

# Chromosome Instability in Carcinomas

## Inestabilidad Cromosómica en Carcinomas

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**SUMMARY:** In the current carcinogenesis models, the occurrence of increasing mutations and selection mechanisms favoring cell survival and higher proliferation rates are taken into account. Epigenetic mechanisms, among which DNA methylation stands out, also take part in oncogenesis. The characteristic of tumor cells that allows the increase of mutations is named genetic instability, encompassing two mechanisms: microsatellite instability, characterized by nucleotide alterations with errors in the DNA repair systems; and chromosomal instability, represented by aberrations occurring in large chromosome segments. Carcinomas are characterized by complex cytogenetic alterations and large gene amalgamations. Telomeric alterations, inadequately repaired DNA breaks, and deficiencies in the mitotic spindle checking systems are events capable of generating the chromosomal instability and aneuploidy which characterize more aggressive neoplasias. A better understanding of the chromosomal instability mechanisms can show the way towards a clinical utilization of such information, like developing more adequate therapeutic strategies, targeted at specific sites involved in the malignization process.

**KEY WORDS:** Chromosomal instability; Carcinomas; Aneuploidies.

## INTRODUCTION

Historically, the search for a better understanding of oncogenesis has been based on a linear model dividing mutations into two categories: those assumed to promote cell growth by oncogene activation and mutations assumed to block growth inhibition by inactivating tumor suppressor genes (Vogelstein & Kinzler, 1993).

Later on, a third category of causal mechanisms for cancer was determined from studies on hereditary nonpolyploid colon cancer (HNPCC), consisting of alterations in DNA repair genes, leading to an increase in the number of mutations in the genome and thus favoring the occurrence of neoplasias. However, the occurrence of an increasing number of mutations alone does not explain the occurrence of neoplasias, which are believed to depend on selective mechanisms resulting from the interaction between chromosome and/or nucleotide alterations and the environment (Breivik, 2005).

Currently, carcinogenesis is considered an evolutionary process involving increasing genetic mutations, followed by selection of those capable of conferring significant proliferation and survival advantages to the cancer cells, thus allowing them to quickly become the dominant population within the tissue (Beckman & Loeb, 2005).

In addition to this process, a participation of epigenetic alterations without gene mutations occurs, through biochemical modifications connected to the modulation of genetic information (Li, 2002). Of these alterations, DNA methylation is the most studied one. It consists of the addition of a methyl radical to the cytosine adjacent to guanine, and its role in the genesis of cancer could be related mainly to gene silencing (Esteller & Herman, 2002).

**Genetic instability.** Neoplasias favor the genesis of mutations. This characteristic is named genetic instability,

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and two mechanisms are considered for its occurrence: microsatellite instability (MI), characterized by an elevated rate of nucleotide errors, and chromosome instability (CI), where the alterations occur in large chromosome segments (Michor *et al.*, 2005; Diaz, 2005).

The discovery of high mutation rates in colorectal cancers, with inactivation of DNA repair genes, represented the first consistent evidence of the role of genetic instability in human neoplasias (Gorringer *et al.*, 2005).

Loss of function of the repair system makes cells become susceptible to the acquisition of somatic mutations in their genome. Repeat sequences (microsatellites) are particularly affected by mutations in the absence of repair genes, and the genetic instability of such tumors is named MI (Yamamoto *et al.*, 2002).

However, cancer cells with MI, even presenting nucleotide mutation rates which are two or three times higher than those of normal cells, remain diploid or with an approximately diploid chromosome content, differently from what occurs with the great majority of human tumors, characterized by aneuploidy (alteration of the number of chromosomes) (Lengauer, 2005; Sieber *et al.*, 2005).

Aneuploidy reflects defects in the mitotic segregation of cancer cells (Rajagopalan *et al.*, 2003), producing an increase in the rate of gains and losses of chromosomes or chromosome segments during cell division (Michor).

