Overview of MDM2 and B-RAF Expression in Gastric Lesions

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Abstract

BACKGROUND: Globally, gastric cancer (GC) is the fourth most common cancer and the third cause of cancer-related deaths. Overexpression of MDM2 and B-RAF appeared to be increased in malignancy and associated with poor prognosis in several human tumours, but their role in gastric cancer remains controversial.

AIM: We had investigated the immunohistochemical expression of MDM2 and B-RAF in 136 gastric lesions with/without H. pylori association.

MATERIAL AND METHODS: Studied specimens include chronic gastritis (32), intestinal type GC (70), diffuse GC (22) and gastrointestinal stromal tumours (GIST) (12).

RESULTS: MDM2 expression increased significantly in intestinal GC compared to other groups (p < 0.001), while B-RAF expression increased significantly in GIST compared to other groups (p < 0.001). H. pylori increased expression of MDM2 in intestinal GC cases but did not affect B-RAF expression. MDM2 expression correlated with high grade of tumor differentiation (p < 0.001), deep invasion (p < 0.05), nodal metastases (p < 0.05) and distant metastases (p < 0.05) in intestinal GC, while B-RAF expression did not correlate with TNM stage (p < 0.1).

CONCLUSION: MDM2 up-regulation was more frequent in intestinal GC, while B-RAF up-regulation was more frequent in GIST compared to other groups; MDM2 expression in intestinal GC was correlated with H. pylori association, high grade of differentiation, deep invasion, nodal and distant metastases, meanwhile, B-RAF expression was correlated with high-grade intestinal GC but did not correlate with H. pylori or TNM stage. The possible role of both MDM2 and B-RAF in predicting progression of gastric tumours and prognosis deserves further investigations.

Introduction

Worldwide, Gastric cancer (GC) is the fourth most common cancer in men (8.5%) and the third cause of cancer-related deaths (10.1%). In the female, it is the fourth most common cancer (4.8%) and the third cause of cancer-related deaths (7.2%) [1]. Although the incidence of gastric cancer has gradually decreased over the last half-century, the prognosis of advanced gastric cancer remains poor and gastric cancer-related mortality rates remain unacceptable in many areas [2].

Gastric carcinogenesis is a multistep and multifactorial process. The intestinal type of gastric cancer is often related to environmental factors such as Helicobacter pylori infection, diet, and lifestyle, while the diffuse type is more often associated with genetic abnormalities [3].

The Helicobacter pylori (H. pylori) bacterium is responsible for 5.5% of all infection-associated cancers and is the major cause of gastric cancer in consequence of chronic inflammation [4]. Persistent gastric mucosa inflammation results in chronic gastritis and progresses through a multistep process to gastric atrophy, intestinal metaplasia, dysplasia, and finally carcinoma [5].

In Egypt, infection with H. pylori is common, and acquisition of infection occurs at a very young age [6]. Also, gastric cancer is the 13th most common cancer in men (1.8%) and the 10th cause of cancer-related deaths (2.2%). In the female, it is the 14th most
common cancer (1.5%) and the 11th cause of cancer-related deaths (2.2%). For both sexes, it is the 12th most common cancer (1.6%) and 11th cause of cancer-related deaths (2.2%) [7].

Several biological markers are tested as potential predictors of the gastric carcinoma outcome, and some of them are essential to developing a malignancy. MDM2 (Murine double minute 2) is an oncogene that has been mapped to chromosome 12q13–14 and encodes a 90 kDa cellular oncoprotein. The gene structure on the human chromosome was identified in 1992 [8]. It binds to, and negatively regulates, transactivation of p53 and was then itself found to be a transcriptional target of p53, defining a negative feedback loop of p53 tumour suppressor gene [9]. The MDM2 oncogene played an important role in cancer progression as overexpression of MDM2 in tumour cells induced cell proliferation and inhibits cell apoptosis [10]. Several studies have shown that MDM2 overexpression was associated with poor survival and was a useful predictive factor for poor prognosis in humans with hepatocellular carcinoma and breast carcinomas [11][12].

V-RAF murine sarcoma viral oncogene homolog B1 (B-RAF) is a member of the RAF family of protein kinases which has three members: A-RAF, B-RAF and Raf-1 [13]. All RAF proteins are serine/threonine kinases located in the RAS/RAF/MEK/ERK cascade as downstream effectors of RAS and can phosphorylate and activate MEK, which in turn activates ERK. B-RAF is the most potent activator of MEK [14][15] and is the only one known to be activated by mutation in human cancer [16]. They are mainly found in melanoma, thyroid papillary carcinoma and colorectal tumours with microsatellite instability [17].

In this study, we investigated immunohistochemical expression of MDM2 and B-RAF in chronic gastritis and malignant gastric lesions; and their correlation with H. pylori association, tumour location, grade, and TNM stage in Egyptian patients.

Material and Methods

This study was conducted on 136 archival gastric paraffin blocks from Pathology Department of Theodor Bilharz Research Institute. All samples had been obtained as endoscopic biopsies or gastrectomy specimens. The study protocol was approved by the Ethics committee of Theodor Bilharz Research Institute, for the protection of human subject and adopted by the 18th world medical assembly, Helsinki, Finland (2013).

