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Molecular and Cytogenetic Analysis of Lymphoblastoid and Colon Cancer Cell Lines From Cotton-top Tamarin (*Sagiunus oedipus*)

Xin Mao, Susan McGuire, and Rifat A. Hamoudi

ABSTRACT: *The cotton-top tamarin (CTT) (Sagiunus oedipus) has been used as an animal model to investigate the etiology and pathophysiology of several human diseases, including ulcerative colitis and its associated colorectal carcinoma (CRC). Little is known, however, about genetic synteny between CTT and humans, and about chromosome aberrations in CTT CRC. To address these issues, we have analyzed CTT lymphoblastoid and CRC cell lines using cytogenetics, fluorescence in situ hybridization (Zoo-FISH), and direct sequencing. The CTT lymphocytes had pseudodiploid chromosomes of 46. The CTT CRC cells showed near-diploid chromosomes of 45. Several clonal structural aberrations were observed, including der(1), a marker chromosome, and double minutes. Zoo-FISH using human chromosome 2, 3, 5, 6, 9, 11, 13, 15, 16, 17, 19, 22, and X paints identified homologous chromosomes and subchromosomal regions in the CTT genome. Fluorescence in situ hybridization with human telomeric probe also detected a homologous sequence in CTT genome. Direct sequencing of CTT genomic DNA using primers amplifying exons 4 and 15 of the human APC gene identified DNA sequences in CTT genome with 99% and 95% homology, respectively. These results provide a basis for further comparative studies of CTT and human genome.*

INTRODUCTION

The cotton-top tamarin (CTT) (*Sagiunus oedipus*) has been widely used as an animal model to investigate the etiology and patho-physiology of several human diseases [1].

Previous cytogenetic studies have described the normal G-banded karyotypes of peripheral lymphocytes and the EBV-transformed lymphoblastoid cell lines of CTT with a diploid chromosome number of 46 (2n) [2, 3]. Subsequent studies have suggested that the development of some malignant diseases in CTT is likely to follow a genetic pathway similar to those of human cancers. For example, cytogenetic analysis of the EBV-induced lymphomas and lymphoblastoid cell lines of CTT has revealed consistent chromosome abnormality and demonstrated the phenotypic similarity between EBV-induced lymphomas in CTT and Burkitt lymphoma in man [4]. CTT lymphocytes also expressed increased SCE and chromosome breakage in MMC-treated cultured conditions, which are also similar to those observed in people with genetic predisposition for malignancy [5]. However, little is known about the comparative cytogenetics of CTT and humans.

Colorectal carcinoma (CRC) is not only one of the most common human malignancies, but one with a high mortality rate [6]. A variety of mechanisms or models have been implicated in the development of CRC. For example, the model put forward by Fearon and Vogelstein proposed that a series of mutations such as the genes of adenomatous polyposis coli (*APC*) and *TP53* occur in the progression from normal cells to CRC, and that these mutations are associated with the histological features of CRC [7]. Another model postulates that a certain proportion of human CRC may be directly developed from ulcerative colitis but not through the adenoma-carcinoma pathway [8]. On the other hand, spontaneous colitis and colon cancer in CTT have been shown to resemble human ulcerative colitis and its associated cancer [9]. Cytogenetics and comparative genomic hybridization (CGH) studies have shown several specific chromosome aberrations in human CRC [10, 11]. However, the molecular defects likely associated with the development of CTT CRC are still ill defined.

To address the issues of whether there is a genetic synteny between CTT and humans and whether there are chromosome aberrations in CTT CRC, we have analyzed CTT lymphoblastoid and CRC cell lines using cytogenetics, fluo-

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rescence in situ hybridization (Zoo-FISH) and direct sequencing. Here we present the findings of our study.

MATERIALS AND METHODS

Chromosome Preparation and Cytogenetic Analysis

The EBV-transformed lymphoblastoid and CRC cell lines isolated from a male CTT were cultured in 10 ml of RPMI 1640 medium (GIBCO BRL) containing 10% fetal calf serum with 100 units/ml penicillin and 100 mg/ml streptomycin, and incubated in 5% CO_2 at 37°C for 72 hours. For cytogenetic analysis, cultured cells were directly harvested without Colcemid treatment. CTT metaphase chromosomes were prepared using standard techniques. At least 25 G-banded metaphase chromosomes were analyzed. The CTT karyotype was described according to the previous publications [2, 3]. The clonal chromosome aberrations were defined according to the standard criteria of human cancer cytogenetics; for example, identically structural aberration emerged in at least two cells and gain or loss of the same chromosome noted in at least three cells [12].

