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Role of Gastrokine 1 in Gastric Cancer

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1. Introduction

Gastric cancer (GC) has high incidence (> 1.000.000 new cases/year) and mortality rate in several countries and is still one of the most frequent and lethal (> 600.000 dead/year) neoplasia with an average survival of five years (less than 20%) (Pisani et al., 1990; Lands et al., 1998). It is already well known that infection of gastric antrum mucosal with the bacterium *Helicobacter pylori* is the cause of the chronic inflammation that leads to intestinal-type gastric cancer in the majority of the cases. The *H. pylori* infection is widespread but only a small number of the total population of infected individuals might eventually develop adenocarcinoma (around 3/10,000 individuals per year or 2.1% for lifetime infection) (Correa & Piazuelo, 2008). Risk factors influencing the outcome of *H. pylori*–associated pathology include bacterial cytotoxic heterogeneity, diet, and geographic differences. The phenomenon of decreased gastric cancer incidence in Africa compared with other regions where *H. pylori* is endemic (Holcombe, 1992) is probably due to the different diet of these populations compared to the western countries. This discrepancy has been partially attributed to helminth co-infection that likely modifies the characteristic proinflammatory type 1 T-helper 1 cell response, to a T-helper 2–predominant response (Whary et al., 2005), typified by the release of non-inflammatory cytokines and reduced incidence of *H. pylori*–associated glandular atrophy, an early marker of cancer development. The identification of novel genes regulated by *H. pylori* in vivo, particularly those contributing to these early stages of gastric cancer, would facilitate improved understanding of the differential susceptibility to this pathogen. The different susceptibility among individuals to *H. pylori* infection is still not yet defined. Some works, however, suggested that the polymorphisms in host genetic factors like the proinflammatory cytokines interleukin-1, interleukin-8, and tumor necrosis factor may play a relevant role (El-Omar et al., 2003).

The evolution of intestinal tumours is characterized by a progression of several sequential steps that starts with gastritis and then progresses to mucosal atrophy (atrophic gastritis), intestinal metaplasia, dysplasia and carcinoma with subsequent metastatic dissemination (Correa, 1992, 1995). The diffuse-type has instead a poorer prognosis and develops through unknown genetic and morphological events from normal gastric epithelium. No preceding steps have been identified in the pathogenesis of diffuse carcinoma other than the chronic gastritis. The pathogenesis of gastric cancer remains poorly understood although it is

evident that several environmental factors, such as *H. pylori* infection can be one of the causes leading to this disease. In fact, the risk to develop gastric cancer is increased in patients with *H. pylori* infections probably as the result of a combination of genetic and environmental factors in which the infection by *H. pylori* is of particular relevance, especially when the inflammation involves the gastric body region with respect to the antrum (Correa, 1995; Goldstone et al., 1996; Nabewera & Logan, 1999). Generally, this condition is associated to different degrees of atrophy and alterations of the secretor function that, in the long term, became associated to gastric carcinoma (Forman et al., 1991; Parsonnet et al., 1997; Watanabe et al., 1998).

Diffuse adenocarcinoma shows an increased propensity for intra and transmural spread and is therefore associated with a poorer prognosis. Unfortunately, the histological classification of an individual gastric adenocarcinoma is not clear-cut with a tumour often comprising a mixture of intestinal and diffuse tissue types. Under these considerations, we think that there is an urgent necessity to dispose of an efficient tool for the detection of early stage gastric cancer like the identification of highly sensitive and specific biomarkers that will aid disease diagnosis and ensure early clinical intervention, thereby preventing mortality and reducing morbidity (Boussioutas & Taupin, 2001). Since most of GC (around 73%) is developed at antrum/pylorus, proteins secreted by antrum/pylorus mucosa might play a critical role in maintaining normal gastric mucosa structure and function.

2. Expression of gastrokine 1 in normal and malignant tissues

From a comparative proteomic analysis of human specimens of gastric mucosa from patients with and without *H. pylori* infection, we have shown the differential expression of several up- and down-regulated proteins (Rippa et al., 2007). One of these proteins, gastrokine 1 (GKN1), a novel stomach-specific protein also known as 18 kDa antrum mucosa protein (AMP-18 or foveolin) (Oien et al., 2004), was highly expressed in normal tissues and markedly down-regulated in samples derived from *H. pylori* infected patients (Nardone et al, 2007). To elucidate the biological function of GKN1, we have characterized its expression in normal and malignant gastric tissues also by immunohistochemical technique. The etiology of most gastric cancers is multifactorial and the pathogenesis is believed to involve a multi-step process in which the normal gastric epithelium evolves through intermediate pre-malignant lesions (intestinal metaplasia and dysplasia) leading to adenocarcinoma. These investigations were based on the hypothesis that GKN1 expression in pre-malignant gastric tissues and in gastric carcinomas is altered in terms of quantity (reduced amount or complete absence of the protein), quality (altered protein size), and distribution (abnormal cell-type and/or subcellular localization) compared to normal gastric tissues.

