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MYC Gene Amplification in Gastric Adenocarcinoma

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Abstract: *MYC* oncogene, which has a key role in the tumorigenic process in several human cancers, has strong homology to cellular oncogenes of several vertebrate and also with the gene v-*Myc* of avian myelocytomatosis virus. The activation of some oncogenes, like *MYC*, can occur by amplification, caused by errors in DNA replication, resulting in a large number of extra copies of the gene and this could lead to the overproduction of the MYC protein. *MYC* gene amplification has been found in gastric adenocarcinoma, cervical squamous cell carcinomas and cell line HL-60 human promyelocytic leukemia, among others. Finally, this review gathered recent publications of a research team from the Federal University of Pará, in Brazil, where the *MYC* gene in gastric carcinogenesis is approached through conventional and molecular cytogenetics. Although still discrete, has increased the production of papers on the large number of copies of the *MYC* gene in gastric adenocarcinoma. This will contribute to important advances in diagnosis, therapeutic and prognosis of gastric adenocarcinoma.

Key words: Cancer · *MYC* Oncogene · Gene Amplification · Gastric Adenocarcinoma

INTRODUCTION

The vast catalog of cancer cell genotypes is a manifestation of six essential alterations in cell physiology that collectively dictate malignant growth: self-sufficiency in growth signals, insensitivity to growth-inhibitory (antigrowth) signals, evasion of programmed cell death (apoptosis), limitless replicative potential, sustained angiogenesis and tissue invasion and metastasis. Each of these physiologic changes – novel capabilities acquired during tumor development – represents the successful breaching of an anticancer defense mechanism hardwired into cells and tissues. These six capabilities should be shared in common by most and perhaps all types of human tumors [1].

As has become appreciated over the years, cancer is not a single disease, but a whole collection of disorders that constitute at least 300 different histological types. On the basis of embryological origin of the initial tumor cells, cancers are classified as follows: carcinomas are epithelial in origin (ectodermal and endodermal); sarcomas are derived from connective tissue (mesodermal); leukemias are from blood-forming cells; melanomas derive from pigment cells (melacocytes); teratomas arise from germ cells or gonadal tissue [2].

The more common types of human cancers - carcinomas - develop from epithelial tissue. These neoplasms are responsible for more than 80% of cancer-related deaths in the Western world. Among the carcinomas are tumors that develop from epithelial cells of the digestive tract - including mouth, esophagus, stomach and intestines - as well as skin, mammary glands, lungs, liver, gallbladder, bladder, ovary and uterus. When these tumors develop from epithelial cells that protect cavities, are known as squamous cell carcinoma; adenocarcinoma is the name reserved for tumors generated from the proliferation of epithelial cells secreting [3].

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Cancer may arise both from genetic or environmental factors that lead to aberrant growth regulation of a stem cell population, or by the dedifferentiation of more mature cell types [4]. Neoplastic transformation is a multicausal process in which the normal controls of cell proliferation and cell-cell interaction are lost. The aberrant activation of proto-oncogenes and the non-regulated inhibition of tumor suppressor genes represent the fundamentals of this process. Several hundred genes have been categorized as belonging to these categories in human cancers, although it is estimated that many more are yet to be identified [5].

Oncogenes are eukaryotic genes coding for proteins, conserved in evolution and presumably fulfills essential physiological functions in the normal cell. These are genes whose normal activity promotes cell proliferation. Gain of function mutations in tumor cells create forms that are excessively or inappropriately active. A single mutant allele may affect the phenotype of the cell. The non-mutant versions are properly called proto-oncogenes. Activation of some oncogenes, like *ERBB2* and *MYC*, can occur by amplification [6].

Myc Gene: Myelocytomatosis MC29 virus, which is known to be capable of inducing the formation of malignant tumors in the bone marrow of chickens, shown carrying a gene acquired in its genome called v-*Myc* oncogene that used to induce rapid growth of tumors in chickens infected [3]. The c-*Myc* gene is the cellular homologue of the retroviral oncogene v-*Myc*, discovered in the mid-twentieth century [7].

