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Research Article

Detection of Human Cytomegalovirus in Tissue Samples of Colorectal Cancer in Brazzaville Hospital University Center, Congo

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Abstract

Background: Colorectal cancer (CRC) is the most common gastrointestinal cancer and the third leading cause of cancer death and morbidity worldwide. Human cytomegalovirus (HCMV) has been implicated as a potential etiopathogenetic agent. He is considered as an oncomodulator virus because of its effects on cell-cycle progression, mutagenesis, angiogenesis, and immune evasion. We conducted this study with the objective of detecting human cytomegalovirus by Nested Polymerase Chain Reaction.

Methods: In this study, 41 colorectal cancer formalin-fixed paraffin-embedded (FFPE) tissue samples diagnosed on histological basis were included. DNA was extracted from these tissues for detection of HCMV DNA virus using Nested Polymerase Chain Reaction. Statistical analysis was performed with Chi-square test.

Results: In 6 cases of colorectal cancer tissue samples (6/41, 14.6%), HCMV DNA was detected. The mean age was 53 years. The histological type of the patients was associated with the positivity of human cytomegalovirus with a statistically significant difference ($p = 0.041$).

Conclusion: The HCMV virus could play a role in creating malignancy and the progression of cancer through the process of oncomodulation. He has a contributing role in colorectal cancer; although more study is required to clearly define its involvement in this type of cancer.

Keywords: Human Cytomegalovirus; Colorectal Cancer; Nested-PCR; Brazzaville; Congo

Abbreviations

ADK: Adenocarcinoma; CRC: Colorectal Cancer; HCMV: Human Cytomegalovirus; CHU-B: Brazzaville University Hospital Center; FFPE: Formalin-fixed Paraffin-embedded; PCR: Polymerase Chain Reaction; WHO: World Health Organization

Introduction

Colorectal cancer (CRC) is the most common gastrointestinal cancer and the third leading cause of cancer death and morbidity worldwide. In general, the most CRC are immunologically silent tumors, grow slowly and often do not produce symptoms until they reach a large size. According to the World Health Organization about 1,849,518 new cases of colorectal cancer have been registered worldwide in 2018 [1]. The incidence of CRC varies worldwide, with rates higher in industrialized countries (Europe, Australia, New Zealand, North America and Japan) and lower in other countries in Asia and Africa [2]. It is generally accepted that colorectal carcinogenesis is a multi-step and multifactorial process with various etiologies including lifestyle, genetic modifications and environmental factors more specifically viral infections [3]. Viral etiology in cancers is a very important question and nowadays, several viruses have already been identified as oncogenic viruses, these are: HTLV-1, EBV, HHV-8, HCV, MCPyV, HBV and HPV [4-7]. The Human Cytomegalovirus (HCMV) is increasingly incriminated as an initiating factor and affects the evolution of human cancers [8]. HCMV is also called human herpesvirus 5 (HHV-5) and belongs to the large family of herpesvirus, to the subfamily of β-herpesvirinae [9]. HCMV prevalence among different populations worldwide varies from 45% to 100%, according to geographical location and socioeconomic level [10]. The HCMV seroprevalence is more prevalent in Asia and Africa than in Western Europe and the United States [11]. HCMV infection causes life-threatening diseases in immunocompromised hosts and can be detected in a certain tumors, including CRC [12]. The HCMV contains many potential viral onco-proteins such as the proto-oncogenes c-foc, c-jun and c-myc which can be activated and regulate the malignant behavior of tumor cells, but has not been categorized as an oncogenic virus till now. This virus is considered as an oncomodulator virus because of its effects on cell-cycle progression, mutagenesis, angiogenesis, and immune evasion [13-15].

Several studies suggest that HCMV may be associated with different types of cancers such as glioblastoma, breast cancer and colorectal cancer [8,16,17]. The association of HCMV with colorectal adenocarcinoma was first reported in 1978 by Huang and Roche, with 40% of CRC samples being positive for HCMV [18] and since then various studies conducted in different countries have confirmed the association between this virus and colorectal cancer. Some previous studies have reported negative detection of HCMV in CRC tumor tissues [19,20]. However, increasingly recent data indicate that HCMV preferentially infects tumor tissue of CRC [21-26]. Such findings imply that HCMV may affect the tumor microenvironment of CRC via a certain immune pathway. Upon infection, HCMV is able to up-regulate different host cellular signal pathways, growth factors, and cytokines, resulting in enhanced cell survival, proliferation, and angiogenesis [14].

The possible contribution of HCMV to the development and progression of CRC is therefore still controversial. This is how we undertook this study aimed at detecting Human Cytomegalovirus by Nested PCR in tissue samples of Colorectal Cancer for better management.

Material and Methods

Study specimens

The study samples were from formalin-fixed, paraffin-embedded (FFPE) tissues of patients with CRC, archived in the Pathological Anatomy and Cytology department of CHU-B between 2012 and 2017. 45 adenocarcinoma biopsies meeting the inclusion criteria were selected from the service register in the above-mentioned period.