2.1 The gastrokine family

GKN1 belongs to a family of genes encoding stomach-specific secreted proteins consisting of 3 known members: gastrokine 1 (GKN1) (Martin et al., 2003), gastrokine 2 (GKN2) (Du et al., 2003), and gastrokine 3 (GKN3) (Menheniott et al., 2010). Although their mode of action remains unclear, the recent demonstration of a GKN2/trefoil factor (TFF1) heterodimer in gastric mucus suggests that gastrokines may regulate the extracellular function of TFFs (Westley *et al.*, 2005).

2.2 Gastrokine 1

GKN1 (also named as AMP18), a member of BRICHOS superfamily, is secreted by antrum mucosa (Oien et al., 2004). *GKN1* gene of about 6 kb was reported to be located at 2p13 and contains 6 exons (Martin et al., 2003). Bioinformatics analysis suggested the secretory signal peptide with 20-amino acids presented at the N-terminal and the processed protein consisted of 165-amino acids after cleavage. Despite several asparagine residues in the N-terminal domain, none seems to be the consensus site of traditional glycosylation (Martin et al., 2003). The *GKN1* gene encodes a small protein of 185 amino acids containing a N-terminal signal peptide (Yoshikawa et al., 2000). The BRICHOS domain consists of about 100 amino acids and it has been found in several unrelated proteins associated with major human diseases (Sanchez-Pulido et al., 2002) like BRI2, related to familial British and Danish dementia; chondromodulin-I (ChM-I), linked to chondrosarcoma; surfactant protein C (SP-C), associated with respiratory distress syndrome; and gastrokine 1 (also known as CA11, AMP18 & foveolin) (Shiozaki et al., 2001; Martin et al., 2003), linked to gastric cancer (Sanchez-Pulido et al., 2002; Shiozaki et al., 2001). Evaluation of GKN1 expression at mRNA and protein level, by RT-PCR and immunhistochemistry (IHC), was found only in stomach, gastric antrum, but not in esophagus, duodenum, or intestine (Oien et al., 2004).

2.3 Evaluation of GKN1 expression levels

The evaluation of GKN1 expression in normal and malignant cells was performed according the following strategy.

2.3.1 Patients enrolment and clinical characterization

In this study a population of 28 patients with gastric cancer (20 intestinal and 8 diffuse types) was enrolled. Interview focusing on diet and lifestyle habits, family history and other toxic environmental factors was performed. A family history positive for gastric cancer was also carefully verified. The biopsy specimens were taken during an upper video-endoscopy in the gastric body or collected immediately after surgical resection and soon delivered for diagnosis and tissue sampling. Specimens were used for culture test, histological and immunohistochemical tests and for molecular approach.

Gastric biopsy specimens were collected from normal subjects and patients with erosive gastritis, peptic ulcer or gastric cancer, either infected or not infected with *H. pylori*. None of the subjects had taken antibiotics, proton pump inhibitors or nonsteroidal antinflammatory drugs during the preceding 3 months. At least 65 gastric biopsy specimens were taken during upper endoscopy: one pinch was used to measure the urease activity (rapid urease test), and one pinch was used for a histological examination of *H. pylori* infection using hematoxylin-eosin stain. Three pinches and four pinches were used respectively for protein and RNA extraction. 36 patients (55%) showed to be negative for *H. pylori* infection and among these, 31 had normal mucosa while the remaining 5 had minimal infiltration of lymphomonocytes in the lamina propria. *H. pylori* infection was detected in 29/65 patients suffering from active gastritis (25 mild-moderate and 4 severe); 10 patients showed mild or moderate atrophy that was associated in 4 cases with focal antral complete IM. Anti-*H. pylori* and anti CagA IgG antibodies were not detected in any of the *H. pylori*-negative subjects but were found in 29 (100%) and 21 (72%) *H. pylori*-positive patients, respectively.

