IL-6 and IL-11 as Markers for Tumor Aggressiveness and Prognosis in Gastric Adenocarcinoma Patients without Mutations in Gp130 Subunits

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Abstract

Background & Aims. A point mutation (gp130Y757F/Y759F) was identified as being responsible for aberrant activation of gp130 in mice and associated with gastric adenocarcinoma induction. As a result, we investigated the possible role of key point mutations in Tyr from IL6ST exon 17 that encode for the catalytic domain of gp130, and of its respective activators (IL-6 family member cytokines) in human gastric cancer initiation and development. **Method**. DNA, protein and plasma from 51 patients with gastric adenocarcinoma have been used in exploring gp130 status. We used sequencing analysis of IL6ST exon 17 in order to identify possible mutations that would lead to constitutive active forms of the receptor. The levels of gp130 activators (IL-6, IL-11, LIF) were analyzed by ELISA in plasma and mucosa of patients with gastric adenocarcinoma. **Results**. Sequencing analysis did not identify mutations in gp130 key positions (Y759, Y767, Y814, Y905 and Y915). An increased IL-6 and IL-11 level in gastric mucosa was observed, correlated with staging, indicating these cytokines as gp130 activators in tumor epithelial cell. Those variations were consistent with increased IL-6 level in plasma. Furthermore, IL-6, but not IL-11 showed a significant correlation with patient's survival time, suggesting that tissue and plasma concentration of IL-6 might be a marker of tumor aggressiveness with prognostic value. **Conclusions**. According to our results, no mutations were detected in gp130 key positions in human gastric adenocarcinoma samples. However, gp130 activation may occur due to the increased level of IL-6 and IL-11 cytokines detected that can become valuable biomarkers.

*The first three authors equally contributed to this work.

Key words

Introduction

Gastric cancer is the second most common cause of cancer-related death in the world. Compared with other cancers, morbidity rates are quite high (~700,000/year), making gastric cancer the second most common cause of cancer death worldwide after lung cancer. Furthermore, a substantial proportion of gastric cancer patients who have undergone curative surgery develop recurrent disease [1-3]. Ten-year relative survival varies worldwide between 7.7% and 23.0% and the only therapeutically scheme applied consists of combined chemo-radiation therapy followed by surgical resection. Therefore, the identification of potential molecular markers as new targets for gastric adenocarcinoma therapy may be useful [4, 5].

Although the molecular mechanisms involved in development of gastric cancer are still unclear, the chronic inflammation triggered by the Gram-negative bacterium *Helicobacter pylori*, which colonizes the epithelium of the gastric mucosa is an important factor in this pathogenesis. Many studies show that *Helicobacter pylori* infection could double the risk of gastric cancer. Accumulation of (epi-) genetic alterations in *p53, tff, E-cadherin, Cox2*, and genes encoding components of the TGF-β/Smad signaling cascade are also linked to gastric cancer [6-9]. More recent studies showed that persistent activation of the latent STAT3 promotes the growth and survival of gastric cells and is associated with increased gastric angiogenesis. This protein has the capacity to induce expression of genes that promote angiogenesis (e.g., VEGF), cell-cycle progression (e.g., cyclin D1), and cell survival (e.g., Bcl-xL, survivin) and could be a prognostic factor for poor survival of gastric cancer patients [10, 11].

In the last decade, the international scientific community has achieved a better understanding of the molecular events leading to tumorigenesis, by identifying mutations in

cytokine receptors and in regulatory proteins of intracellular signaling. Data published in the last years, suggest that abnormal signaling through gp130 (IL6ST) protein might play a role in gastric epithelium hyperplasia. Gp130 is a transmembrane protein that forms one subunit of type I cytokine receptors within the IL-6 receptor family, which interacts with Janus kinases and induces an intracellular signal following ligand-receptor binding.

Ernst et al, using mouse genetic models in which the *gp130Y757F/Y759F* mutation was bred onto either an IL-6– or IL-11α receptor–null background, showed that IL-11 is responsible for the aberrant activation of mutant receptor gp130 in gastric tumorigenesis [6, 12-14]. The critical Y757 residue in gp130 (pY757 in mouse, pY759 in human) is a binding site for the tyrosine phosphatase Shp2 which upon phosphorylation, mediates activation of the Ras/ERK and PI3K/Akt pathways [15-17].

