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Analysis of Telomeric Repeats and Telomerase Activity in Human Colon Carcinoma Cells with Gene Amplification

Alejandro D. Bolzán, Gerardo L. Páez, Martha S. Bianchi, and Néstor O. Bianchi

ABSTRACT: *COLO320DM and COLO320HSR are cell lines derived from a human malignant neuroendocrine colon carcinoma. Both lines have a 30–40-fold amplification of a large DNA domain containing the MYC oncogene. By using fluorescence in situ hybridization techniques with a MYC probe, we could demonstrate that MYC amplicons are contained in a large marker chromosome in COLO320HSR cells, in double minutes (dmin) of COLO320DM cells, and in the interstitial regions of 3–4 additional chromosomes in both cell lines. Amplicons in homogeneous staining regions (HSRs) comprise normal MYC genes, while dmin chromosomes contain PVT/MYC chimeras. Although both cell lines showed similar levels of telomerase activity, the telomere length and telomere distribution in chromosomal termini were considerably lower in COLO320DM than in COLO320HSR cells. This indicates that the average telomere length in cancer cells is regulated no only by the rates of telomerase activity but also by some other non-enzymatic mechanisms.*

INTRODUCTION

COLO320DM and COLO320HSR are cell lines derived from a human malignant neuroendocrine colon carcinoma [1]. Both lines have a 30–40-fold amplification of a large DNA domain containing the *MYC* (*C-MYC*) oncogene [2]. In 1983, Alitalo et al. [2] studied the chromosomal location of amplified *MYC* sequences by G-banding and isotopic in situ hybridization. In COLO320HSR cells, the amplified *MYC* oncogene was found exclusively in an extended homogeneous staining region (HSR) in a large marker chromosome. On the other hand, due to the limitations of the method, Alitalo et al. [2] suggested, but did not prove, that *MYC* amplicons were located in double minutes (dmin) of COLO320DM cells.

Normal somatic cells from adult individuals undergo a progressive shortening of telomeres due to the lack of telomerase activity. On the other hand, most cancer and immortal cells have telomerase activity, suggesting that the reactivation of this enzyme and the maintenance of telomere lengths above a certain threshold is an essential step in the process of cell transformation [3–5].

In the present report, we analyze the distribution of *MYC* amplicons, the telomere structure, and the level of telomerase activity in COLO320HSR and dmin cells, to detect similarities and differences between the two cell lines.

MATERIALS AND METHODS

COLO320HSR and COLO320DM cells were grown in RPMI-1640 medium supplemented with 1% glutamine, 20% fetal calf serum, and antibiotics. Colchicine treatments lasted 3 hours, harvesting and chromosome preparations were performed as usual.

Digoxigenin-labeled probes (*MYC*- and telomere-specific) were obtained from Oncor (Gaithersburg, MD, USA). Fluorescence in situ hybridization (FISH) was performed according to the instructions provided by the supplier. The bound probes were detected by rhodamine-labeled anti-digoxigenin. Metaphases were counterstained with DAPI and photographed using a fluorescence microscope (Carl Zeiss) equipped with a filter set suitable for the simultaneous viewing of DAPI and rhodamine signals (Chroma Technology, USA). Photographs were digitally processed with the Adobe Photoshop 4.0 software™.

Telomere length was measured by detecting the length of telomere restriction fragments (TRF) by Southern blot as reported elsewhere [6]. Five micrograms of genomic DNA from each cell line were digested with *Hinf*I (2 U/μg

From the Multidisciplinary Institute of Cell Biology (IMBICE), La Plata, Argentina.

Address reprint requests to: Dr. Néstor O. Bianchi, IMBICE-CC 403, 1900 La Plata, Argentina.

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655 Avenue of the Americas, New York, NY 10010

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DNA), electrophoresed in 0.8% agarose gels, capillary blotted to nylon membranes (Zetaprobe, Bio-Rad) and hybridized with a 5′ ^{32}P-labeled $(TTAGGG)_n$, oligonucleotide probe generated by PCR [7]. Hybridization was carried out in 5 × SSC, 5 × Denhart's solution, and 0.1% sodium duodecyl sulfate (SDS), at 56°C for 16 hours. The hybridized membranes were washed twice with 4 × SSC/0.1% SDS at 56°C for 20 minutes. Autoradiograms were exposed for 16 hours at −70°C with intensifying screens. The maximum peak of absorbance in each of the autoradiogram lanes was determined by densitometry. The position of peaks of maximal absorbance was determined by comparison with a suitable size marker.