2.3.2 Proteomic analysis

In order to identify proteins differently expressed in human gastric mucosa without and with *H. pylori* infection, proteins extracted from biological specimens were analyzed by 2D electrophoresis in the pH range 3-10. Protein samples (100 μg) were focused on IPG strips and then separated by 12% SDS-PAGE. Proteins were stained with Comassie Brilliant Blue G-250. Comassie-stained protein spots were excised from 2-D gels and treated with 10 ng/μl of trypsin and digested overnight at 37°C according to Schevechencko et al., 1996. The peptide mixture obtained was then analyzed using a MALDI-TOF mass spectrometer Voyager DE™ PRO (Applied Biosystems), operating in positive-ion reflectron mode. Protein identification was achieved using MALDI mass spectral data for a data-base search against the NCBInr database using the Mascot search algorithm, a parameter representing the reliability of protein identification (see also http://www.matrixscience.com/) (Nardone et al., 2007).

Figure 1, shows the protein profiles observed in human gastric mucosa specimens. GKN1 was identified by MALDI-TOF analysis of tryptic fragments (Rippa et al., 2007). On 2D gels, the GKN1 protein spot was found in all samples from *H. pylori* non-infected patients and it was generally drastically reduced in several samples of *H. pylori* infected tissues. The amino acid sequence of GKN1 isolated from gel covered about 38% of the entire protein sequence. Mature GKN1 did not contain the first 20 amino acid leader peptide as confirmed by the amino acid sequence of the N-terminal performed by Edman's degradation. As expected from the isoelectric point of the mature protein (pI = 5.32), the migrating position of GKN1 on the 2D gel was toward acidic pH.

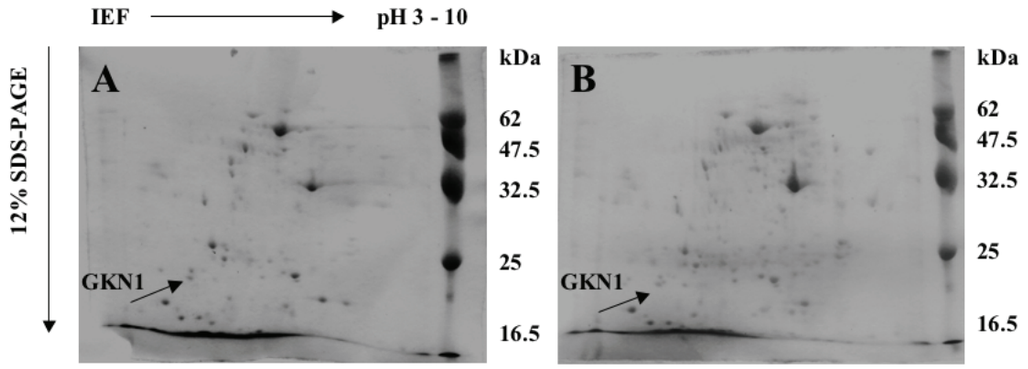

Fig. 1. Bidimensional gel electrophoresis of proteins extracted from gastric endoscopic mucosal samples. A) *H. pylori*-negative sample, B) *H. pylori*-positive sample. The first-dimension (isoelectric focusing, IEF) was performed using the Ettan IPGphor (Amersham Biosciences) equipped with 7 cm strip holders.

To analyze more quickly the expression levels of GKN1 in our collection of samples from patients with and without *H. pylori* infection (45 cases), we first tried to use mono-dimensional SDS-PAGE followed by staining of the gel with comassie blue. Figure 2 shows

the position of the GKN1 stained band after electrophoresis at around 18 kDa however this approach resulted only qualitative and not quantitative.

