

FULL LENGTH ARTICLE

Upregulation of the *APE1* and *H2AX* genes and miRNAs involved in DNA damage response and repair in gastric cancer

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Received 30 December 2018; accepted 28 March 2019

Available online 5 April 2019

KEYWORDS

DNA damage
response;
DNA repair;
Gastric cancer;
Gene expression;
microRNA

Abstract Gastric cancer remains one of the leading causes of cancer-related death worldwide, and most of the cases are associated with *Helicobacter pylori* infection. This bacterium promotes the production of reactive oxygen species (ROS), which cause DNA damage in gastric epithelial cells. In this study, we evaluated the expression of important genes involved in the recognition of DNA damage (*ATM*, *ATR*, and *H2AX*) and ROS-induced damage repair (*APE1*) and the expression of some miRNAs (miR-15a, miR-21, miR-24, miR-421 and miR-605) that target genes involved in the DNA damage response (DDR) in 31 fresh tissues of gastric cancer. Cytoscape v3.1.1 was used to construct the postulated miRNA:mRNA interaction network. Analysis performed by real-time quantitative PCR exhibited significantly increased levels of the *APE1* (RQ = 2.55, $p < 0.0001$) and *H2AX* (RQ = 2.88, $p = 0.0002$) genes beyond the miR-421 and miR-605 in the gastric cancer samples. In addition, significantly elevated levels of miR-21, miR-24 and miR-421 were observed in diffuse-type gastric cancer. Correlation analysis reinforced some of the gene:gene (*ATM/ATR/H2AX*) and miRNA:mRNA relationships obtained also with the interaction network. Thus, our findings show that tumor cells from gastric cancer presents deregulation of genes and miRNAs that participate in the recognition and repair of DNA

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Peer review under responsibility of Chongqing Medical University.

damage, which could confer an advantage to cell survival and proliferation in the tumor micro-environment.

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Introduction

Gastric cancer, one of the most common types of cancer, is the third leading cause of cancer-related death worldwide¹ and presents wide variations in incidence throughout the world.² In Brazil, gastric cancer ranks fourth in incidence and second in death, with an estimated 23,290 new cases in 2018.³ Diets high in food preservatives (salts and nitrates), alcohol, and smoking are among the main risk factors for gastric carcinogenesis.⁴ However, chronic inflammation induced by infection with *Helicobacter pylori* (*H. pylori*) is the most important factor.⁵ This bacterium, which colonizes the gastric mucosa, and its virulence factors, such as cytotoxin-associated gene A (CagA) and vacuolating cytotoxin A (VacA), are responsible for increased levels of reactive oxygen species (ROS) and reactive nitrogen species (RNS) produced by immune and epithelial cells in an attempt to kill the bacteria.⁶ The excessive production of ROS is believed to be a major cause of gastric mucosal DNA damage in the infected mucosa,⁷ thus promoting genomic instability and tumorigenesis.⁸

Furthermore, ROS produced during *H. pylori* infection can cause DNA oxidation, leading to the generation of abasic sites,⁶ which are recognized and processed by human apurinic/apyrimidinic endonuclease 1 (APE1), an essential enzyme that is involved in the base excision repair (BER) pathway.⁹ Previous studies have shown increased APE1 expression in various types of cancer,^{10–12} including gastric cancer,¹³ thus suggesting that APE1 could be associated with survival outcome, lymph node status, proliferation index and resistance to chemotherapy or radiotherapy¹⁴ and that upregulation of BER in solid cancers may represent an adaptive survival response.¹⁵

In addition, *H. pylori* can also induce DNA double-strand breaks (DSBs),¹⁶ activating the DDR (DNA damage response), a complex network that includes specialized sensor proteins to recognize DNA damage and transducer proteins to recruit subsequent effector proteins, which in turn are responsible for cell cycle arrest, apoptosis, transcription arrest, and DNA repair.¹⁷ In response to DSBs, the activation of proteins such as ataxia telangiectasia mutated (ATM) and Rad3-related (ATR) occurs to recognize DNA damage, resulting in H2AX histone phosphorylation at Ser 139 (γ H2AX) as an initial step toward DNA repair.¹⁸ If the lesion cannot be repaired, apoptosis or premature senescence is promoted.¹⁹

