TELOMERE, TELOMERASE AND MALIGNANT MELANOMAS IN HUMAN AND DOMESTIC MAMMALS

TELÓMERO, TELOMERASA Y MELANOMAS MALIGNOS EN HUMANOS Y ANIMALES DOMÉSTICOS

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Additional keywords

Telomere. Telomerase. Malignant melanomas. Human. Domestic animals.

Palabras clave adicionales

Telómero. Telomerasa. Melanomas. Especie humana. Animales domésticos.

SUMMARY

1. Nonrandom chromosome abnormality associated with cancer predisposition can be identified in lymphocytes.

2. Tumor markers (cytogenetic) can be identified in the lymphocytes of cancer patients and in some of their asymptomatic family members.

3. Reduction in telomeric repeats may induce genetic instability in somatic cells and may serve as an early manifestation of cell death.

4. The telomere controls the function of the centromere and plays an important role in tumor regression.

5. Telomerase activation or centromere inactivation in compound chromosomes may bypass cell death.

6. Multicentric chromosomes and ring configurations could be formed due to the progressive loss of telomeric DNA. Such abnormalities can be observed in cells that have not been exposed to clastogens.

RESUMEN

1. En los linfocitos pueden identificarse anormalidades cromosómicas, no debidas al azar, asociadas con la predisposición al cáncer.

2. En los linfocitos de pacientes cancerosos, así como en algunos de los miembros asintomáticos de su familia, pueden identificarse marcadores tumorales (citogenéticos).

3. La reducción de las repeticiones teloméricas puede inducir inestabilidad genética en las células somáticas y puede servir como una manifestación precoz de la muerte celular.

4. El telómero controla la función del centrómero y juega un importante papel en la regresión del tumor.

5. La activación de la telomerasa o inactivación del centrómero en los cromosomas compuestos puede evitar la muerte celular.

6. A causa de la pérdida progresiva del DNA

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telomérico podrían formarse cromosomas multicéntricos y configuraciones en anillo. Esas anormalidades pueden observarse en células que no han sido expuestas a clastógenos.

INTRODUCTION

The chromosomes whose number and morphology are the characteristics of a given species carry cellular genes that control most of the mitotic and meiotic processes. With the renewed interest in the mechanism of programmed cell death (PCD) or apoptosis and the parts played by telomeres and the enzyme telomerase that elongates them, there has been much recent excitement in chromosome research among developmental biologists, cell biologists, apoptologists, and lately by oncologists. In a 1995 issue of *Science* (December 8, volume 270), Joseph G. Gall wrote *Homage to the Chromosome*. His last sentence of this brief write-up reads, *Chromosome research is alive and well!* A number of articles that are devoted to the functional aspects of the centromere, telomere, dosage compensation, and roles of telomerase in cancer are published in this volume.

Muller (1938) coined the term *telomere* from the Greek words *TELOS* (end) and *MERAS* (part). McClintock (1941) noted that without these terminal caps, chromosomes stick to one another, even forming ring configurations, and follow the breakage-fusion-bridge cycles during subsequent cell divisions. These breakage-fusion-bridge cycles eventually will disintegrate such chromosomes and consequently cell death occurs (Pathak *et al.*, 1994 a and b). Telomeres of human chromosomes contain repeats of (TTAGGG)n, sequences that are conserved in most mammalian species. Telomeres serve as a *mitotic signal* in somatic cells and determine whether the cell will die *(PCD)* after a certain number of divisions or continue to divide as cancerous growth. Contemporary cytogeneticists have given too much importance to the centromere and have mostly neglected the functions of telomeres.