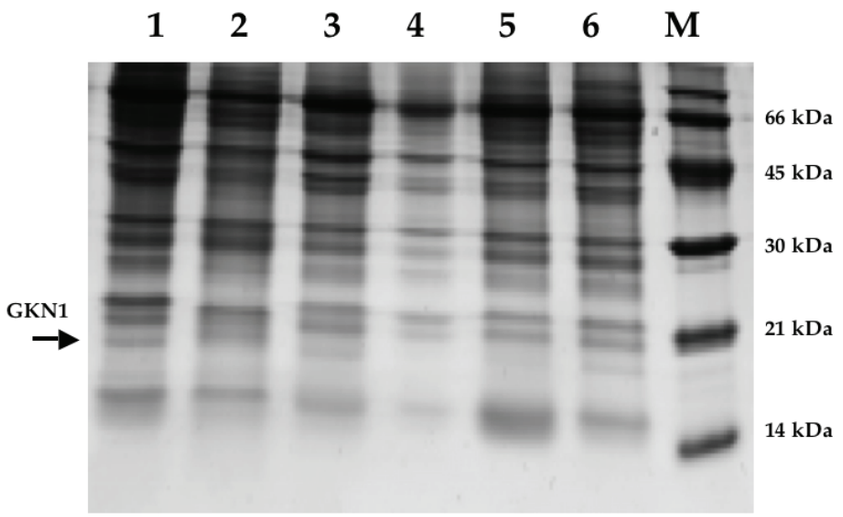

Fig. 2. Monodimensional gel electrophoresis of proteins extracted from gastric endoscopic mucosal samples. SDS-PAGE (14%) of proteins extracted from gastric endoscopic mucosal samples from *H. pylori*-negative patient (Lanes 1, 3, 5) and *H. pylori*-positive patient (Lanes 2, 4, 6). M, size markers.

2.3.3 Western blotting analysis of GKN1 expression

To detect more precisely the GKN1 expression levels, we adopted the Western blot procedure using a specific anti-GKN1 antibody (Abnova) raised against the intact protein. The analysis was carried out starting from three biopsies. Specimens were mixed with proteases inhibitor (Roche), homogenized and equivalent amounts of protein (10 μg) were separated by electrophoresis on sodium dodecyl sulfate-12% polyacrylamide gels. After electrophoresis, the proteins were electroblotted to a nitrocellulose membrane (Immobilon P^{QS}). GKN1 protein was identified using a specific rabbit polyclonal antibody (Abnova, diluted 1/50000). Visualization was obtained with an ECL kit (Pirce).

As reported in Figure 3, the reduction in expression levels of GKN1 was not always observed in all patients with *H. pylori* associated gastritis but only in about 20% of samples. The evaluation has been based on the expression levels of β-tubulin. In all cases, the reduction of GKN1 levels was about two times that of non-infected samples. Densitometric evaluation of the bands showed a lower level in *H. pylori*-positive patients compared to the *H. pylori*-negative subjects (average: 0.19 ± 0.02 vs 0.44 ± 0.07, $p < 0.005$). This finding is in agreement to what observed also in another proteomic analysis regarding other proteins/enzymes differentially expressed by *H. pylori* infection (Baek et al., 2004).

We also analyzed the expression of GKN1 in human specimens derived from patients with gastric cancer. With respect to the non-tumoral area, it was observed that GKN1 expression was consistently down regulated or absent in tumoral area (Nardone et al., 2008). The 28 patients with Gastric Cancer (GC) (20 intestinal and 8 diffuse) showed the following characteristics: the intestinal type was well differentiated in 4, moderately differentiated in 8

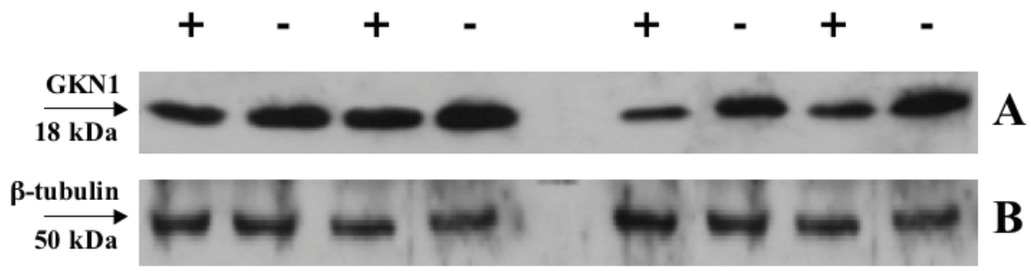

Fig. 3. Western blot analysis of human gastric mucosa samples. About 10 μg of protein extracts from samples of gastric mucosa from non-infected patient (-) and patients with *H. pylori* infection (+) were separated on SDS-PAGE and blotted against commercial antibody anti-GKN1 (A) and anti-β-tubulin (B) (control).

and poorly differentiated in the remaining 8 cases, while four were in the early stage and the remaining 16 in the advanced stage. Diffuse type GC was poorly differentiated and advanced in all cases. The non tumoural areas of intestinal type GC showed a variable degree of gastric atrophy with diffuse IM, instead, the peritumoural areas of diffuse type GC showed a variable degree of non-dysplastic inflammation. *H. pylori* infection was not revealed in all cases of tumoural areas. It is interesting to point out, however, that *H. pylori* infection was detected, in the peritumoral areas, in 6 cases (4 early and 2 advanced). Anti-*H. pylori* IgG antibodies were detected in 16/20 patients with intestinal histotype GC and in 6 out of 8 with diffuse type GC. Anti-CagA antibodies were detectable in 17/28 cases (14 intestinal and 3 diffuse histotype). Using Western blot (WB) analysis, GKN1 protein expression was not detected in any of the tumoural areas but was revealed instead in non-tumoural areas (Fig. 4).

