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Analysis of Chromosome 8 Copy Number Changes in Colorectal Cancers by Fluorescence In Situ Hybridization (FISH).

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ABSTRACT

Objective: Colorectal cancer (CRC) is one of the most common malignancies worldwide. Colorectal carcinogenesis has associated with the progressive acquisition of a variety of genomic alterations by neoplastic cells, some of these have been linked to early stages of CRC development. The aims of this study (1) to identify alterations of chromosome 8 in primary colorectal carcinomas from Turkish patients and (2) to determine which alterations of chromosome 8 are early events during the development of colorectal carcinoma using fluorescence in situ hybridization (FISH).
Materials and Methods: To reveal the significance of genetic abnormalities of the chromosome 8, 28 colorectal tumors were analyzed using FISH. The centromeric-probe for chromosome 8 was used for FISH. In each case, at least 200 nuclei were scored for each hybridization.
Results: Monosomy in 3.6%, disomy in 39.3%, trisomy in 53.6% and tetrasomy in 3.6% of the analyzed adenomas were determined. Chromosome 8 gain was found in 5 of 8 (62.5%) nonpolypoid and 3of 9 (33.3%) polypoid cancers. There was statistically significant correlation between chromosome 8 gain and stage of CRC.
Conclusions: There are several reports of chromosome 8 gain in solid tumors. FISH is a useful method to detect genetic abnormalities in solid tumors. It was shown that chromosome 8 gain FISH associated with the stage of CRC. Chromosome 8 monosomy may be a early event in CRC. Further studies involving more patients need to determine the importance of this alteration in CRC. *©2007, Firat University, Medical Faculty*

Key words: *Colorectal cancer, fluorescence in situ hybridization , chromosome 8.*

ÖZET

Kolorektal Kanserlerde Floresans in situ Hibridizasyon (FISH) Kullanılarak Kromozom 8 Kopya Sayı Değişimlerinin Analizi

Amaç: Kolorektal kanser (KRK) dünyadaki en yaygın malignansilerden biridir. Kolorektal karsinogenezis neoplastik hücrelerde meydana gelen çeşitli genetik değişikliklerin birikimiyle beraberlik göstermektedir. Bunlardan bazıları KRK gelişiminin erken safhalarında gerçekleşmektedir. Bu çalışmanın amacı, Floresans in situ Hibridizasyon (FISH) kullanılarak Türk primer kolorektal kanserli hastalarda kromozom 8 değişimlerini tanımlamak ve kromozom 8 değişimlerinin kolorektal kanserin gelişiminde erken bir olay olup olmadığını tespit etmektir.
Gereç ve Yöntem: Kromozom 8'in genetik anomalilerinin anlamını göstermek için 28 kolorektal kanser FISH'le analiz edildi. FISH için kromozom 8 sentromerik prob kullanıldı. Her bir hasta için en azından 200 hücredeki sinyaller incelendi.
Bulgular: Analiz edilen tümörlerin %3.6'sında monozomi, %39.3'ünde dizomi, %53.6'sında trizomi ve %3.6'sında tetrazomi saptandı. Kromozom 8 kazancı 8 nonpolipoid kanserin 5'inde (%62.5) ve 9 nonpolipoid kanserin 3'ünde (%33.3)'de bulundu. Kromozom 8 kazancı ve KRK evresi arasında istatistiki olarak anlamlı korelasyon vardı.
Sonuç: Solit tümörlerde kromozom 8 kazancıyla ilgili birkaç çalışma vardır. FISH solit tümörlerdeki genetik anomalilerin tespiti için faydalı bir yöntemdir. Kromozom 8 kazancı ve KRK evresi arasında ilişki olduğu gösterildi. Kromozom 8 monozomisi KRK'da erken bir olay olabilir. Bu değişimin öneminin ortaya konması için KRK'da daha fazla hasta sayısı içeren çalışmaların yapılması gerekmektedir. *©2007, Fırat Üniversitesi, Tıp Fakültesi*

Anahtar kelimeler: *Kolorektal kanser, floresans in situ hibridizasyon, kromozom 8.*

Colorectal cancer is the second leading cause of cancer-releated death in the European countries and USA. In recent years our understanding of the cellular and molecular events underlying the development of colorectal cancer has improved immeasurably (1). However, most colorectal cancers arise from adenomas through a process described as the adenoma-carcinoma sequence (2). It is now widely accepted that the development of cancer is caused by the accumulation of the large amount of genetic alterations during the pathogenesis of cancer (3). Many detailed reports have been published on the carcinogenesis of colorectal cancer. Several genetic aberrations are required for tumor iniation and progression (4). Over 90 percent of all colorectal cancers show chromosomal aberrations; only a minority have a normal karyotype (1).

