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Original article

Evidence of merkel cell carcinoma polyomavirus in prostate cancer tissue using nested PCR assay

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ABSTRACT

Prostate cancer, the most common cancer among men in United Kingdom, is considered to be a multifactorial disease. The role of infectious agents, particularly viruses, in prostate cancer development is a controversial issue. Polyomaviruses are world distributed DNA viruses which are suspected to induce malignancies in human. They have been identified in several types of human cancers such as prostate carcinomas. This study was performed to investigate presence of Merkel cell carcinoma polyomavirus (MCV) and Karolinska Institute polyomavirus (KIV) in prostate cancer tissues. Nested PCR was used in order to detect MCV and KIV conserved DNA sequences in total 23 purified genomic DNA samples from human prostate cancer tissues. The presence of MCV DNA was shown in 26% (6 out of total 23) of our samples while KIV DNA was undetectable in our nested PCR assay. These findings suggest MCV infection can occur in prostate tissue. However, further in-depth investigations of MCV gene expression and protein localization in prostate cancer cells are required to inspect probable association of this virus with prostate cancer development. In addition, larger scale studies shall be performed in order to investigate association of KIV and prostate cancer in more depth.

1. Introduction

Prostate cancer is the most frequent cancer besides the skin cancer and second cause of cancer death among men in United States (American Cancer Society, 2013). Its severity is varied from carcinomas without significant clinical symptoms to fatal metastatic tumors. It is a race, age and environment dependent disease which is occurred more frequently in men with westernized life style and diet (Kumar et al., 2010). Plus, the role of familial history and obesity in increasing the chance of developing prostate cancer is shown to be remarkable (American Cancer Society, 2013). Recently, several attempts have been done in order to investigate association of prostate cancer with viral infections.

Polyomaviruses are world distributed non-envelope DNA viruses which contain small double stranded circular DNA molecules as their genome (Krumbholz et al., 2019). Some of them are proved to be able to induce tumor formation in laboratory animals and there is accumulating data regarding their presence in human malignant tissues (Dalianis and Hirsch, 2013). They have been detected in several types of human malignancies such as prostate carcinoma tissues (Abend et al., 2009; Jiang et al., 2009). Polyomaviral infection in non-permissive cells can results in continuous expression of their early proteins, large T and small t antigens, which may lead to host cell transformation. Large T antigen stimulates cells to undergo uncontrolled growth by binding and inactivating host cell tumor suppressor proteins such as P53, retinoblastoma susceptibility protein and members of Retinoblastoma protein family such as P130 and P107 (Abend et al., 2009).

Merkel cell polyomavirus (MCV) is almost a newly identified human polyomavirus which has been frequently isolated from merkel cell carcinoma tissue samples. Its common presence in human population has also been reported previously (Chang and Moore, 2012). Presence of this virus in various types of human malignancies has been investigated, but; there is not sufficient data about its presence in prostate cancer tissues to make a conclusion about its role in this type of cancer (Dalianis and Hirsch, 2013). Due to significant prevalence of MCV in merkel cell carcinoma tissues, this virus is considered to be related to this type of cancer (Johnson, 2010).

Karolinska Institute virus (KIV), another recently identified human polyomavirus, has not been associated with any particular human disease yet (Jiang et al., 2009). This virus has been found mainly in human respiratory tissues but not in human blood or urine samples (Dalianis et al., 2009). Then, several studies consider a probable association of this virus with some acute respiratory diseases (Jiang et al., 2009). This virus has also been detected from lung cancer tissues (Babakir-Mina et al., 2009), however; there is no published report about presence and probable association of KIV and prostate cancer pathogenesis.

The homology among polyomaviral genomes is significant particularly among their early genes which encodes their putative oncoproteins. This great homology indicates that all polyomaviruses, including mentioned viruses, may associate with cancer development by approximately same molecular mechanisms (Barbanti-Brodano et al., 2004). This significant homology in addition to frequent detection of these viruses from various human cancer tissues persuaded us to investigate presence of MCV and KIV as new members of *polyomaviridae* in prostate cancer tissues. In order to provide some clues to illuminate association of these viruses with development of prostate cancer in human this study aimed specifically to find evidences for presence of these viruses in prostate cancer tissue by detecting their DNA.

