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Research Article

Cytogenetic Screening in Colorectal Cancer Patients of Tamil Nadu Population

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Abstract

Colorectal cancer (CRC) is one of the most common types of cancer worldwide and harbors distinct chromosomal alterations. This study tries to investigate clinic-pathological, chromosomal alterations to identify the novel genes and expression pattern in Tamil Nadu population of CRC patients. Patients with CRC (*n*= 13) and healthy controls (*n*= 13) were evaluated by analyzing the chromosomal alterations by standard protocols. Higher percentage of deletions was found with 46, XY, del 18p- and translocation was 46, XY. In conclusion, correlation of focal chromosomal alterations enriched in cancer genes with its clinical outcome indicates a prognostic index contributing to drive the process of CRC.

Keywords: Colorectal Cancer (CRC), Chromosomal aberrations (CAs), deletion, Tamil Nadu

INTRODUCTION

Colorectal cancer (CRC) is one of the most widespread human melanoma, predominant in industrialized countries. The condition of metastasis of these tumors to the liver is a foremost foundation of death among patients with CRC. The frequency of CRC is mostly sporadic with 25% being familial, suggesting a contribution of shared genes and environment. However, only 5-6% of CRC is due to inherited mutations in major CRC genes whilst the remaining familial forms are likely to result from gene environment interactions [1]. CRC is most frequent in Chinese, followed by Malay and Indian with 1141, 805, and 118 new cases in 2007 [2]. CRC rates are about 2 to 5 times pronounced in the urbanized countries in comparison with the developing countries which may be attributed to an assortment of variations in the contrasting set of risk factors and analytical practices [3, 4]. Adopting globalized Western-style diet high in fat, animal protein and a sedentary lifestyle are believed to be the underlying reasons for an increase in CRC cases, although an interlink between these factors and genetic characteristics of the Asian populations might also have an essential role [3, 5]. The sex hormones and their activation of transcription in target tissues are determined by biosynthetic and metabolizing enzymes whereas the steroid receptors, genetic and epigenetic modifications of the genes and proteins may enhance the risk of cancer [6]. Some of the studies have also supported the hypothesis that lower testosterone may increase men's

risk of developing CRC [7, 8].

Chromosome breaks appear to be important in nearly all human cancers and are especially common in colorectal cancer [9]. Development and progression of the tumor are closely related with the chromosome quantities and/or structural abnormalities. The activation of oncogenes and inactivation of tumor suppressor genes are the major mechanisms of carcinogenesis [10]. They are caused by mutations in genes that are indispensable to maintain chromosomal stability, leading to the accumulation of gains and losses of chromosomes (aneuploidy) as well as by structural chromosomal rearrangements [11-13]. The gains of chromosome arms 8q, 17q, 20q, and the losses of 8p and 17p are also unbalanced chromosomal alterations which have been identified earlier [14, 15].

Based on the earlier reports, chromosomal alterations (CA) were determined and assessed using the standard protocols in our study to analyze whether this genetic compensation might be associated with CRC susceptibility. The current study mainly aimed to investigate the chromosomal alteration in the CRC subjects recruited from Tamil Nadu population. The surveillance of identical karyotype abnormalities in all cells within a tumor provides strong indication for a clonal beginning of the tumor. In turn, the CAs serves as markers of a common malignant state in the individual cells to observe the risk of CRC.

Materials and methods

Subject Recruitment

In the present study, we collected 13 blood samples from CRC patients of Thanjavur and Coimbatore population. An equal number of normal and healthy individuals were selected as controls including those who have not exposed themselves to any kind of chemicals or radiation. The patients and the controls were divided into two groups based on age (Group I ≤ 55 years and group II > 55years). Average patient age in group I was *n*=5 (48.22 ± 3.67) and in group II was *n*=8 (60.89 ± 3.63) respectively. All the subjects were recruited consecutively with controls being matched to the respective CRC subjects in terms of age (±2 years relaxed) and an informed consent was obtained. The study procedure and institutional human ethical clearance has been obtained and Helsinki (1966) declaration was followed throughout the study. Peripheral blood samples of patients and control subjects were collected using heparinised syringe for leucocyte culture. Chromosomal preparations obtained were processed and stained with Giemsa to obtain G-bands. Clinical data were obtained by reviewing patient charts and medical records, and cancer stages were determined using the American Joint Committee on Cancer (AJCC).