Isolation of genomic DNA from paraffin-embedded tissues

5 µm paraffin sections were obtained from biopsy samples and collected in 1.5 ml Eppendorf tubes. Samples were dewaxed with xylene and ethanol as described by Maryam Karimi [27].

Genomic DNA extraction

Genomic DNA extraction was performed using the ReliaPrep™ gDNA Tissue Miniprep System Kit (PROMEGA, FR) following the protocol established by the manufacturer. The eluate was collected and stored at -20° C, prior to the PCR amplification procedure.

Verification of DNA extraction

All samples were examined for DNA integrity using amplification of a 268 bp β-globin gene fragment. Sequences of primers were: PC04: 5' CAA CTT CAT CCA CGT TCA CC 3'; GH20: 5' GAA

GAG CCA AGG ACA GGT AC 3'. PCR were carried out in a final volume of 25 µl containing 12.5 µl of Amplicon master mix, 0.5 µl of forward and reverse primers, 1.5 µl of water PCR grade and 10 µl of DNA template. Amplification was carried out in Thermal Cycler (Applied Biosystems™,GeneAmp™) according to the following program: After an initial denaturation step at 95°C for 3 min, 45 cycles were programmed as follows: denaturation step at 95°C for 30 sec annealing step at 53°C for 40 sec, primer extension at 72°C for 40 sec and final extension step at 72°C for 5 min. PCR products were determined by visualization of amplicons coupled to bromophenol blue (in equal quantity) on 2% agarose gels stained with gel red.

Nested PCR amplification of the HCMV UL55 gene

The nested PCR reaction for amplification of the 268 bp fragment of the HCMV UL55 gene encoding the IE-2 protein was performed on 41 extracted human DNA samples. The oligonucleotide primer sequences were: 5'TCCAACACCCACAGTACCCGT-3 and 5'CGGAAACGATGGTGTAGTTCG-3, as previously described [28]. PCR program was performed as follows: pre-denaturation at 95°C for 5 minutes, 1 cycle; denaturation at 94°C for 30 seconds, annealing at 55°C to for 30 seconds, extension at 72°C for 30 seconds, 20 cycles; post- extension at 72°C for 5 minutes, 1 cycle. The second-round PCR amplification was carried out by using 12.5 µl of Amplicon master mix, 0.5 µl of forward and reverse primers and 5 µl of the first round PCR products as templates in 25 µL reactions. The sequences of the inside primer pair were: 5'-GCCCGCCGCGGCAGCACCTGGCT-3' and5'-GTAAACCACATCACCCGTGGA-3' [28]. PCR cycling protocol was used: 95°C for 5 minutes, 1 cycle; 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds, 30 cycles, 72°C for 5 minutes, 1 cycle. At the end of amplification, 5 µL of the PCR products mixed with 5 µl of bromophenol blue was analyzed on 2% agarose gel. The resultant product was expected to be a 174 bp fragment.

Statistical analysis

Data processing and statistical analyses were performed using the Microsoft Excel 2016. Frequency tables were analyzed using the Chi-square test, with GraphPad Prism version 7 software. $P < 0.05$ was considered statistically significant.

Results

A total of 45 formalin-fixed, paraffin-embedded (FFPE) tissues from patients with CRC diagnosed on histological basis were included in this study. Four blocks FFPE were excluded because they were degraded and unusable. Analysis of the sociodemographic characteristics (Table 1) indicated that 21 (51%) were female, ie a sex ratio of 0.95 in favor of women. The mean age was 53 ± 27,58 years (minimum: 20 years old; and maximum: 80 years old). Note that, the most represented age group was 44 - 55 years old. The histological types encountered were lieberkühnian adenocarcinoma, mucosal colloid adenocarcinoma and cell independent adenocarcinoma. The histological type of the patients was associated with the positivity of human cytomegalovirus with a statistically significant difference ($p = 0.041$). The tissues studied were classified into one of three stages or histological grades: (i) well differentiated (G1), (ii) moderately differentiated (G2), and (iii) poorly differentiated (G3). No statistically significant difference was found between the different histological grades and the positivity of human cytomegalovirus ($p = 0.79$). Finally, In 6 cases of colorectal cancer tissues (6/41, 14.6%) human cytomegalovirus was detected (Figure 1).

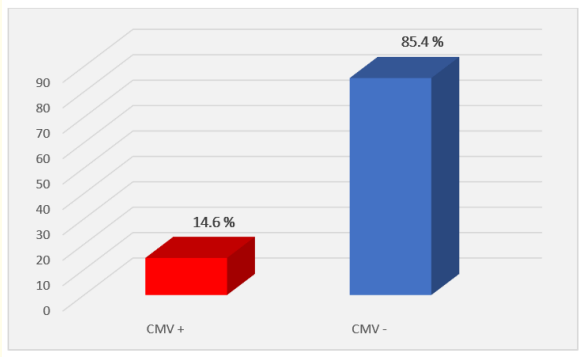

Figure 1: General frequency of the detection of HCMV DNA.