Several studies have shown that the microRNA (miRNA) expression profiles are altered when cells are treated with different types of genotoxic agents and chemical mutagens.^{20–22} DNA repair genes are directly inhibited by miRNAs,²³ such as miRNA-421 that suppresses ATM expression²⁴ and miRNA-24 that target H2AX.²⁵

Therefore, the interactions of miRNAs and their ability to target DDR components control the cellular response to DNA-damaging agents,²⁶ indicating a pivotal role in DDR regulation.^{27,28}

Therefore, considering the importance of the DDR in the recognition and repair of DNA damage to guarantee genomic stability, the present study evaluated the expression of important genes involved in recognition (ATM, ATR, and H2AX) and ROS-induced damage repair (APE1) and the miRNAs (miR-15a, miR-21, miR-24, miR-421, and miR-605), selected from public databases (TargetScan, TarBase, and MirTarBase), that target genes of the DDR pathway in gastric cancer tissue samples. The goal of this study was to identify genes and miRNAs that may be modulated in response to induced damage in the gastric mucosa and to construct the interaction network among them.

Materials and methods

Ethics statement and study population

This study was approved by the Research Ethics Committee of IBILCE/UNESP (n° 2.197.528) for the use of DNA/RNA samples stored in our laboratory from a previous study.²⁹ Written informed consent was obtained from all participants.

RNA/DNA were extracted using TRIzol reagent (Invitrogen, Carlsbad, California, USA) from fresh biopsies or surgical fragments collected from 35 individuals recruited at the Service of Endoscopy or the Surgery Center at the Hospital de Base, São José do Rio Preto, SP, Brazil. Thirty-one tissue samples were histopathologically diagnosed as gastric adenocarcinoma (GA) according to Lauren's classification,³⁰ and four tissue samples were diagnosed as histologically normal, *H. pylori*-negative (Hp-), gastric mucosa (NM). These normal tissues were collected from healthy individuals who had no previous history of gastric dyspepsia and were cancer free and were analyzed in qPCR experiments as a pool (prepared from equal amounts of each normal mucosa RNA). Epidemiological data for both GA and NM samples are presented in Table 1.

Molecular diagnosis of *H. pylori* infection

The molecular diagnosis was performed according to the protocol of Singh et al.,³¹ based on nested PCR to amplify the *HSP60* gene. The PCR was carried out in a 25- μ L volume using 1 $\times$ PCR buffer, 2.5 mM MgCl₂, 0.25 mM (each) deoxynucleotide triphosphate, 10 μ M each primer (HSP1: 5'-AAGGCATGCAATTGATAGAGGCT-3' HSP2: 5'-TTTTTTCTCTTTCATTCCACTT-3'), 1 U of Platinum Taq polymerase

Table 1 Epidemiological data from individuals with normal mucosa (NM) and gastric adenocarcinoma (GA).

Variable	NM N (%)	GA N (%)
Gender		
Female	3 (75)	6 (19.4)
Male	1 (25)	25 (80.6)
Total	4	31
Age (years)		
Mean $\pm$ SD	30 $\pm$ 12.5	65 $\pm$ 13.8
	<30 3 (75)	<65 16 (51.6)
	$\geq$ 30 1 (25)	$\geq$ 65 15 (48.4)
Total	4	31
Smoking		
Yes	0 (0)	18 (64.3)
No	4 (100)	10 (35.7)
Total	4	28 ^a
Drinking		
Yes	0 (0)	14 (50)
No	4 (100)	14 (50)
Total	4	28
H. pylori		
Positive	0 (0)	15 (50)
Negative	4 (100)	15 (50)
Total	4	30 ^a
Histological type		
Intestinal	—	26 (86.7)
Diffuse	—	4 (13.3)
Total		30 ^a

^a Data not available for some individuals; N = number of individuals.

(Carlsbad, California, USA), and 100 ng of DNA. For internal amplification, the PCR product from the primary cycle was diluted 1:10, and 10 μ L was used as the template in the nested PCR using the primers HSPN1 (5'-TTGATA-GAGGCTACCTCTCC-3') and HSPN2 (5'-TGTCATAATCGCTTCTCGTGC-3'). All the amplification reactions were carried out in a thermal cycler (LabNet Inc) over 30 cycles, changing the temperature from 94 °C to 56 °C to 72 °C, holding each temperature for 30 s. In all the reactions, a negative control without DNA and a negative control containing intestinal DNA sample with a negative diagnosis for *H. pylori* were used. The nested PCR products were analyzed by electrophoresis on a 1.5% agarose gel and staining with ethidium bromide. A 501-bp fragment was observed in only the *H. pylori*-positive samples.

