissues, the expression levels of P504s, PSA and AR were significantly increased, while the low expression levels of P63, 34βE12, P53, PTEN and RB1 (figure 2, table 3). The three groups all had high expression levels of AR and low expression levels of P53, PTEN and RB1.There was no significant difference between the prostate tissue of cynomolgus monkey and human prostate hyperplasia by immunohistochemical evaluation ($P>0.05$), but the difference between the prostate tissue of cynomolgus monkey and human prostate tumor tissue (P504s, P63, 34βE12, PSA) was statistically significant ($P<0.05$). These results suggest that the natural prostate tissues of cynomolgus monkeys are similar to normal human prostate tissues in both tissue morphology and prostate specific antibody expression, suggesting that cynomolgus monkeys have a high homology with human genes and are more suitable as animal models for the study of human prostate tumors than mice and other animals.

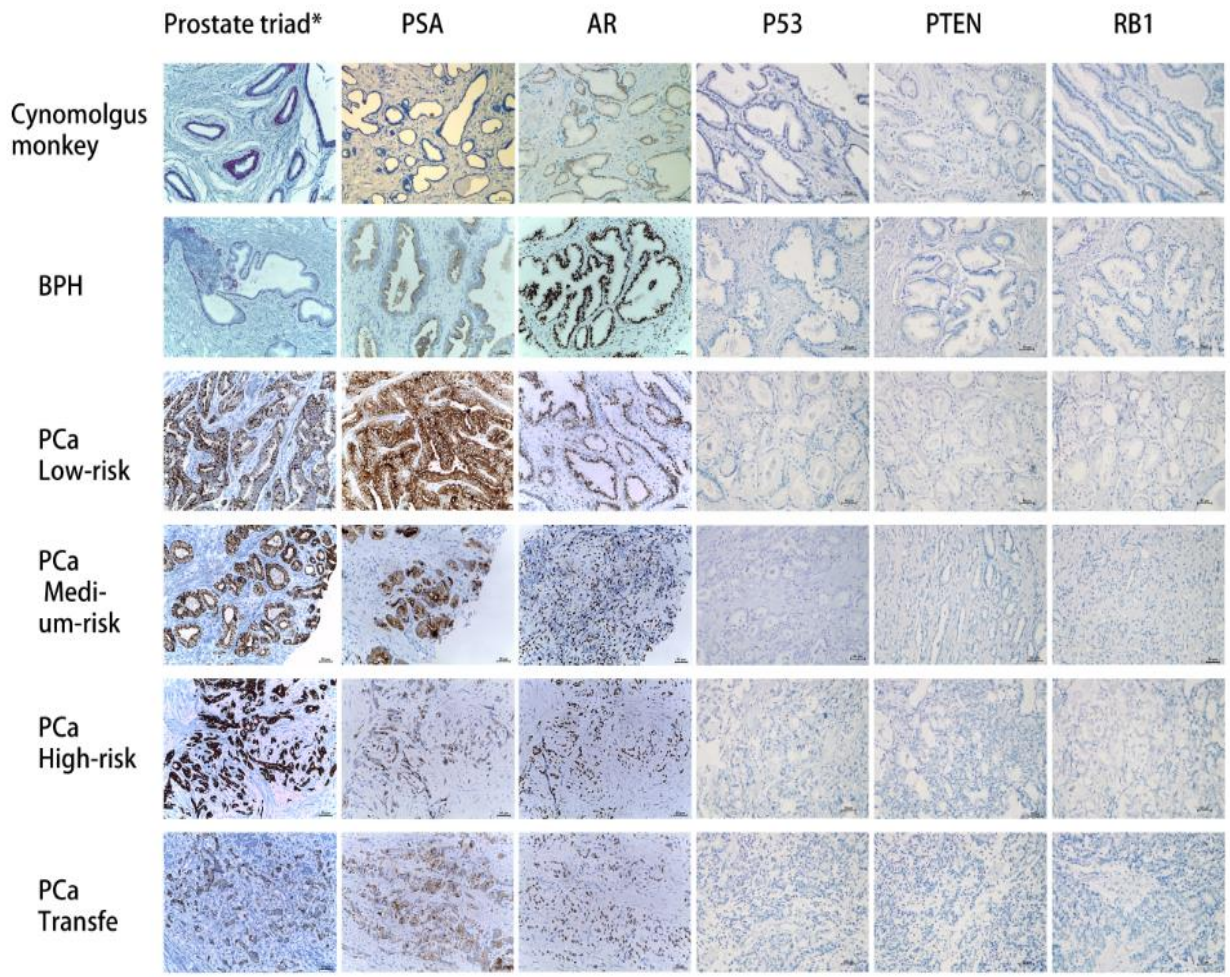

Figure.2: Comparison of immunohistochemical results of prostate markers in cynomolgus monkey and human (s-p method,×200). *Prostate traid means P504s,P63,34βE12.

Table 3. Immunohistochemical scores（mean±SD, n=9）

Group	Prostate-related Gene Expression							
	P504s	P63	34βE12	PSA	AR	P53	PTEN	RB1
Cynomolgus monkey	1	3.78±0.42	3.78±0.42	1.11±0.31	3.89±0.31	1	1	1
BPH	1	3.89±0.31	3.89±0.31	1.78±0.63	4	1	1	1
PCaLow-risk	3.89±0.31	1.22±0.42	1.33±0.47	3.89±0.31	4	1	1	1
PCa Medium-risk	4	1.11±0.31	1.11±0.31	3.89±0.31	4	1	1	1
PCaHigh-risk	4	1	1	3.67±0.47	4	1	1	1
PCaTransfe	4	1	1	3.78±0.42	3.89±0.31	1	1	1
	* P＞0.05 ** P＜0.05	* P＞0.05 ** P＜0.05	* P＞0.05 ** P＜0.05	* P＞0.05 ** P＜0.05	P＞0.05	P＞0.05	P＞0.05	P＞0.05

