ORIGINAL ARTICLE

Detection of Polyomaviral Large T Antigen in Benign Prostatic Hyperplasia and Prostate Carcinoma Tissues

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Abstract:

Background: The role of infectious agents in prostate cancer development has been considered for many years. Polyomaviruses are suspected to be related to human malignancies and they have been frequently detected from various human cancers. *Aim:* This study was performed to investigate presence of large t antigen encoded by Merkel Cell polyomavirus (MCV), JC polyomavirus (JCV), BK polyomavirus (BKV) and Simian Virus 40 (SV40) in prostate carcinoma and Benign Prostatic Hyperplasia (BPH) tissues. *Material and Methods:* 20 human BPH and 6 human prostate carcinoma samples (from archival formalin fixed paraffin embedded tissues) were collected and immunohistochemically examined for presence of large t antigen encoded by mentioned polyomaviruses. *Results:* The obtained results indicated presence of large t antigen encoded by SV40, BKV and/or JCV in 40% (8/20) and 50% (3/6) of examined BPH and prostate cancer tissues respectively. However, MCV large t antigen was not detectable in the mentioned tissues. *Conclusion:* The obtained results indicate probable contribution of SV40, BKV and/or JCV in development of BPH and prostate carcinoma. However, to create a relation between these viruses and the mentioned diseases, further in-depth investigations on protein expression and localization of these polyomaviruses in normal and diseased prostate tissues seem to be required. In addition, our findings propose no association between MCV and these diseases.

Keywords: BK polyomavirus (BKV), Benign Prostatic Hyperplasia (BPH), Cancer, JC polyomavirus (JCV), Hyperplasia, Prostate, Merkel Cell polyomavirus (MCV), Simian Virus 40 (SV40)

Introduction:

Benign Prostatic Hyperplasia (BPH) which is also known as nodular hyperplasia is the most common prostate problem in human and its development is considered to be related to prostatic cell death inhibition. Prostate cancer, the other problem related to prostate, is the most common cancer among men and mostly arisen in men over 50. The exact mechanism of prostate cancer development has not been understood yet; however, it is considered as a multi stage, race and environment dependent process which has more incidence among men with westernized life style, obesity and previous familial history of this disease [1, 2].Viral infections have been considered to be connected to more than 10% of human cancers. This caused several attempts to be done in order to investigate association of prostate cancer with viral infections [3, 4].

Polyomaviruses are world distributed non-envelope DNA viruses which contain a small double stranded circular DNA molecule as their genome [5]. Some of them such as JC polyomavirus (JCV), BK polyomavirus (BKV) and simian virus 40 (SV40) are proved to be tumourigenic in rodents and cell cultures [6, 7]. Simian virus 40 is not classified as a human polyomavirus; however, it has been spread extensively in human population following to administration of contaminated polio vaccine during 1960s [6]. SV40 has been detected in several human malignant tissues such as mesothelioma, lymphoma, brain tumours, bone tumours,

thyroid gland tumours, parotid gland tumours, pituitary gland tumours and also in lung cancer tissues [8, 9]. JCV and BKV are human polyomaviruses which infect more than 80% of human population. JCV has been detected in different types of human malignant tissues including colorectal cancer, lymphoid tumours and particularly in nervous system malignancies. BKV, the other well studied polyomavirus, has been frequently isolated from variety of human tumours such as lymphomas, colorectal cancer, brain tumours, urothelialtumours and prostate cancers [6, 10, 11]. Merkel cell polyomavirus (MCV) is almost newly identified polyomavirus which is considered to be causative agent for development of merkel cell carcinoma (MCC), an aggressive and rare type of skin cancer [12].