Pathak (1996) has recently proposed a hypothesis by asking the question, *Centromere or telomere: Who is the boss?* Classical and molecular cytogenetic data collected in his laboratory seem to support that the telomere is the boss. A number of important functions could be assigned to the telomeres: (a) protection of the ends of the chromosomes from disintegration; (b) attachment of individual chromosomes to the nuclear membrane in their respective domains (each chromosome has its own domain within a nucleus); (c) initiation of an active participation in the pairing of homologous chromosomes during meiosis, which starts from the telomeric ends; (d) help in somatic recombinations; (e) causation of a transient cell division arrest if defective (cell cycle check point); (f) help the cell in distinguishing between the intact and the broken chromosomes; (g) regulation of the expression of certain genes called telomere position effect (TPE); (h) controlling the activity of the centromere in a compound chromosome; and (i) playing an important role in cell senescence and programmed cell death (Pathak *et al.*, 1994 a and b).

In subsequent pages, we will briefly review our observations on the cytogenetic defects in malignant melanomas of man, horse, pig, dog, and the gray, short-tailed opossum *(Monodelphis domestica)*. An

animal model system of the Sinclair swine will briefly be presented for the study of spontaneous regression of melanoma.

CHROMOSOMES OF HUMAN MELANOMA

After examining cytogenetically a number of primary and metastatic melanomas, it has been shown that human chromosomes 1, 6, and 9 are often involved in structural alterations. Specifically, lp, 6q, and 9p and 9q are either involved in translocations with other chromosomes or deleted in different melanoma samples (Dave *et al.*, 1994). In a recent report, Borg and associates (1996) reported a germ-line mutation in the p16 *(MTS1)* tumor suppressor gene located in the 9p21 region in Swedish families with malignant melanomas. Another tumor suppressor gene called *ptc,* (homolog of the *Drosophila* patched gene associated with wing development) has recently been identified in patients with the basal cell nevus syndrome (Johnson *et al.,* 1996). Both tumor suppressors are located on human chromosome 9. Still another gene, p15 located on 9p, is implicated in a variety of solid neoplasms.

To identify if chromosomal alterations in lp, 6q and 9p are primary (early) events in melanoma development, we have examined lymphocyte cultures from 20 untreated melanoma patients and some of their first degree relatives. In at least 45 p. cent of samples, each of these chromosomes alone or in combination was altered structurally in 2 p. cent to 3 p. cent of metaphases. By challenging the lymphocyte cultures of melanoma patients with clastogens, Dave *et al.*

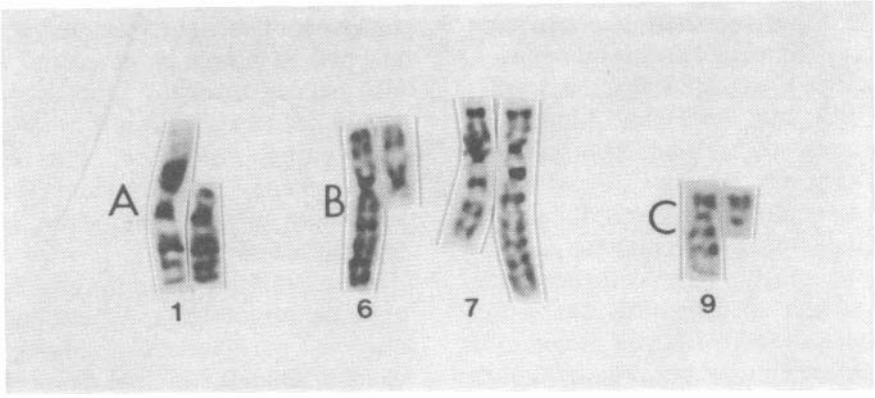

Figure 1*. Giemsa (G-)-banded partial karyotypes from lymphocyte cultures of a melanoma, basal cell carcinoma and from an asymptomatic family member showing structural defects in chromosomes 1, 6 and 9, respectively. Deletion of 1p (A); translocation between chromosomes 6 and 7 (B); Deletion of 9q (C).* (Cariotipos parciales, bandeados con Giemsa (G-), procedentes de cultivos de linfocitos de un melanoma, carcinoma de células basales y de un miembro asintomático de la familia mostrando defectos estructurales en los cromosomas 1, 6 y 9 respectivamente. Delección de 1p (A); Translocación entre los cromosomas 6 y 7 (B); Delección de 9q (C)).

