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Letter to the editor

MYC in gastric carcinoma and intestinal metaplasia of young adults

MYC has a key role in gastric carcinogenesis. We evaluated *MYC* copy number and protein expression in non-neoplasic, intestinal metaplasia, and gastric cancer samples from five young adults. We observed 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. *MYC* copy number and expression can be biomarkers of gastric malignance.

Gastric cancer (GC) is the fourth most frequent cancer type and the second highest cause of cancer mortality worldwide [1]. In Pará state, northern Brazil, elevated incidence of GC has been verified [2].

Gastric adenocarcinoma is divided mainly into intestinal and diffuse types according to Laurén classification [3]. The intestinal type progresses through a number of sequential steps, beginning with atrophic gastritis, followed by intestinal metaplasia (IM), intraepitelial neoplasia, and carcinoma [4]. On the other hand, diffuse-type generally does not evolve from precancerous lesions [5], even though some patients presented diffuse-type together with IM, which raised doubts about the association between IM and GC development [6]. Thus, it remains unclear whether IM is a premalignant condition or a marker for an increased risk of malignancy.

GC affects mainly older patients. Early-onset GC ($\leq$ 40 years) is rare, and genetic factors may be more important in these patients. It is suggested that young patients with GC have a poorer prognosis than elderly patients [7].

MYC is a proto-oncogene commonly deregulated in gastric cancer, which is involved mainly in cell cycle regulation and growth arrest [8]. To investigate whether *MYC* alterations can be used as a cancer biomarker, we evaluated *MYC* copy number and protein expression in formalin-fixed, paraffin-embedded samples microdissected into non-neoplasic mucosa, IM, and neoplastic tissue from five patients with early-onset GC. These samples were obtained at Pará State João de Barros Barreto University Hospital, with the approval of the hospital Ethics Committee. Table 1 shows the cases along with their clinicopathologic characteristics.

Fluorescence in situ hybridization (FISH) was performed according to the method of Raiol et al. [9], using the *MYC* region probe (ONPON0824; Bioagency Biotechnology, São Paulo, Brazil) to evaluate this gene copy number. Non-neoplasic gastric mucosa from each patient and lymphocyte nuclei were used as negative controls. In IM samples, *MYC* gain was observed in up to 11%. In GC samples, *MYC* gain ranged from 41% to 54% of cells (Table 1, Fig. 1). *MYC* gain frequency was higher in IM than non-neoplasic tissue and was higher in GC than IM ($P < 0.0001$, chi-square test).

In our previous FISH results with advanced GC in patients older than 40 years, we observed that *MYC* gain was presented in all tumor samples, ranging from 43% to 83% of cells [10,11]. These results suggest that more than 40% of cells can be used to identify an advanced GC in our population.

To our knowledge, this is the first study concerning *MYC* copy number in IM samples and the first that evaluated this alteration in non-neoplasic, IM, and GC samples from the same patients by FISH. We observed a higher frequency of *MYC* gain in neoplastic tissue than IM samples from the same patients. The frequency of this alteration was also higher in early GC samples [9] than the IM samples from the present study ($P < 0.001$). In non-neoplasic tissue, gains or losses were observed in up to 5% of cells, which is usually used as a cut-off for clonal alterations in cytogenetic analysis. These results show an increase of *MYC* gain with the evolution of carcinogenesis process and suggest that IM and GC samples share the same genetic alteration, supporting the hypothesis that IM is a premalignant condition for GC.

Since *MYC* amplification is a common genetic alteration in GC samples from individuals of northern Brazil, we suggest that patients with gastric samples presenting clonal *MYC* alteration must be included in a risk group for GC. Moreover, the presence of *MYC* amplification evaluated on endoscopic biopsy specimens from individuals of northern Brazil can provide valuable information for making a preoperative genetic diagnosis of GC or a preneoplastic lesion.

MYC overexpression is described as ranging from 15.6% to 100% in primary GC [8]. This alteration was also observed in precancerous lesions [12–14]. However, few studies have evaluated *MYC* amplification and expression in GC [9–11,15,16]. Therefore, we performed immunohistochemical staining according to Raiol et al. [9] with *MYC* primary antibody (clone9E10, dilution 1:150; Zymed Laboratories, San Francisco, CA) to analyze this protein expression. We observed that all non-neoplasic gastric samples

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Table1
Immunohistochemical staining for MYC protein and *MYC* signal number in gastric sample