Sample collection

For each study, 10 mL of blood was drawn from the participants by vein puncture and collected in heparin tubes to be used for cytogenetic assay.

Chromosome Aberration Assay

Cytogenetic techniques such as conventional chromosomal analysis (karyotyping) using Trypsin G-Banding were studied. Cultures of leucocytes obtained from peripheral blood were set-up by a standard protocol. Briefly, 0.5 mL whole blood was added to 4.5 mL RPMI 1640 medium supplemented with 10 % fetal bovine serum, 2 mM L-glutamine, 1% streptomycin–penicillin antibiotics, and 0.2 mL reagent-grade phytohemagglutinin and incubated at 37°C. At 71 h, cultures were treated with 0.1 mg/mL colcemid to block cells at mitosis. Lymphocytes were harvested at 72 h, by centrifuging cells to remove culture medium (800–1,000 rpm/7 min) and adding hypotonic solution (KCl 0.075 M) at 37°C for 20 min to swell the cells after treating twice with a fixative (methanol and acetic acid [3:1 vol/vol]). Cytological preparations were made by placing two to three drops of the concentrated cell suspension onto slides wetted with ice-cold acetic acid (60 %). Slides were carefully dried on a hot plate (56°C for 2 min). For CAs analysis, 100 complete metaphase cells of the first cell cycle were evaluated under a microscope (100X) to identify numerical and structural CAs according to the norms of International System for Human Cytogenetic Nomenclature (ISCN). The collected data were registered on master tables and later transferred to a computer file.

Statistical Analysis

The statistical significance of the differences in the frequencies of chromosomal alterations between groups was calculated by *t* test. Odds ratios (OR) and confidence intervals (CI) were calculated to estimate the strength of CA in CRC patients and

controls [41]. Mean and standard deviation were calculated to assess the difference between the patients and controls and the level of significance was calculated by one way-ANOVA. All the analyses were performed with IBM-SPSS software 22.0 version.

Results

A total of 26 subjects including 13 CRC patients and 13 controls were recruited. Chromosomal damage of CRC patients and controls are exhibited in Table 1. CTAs in group I and group II CRC subjects were 2.56 ± 0.81and 6.9 ± 1.03, which were found to be significant when compared to their group I and II controls (1.23 ± 0.67 and 1.97 ± 0.83). The CSAs of group I and II CRC were 3.93 ± 0.90 and 8.95 ± 1.76 which were significant compared to their group I and II controls (2.4 ± 0.62 and 3.25 ± 0.66), respectively. The mean values of total CAs in group I and II CRC were 6.43 ± 1.56 and 15.85 ± 2.65 and showed statistical significance when compared to their controls (3.63 ± 1.06 and 5.12 ± 1.30), respectively. All the CRC subjects showed significant values by ANOVA at $P < 0.05$ level. The higher percentage of deletions found were 46, XY, del 18p- and translocation was 46, XY.

Table 1 Depicts the frequency of chromosomal alterations observed in CRC patients and controls

Sl. no.	Groups	Total subjects	Age	CA/100 cells		
				CTAs	CSAs	TCA
1	**CRC patients**					
	Group I ≤ 55	5 (43.47)	48.22 ± 3.67	2.56 ± 0.81	3.93 ± 0.90	6.43 ± 1.56
	Group II > 55	8 (56.52)	60.89 ± 3.63	6.9 ± 1.03	8.95 ± 1.76	15.85 ± 2.65*
2	**Controls**					
	Group I ≤ 55	5 (43.47)	49.44 ± 5.44	1.23 ± 0.67	2.4 ± 0.62	3.63 ± 1.06
	Group II > 55	8 (56.52)	59.7 3± 3.11	1.97 ± 0.83	3.25 ± 0.66	5.12 ± 1.30