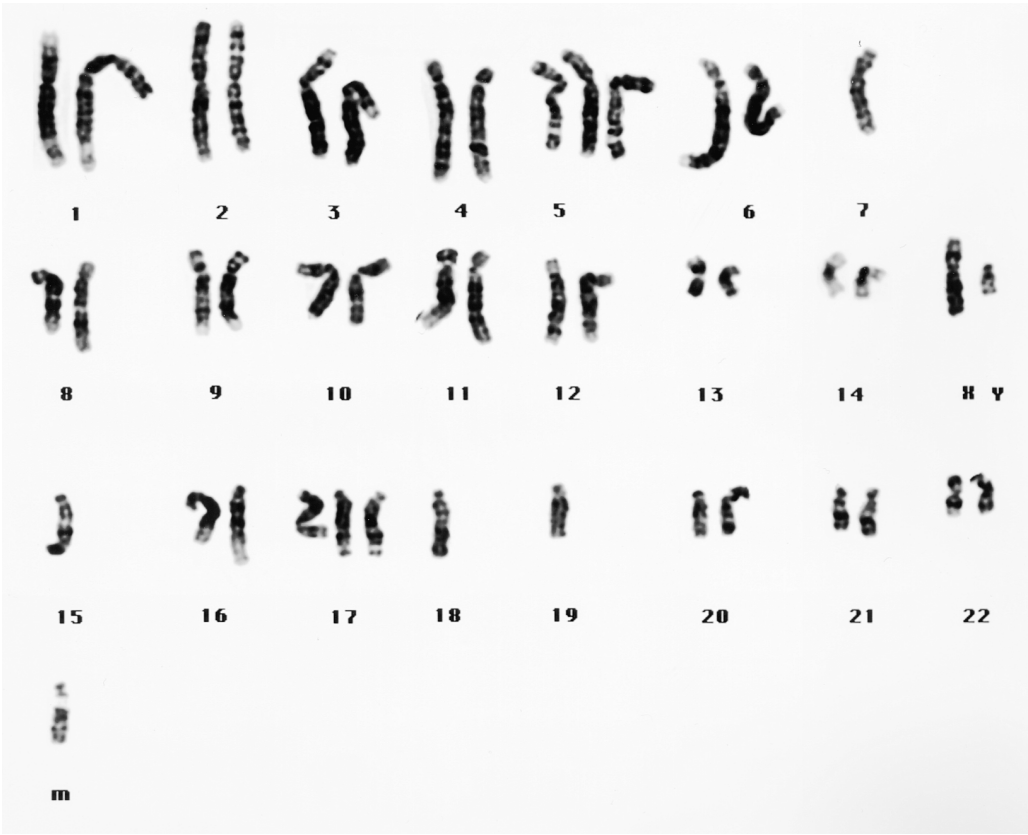
Zoo-FISH

Thirteen available human whole chromosome paints (WCP) including chromosomes 2, 3, 5, 6, 9, 11, 13, 15, 16, 17, 19, 22, and X (Cambio, UK) were hybridized with metaphase chromosomes prepared from lymphoblastoid and CRC cell lines of CTT using human Cot-1 blocking DNA to suppress the repeat sequences in the CTT genome [13]. Zoo-FISH using the human telomeric sequence (TTAGGG)n was conducted on the CTT chromosome preparations without blocking DNA. Chromosome banding and digitized image analysis were conducted according to the supplier's instructions [Vysis (UK), Ltd.]. At least 25 metaphase spreads were analyzed.

Sequencing

Exons 4 and 15 of the human *APC* gene were sequenced in CTT genomic DNA. The nested PCR was performed in a mixture consisting of 2.5 μl of 10× buffer, 4 μl dNTP (1.25 mM), 1 μl of primer amplifying exons 4 or 15 of *APC*, 0.5 μl of Taq polymerase, 15 μl of H_2O, and 2.5 μl of DNA under standard conditions. The second PCR was then carried out in a mixture containing 8 μl of sequencing reaction mix, 1 μl of above primer, 4 μl of PCR products, and 7 μl of H_2O, under standard conditions. The purified PCR products were sequenced using dRhodamine dye terminators on an ABI 377 automated fluorescent DNA sequencer according to the supplier's instructions (Applied Biosystems, USA).