Relative quantification of mRNA and miRNA expression levels by quantitative PCR (qPCR)

First, complementary DNA (cDNA) was synthesized for mRNA and miRNA with the High Capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA, USA) and TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA), respectively, according to the manufacturer's protocols. qPCR was performed in a

StepOnePlus real-time PCR system (version 2.2.3) (Applied Biosystems, Foster City, CA, USA) with TaqMan assays using specific probes for the target genes *APE1* (Hs00959050_g1), *ATM* (Hs00175892_m1), *ATR* (Hs00992123_m1), and *H2AX* (Hs00266783_s1) and for the miRNAs hsa-miR-15a-5p (MIMAT0000068), hsa-miR-21-5p (MIMAT0000076), hsa-miR-24-3p (MIMAT0000080), hsa-miR-421 (MIMAT0003339), and hsa-miR-605-5p (MIMAT0003273). The reference genes *ACTB* (Hs99999903_m1) and *GAPDH* (Hs03929097_g1) were used as endogenous control genes and *RNU6B* (001093) and *RNU48* (001006) were used as control miRNAs in all the analyses, according to the validation performed in a previous study.³² All reactions were performed in triplicate in a final volume of 10 μ L using GoTaq Probe qPCR Master Mix 2 $\times$ (Promega, Madison, Wisconsin, USA). Raw cycle quantification (Cq) data were generated by StepOnePlus™ Software (Applied Biosystems, Carlsbad, California, USA) and normalized to the reference control genes. Relative quantification (RQ) of mRNA and miRNA expression was calculated using the $2^{-\Delta\Delta C_t}$ method according to the model proposed by Livak and Schmittgen,³³ using the pool of Hp-normal mucosa samples as calibrator (RQ = 1.0). qPCR experiments followed the MIQE guidelines,³⁴ and RQ values were expressed as medians of the genes and miRNAs for the GA group.

In silico analysis for prediction of miRNA targets and the miRNA:mRNA interaction network

Considering only the genes and miRNAs evaluated in the present study, an *in silico* analysis for the search of genes considered as predicted targets was performed from the RNA22-HAS (<https://cm.jefferson.edu/rna22>), TARGETSCAN-VERT (http://www.targetscan.org/vert_71), MICRORNA.ORG (<http://www.microrna.org/microrna/home.do>), and MIRDB (MirTar2 v4.0) (<http://mirdb.org/miRDB/index.html>) databases, while those genes considered as validated targets were collected from the MIRTARBASE (<http://mirtarbase.mbc.nctu.edu.tw/>) and TARBASE (<http://diana.imis.athena-innovation.gr/DianaTools/index.php?r=tarbase/index>) databases (Supplementary Table 1). For predicted targets, only genes identified by at least three databases were considered. Then, data were integrated using bioinformatic methods, and genes were mapped into proteins, which were then used to construct protein–protein interaction (PPI) networks. The PPI networks were generated using the Metasearch STRING platform v10.5.³⁵ Data integration and visualization were performed using Cytoscape v3.1.1.³⁶

Statistical analysis

Initially, the data were evaluated using the D'Agostino-Pearson normality test. All data analyzed were considered non-parametric and the values for the relative expression (RQ) of mRNA and miRNA were expressed as medians with interquartile range. The One-sample Wilcoxon signed-rank test was used to assess changes in mRNA or miRNA expression levels compared to those in a pool of normal

mucosa samples (RQ = 1.0), while the correlation between mRNA and miRNA expression was analyzed using Spearman's correlation. The analysis was performed by GraphPad Prism software (version 6.01). $P < 0.05$ was considered statistically significant.

Results

Deregulated expression of genes and miRNAs involved in the DDR in gastric cancer tissues

qPCR analysis showed significantly upregulated expression of the *APE1* (RQ = 2.55, $p < 0.0001$) and *H2AX* (RQ = 2.88, $p = 0.0002$) genes in the gastric cancer samples compared to the expression in the normal mucosa. However, the expression level of the *ATM* (RQ = 0.46, $p = 0.45$) and *ATR* (RQ = 0.94, $p = 0.41$) genes were not significantly altered in the gastric cancer samples (Fig. 1).