Telomerase activity was detected by using the TRAPeze™ telomerase detection kit from Oncor following the instructions of the supplier. At the end of the reaction, 25 μl of the polymerase chain reaction (PCR) products were electrophoresed on a 12.5% nondenaturing polyacrylamide gel and visualized by staining with SYBR-Green (1%) (Molecular Probes, USA).

RESULTS

Chromosome Distribution of Amplified *MYC* Domains

In COLO320DM, FISH techniques with a MYC probe showed intense fluorescence signals in all dmin and also in the interstitial regions of 3–4 chromosomes (Fig. 1a, 1b). Moreover, in COLO320HSR, we observed *MYC* amplification in both arms of a large marker chromosome, similar to that reported by Quinn et al. [1] and Alitalo et al. [2], and also in 3–4 additional medium-sized chromosomes similar to those found in COLO320DM cells (Fig. 1c, 1d).

Telomere Identification

When we used FISH with a telomere-specific probe, even after six rounds of signal amplification, no more than 25–30% of COLO320DM chromosomal ends showed weak fluorescence; moreover, no telomeric-specific signals were found in dmin (Fig. 2a, 2b). Conversely, after four rounds of signal amplification, most chromosomal termini of COLO320HSR cells showed clear fluorescence signals (Fig. 2c, 2d). Furthermore, no interstitial telomeres were detected in the HSR regions of either of the two cell lines (Fig. 2b, 2d).

Average Size of Telomeric Repeats

Southern blotting with a telomere-specific probe produced smears in both cell lines, with a peak of absorbance at 6.5- and 4-kb positions for COLO320HSR and COLO320DM cell lines, respectively (Fig. 3). This finding agrees with the data obtained with FISH, in showing that the average number of telomere repeats is considerably lower in COLO320DM than in COLO320HSR chromosomes.

Telomerase Activity

The results of the Telomerase Repeat Amplification Protocol (TRAP) assay in COLO320HSR and COLO320DM cell lines showed similar levels of telomerase activity in both cell lines (Fig. 4).

DISCUSSION

Chromosomal Location of Amplified *MYC* Domains

Previous studies using G-banding reported that genome amplified regions in the COLO320HSR cell line are comprised in an extended G-band-negative segment located in a rearranged X chromosome. On the other hand, because no HSR region was detected with G-banding in the COLO320DM, genome amplification in this cell line was assumed to occur only as dmin [1, 2]. Our results using an MYC probe and FISH techniques are coincident with previous results, in showing *MYC* amplicons in a large marker chromosome in COLO320HSR cells and in showing a strong *MYC* signal in all dmin elements of the COLO320DM line. However, the high sensitivity of FISH in comparison to G-banding techniques allowed us to identify, in both cell lines, the presence of 3–4 additional chromosomes showing interstitial regions containing *MYC* amplicons.

Alitalo et al. [2] found that 95–99% of amplified *MYC* copies in COLO320HSR cells are normal and expressed. Conversely, in COLO320DM cells, approximately half of the amplified *MYC* oncogenes are normal and half are *PVT/MYC* chimeric genes, in which exon 1 and most of intron 1 of the *MYC* gene have been replaced by the first exon of the *PVT* gene that normally lies 50 kb downstream of *MYC* oncogenes. The cytogenetic study of COLO cells reported by Alitalo et al. [2] seems to indicate that normal *MYC* genes are specific of amplicons in the HSR chromosomes of COLO320HSR cells. Furthermore, because no HSR chromosomes were detected by these authors [2] in COLO320DM cells, it should be assumed that dmin elements were formed by amplicons having normal and rearranged *MYC* genes in a 1/1 ratio.

Our findings in both cell lines of 3–4 chromosomes carrying amplicon clusters compatible with HSRs seem to indicate that: (1) normal *MYC* genes are specific of amplicons in HSR segments of both cell lines; (2) dmin very likely contain only *PVT/MYC* rearranged genes.

Mechanism of Maintenance of Appropriate Levels of Terminal Telomere Repeats in COLO320 Cells

The presence of telomerase activity is considered essential in maintaining the average number of telomere repeats above a given threshold that assures persistent cell division during early embryogenesis and in cancer transformed cells [8, 9].

The analysis of chromosome ends in malignant human tumors revealed that different tumors have distinct levels of hexamer repeats, producing telomeres ranging from 1 to 20 kb in length [10]. In this report, we demonstrate that the telomere length in COLO320HSR and dmin cells is 6.5 and 4 kb, respectively. Because the level of telomerase activity was similar for the two cell lines, differences in telomere length do not depend on telomerase rates. Previous reports show that in some cases, telomere elongation occurs through a mechanism not involving telomerase activity [11, 12]. Accordingly, our results can be one more example of the above phenomena.