Our studied lesions were classified into four groups: chronic gastritis: 52 specimens; intestinal GC: 70 specimens; diffuse GC: 22 specimens; GIST: 12 specimens.

Gastric tissue sections were stained by Hematoxylin-eosin for routine diagnosis, grading and staging of tumours. Giemsa stain was used to detect H. pylori in gastric sections.

Immunohistochemistry for MDM2 and B-RAF was performed on tissue sections cut from the paraffin blocks at 4μm onto positively charged slides (Superfrost Plus, Menzel-Glaser, Germany) and stained on an automated platform (Dako Autostainer Link 48) using: anti-human MDM2 monoclonal primary antibodies (Clone MSP14, NeoMarkers, Fremont, CA, USA) and anti-B-RAF pV600E (Spring Bioscience, Pleasanton, CA; purchased from Zytomed Systems, Berlin, Germany) at 1:200 dilution. Heat-induced antigen retrieval was used for 30 min at 97°C in the high-PH EnVision™ FLEX Target Retrieval Solution.

For each setting, positive and negative control slides were included. As a negative control, gastric tissue was processed, but the primary antibodies were not added and instead add non-immune immunoglobulin G (IgG; DAKO, Glostrup, Copenhagen, Denmark). The positive control was a section of liposarcoma for MDM2 and colorectal carcinoma for B-RAF.

All sections were assessed and scored. The sections were examined by using light microscope [Scope A1, Axiol, Zeiss, Germany]. Photomicrographs were taken using a microscope-camera [Axiocam, MRC5, Zeiss, Germany]. All procedures were done at the pathology department of Theodor Bilharz Research Institute, Cairo, Egypt.

Scoring of MDM2 immunostaining was performed semiquantitatively, using digital images and 22-in monitor with hardware calibration capabilities. Staining was considered to be negative (0) if no staining was seen within a tumour, weakly positive (1+) if focal staining was seen, and strongly positive (2+) if there was diffuse staining in more than 80% of tumour cells [18]. Nuclear staining could be detected in very few cases, and the vast majority of positive cases showed only cytoplasmic staining.

The intensity of cytoplasmic immunostaining was scored from zero to 3 (0: no staining, 1: weak, 2: moderate and 3: strong) [19]. Cases with moderate and strong immunostaining were considered positive [20].

We have also counted the percentage of cells with positive expression in 5 successive high power fields.

The immunohistochemical results were analysed using SPSS version 20 (IBM Corporation, Armonk, New York, USA). Data are presented as the mean ± S.D. Two-tailed Student’s t-tests and one-way
ANOVA were used to evaluate the data. Comparison of difference in percentage between groups was evaluated using two-tailed Fischer’s exact test. Differences were considered statistically significant at P < 0.05.

Results

Different studied gastric lesions were more common in males (73.5%) than females (26.5%). The differences were statistically significant (p < 0.05) in cases of chronic gastritis and intestinal GC, while non-significant in cases of diffuse GC and GIST (p > 0.05) (Table 1).

Table 1: Gender in different studied lesions

| Lesion              | Female no. (%) | Male no. (%) | Total no. (%) |
|---------------------|----------------|--------------|---------------|
| Chronic gastritis   | 23 (101)       | 32 (138)     | 55 (233)      |
| Intestinal GC       | 24 (66.7)      | 46 (46)      | 70 (51.5)     |
| Diffuse GC          | 6 (16.7)       | 16 (16)      | 22 (16.2)     |
| GIST                | 2 (5.6)        | 10 (10)      | 12 (8.8)      |
| Total               | 36              | 100          | 136           |

GC: gastric cancer, GIST: gastrointestinal stromal tumor.

Endoscopically, cases of chronic gastritis represented usually as diffuse mucosal lesions, cases of intestinal and diffuse GC represented as fungating or ulcerative lesions and usually located at the gastro-esophageal junction (GEJ) or pylorus, while GIST cases represented as mass lesions. No significant differences were found considering endoscopic appearance or location of studied gastric lesions (Table 2).

Table 2: Endoscopic appearance and location of studied gastric lesions

| Lesion                   | Chronic gastritis no. (%) | Intestinal GC no. (%) | Diffuse GC no. (%) | GIST no. (%) | Total no. (%) |
|--------------------------|---------------------------|-----------------------|-------------------|-------------|---------------|
| Diffuse                  | 32 (100)                  | 0                     | 0                 | 32 (100)    | 32 (100)      |
| Fungating                | 0                         | 64 (91.4)             | 20 (90.9)         | 0           | 84 (61.8)     |
| Mass                     | 0                         | 0                     | 0                 | 86 (66.7)   | 86 (66.7)     |
| Ulcer                    | 0                         | 0                     | 0                 | 8 (5.9)     | 8 (5.9)       |
| Wall thickening          | 0                         | 0                     | 4 (2.9)           | 33 (33.3)   | 34 (2.9)      |
| Unavailable              | 32 (100)                  | 56 (86)               | 14 (63.6)         | 12 (100)    | 114 (83.8)    |
| Carcix                   | 0                         | 0                     | 0                 | 0           | 2 (1.5)       |
| Diffuse                  | 0                         | 0                     | 0                 | 0           | 2 (1.5)       |
| Fundus                   | 0                         | 2 (5.7)               | 0                 | 0           | 2 (5.7)       |
| GEJ                      | 0                         | 0                     | 4 (18.2)          | 0           | 4 (18.2)      |
| Pyramid                  | 0                         | 0                     | 4 (18.2)          | 0           | 4 (18.2)      |
| Total                    | 32 (100)                  | 70 (100)              | 22 (12.3)         | 136 (100)   | 230 (100)     |

GC: gastric cancer, GEJ: gastro-esophageal junction, GIST: gastrointestinal stromal tumor.