TCA - Total Chromosomal Alterations; CTA - Chromatid Type aberrations; CSA - Chromosomal Type aberration; CA - Chromosomal Alterations; * Values significant at p<0.05 level by ANOVA

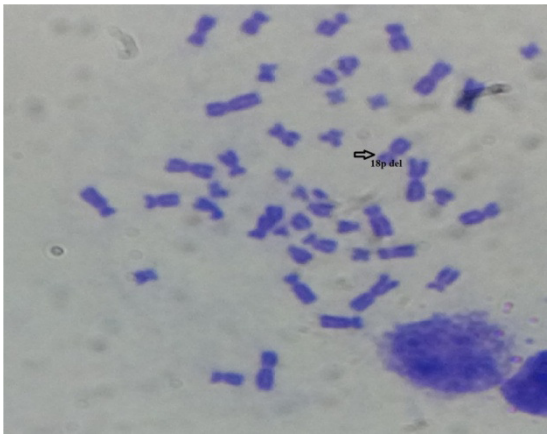

Figure 1: Chromosomal aberration in CRC patient

Discussion

CRC is a disease in which normal cells in the lining of the colon or rectum begin to change, start to grow uncontrollably, and no longer die. Genetic and environmental factors including diet and lifestyle may play a major role in the carcinogenesis of CRC [16]. In disparity, the predictions of a study from Europe about chronological transmutation and accumulation of genes, present quite a different view, in which the gene mutation spectrum in a large cohort of CRC patients was correlated to only 6.6 % [17]. The heterogeneous prototypes of tumor mutations suggest the presence of multiple alterna-

tive genetic pathways for CRC and it was also speculated that the widely accepted genetic model of cancer development is not a representative of the majority of CRC [18].

CRCs are characterized by multiple chromosomal abnormalities. Recent studies addressing the characterization and identification of distinct pathways of tumor progression suggests that, there are several important correlations between selection of any specific type of genetic pathway and variations of the clinical outcome in stage I to IV CRC patients. This has been suggested as a reflection of different etiological factors involved in the pathogenesis of right and left sided CRCs [19]. Moreover, type of the genetic instability plays an important role both in the tumor location and prognosis [20]. Each alteration whether an initiation or a progression associated event, may be mediated through gross chromosomal change and hence has the potential to be detected cytogenetically [21]. These broadly defined alterations are in perfect agreement with chromosome specific trends in our expression data, especially the exclusive presence of alterations on chromosome 4, 8, 13, 18, and 20. In our study, an increase in CA frequencies in CRC patients compared to normal controls was highly significant. CTAs and CSAs in group I and II CRC subjects were found to be significant when compared to their controls. Also, the mean values of total aberrations in groups I and II CRC displayed statistically significant results compared to their controls. The higher percentage of deletions found were 46, XY, del 18p- and translocations were 46, XY t (1; 21p) and t (4; 6) which confirm that our results support the previous findings and most of the studies reported frequent gains of chromosome 7, 8q, 13q, 20q and losses of 4 and 18q in CRC [22]. Previous epidemiology data with sporadic CRC found elevated Loss of Heterozygosity (LOH) for regions of chromosomes 5, 8, 11, 12, 17, and foremost 14 and 18; these are the same chromosomes that were seen in those cases with either whole chromosome or segmental uniparentalism on the SNP arrays [22].

Conclusion

The progress of specialized markers for the finding of CRC could have an impact on cancer mortality and significant implications on public wellbeing. Karyotypic investigation has provided precious information on chromosomal aberrations in CRC, which is supportive in assessing organization and monitoring treatment regimens. In conclusion, the present investigation reveals that mutational analysis should be performed; suggesting that assessment of genes for hormone-metabolizing enzymes and receptors may offer an approach for identifying individuals at high CRC risk.

Conflict of Interest

The authors declare that they have no conflict of interest.

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