Figure 1 Illustration of a karyotype of the cell line of CTT CRC with der(1) and marker chromosome.



RESULTS

Cytogenetics

The EBV-transformed lymphoblastoid cells of CTT were observed to have a pseudo-diploid cell population of 46. All 46 chromosomes can be recognized individually in G-banding, and contain 4 metacentrics, 26 submetacentrics, 14 acrocentrics, a submetacentric X, and an acrocentric Y. Random gains of chromosomes 1, 3, 7, 10, and 19, and losses of chromosome 3, 5, 13, 16, and 17 were noted. However, no clonal chromosome changes were seen in this cell line. The CRC cells showed a near-diploid cell population of 45 with the same karyotypic pattern as that of lymphoblastoid cells. Clonal chromosome aberrations were found in this tumor cell line. The most common structural aberrations were a rearranged chromosome 1, a marker chromosome, and double minutes (dmin). Non-clonal aberrations, including −2,del(3)(q),−4,der(12)t(12;?)(p;?),+13 were also seen in CTT CRC cells. The typical karyotype was described as 45,XY,−1,+der(1),+5,−7,−15,+17,−18,−19, +mar (Fig. 1).

Zoo-FISH

All of the 13 human WCPs showed that synteny has been conserved for large segments of the CTT genome. For example, human WCPs 2, 5, 19, and X identified CTT chromosomes 4, 5, 21, and X as their homologous chromosomes (Fig. 2). In addition, human WCPs 3, 6, 9, 11, 13, 15, 16, 17, and 22 assign subchromosomal regions on CTT chromosomes 18, 19, 15, 3, 11, 3, 7, 20, 9, and 6 (Fig. 2). A human telomeric probe also identified a homologous sequence in the CTT telomeric region. Zoo-FISH with these human WCPs on metaphase chromosomes made from both lymphoblastoid and CRC cell lines of CTT showed the same results.