The normal gp130 protein is able to activate both the ERK and STAT3 pathways but in the presence of the Y757F mutation, Shp2 phosphorylation and subsequent activation of the proapoptotic Ras/Erk and PI3K/AKT pathways do not occur. Instead, phosphorylation of the four C-terminal tyrosine residues in the gp130 protein results in STAT activation. Consequently, a molecular mechanism was proposed whereby gastric tumorigenesis is initiated through the occurrence of an imbalance between Shp2/Ras/ERK pathway target and gastric tumor suppressor gene *tff1*. IL-11 was proposed as the primary cytokine that induced gp130-mediated gastric tumorigenesis and highlights the potential oncogenic implication of STAT3 in the progression of inflammation-associated gastric tumors [13, 18].

Here, we investigate the possible role of key point mutations in Tyr from IL6ST exon 17 that encode for the catalytic domain of gp130 and of IL-6 and IL-11 in gastric cancer initiation and development.

Methods

Sample collection, processing and storage

Plasma and tumoral and adjacent non-malignant tissue fragments were obtained from 51 patients (33 men and 18 women, mean age 62 years) during surgery at the Center of General Surgery and Liver Transplantation of the Fundeni Clinical Institute and Clinical Emergency Hospital Floreasca. The study was approved by the local research ethical committee, and all participants gave informed written consent. None of the patients had received preoperative chemotherapy or radiotherapy. Tumor tissue for experiments was obtained from tumor mass core, and the corresponding non-malignant tissue was selected from the resection rim (≥ 4 cm from gross tumor), verified by a pathologist and classified as R0.

Each tumor was assigned a histological type and a depth grading of infiltration; gastric adenocarcinoma samples were classified according to the American Joint Committee on Cancer (AJCC) TMN (tumor, node, and metastasis) staging system. The plasma and all tumoral and corresponding non-

tumoral gastric tissues collected after surgical resection were frozen and stored at –80°C until the assay was performed. Finally, for cytokine level measurements in plasma, the study included a control group that consisted of 24 healthy, cancer-free volunteers with no inflammatory condition (median age 57 years; range: 35–70 years). No more than two freeze-thaw cycles were allowed for each sample.

DNA purification and sequencing analysis

Genomic DNA was isolated from tissue by standard treatment with SDS and EDTA in the presence of 200 μg/mL proteinase K (Sigma), followed by NaCl extraction and isopropanol precipitation [19].

Exon 17 of IL6ST gene was analyzed by PCR amplification of 20 genomic DNA (30 ng each) and direct sequencing of the PCR products using IL6ST VariantSEQr kit (Applied Biosystem), according to manufacturer protocol. PCR products were purified using the QIAquick Gel Extraction Kit (QIAGEN), sequenced using BigDye Terminator v3.1 chemistry and purified with the BigDye® XTerminator TM Purification Kit (Applied Biosystem) according to manufacturer protocols.

Purified DNA fragments were sequenced in duplicate using the corresponding forward PCR primers. Samples were subsequently sequenced in the reverse direction to confirm the results, using the reverse PCR primers. Analysis was performed on an Applied Biosystems 3130 Genetic Analyzer.

Measurement of cytokine level

Level of IL-6, IL-11 and LIF cytokines expression in plasma, malignant, and adjacent non-malignant tissue extracts from the 51 patients was measured using commercially available ELISA kits (Quantikine, R&D Systems). 100 mg of each snap-frozen tissue sample were transferred to different tubes containing 1 ml of T-PER (Tissue protein extraction reagent, Pierce) with Complete Mini Protease Inhibitor Cocktail tablets (Roche). The tissues fragments were homogenized on ice and then centrifuged at 9,000 × g for 10 minutes at 4°C. Supernatants were collected and analysed for total protein concentrations using Coomassie Protein Assay Kit (Pierce – Thermo Scientific). The tissue extracts were diluted with T-PER reagent to a final protein concentration of 1mg/ml. Finally, tissue lysates and plasma were subjected to cytokine detection by ELISA assay according to the manufacturer's instructions. The dynamic range of the tests was 0 – 2000 pg/ml with $R^2 = 0.999$ (IL-6), $R^2 = 0.996$ (IL-11) and $R2\ 2 = 0.998$ (LIF).