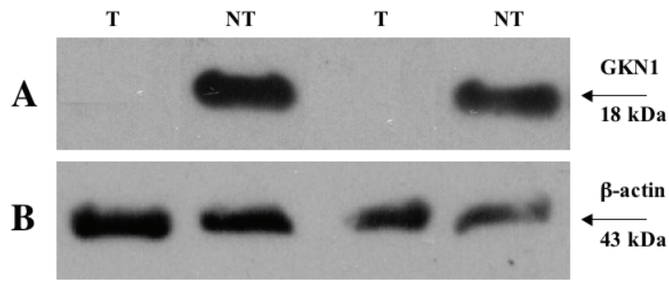

Fig. 4 Western blot analysis of GKN1 expression in human gastric specimens. About 20 μg of protein extracts from samples tumoral area (T) and non-tumoral area (NT) were separated on SDS-PAGE and blotted against commercial antibody anti-GKN1 (A) and anti--β-actin (B) (control).

2.3.4 Immunohistochemical analysis of GKN1 expression

The Immunohistochemical evaluation of GKN1 on surgical specimens was performed on 7-micron sections of frozen unfixed gastric tissues. The percentages of immunoreactive cells

was scored as follows: 0-5% = negative; 5-25% = low staining; 25-50% = moderate staining; >50% = intense staining. The results obtained, summarized in Fig. 5 showed the absence of GKN1 in tumor tissues (our actual collection of about 30 cases). In fact, the GKN1 staining was intense in normal gastric tissues, low or completely negative in intestinal metaplasia and gastric cancer, respectively (Fig. 5) (Rippa et al., unpublished results).

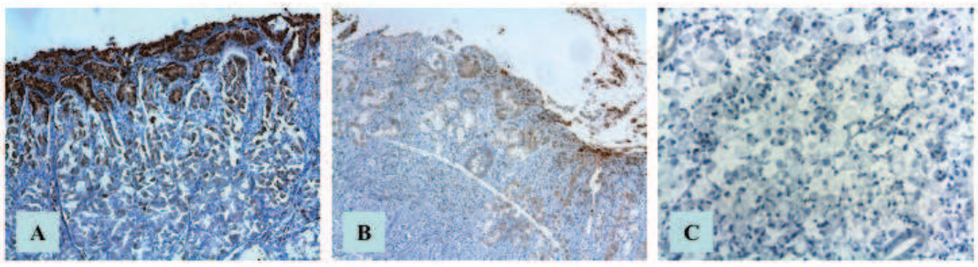

Fig. 5. Representative immunohistochemical images of surgical specimens from: A) Normal gastric tissue (LSAB, orig. magn. X200); B) Intestinal metaplasia (LSAB, orig. magn. x100); C) Intestinal type gastric cancer (LSAB, orig. magn. X200).

In addition, the hystopathological aspect of the tumor specimens correlated to the GKN1 expression with tumor subtype. This observation suggested that GKN1 could play an important role in normal gastric function thus and strongly suggested that GKN1 could be a biomarker for gastric cancer (Yoshihara et al., 2006; Moss et al., 2008).

3. Functional role of GKN1

Regarding the functional role of GKN1, there are two questions that are still to be clarified. The first important question is whether GKN1 is secreted from gastric cells. Although data from bioinformatics analysis and the presence in the protein of a leader peptide sequence, there is not a conclusive answer to this point. If GKN1 is secreted from the gastric mucosa cells, other questions will raise up concerning the sequence of the secreted protein and how it interacts with its target cells. The second question is whether and how the GKN1 protein modulates the behavior of gastric cells. To this aim, we started to elucidate the role of the protein in the processes of proliferation and signalling transduction analyzing the expression of GKN1 in gastric carcinoma cells and its effects on apoptosis.

