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Telomeric Association in Women with Breast and Uterine Cervix Cancer

César Paz-y-Miño, Ma. Eugenia Sánchez, Mercedes Del Pozo, Ma. Augusta Baldeón, Augusta Córdova, Sara Gutiérrez, Ma. Serena Peñaherrera, Marco Neira, Ligia Ocampo, and Paola E. Leone

ABSTRACT: *This study compares the frequency of telomeric associations in the peripheral blood of women suffering breast and cervix uterine cancer with a healthy control group. Two kinds of cultures were developed for each individual: with and without aphidicolin. In the normal cultures, the number of telomeric associations observed was 95.5 times higher in individuals affected by breast cancer and 41.3 times higher in those affected by cervix uterine cancer when compared to the control group ($p < 0.001$). In the cultures with aphidicolin, higher numbers of altered metaphases were observed in both groups as compared to the control groups ($p < 0.001$). Statistically significant differences ($p < 0.001$) could also be observed when comparing telomeric associations between the two types of cancer in both cultures. When we compared individuals affected by breast cancer in both types of cultures statistical differences were found ($p < 0.05$), and similar results were found in individuals affected by uterine cervix cancer ($p < 0.001$). The findings suggest that telomeric associations may be reflecting chromosome instability observed in cancer and that this instability behaves differently for various types of cancer.*
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INTRODUCTION

Telomeres consist of a variable number of repetitions of the TTAGGGG sequence located in the terminal region of the chromatids of the 46 human chromosomes [1, 2], which they protect from degradation processes, fusion, and incomplete replication [3, 4].

In some pathologies chromosomes tend to fuse by their telomeres, forming dicentric chromosomes. This phenomena, known as telomeric association (TA), has been reported in various kinds of leukemia [5–7], malignant histiocytoma [8], meningioma [9], cardiac myxoma [10], renal tumors [11], Thiberg-Weissenbach Syndrome [12–14], squamous cell carcimona lines [15], senescent and SV-40 infected fibroblasts [16–19], and in human embryonic fibroblast undergoing senescence [20].

The processes that cause telomeric associations are unknown, but it has been reported that it could be because of failure in the enzymatic activity in charge of telomere replication [9]. It has been reported that deregulation of this enzyme, called telomerase, may participate in cellular immortality and oncogenesis [21–23]. Another cause could be because of the importance of deletions in the repetitive sequences of the telomeres [24].

It has been reported that aphidicolin can induce telomeric associations in the cells of normal individuals [25, 26].

The purpose of this study was to detect the frequency of telomeric associations in peripheral blood of individuals suffering from breast and uterine cervix cancer as compared with the frequency in control individuals. This study introduces a new element of analysis, aphidicolin, which is a mycoditerpenoid toxin that competes with DNA polymerases [27].

MATERIALS AND METHODS

Four groups of women of similar ages were analyzed in the present study: One group of 20 individuals affected by

From the Laboratorio de Genética Molecular y Citogenética Humana, Departamento de Ciencias Biológicas (C. P-y-M., Ma. E. S., M. D. P., Ma. A. B., A. C., S. G., Ma. S. P., M. N., P. E. L.), Facultad de Ciencias, Pontificia Universidad Católica del Ecuador, and the Laboratorio de Citología (L. O.), Instituto Nacional del Cáncer, SOLCA, Quito, Ecuador.

Address reprint requests to: César Paz-y-Miño, M.D., Prof. Laboratorio de Genética Molecular y Citogenética Humana, Departamento de Ciencias Biológicas, Facultad de Ciencias, P. Universidad Católica del Ecuador, P.O. Box 17-01-2184, Quito-Ecuador.