Helicobacter pylori and *CagA* detection

Detection of *H. pylori* in gastric samples was performed for PCR amplification with primers specific to *H. Pylori urease A* (*ureA*) gene. The primer sequence used (5'-CGTTGTCTGCTTGCCTATCA-3', sense and 5'-CGGCTCACACTTCCATTTCT-3', antisense) amplifies a 203 bp fragment. To detect *cagA*, the primer set 5'-AGCAAAAAGCGACCTTGAAA -3' (sense) and 5'-AGCCAATTGCTCCTTTGAGA-3' (antisense) was used to amplify a 172 bp fragment. Each primer set (*ureA* and *cagA*)

was used in an independent PCR reaction in a final volume of 25 μL containing 12.5μL PCR Master Mix (Fermentas), 9.5 μl water, 4 pmol of each primer set, and 200 ng of DNA, under the following conditions: for *ureA* gene 35 cycles of denaturation at $94^0C/30$ s, annealing at $60^0C/30$ s and extension at $72^0C/2$ min; for the *cagA* gene 35 cycles of denaturation at $94^0C/30$ s, annealing at $57^0C/30$ s and extension at $72^0C/2$ min. PCR products were separated by 10% agarose gel electrophoresis.

Statistical analysis.

All statistical analyses were performed using GraphPad Prism v.5.03 software version 16.0. The comparisons between groups were performed using Student's test. Data are expressed as the mean ± SD. Univariate analyses of survival were performed using the log-rank (Mantel Cox) test. The data were censored from the analysis for the surviving patients at the date of last follow-up. Cut-off points for dividing patients into subgroups based on cytokine levels were identified using the method of Contal and O'Quigley [20]. All differences and associations were considered statistically significant if the 2-sided p-value was below 0.05 (95% Confidence Interval).

Results

The characteristics of the patients included in the study are shown in Tabel I.

Table I. Clinical and pathological details of the patients included in the study.

Group characteristics	Patients (n=51)
Age (years): median	62±11.79
Sex (%)	
Men / Women	33 (64.7) / 18 (35.3)
Tumor size (%)	
≤5 cm	21 (41.2)
5-7 cm	17 (33.3)
>7 cm	13 (25.5)
Site of tumor (%)	
cardia	3 (5.9)
stomach body	32 (62.7)
antrum	16 (31.4)
Type of gastrectomy (%)	
Total / Subtotal	35 (68.6) / 16 (31.4)
AJCC Stage (%)	
IB	1 (2)
II	14 (27.4)
IIIA	16 (31.4)
IIIB	5 (9.8)
IV	15 (29.4)
Histological type (%)	
Differentiated / Undifferentiated	6 (11.8) / 45 (88.2)