The ability of polyomaviruses to induce transformation in host cells is linked to their large and small t antigens, two phosphorylated oncoproteins which are responsible for normal DNA replication of these viruses. Large t antigen stimulates cells to undergo uncontrolled cell growth by binding and inactivating host cell tumour suppressor proteins such as P53, retinoblastoma susceptibility protein and members of retinoblastoma protein family such as P130 and P107 [13, 14]. Accumulating data is being available regarding the presence of their genome or encoded proteins in human malignant tissues [6]. This has persuaded us to investigate presence of proteins encoded by these viruses in prostate cancer and BPH tissues by using immunohistochemical staining.

Material and Methods:

Sample Collection:

20 human BPH tissue samples and 6 human prostate carcinoma tissue samples (derived from corresponding archival formalin fixed paraffin embedded tissues) were collected from Middlesex University tissue bank. The study protocol was approved by Natural Sciences Ethics sub-committee (NSESC), Middlesex University.

Antibodies and reagents:

In order to detect MCV large t antigen, mouse monoclonal antibody, CM2B4, was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Another mouse monoclonal antibody, pab-416, was purchased from Abcam (Cambrige, UK) to detect large t antigen of SV40, JCV and BKV. Pab-416 is a cross reactive antibody which is able to respond to large t antigen encoded by SV40, JCV and BKV [15, 16, 17]. Biotinylateduniversal antibody and Vectastain Elite ABC reagent were purchased from Vector lab (Peterborough, UK).

Immunohistochemistry:

Total number of 20 BPH and 6 prostate carcinoma tissue sections were examined for detecting polyomaviral large t antigens. Briefly, tissue sections were deparaffinized and subsequently rehydrated by a series dilutions of absolute to 70% alcohol. Endogenous peroxidase activity was inhibited by incubating tissue sections with 3% H_2O_2 solution in methanol for 10 minutes. Then, microwave antigen retrieval was performed by boiling sections in preheated sodium citrate with EDTA for two minutes with 10 seconds break and two other minutes of heating in a microwave. Tissue sections were blocked with 50% normal horse serum in PBS for 10 minutes and then they were incubated with diluted primary antibodies in PBS (1:50 CM2B4 and 1:100 pab-416) for 60 minutes in the laboratory temperature. Then, tissue sections were incubated with biotinylated universal antibody as secondary antibody followed by treating with Vectastain Elite ABC reagent contained avidin-biotin complex, for 20 minutes at laboratory temperature. Tissue sections were colour developed by incubating them with diaminobenzidine. They were washed with PBS and counterstained in haematoxylin. Finally, all immunohistochemically stained slides were

dehydrated, mounted and covered with coverslips, then examined under the light microscope.

Results:

Immunohistochemistry:

The immunohistochemistry results are shown in (Table 1).

Discussion:

Polyomaviruses have been frequently addressed as probable etiological agents of several human cancers. Accumulative data has been available about association of MCV with development of merkel cell carcinoma in human [6]. Infection of non-permissive cells with some polyomaviruses such as SV40, BKV and JCV can result in continuous expression of polyomaviral early proteins, large and small t antigens, which may lead to host cell transformation. However, some polyomaviruses such as MCV are able to induce tumourigenicity in permissive cells [7, 13]. These viruses, particularly BKV, JCV and SV40, are greatly homologous in their genomic structure. This great homology in DNA sequence, particu-

Table 1: Comparison of the Results of 20 PBH Tissue Samples and 6 Prostate Cancer Tissue Samples for Reactivity with Pab-416 and CM2B4

Sample	Reactive with pab-416 (%)	Reactive with CM2B4 (%)	Total (%)
Benign prostatic hyperplasia	8 (40%)	0 (0%)	20 (100%)
Prostate cancer	3 (50%)	0 (0%)	6 (100%)

Briefly, MCV large t antigen was detected neither in 20 BPH nor in 6 prostate carcinoma tissue samples by using CM2B4 antibody. However, SV40 large t antigen was detected in 8 of studied BPH and in 3 of prostate carcinoma tissue samples by using pab-416 as primary antibody (Fig. 1)