2. Materials and methods

2.1. Sample collection

23 extracted and purified genomic DNA samples derived from human prostate cancer tissues were collected from Middlesex University molecular biology laboratory archive, London, UK. Plasmids contained MCV (kindly gifted by Prof. Patrick S Moore of NIH, United States) and KIV genomes (kindly gifted by Prof. Torbjörn Ramqvist of Karolinska Institute, Stockholm, Sweden) were used as positive controls for our nested PCR reactions. The study protocol was approved by Natural Sciences Ethics sub-committee (NSESC), Middlesex University, London, UK.

2.2. PCR reactions

PCR master mixture for all reactions contained 1X PCR reaction buffer, 0.2mM of each dNTP, 1.5mM $MgCl_2$, 0.025u/µl (Thermo Scientific, Epsom, UK) Red Hot Taq DNA polymerase and 0.5µM of each primer (Sigma-Aldrich, UK). Genomic DNA derived from total 23 prostate cancer tissues were used as templates for the first round of

nested PCR reaction. Then, the PCR products were used as DNA templates for the next round of nested PCR. TECHNE TC-512 and TC-3000G PCR thermocyclers were used to perform nested PCR assay. PCR products were transferred and electrophoresed on 1.4% agarose (Fisher Scientific, UK) gels, stained with ethidium bromide and visualized by UVP Bioimaging System UV trans-illuminator.

2.3. Detection of MCV

In order to detect presence of MCV genome in all 23 samples by nested PCR, outer and inner primers targeting large T antigen sequence were used. The outer primers (LT1F and LT1R) and inner primers (MCPyLT1709.F and MCPyLT1846.R) were previously reported to yield PCR products of expected size in separate conventional PCR assays (Feng et al., 2008; Giraud et al., 2008). Here we utilize them to perform a nested PCR assay for detecting MCV DNA since MCPyLT1709.F and MCPyLT1846.R are able to anneal the sequence amplified by the LT1F and LT1R. The outer reaction consisted of a single step of 10 minutes at 94° followed by 20 cycles of 94° for 45 seconds, 58° for 30 seconds, 72° for 45seconds and a final extension step of 72° for 15 minutes. The primers LT1F 5'-TACAAGCACTCCACCAAAGC-3' and LT1R 5'-TCCAATTACAGCTGGCCTCT-3' were used in the outer reaction (Feng et al., 2008). The inner reaction was started with 10 minutes at 94° followed by 35 cycles of 94° for 30 seconds, 53° for 30 seconds, 72° for 45 seconds and terminated with 5 minutes at 72° using MCPyLT1709.F 5'-CAGGCATGCCTGTGAATTAGGATG-3' and MCPyLT1846.R 5'-TCAGGCATCTTATTCACTCC-3' as inner primers (Giraud et al., 2008).

2.4. Detection of KIV

A nested PCR was carried out to identify KIV genome in all 23 samples. POLVP1-39F 5'-AAGGCCAAGAAGTCAAGTTC-3' and POLVP1-363R 5'-ACACTCACTAACTTGATTTGG-3' were used as outer primers to amplify a sequence from KIV regulatory region (Allander et al., 2007). The outer reaction programed as 10 minutes at 94° and 20 cycles consisted of 94° for 1 minute, 54° for 1 minute, 72° for 2 minutes and a final extension of 10 minutes at 72°. POLVP1-118F 5'-GTACCACTGTCAGAAGAAAC-3' and POLVP1-324R 5'-TTCTGCCAGGCTGTAACATAC-3' were used as inner primers (Allander et al., 2007). The inner reaction initiated with one cycle at 94° for 10 minutes, continued with 35 cycles of 94° for 1 minute, 54° for 1 minute, 72° for 2 minutes and a final extension of 10 minutes at 72°.

3. Results

PCR reactions

In the present study total 23 genomic DNA samples derived from prostate cancer tissues were analyzed for presence of MCV and KIV DNA sequences. The results showed that following to nested PCR and agarose gel electrophoresis amplification of MCV DNA with expected size was confirmed in 6 out of total 23 specimens (Fig. 1). In addition, KIV DNA was not detectable in all of the 23 samples by our nested PCR assay (Fig. 2). Absence of PCR products in negative control confirmed no contamination was occurred in all nested PCR experiments. In addition, PCR products of expected size were visualized on agarose gel electrophoresis for both MCV and KIV positive controls.