Sequencing analysis

In order to find possible factors involved in gp130 abnormal activation that can contribute to gastric cancer initiation and development, 20 samples of human gastric adenocarcinoma were sequenced for the exon 17 of IL6ST
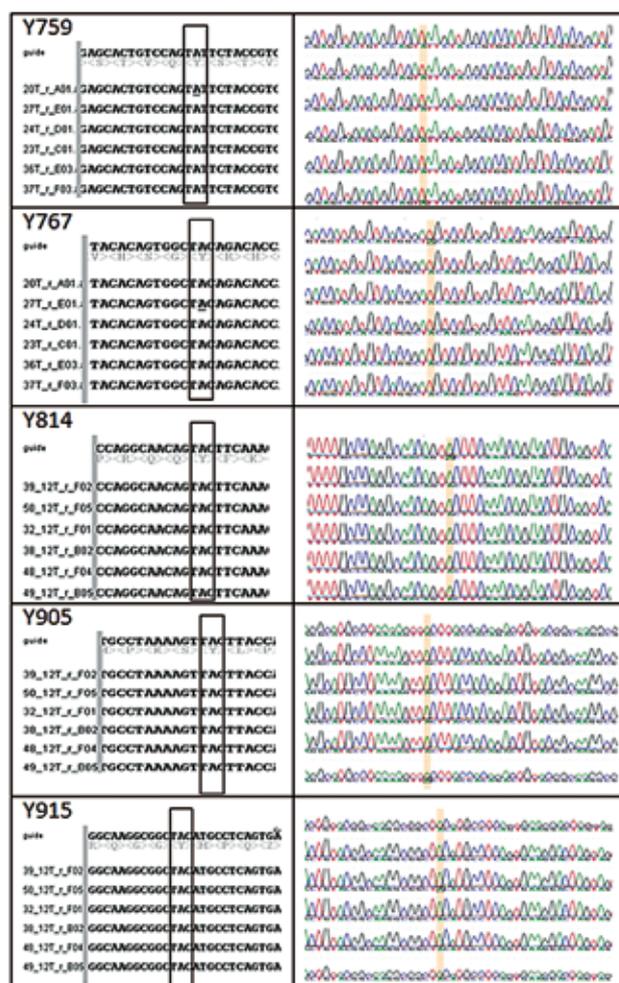

Fig 1. Results of sequence analysis showing sample chromatograms aligned to IL6ST gene reference sequence (NCBI NM_002184.3). Codons corresponding to Y759, Y767, Y814, Y905 and Y915 were evidenced.

gene. Samples were selected according to AJCC status: stage IB (1 sample), stage II (7 samples), stage III (6 samples) and stage IV (6 samples). Half of samples (n=10) were positive for *H. pylori* infection and half of them negative.

No point mutations in key positions of IL6ST (Y759, Y767, Y814, Y905 and Y915) could be detected in any of the gastric adenocarcinoma samples, mutations that could lead to constitutive activation of gp130 protein and STAT3 (Fig. 1). Furthermore, no mutations at all were observed in the IL6ST catalytic domain.

Measurement of cytokine level

The ELISA technique was used to determine the IL-6, IL-11 and LIF level in malignant and adjacent non-malignant tissue, as well as in plasma from 51 gastric adenocarcinoma patients. Results showed a significant correlation between IL-6 and IL-11 tissue level and tumor progression, suggesting that IL-6 and IL-11 might play an important role in inflammation, carcinogenesis and invasion. A significant increase in plasma levels of IL-6 ($p<0.05$) was observed, above the mean concentration observed in healthy donors. IL-11 plasma
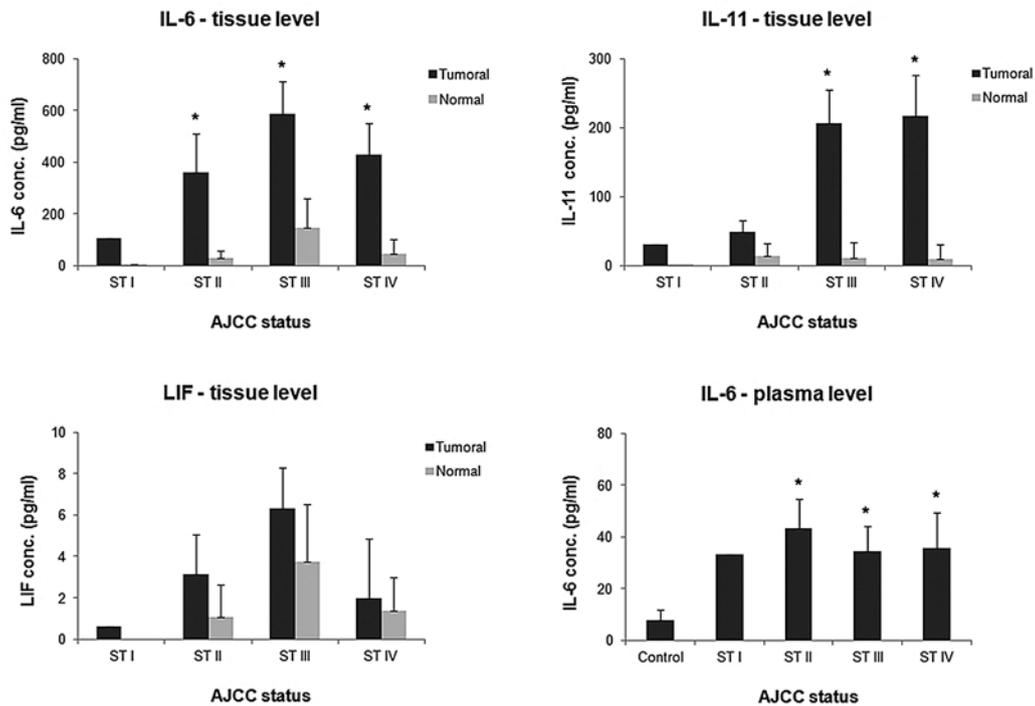
Fig 2. IL-6, IL-11 and LIF expression in gastric tumors and adjacent non-malignant tissue lysates. Results showed increased expression of IL-11 and IL-6 in tumoral tissue lysates and of IL-6 level in plasma, correlated with tumor stage progression. * p<0.05 (Student *t* test).