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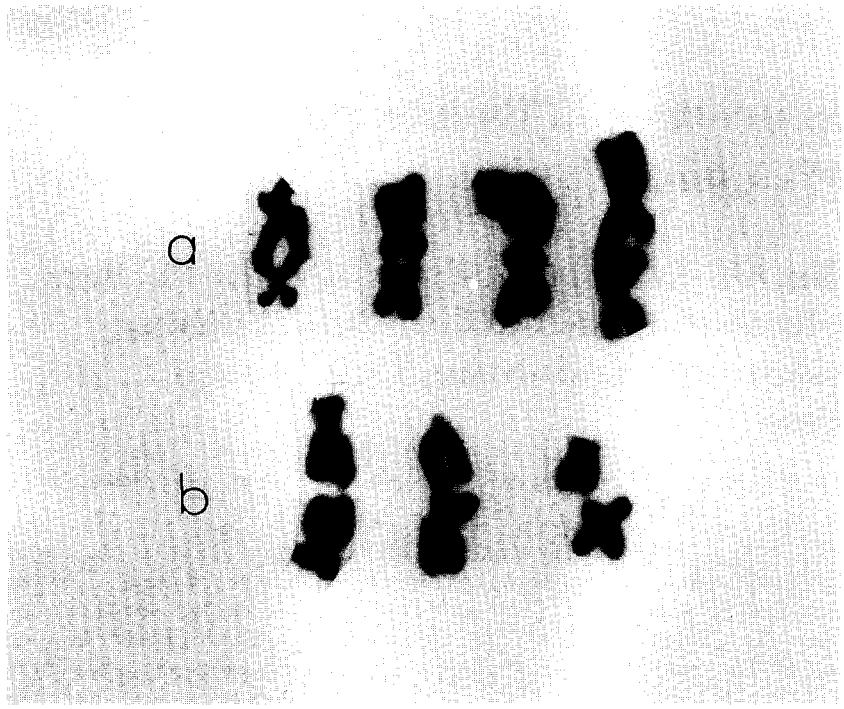

Figure 1 Different types of telomeric associations observed with Giemsa staining. (a) TA between double chromatids. (b) TA between single chromatids.

ductal invasive or infiltrant unilateral breast cancer; one group of 20 individuals affected by squamous cell uterine cervix cancer (none of the individuals of these first two groups had received previous chemotherapy, radiotherapy, or surgery treatment); and two control groups (one for each kind of analyzed cancer), each one consisting of 20 healthy women not exposed to any known genotoxic agents.

Cytogenetic Analysis

Peripheral blood cultures of T lymphocytes (TL) were carried out using two types of culture mediums for each individual: a) Normal culture medium that contains 5 ml of RPMI-1640 medium supplemented with fetal bovine serum (10%), penicillin (10,000 IU/ml), streptomycin (10mg/ml), L-glutamine (200mM), HEPES buffer (1mM/ml), and phytohemagglutinin (1mg/ml), and b) The same normal culture medium with Aphidicolin (Ap) added at a final concentration of 0.12 μM/ml. Peripheral blood cells were harvested after 72 hours of culture at 37°C [28]. Harvesting was carried out using standard techniques [29]. The slides were air dried and stained with a 5%. Giemsa solution. One hundred metaphases were analyzed per individual in coded slides. The aberrations were classified according to the International System for Human Cytogenetic Nomenclature [30]. The TA observed were analyzed with Giemsa staining (Fig. 1).

Chi-square test was used for statistical analysis.

RESULTS

The results obtained in this study are summarized in Table 1. In normal cultures, the percentage of telomeric association is 95.5 times higher in individuals suffering from breast cancer and 41.3 times higher in those suffering with cervix cancer than those in the control group. The difference is highly significant ($p < 0.001$).

In regard to the aphidicolin cultures, the percentage of altered metaphases was 113.5 times higher in individuals affected by breast cancer and 24.7 times higher in those affected by cervical cancer than in the control group and the difference is also significant ($p < 0.001$).

When comparing the two types of cancer in the two culture mediums analyzed, a highly significant statistical difference was found ($p < 0.001$). Also, the percentage of telomeric associations observed in individuals affected by breast cancer when compared to that observed in the cultures with aphidicolin showed a statistical difference ($p < 0.05$). Similar results were found in individuals affected by uterine cervix cancer ($p < 0.001$). There were no statis-

Table 1 Percentage of metaphases with telomeric associations in the analyzed groups

		No. metaphases analyzed		No. metaphases with TA		% metaphases with TA	
Group	Culture	Breast	Cervix	Breast	Cervix	Breast	Cervix
Affected	N	2,000	2,000	191	124	9.55	6.2
Affected	Ap	2,000	2,000	227	173	11.35	8.65
Control	N	2,000	2,000	2	3	0.1	0.15
Control	Ap	2,000	2,000	2	7	0.1	0.35

Abbreviations: N, normal medium; Ap, medium with aphidicolin; TA, telomeric associations.