Case	Age	Gender	Histopathological	TNM*	Tissue	Immunoreactivity	Nuclei exhibiting *MYC* signals, no. (%) out of 200				
							1	2	3	4	≥5
1	37	Male			Normal	+	6 (3%)	190 (95%)	4 (2%)		
					Metaplasia	−	13 (6.5%)	165 (82.5%)	14 (7%)	8 (4%)	
			Diffuse	T3N2Mx	Tumor	−	17 (8.5%)	96 (48%)	43 (21.5%)	24 (12%)	20 (10%)
2	40	Female			Normal	+	3 (1.5%)	192 (96%)	5 (2.5%)		
					Metaplasia	+	7 (3.5%)	172 (86%)	18 (9%)	3 (1.5%)	
			Intestinal	T3N2M1	Tumor	+	10 (5%)	108 (54%)	44 (22%)	19 (9.5%)	19 (9.5%)
3	37	Male			Normal	+	10 (5%)	187 (93.5%)	3 (1.5%)		
					Metaplasia	+	10 (5%)	174 (87%)	12 (6%)	4 (2%)	
			Intestinal	T4N1Mx	Tumor	+	9 (4.5%)	100 (50%)	50 (25%)	23 (11.5%)	18 (9%)
4	37	Male			Normal	+	7 (3.5%)	188 (94%)	5 (2.5%)		
					Metaplasia	−	11 (5.5%)	176 (88%)	7 (3.5%)	6 (3%)	
			Diffuse	T3N0Mx	Tumor	−	15 (7.5%)	95 (47.5%)	38 (19%)	30 (15%)	22 (11%)
5	36	Male			Normal	+	5 (2.5%)	191 (95.5%)	4 (2%)		
					Metaplasia	−	25 (12.5%)	168 (84%)	7 (3.5%)		
			Diffuse	T3N3M0	Tumor	−	6 (3%)	86 (43%)	73 (36.5%)	26 (13%)	9 (4.5%)

TNM* is a system of cancer staging (tumor, nodes and metastases).
Less than 2% nucleus analyzed did not show MYC signal.

showed standard staining for *MYC* protein expression. *MYC* is usually immunoreactive in non-neoplasic gastric tissue [17] because of its role in continuous cell renewal, which is essential for maintenance of gastric mucosa.

Here, *MYC* overexpression was only detected in IM and neoplastic tissue from patients with the intestinal-type GC (Table 1; Fig. 1), corroborating our previous results, which described an association between *MYC* immunostaining in intestinal-type GC [11]. On the other hand, IM and neoplastic samples from patients with diffuse-type did not present *MYC* staining (Table 1; Fig. 1). The lack of staining in these patients can result from *MYC* insertion or translocation, which could lead to an abnormal MYC protein not identifiable by the antibody. Our research group previously

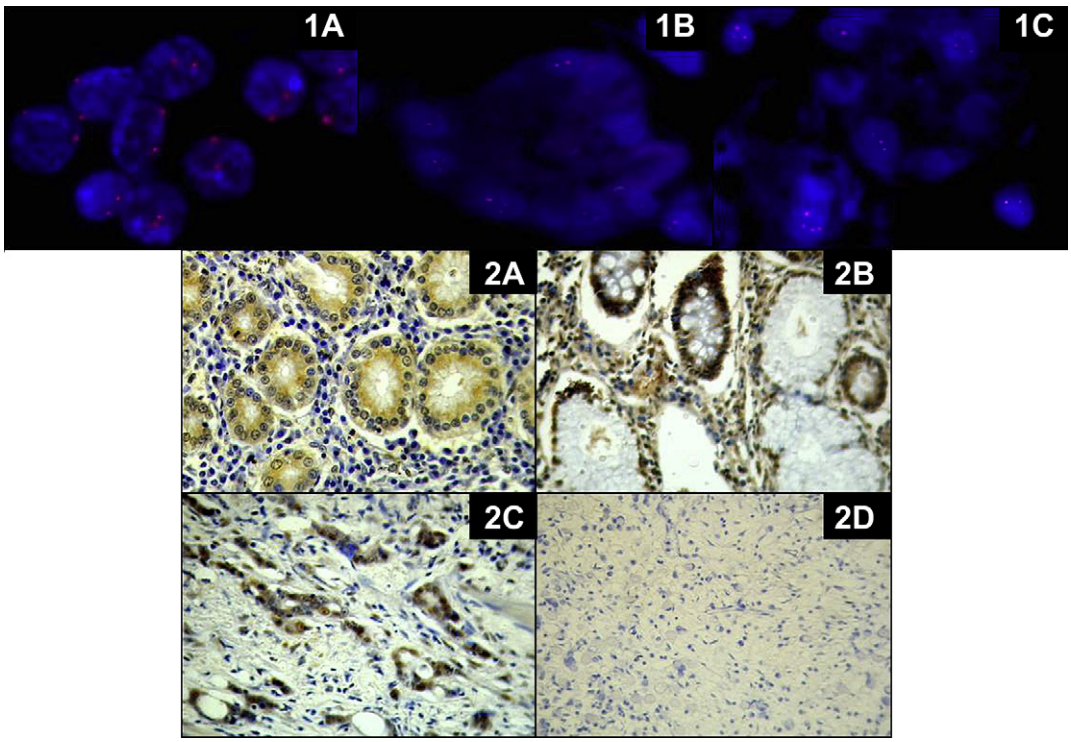

Fig. 1. (1) FISH assay (A) Interphase nuclei with two *MYC* signals in normal mucosa; (B) Interphase nuclei with *MYC* signal number alteration in IM; (C) Interphase nuclei with *MYC* signal number alteration in GC. (2) Immunostaining (×400) (A) MYC staining in non-neoplasic tissue; (B) MYC immunopositivity in IM; (C) MYC immunoreactivity in intestinal-type GC; (D) MYC-negative staining in diffuse type.

described that all diffuse-type and no intestinal-type GC samples showed possible rearrangements between *MYC* and chromosome 8 centromere [10,11]. This hypothesis was confirmed by FISH analysis in metaphase cells of diffuse-type GC [18].

Taking our results together with those from the literature, we suggested that MYC immunoreactivity pattern and gene copy number can be biomarkers of gastric malignance and potential therapeutic targets.

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