In scientific papers it is important to use terms that convey ideas of the author to readers of way clear and concise. For this there are naming conventions that, when known and used by everyone, facilitate understanding among professionals. Thus, the non-human oncogenes are normally written as words of three letters, with a capital initial letter, italicized (e.g., *Myc*), whereas their protein products are written in roman font (e.g., Myc protein) [8]. On the other hand, human genes follow a different nomenclature, so that the human Myc gene is written *MYC* and its product is the MYC protein [3, 8].

The protein product of the c-*Myc* gene has been shown to play a causal role in tumor formation in several avian and mammalian systems [9]. DNA sequences related to c-*Myc* are readily detected in the genomes of various species in the evolutionary tree of vertebrates. Van Beneden *et al*. [10] have isolated, cloned and sequenced the rainbow trout (*Salmo gairdneri*) c-*Myc* gene. The presumptive coding region of the trout c-*Myc* gene shows extensive homology with the of chicken, mouse and human.

***MYC* Gene in Humans:** *MYC* is an oncogene involved in cell cycle regulation, cell growth arrest, cell adhesion, metabolism, ribosome biogenesis, protein synthesis and mitochondrial function. It has been described as a key element of several carcinogenesis processes in humans [11].

The *MYC* gene located on chromosome 8 at band 8q24.1 and encodes a nuclear phosphoprotein [12]. *MYC* expression might be regulated transcriptionally (initiation and elongation), post-transcriptionally (mRNA stability and translation) or post-translationally (protein stability) [13].

Deregulation of this gene is cited as a major event in the pathogenesis of cancer, founded in 15 to 30% of the human cancer cases [3]. Recent studies have implicated MYC oncoprotein as a mediator of genomic instability, since it induces reactive oxygen species (ROS), causing aneuploidies [14].

The inappropriate activation of the *MYC* gene, which contributes to the development of human tumors, can occur by different mechanisms: chromosomal translocations, as found in Burkitt's lymphoma; through the juxtaposition of the promoter region of the immunoglobulin heavy chain gene, highly expressed in cells B, adjacent to the site of the *MYC* gene; stimulation of gene transcription, as observed in colon carcinoma cells; insertion of retrovirus adjacent to the MYC gene locus, activates its expression via the retroviral regulatory sequences; gene amplification, increasing the number of copies of the *MYC* and consequently, its expression, among others [15]. Moreover, in study of cytogenetic characterization of PG100, a new Brazilian gastric cancer cell line, the most frequent alteration was chromosome 8 trisomy [16].

MYC Protein: MYC is located in the nucleus, which functions as a transcription factor, a growth promoter. MYC is included in the family of bHLH transcription factors that form homo and heterodimers with themselves and with other family members. These dimeric complexes are associated then the regulatory sequences called E-boxes (composed by the sequence CACGTG) found in promoters of target genes that they regulate [3].

One of the ligands recognized as essential for most biological activities exerted by MYC is the MAX protein. This, when dimerized with MYC protein, functions as activating transcriptional [17]. Virtually every cell MYC protein is complexed with MAX that, unlike the MYC is expressed constitutively [18].

While the levels of MYC are heavily influenced by mitogenic signals, MAX levels are kept relatively constant within the cells. Similarly, when normal cells are grown in the presence of serum mitogens, MYC accumulates substantially; in contrast, the levels of MYC fall in the absence of mitogenic components. This means that the levels of MYC-MAX heterodimer are continuously controlled by the flow of mitogenic signals that normal cells are receiving [3].

In fact, the *MYC* gene has three exons, whose products (p64 and p67, "p" for protein, followed by weight in kDa) consist of highly conserved nuclear phosphoproteins [19], with relative abundance of p67 in relation to p64, also known as MYC-1 and MYC-2, respectively.

