 Int J Hum Genet, 5(3): 199-203 (2005)

Cytogenetic Findings in Cancerous and Non-Cancerous Lesions of the Digestive System

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KEYWORDS Digestive system; cancer; non-cancerous lesion; chromosomal aberration

ABSTRACT Chromosome instability provides a predisposing background to malignancy, contributing to the crucial genetic changes in multistep carcinogenesis. It is generally accepted that cancer is a genetic disease resulting from multiple genome rearrangements. A variety of chromosomal aberrations have been identified in various cancerous and non-cancerous lesions of the gastrointestinal tract. Certain aberrations observed in non-cancerous lesions were identical to those of cancerous lesion.

INTRODUCTION

Cancers are essentially genetic diseases, usually acquired, with mutation, chromosomal deletions and reduplications and epigenetic phenomena such as DNA methylation leading to inactivation of tumour suppressor genes or activation of oncogenes. Multistep genetic alterations at the nucleotide and chromosome levels underlie carcinogenesis. The Gastrointestinal Tract (GIT) is a frequent site for the development of cancers. Malignant neoplasms of the GIT and accessory organs account for approximately 25% of all cancers and 28% of cancer deaths (Gerald 1977). There are a large variety of inherited conditions in gastroenterology ranging from dominantly inherited predispositions to neoplasms and to relatively recessive metabolic diseases. There are also conditions that show familial aggregation where until now it has not been clear whether shared environment or genetic predisposition is responsible.

It is now widely accepted that cancer results from the accumulation of mutations in the genes that directly control cell birth or cell death. But the mechanisms through which these mutations are generated are the subject of continuing debate. It has been argued that an underlying genetic instability is absolutely required for the generation of multiple mutations that underlie cancer (Loeb 1991; Hartwell 1992). The instability exists at two distinct levels; in a small subset of tumours, the instability is observed at the nucleotide level and results in base substitutions or deletions or insertions of a few nucleotides. In most other cancers, the instability is observed at the chromosome level, resulting in losses and gains of whole chromosomes or large portions of chromosomes (Christoph et al. 1998).

In the last 50 years, interest has turned increasingly to the study of genetics in relation to medicine. It has been realised that many prevalent diseases can partly be determined by genetic factors. Hitherto there have been different findings in the knowledge of the cytogenetic aspects related to digestive diseases which may be because of the extremely complex interrelationship of biological activities within the cell.

The objective of the study was to identify chromosomal alterations and rearrangements in cancerous and non-cancerous lesions of the Digestive System.

MATERIALS AND METHOD

Samples for the study were collected from patients comprising of an admixture of native and neighboring district population attending a gastrointestinal tertiary care hospital at Coimbatore, Tamilnadu, India. 80 subjects with cancers and 60 subjects with non-cancerous lesions of the digestive system were selected for the cytogenetic analysis.

0.5ml of the sample blood was inoculated under aseptic conditions into a culture vial containing 5.0 ml of culture medium, 1.0ml of AB serum and 0.2 ml of Phytohaemagglutinin (PHA). The cultures were incubated at 37°C for a period of 72hrs. The dividing cells were arrested at the metaphase stage by adding 0.05 ml of colchicine solution (0.01%) 30 minutes before harvesting the culture. The contents of the vials were centrifuged at 1000rpm for 10 minutes at the end of colchicine treatment. The supernatant was discarded and 6ml of hypotonic solution was added to the test tube after disturbing the cell button. The contents of the test tubes were incubated for 7 minutes and freshly prepared fixative [Methanol : Glacial Acetic acid (3:1 v/v)] was added and centrifuged at 1000 rpm for 10 minutes. Later the supernatant was discarded and two or three changes of the fixative were given to obtain a colourless cell pellet.

The slides bearing chromosome spreads were treated with 0.25% trypsin for 3 to 10 seconds and were stained in 4% buffered Giemsa solution for 3 minutes. Fifty well spread metaphase plates of each subject were analysed.

