Original Article

The possible role of EBV in carcinogenesis of colorectal carcinoma

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Summary:

Background: Except for the tight correlation to nasopharyngeal carcinoma, accumulating evidences show that Epstein-Barr virus (EBV) is correlated to other carcinomas. This study was to investigate the association of EBV with colorectal tumors.

Materials & methods: Forty paraffin embedded blocks of colorectal tumors (thirty were adenocarcinoma and ten were benign tumors) were all examined for the presence of EBV DNA with the application of In Situ hybridization.

Results: In Situ EBV DNA signals was detected in 6 out of 30 (20%) of colorectal carcinoma with no observed signals in the sections from benign group.

Conclusion: Our results showed that infection of EBV exists in human colorectal adenocarcinoma, which indicates that EBV may be involved in the carcinogenesis process.

Key words: EBV, In Situ Hybridization, colorectal tumors.

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Introduction:

EBV is a DNA virus belonging to the herpes family; its portal of entry is considered the oropharyngeal epithelium (1). Following primary infection, the virus establishes a life-long latent infection in the B-cell lymphocytes where it expresses some antigens in latent phase, which are proved to have some oncogenic properties (2). Except for the tight correlation to nasopharyngeal carcinoma, accumulating evidences show that Epstein-Barr virus (EBV) is correlated to other carcinomas. More recently, there have been scattered reports linking EBV with conventional epithelial cancers of other primary sites including breast, lung, gastric and colorectal carcinomas (2, 3, 4, and 5). The development of each type of EBV-associated malignancy requires a complex interplay between a specific cellular context and a specific mode of viral expression. Even in the restricted field of EBV-associated epithelial malignancies, there are major differences for example between EBV-associated gastric carcinomas and nasopharyngeal carcinoma in terms of cell differentiation and viral gene expression (6). In addition, the association of EBV with some epithelial neoplasm has been reported to depend on ethnic and/or regional background (3, 7).

Materials & Methods:

Forty paraffin-embedded blocks of colorectal tumors were collected, of those thirty were adenocarcinoma and ten were benign tumors (polyps & adenomas). The resection margins of the tumor (which are tumor free) were considered as a control group. Those archieved paraffin embedded blocks with their histopathological reports were taken from histopathological laboratories of Al-kadhymia, Gastroenterology and Hepatology and Baghdad teaching hospitals from November2005 to March 2006. Slides with H and E staining were prepared from each block in Pathology Department of Al-Nahrain College of Medicine and were reexamined by histopathologist to confirm the diagnosis. In situ Hybridization: Thin tissue sections (4um) were prepared on positively charged slides, which were baked overnight at 65°C. In Situ Hybridization, procedure was conducted in Microbiology Department of Al-Nahrain College of Medicine. In this study we followed the instruction of the manufacturer using DNA probe Hybridization/detection System In Situ Kit (Maxim Biotech, USA).After deparaffinization in xyelene for 5 minutes and rehydration through a series of ethanol dilutions, digestion with 1X proteinase K at 37°C for 30 minutes was done and the sections were quickly dehydrated in ethanol. Hybridization was carried out by applying 10 µl of heat denatured properly diluted probe (0.8 µl of biotin labeled DNA probe {BNRF1 and IR3 EBV specific probe, Maxim Biotech, USA} diluted in 9.2 µl hybridization solution). Slides were covered with cover slips and incubated at 95°C for 10 min. followed by overnight incubation in humid chamber. The slides then were soaked for 10 minutes in 1X detergent wash at 37°C, followed by RNAase treatment at 37°C for 30 minutes, washed for 5 minutes in 1X protein blocking buffer. The detection was performed using the avidin-biotin-Alkaline phosphatase complex technique and NBT (Nitoblue tetrazolium), yielding a blue to black signal at the specific site of hybridization and Nuclear Fast Red was used for counterstaining. Positive control was made with housekeeping gene probe while the negative control

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with hybridization solution without probe. Slides were examined at high power (X400 magnification) for estimation of percentage of tumor cells with positive blue to black signals and more than 5% considered as convincing EBV reactivity[8]. Statistical analysis of observed data was performed utilizing SPSS with the application of percentage, Chi-Square and Fisher Exact test.