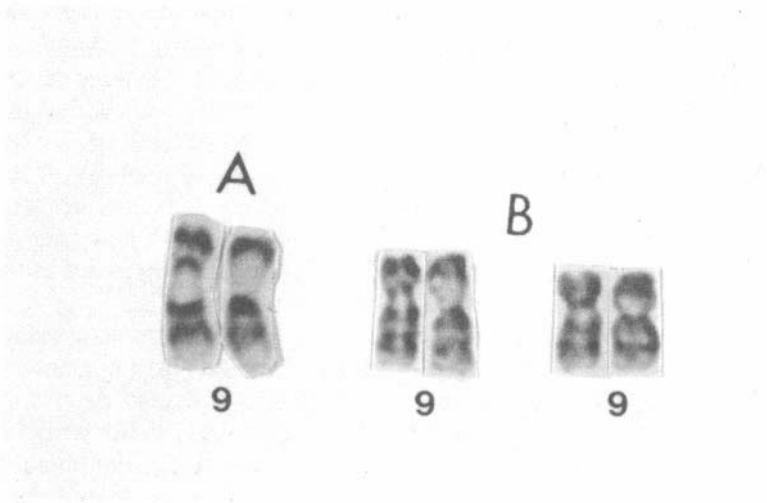

Figure 2. *G-banded partial karyotypes from lymphocytes of a patient with lung cancer (A) and a young healthy volunteer (B) showing pericentric inversion in one homolog of chromosome 9 (left).* (Cariotipos parciales, G-bandeados, procedentes de linfocitos de un paciente con cancer de pulmón (A) y de un voluntario joven y sano (B) mostrando la inversión pericéntrica en un homólogo del cromosoma 9 (izquierda)).

(1994) have reported a significant clustering of breaks in chromosomes 1, 6, and 9 (lp32 and lq32; 6p21 and 6q21; and 9ql2 and 9q22). A number of important genes are mapped on these *hot spots* of chromosomes 1, 6, and 9 (see Dave *et al.,* 1994). A G-banded partial karyotype from the lymphocyte cultures of melanoma and basal cell carcinoma patients and an asymptomatic family member is shown in **figure 1**. Deletion of 1p, translocation between chromosomes 6 and 7, and deletion of 9q are the characteristic features of this **figure**. It is interesting to mention here that murine chromosome 4, which has shown an interstitial deletion in a UVRinduced melanoma cell line (Pathak *et al.,* 1991), has a close homology with human chromosomes 1, 6, and 9. Two tumor suppressor genes (*p15* and *p16)* that are mapped on human chromosome 9p are also mapped on mouse chromosome 4. It would not be surprising if the recently implicated *ptc* gene, located on chromosome 9, in the basal cell nevus syndrome is also located on mouse chromosome 4.

In a preliminary study of a human prostate tumor-derived cell line, we observed an inversion involving a C-banded segment in one homolog of chromosome 9 and predicted a mutation in the *p16* gene. Molecular analysis indicated concurrent mutations of the p53 and *p16* genes in this prostate cancer cell line (Greene *et al.,* 1996). Since the *p16* tumor suppressor gene is frequently mutated in a wide variety of human tumors, including melanoma, glioma,

