

Detection of Human Papillomavirus Type 16 Oncogen E7 in Surgical Materials from Russian Prostate Cancer Patients

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Research Article

Received date: 06/04/2017

Accepted date: 21/04/2017

Published date: 28/04/2017

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Keywords: Prostate cancer, Etiology, Human papillomaviruses, Oncogene E7

ABSTRACT

Prostate cancer (PC) is the second most common malignant tumor of men the world over. There is no clarification on causes of PC development yet. The long-discussed item of possible PC association with oncogenic human papillomaviruses (HPV) is still being disputable. The aim of the study was to test by PCR whether surgical materials from PC patients in Russia harbour E7 oncogene of HPV16, the main HPV type responsible for cervical cancer. Cryostate cuts of prostates excised in the course of radical prostatectomy from 17 PC patients were microdissected. HPV16 E7 was found in 7 patients including all those five cases for which DNA had been isolated from homogeneous sites of cancer cells. The results obtained seem to be significant for clarification of PC etiology as well as for development of preventive measures.

INTRODUCTION

Prostate cancer (PC) is the second most common malignant tumor of men the world over including the Russian Federation^[1,2]. Lifting rates of PC incidence and mortality were the highest among all male tumors in Russia for the last decade^[2]. There is no comprehensive clarification on causes of PC development yet^[3]. One group of possible causative agents of PC may be oncogenic human papillomaviruses (HPV)^[4-6]. It is appropriate to mention here that oncogenic HPVs were revealed in genitourinary system of 42% of healthy Russian men^[7]. Despite that possible association of PC with HPV is a long-discussed problem it remains to be unresolved. Its topicality cannot be overestimated: in case association of PC with high-risk HPVs is proven an outlook appears to prevent this cancer by immunization of boys with vaccines made for prophylaxis of cervical cancer (CC).

As to malignant conversion of cervical epithelial cells infected with oncogenic HPVs it occurs due to activities of two viral proteins, E6 and E7, coded by two corresponding viral oncogenes. These proteins inactivate cellular tumour suppressor's p53 and pRB respectively. Cervical cell loses its ability for controlled proliferation, apoptosis, DNA reparation and some other important functions. The most common oncogenic HPV type responsible for more than 50% of CC cases is HPV16^[8,9]. The aim of this work was to test by PCR whether HPV16 oncogene E7 is present in surgical materials removed from PC patients at radical prostatectomy. For a better DNA preservation the study was carried out on cryopreserved (not treated with either formalin or paraffin) prostate tissues. As far as a multifocal growth is characteristic for PC pathologically altered regions of prostate were firstly detected by the morphologist (V.D.E.) macroscopically in the course of cutting, then micro dissections were performed as described below. To our knowledge this is the pioneering attempt to detect HPV DNA in PC using cryopreserved PC specimens in combination with micro dissections.

MATERIALS AND METHODS

Prostate glands of 17 PC patients were used. The glands were surgically excised at the Urological Department of the Blokhin Russian Cancer Research Centre. Patients' age varied from 52 up to 69 years, serum PSA indices were within 4.6 – 42.0 ng/ml and Gleason scores made up 6-8. These characteristics as well as TNM tumour stage meanings are presented in **Table 1**, columns 2-5. Cutting of pathologically altered region of prostate was done within 1-2 hours after surgery; tissue fragment with a macroscopically detected abnormality intended for HPV E7 test was placed into a freezer (-60°C). Micro dissection of cryostate cuts and DNA isolation were carried out according to the slightly modified procedure by Guo et al. [10]. Two serial cuts 5 µm thick were made from each frozen tissue fragment and placed on two different object glasses. One of them was stained with hematoxylin-eosin and then used for microscopic revelation of zones of normal epithelium, displasia and PC. Corresponding marks were made on its cover glass. The second cut was left unstained and uncovered for micro dissection which was performed under magnifier with taking into account marks on the first slide. To avoid contamination new scalpel was taken for every next zone. In predominant majority of cases single zone was micro dissected on a given slide. The only exception made patient N1. For this case it turned out to be possible to isolate DNA from two zones corresponding to Pin I and Pin III.