* Cynomolgus monkey groupvsBPH group； ** Cynomolgus monkey group vs PCa group.

4. Dicussion

Animal model of prostate is an important tool for in-depth study of prostate-related diseases, especially tumorigenesis, development and treatment methods. Currently, the commonly used experimental animal research mainly includes mice, rats, dogs and orangutans. [13]. Animal models of prostate tumors, including transgenic, gene knockout and xenotransplanted mice, have shown greater significance in human understanding of the rules of disease occurrence and development[14]. However, prostate cancer has a multi-factor and complex progression process. Although animal models provide new research advances, existing animal models do not fully simulate all the characteristics of prostate tumors [15]. Compared with other animal models, NHP own high homology with human genetic material, and are highly similar to humans in terms of anatomical structure, body immunity, and functional metabolism. So on the construction of animal models, it is close to human diseases to the greatest extent. James N [16] measured the PSA levels of 66 cynomolgus monkeys and found that the serum PSA concentration was 0.04 to 6.2 ng/ml, with a median of 1.2 ng/ml, which was similar to the PSA results of cynomolgus monkeys measured in this study. Other studies have shown that the PSA gene exists in non-human primates such as orangutans, chimpanzees, cynomolgus monkeys, and rhesus monkeys, but not in dogs, mice, or cows [17]. This is also an advantage of the cynomolgus monkey model as opposed to the animal model commonly used for prostate cancer.