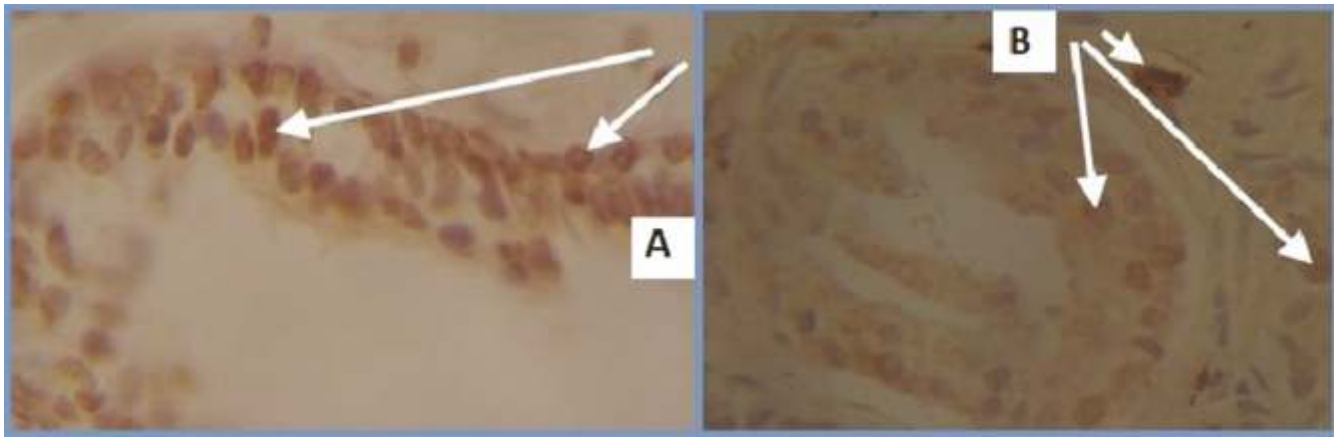

Fig. 1: Immunohistochemical Staining For Detecting SV40, BKV and JCVLARGE T Antigen Using Pab-416. Panel A and B represent Expression of Large T Antigen in BPH and Prostate Cancer Tissues Respectively

Interestingly, accumulation of SV40 large t antigen in nucleus of epithelial cells in prostate glands was observed.

larly in their early genes that encode polyomaviruses oncoproteins, indicates all polyomaviruses may be associated with cancer development by approximately same molecular mechanisms [8]. Among polyomaviral early proteins, large t antigen is considered to have greater association with oncogenicity of these viruses [18]. Several studies have been performed to trace presence of SV40 in human related diseases. Since this virus is a potent tumourigenic infectious agent, most studies have focused on its association with different human malignancies and there are various reports about its presence in several types of brain tumours and colon cancers [19]. Additionally, BKV and JCV are known for being able to cause latent infections in human urogenital tracts[20]. Our immunohistochemical analysis showed that 40% (8/20) of BPH and 50% (3/6) of prostate carcinoma tissue samples was immunoreactive to pab-416 antibody, a monoclonal antibody against SV40, JCV and BKV large t antigen. This is in accordance with other researches which have reported a significant expression of SV40 large t antigen in different tissues such as a recent immunohistochemical study of osteosarcoma tissues which reported presence of SV40 large and small t antigens in 55.4% (31/56) of the examined samples [21]. However, in contrast to our results, Martinez-Fierro and colleagues were not able to find evidences of SV40 presence in prostate cancer tissues [22]. These different findings can result from difference in applied methods and their sensitivity to detect SV40 presence in cancerous tissues. Moreover, it is more probably a result of difference in target populations of these studies and various prevalence of SV40 in different human communities. The current study also shows similar outcomes with those conducted by Delbue and colleagues in 2013, indicating presence of BKV in prostate cancer and BPH tissues [20].