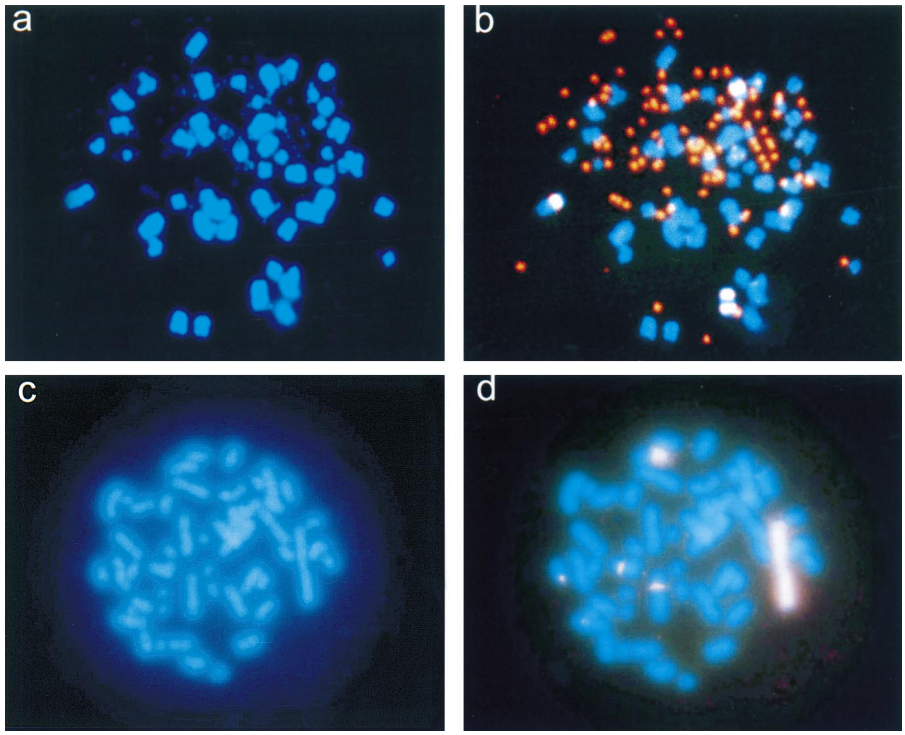
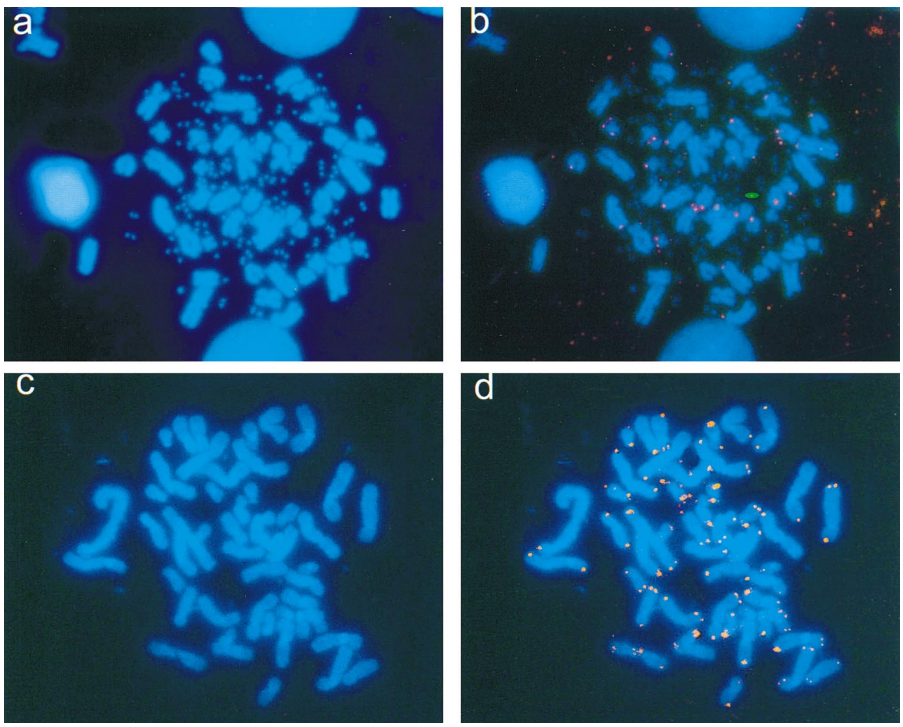


Figure 1 Fluorescence in situ hybridization using a digoxigenin-labeled MYC probe. (a) COLO320DM chromosomes stained with DAPI. (b) The same metaphase visualized with a filter set suitable for the simultaneous viewing of DAPI and rhodamine signals show the location of *MYC* amplicons in all dmin and in several medium-sized chromosomes. (c) COLO320HSR chromosomes counterstained with DAPI. (d) The same metaphase viewed with a filter set for DAPI and rhodamine show *MYC* amplicons in a large marker chromosome and in several medium-sized chromosomes.