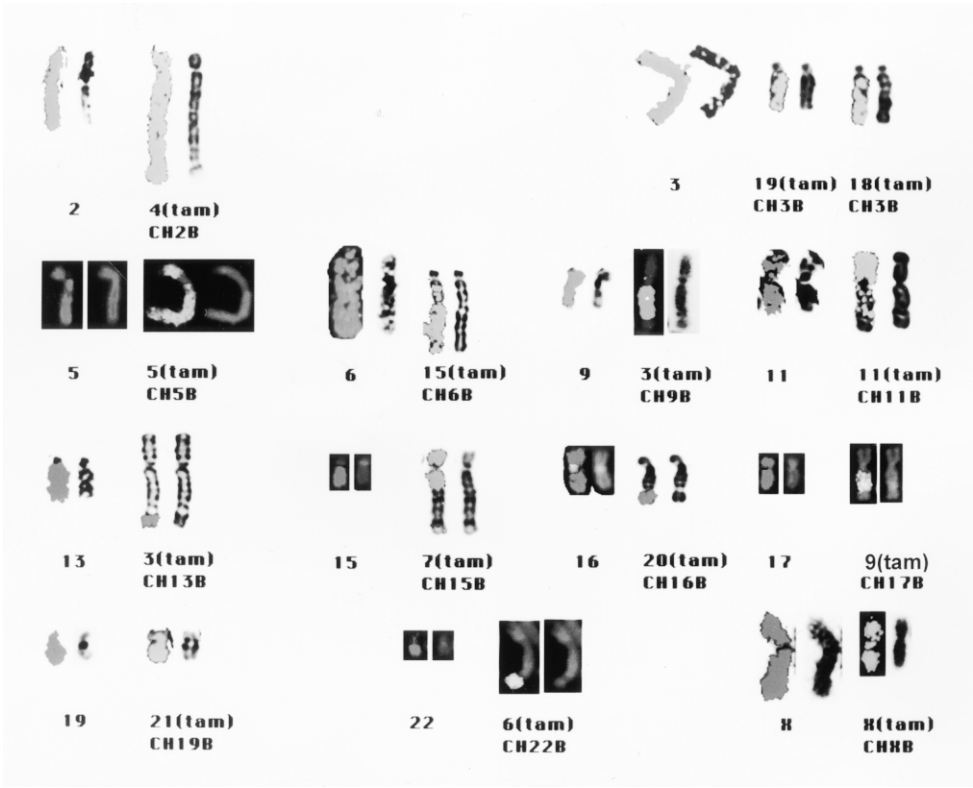
Sequencing

Direct sequencing of CTT genomic DNA using the primers amplifying exon 4 of human *APC* revealed a 99% homology of DNA sequence in this region between human and CTT. A single base pair change (C→T) was seen at codon 495 of *APC* in CTT genome (Fig. 3). Sequencing with exon 15 primers of human *APC* showed 95% sequence homology between human and CTT in this region. Five base pair changes were observed at codons 2586 (C→A), 2595 (T→C), 2634 (T→C), 2697 (A→G), and 2793(C→T).

DISCUSSION

Previous cytogenetic studies have reported that the normal somatic cells of CTT have a diploid chromosome

Figure 2 Illustration of a partial Zoo-FISH karyotype of CTT cells. Human chromosomes and WCPs are shown on the left side of each set of chromosomes. Homologous chromosomes and subchromosomal regions of CTT identified by using human WCPs are listed on the right side. For example, in this partial Zoo-FISH karyotype, human chromosome 2 and human WCP 2 (2) are illustrated on the left (column 1 and row 1), while CTT chromosome 4 and homologous chromosome [4(tam)] identified by human WCP 2 (CH2B) are shown on the right.