Among the miRNAs evaluated for involvement in the regulation of the DDR, significant upregulation for miR-421 (RQ = 1.27, $p = 0.04$) and miR-605 (RQ = 1.47, $p = 0.02$) was observed, while no significant change in the expression of miR-15a (RQ = 0.78, $p = 0.50$), miR-21 (RQ = 0.89, $p = 0.91$), and miR-24 (RQ = 0.43, $p = 0.82$), was detected in the gastric cancer group compared to the expression of a pool of normal mucosa (Fig. 2). In contrast, when we stratified the samples by the histological type of cancer, we identified significantly higher expression of miR-21, miR-24, and miR-421 in diffuse-type cancer than in intestinal-type cancer (Fig. 3). Regarding the mRNA expression levels of the evaluated genes (*APE1*, *ATM*, *ATR*, and *H2AX*), no significant association was observed with risk factors such as gender, age, *H. pylori* infection, and histological type of gastric cancer (Fig. 4).

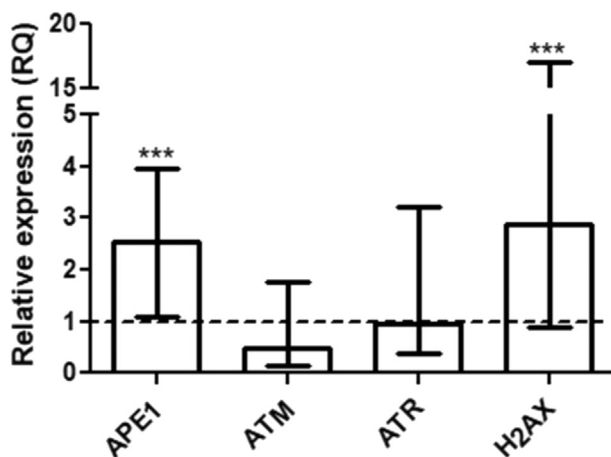


Figure 1 Relative expression of the mRNA of the *APE1*, *ATM*, *ATR*, and *H2AX* genes in the gastric cancer samples in comparison to the expression of a pool of normal mucosa and the expression of the reference genes (*ACTB* and *GAPDH*). The dashed line represents the RQ value of a pool of normal gastric mucosa samples used as calibrator (RQ = 1.0) and error bars represent the interquartile range.

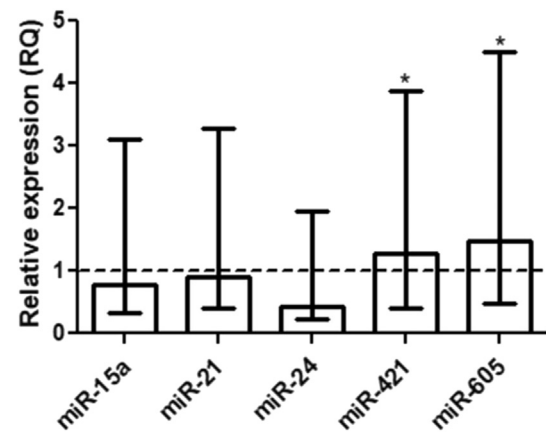


Figure 2 Relative expression of the miRNAs (miR-15a, miR-21, miR-24, miR-421, and miR-605) in the gastric cancer samples in comparison to the expression of a pool of normal mucosa and the expression of endogenous RNUs (RNU6B and RNU48). The dashed line represents the RQ value of a pool of normal gastric mucosa samples used as calibrator (RQ = 1.0) and error bars represent the interquartile range.

Correlation analysis and interaction network involved in the DDR

The expression of the *ATM*, *ATR*, and *H2AX* genes was positively correlated in gastric cancer samples (Table 2). The strongest correlation was observed between *ATM* and *ATR* ($r = 0.91$, $p < 0.0001$), followed by the correlations between *ATM* and *H2AX* ($r = 0.76$, $p = 0.0002$) and between *ATR* and *H2AX* ($r = 0.72$, $p < 0.0001$). However, the expression of the *APE1* gene did not correlate with that of any of these genes. In addition, significant negative correlations were observed among the expression of miR-21, miR-421, and miR-605 and the *ATM* and *ATR* genes (Table 3).