levels were mostly not detectable, except in 4 patients (7.8%). Details of these results are presented in Fig. 2.

Survival Analysis

Survival analysis was performed on the 51 gastric adenocarcinoma patients using the Cox proportional hazards model. At the time of censoring (3 years), 32 patients had died. Overall median survival was 17.6 months for the entire group of patients, with a median survival of 22.7 months
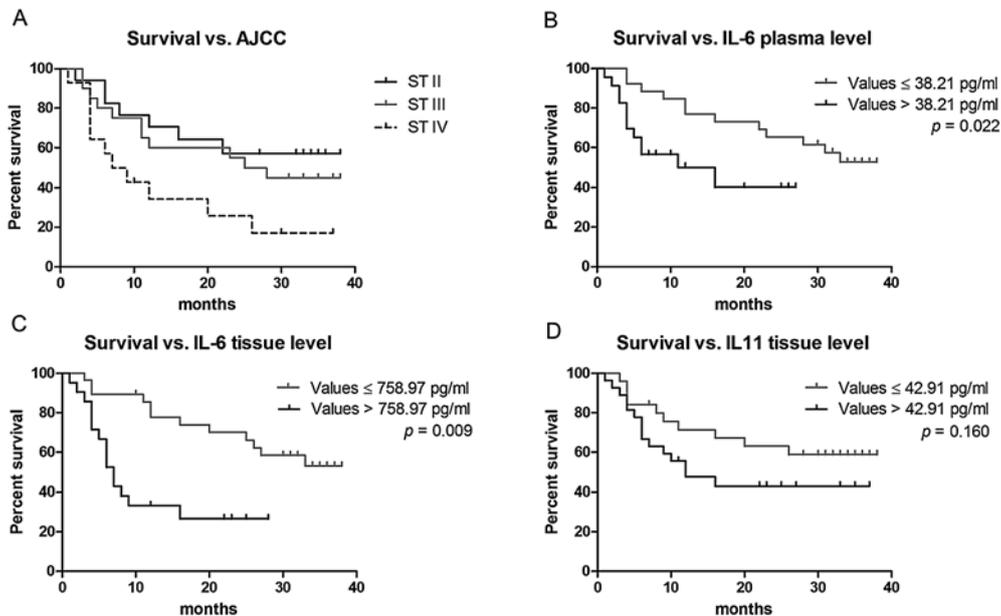

Fig 3. Kaplan-Meier survival plots for gastric adenocarcinoma patients according to AJCC status, IL-11 and IL-6 levels. A: Poor survival associated with stage IV disease (13 months); B: High IL-6 plasma levels (>38.21 pg/ml) were significantly associated with shorter survival in gastric adenoacarcinoma patients; C: High IL-6 tissue levels (>758 pg/ml) were significantly associated with shorter survival in gastric adenoacarcinoma patients; D: No statistically significant difference between survival curves of patients with high versus low level of IL-11. Survival distributions estimated using the Kaplan and Meier method. Tick marks represent the time of last follow-up.

for stage II disease (mortality rate 26.67%), 19.4 months for stage III disease (mortality rate 64.7%), and 13 month for stage IV disease (mortality rate 84.64%) as shown in Fig. 3A.