Normally, prostate contains several prostatic glands which are lined by a basal cuboidal epithelium surrounding a layer of columnar secretory cells. The spaces between prostatic glands are occupied by fibromuscular stroma. The epithelial cells in prostate cancer tissues are proliferated while they show nuclear alterations and also basal epithelial cells lost some of their cellular components [2]. Interestingly, our immunohistochemistry results showed accumulation of large t antigen in nucleus of epithelial cells in prostatic glands of BPH and prostate cancer tissues. Nuclear localization of large t antigen in our prostate cancer and BPH samples suggests probable interaction of large t antigen with p53, pRB and other cell cycle regulatory proteins located in the nucleus of host cells. It is important to be mentioned that pab-416 is a cross-reactive antibody capable of binding and detecting SV40, JCV and BKV native large t antigen plus JCV and SV40 denatured large t antigen. This antibody has been used frequently in previous studies and seems to have acceptable sensitivity in detecting large t antigen [23]. Nevertheless, extensive presence of large t antigen in PBH and prostate cancer tissues showed by current investigation suggests a probable association of SV40 and/or JCV and/or BKV with development of these diseases. But, further studies are necessary to identify exact cellular distribution and accumulation of p53 and pRb plus their co-localization with SV40, JCV and BKV oncoproteins within large number of prostate cancer and BPH samples. Furthermore, using non-cross-reactive monoclonal antibodies in immunohistochemical analysis or utilizing quantitative real-time PCR will separately draw correlations between detected large t antigens and SV40, BKV or JCV.

MCV was first isolated from merkel cell carcinoma tissues, an aggressive neuroendocrine malignancy of human skin. Previous reports

showed that it commonly infects human population (up to 80% of healthy individuals) and initial contact with this virus seems to have occurred in the childhood. Infection with this virus is considered to have extensive association with MCC. However, similar to other cancers with viral origin, MCC is not an exclusive result of MCV infection and various other factors are required for MCC development. Several attempts have been performed in order to investigate probable association of MCV with other types of human malignancies; but, to date there is no report significantly supporting these associations [12, 24]. In a large scale study of 1241 tumour samples to detect MCV DNA, only 10 MCC samples were positive for MCV and other tumours including ovary, large bowel, uterine cervix, breast, soft tissue tumours, melanoma, and basal cell carcinoma, were negative for MCV [6]. In another research, its DNA was detected only in 1 of 54 breast cancer tissues [25]. In our immunohistochemistry study, MCV large t antigen was not detected in 20 BPH and 6 prostate carcinoma tissue samples by using CM2B4 antibody. CM2B4 was previously used in several studies to detect MCV large t antigen and this antibody seems to present acceptable sensitivity in MCV large t antigen detection [26]. To our knowledge there is no published study available about immunohistochemical detection of MCV large t antigen in prostate cancer and BPH tissues. However, similar to our results, Bluemn and colleagues did not detect MCV large t antigen RNA in prostate cancer cells by using quantitative real-time PCR [27]. Failure to detect MCV large t antigen in prostate cancer and BPH tissues in our study is proposed to be resulted from inability of MCV to express its early proteins in non-permissive host cells such as prostatic cells.

Conclusion:

This study showed the ability of SV40 and/or BKV and/or JCV to infect prostatic cells and express their oncoprotein, large t antigen, in prostate cancer and hyperplasia tissues. In addition, considerable association of these viruses with prostate cancer and BPH can be concluded. However, due to limitations in our utilized techniques and the number of samples, these associations shall be confirmed by performing further large scale investigations using more advanced methods. Also, non-cross reactive monoclonal antibodies and qualitative real-time PCR are proposed to be used for detection of small and large t antigens of these viruses to enhance specificity of further investigations. Seeking genomic DNA sequences belong to these viruses by using polymerase chain reaction and specific pairs of primers is another option for clarifying association of these viruses with prostate cancer and BPH. Moreover, according to our research outcomes, if any probable association of MCV with prostate cancer and hyperplasia is assumed, it is surely not related to expression of MCV large t antigen.

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