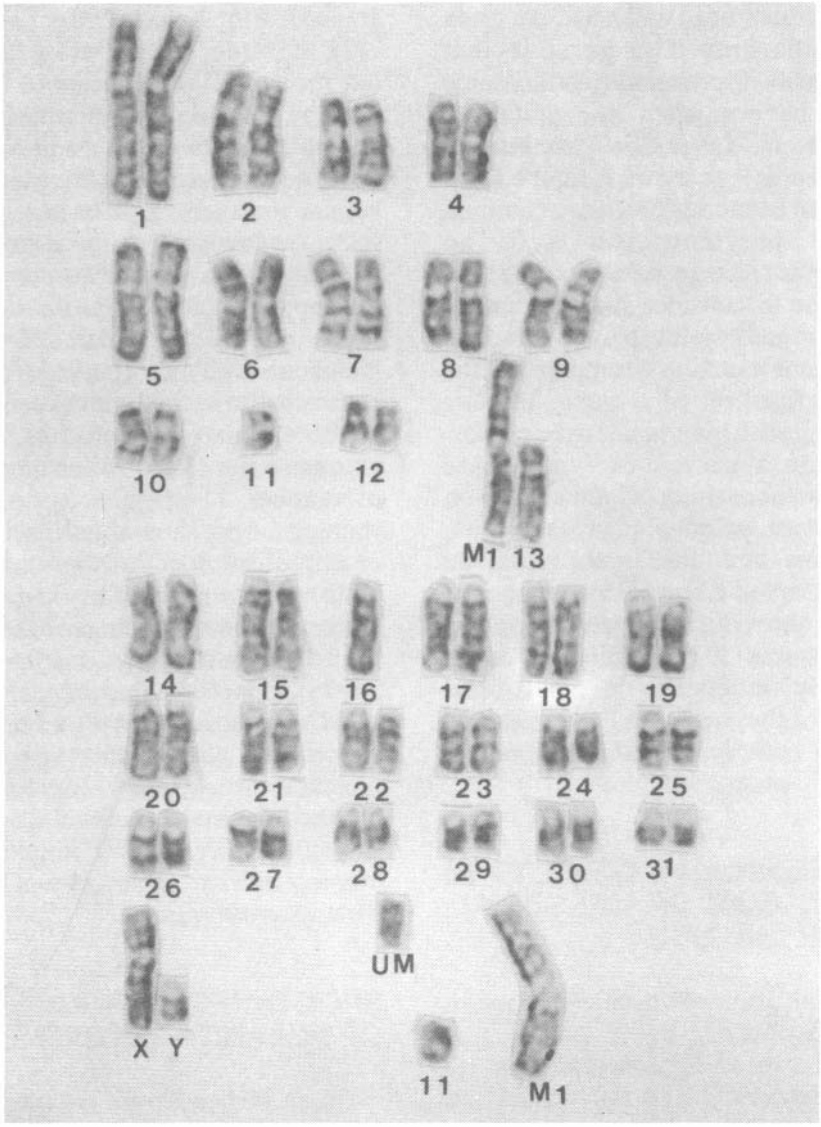

***Figure 3**. A G-banded karyotype of horse melanoma cell line showing a marks (M1) chromosome. Chromosomes 11, 13 and 16 are apparently monosomic. M1 which is a translocation product of chromosomes 13 and 16 from another metaphase is shown at the bottom. Unidentified marker (UM) is also shown.* (Cariotipo, G-bandeado, de una línea celular de melanoma de caballo mostrando un cromosoma marca (M1). Los cromosomas 11, 13 y 16, son aparentemente monosómicos. En la parte baja, se muestra M1 que es el producto de translocación de los cromosomas 13 y 16 de otra metafase. Se muestra igualmente un marcador no identificado (UM)).

lung, bladder, head and neck, pancreas and esophagus, it is possible that individuals with inversion in chromosome 9 may have a germ line mutation. Pericentric inversion in human chromosome 9, as shown in **figure 2**, has in the past been considered as a common C-band polymorphism with no phenotypic consequences. Now the time has come to consider such inversions important and possibly playing an active role in gene mutation or suppressing the normal function of a gene, thereby rendering such individuals to be cancer-prone. In a survey of lymphocyte chromosome analyses of individuals with lung, breast, prostate, pancreas, colon, melanoma, and other tumor types, we have observed a reasonable number of cases showing an inversion in chromosome 9 (unpublished data). Similarly, inversions in the C-band regions of chromosomes 1 and 16 should also be considered indicators of yet unidentified gene mutations.