4. Discussion

Prostate cancer is the most common cancer among men in United Kingdom and it is estimated to be the second cause of cancer death among the men in United States (American Cancer Society, 2013; Groom et al., 2012). It is considered to be a multifactorial disease related mostly to familial history and environmental factors such as diet and lifestyle. The role of infectious agents in prostate cancer development has been studied since 1950s, when association of infectious diseases with prostate cancer was suggested for the first time. Epidemiological studies showed there is a higher probability for developing prostate cancer in patients who have previously acquired sexually transmitted diseases. However, microbiological and pathological studies have not been successful in drawing a relation between prostate cancer and infectious diseases (Groom et al., 2012). MCV was primarily detected from merkel cell carcinoma (MCC), an aggressive and rare type of skin cancer. Due to the

extensive presence of this virus in MCC tissues, it is proposed as etiological agent for MCC (Johnson, 2010; Feltkamp et al., 2013).

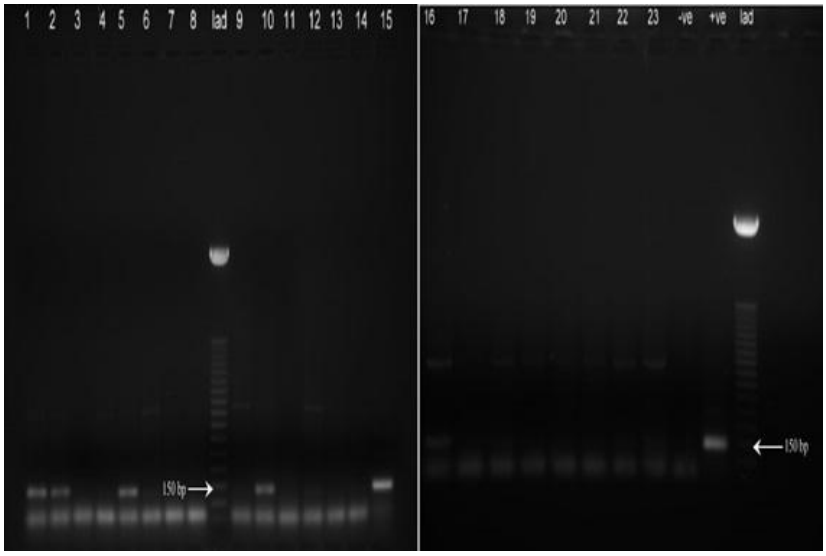

Fig. 1. Agarose gel electrophoresis of MCV nested PCR products. Lane 1 - 23 show investigated samples. Lad indicates 50 bp DNA ladder. –ve and +ve are negative and positive controls respectively.

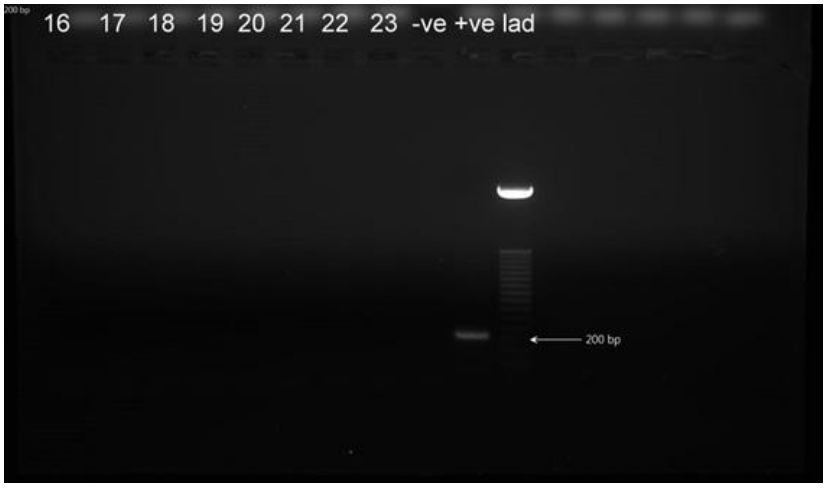

Fig. 2. Agarose gel electrophoresis of KIV nested PCR products. 16-23 indicate the samples while lad shows 50 bp DNA ladder. Lanes –ve and +ve show negative and positive controls respectively.