A correlation between survival and plasma and/or tissue level of these cytokines was assessed in order to consider if they could become a valuable prognostic factor in patients with gastric adenocarcinoma. A cut-off point was determined for each marker using the Contal and O'Quigley method. According to each independent marker and its cut-off point, survival curves were determined using the Kaplan-Meier method. The results showed that even if both cytokines, IL-6 and IL-11, are increased in gastric adenocarcinoma tissue in a stage dependent manner, only IL-6 was significantly correlated with the survival in both plasma and tissue (Fig. 3B, 3C). Patients with IL-6 levels of less than 38.21 pg/ml in plasma had significantly better survival than those with levels over 38.21 pg/ml (log-rank Mantel Cox, p = 0.022). Therefore, circulating IL-6 level appears to be an important prognostic factor in patients with gastric adenocarcinoma. If confirmed in further studies, it could be considered for treatment decisions in these patients.

Helicobacter pylori detection

Helicobacter pylori infection is an important factor in inflammation triggered tumorigenesis and therefore associated with an increase of proinflammatory cytokines level in gastric epithelia. In order to find out if *H. pylori* presence is responsible for the elevated levels of IL-6 and IL-11 detected in patients' samples, PCR was performed on DNA samples: 52.27% samples were positive for H. pylori, and 73.91% were CagA positive. Analyzing the cytokine level according to *H. pylori* presence, results showed an elevated level of IL-6 in plasma of patients with gastric adenocarcinoma that was not associated with H. pylori presence (p=0.02) (Fig. 4A). Comparing the IL-6 levels from H. pylori negative tumor and normal tissue, we observed higher levels of IL-6 in gastric adenocarcinoma samples (p=0.04). Moreover, there was no statistically significant difference between IL-6 level in tumors with *H. pylori* compared with tumors H. pylori negative (Fig. 4B). IL-6

high level seems to be mainly associated with the presence of tumor epithelial cells, and could be a valuable marker for tumor progression and prognosis.

Discussion

Gastric cancer is one of the most common human cancers worldwide and the 5-year survival rate of gastric carcinoma is still low. The etiology and pathogenesis are not fully known but it seems to involve numerous genetic and epigenetic alterations of growth factors/receptors, angiogenic factors, cell cycle regulators, DNA mismatch repair genes, etc [21, 22].

Cytokine signaling pathways using gp130 are deregulated in several epithelial cancers including gastric cancer.

Mutation of tyrosine 759 to phenylalanine was shown to enhance signal transduction of IL-6, leukemia inhibitory factor and oncostatin M and Shp2 phosphorylation and subsequent activation of the proapoptotic Ras/Erk and PI3K/AKT pathways do not occur [23, 13]. Even in the absence of Tyr759 phosphorylation, the phosphorylation of the other four C-terminal tyrosine residues in the gp130 protein results in STAT3 pathway activation, thus inducing proproliferative and oncogenic signals [13].

Our sequencing results do not reveal any point mutations at Tyr positions (Y759, Y767, Y814, Y905 and Y915) from IL6ST exon 17. These results do not exclude the possibility that these mutations exist in a small group of cells (presumably transformed cells), but because of tumor stroma, they could not be detected. To verify this hypothesis would require purification of tumor cells using a laser capture microdissection or by repeated passages in laboratory immunodeficient animals.

Results of Ernst et al revealed that cancer development in the *gp130Y757F/Y757F* animals occurs fast and penetrates deeply, in comparison with gastric cancer in humans. Other important observation is that these animals do not develop chronic gastritis and /or other preneoplastic lesions, implying that there might be several parallel mechanisms which target gp130 pathway up- or downstream [13].