RESULTS

Cytogenetic analysis revealed various chromosomal aberrations and rearrangements in 19 of the 60 subjects with non-cancerous lesions and 26 of the 80 subjects with cancerous lesions of the digestive system. The percentage of aberrations accounted to a higher score in the group with cancerous lesions than the group with non-cancerous lesions.

The types of chromosomal aberrations observed in male patients with non-cancerous lesions were $4p^-$ and $8q^+$ in patients with Barrett's esophagus; translocations t ($5q^+$;$21p^-$), t ($8q^-$;$13q^+$) and mosaic karyotype with t ($9p^-$;$12p^+$) in patients with pancreatitis; inversion of chromosome 3 in a patient with colonic polyp; mosaic karyotype with addition of Y chromosome in a patient with duodenal ulcer; deletion $5p^-$ in a patient with ulcerative colitis; addition in chromosome 16 and deletion of the long arm of chromosome 8 ($8q^-$) in patients with gastric ulcer and $14q^-$ in a patient with gastritis (Table 1).

Among female patients with non cancerous lesions, a patient with duodenal ulcer expressed a mosaic karyotype with addition of X chromosome; a patient with chronic colitis expressed translocation t ($13q^-$;$22q^+$); a patient with Barrett's esophagus displayed a mosaic karyotype with total deletion of an X chromosome; a patient with colonic polyp expressed inversion of chromosome 3, two patients with ulcerative colitis displayed deletion of the short arm of chromosome 5 ($5p^-$); a patient with gastric ulcer displayed $12q^-$ and a patient with gastritis expressed $9p^-$ (Table 1).

Table 1: Chromosomal aberrations in subjects with non-cancerous lesions of the digestive system

S. No.	*Particulars*	*Case ID*	*Chromosomal aberration in males*
1	Group I	NCBE2	46,XY, del ($4p^-$)
2	Group I	NCCP29	46,XY, t ($5q^+$; $21p^-$)
3	Group I	NCCOP18	46,XY, inv (3)
4	Group I	NCDU3	46,XY / 47,XYY
5	Group II	NCCP13	46,XY, t ($8q^-$; $13q^+$)
6	Group II	NCUC9	46,XY, del ($5p^-$)
7	Group II	NCBE8	46,XY, ($18q^+$)
8	Group II	NCGU7	46,XY, ($16q^+$)
9	Group II	NCCP6	46,XY / 46,XY, t ($9p^-$; $12p^+$)
10	Group II	NCGU13	46,XY, del ($8q^-$)
11	Group II	NCAG23	46,XY, del ($14q^-$)
S.No.	*Particulars*	*Case ID*	*Chromosomal aberration in females*
1	Group I	NCDU7	46,XX / 47,XXY
2	Group I	NCCC19	46,XX, t ($13q^-$; $22q^+$)
3	Group II	NCBE4	46,XX / 45, XO)
4	Group II	NCCOP9	46,XX, inv (3)
5	Group II	NCUC6	46,XX, del ($5p^-$)
6	Group II	NCGU2	46,XX, del ($12q^-$)
7	Group II	NCAG12	46,XX, del ($9p^-$)
8	Group II	NCUC25	46,XX, del ($5p^-$)

Group I: Subjects < 55 years of age Group II: Subjects >55 years of age

Chromosomal aberrations observed in male patients with cancerous lesions were $13p^-$ and a mosaic karyotype showing addition of chromosome 22 in patients with cancer in the ampulla of Vater; deletions $11p^-$ and $13p^-$ in two patients with cancer of the liver; deletion ($3p^-$), translocation t ($5p^-;13q^+$) and inversion in chromosome 3 in patients with cancer of the colon; translocation t ($3p^-;16q^+$), inversion of chromosome 9 and deletion ($7p^-$) in patients with cancer of the stomach; translocation t ($9q^-;12q^+$) in a patient with cancer of the pancreas; translocation t ($14p^-;22q^+$) in a patient with cancer of the bile duct; a mosaic karyotype with addition in the long arm of chromosome 8 and inversion of chromosome 9, and translocation t ($1q^-;13q^+$) in patients with cancer of the esophagus (Table 2).