The measurement of the rate of chromosome gains and losses in different cell lines, and the discovery of mutations in mitotic spindle checking genes made it possible to prove the existence of CI, which is currently considered to be the most frequent genetic instability mechanism in human tumors (Lengauer).

CI may accelerate the rate of tumor suppressor gene loss, and/or actually amplify oncogenes by duplication of the chromosomes on which they are located, contributing to tumorigenesis (Rajagopalan *et al.*). The acquisition of numerical chromosome aberrations appears to be the mechanism by which several relevant genes can be simultaneously affected, drastically reducing the number of events necessary for carcinogenesis, and corresponding to a kind of “jumping” evolution, in contrast with the sequential accumulation of mutations described on the gene level (Teixeira & Heim, 2005).

CI might also favor the ability of cancer cells to acquire resistance to chemotherapies, such as the one related to imatinib mesylate, secondary to chromosome

amplifications and duplications in cells with CI (Rajagopalan & Lengauer, 2004); and to 5-fluorouracyl, resulting from amplification of the thymidylate synthetase gene in aneuploid cells (Wang *et al.*, 2004).

Several cell pathways lead to numerical and structural CI in cancer cells, including defects in chromosome segregation, in abnormal cell reproduction control checking systems, in the stability of telomeres (chromosome ends) and in the answer to DNA damage. These mechanisms are closely connected, and their understanding will be of fundamental importance, not only for the understanding of carcinogenesis, but also of the reasons for pitfalls in cancer treatment (Gollin, 2005).

**Chromosome instability in carcinomas.** The great majority of human cancers are carcinomas. These tumors present karyotypes with a great number of rearrangements and a high frequency of translocations and other events related to malignization (Desmaze *et al.*, 2003; Calcagno *et al.*, 2005). Cells of the same tumor show differences in chromosome number, which seem to result from continuous gains and losses during mitosis (Li). This plasticity characterizes CI, occurring continuously and generating extensive genomic mixture, differently from what is observed in lymphomas and leukemias (Pihan & Doxsey, 2003).

Telomeres cover the ends of the chromosomes and diminish at each cell cycle. In the absence of telomerase (the enzyme capable of synthesizing telomeres, that is inactive in most tissues after birth), they undergo progressive shortening and, when they reach a critical size, they lead the cell to leaving the cycle and entering into replication senescence (Assumpção & Burbano, 2005).

It is well known that there is progressive telomere shortening in normal and accelerated aging in vitro and in vivo. A telomere hypothesis of cellular aging has been proposed, where the loss of telomere sequences provides the signal for cells to enter into senescence, leading to the formation of dicentric chromosomes and classical breakage-fusion-bridge (BFB) cycles followed by genome rearrangements and aneuploidy (Pathak & Multani, 2006).

In cancer cells, chromosomes with short telomeres remain unstable until they are “covered”. Sometimes, the addition of telomeres to the unprotected end occurs by translocation of another chromosome fragment. If the chromosomes do not receive telomeres, they may fuse with sister chromatids by means of a non-homologous end fusion mechanism, forming chromosomes with two centromeres. During the progression of the cell cycle, these dicentric chromosomes will be competitively pulled towards the opposite poles of the mitotic spindle (each centromere

towards one pole), and their stretching will result in the formation of bridges during anaphase (the step that precedes cell division). The "stretched" chromosome will suffer breaks, generating unstable ends (similar to short telomeres), which will undergo new fusions, resulting in additional rearrangements and continuous BFB cycles (Desmaze *et al.*).

A CI model induced by telomere dysfunction was described, in an attempt to explain the origin of epithelial tumors. Telomere dysfunction results in gains and losses of segments, directing epithelial carcinogenesis in animal models. These alterations resemble those observed in human epithelial tumors, reinforcing the hypothesis of short telomeres and BFB cycles producing CI in cancer cells (O'Hagan *et al.*, 2002).

It is further pointed out that cancer predisposition syndromes lead to telomere dysfunction and CI, and that both telomere shortening and the probability of cancer increase with age (Feldser *et al.*, 2003).

