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Th17 cytokine profiling of colorectal cancer patients with or without enterovirus 71 antigen expression

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ABSTRACT

Object.

Th17 cytokines have been identified in several types of human cancers. In this pilot study, the expression of Th17 cytokines profiling in enteroviruses 71 (EV71) associated colorectal cancer (CRC) were explored.

Methods: 66 patients with CRC were enrolled in this study; immune- histochemical analyses were performed on cancerous tissues and adjacent non- cancerous tissues of the patients. Serum Th17 cytokines of CRC patients and healthy controls were measured using a Luminex 200 analyzer.

Results: Cancerous tissues had more positive EV71 antigen expression than adjacent non- cancerous tissues. In TNM II-III CRC, 59.9% of cancerous tissues were observed to be EV71 positive; on the contrary, 65.2% of the adjacent non- cancerous epithelium was EV71 negative. In TNM I CRC, all adjacent non- cancerous epithelium was virus negative, but in TNM IV, half of adjacent non- cancerous tissues were virus positive. Serum IL-10 were significantly higher in CRC patients than in healthy controls, and IL-10 concentrations in the EV71 positive group were higher than those of the EV71 negative group, with the highest IL-10 levels being observed in CRC patients with strong positive group ($P < 0.05$). Similar results were found for IL-21 and IL-23. IL-17 levels were higher in CRC patients than in healthy controls, there was no significant difference in IL-17 between the viral positive and viral negative groups ($P > 0.05$).

Conclusion: Persistent existing EV71 viral antigens in intestinal tissues are positively associated with TNM III/IV CRC. EV71 latent infection recruits Th17 cells in the colorectal tumor site, stimulating Th17 cytokine production that closely associated with CRC carcinogenesis.

1. Introduction

Colorectal cancer (CRC) is the third most common malignancy worldwide, and long-term survival for patients with metastatic disease remains poor [1,2]. The carcinogenesis of CRC depends on genetic background, lifestyle choices, and chronic inflammation [3]. The microbial communities presented in the intestinal tract have known associations with colon health [4], where the microbiota mutually interact with the immune system to maintain homeostasis in the intestine. Microorganisms can also alter this balance and promote chronic inflammation, which can induce intestinal tumor development [3]. The connection between inflammation and tumorigenesis has extensively been studied, it is estimated that 16–18% of the global cancer burden can be associated with oncogenic viruses, including Human polyomavirus (JCV), Epstein Barr Virus (EBV), human cytomegalovirus (HCMV), and human papillomavirus (HPV) [5,6]. Viruses can have a direct role in carcinogenesis through their contribution to dramatic changes in the microenvironment and immunosurveillance [7,8]. Furthermore, inflammatory cells stimulated by a virus can release chemicals, notably reactive oxygen species that are actively mutagenic for nearby cells, thus accelerating their genetic evolution toward states of heightened malignancy [9]. Currently, investigation into the role of viral infection in carcinogenesis has just been initiated. Enterovirus, may similar with the pathogens mentioned above, participate in CRC carcinogenesis, but the clinical data and the underlying mechanisms remain to be revealed.

EV71, a single-positive-stranded RNA virus, belongs to the enterovirus B genus of the Picornaviridae family [10]. Since 1998, when a large EV71 outbreak resulted in 130,000 children being infected and led to 78 fatalities, EV71 infection has recurred every year and EV71

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outbreaks have been reported periodically throughout the world [11,12]. The virus is known to survive in the acidic of the stomach, entering the host through the intestines and spreading to other regions of the body via the circulatory system [13]. In human, EV71 infection is either acute or latent. Although there is no data regarding EV71 infection in adults, our previous epidemiological analyses demonstrated the ubiquitous presence of EV71 infection in both children and adults, where the seroprevalence of EV71 is from 50–60% in children and 60–90% in adults. Moreover, we found that EV71 specific receptors SCARB2 and PSGL-1 were distributed widely in the human gastrointestinal tract, which correlated with the distribution of pathological changes seen in EV71 infection [14]. Virus receptors have essential roles in the early steps of viral infection, when viruses attach to cell surface molecules of the host [12]. Unlike other animal viruses that have evolved strategies to evade or delay early apoptosis to allow the production of high yields of progeny virus, EV71 virus replication is associated with the apoptosis of infecting cells [15], which may interfere with cellular and immunologic functions in a complex manner. Like other cancer related viruses, EV71 may have the ability to establish a persistent infection in host cells, existing either in the form of a silent or productive infection [16]. Since most EV71 infections in human adults remain inactive or latent and there are no effective therapies to treat EV71 infection, the EV71 infection may continue to exist in the intestine and could easily be ignored in the clinic. The high seroprevalence of EV71 in children and adults suggests that most people have been infected by EV71 once or repeatedly. Persistent EV71 infection may lead to malignant changes in some patients, especially those with immunosuppression status. Therefore, the relationship of EV71 infection and gastrointestinal disease needs to be elucidated.