Cases of intestinal GC and diffuse GC showed the significantly higher percentage of H. pylori positivity compared to chronic gastritis and GIST (p < 0.05) (Table 3).

All studied chronic gastritis and GIST cases were negative for MDM2 expression. MDM2 positivity was identified in 31.4% of intestinal GC and 9.1% of diffuse GC, with the statistically significant difference between intestinal GC and other groups (p < 0.001) as well as between diffuse GC and both chronic gastritis and GIST (p < 0.05).

Table 3: Association between H. pylori and different studied lesions

| Lesion              | Chronic gastritis no. (%) | Intestinal GC no. (%) | Diffuse GC no. (%) | GIST no. (%) | Total no. (%) |
|---------------------|---------------------------|-----------------------|-------------------|-------------|---------------|
| Positive            | 12 (92.3)                 | 84 (61.8)             | 16 (63.6)         | 12 (100)    | 114 (83.8)    |
| Negative            | 0                         | 20 (25.5)             | 8 (3.9)           | 0           | 28 (21.2)     |
| Total               | 32 (100)                  | 104 (100)             | 24 (100)          | 12 (100)    | 154 (100)     |

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Mean percentage of MDM2 positive cells and intensity of expression were significantly higher in intestinal GC followed by diffuse GC compared to chronic gastritis and GIST cases (p < 0.001), while mean percentage of B-RAF positive cells and the intensity of expression were significantly higher in GIST followed by intestinal GC compared to chronic gastritis and diffuse GC cases (p < 0.001) (Table 5).

Table 4: MDM2 and B-RAF immunoreactivity in different lesions

| Lesion              | MDM2 | B-RAF |
|---------------------|------|-------|
| Chronic gastritis   | 0.50 | 0.36  |
| Intestinal GC       | 0.94 | 0.08  |
| Diffuse GC          | 0.19 | 0.09  |
| GIST                | 0.74 | 0.10  |
| Total               | 0.50 | 0.00  |

For statistical purposes, we separately studied the relation between clinic-pathological features of intestinal GC cases and immunohistochemical expression results of MDM2 and B-RAF.

As regards the endoscopic appearance of intestinal GC; fungating lesions exhibited a higher percentage of MDM2 positive cells and MDM2 intensity of expression, while ulcerative lesions...
exhibited a higher percentage of B-RAF positive cells and B-RAF intensity of expression. However, these relations did not reach a significant difference between examined groups (p > 0.1) (Table 6).

Table 6: Relationship between the expression of MDM2 and B-RAF with the Endoscopic appearance of intestinal GC

| Endoscopic appearance | MDM2 | B-RAF |
|-----------------------|------|-------|
| Percent | Intensity | Percent | Intensity |
| (no. Of lesions) | Mean ± Std. Error of mean | Mean ± Std. Error of mean | Mean ± Std. Error of mean |
| Fungating (64) | 8.94 ± 1.38 | 0.97 ± 0.06 | 15.38 ± 3.33 | 0.72 ± 0.10 |
| Ulcer (6) | 4.00 ± 1.26 | 0.07 ± 0.21 | 16.07 ± 9.01 | 1.00 ± 0.37 |
| P value | P > 0.1 | P > 0.1 | P > 0.1 | P > 0.1 |

Considering the tumour location, the mean percentage of MDM2 positive cells and intensity of expression were significantly higher in tumours with the diffuse location, followed by GEJ compared to other sites (p < 0.001). On the other hand, the mean percentage of B-RAF positive cells and intensity of expression were higher in tumours at GEJ followed by fundus compared to other sites; the difference was statistically significant for B-RAF intensity score (p < 0.001) but non-significant for B-RAF per cent (p > 0.1) (Table 7).