Another important finding is obtained during immortalization of human epithelial cells: in parallel to telomere erosion, a gradual increase of anaphase bridges and multipolar mitoses occur, and these phenomena regress with the increase of telomerase (Gisselsson & Höglund, 2005).

Frequent BFB events are observed in human tumors with chromosomal aberrations. They have been implied in gene amplification and may result in an imbalance of factors which are critical for cell homeostasis (Dey, 2004).

In highly aggressive tumors, BFB events produce a generalized instability. The occasional formation of one or more mitotically stable aberrations triggers an endless chain of BFB events, constantly producing new chromosome variations and genome imbalance. However, for BFB events to occur, there has to be inadequate functioning of one or more cell cycle checking systems (Maser & DePinho, 2003).

In the presence of short telomeres or DNA breaks of any origin, such as those resulting from exposure to carcinogens, alterations in the DNA damage checkpoints are necessary to prevent the imprisonment of cells in mitosis (allowing to repair the damage) or apoptosis (Maser). A combination of forces between eroded telomeres and an altered functioning of the checking systems is fundamental for CI to occur (Gisselsson & Höglund).

It is still obscure whether the breaks generated by the BFB cycles are random or occur in defined regions, contributing for cancer to arise. A possibility would be that the breaks are random, but with an overrepresentation of those which position oncogenes, tumor suppressor genes and nullizygous genes (indispensable for cell survival) in linkage balance, due to a selective advantage. The extensive gene mixture typical of carcinomas could result from an evolution towards a state of "linkage balance" produced by CI and oriented by natural selection (Pihan & Doxsey).

Our research team has performed cytogenetic investigations in gastric adenocarcinomas and has verified the occurrence of several chromosome aberrations, especially the presence of chromosome 8 trisomy (Assumpção *et al.*, 2001; Lima *et al.*, 2004), amplifications of gene C-MYC in Lauren's intestinal-type tumors, and translocations of this gene in Lauren's diffuse-type adenocarcinomas (Calcagno *et al.*). We have further observed differences between carcinomas presenting metastases and those of less advanced staging and higher amplification levels of gene C-MYC in more advanced tumors. These differences between histological subtypes and different staging levels encourage us to pursue a better understanding of the mechanisms involved in carcinogenesis, with the prospect of offering data which can be helpful in the clinical use of such knowledge.

A better understanding of the CI mechanisms can provide a possibility of clinical use of such information in the development of more adequate therapeutic strategies, oriented towards specific targets involved in the malignization process.

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**RESUMEN:** Los modelos actuales de carcinogénesis consideran la ocurrencia de mutaciones crecientes y mecanismos selectivos, favoreciendo la sobrevivencia y proliferación celular aumentada. Mecanismos epigenéticos también participan en la oncogénesis, destacando la metilación del DNA. La característica de las células tumorales que permite el aumento de la ocurrencia de mutaciones es denominada inestabilidad genética, donde son identificados dos mecanismos: inestabilidad de microsatélites, caracterizada por alteraciones nucleotídicas con errores en los sistemas de reparación del DNA; inestabilidad cromosómica, en la cual las aberraciones suceden en grandes segmentos cromosómicos. Los carcinomas son caracterizados por alteraciones citogenéticas complejas y grandes mezclas génicas. Alteraciones teloméricas, quiebres de DNA reparados inadecuadamente y deficiencia en los sistemas de chequeo del huso mitótico, son eventos capaces de generar inestabilidad cromosómica y aneuploidía que caracterizan estas neoplasias más agresivas. El conocimiento de los mecanismos que provocan la inestabilidad cromosómica puede permitir la utilización clínica de información en el desarrollo de estrategias terapéuticas más adecuadas, dirigidas a puntos específicos involucrados en procesos de malignización.

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**PALABRAS CLAVE:** Inestabilidad cromosómica; Carcinomas; Aneuploidías.

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