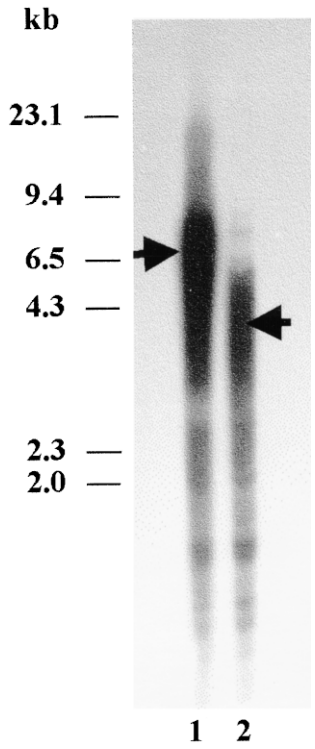


Figure 3 Southern blot autoradiograms obtained by hybridization of a ^{32}P-labeled telomere-specific probe $(TTAGGG)_n$ with *Hinf*I-restricted DNA (5 μg) from COLO320HSR (lane 1) and COLO320DM (lane 2) cells. Arrows indicate the peaks of maximal absorbance.

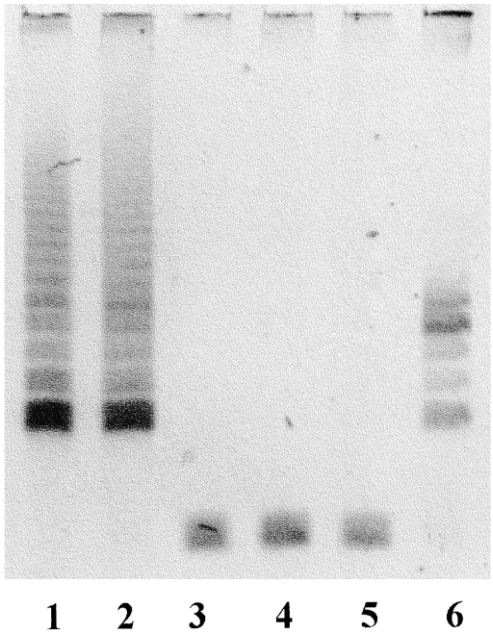


Figure 4 TRAP assay of COLO320DM and COLO320HSR cell lines. Lane 1: COLO320DM cells (2 μl of extract from 5×10^5 cells). Lane 2: COLO320HSR cells (2 μl of extract from 5×10^5 cells). Lane 3: heat inactivated extract from COLO320DM cells. Lane 4: heat inactivated extract from COLO320HSR cells. Lane 5: primer-dimer control. Lane 6: telomerase quantification control template. The number of bands in the ladder represents the level of telomerase activity.

Lack of Telomeric Sequences in HSR and DM Chromosomes

Information about the DNA structure within the amplified domains is scarce. In 1991, Bianchi et al. [13], based on the finding of DNA discontinuities in the amplified segment of COLO320HSR cells, proposed the existence of telomeric sequences at both ends of amplicons with the subsequent formation of hairpins or Hoogsteen structures connecting individual amplicons to give rise to HSR. Several reports have demonstrated the presence of interstitial telomere-like sequences in several cell lines [14–16]. In this report, the use of FISH with a telomere probe produced no fluorescence in COLO320HSRs. Therefore, we could not confirm the existence of telomere repeats at the amplicons ends. Moreover, the lack of telomere signals in DM chromosomes support previous data, indicating that these elements comprise circularized DNA [17].

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Figure 2 Fluorescence in situ hybridization with the digoxigenin-labeled telomeric probe on metaphase chromosomes of COLO320HSR and COLO320DM cells. (a) COLO320DM metaphase chromosomes stained with DAPI. (b) The same metaphase visualized with a filter set suitable for the simultaneous viewing of DAPI and rhodamine signals show weak telomere signals in the terminal regions of several chromosomes. Note the lack of telomeric-specific signals in dmin. (c) COLO320HSR metaphase chromosomes counterstained with DAPI. (d) The same metaphase viewed with a filter set for DAPI and rhodamine to show the telomeric signals in the terminal regions of most chromosomes.

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