The interaction network depicted in Fig. 5 shows strong relationships among genes and miRNAs participating in the DDR and reinforces the negative correlations observed between the expression of the miR-21, miR-421, and miR-605 and the *ATM* and *ATR* genes (Table 3). Furthermore, we also observed interactions among the *ATM*, *ATR*, and *H2AX* genes (Fig. 5), confirming the strong positive correlations observed among the expression levels of these genes in gastric cancer samples (Table 2).

Discussion

Our results show that gastric cancer tissues exhibit elevated expression of the genes (*APE1* and *H2AX*) and miRNAs (miR-421 and miR-605) involved in DDR and DNA repair, independent of *H. pylori* infection, histological type, gender or age. However, upon considering the histological type, miR-21, miR-24, and miR-421 presented upregulation in diffuse-type gastric cancer tissues. Our findings also showed significant positive correlations in mRNA expression among *ATM*/*ATR*/*H2AX*, as well as negative correlations among miR-605/*ATM*/*ATR*, miR-21/*ATM*/*ATR*, and miR-421/*ATM*/*ATR* demonstrating gene:gene and gene:miRNA

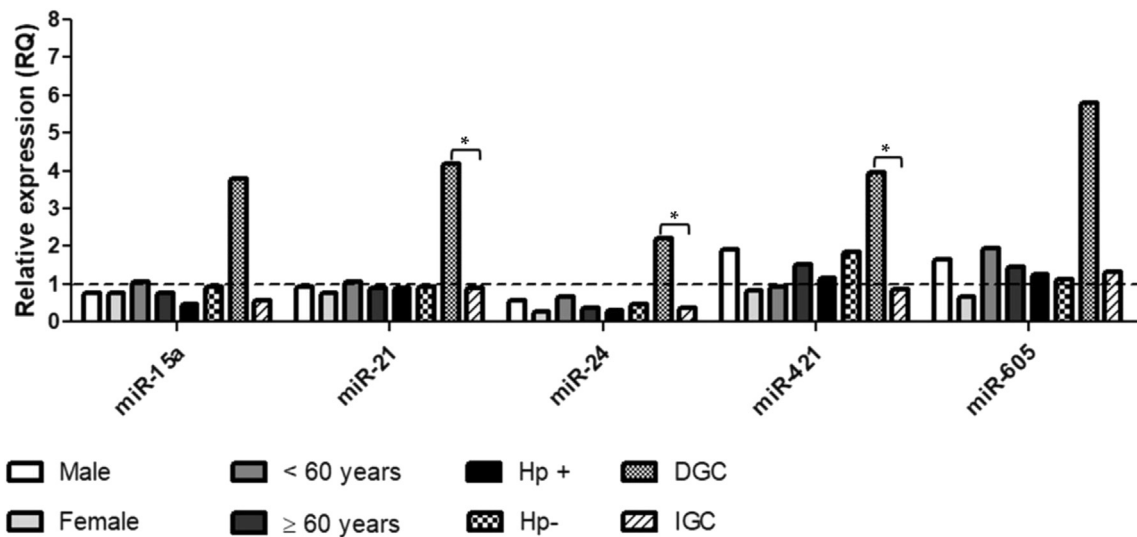


Figure 3 Comparison of the relative expression levels of the microRNAs (miR-15a, miR-21, miR-24, miR-421, and miR-605) according to the risk factors gender, age, *H. pylori* status, and histological tumor type, in the gastric cancer group. The dashed line represents the RQ value of a pool of normal gastric mucosa samples used as calibrator (RQ = 1.0). Hp+ (*H. pylori* positive); Hp- (*H. pylori* negative); DGC (diffuse-type gastric cancer); IGC (intestinal-type gastric cancer).