3.1 Over-expression of GKN1 in gastric cancer cell lines

The knowledge about the biological function of GKN1 is not completely clarified. What is known at the moment is that GKN1 appears to be important in maintaining mucosal integrity and could play a role in cell proliferation and differentiation. In fact, GKN1 might have a protective effect by increasing accumulation of specific tight and adherens junction proteins and also protecting their loss after injury (Walsh-Reitz et al., 2005). The presence in GKN1 of the BRICHOS domain might explain its protective role. In fact, the BRICHOS is

present also in another gastric protein GKN2, known also TFIZ1, because of its homology to GKN1. This protein is involved in the binding of tumor suppressor proteins such as the trefoil protein 1 (TFF1). Such interaction could be important in the regulation of the integrity of the mucosa (Bruce et al., 2005; Otto et al., 2006; Baus-Loncar et al., 2007).

The strong down-regulation of GKN1 expression in patients with *H. pylori* infection (Nardone et al., 2007) its absence in gastric cancer (Nardone et al., 2008; Moss et al., 2008) and its absence in human gastric cancer cell lines (AGS and MKN28) (Motoyama et al., 1986; Segal et al., 1996; Oien et al., 2004) are factors that suggest the participation of GKN1 in the host response to *H. pylori* and suggest also that GNN1 can act as gastric tumor suppressor gene (Du et al., 2003). Regarding this point, since GKN1 is highly down-regulated or absent in gastric cancer, one possible explanation of its disappearance could be linked to epigenetic events or loss of heterozygosity, as it was observed for other tumor suppressor genes such as TFF1 (Carvalho et al., 2002). However, as recently reported by Yoon et al., 2010, no significative mutations nor methylation in the GKN1 gene in gastric tumors were found, thus the down-regulation of GKN1 it is likely not depending by epigenetic events.

Toback et al., (2003) proposed that GKN1 could have some mitogenic effects on intestinal epithelial cells (IEC) as compared with EGF 5. Shiozaki et al., 2001, instead found that this protein was able to inhibit proliferation of a human carcinoma cancer cell line (MKN28) cells after transfection. From this finding it emerges that it is important to define the role of GKN1 in the modulation of inflammatory damage or tumorigenesis in the gastric mucosa. Attempts to reduce cancer survival rates include strategies directed towards the identification of specific targets inducing apoptosis in tumor cells.

3.1.1 Evaluation of transient expression of GKN1 in gastric cancer cell

AGS or MKN28 cancer cells (Motoyama, *et al.*, 1986) were transfected with the eukaryotic expression vectors pcDNA 3.1 containing the entire GKN1 cDNA. Cells were grown in 5% CO2 at 37° in DMEM (Dulbecco's modified Eagle's medium) and transfected using lipophectamine 2000. After transfection, cell growth and apoptosis was evaluated by cytofluorimetry.

The overexpression of GKN1 in MKN28 or AGS cells, with respect to control cells, reduced cell growth (Rippa et al., 2010). A similar effect was also reported by Shiozaki et al., 2001, who observed a reduction in MKN28 colony formation in cells transfected with CA11 gene with respect to control cells. Also GKN3, the new gastrokine found in mammals, strongly attenuated the growth of GKN3-overexpressing MKN28 cells (Menheniott et al., 2010). Because tissue repair is determined by many signals coming from the local environment, central to this process is the commitment of gastric cells to undergo apoptosis, survive, or proliferate, following inflammation. Therefore, gastric epithelial cell apoptosis could be influenced by GKN1 during the inflammatory process. The Fas-Fas ligand (FasL) system has been recognized as the major pathway for the induction of apoptosis in a variety of human normal and neoplastic cells (Itoh & Nagata, 1993; Suda et al., 1993; Nagata 1996). The Fas antigen (CD95, APO-1) belongs to a conserved family of membrane receptors known as the tumor necrosis factor receptor or TNFR family (Itoh et al., 1991). Therefore, overexpression of GKN1 could activate Fas, normally expressed at extremely low levels at least in MKN28 cells (Osaki et al., 2001). In fact, the expression of Fas receptor, evaluated by flow cytometry (Figure 6) Western blotting and RT-PCR showed a significant increase.