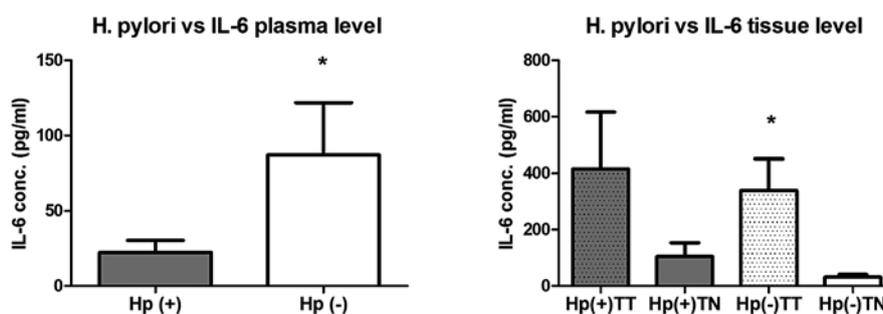This study evaluated the level of some cytokines that use

Fig 4. IL-6 level in plasma and tissue of gastric adenocarcinoma patients according with H. pylori infection. A: IL-6 levels are significant higher in *H. pylori* negative samples (p=0.02); B: Elevated levels of IL-6 could be observed in tumor samples (TT) compared with normal ones (NT), with no significant differences according to *H. pylori* presence (p=0.725).
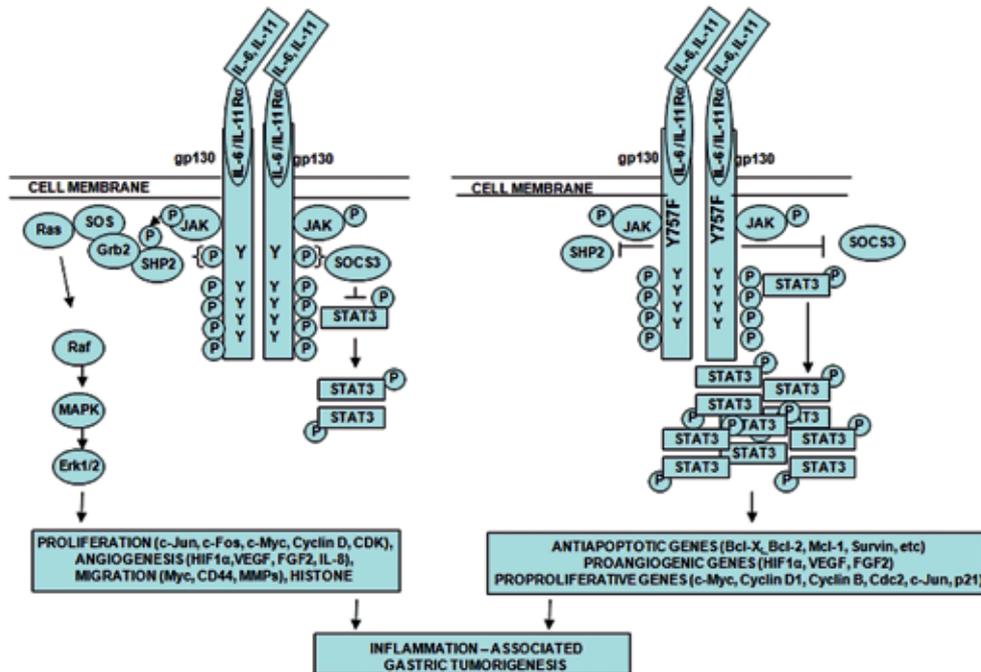
Fig 5. Mechanism proposed for IL-6 and/or IL-11–mediated induction of gp130 and gastric tumorigenesis.

the subunit gp130 (IL-6, IL-11, LIF) for signal transduction, in serum and tissue fragments from patients with gastric adenocarcinomas. The study was performed on 51 pairs of samples (normal and tumor tissue) obtained from patients with gastric adenocarcinoma. Our results revealed elevated tissue levels of IL-6 and IL-11 correlated with tumor progression. Both cytokines use gp130 protein for signaling. It is well known that IL-6 can cause carcinogenesis through several signal pathways involved in carcinogenesis and metastasis [24]. IL-6 activates target genes involved in differentiation, survival, apoptosis, proliferation and manipulates the process of tumorigenesis and tumor progression [25]. IL-6 and IL-11 mediate transcription of target genes after binding their specific α-receptors, and dimerization of the signal transducing β-receptor subunit gp130. Transcriptional regulation occurs via ERK and STAT3-dependent pathways [26]. This is illustrated schematically in Fig. 5.