Discussion

HCMV is a human herpes beta virus whose host immune system is unable to clear the next initial infection, the virus remains latent in the body and can be reactivated at any time during life. In our study, the presence of HCMV DNA in CRCs was confirmed by the nested PCR method, the results showed that the frequency of detection of HCMV DNA was 15%, this result was opposite to those of Akintola-Ogunremi., *et al*. in the USA (2018) who detected no distinct band of genomic DNA in the 13 adenocarcinomas, although the expected band (106 bp) was demonstrated in 2 cases of CMV colitis [19]. Our results are significantly lower than those

Variable	Total specimens of FFPE (N = 41)		HCMV positive (n = 6)		HCMV Négative (n = 35)		P Value
	N	%	n	%	n	%	
Sex							
Male	20	48.78	2	33.33	18	51.43	0.66
Female	21	51.22	4	66.66	17	48.57	
Age							
20 - 31	4	9.76	0	0	4	11.43	0.52
32 - 43	8	19.51	2	33.33	6	17.14	
44 - 55	12	29.27	2	33.33	10	28.57	
56 - 67	8	19.51	0	0	8	22.86	
≥ 68	9	21.95	2	33.33	7	20	
Histological type							
Lieberkühnian ADK	37	90.2	5	83.33	32	91.43	0.041
Mucous colloid ADK	3	7.3	0	0	0	0	
Independent cell ADK	1	2.4	1	16.66	3	8.57	
Histological grade							
Grade 1	34	82.93	5	83.33	29	82.86	0.79
Grade 2	2	4.88	0	0	2	5.71	
Grade 3	5	12.19	1	16.66	4	11.42	

Table 1: Distribution of HCMV based on sex, age, type and histology grade in patients with CRC.

reported by Sahar Mehrabani-Khasraghi., *et al*. in Iran (2016) with a frequency of 53.3% of HCMV DNA in CRCs (8/35) by PCR [29], as well as with Cai., *et al*. in China (2016) with a detection frequency of 38.8% (40/103) in colorectal ADK [21]. The large part infected with HCMV were women (66.7%) with a sex ratio of 0.5. These results are slightly greater to those found in a study of the prevalence of cytomegalovirus infections in patients with colorectal cancer with 40% of women; with no statistically significant relationship (p = 0.6628). This female predominance could be explained on the one hand by the fact that the woman has a genital anatomy favorable to sexually transmitted infections compared to the man and on the other hand, by the female predominance of the general study population according to the census [17,29].

In addition, Harkins., *et al*. also demonstrated the presence of the HCMV UL55 gene (6/8 tumor cases) by amplified nested PCR, which was confirmed by direct sequencing of the PCR products in breast cancer, this shows the ability of the virus to infect different cancer cells and that HCMV may play a causal role in the process of carcinogenesis and cancer progression, but the mechanism of carcinogenesis needs to be further clarified [21]. Kalejta., *et al*. reported that the HCMV UL82 pp71 gene product stimulates cell cycle progression by inducing degradation of tumor suppressor proteins Rb and its family members p107 and p130. Taken together, these experimental observations strongly suggest that HCMV is a potential carcinogen [30]. However, the possible association of HCMV with human colorectal ADKs has also been reported by Huangand who detected HCMV DNA in 4/7 adenocarcinomas of the colon by RNA-complementary membrane DNA hybridization [18]. *In vitro* studies have shown that various proteins (E1 and E2) encoding HCMV can initiate cell transformation through signaling pathways, contribute to tumor development, and provide mechanisms associated with cancer characteristics [31]. In addition, it is also able to act on the cell cycle, apoptosis, angiogenesis [12,32].

In our study series, we found 5/6 (83.33%) of grade 1 ADKs in the infected population. However, Bender., *et al*. (2009) in Italy found viral DNA in 5/24 (21%) of grade 2 ADKs [33]. On the histological level of the CRC, we found 5/6 (83.33%) of the HCMV DNA in the lieberkünhian ADKs, this result was superior to those of Hsin-Pai Chen., *et al*. (2016) in China, with 34.8% infection in lieberkünhian ADKs [17]. The observed difference was statistically significant (p = 0.04). Indeed, the plausive explanation for these results would be that the presence of HCMV at early histological stages proves that the virus could theoretically play a role of initiating factor in the oncogenesis of CRC (oncomodulator) and other elements would then promote the tumor progression hence its oncogenic [33]. Our results show the hypothesis that HCMV has the potential to contribute to oncogenesis through a "hit and run" mechanism by inducing mutations in genes. Cellular.

Conclusion

The present study has shown the presence of DNA HCMV in CRC by nested PCR method reflects the ability of the virus to infect of the different colon cells, These findings suggest that further research in this area is warranted to determine whether HCMV infection of colorectal epithelium represents an important factor in the initiation and promotion of colorectal cancer, and raise the possibility that in the future, antiviral based strategies may play a role in the management of this disease.

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Conflict of Interest

None of the co-authors has a conflict of interest related to this work.

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