Cells thus collected were lysed with proteinase K solution (500 µg/ml, "Helicon", Russia) in Tris-HCl buffer (0.01 M Tris-HCl, pH 8.0, 0.001 M EDTA, 0.5% Tween20) under 55°C overnight. The enzyme was inactivated by heating lysate at 95°C for 10 min. Successfulness of DNA isolation was controlled in PCR with the following primers to GAPDH gene: forward (5'- ACC ACA GTC CAT GCC ATC AC - 3') and reverse (5'- TCC ACC ACC CTG TTG CTG TA - 3'). The amplification product length was 450 bp. The amplification program was like this: 94°C – 5 min, 28 cycles of 94°C – 30 sec, 58°C – 30 sec, 72°C – 60 sec; final extension for 2 min.

For detection of HPV16 E7 the procedure described earlier was applied [11]. The following type-specific primers were used: sense, 5'- CGG ACA GAG CCC ATT ACA AT- 3'; antisense, 5'-GAA CAG ATG GGG CAC ACA AT - 3'. The amplification product length was 144 bp. The program was like this: 94°C – 4 min; then 35 cycles of three steps: 94°C – 30 sec, 58°C – 30 sec, 72°C – 90 sec; concluding elongation for 6 minutes.

For positive control in PCR with HPV16 E7-specific primers DNA from HPV16-harboring CC specimen was used; reaction mixture without DNA was taken as negative control. The data were registered if adequate results had been obtained in both controls. The amplified DNA fragments were detected by means of electrophoresis in 1.5% (for GAPDH) or 2.0% (for HPV16 E7) agarose gel in presence of ethidium bromide (0.5 µg/ml).

PCR was carried out in the apparatus "Tertsik" ("DNK tekhnologii", Russia); results of electrophoresis of PCR-products were analysed and registered with the help of Image Quant Las 4000 ("GE Healthcare").

RESULTS AND DISCUSSION

Presence of PC in prostates of 16 patients under study was confirmed by results of histological analysis of the gland after surgery (**Table 1**, column 6). Small acinar cancer was revealed in 15 of them, while one specimen (patient N2) turned out to be a moderately differentiated adenocarcinoma. Cancer was not detected in prostate tissue of patient N14: large zones of fibrosis, scarce lymphoid infiltrates and rare atrophic ducts made a picture of a total therapeutic pathomorphosis of cancer. Data of microscopic analysis of one of two serial cuts intended for the procedure of micro dissection are presented in **Table 1**, column 7. Homogeneous zones of PC were found in five specimens (patients 6, 8, 10, 11 and 17). There were Pins in seven other cases (patients 1-5, 7 and 9). The rest specimens were as follows: normal prostate tissue (patients 12 and 14), benign hyperplasia (patients 15 and 16) and adenosis (patient 13). Presence of HPV16 E7 was registered in 7 out of 17 patients by PCR. All five PC cases turned out to be positive as well as both Pins from patient 1 (**Table 1** and **Figure 1**).

Table 1. Patients' clinical data; results of the HPV 16 oncogene E7 detection in their PC.

Patient No	Age, years	TNM stage	Serum PSA (ng/ml)	Glea-son score	Postoperational histology data	Cryostate slide histology data	Results of HPV16 E7 detection by PCR
1	52	T2N0M0	5,9	7	small acinar cancer	Pin I	+
						Pin III	+
2	60	T2N0M0	8,1	7	moderately differentiated adenocarcinoma	Pin III	-
3	56	T2N0M0	9,3	6	small acinar cancer	Pin III	-
4	64	T2N0M0	5,2	7	-«-	Pin III	-
5	52	T2N0M0	42,0	6	-«-	Pin III	-
6	63	T2N0M0	4,7	6	-«-	PC	+
7	63	T2N0M0	6,0	6	-«-	Pin III	-
8	66	T2N0M0	12,6	6	-«-	PC	+
9	63	T3N0M0	4,9	6	-«-	Pin III	-