Table 2 Comparative analysis between different groups

Culture	Af.br./br	Af.br./C	Af.cx/Af.cx	Af.cx/C	Af.br./Af.cx	C/C
N		$p < 0.001$		$p < 0.001$	$p < 0.001$	
	$p < 0.05$		$p < 0.001$			$p > 0.05$
Ap		$p < 0.001$		$p < 0.001$	$p < 0.001$	

Abbreviations: N, normal medium; Ap, medium with aphidicolin; Af, affected; br, breast cancer; cx, uterine cervix cancer; C, control.

tical differences when comparing both types of culture with their respective control groups ($p > 0.05$) (Table 2).

DISCUSSION

An unusually high frequency of telomeric associations has been reported in several types of malignant processes [5–7, 9–11]. The results obtained in this study show similarities with those published by other investigators. A highly significant incidence of TA was observed in both types of cancer when compared to the healthy individuals used as controls. In previous studies, it has been suggested that telomeric associations may contribute to chromosome instability; though it is still unknown to what extent this instability can determine malignancy of a cellular line [5–7, 9–11, 31].

Lima de Faria and co-workers [1], showed that most human retroviral oncogenes (among which are the oncogenes MYC, ABL, and HRAS1, implicated in the development of several types of cancer) are located in regions very close to the telomeres, a location that has been highly preserved during the evolution process. On the other hand, it has been observed that telomeres showing TA have suffered important deletions that caused them to lose functionality [10]. With these facts, we could assume that deletions of telomeric regions or an abnormally increased association, could somehow influence the activation of specific oncogenes.

Cytogenetic analysis in T lymphocytes revealed that a high proportion of metaphases presenting TA could be considered as an associated indicator of malignant processes (Table 1). The percentage of metaphases with TA for healthy individuals is not higher than 0.35%, whereas in affected individuals it could reach 11.35%.

The use of aphidicolin enhances the expression of TA in T lymphocytes, 1.18 times in individuals suffering breast cancer and 1.39 times in those suffering cervical cancer. Even though the percentage of altered metaphases in cultures from control individuals increased 2.3 times with the use of aphidicolin, it never exceeded 0.35%. These results resemble those obtained by Fuster et al. [25] and Kuwano et al. [26]. Their result reveal that the use of aphidicolin in cultures from healthy individuals induced low frequencies of TA. In this study aphidicolin increased the percentage of TA in peripheral blood from individuals affected by cancer.

High percentages of TA expression found in T lymphocyte cultures of individuals affected by breast and cervical uterine cancer could be explained as follows: In the transformation process of a normal cellular line into a tumoral one, a certain genomic instability originates, which is enhanced when Aphidicolin inhibits the action of alpha and delta polymerases [28]. When telomeres are affected by instability, they lose functionality, which is expressed by telomeric associations in a cytogenetic study. This loss of functionality produces an increase in the instability of genetic material, which in order to complete the cycle, accelerates the cellular malignant process. Fuster et al. [25] associated the high percentages of TA found in phenotypically healthy individuals to a "constitutional telomere anomaly that is more often expressed in the presence of aphidicolin." These results agree with the observations in this study that there is marked increase of TA in T lymphocyte cultures of individuals suffering from cancer by using aphidicolin. This leads us to conclude that chromosomal fragility and the frequency of altered metaphases in peripheral blood lymphocytes of breast cancer and cervical uterine cancer patients can be considered as a reliable marker for genetic behavior to both types of human neoplasia as has been previously reported by other authors [32–34].

The TA differences found in peripheral blood T lymphocytes between breast and cervix uterine cancer patients ($p < 0.001$) support an individual genetic response that is different in each type of cancer. This idiosyncratic phenomenon has been reported by other investigators [35] and is supported by the results of this study. The idiosyncratic response could be genetically determined and expressed by metabolic variations, detoxification processes, enzymatic processes, repair gene action, protein synthesis inhibition, or changes in DNA [35].

Our study opens the possibility of evaluating the development of malignant processes with cytogenetic analysis of peripheral blood, adding TA analysis as part of the genetic instability phenomena in cancer. Instability is reflected mostly as chromosomal fragility.

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