Among the female patients, a mosaic karyotype with deletion ($8p^-$), mosaic karyotype showing loss of an X chromosome, addition of chromosome 13, deletion ($11q^-$) and translocation t ($1q^-;13q^+$) were observed in various patients with cancer of the esophagus; deletion ($5q^-$) and inversion of chromosome 3 were observed in patients with cancer of the colon; translocation t ($7p^-;8q^+$) was displayed by a patient with cancer of stomach; deletion ($4q^-$) was expressed by a patient with cancer of liver; addition in chromosome 20 was seen in a patient with cancer of the bile duct; a patient with cancer of the ampulla of Vater expressed a mosaic karyotype with addition of the whole chromosome 21 and another patient expressed additions in chromosome 6 (Table 2).

DISCUSSION

According to Gerald (1977), malignant neoplasms of the gastrointestinal tract and accessory organs had accounted for approximately 25% of all cancers and 28% of cancer deaths. The major sites of involvement included the esophagus, stomach, large intestine and pancreas. In the present study, of the various aberrations observed in chromosomes, certain aberrations were corroborative to the findings of earlier studies.

The primary chromosome change in malignancy is the initial one occurring in the development of the disease. Secondary

Table 2: Chromosomal aberrations in subjects with cancerous lesions of the digestive system

S.No.	*Particulars*	*Case ID*	*Chromosomal aberration in males*
1	Group I	CAV23	46,XY, del ($13p^-$)
2	Group I	CL11	46,XY, del ($11p^-$; $13p^-$)
3	Group I	CL5	46,XY, del ($11p^-$; $13p^-$)
4	Group I	CCR4	46,XY, del ($3p^-$)
5	Group I	CCR2	46,XY / 46,XY, t ($5p^-$; $13q^+$)
6	Group I	CS10	46,XY, t ($3p^-$; $16q^+$)
7	Group II	CAV17	46,XY / 47,XY, +22
8	Group II	CP2	46,XY, t ($9q^-$; $12q^+$)
9	Group II	CCBD7	46,XY, t ($14p^-$; $22q^+$)
10	Group II	CS5	46,XY, inv (9)
11	Group II	CCR1	46,XY, inv (3)
12	Group II	CS7	46,XY, del ($7p^-$)
13	Group II	CE9	46,XY / 46,XY, ($8q^+$), inv (9)
14	Group II	CE14	46,XY ($1q^-$; $13q^+$)
S.No.	*Particulars*	*Case ID*	*Chromosomal aberration in females*
1	Group I	CE18	46,XX / 47,XXY, del ($8p^-$)
2	Group I	CE11	46,XX / 45,XO
3	Group I	CCR10	46,XX, del ($5q^-$)
4	Group II	CS13	46,XX, t ($7p^-$; $8q^+$)
5	Group II	CL12	46,XX, del ($4q^-$)
6	Group II	CCBD4	46,XX, ($20q^+$)
7	Group II	CE5	47,XX, +13
8	Group II	CE7	46,XX, del ($11q^-$)
9	Group II	CAV6	46,XX / 47,XX, +21
10	Group II	CE15	46,XX, t ($1q^-$; $13q^+$)
11	Group II	CCR7	46,XX, inv (3)
12	Group II	CAV3	46,XX, ($6q^+$)

Group I: Subjects < 55 years of age Group II: Subjects >55 years of age

chromosome changes occur during karyotype evolution in malignancy and may play a crucial role in the aggressiveness of the disease and other aspects of its biology (Grant and Frederick 1985).

Subjects with esophageal cancer displayed karyotypes such as addition of the whole chromosome 13, $8q^+$ and $11q^-$ (as observed by Atiphan et al. 2000). Another subject with a mosaic karyotype displaying $8p^-$ also presented with esophageal cancer. Two subjects with esophageal cancer showing translocation ($1q^-$; $13q^+$) was also observed. The manifestation of esophageal cancer in these subjects may be due to the participation of genes located on the altered chromosomes that are involved in the process of oncogenesis. The altered chromosomes are some of the many chromosomes involved in the development of esophageal cancer observed by Atiphan et al. (2000).