Table 7: Relationship between the expression of MDM2 and B-RAF with anatomical site of intestinal GC

| Anatomical site | MDM2 | B-RAF |
|----------------|------|-------|
| Percent | Intensity | Percent | Intensity |
| (no. Of lesions) | Mean ± Std. Error of mean | Mean ± Std. Error of mean | Mean ± Std. Error of mean |
| Undefined (56) | 5.18 ± 0.50 | 0.79 ± 0.06 | 14.71 ± 3.51 | 0.68 ± 0.10 |
| Cardia (2) | 5.00 ± 0.00 | 1.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Diffuse (2) | 40.00 ± 0.00 | 3.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Fundus (4) | 17.50 ± 7.22 | 1.00 ± 0.00 | 20.00 ± 11.38 | 1.00 ± 0.58 |
| GEJ (4) | 20.60 ± 12.08 | 2.00 ± 0.58 | 45.60 ± 144.30 | 2.50 ± 0.29 |
| Pylorus (2) | 15.00 ± 0.00 | 1.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| P value | P < 0.001 | P < 0.001 | P < 0.001 | P < 0.001 |

Regarding \( H. pylori \) association, the mean percentage of MDM2 positive cells and intensity of expression were higher in \( H. pylori \)-associated intestinal GC compared to \( H. pylori \) non-associated tumours, without a statistically significant difference (p > 0.1). On the contrary, mean percentage of B-RAF positive cells and intensity of expression were higher in \( H. pylori \) non-associated intestinal GC, without statistical significance (p > 0.1) (Table 8).

Table 8: Relationship between the expression of MDM2 and B-RAF with \( H. pylori \) association of intestinal GC

| \( H. pylori \) (no. Of lesions) | MDM2 | B-RAF |
|-----------------------------|------|-------|
| Percent | Intensity | Percent | Intensity |
| Mean ± std error of mean | Mean ± std error of mean | Mean ± std error of mean |
| Positive (44) | 9.41 ± 1.85 | 1.00 ± 0.11 | 14.55 ± 3.97 | 0.73 ± 0.13 |
| Negative (26) | 7.00 ± 3.1 | 0.85 ± 0.07 | 17.08 ± 5.36 | 0.77 ± 0.14 |
| P value | P > 0.1 | P > 0.1 | P > 0.1 | P > 0.1 |

Table 9: Relationship between the expression of MDM2 and B-RAF with intestinal GC grade of differentiation

| Grade (no. Of lesions) | MDM2 | B-RAF |
|-----------------------|------|-------|
| Percent | Intensity | Percent | Intensity |
| Mean ± Std. Error of mean | Mean ± Std. Error of mean |
| High (12) | 21.83 ± 5.77 | 1.00 ± 0.34 | 25.83 ± 8.28 | 1.17 ± 0.37 |
| Low (58) | 7.76 ± 0.52 | 0.83 ± 0.05 | 13.34 ± 3.33 | 0.86 ± 0.09 |
| P value | P > 0.0001 | P < 0.01 | P > 0.1 | P > 0.05 |

In addition, mean percentage of B-RAF positive cells and the intensity of expression were higher in high-grade intestinal GC compared to low-grade tumours; the difference was statistically significant for B-RAF intensity score (p < 0.05) and non-significant for B-RAF per cent (p > 0.1) (Table 9); moreover, these parameters were higher in T3 intestinal GC compared to T2 and T4 without statistical significance (p > 0.1) (Table 8). Also, B-RAF parameters were higher in N1 stage compared to N0 and N3 and in M0 compared to M1 without statistical significance (Table 10).

Table 10: Relationship between the expression of MDM2 and B-RAF in intestinal GC with TNM stage

| Item (no. Of lesions) | MDM2 | B-RAF |
|-----------------------|------|-------|
| Percent | Intensity | Percent | Intensity |
| Mean ± Std. Error of mean | Mean ± Std. Error of mean |
| T | | |
| 2 (12) | 2.50 ± 0.75 | 0.50 ± 0.15 | 13.83 ± 7.62 | 0.67 ± 0.22 |
| 3 (36) | 9.21 ± 2.06 | 1.00 ± 0.12 | 18.84 ± 4.85 | 0.79 ± 0.13 |
| 4 (20) | 10.80 ± 1.76 | 1.00 ± 0.00 | 10.10 ± 3.76 | 0.70 ± 0.18 |
| P value | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 |
| N | | |
| 0 (26) | 5.14 ± 1.09 | 0.57 ± 0.10 | 7.07 ± 3.40 | 0.50 ± 0.12 |
| 1 (26) | 8.15 ± 1.29 | 1.00 ± 0.00 | 22.46 ± 5.67 | 0.92 ± 0.15 |
| 3 (16) | 15.00 ± 4.52 | 1.50 ± 0.22 | 18.88 ± 7.64 | 0.88 ± 0.27 |
| P value | P > 0.05 | P > 0.0001 | P > 0.05 | P > 0.05 |
| M | | |
| 0 (52) | 8.04 ± 1.47 | 0.85 ± 0.08 | 17.00 ± 3.67 | 0.85 ± 0.12 |
| 1 (18) | 9.89 ± 2.06 | 1.22 ± 0.15 | 11.11 ± 5.05 | 0.44 ± 0.12 |
| P value | P > 0.1 | P > 0.05 | P > 0.1 | P > 0.05 |

Each subscript letter denotes a subset of gender categories whose column proportions do not differ significantly from each other at the 0.05 level.

Each subscript letter denotes a subset of lesion categories whose column proportions do not differ significantly from each other at the 0.05 level.

Discussion

Gastric cancer is still a serious public health problem in the world. The high mortality rate that is seen globally is mainly due to the advanced stage at...
diagnosis with the availability of few biomarkers for early detection [21].

