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LETTER TO THE EDITOR

hTERT and *TP53* deregulation in intestinal-type gastric carcinogenesis in non-human primates

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Abstract Despite the high incidence, the molecular events involved in intestinal-type gastric carcinogenesis remains unclear. We previously established an intestinal-type gastric carcinogenesis model in *Cebus apella*, a New World monkey. In the present study, we evaluated *hTERT* and *TP53* mRNA expression, as well as their protein immunoreactivity, in normal mucosa, non-atrophic gastritis, atrophic gastritis, intestinal metaplasia, and intestinal-type gastric cancer samples of non-human primates treated with N-methyl-nitrosourea. In addition, we evaluated the number of *TP53* copies in these samples. Although hTERT immunoreactivity was only detected in gastric cancer, a continuous increase of *hTERT* mRNA expression was observed from non-atrophic gastritis to gastric tumors. No sample presented p53 immunoreactivity. However, we also observed a continuous decrease of *TP53* mRNA expression during the sequential steps of gastric carcinogenesis. Moreover, loss of *TP53* copies was observed in intestinal metaplasia and gastric cancer samples. Our study highlights that *hTERT* and *TP53* have a key role in intestinal-type gastric cancer initiation.

Keywords *hTERT* · *TP53* · Gastric carcinogenesis · Non-human primates · Precancerous lesions · Animal model

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Dear Editor,

Gastric cancer (GC) is one of the most common neoplasias in the world and can be classified into intestinal and diffuse subtypes [1]. Intestinal-type GC is progressed through multiple steps beginning with atrophic gastritis that is followed by intestinal metaplasia, dysplasia, and carcinoma [2, 3]. The exact mechanism of intestinal-type GC development remains unclear.

Non-human primates offer a useful model for carcinogenic studies due to their close phylogenic relationship to humans and greater similarities with regard to anatomy, physiology, biochemistry, and organ systems, as compared to rodents [4]. We recently established an intestinal-type gastric carcinogenesis model in non-human primates [5]. We treated 6 *Cebus apella,* a New World monkey, with N-methyl-nitrosourea (MNU) for about 2.5 years. All animals developed pre-neoplastic lesions and five died of drug intoxication before the development of GC. All animals presented non-atrophic gastritis on the 90th day. On the 110th day, one animal died from drug intoxication and the other five animals presented atrophic gastritis on the 120th day. On the 300th day, the two surviving *C. apella* presented intestinal metaplasia in gastric mucosa. On the 940th day, the last surviving animal developed intestinal-type adenocarcinoma in the antral region of the stomach.

In a previous study, we observed that *MYC* mRNA expression and copy number increased during the

sequential steps of intestinal-type gastric carcinogenesis and immunoreactivity was only observed in intestinal metaplasia and GC [4]. The findings in *C. apella* GC corroborate our observations in human gastric carcinogenesis, in which the presence of *MYC* amplification was detected in all intestinal-type GC [6–11] and a significant increase of *MYC* copy number was seen with the evolution of human carcinogenesis process: normal mucosa, intestinal metaplasia, and GC [8]. These findings also corroborate our results showing MYC protein overexpression in intestinal metaplasia and neoplastic tissue from all patients with intestinal-type GC [8, 9, 11]. Thus, the established animal model may be an interesting tool for studying intestinal-type gastric carcinogenesis.

The MYC protein has an effect on about 15 % of the genes in the human genome [12]. Among the *MYC* target genes are *hTERT* (the catalytic subunit of telomerase) and *TP53* tumor suppressor gene [13]. We previously described that alteration in *TP53* was a common finding in intestinal-type GC [14], as well as of *hTERT* [15]. To elucidate whether *hTERT* and *TP53* have a key role in intestinal-type gastric tumor initiation, we evaluated their expression in normal, non-atrophic and atrophic gastritis, intestinal metaplasia, and GC samples of *C. apella* obtained during the study of Costa & Leal et al. [5]. In addition, we analyzed the *TP53* copy number in these samples.

Immunohistochemistry was performed on formalin-fixed, paraffin-embedded sections according to Calcagno et al. [9] with primary mouse monoclonal antibody against *hTERT* (dilution 1:50; clone 44F12, Novocastra Laboratories Ltd, UK) and p53 (dilution 1:50; Dakocytomation, USA). In the present study, only the GC sample presented hTERT immunoreactivity and no sample presented p53 immunoreactivity (Table 1).