The exact mechanisms of MCV transmission and spread among human population have not been identified yet. The probability of MCV to be associated with prostate cancer increases if it has the ability to transmit to individuals through urinary tract, like the way that some other polyomaviruses transmit (Bluemn et al., 2009). Serological studies showed that MCV infection is almost common in human population and there are some reports indicating 80% seropositivity in adults (Chang and Moore, 2012). Several attempts to find evidences of association of MCV with other types of human tumors were unsuccessful (Giraud et al., 2008; Andres et al., 2010; Giraud et al., 2009). In a large study of 1241 tumor samples to detect MCV DNA, only 10 MCC samples were positive for MCV and other tumor samples including ovary, large bowel, uterine cervix, breast, soft tissue tumors, melanoma, and basal cell carcinoma, were negative for MCV (Dalianis and Hirsch, 2013). In another research its DNA was detected only in 1 of 54 breast cancer tissues (Antonsson et al., 2012). There are various reports about isolation of unintegrated MCV DNA from different types of tissues. These infected tissues exhibit low MCV DNA replication which suggests MCV to be in latent phase and cause a persistent infection in these tissues (Chang and Moore,

2012). Integration of linear MCV genome to MCC cells has reported previously. Plus, Expression of both MCV large and small T antigens is required for MCC cell transformation (Feltkamp et al., 2013). Our findings showed that MCV genome is present in 26% of the examined prostate cancer samples. However, Bluemn and colleagues in the only published study about presence of MCV in prostate cancer cells were not able to detect MCV encoded RNA in prostate cancer tissues (Bluemn et al., 2009). These contradictory results can be interpreted by probable ability of MCV to infect prostate cells and cause latent infection in these cells; but, its inability to express proteins such as large T antigen in prostate cells. Then, probability of insertional mutations due to integration of MCV DNA in prostate cells genome shall be investigated to clarify association of MCV infection with prostate cancer. In addition to probable inability of MCV to express its oncoproteins in prostate tissue, the significant difference between these findings can be resulted from using different prostate cancer samples in these two separate experiments. Therefore, further studies shall be performed using high number of prostate cancer and normal prostate samples.

KIV has been identified for the first time in nasopharyngeal aspirates from children with respiratory infections. However, further studies showed prevalence of KIV does not differ between patients with respiratory diseases and healthy individuals (Dalianis and Hirsch, 2013). Serological studies suggested an age dependent seropositivity pattern against KIV due to increase of seropositivity to maximum of 70% in older adults (White et al., 2013). There is contradictory data about frequency of KIV DNA in different human tissues. KIV DNA was found in 2.4% (7/291) of saliva samples in a recent study (Robaina et al., 2013), however; Bergallo and colleagues detected KIV in 31% (26/84) and 13.2% (12/91) of stool and tonsil samples respectively using newly designed primers for their real time PCR assay. They claimed results are different when using different sets of primers. They suggested that different results in KIV detection may contribute to the sensitivity of the assay and the sequences which primers target (Bergallo et al., 2009). Attempts to detect this virus in cancer tissues were not successful. However, KIV VP1 sequences have been recently detected in 45% of samples in a study on lung cancer tissues (Babakir-Mina et al., 2009). There has been no correlation proved between KIV infection and any particular human disease including cancer (Dalianis and Hirsch, 2013). Our negative results for presence of KIV in prostate cancer tissue seem to be similar to some other reports about absence of KIV DNA in cancer tissues (Giraud et al., 2009; Giraud et al., 2008). Then it seems there is no relation between KIV and prostate cancer development in our samples. However, in order to draw a more certain conclusion, this investigation may be repeated in future using more prostate carcinoma samples. To our knowledge, there is no published report about presence or absence of KIV genome in prostate cancer tissues.

5. Conclusions

To conclude, this study showed that human prostate tissue can be infected with MCV. However, due to limitation of our utilized technique in analyzing expression of MCV proteins specifically its Large T antigen we cannot conclude a robust association between this virus and prostate cancer. Further investigations using larger number of samples and more advanced methods seem to be required in order to clarify probable association of MCV and prostate cancer. Moreover, due to inability to detect KIV DNA in few number of samples used in current study, there is a need to design a larger scale experiments in order to confirm or refuse the association of KIV with prostate cancer development.

List of abbreviations

KIV - Karolinska Institute virus
MCV - Merkel cell carcinoma polyomavirus

Conflict of interest

The author has no conflicts of interest related to this work.

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