Endoscopically, our studied data sheet showed that cases of chronic gastritis usually represented as diffuse mucosal lesions, cases of intestinal and diffuse GC represented as fungating or ulcerative lesions, while GIST cases represented as mass lesions. Anatomically, no significant difference was detected considering the location of studied gastric lesions. Anatomical site of most of our studied lesions had not been mentioned. However, GEJ was the most frequent site mentioned for GCs; and this could be related to gastro-oesophageal reflux.

In the present work, male predominance was reported which is similar to the worldwide trend (2:1) [22], as 73.5% of gastric lesions belonged to males compared to 26% belonged to females, with incidence 2.8:1. A percentage lower than ours reported by Gaballah et al., [23] and Darwish et al., [24] who reported male to female ratio of 1.2:1 and 1.3:1 respectively.

Wade et al., [30] and Li and Lozano [10] reported that MDM2 oncogene played an important role in cancer progression and MDM2 overexpression in tumour cells induced cell proliferation inhibited cell apoptosis. We found MDM2 positivity in 31.4% of intestinal GC cases. Gunther et al., [31] found MDM2 expression in 45% of intestinal GCs. However, Ye et al., [32] reported a much higher per cent, as they detected MDM2 immunopositivity in 70.4% of their GC cases. Moreover, intestinal GC exhibited a significantly higher percentage of MDM2 positive cells (8.51%) and higher intensity of expression compared to other groups. This matches the findings of Gunther et al., [31] and Nakajima [33] who detected MDM2 positivity in 10% and 7.76% of gastric cancer cells respectively. Shen et al., [34] stated that MDM2 expressed at higher levels in GC tissues than in non-cancerous gastric mucosa. On the contrary, Busuttil et al., [21] observed negligible levels of MDM2 staining in GC samples. Variable results between studies may be attributed to different risk factors promoting to gastric cancer including H. pylori, obesity, tobacco smoking, red meat, a high-salt diet, alcohol, and low socioeconomic status, genetic polymorphisms, the age of cancer onset and gender.

On the other hand, B-RAF was expressed in
all GIST specimens that showed a significantly higher mean percentage of $B$-$RAF$ positive cells (86.67%) and higher intensity of expression compared to other groups. This matches with findings of Holstein et al., [35] who observed $B$-$RAF$ expression in all GIST cases in more than 80% of cells. On the contrary, several other studies reported a much smaller percentage of $B$-$RAF$ positivity in GIST than ours [36] [37] [38] as they detected $B$-$RAF$ mutation in 7%, 3.8% and 3.5% of GISTs respectively. Furthermore, intestinal GC cases showed significantly higher expression of $B$-$RAF$ (higher number of positive cases, the percentage of positive cells and intensity of expression) compared to chronic gastritis and diffuse GC. Many previous studies reported the presence of a $B$-$RAF$ mutation in patients with gastric adenocarcinoma [27] [39] [40].

Considering cases of intestinal type GC, no statistically significant difference was achieved when comparing fungating and ulcerating intestinal GC for parameters of $MDM2$ and $B$-$RAF$ expression (mean percentage of positive cells and intensity of expression). Tumours with diffuse location and at GEJ showed significantly higher mean percentage of $MDM2$ positive cells and $MDM2$ intensity of expression. On the other hand, tumours at GEJ and fundus showed non-significantly higher mean percentage of $B$-$RAF$ positive cells and significantly higher $B$-$RAF$ intensity of expression. To our knowledge, no other studies demonstrated $MDM2$ or $B$-$RAF$ expression about endoscopic appearance or anatomical site of intestinal GC.

In the present study, $MDM2$ parameters were non-significantly higher in $H.~pylori$-associated intestinal GC than in $H.~pylori$ non-associated ones. This goes with many previous studies reporting that $H.~pylori$ infection was associated with higher expression of $MDM2$ in intestinal metaplasia and gastric carcinoma [33] [41] [42]. Furthermore, Kodama et al., [43] reported that successful eradication of $H.~pylori$ dramatically reduced $MDM2$ levels. On the contrary, $B$-$RAF$ parameters were non-significantly higher in $H.~pylori$-non-associated intestinal GC than in $H.~pylori$-associated ones; however, Sabry et al., [27] found a significant positive relationship between the qPCR of $H.~pylori$ and quantitative $B$-$RAF$ in GC cases.

As regards different grades of differentiation in intestinal GC, we found a statistically significant higher percentage of $MDM2$ positive cells and non-significant higher percentage of $B$-$RAF$ positive cells in high-grade tumours compared to low-grade ones. This goes with findings of Sabry et al., [27] who detected a significant positive correlation between grades of GC and qPCR of $B$-$RAF$.

Our current results showed an increase in $MDM2$ expression parameters with increasing depth of invasion, the presence of distant metastases and lymph node metastases. This matches with Ye et al., [32] results which reported that $MDM2$ expression was associated with depth of invasion, lymph node metastases and distant metastases. Sepideh et al., [44] found a direct correlation between lymph node metastases and $MDM2$ staining intensity; meanwhile, they did not find a remarkable correlation between $MDM2$ expression and nodal involvement.