Results:

The median age of the 30 patients enrolled in this study was 58 years ranging between 26 and 73 years with mean ± S. D.(56.6 ± 13.56) years. Twenty of patients were males (66.7%) and the females were ten (33.3%). Nuclear hybridization signals for EBV were observed in 6 out of 30 (20%) of colorectal carcinoma paraffin-embedded tissues (table 1) with no detectable signals in the sections from the benign group. In all the EBV-associated carcinomas, the virus was detected in the neoplasm cells but not in the normal colorectal epithelium (figure 1).We further analyzed the possible association between EBV expression and histopathological criteria of colorectal tumor considering tumor site, Grade, stage or metastasis to lymph nodes with no significant difference (p=0.690, p=0.338, p=1.000 and p=0.372 respectively) and as shown in table (2), out of the 6 EBV-associated carcinomas, 5 (83.3%) were moderately differentiated and 3 (50%) associated with lymph node metastasis.

Table (1): Frequency of EBV in Situ hybridization signals in cases of colorectal cancer.

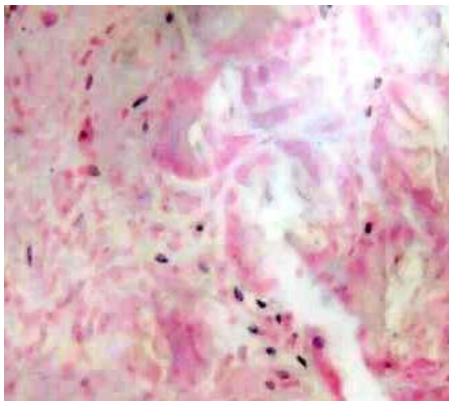
EBV signals	Number (%)
Positive	6 (20)
Negative	24 (80)
Total	30

Table (2): EBV positivity in association with histopathological criteria of colorectal carcinoma.

Histo-pathological criteria		EBV Positive (%)	EBV Negative (%)	P value
Tumor site	Caecum Colon Sigmoid & rectum Total	0 2(33.3) 4(66.6) 6	1(4.2) 5(20.8) 18(75) 24	0.690
Tumor grade	Well differentiated Mod. Differentiated Poorly differentiated Total	1(16.6) 5(83.3) 0 6	5(20.8) 12(50) 7(29.2) 24	0.338
Tumor stage	Stage A&B Stage C&D Total	3(50) 3(50) 6	11(45.8) 13(54.17) 24	1.000
Lymph nodes involvement	Positive Negative Total	3(50) 3(50) 6	7(29.2) 17(70.8) 24	0.372

Fisher's Exact Test

A



B

Figure (1): EBV in Situ Hybridization (A): Positive signals were shown in nuclei of the tumor cells (B): negative control. Nuclear Fast Red counterstaining, ×400.

Discussion:

Throughout the world, EBV is detected in the tissues of about 10 % of gastric carcinoma cases (9). Though colorectal epithelium is similar to that of gastric, and colorectal carcinoma is similar to gastric carcinoma, too, the association of EBV and colorectal tumors remained controversial. Recently, a correlation between EBV infection and gastric and colon cancer has been proposed (10, 11, 12, 13).Many authors had investigated Colorectal tumors for the presence of EBV using immunohistochemistry, polymerase chain reaction and In Situ Hybridization. EBV was detected by each method, but the positive rates were different with different methods (8, 14, and 15). Among the three methods, In Situ Hybridization was considered as the golden standard (12, 16). In this study EBV In Situ Hybridization was detected in 20% of the studied colorectal cancer a finding that is supported by many authors who reported that EBV might play an oncogenic role in frequent epithelial cancers, including colorectal cancers, and possibly also in hyperplasias and certain dysplasias preceding carcinomas (8,11,14). However, other investigators reported that none of the studied colorectal carcinomas showed a positive signal for EBV (17, 18). We can offer no satisfactory explanation for these variable findings by different investigators, as to clarify the infection of EBV, more than one kind of method should be used, in addition CD21 reactivity (which was not able to be evaluated in this study) need to be investigated as it has been established as the receptor for EBV in lymphoid B

cells. In this study, as Benign tumor counterparts were negative for EBV Hybridization, we can conclude that infection of EBV exists in human colorectal carcinoma, which may shed light on the possible role of EBV in the carcinogenesis of colorectal cancer, however, the mechanism needs to be clarified further at molecular level and perhaps larger number of cases need to be investigated.

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