Van Grieken et al. (2000) justified that a complex of chromosomal aberrations are involved in gastric cancer. Subjects with gastric cancer in the present study presented with aberrations involving chromosomes 9 with inversion, chromosome 7 with deleted short arm and translocations causing deletion of short arm of chromosome 7 and gain of long arm of chromosome 8. These aberrations might have played a major part in the development of cancer in these patients (as observed by Katayama et al. 2000).

In the present study, a subject with cancer of the colon displayed the chromosomal aberration $5q^-$, which is an important site of location of tumour supressor genes (Grandjouan 1996), and this may be the reason for the manifestation of cancer in the subject. Karyotype analysis by Koji Sasajima et al. (1993) in a patient with rectal cancer accompanied by multiple polyps in the digestive tract showed 46,XX, inv (3) genotype. This condition was noted in two subjects presenting with colon cancer in the present study suggesting that the development of carcinoma of the rectum results from the allelic loss in chromosome 3p.

In the present study, one subject with cancer of the liver presented with deletion of the long arm of chromosome 4. In support of the above finding, loss of 4q was observed in a study by Sakakura et al. (1999) involving hepatocellular cancer.

Certain benign lesions (e.g., Barrett's esophagus, gastritis, gastric ulcer, polyps, cirrhosis, pancreatitis and ulcerative colitis) were marked by chromosome rearrangements characteristic of cancerous lesions suggesting possible cancer predisposition or a pre-cancerous condition. Two of the twelve patients with Barrett's esophagus expressed the chromosome aberrations $4p^-$ and $18q^+$ that are characteristic of those aberrations observed in cases of Barrett's esophagus by Peter Riegman et al. (2001).

A mosaic karyotype 46,XX / 45,XO was observed in a patient with Barrett's esophagus and in a patient with esophageal cancer suggesting the possible role of the missing chromosome in the suppression of dysplasia in female subjects. Patients with antral gastritis presented with chromosomal aberrations $9p^-$ and $14q^-$ and a patient with gastric ulcer displayed $12q^-$ karyotype [aberrations characteristic of cancer of the stomach observed by Van Grieken et al. (2000)]. Two subjects with polyps in the large intestine displayed inversion of chromosome 3 which is one of the characteristic aberration in colorectal cancer (Koji Sasajima et al. 1993). A subject with cirrhosis of the liver exhibited chromosomal translocation involving the long arm of chromosome 13 and three patients with chronic pancreatitis presented with translocations involving chromosomal arms 5q, 8q and 12p which are characteristic aberrations of corresponding cancerous lesions (Kusano et al. 1999; Rijken et al. 1999) involving the respective digestive structures. These aberrations might be markers suggesting possible underlying malignant process. According to Richard et al., 1994, despite considerable inter-individual variations, increased chromosome breakage and rearrangement may be the signs of chromosome instability in the predisposition to colorectal cancer. It was interesting to note the chromosomal aberration $5p^-$ in three patients with ulcerative colitis suggestive of a possible precancerous condition when noting the loss of the short arm of chromosome 5 during translocation in a patient with cancer of the colon.

CONCLUSION

The identified altered chromosomal regions may harbour tumour supressor genes or oncogenes that are involved in the multistep process of carcinogenesis or disease pathology.

These data may provide evidence for the occurrence of characteristic genomic alterations which are of biological relevance for the genesis of digestive system cancers. The results of this study might help in providing important clues and to add better knowledge in the location of relevant genes on specific altered regions of chromosomes.

ACKNOWLEDGEMENTS

The author (Calistus Jude) is thankful to the Council of Scientific and Industrial Research, New Delhi, India for the award of Senior Research Fellowship, the authorities of Bharathiar University, Coimbatore, India and to the medical and para-medical staff of MedIndia Hospitals, Coimbatore, India.

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