CHROMOSOMAL DEFECTS IN MELANOMAS OF MARSUPIAL, HORSE AND DOG

In the gray, short-tailed opossum *(Monodelphis domestica;* 2n=18), it is possible to induce melanoma by ultraviolet radiation (UVR) alone (Pathak *et al.,* 1992). Detailed cytogenetic studies of a number of such melanoma cell lines were described by our group (Pathak *et al.,* 1992). The chromosome number in UVR induced melanomas varies between 27 and 29 with a peak at 28. A number of marker chromosomes are consistently present in marsupial melanomas. Tentative identifications of these markers are: M1=t(1q; Xq); M2=der (3); t (3p; ???); M3=t(4q; Xq); M=t(5q; 8q) (data not shown). There appears to be a G-banding homology between the 1p of this opossum and human chromosome 6q. Since 6q is often deleted/translocated in human melanomas, it is important to search for a melanoma suppressor gene(s) in the short arm of chromosome 1 of this marsupial species. From the domestic horse *(Equus caballus;* 2n=64), a melanoma cell line (passage 10) was determined to have 63 chromosomes with a Robertsonian translocation between chromosomes 13 and 16 and monosomy of number 11 **(figure 3)**. Ag-NOR staining did not show abnormal location or amplification of the ribosomal genes (data not shown). We also examined the chromosome constitutions of a melanoma from the domestic dog *(Canis familiaris;* 2n=78). This cell line has a modal number of 61 chromosomes with a number of structurally altered chromosomes. A typical Y chromosome was present in all 50 metaphases examined (data not shown). Typical double minute (DM) chromosomes were not present in these melanoma samples.

SPONTANEOUS REGRESSION OF MELANOMA IN SWINE

In an earlier report, we proposed a hypothesis that extensive telomeric associations (TAs) of chromosomes are an early manifestation of programmed cell death (Pathak *et al.,* 1994 a and b). Recently, we have extended our observations to a special breed of the Sinclair swine. These animals develop malignant melanoma *in utero,* and as the piglets grow older, melanomas regress