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10	68	T2NOMO	42,0	7	-«-	PC	+
11	69	T2NOMO	32,8	7	-«-	PC	+
12	67	T2NOMO	8,4	6	-«-	normal tissue	-
13	58	T2NOMO	22,8	8	-«-	adenosis	+
14	55	T3NOMO	34,0	7	therapeutic pathomorphosis	normal tissue	-
15	68	T2NOMO	5,5	7	small acinar cancer	benign hyperplasia	-
16	63	T2NOMO	8,4	8	-«-	-«-	-
17	65	T2NOMO	4,6	7	-«-	PC	+

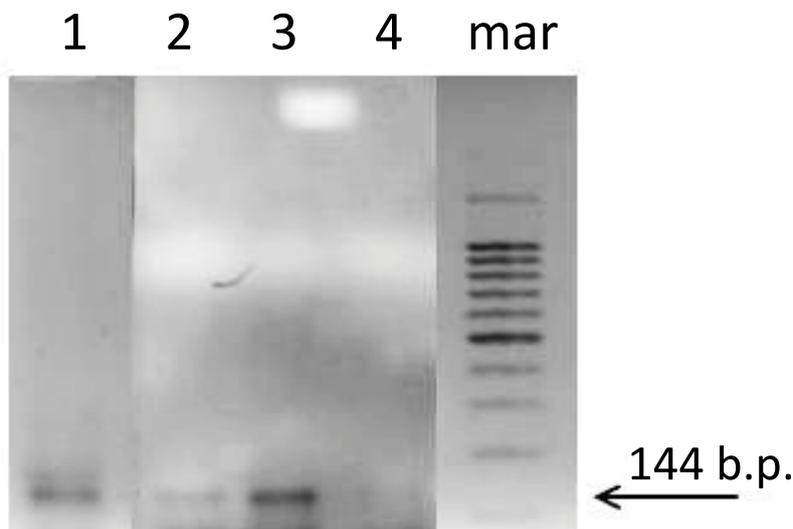


Figure 1. Electrophoregram of amplification products of PCR with E7 HPV16-specific primers. 1-HPV16 – positive CC (positive control); 2 – Pin I of patient 1; 3 – Pin III of patient 1; 4 – negative control.

The results of our study carried out on the restricted number of PC patients with special attention to maximal DNA preservation as well as to examination of homogeneous tissue zones testify to the fact that oncogenic HPV16 may be frequently found in abnormal sites of prostates. The results of meta-analysis of literature data by L Yang et al. confirm the link between worldwide prevalence of HPV and relative risk of PC but stress the inconclusiveness of results due to many parameters such as geographic region, specimen type, detection method, Gleason score, etc. [12]. In view of the urgency of the given problem for practical healthcare it deserves further investigation. Our current understanding of the link of global cancer burden with infectious agents including viruses may be rather far from completeness as was stressed by the pioneer of research into papillomavirus carcinogenesis Prof. H zur Hausen in his Nobel lecture [13]. In this connection it seems worthwhile to mention here publications indicating to presence of oncogenic HPVs in some widespread cancer types: lung cancer [14,15], breast cancer [16-18], bladder cancer [19,20]. Our previous experience shows that for disputable situations it seems reasonable to verify the fact of viral genome expression at mRNA and/or protein level [11,19]. Importance of such discoveries for practical oncology regarding means of prophylaxis or application of tumor HPV status as a predictive marker may become clear afterwards in the course of further studies.

CONCLUSION

The results obtained testify to the not uncommon occurrence of oncogenic HPV16 in Russian PC patients' prostates. They are of interest for both PC etiology elucidation and for working out of PC prevention measures. Possible participation of oncogenic HPVs in PC genesis deserves close attention and further study.

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