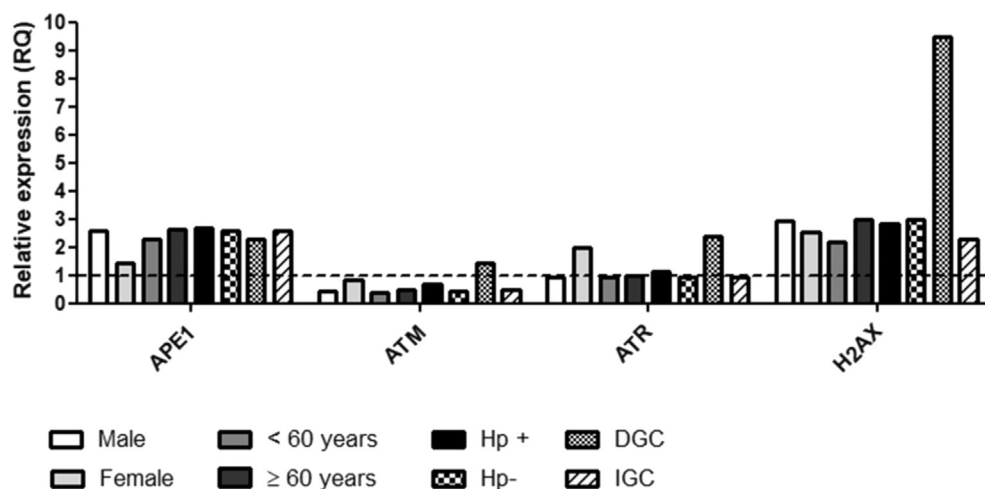


Figure 4 Comparison of mRNA expression levels of the *APE1*, *ATM*, *ATR*, and *H2AX* genes according to the risk factors gender, age, *H. pylori* status, and histological tumor type, in the gastric cancer group. The dashed line represents the RQ value of a pool of normal gastric mucosa samples used as calibrator (RQ = 1.0). Hp+ (*H. pylori* positive); Hp- (*H. pylori* negative); DGC (diffuse-type gastric cancer); IGC (intestinal-type gastric cancer).

Table 2 Correlation analysis among the relative expression levels of DDR genes in gastric cancer samples. Data are presented as the Spearman correlation coefficient (*r*), with significant correlations highlighted in color.

	<i>ATM</i>	<i>ATR</i>	<i>H2AX</i>
<i>ATR</i>	0.91		
<i>H2AX</i>	0.76	0.72	
<i>APE1</i>	-0.09	-0.11	0.14

Red: $P < 0.001$

interrelations, which were supported by the *miRNA:mRNA* interaction network (Fig. 5).

It has been suggested that BER factors may be preferentially upregulated in tumors to repair DNA damage induced by oxidative stress.³⁷ Accordingly, several studies have reported increased expression of *APE1* in gastric cancer, being correlated with poor prognosis and development,¹³ poor overall survival,³⁸ and lymph node metastasis,³⁹ acting as a marker for prognosis in patients with gastric cancer.^{11,13} In our study, we also observed elevated expression of *APE1* in fresh samples of patients with gastric cancer, reinforcing the hypothesis that upregulation of BER in solid tumors may represent an adaptive survival response in the tumor microenvironment.¹⁵

Table 3 Correlation analysis among the relative expression levels of genes and miRNAs associated with the DDR in gastric cancer samples. Data are presented as the Spearman correlation coefficient (r), with significant correlations highlighted in color.

	miR-15a	miR-21	miR-24	miR-421	miR-605
<i>APE1</i>	0.57	0.42	0.30	0.50	0.42
<i>ATM</i>	-0.33	-0.46	-0.34	-0.41	-0.60
<i>ATR</i>	-0.30	-0.42	-0.35	-0.40	-0.52
<i>H2AX</i>	-0.05	-0.10	-0.13	-0.06	-0.13

Yellow: $P < 0.05$; Orange: $P < 0.01$; Red: $P < 0.001$

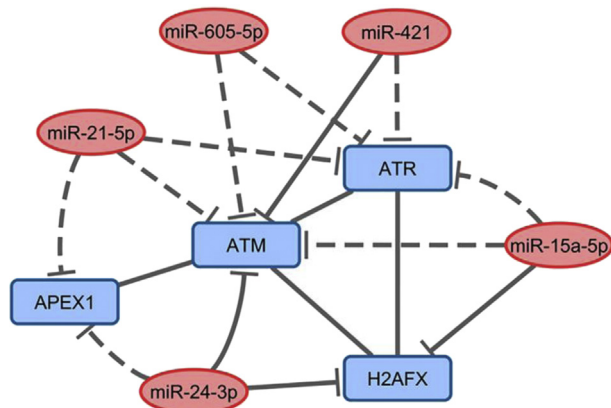


Figure 5 Interaction networks among the proteins encoded by target genes that are regulated by the miRNAs. Rectangles represent the genes, and the ellipses represent the miRNAs. Dashed lines represent predicted interactions, and the continuous line represents validated interactions. *APEX1* = *APE1*; *H2AFX* = *H2AX*.