Gene Amplification: The amplification increases the number of copies of a gene in a genome and can give rise to karyotype abnormalities called double minutes (DM) and homogeneously staining regions (HSR), both of which have been widely observed in human tumors but are also known to play a major role during embryonic development due to the fact that they are responsible for the programmed increase of gene expression [20].

Despite being most studied in amphibians due to the size of chromosomes, the feathery chromosomes appear in prophase of meiosis of oocytes from different animals. The ultrastructural studies performed during this period suggest a massive transfer of ribosomal RNA from nucleus to cytoplasm. This process thus represents a considerable amplification of genes that form the template for synthesis of rRNA [21].

Gene Amplification in Carcinogenesis: The events of gene amplification, such as might be caused by errors in DNA replication, may result in a large number of extra copies of the gene and this could lead to the overproduction of the protein [5]. Oncogenes such as *CCND1*, *MET*, *MYC*, *ERBB2*, *EGFR* and *MDM2* are amplified in human tumors and can be associated with increased expression of their respective proteins or not [20].

According to Weinberg [3], one of the first findings of *MYC* gene amplification occurred in the DNA of cell line HL-60 human promyelocytic leukemia. These extra copies of the *MYC* gene (about 10 to 20 per diploid genome) were the result of gene amplification process and suggested that they caused proportionally high levels of its protein product, which somehow favored the proliferation of cancer cells. In some human tumors, for example, the *MYC* gene expression continues to be driven by its own natural transcriptional promoter, but the number of copies of this gene was increased to levels many times higher than the two copies present in the normal human genome.

Studies of *MYC* activation in cervical carcinomas have reported that gene over-expression (mainly gene amplification) are common in cervical squamous cell carcinomas and may correlate with the biologic behavior of the neoplasm. Abba *et al*. [22] showed that the average *MYC* copy number increased according to the histological grade of the lesion. Also, their results showed that the infection with HPV 16 was tightly associated with *MYC* amplification. These results could indicate that oncogene amplification take place in pre-invasive stages of cervical disease and could cooperate not only in tumor progression but also in cell transformation.

Myc Gene Amplification in Gastric Adenocarcinoma: The research team from the Human Cytogenetic Laboratory of Federal University of Pará (UFPA), Brazil, has performed cytogenetic investigations in gastric adenocarcinoma and found the occurrence of different chromosomal aberrations, especially the presence of aneuploidies of chromosome 8 [23, 24, 25], *MYC* gene amplifications [25-30] and translocations of this gene in adenocarcinoma diffuse type (Laurén classification) [25]. In these investigations were also observed differences between carcinomas presenting metastases and those of less advanced staging and higher amplification levels of *MYC* gene in more advanced tumors.

Lima *et al*. [24] described the establishment of the cell line ACP01 and characterize it cytogenetically by means of *in vitro* immortalization. Cells were transformed from an intestinal-type gastric adenocarcinoma (T4N2M0). This was the first gastric adenocarcinoma cell line established in Brazil. The ACP01 cell line is triploid, grows as a single, non-organized layer, similar to fibroblasts, with focus formation, heterogeneous division and a cell cycle of approximately 40 h. Chromosome 8 trisomy, present in 60% of the cells, was the most frequent cytogenetic alteration.

The fluorescence in situ hybridization (FISH) method was performed by Calcagno *et al*. [25] for the *MYC* gene and chromosome 8 centromere in gastric adenocarcinomas from 11 male African-Brazilian patients at the Pará State, Brazil. All cases showed aneuploidy of chromosome 8 and *MYC* amplification, in both the diffuse and the intestinal histopathological types of Laurén. *MYC* amplification, like homogeneously-stained regions (HSRs) and double minutes (DMs), was observed only in the intestinal type. Translocation of *MYC* was observed only in the diffuse type.