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The usage of fluorescence in-situ hybridization (FISH) techniques has enabled the rapid analysis of cytogenetic specimens as an adjunct to conventional cytogenetic analysis (5). In most FISH studies of solid tumors, changes in the copy number of specific gene for chromosomes were evaluated in interphase cell nuclei (6, 7). However, genetic rearrangements can not be demonstrated by interphase- FISH (8,9). Interphase-FISH studies related to chromosome 8 gain in colorectal cancer are very rare in literature. In the present study, FISH was used to analyze alterations of chromosome 8 in 28 primary colorectal carcinomas. The aims of this study (1) to identify alterations in chromosome 8 in primary colorectal carcinomas from Turkish patients and (2) to determine which alterations of chromosoma 8 are early events during the development of colorectal carcinoma.

MATERIALS AND METHODS

Sample Collection

Tissue specimens for FISH were obtained from fresh surgically resected primary tumors of patients hospitalized at Fırat Medical Center, Elazığ. Primary tumors from 28 cancer patients with colorectal cancer were classified according to the TNM classification system of the Union International Center of Cancer (UICC). We have obtained informed consent from each subject or the subject's guardian. All samples were obtained before the administration of chemo/radiation therapy. Apart of each specimen was used for rutine histopathological examinations. Peripheral blood lymphocytes obtained from healthy adults were used as negative control.

Slide Preparation

Slides were prepared to use touch preparation protocol (10). Specimens for normal tissue and malignant tumour tissue were touched lighty on precleaned slides, which can be performed in a short time. After air-drying at room temparature, these slides were fixed with fixing solution (3:1 methonol:aceticacid) and stored at -20^{0}C until subsequent analysis.

Probe and Hybridization

A directly labelled centromeric probe for the chromosome 8 centromere (Spectrum Green; Cytocell, Oxfordshire, UK), as well as reagents necessary for hybridization, were purchased. Hybridization was performed in according to the manufacturer's instructions. Slides prepared with blood, normal tissue and malignant tissue for each patient were denatured in 2XSSC /70% formamide, pH 7, at 67 ^{0}C for 6 minutes and dehydrated in graded ethanol. Hybridization was performed with 10µl of the hybridization mixture which contains 7µl probe and 3µl hybridization buffer. Probes were denatured at 67 ^{0}C for 10 minutes and applied to the target slides. Hybridization was performed overnight at 37 ^{0}C in humidified chamber. Posthybridization washes were performed with %50 formamide/ 2XSSC three times for 10 minutes, 2XSSC for 5 minutes, and 2XSSC/Nonidet P-40 for 5 minutes at 42 ^{0}C. Counterstaining was fresly prepared by mixing 2µl of PI to 8µl of DAPI and then used. The number of FISH signals were counted with a Nicon microscope equipped with a color filter. At least 200 nuclei were scored for each hybridization , depending on availability and appropriateness of nuclei. Presence of aneusomy was defined in correspondence with the presence of at least 20% of the abnormality, i.e., with one centromeric signal monosomie, with three and more centromeric signal polysomies. The threshold value of 20% was in keeping with that of previous publications (11, 12).

Statistical analysis

Statistical analysis was performed with the SPSS software (SPSS version 11). Alterations of chromosome 8 were compared between various parameters by using Linear Correlation, Spearsman's and chi square tests. $P<0.05$ was considered to indicate statistical significance.

RESULTS

The number of the patients included in this study of 28 patients 9 (32.1%) were female and 19 (67.9%) were male and their ages were 44.5±14.9 and 41.5±13.8 years, respectively. At least 20% of nuclei having a number of centromeric two signals corresponding to disomy was considered as aneusomy. Chromosome 8 aneusomy was detected in 17 of 28 tumor analyzed. Among analyzed adenomas in 3.6%, disomy in 39.3%, trisomy in 53.6% and tetrasomy in 3.6% were found. Figure 1 shows typical FISH results for chromosome 8. An interesting example is presented in tumor 5 in which tetrasomy was dominant in 96% of nuclei. Samples from tumor 26 showed monozomic dominancy was more than 50% of nuclei.

Table1. Characteristics of the patients.

Characteristic	Patients	Gain of Chromosome 8	Loss of Chromosome8
Mean age (yr)	54.2		
Sex (no.)	28		
Male	19	57.89% (11/19)	ND
Female	9	55.55%(5/9)	11.11%(1/9)
UICC Classification			
Stage1	1	ND	100%(1/1)
Stage2	8	62.50%(5/8)	ND
Stage3	5	60.00%(3/5)	ND
Stage4	14	50.00%(7/14)	ND

Significant difference was found in the statistical analysis with Spearman's test between patients age and alterations of chromosome 8. There was statistically significant correlation between chromosome 8 gain and stage of colorectal cancer which gain of chromosome 8 was excepted to be presence three signal at least in 40% of nuclei. Table 1 shows the clinical characteristics of the 28 patients and tumors. We have investigated 17 tissue specimens for chromosome 8 alterations consisting of 8 nonpolypoid and 9 polypoid adenomas. Chromosome 8 gain was found in 5 of 8 (62.5%) nonpolypoid and 3of 9 (33.3%) polypoid cancers. However, correlation of this alteration to the cancer type statically was in significant.