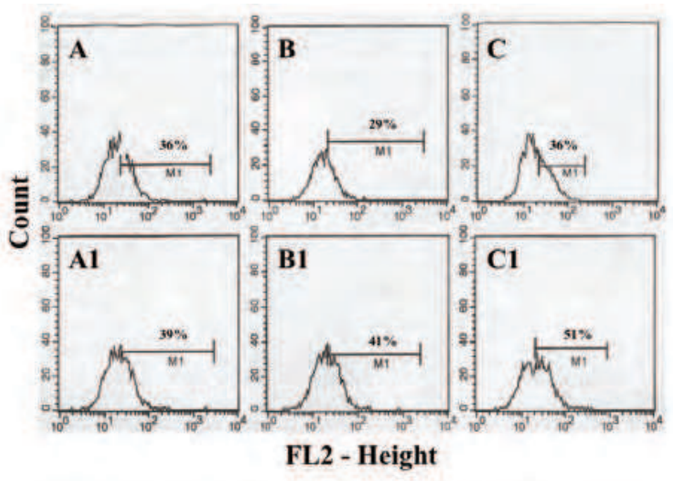

Fig. 6. Flow cytometric analysis of apoptosis in AGS cells. Representative flow cytometry of cells transfected with empty pCDNA 3.1, lipophectamine and GKN1 before (A, B, C) and after (A1, B1, C1) incubation with a monoclonal antibody (IgM) anti-FasL.

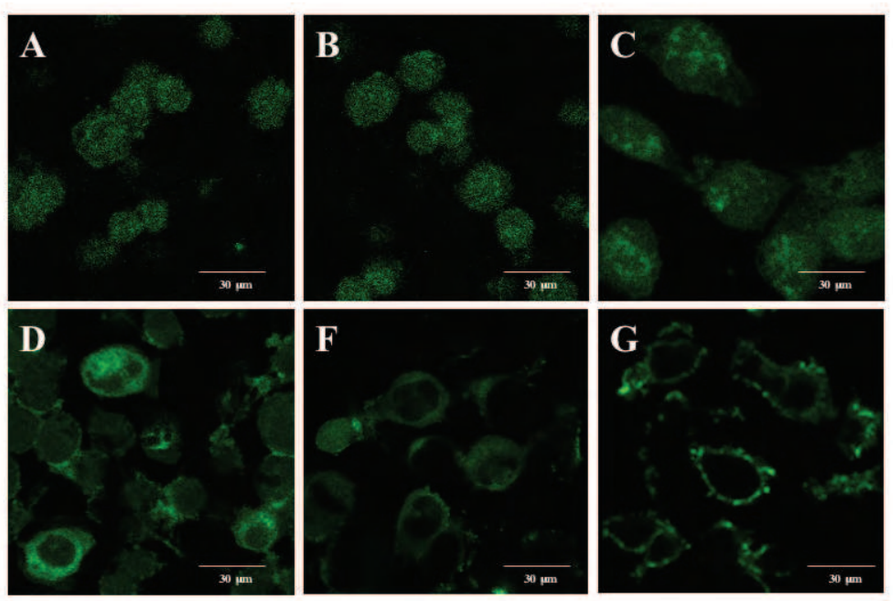

Fig. 7. Confocal microscopy of AGS cells. AGS cells were transfected with pCDNA 3.1 containing the GKN1 cDNA and after 48 hours from transfection cells were analyzed at confocal microscopy in the presence of antibody anti-GKN1 and anti-Fas. A) Untransfected cells. B) Cells treated with lopophectamine. C) Non-premealized transfected cells after incubation with anti-GKN1 antibody. D) Premeabilized transfected cells after incubation with anti-GKN1 antibody. E) Non-premeabilized untransfected cells after incubation with anti-Fas antibody. F) Premeabilized transfected cells after incubation with anti-Fas antibody.

Fas expression was also analyzed by confocal microscopy. As shown in Fig. 7, compared to the control cells, cells treated with lipophectamine and cells transfected with GKN1 showed, exposure of the permeabilized transfected cells to anti-GKN1 antibody showed a gain in the fluorescent signal that indicated mainly a cytoplasmic distribution of GKN1 protein whereas, the exposure of the transfected cells to anti-Fas antibody showed with respect to untransfected cells a gain in the fluorescent signal that was localized mainly at the level of cell membrane.