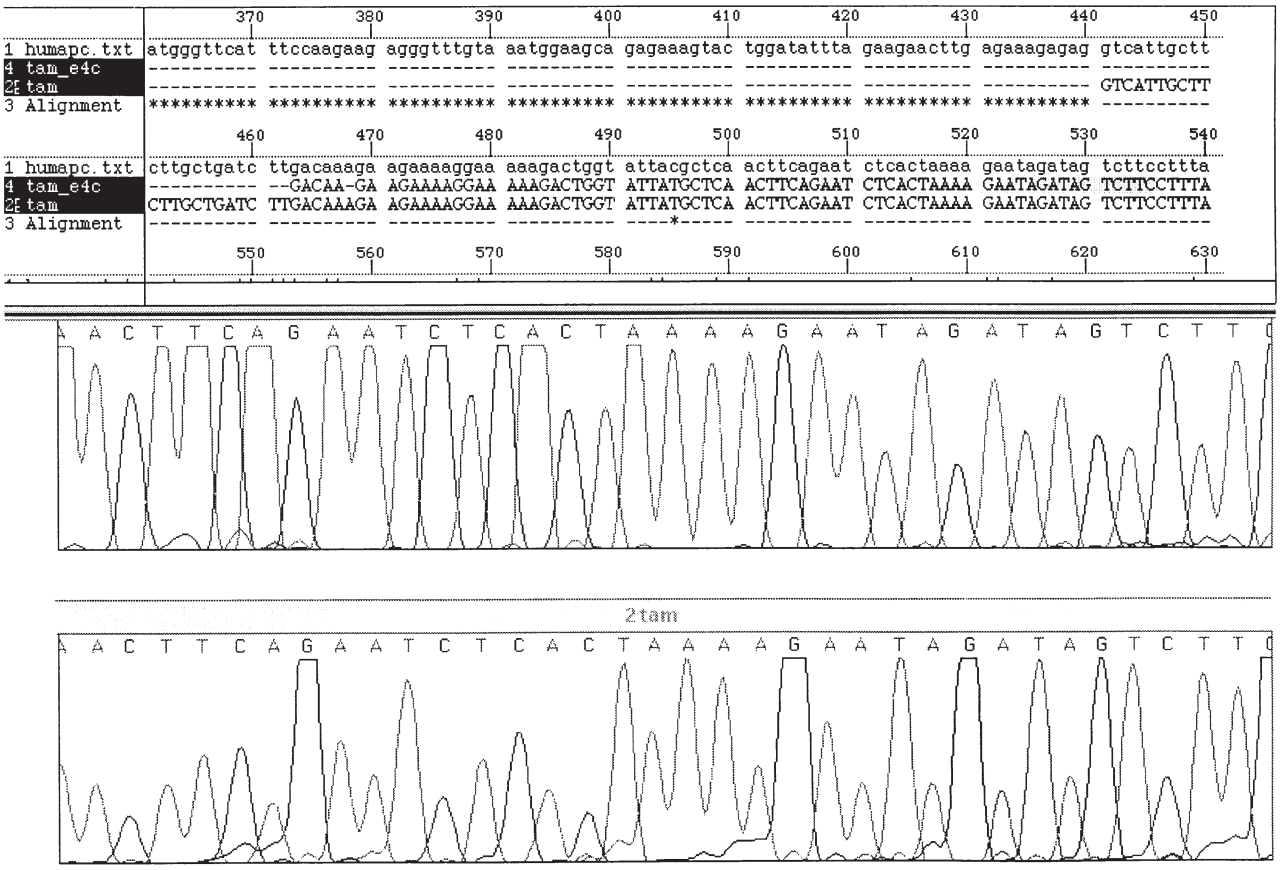


number of 46 (2n) [2, 3]. Subsequent cytogenetic studies of the EBV-induced lymphomas and lymphoblastoid cell lines of CTT have detected the common loss of a single chromosome [4]. MMC-treated lymphocytes of CTT have also shown increased SCE and chromosome breakage [5]. In this study, our cytogenetic findings of CTT lymphoblastoid cells were similar to those noted by others [2, 3].

Chromosome aberrations involved in the initiation and progression of human CRC have been documented [10, 11]. Our recent molecular studies of hundreds of dissected multiple adenomas from patients with familial and sporadic adenomatous polyposis have also shown frequent allelic losses on several chromosomes (Mao et al., in preparation). Although there appears to be no one to one morphological match between human and CTT chromosomes as discussed below, in this study, CTT CRC cells did show clonal chromosome aberrations such as der(1), an unknown marker chromosome, and DMs (Fig. 1). However, cytogenetic studies of lymphocytes of red-bellied marmosets (*Saguinus labiatus labiatus*) have shown chromosome rearrangements and DMs [14]. Therefore, the pathological significance of these clonal aberrations observed in CTT CRC cells remains to be investigated. To further address this issue, we are currently conducting alleletyping and CGH studies of CTT CRC.

Zoo-FISH has been proved to be a powerful cytogenetic technique for identification of homologous chromosomes and subchromosomal assignments of chromosome regions between different species [15]. In this study, although only 13 human WCPs were available, they did identify homologous chromosomes (4, 5, 21, X) and assign subchromosomal regions (3, 6, 7, 9, 11, 15, 18, 19, 20) in the CTT genome (Fig. 2). In addition a human telomeric sequence was seen presenting on a chromosome telomeric region of CTT. Moreover, human chromosome 10 and 12 centromere-specific primers amplified homologous sequences on CTT chromosomes when using primed in situ labeling (Mao et al., unpublished data). All of these results indicate that there is, to a large extent, a synteny between human and CTT genomes. Further studies using a fluorescence-activated flow sorting system to isolate each CTT chromosome complement and multi-color FISH to hybridize fluorescently labeled CTT chromosomes with normal human chromosomes may allow us to gain more insight into genomic evolution and conservation between CTT and humans.

Figure 3 Illustration of a partial sequence of exon 4 of the human *APC* gene and its CTT homologous. Top shows the alignment of the human (humapc.txt) and the CTT (tam-e4c and tam) *APC* sequences. Bottom demonstrates a fluorescent gel of exon 4 (forward and reverse sequences) of the CTT *APC* gene. A single base pair change (C→T) presents at coden 495 of *APC* in CTT genome.



Mutation of the human *APC* gene has been shown to be important for the pathogenesis of human CRC [8, 16]. To investigate whether *APC* plays a role in the development of CTT CRC, the first step is to identify the homologous DNA sequence of human *APC* in CTT genome. In this study, we observed that DNA sequences of exons 4 and 15 of CTT APC showed 99% and 95% homology with exons 4 and 15 of human *APC*, respectively. Both our Zoo-FISH and direct sequencing data indicate that the *APC* gene and its carrier, chromosome 5, are conservative in the evolution. These results provide us a basis for further analysis of the entire human *APC* gene in both normal genomic and tumor DNAs of CTT.

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