CAC is characterized by production of pro-inflammatory cytokines that can induce mutations in oncogenes (KRAS) and/or tumor suppressor genes (APC, p53, etc.) and genomic instability. Th17 cell is an essential part of the immune response to control microbial invasion in intestine. Th17 cells can also induce chronic inflammation and autoimmune disease when its compensatory mechanisms fail. A growing body of evidence suggests that Th17 cells have a strong pro-tumorigenic potential in the intestine [3], and a high number of Th17 cells have been observed at advanced tumor stages [17]. The endogenous microbiota is crucial for the induction of Th17 cells and then plays a critical role in cancer development. Th17 cells are characterized by their ability to secrete specific inflammatory cytokines including IL-6, IL-17, IL-21, IL-22, IL-23, and IL-26. When Th17 cells are over- stimulated by a specific component of the microbiota, they can promote a state of chronic inflammation mediated by the release of IL-17 and IL-22, which likely favors intestinal tumor growth [3]. Elevated levels of pro-inflammatory Th17 cytokines are found in CRC patients, illustrating a key role for these cytokines in facilitating the survival and growth of CRC cells [18].

Inflammatory cells in the tumor microenvironment release inflammatory mediators (include chemokines and cytokines, such as TNF-α and interleukins, key transcription factors involved in inflammation (such as NF-κB and STAT3))that in turn activate local immune networks to promote the development and growth of malignant cells [19]. Although the intestinal microbiota is mainly localized to the gut and was originally thought to display regulatory functions within the intestinal mucosa, recent evidence indicates that functional microbiota alterations may also have systemic effects that impact other parts of the body [20]. Systemic effects of virus associated tumors usually comprise alterations in circulating cytokine concentration or frequency of cells in the peripheral blood of patients [21]. Accordingly, serum cytokine levels may mirror the ongoing inflammatory reaction at the tumor site [22]. Individuals with high circulating levels of inflammatory mediators (like IL-6, CRP, etc.) are at greater risk for developing CRC [23,24]. The Th17 cytokine is specifically up-regulated in patients affected by specific types of CRC (e.g. sporadic vs. colitis-associated). Therefore, circulating biomarkers of inflammation have varying ability as risk predictors of incident cancer [23]. Abnormal cytokine expression was reported in patients with EV71 infection [25]; however, Th17 cytokine profiles are rarely reported in these cases.

Table 1
Clinical features of CRC patients and controls.

	CRC patients(n)	Controls(n)
Gender		
Male	45	8
Female	21	4
Age		
Median(year)	63.5	55.5
Range(year)	38–82	46–70
Location		
Right side of colon	9	
Left side of colon	5	
Transverse colon	7	
Sigmoid colon	19	
Rectum	24	
Splenic flexure	1	
Hepatic flexure	1	
Clinical stage		
I	4	
II	27	
III	27	
IV	8	
Adenoma histological grade		
I	1	
II	54	
III	11	
Biomarker		
Her-2(+ ~ + +)	49	
CgA(+ ~ + +)	8	
Syn(+ ~ + +)	11	
Ki-67(+)	19	
CD31(+)	30	
D2-40(+)	31	

Her-2: human epidermal growth factor receptor 2; CgA: chromogranin A; Syn: synaptophysin.

To investigate whether there is any association between the EV71 infection and CRC, immunohistochemical analyses were used to detect whether EV71 viral antigens existed in CRC tumor tissue. Our study found that EV71 antigens were detected in colorectal cancer tissues, but evidence clarifying the nature of this association was still limited. Whether microbial dysbiosis observed in CRC patients is a consequence of the pathology or is a causal, active modifier of disease outcome remains to be defined [5]. Inflammatory mediators detected in the serum of patients may mirror the ongoing inflammatory reaction at the tumor site [22], thus a serum array of Th17 cytokines was measured to investigate the associations between EV71 infection and CRC pathogenesis.

2. Methods

2.1. Patients

All clinical samples were obtained from patients admitted to the First Affiliated Hospital of Shantou University Medical College. An informed consent of each patient was obtained before they do surgery of tumor resection. The patients with CRC were selected based on the following criteria: patients with TNM stage I- IV disease with or without distant metastases, clinical and histopathologic staging at diagnosis was determined in all patients by combining histopathologic findings with surgical records and perioperative imaging. Non- cancerous adjacent tissue was collected from an area that was 15 cm distal to the cancerous tissue. Fresh tissue was stored at −80 °C. A blood sample from each patient was withdrawn, after the patients underwent biopsy at the time