Therefore, the increase of Fas expression observed in cells transfected with GKN1 appeared to be specific as also demonstrated by the increase of the Fas mRNA transcription evaluated by RT-PCR (not shown). Fas-mediated apoptosis in gastric cells was also described upon infection by *H. pylori*, however in this case other apoptotic factors are involved as well. These include TRIAL and its receptor subtypes (Yang, et al. 2003; Martin et al., 2004). Because the binding of Fas ligand (FasL) to the Fas antigen results in the transduction of a cytolytic signal into the cell followed by apoptosis, cells overexpressing GKN1 showed an increase in apoptosis that is mainly due to the exposure of the MKN28 cells to FasL with respect to that observed in the absence of FasL. In addition, when FasL binds to Fas, intracellular death caspases are activated, resulting in apoptotic demise of the cell (Chen et al., 1999). Also in cells transfected with GKN1, we observed the proteolytic activation of caspase-3, normally present as a 32 kDa inactive precursor (Zou et al., 1997), as evaluated by Western blot and fluorimetric assay, was observed thus indicating that GKN1-transfected cells were signaled to die (Nicholson et al., 1995; Schlegel et al., 1996; Wang et al., 1996).

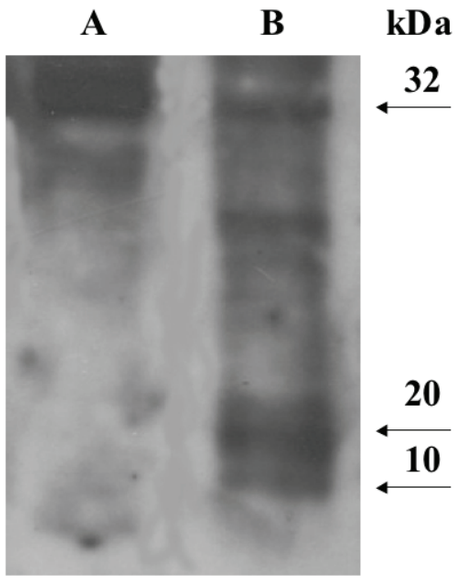

Fig. 8. Western blot of caspase-3. Evaluation of caspase-3 activation in AGS cells before (A) and after (A) 48 h transfection with GKN1 and exposure to FasL.

In addition, Yoon et al., 2011, found that transfection of GKN1 in AGS cells showed activation of apoptosis related proteins, including cleaved caspase 3, caspase 8 and PARP. The GKN1-induced apoptosis is suppressed by the presence of caspase-3 and caspase-8 inhibitors.

4. Conclusion

We have evaluated the expression of GKN1 in normal and unhealthy tissues and found that the GKN1, both at protein and mRNA levels, is down-regulated in human specimens from *H. pylori* infected gastric mucosa or absent in gastric GC tissues or cells. GKN1 is instead normally present in normal tissues and cells or in the peritumoral area of GC. Also the immunohistochemical analysis of human specimens (normal tissues, intestinal metaplasia, GC) showed a progressive decrease of GKN1 expression thus suggesting that GKN1 could be a good biomarker candidate for gastric cancer progression.
In addition, our studies on the overexpression of the protein in gastric cancer cell lines showed that GKN1 could act as modulator of apoptosis and suggested a possible role of GKN1 as a tumor suppressor gene. The modulation of apoptotic signals by GKN1 during the early stages of acute gastric injury may have a profound influence on tissue repair and be instrumental in determining the individual host response.

5. Acknowledgements

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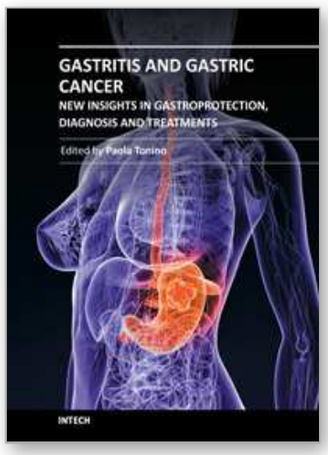

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This book is a comprehensive overview of invited contributions on Helicobacter pylori infection in gastritis and gastric carcinogenesis. The first part of the book covers topics related to the pathophysiology of gastric mucosal defense system and gastritis including the gastroprotective function of the mucus, the capsaicin-sensitive afferent nerves and the oxidative stress pathway involved in inflammation, apoptosis and autophagy in H. pylori related gastritis. The next chapters deal with molecular pathogenesis and treatment, which consider the role of neuroendocrine cells in gastric disease, DNA methylation in H. pylori infection, the role of antioxidants and phytotherapy in gastric disease. The final part presents the effects of cancer risk factors associated with H. pylori infection. These chapters discuss the serum pepsinogen test, K-ras mutations, cell kinetics, and H. pylori lipopolysaccharide, as well as the roles of several bacterial genes (cagA, cagT, vacA and dupA) as virulence factors in gastric cancer, and the gastrokine-1 protein in cancer progression.

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