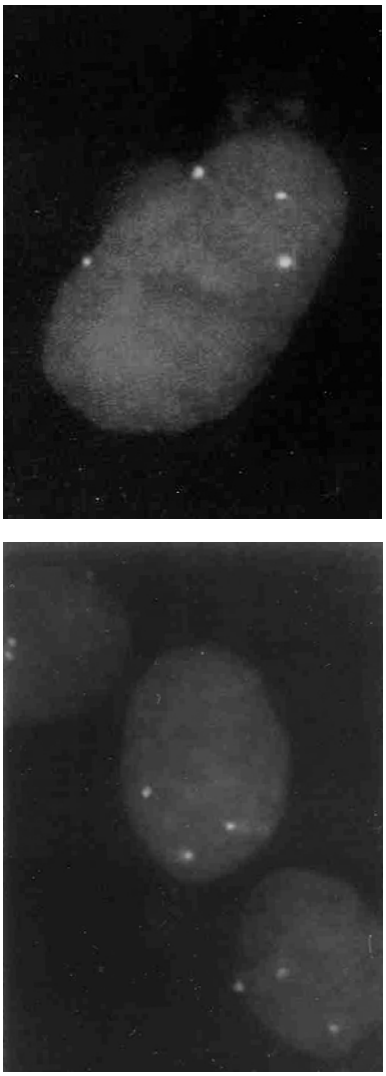

Figure 1. Single color FISH with chromosome 8 centromere (green signal). A: Nucleus of colorectal cancer cell with 3 signals, B: Nucleus of colorectal cancer cell with 4 signals, indicating gain of chromosome 8.

DISCUSSION

The evaluation of chromosome 8 alteration in 28 human sporadic colorectal adenocarcinom analysis enabled to understand to the aneuploidization of chromosome 8 in human colorectal cancer better with the aim to better understand the aneuploidization of chromosome 8 in human colorectal cancer. Chromosome aberrations involving chromosome 8 have beeen reported in various solid tumors, such as stomach, lung, prostate and bladder cancer (6, 7). Takahashi et al. reported gain of chromosome 8 in all colorectal cancer evaluated by interphase-FISH (13). Aragane et al. and He et al. have found a gain at 8q in 43% and 54% of tumors analyzed by Comparative Genomic Hybridization (CGH), respectively (14, 15). Nakao et al. determined gain involving 8q in 42% of patients by array-based CGH (16). Pirc-Danoewinata et al. reported one of the most chromosomal gain of chromosome 8 using cytogenetic evaluation in 26 patients with colorectal carcinoma (17). We found chromosome 8 trisomies in 53% of CRC by interphase-FISH. This ratio is similar with other studies. However, we founded different results in respect of chromosome 8 monosomies and tetrasomies. The number of centromeric signals is reportedly unchanged throughout the cell cycle (18). Therefore, the increase in chromosome 8 centromeric signals suggests an abnormally increased chromosome 8 copy number in these cases. A model of tetraploidization occured from a diploid status and loss of chromosomes may be common in other tumor types but apparently not during colorectal tumor progression (19). Tetraploidization and monosomies for chromosome 8 was very rare events during adenom-carcinom progression in colorectal cancer. We found chromosome 8 monosomy in the patient with stage I and it could be suggested that chromosome 8 monosomy can be an early event in colorectal carcinogenesis. The importance of chromosome 8 monosomy in colorectal cancer is unclear (20). Because of this, further studies involving more patients are needed to identify biological significance of chromosome 8 monosomy for the development and progression of colorectal cancer.

It has been reported that there may be a different clinical outcome and histopathological character between nonpolypoid and polypoid adenomas suggesting an alternative pathway in the genesis of colorectal cancer. However, the variations the clinical and in the molecular genetic findings of nonpolypoid neoplastic lesions still remain rather unclear (20). Richter et al reported that gains on chromosomes 2q, 5q, 6, 8q and 12q occured exclusively in nonpolypoid adenomas (20). Although, we didn't found statistically significant correlation attributable to chromosome 8 alteration in polypoid and nonpolypoid colorectal cancers, but our results showed that chromosome 8 gain are more frequent nonpolypoid carcinomas. This represents another indication for a different carcinogenic pathway in both lesions. More striking evidence for this hypothesis comes from a detailed analysis of other single aberrations.

FISH results are often unsatisfactory in solid tumors compared with blood or cell lines, because the abundant connective tissue in solid tumors hampers separation into single cells and produces excessive background debris. It make difficult the preparation of clear specimens when interphase-FISH studies performed using touching protocol (6, 7, 9). For this reason, we advice that presence of aneusomy is defined in correspondence with the presence of 20%-40% of centromeric signals different from two signals corresponding to disomy. Thus, most FISH studies of solid tumors have assessed only changes in the copy number of chromosomes in interphase cell nuclei. However, all chromosomal rearrangements can not be detected, because FISH can not be used to full advantage, employing only interphase analysis. We have achieved reliable hybridization in interphase cell nuclei from surgically resected solid tumors. We avoided changes in the cell population harboring the original mutation due to selective pressure by using primary samples rather than subcultures of cell lines. Thus confidently can be stated that the chromosome 8 gain and loss observed in this study was present in the original cancer cells, rather than induced artifacts.

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