Moreover, oxidative stress in tumors can promote DNA damage that generate oxidative base damage, AP sites, DNA single strand (SSBs) and double strand-breaks (DSBs).³⁷ In our study, we observed upregulation of the mRNA of *H2AX* in gastric cancer samples, possibly indicating the occurrence of DSBs in the DNA of tumor cells. Furthermore, other studies observed high expression of γ H2AX in several types of cancer,^{40–42} including gastric cancer and gastric precancerous lesions.⁴³ Considering that *H2AX* acts as a key factor in the repair process of damaged DNA and in the maintenance of DNA stability, *H2AX*/ γ H2AX has been proposed as marker for early cancer detection, prognosis, and therapeutics.⁴⁰

H2AX phosphorylation is catalyzed by ATM, ATR and DNA-dependent protein kinase (DNA-PK), which are kinases that belong to the phosphatidylinositol-3-kinase (PI3K) family.⁴⁴ While ATM is a key player in the activation of cell cycle checkpoints in response to radiation-induced DSBs,⁴⁵ ATR is activated during every S-phase in response to a wide range of DNA damage, such as single-stranded DNA (ssDNA) and DNA replication errors.⁴⁶

Although in our study we observed low expression of *ATM* level ($RQ = 0.46$) in gastric cancer samples, no significant

difference was observed. However it has already been described decreased expression of *ATM* in gastric cancer cell line exposed to ionizing radiation,⁴⁷ and in gastric cancer tissues correlated with poor prognosis.⁴⁸ In addition, we also found no alterations in the mRNA expression of *ATR* gene ($RQ = 0.94$) in samples of gastric cancer, but mutations in the *ATR* gene have been observed in colon cancers.⁴⁹ There is only one study in gastric cancer that observed loss of ATR protein expression by immunohistochemical analysis.⁵⁰ Therefore, our study adds information about mRNA expression level of this gene in gastric cancer, indicating the need for further studies on this important gene involved in DNA damage-associated signaling. Furthermore, we observed a strong positive correlation among *ATM*/*ATR*/*H2AX* gene expression levels, further highlighting the role of interactions among these genes in the recognition of DNA damage.

Notably, the complex DNA repair machinery can be regulated by miRNAs.⁵¹ It has been suggested that there is a bidirectional connection between miRNAs and the DDR; while some DDR proteins appear to regulate miRNA expression, miRNAs also influence DDR protein expression.²³ A large number of miRNAs are transcriptionally induced by different doses of DNA-damaging agents, and the level of induction is variable depending on cell type and the nature and intensity of DNA damage.²³

In gastric cancer, upregulation or downregulation of specific miRNAs has been observed,⁵² which may be associated with progression and prognosis of this cancer.⁵³ We evaluated the expression levels of five miRNAs (miR15a, miR-21, miR-24, miR-421, and miR-605) that target some key proteins involved with the DDR (*BCL2*, *CDC25A*, *H2AX*, *ATM*, and *MDM2*)^{51,54} in gastric cancer samples, and we found significantly increased expression of miR-421 and miR-605 in these samples, besides upregulation of miR-21, miR-24, and miR-421 in diffuse-type gastric cancer samples compared to the expression in intestinal-type gastric cancer samples, while no change in the expression of the other miRNAs was observed.

Increased expression of miR-421 has been observed in gastric cancer tissues compared to the expression in adjacent tissues and normal tissues.^{55,56} Therefore, acting as an oncogene to facilitate tumor growth in gastric cancer,⁵⁶ mainly in the early stage of stomach carcinogenesis, and being indicated as an efficient diagnostic biomarker.⁵⁷ Recently, increased expression of miR-421 was also associated with lymph node metastasis and the clinical stage of gastric cancer.⁵⁸ Thus, our study further indicates the occurrence of high expression of this miRNA, mainly in diffuse gastric cancer. In addition, miR-421 showed negative correlations with the expression levels of the *ATM* and *ATR*, their validated and predicted targets, respectively (Fig. 5).

To the best of our knowledge, there are no reports on the expression of miR-605 in gastric cancer. Our study is the first to show significant increase of the miR-605 ($RQ = 1.47$) in the gastric cancer samples. Furthermore, our study identified negative correlations between miR-605 and the mRNA level of the *ATM* and *ATR*, which are considered predicted targets of this miRNA (Fig. 5). These results are interesting and should be confirmed in future studies. Moreover, a previous study from our laboratory showed that

the miR-605 rs2043556 (A > G) polymorphism confers an increased risk for the development of gastric cancer in individuals from the southeastern region of Brazil,⁵⁹ thus indicating a possible involvement of this miRNA in gastric carcinogenesis.