In a study that aimed to evaluate chromosomal aberrations implicated in gastric carcinogenesis, Guimarães *et al*. [27] analyzed three different passages (6th, 12th and 35th) of ACP01 cell line gastric adenocarcinoma by FISH method using chromosome 8 alpha-satellite probe. Most of the chromosome 8 alterations found involved a numerical increase of this chromosome. Chromosome 8 trisomy was detected in all cases, varying from 37% (6th passage) to 67% (35th passage). The presence of four or five signals for chromosome 8 also was observed. These results confirmed that trisomy of chromosome 8 is a common biological phenomenon in adenocarcinoma of stomach and can be used as a gastric mucosa malignancy marker.

Burbano *et al*. [26] investigated 21 primary gastric adenocarcinomas by comparative genomic hybridization (CGH) and evaluated the relationships between genomic abnormalities and histopathological features. 81% of cases presented DNA copy-number changes. Chromosomal gains were the most frequent finding, losses occurring only in the diffuse type. The main copy-number gains were on chromosome 8, principally on 8q24.1 (8/21 cases), 8p21 (3/21) and 8p23.2-8p12 (2/21). Gain of region 8q24.1, where *MYC* is located, was the main finding, exclusively in the intestinal type with metastasis. *MYC* locus amplification may be predictor of aggressiveness in intestinal type gastric cancer, playing an important role in its development and progression.

Whereas there are few studies on cytogenetic alterations in early gastric cancer, Raiol *et al*. [28] measured the number of *MYC* copies and the expression of its protein, by fluorescence in situ hybridization and immunohistochemistry in five early gastric adenocarcinomas of patients from northern Brazil. Three signs of *MYC* and MYC immunoreactivity were observed in all five samples, regardless of histological type, tumor extension, or lymph node status. These new discoveries about the *MYC* copy number changes in early gastric cancer suggest that the change is observed *MYC* in early gastric carcinogenesis and may be used as a therapeutic target.

Calcagno *et al*. [29] evaluated *MYC* copy number and protein expression in non-neoplasic, intestinal metaplasia and gastric cancer samples from five young adults. To our knowledge, this was the first study concerning *MYC* copy number in intestinal metaplasia samples and the first that evaluated this alteration in non-neoplasic, intestinal metaplasia and gastric cancer samples from the same patients by FISH. There was a significant increase of *MYC* amplification with the evolution of carcinogenesis process. MYC overexpression was observed in intestinal metaplasia and neoplastic tissue from all patients with intestinal-type gastric cancer and from no patients with diffuse type. Distinct patterns of alterations support the hypothesis that these tumor types follow different genetic pathways.

Leal *et al*. [30] evaluated whether *MYC*, *TP53* and chromosome 17 copy-number alterations occur in ACP02, ACP03 and AGP01 gastric cancer cell lines and in their tumor counterpart. Fluorescence in situ hybridization for *MYC* and *TP53* genes and for chromosome 17 was applied in the 6th, 12th, 60th and 85th passages of the cell lines and in their parental primary tumors. Three and four *MYC* signals were the most common alterations in gastric cell lines and tumors. The findings reveal that these cell lines retain, *in vitro*, the genetic alterations presented in their parental primary tumors.

CONCLUSION

MYC is a human oncogene in different ways involved in cell cycle regulation and cell growth. Their homologous are found in the various classes of vertebrates and also in the avian myelocytomatosis virus. The inappropriate activation of the *MYC* gene, which contributes to the development of human tumors, can occur by different mechanisms. Gene amplification is one of the most common mechanisms.

A growing number of studies of conventional and molecular cytogenetics about the performance of the *MYC* gene in gastric carcinogenesis have demonstrated their increased number of copies. The cytogenetic findings are trisomy 8, homogeneously-stained regions (HSRs) and double minutes (DMs). This knowledge produced will might help in diagnosis of gastric adenocarcinoma and suggest MYC gene as a potent therapeutic target. Inhibiting MYC expression can be a potential tool for gastric adenocarcinoma treatment in tumors with MYC overexpression.

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