As regards $B$-$RAF$ expression parameters in intestinal GC, no significant differences were achieved with different tumour stages, different stages of lymph node metastasis and state of distant metastases. These findings match results of other previous studies which did not find a relationship between $B$-$RAF$ expression and histopathological variables of GC [45] [46] [47].

In conclusion, we found that: (1) $MDM2$ up-regulation was more frequent in intestinal GC compared to other groups, while $B$-$RAF$ up-regulation was more frequent in GIST compared to other groups; (2) $H.~pylori$ induces $MDM2$ up-regulation in intestinal GC; (3) In intestinal GC cases, $MDM2$ expression was correlated with high grade of differentiation, deep invasion, nodal and distant metastases, meanwhile, $B$-$RAF$ expression was correlated with high-grade tumours but had no association with TNM stage. The possible role of both $MDM2$ and $B$-$RAF$ in predicting progression of gastric tumours and prognosis deserves further investigations.

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References

1. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F. Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer. 2015; 136(5):E359–E386. https://doi.org/10.1002/ijc.29210 PMid:25220842

2. Ferro A, Peleteiro B, Malvezzi M, Bosetti C, Bertuccio P, Levi F, Negri E, La Vecchia C, Lunet N. Worldwide trends in gastric cancer mortality (1980–2011), with predictions to 2015, and incidence by subtype. Eur J Cancer. 2014; 50:1330–44. https://doi.org/10.1016/j.ejca.2014.01.029 PMid:24650579

3. Monograph of the incidence of Gastric carcinoma in Middle East: Middle East Cancer Consortium (MECC). Available at: http://www.cancer.gov/cancertopics/pdq/treatment/gastric/HealthProfessional/page4#Reference1. [accessed on 2014 Jan 10]

4. Parkin DM. The global health burden of infection-associated cancers in the year 2002. International Journal of Cancer. 2006; 118(12):3030–3044. https://doi.org/10.1002/ijc.21731
5. Poteca T, Poteca A, Sajin M, Comanescu M. Biological prognostic parameters in gastric carcinomas. Chirurgia (Bucur). 2014; 109(3):347–54.

6. Mohammad MA, Hussein L, Coward A, Jackson SJ. Prevalence of Helicobacter pylori infection among Egyptian children: impact of social background and effect on growth. Public Health Nutr. 2008; 11(3):230–36. https://doi.org/10.1017/S1368980007000481

7. GLOBOSCAN 2012: Estimated cancer incidence, mortality and prevalence worldwide 2012. Population fact sheets, Egypt, available at http://globoscann.fr/ Pages/fact_sheets_population.aspx

8. Oliner JD, Kinzler KW, Melzer PS, George D, Vogelstein B. Amplification of a gene encoding a p53-associated protein in human sarcomas. Nature. 1992; 358:80–83. https://doi.org/10.1038/358080a0

9. Moll UM and Petrenko O. The MDM2-p53 interaction. Mol Cancer Res. 2003; 1:1001–1008. PMid:14770283

10. Li Q, Lozano G. Molecular pathways: targeting Mdm2 and Mdm4 in cancer therapy. Clin Cancer Res. 2013; 19:34–41. https://doi.org/10.1158/1078-0432.CCR-12-0053

11. Rahman MA, Salajegheh A, Smith RA, Lam AK. B-Raf mutation: a key player in molecular biology of cancer. Exp Mol Pathol. 2013; 95:336–42. https://doi.org/10.1016/j.yexmp.2013.10.005

12. Keime J, Hetu G, Arpin S, Falardeau M, Tremblay S. The Mdm4 gene and its role in cancer. J Cell Physiol. 2012; 227(9):3464–71. https://doi.org/10.1002/jcp.241954

13. Emuss V, Garnett M, Mason C, Marais R. Mutations of C-RAF are rare in human cancer because C-RAF has a low basal kinase activity compared with B-RAF. Cancer Res. 2005; 65:9719–26. https://doi.org/10.1158/0008-5472.CAN-05-1683

14. Wellbrock C, Karasarides M, Marais R. The RAF proteins take centre stage. Nat Rev Mol Cell Biol. 2004; 5:875–85. https://doi.org/10.1038/nrm14198

15. Dhomne N, Marais R. New insight into BRAF mutations in cancer. Curr Opin Genet Dev. 2007; 17:31–9. https://doi.org/10.1016/j.gde.2006.12.005

16. Davies H, Bignell GR, Cox C, et al. Mutations of the RAF genes in human cancer. Nature. 2002; 417:949–954. https://doi.org/10.1038/nature00766

17. Turbin DA, Cheang MC, Badik JD, Gelmon KA, Yorida E, De Luca A, Nielsen TO, Huntsman DG, Gilks CB. MDM2 protein expression is a negative prognostic marker in breast carcinoma. Mod Pathol. 2006; 19(1):69–74. https://doi.org/10.1038/modpathol.3800484

18. Bossmuller H, Fischer A, Pham DL, Fehm T, Capper D, v. Deimling A, Bonzheim I, Staebler A, Fend F. Detection of the B-RAF V600E mutation in serous ovarian tumors: a comparative analysis of immunohistochemistry with a mutation-specific monoclonal antibody and allele specific PCR. Hum Pathol. 2013; 44:329–35. https://doi.org/10.1016/j.humpath.2012.07.010