In our study, the HE staining of cynomolgus monkey prostate tissue showed that the basal cell layer was present and continuous, with few enlarged nucleoli in the glandular epithelial cells. Compared with the human prostate hyperplasia, the tissue morphology of the two was quite similar. In the immunohistochemistry of the markers, P504s, P53, PTEN and RB1 were expressed at low levels in both, and P63, 34βE12, PSA and AR were expressed at high levels ($P>0.05$). When the normal basal cells of the prostate turn into tumor cells, they secrete unique luminal proteins, such as prostate-specific antigen (PSA) and androgen antibody (AR), which are commonly used tools to diagnose prostate tumors [18]. The mutation or deletion of tumor suppressor genes has been a hot topic in the research and discussion of cancer-related aspects, and the retinoblastoma gene (*rb*), the tumor protein 53 (*tp53*) gene and the phosphatase tensin homolog gene (*pten*) with the deletion of chromosome 10 are the most concentrated in the study of prostate cancer. The dysfunction of PTEN leads to the failure of the phosphoinositol 3-kinase (PI3K) signaling pathway, leading to prostate adenocarcinoma (PADC) [19]. P53 mutation is more common in prostate tumors, especially in CRPC [20], which can promote the transformation of normal cells into tumor cells by regulating the chromatin pathway, and also lead to the drug resistance of anti-androgen therapy in prostate cancer patients [21]. IHC was used to detect missense mutation of TP53 with the specificity of 85% and the sensitivity of 100%, which was a good evaluation method [22]. The functional loss of RB1 and TP53 genes together promoted the transition from AR-dependent luminal epithelial cells to AR-independent basal-like cells, and promoted PADC' s progression and lineage plasticity, partly due to the activation of SOX2 [23, 24]. Therefore, like AR and PSA, PTEN, RB1, and TP53 are all tumor markers indicating prostate tumor. In recent years, AMACR (P504s) has been proven to be a high-quality immune tissue biomarker for PCa, with a sensitivity of up to 97%, a specificity of 92%, and is undetectable or low expression in hyperplasia and normal prostate [25, 26]. Both P63 and high molecular weight keratin (34βE12) are markers of prostate basal cells. They often indicates that prostate cells are not cancerous [27]. Anatomic data show that the prostate of cynomolgus monkeys is similar in appearance and size to that of human beings, indicating the close proximity of the two species. The results showed that the expression of PSA in normal cynomolgus monkey prostate was low, which was the same as that in normal humans (0-4ng/ul), suggesting that it is similar to human expression. Therefore, PSA level in vivo could be used as an indicator to monitor the occurrence and development of prostate cancer in cynomolgus monkeys. Xiaoqian Ma [28] established a mouse model of prostate cancer in which pten was completely knocked out by targeting the inactivated pten gene, and it could progress to aggressive prostate cancer and eventually metastatic prostate cancer. Yao Wang [29] simultaneously

knocked out p53 and eaf2 genes in mice in the study of prostate tumors, which resulted in the formation of prostate tumors. These hints that we may induce prostate tumors in cynomolgus monkeys by knocking out tumor suppressor genes such as pten and p53. Normal cynomolgus monkeys rarely develop prostate tumors naturally , which provides convenience for us to use CRISPR-Cas9 to knockout pten, p53, rb1 gene to induce spontaneous tumorigenesis of prostate. At the same time, this is also the reason why the IHC results of various prostate tumor markers in cynomolgus monkeys are similar to BPH sampers, suggesting that we can evaluate the success of prostate tumor model construction by observing the IHC changes of these markers. It is worth noting that our current model construction should be based on the premise of large-scale breeding. Although the NHP model has been gradually used in cancer research, the physiological health of animals will also have a certain impact on the experimental results, which also suggests that we should further optimize the breeding and management mode [30, 31]. Non-human primates are the most ideal substitutes for humans and important research and experimental objects. They are widely used in scientific research, medicine, national defense, and non-clinical trials of drugs, safety assessment, etc. All through the cynomolgus monkey marker gene associated with prostate tissue morphology and immunohistochemical contrast, can be concluded that the cynomolgus monkey gene has high homology with humans, which is expected to become a human model as an ideal model of prostate tumor animal research.

5. Conclusion

The prostatic tissue of cynomolgus monkey was similar to that of human benign prostatic hyperplasia，and different from that of prostate tumors of different grades. The expression of P504s, PSA, P53, PTEN and RB1 in immunohistochemistry were low, while the expression of P63, 34βE12 and AR were high. The expression of related markers in benign prostatic hyperplasia tissues of cynomolgus monkeys was similar to that of human, but significantly different from tumor in P504s, P63, and 34βE12. Cynomolgus monkeys could be used as one of the best model for the study of prostate cancer.

Conflict of interest

The authors declare that they have no conflict of interest.

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