Real-time quantitative reverse transcription PCR (qRT–PCR) was performed to evaluate *hTERT* and *TP53* mRNA expression. All the reactions were performed in triplicate for the target genes (hTERT: Hs00972656_m1; TP53: Hs01034249_m1) and the internal control (GAPDH: NM_002046.3). Relative quantification (RQ) of the gene expression was calculated according to Livak and Schmittgen [16] using the baseline values (from day 0) of each animal as a calibrator. The Friedman test showed that *hTERT* and *TP53* mRNA expression differed among normal mucosa, non-atrophic gastritis, and atrophic gastritis samples ($p = 0.022$ for both analyses). The Wilcoxon test detected that the expression of *hTERT* and *TP53* differed between atrophic gastritis and normal mucosa samples ($p = 0.043$ for both analyses), and between atrophic and non-atrophic gastritis ($p = 0.043$ for both analyses). However, the three groups of samples did not differ after Bonferroni correction of Wilcoxon analyses considering an alpha level of 0.167.

Due to the small number of samples, we did not include the intestinal metaplasia and GC samples in the statistical comparison among the different stages of gastric carcinogenesis. However, we were able to observe a continuous increase of *hTERT* mRNA expression from non-atrophic gastritis to GC, which showed about a fourfold increase in mRNA levels, even with only GC sample presenting hTERT immunoreactivity (Table 1). Some studies also reported that the *hTERT* expression increases with the sequential steps of intestinal-type gastric carcinogenesis in humans [17–21], suggesting that *hTERT* deregulation represents an important step in the carcinogenesis progress.

In the present study, almost 1.5- and two-fold reduction of *TP53* expression was observed in intestinal metaplasia and in the GC sample, respectively (Table 1). Moreover, we also observed a continuous decrease of *TP53* mRNA expression during the sequential steps of gastric carcinogenesis in MNU-treated monkeys, which corroborates previous studies in humans [17, 22].

Table 1 Relative quantitation *hTERT* and *TP53* mRNA expression and their protein immunoreactivity, and *TP53* gene copy number variation in biopsies of MNU-treated animals

Treatment	Histology	Number of animals	*hTERT*		*TP53*		
			IHC	RQ (median ± IQR)	IHC	RQ (median ± IQR)	CNV [number (median ± IQR)]
Baseline	Normal mucosa	6	Negative	1	Negative	1	2 (2.09 ± 0.12)
MNU/90th day	Non-atrophic gastritis	6	Negative	1.35 ± 0.13	Negative	1.04 ± 0.09	2 (1.16 ± 0.15)
MNU/120th day	Atrophic gastritis	5	Negative	1.56 ± 0.13	Negative	0.88 ± 0.07	2 (2.14 ± 0.18)
MNU/300th day	Intestinal metaplasia	2	Negative	1.88 ± 0.01	Negative	0.62 ± 0.03	1 (1.29 ± 0.18)
MNU/940th day	Gastric cancer	1	Positive	3.98	Negative	0.55	1 (1.11)

IHC immunohistochemistry, *RQ* Relative quantification of mRNA expression, *CNV* copy number variation, *IQR* interquartile range

In addition, we detected an inverse correlation between *hTERT* and *TP53* mRNA expression ($p < 0.001$, Spearman test; $\rho = -0.818$) in agreement with the role of hTERT as a p53 repressor [23].

Since the loss of *TP53* is a frequent finding in gastric tumors [14, 24] and GC cell lines [25–27], we also evaluated this gene copy number. For this analysis, duplex quantitative PCR (qPCR) was performed in quadruplicate using TaqMan probes for *TP53* gene (Hs06423639_cn) and *RNAse P* (#4403326, internal control). Relative quantification using a known human gDNA (Promega, USA) as calibrator was analyzed by Copy Caller Software V1.0 (Applied Biosystems, USA). We observed that all normal mucosa, non-atrophic gastritis, and atrophic gastritis samples presented two *TP53* copies. Loss of *TP53* copies was only detected in intestinal metaplasia and GC samples (Table 1). Our results corroborate Williams et al. [24] study, which described the *TP53* deletion as a common event in premalignant stages of human gastric carcinogenesis. Although few studies evaluated the number of copies of *TP53* in pre-neoplastic gastric lesions, our results showed that this gene loss can contribute to intestinal-type tumor initiation.

Our study highlights that *hTERT* and *TP53* have a key role in intestinal-type GC initiation and may be used as biomarkers to help in monitoring populations with this high-risk neoplasia subtype.

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Conflict of interest All authors declare that they have no conflicts of interest.

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