19. Hess S, Pasterneck H, Ihle MA, Merkelbach-Bruse S, Heikötter B, Hartmann W, Trautmann M, Gevensleben H, Böttner R, Schildhaus HU, Wardelmann E. Clinicopathological and molecular features of a large cohort of gastrointestinal stromal tumors (GISTs) and review of the literature: BRAF mutations in KIT/PDGFRα wild-type GISTs are rare events. Hum Pathol. 2017; 62:206–214. https://doi.org/10.1016/j.humpath.2017.01.005

20. Busuttil RA, Zapparoli GV, Haupt S, Fennell C, Wong SQ, Pang JM, Takeno EA, Mitchell C, Di Costanzo N, Fox S, Haupt Y, Dobrovic A, Boussioutas A. Role of p53 in the progression of gastric cancer. Oncotarget. 2014; 5(23):12016–12026. https://doi.org/10.18632/oncotarget.2434

21. Northern Ireland Cancer Registry. Cancer incidence and mortality cancer research united kingdom (online) Available: http://info.cancerresearchuk.org/. (Accessed January, 2012).

22. Amieva M and Peek RM Jr. Pathobiology of Helicobacter pylori-induced gastric cancer. Gastroenterology. 2015; 150:64–78. https://doi.org/10.1053/j.gastro.2015.09.004

23. Lázaro MJ, Lario S, Casalots A, Sanfeliu E, Boix L, García-Iglesias P, Sánchez-Delgado J,Montserrat A, Bella-Cueto MR, Gallach M, Sanfeliu I, Segura F, Calvet X. Real-time PCR improves Helicobacter pylori detection in patients with peptic ulcer bleeding. PLoS One. 2011; 6(5):e20009. https://doi.org/10.1371/journal.pone.0020009

24. Wu WK, Cho CH, Lee CW, Fan D, WuK, Yu J, Sung JJ. Dysregulation of cellular signaling in gastric cancer. Cancer Lett. 2010; 295:144–53. https://doi.org/10.1016/j.canlet.2010.04.025

25. Wadie M, Li YC, Wahl GM. MDM2, MDMX and p53 in oncogenesis and cancer therapy. Nat Rev Cancer. 2013; 13:83–96. https://doi.org/10.1038/nrc3430

26. Ramírez-Lázaro MJ, Lario S, Casalots A, Sanfeliu E, Boix L, García-Iglesias P, Sánchez-Delgado J,Montserrat A, Bella-Cueto MR, Gallach M, Sanfeliu I, Segura F, Calvet X. Real-time PCR improves Helicobacter pylori detection in patients with peptic ulcer bleeding. PLoS One. 2011; 6(5):e20009. https://doi.org/10.1371/journal.pone.0020009

27. Sabry D, Ahmed R, Abdalla S, Fathy W, Eldemery A, Elamir A. Braf, Kras and Helicobacter pylori epigenetic changes-associated chronic gastritis in Egyptian patients with and without gastric cancer. World Journal of Microbiology and Biotechnology. 2016; 32(6):92. https://doi.org/10.1007/s11274-016-2048-x

28. Amieva M and Peek RM Jr. Pathobiology of Helicobacter pylori-induced gastric cancer. Gastroenterology. 2015; 150:64–78. https://doi.org/10.1053/j.gastro.2015.09.004

29. Wu WK, Cho CH, Lee CW, Fan D, WuK, Yu J, Sung JJ. Dysregulation of cellular signaling in gastric cancer. Cancer Lett. 2010; 295:144–53. https://doi.org/10.1016/j.canlet.2010.04.025

30. Wadie M, Li YC, Wahl GM. MDM2, MDMX and p53 in oncogenesis and cancer therapy. Nat Rev Cancer. 2013; 13:83–96. https://doi.org/10.1038/nrc3430

31. Gunther T, Schneider-Stock R, Hackel C, Kasper HU, Pross M, Hackelsberg A, Lippert H, Roessner A. Mdm2 gene amplification in gastric cancer correlation with expression of Mdm2 protein and p53 alterations. Mod Pathol. 2000; 13:621–626. https://doi.org/10.1038/modpathol.3800107

32. Ye Y, Li X, Yang J, Miao S, Wang S, Chen Y, Xia X, Wu X, Zhang J, Zhuo Y, He S, Tan Y, Yang F, Li G, Rhee OD, Zhou J. MDM2 is a useful prognostic biomarker for resectable gastric cancer. Cancer Sci. 2013; 104:590–598. https://doi.org/10.1111/cas.12111

33. Nakajima N, Ito Y, Yokokawa K, Uno A, Kinukawa N, Nemoto N and Moriyama M. The Expression of Murine Double Minute 2 (MDM2) on Helicobacter pylori-infected Intestinal Metaplasia and Gastric Cancer. Journal of clinical biochemistry and nutrition. 2009; 44:196-202. https://doi.org/10.3164/jcbn.2009.08-254

34. Shen J, Niu W, Zhou M, Zhang H, Ma J, Wang L, Zhang H. MicroRNA-410 suppresses migration and invasion by targeting MDM2 in gastric cancer. PLoS One. 2014; 19(9):e104510.