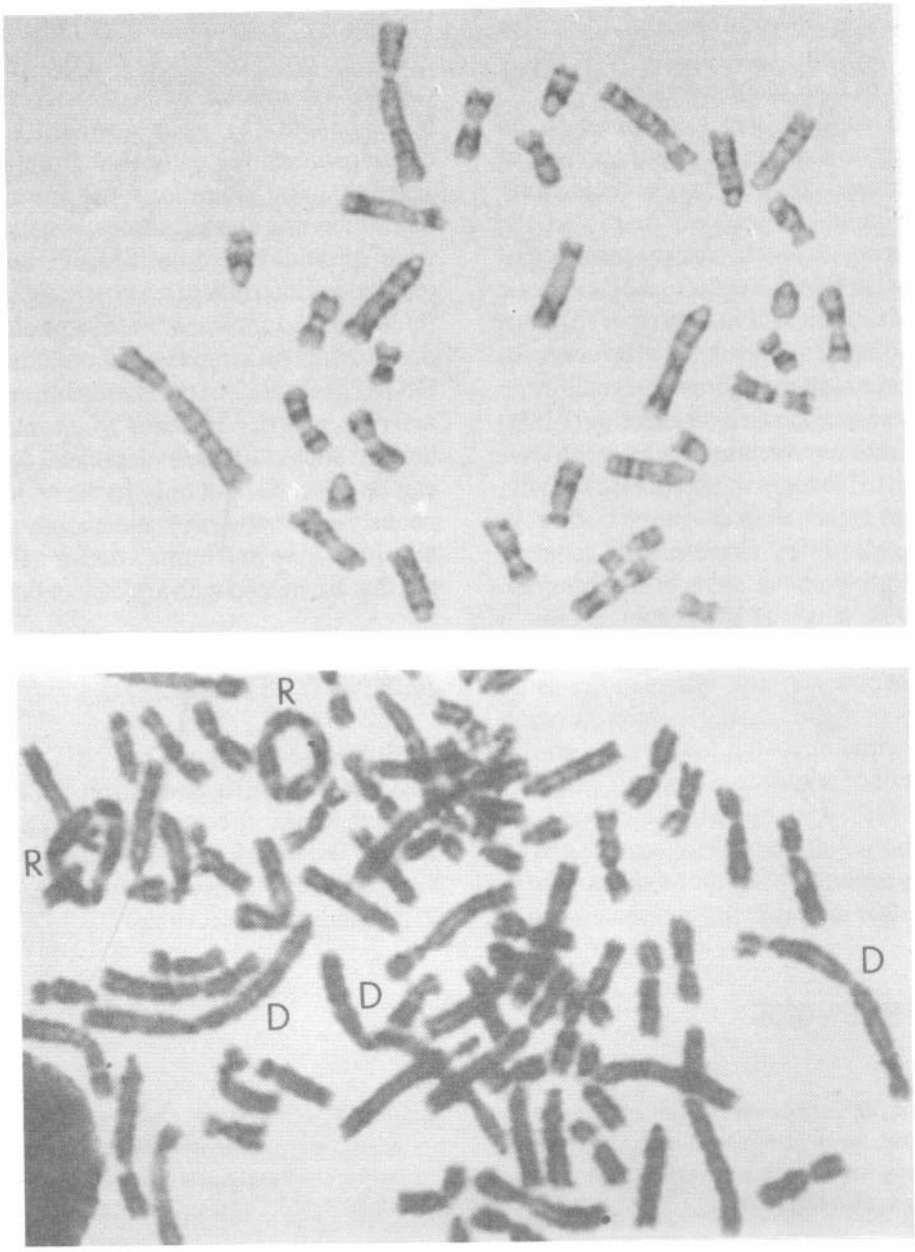

***Figure 4**. G-banded metaphases from embryonic and adult Sinclair swine melanoma cells showing absence and presence of rings (R) and dicentric (D) chromosome configurations respectively.* (Metafases, G-bandeadas, de células embrionarias y adultas de del melanoma porcino de Sinclair, mostrando configuraciones de ausencia y presencia de anillos (R) y cromosomas dicéntricos).

spontaneously, resulting in a cancer-free adult animal. This tumor very rarely becomes fatal to the animals.

To support our hypothesis in an attempt to explain this phenomenon of spontaneous regression of melanomas in the Sinclair swine, we predicted the following observations: (a) presence of extensive TAs due to the loss of telomeric repeats, as shown in **figure 4**; (b) poor spreading of analyzable metaphases; (c) absent or reduced telomeric signals in the fluorescence *in situ* hybridization (FISH) preparations with human telomeric DNA probe; (d) absence of telomerase activity, and (e) presence of apoptotic bodies. In our preliminary experiments, most of these phenomena have been observed. Early passages of swine melanomas in *vitro* do not show TAs but have shown telomerase activity, whereas the same tumor at higher passages starts showing TAs with increasing frequencies and an absence of telomerase activity (Pathak *et al.*, 1996). Reduction in the intensity of telomeric signals has also been observed in regressing swine melanomas. These and other experiments performed in our laboratory have shown a panel of cytogenetic characteristics of the dying cell: (a) low mitotic index of analyzable metaphases; (b) poor spreading of chromosomes; (c) extensive structural and numerical alterations; (d) presence of multicentric chromosomes, rings, and fragments due to telomeric associations; (e) reduced intensity of telomeric signals; (f) condensed chromosome morphology due to reduction in repeats of the telomeric DNA; (g) absence of telomerase enzyme activity; and (h) presence of apoptotic bodies. Such characteristics of cell death can be observed not only in the spontaneously regressing swine melanomas, but also in murine and human tumor cell lines that are treated with anticancer drugs.

ACKNOWLEDGMENTS

Supported in part by NIH grant RRO499901 and a grant from the John S. Dunn Research Foundation, Houston, Texas. We thank Joyce Benjamin for expert secretarial assistance and Leslie Wildrick for editorial help.

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