Cumulative evidence indicates that miR-21 plays a significant role in the progression of gastric cancer, suggesting that this miRNA can be used as a candidate for early detection and prognosis prediction^{56,60} and for prognosis of lymph node metastasis.⁶¹ Recently, Gu et al.⁶² reported high levels of miR-21 in human gastric adenocarcinoma cells, supporting the potential of this miRNA as a marker for early diagnosis and target treatment. In the present study, increased expression of miR-21 and miR-24 was observed only in diffuse-type gastric cancer. It has been shown that miR-24 can be potentially used for early diagnosis and as a tumor molecular marker to assess the stage of gastric lesions and lymph node and liver metastasis.⁶³ This miRNA can regulate gastric carcinogenesis by modulating proliferation, migration, and local invasion and has been suggested as a therapeutic target for gastric cancer.⁶⁴ Moreover, miR-24 regulates the histone variant H2AX, which plays a key role in DNA damage signaling via phosphorylation of its C terminus. Lal et al.²⁵ showed that upregulation of miR-24 in postreplicative cells reduces H2AX and thereby renders the cells highly vulnerable to DNA damage induced by genotoxic agents.

It has been suggested that circulating miRNAs exhibit unique profiles for each tumor and histopathological subtype.⁶⁵ Using a mouse model of diffuse-type gastric cancer (DGC) similar to that in humans, Rotkrue et al.⁶⁶ identified circulating miRNAs upregulated in the sera of mice with both early- and advanced-stage DGC compared with controls, suggesting that these miRNAs can serve as noninvasive biomarkers for DGC diagnosis. Accordingly, our results also suggest that miR-21, miR-24, and miR-421 can be potential biomarkers for DGC diagnosis, which needs to be investigated in future studies.

miR-15a has previously been identified as a tumor suppressor that promotes apoptosis and inhibits cell proliferation, and reduced expression of miR-15a is predictive of a poor survival outcome.⁶⁷ The expression of this miRNA has been found to be significantly reduced in gastric cancer,⁶⁸ with ectopic expression of miR-15a being linked to reduced gastric cancer cell growth and invasion.⁶⁹ However, our study found no significant change in the expression level of miR-15a (RQ = 0.78) in gastric cancer samples.

Although half of the gastric cancer samples evaluated in the present study were *H. pylori* positive, we did not observe an association of *H. pylori* infection with the expression levels of the genes and miRNAs investigated. It is possible that the small number of samples obtained after stratification according to the presence and absence of the bacteria may have influenced the statistical analysis. In addition, it is known that *H. pylori* shows a stronger relationship with the early stage of gastric cancer than with the advanced stage,⁷⁰ *H. pylori* infection is often acquired in childhood and persists for years, but the infection tends to disappear with advanced atrophy and intestinal metaplasia, rendering the gastric mucosa inhospitable to the bacterium but increasing the cancer risk even after loss of infection.⁷¹ Moreover, the incubation period of *H. pylori* infection is

longer in advanced cancer patients than in early cancer patients, and this factor may lead to frequent positive-to-negative changes in serology in advanced cancer patients, which is consistent with the weak correlation between advanced gastric cancer and *H. pylori* infection.⁷² Therefore, all of these factors may influence the analysis of the association between *H. pylori* infection and gastric cancer.

Conclusion

In conclusion, our findings show the elevated expression of genes and miRNAs that act in response to DNA damage and repair, such as *APE1*, *H2AX*, *miR-421* and *miR-605* in gastric cancer tissues, which might be involved with the survival of the cells in the tumor microenvironment. However, additional studies are needed to better understand the performance of these genes and miRNAs in response to the treatment of gastric tumor cells with DNA-damaging agents in an attempt to identify possible therapeutic targets for the treatment of this type of neoplasia.

Conflict of interest

The authors declare that they have no conflicts of interest.

Acknowledgments

This study was financed by São Paulo Research Foundation (FAPESP, grant number 2015/21464-0), Coordination for the Improvement of Higher Education Personnel (CAPES, grant number 1460154) and the National Council for Scientific and Technological Development (CNPq, grant number 310120/2015-2).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.gendis.2019.03.007>.

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