35. Hostein I, Faur N, Primois C. Amplification of a gene encoding a p53-associated protein in human sarcomas. Nature. 1992; 358:80–83. https://doi.org/10.1038/358080a0

36. Mohammad MA, Hussein L, Coward A, Jackson SJ. Prevalence of Helicobacter pylori infection among Egyptian children: impact of social background and effect on growth. Public Health Nutr. 2008; 11(3):230–36. https://doi.org/10.1017/S1368980007000481
36. Agaimy A, Terracciano LM, Dimhofer S, Tornillo L, Foerster A, Hartmann A, Bihl MP. V600E BRAF mutations are alternative early molecular events in a subset of KIT/PDGFRα wild-type gastrointestinal stromal tumours. J Clin Pathol. 2009; 62(7): 613-6. https://doi.org/10.1136/jcp.2009.064550 PMid:19561230

37. Martinho O, Gouveia A, Viana-Pereira M, Silva P, Pimenta A, Reis RM, Lopes JM. Low frequency of MAP kinase pathway alterations in KIT/PDGFRA wild-type GISTs. Histopathology. 2009; 55(1):53-62. https://doi.org/10.1111/j.1365-2559.2009.03323.x PMid:19614767

38. Daniels M, Lurkin I, Pauli R, Erbstößer E, Hildebrandt U, Hellwig K, Zschille U, Lüders P, Krüger G, Knolle J, Stengel B. Spectrum of KIT/PDGFRα/BRAF mutations and Phosphatidylinositol-3-Kinase pathway gene alterations in gastrointestinal stromal tumors (GIST). Cancer letters. 2011; 312(1):43-54. https://doi.org/10.1016/j.canlet.2011.07.029 PMid:21906875

39. Lee SH, Lee JW, Soung YH, Kim HS, Park WS, Kim SY, Lee JH, Park JY, Cho YG, Kim CJ, Nam SW, Kim SH, Lee JY, Yoo NJ. BRAF and KRAS mutations in stomach cancer. Oncogene. 2003; 22(44):6942-6945. https://doi.org/10.1038/sj.onc.1206749 PMid:14534542

40. Kim TM, Jung SH, Kim MS, Baek IP, Park SW, Lee SH, Lee HH, Kim SS, Chung YJ, Lee SH. The mutational burdens and evolutionary ages of early gastric cancers are comparable to those of advanced gastric cancers. J Pathol. 2014; 234:365-74. https://doi.org/10.1002/path.4401 PMid:25042771

41. Moradi MT, Salehi Z, Aminian K, Hashtchin AR. Helicobacter pylori infection and MDM2 SNP309 association with gastric cancer susceptibility. Genetic testing and molecular biomarkers. 2013; 17(11):794-8. https://doi.org/10.1089/gtmb.2013.0173 PMid:24010568

42. Fenouille N, Puissant A, Tichet M, Zmiriak G, Abbe P, Mallavialle A, Rocchi S, Ortonne JP, Deckert M, Ballotti R, Tartare-Deckert S. SPARC functions as an anti-stress factor by inactivating p53 through Akt-mediated MDM2 phosphorylation to promote melanoma cell survival. Oncogene. 2011; 30:4887-4900. https://doi.org/10.1038/onc.2011.198 PMid:21689397

43. Kodama, M, Fujikata T, Murakami K, Okimoto T, Sato R, Watanabe K, Nasu M. Eradication of Helicobacter pylori reduced the immunohistochemical detection of p53 and MDM2 in gastric mucosa. J Gastroenterol Hepatol. 2005; 20:941–946. https://doi.org/10.1111/j.1440-1746.2005.03323.x PMid:15946145

44. Sepideh S, Mohammadreza JN, Ali D, holamreza TP, Samira G. Study of the Murine Double Minute 2 status in patients with gastric and colorectal carcinomas and its correlation with prognostic factors. Indian J Pathol Microbiol. 2012; 55:192-5. https://doi.org/10.4103/0377-4929.97866 PMid:22771642

45. Corso G, Velho S, Paredes J, Pedrazzani C, Martins D, Milanezi F, Pascale V, Vindigni C, Pinheiro H, Leite M, Marrelli D, Sousa S, Carneiro F, Oliveira C, Roviello F, Seruca R. Oncogenic mutations in gastric cancer with microsatellite instability. Eur J Cancer. 2011; 47:443–451. https://doi.org/10.1016/j.ejca.2010.09.008 PMid:20937558

46. Stella G, Rojas Limpe F, Barone C, Falcone A, Di Fabio F, Martoni A, Lamba S, Ceccarelli C, Siena S, Bardelli A, Pinto C. KRAS and BRAF mutational status as response biomarkers to cetuximab combination therapy in advanced gastric cancer patients. Journal of Clinical Oncology. 2009; 27(Suppl. 15):e15503.

47. Sasao S, Miyama T, Tanaka S, Yoshihara M, Yasui W, Chayama K. Clinicopathologic and genetic characteristics of gastric cancer in young male and female patients. Oncol Rep. 2006; 16:11–15. https://doi.org/10.3892/or.16.1.11