Another important element of our findings is that the IL-6 level was increased in the tissue and plasma of gastric adenocarcinoma patients, independently of *H. pylori* presence. IL-6 may perpetuate the inflammation inducing anti-apoptotic signals mediated by STAT3. Increased expression of IL-6 and IL-11 in tumor tissue when compared with non-malignant gastric tissue counterpart contributes to persistent cellular proliferation, overcoming the negative regulation of SOCS3 and the SHP2/ERK pro-apoptotic program. The IL-6 level found in *H. pylori* negative tumor tissue was higher than the one in normal tissue and comparable with IL-6 level from *H. pylori* positive tumors, indicating that IL-6 presence and high level is mainly associated with the epithelial cells transformation. In addition, tissue and blood levels of IL-6 were significantly correlated with survival, and therefore

IL-6 level may possibly become an important prognostic factor in gastric adenocarcinoma.

Conclusion

Our results sustain the hypothesis on the role of IL-6, IL-11 and gp130 in the oncogenic transformation of gastric epithelial cells. It might be possible that all these anomalies are triggered by mutations in gp130 protein that obstruct a normal signaling pathway but our study did not identify mutations in the key positions of gp130 in patients with gastric adenocarcinoma, leaving IL-6 and IL-11 as main activators of signaling pathways. Our results suggest that the IL-6 cytokine family plays a role in tumor progression in gastric carcinoma and is one of the steps required in carcinogenesis and the development of invasive characteristics.

Conflicts of interest

No conflict to declare.

Authors' contribution

The work presented here was carried out in collaboration between all authors. MCE, LGN, CCD, IP and GC defined the research theme, designed methods and experiments; ELS, AD, GC and SOD collected tumor samples from patients; LGN, MCE, CCD performed the sequencing analysis and PCR reactions; CB and IA were involved in detection of cytokine levels; ELS collected patients' data and performed statistical analysis on patients survival; LGN, MCE and ELS analyzed the data, interpreted the results and wrote the paper, together with CCD. Final approval of manuscript: all authors.

Acknowledgements

References

1. Jackson CB, Giraud AS. STAT3 as a prognostic marker in human gastric cancer. J Gastroenterol Hepatol 2009; 24: 505–507.

2. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin 2005; 55: 74–108.

3. Ahn HS, Lee HJ,Yoo MW, et al. Changes in clinicopathological features and survival after gastrectomy for gastric cancer over a 20-year period. Br J Surg 2011; 98: 255–260.

4. Economescu MC, Necula LG, Dragu D, et al. Identification of potential biomarkers for early and advanced gastric adenocarcinoma detection. Hepatogastroenterology 2010; 57: 1453-1464.

5. Grady WM, Tewari M. The next thing in prognostic molecular markers: microRNA signatures of cancer. Gut 2010; 59: 706-708.

6. Ernst M, Najdovska M, Grail D, et al. STAT3 and STAT1 mediate IL-11–dependent and inflammation-associated gastric tumorigenesis in gp130 receptor mutant mice. J Clin Invest 2008; 118: 1727-1738.

7. Maekita T, Nakazawa K, Mihara M, et al. High levels of aberrant DNA methylation in helicobacter pylori infected gastric mucosae and its possible association with gastric cancer risk. Clin Cancer Res 2006; 12: 989-995.

8. Handa O, Naito Y, Yoshikawa T. CagA protein of Helicobacter pylori: A hijacker of gastric epithelial cell signaling. Biochem Pharmacol 2007; 73: 1697-1702.

9. Tamura G. Alterations of tumor suppressor and tumor-related genes in the development and progression of gastric cancer. World J Gastroenterol 2006; 12: 192-198.

10. Gong W, Wang L, Yao JC, et al. Expression of activated signal transducer and activator of transcription 3 predicts expression of vascular endothelial growth factor in and angiogenic phenotype of human gastric cancer. Clin Cancer Res 2005; 11: 1386-1393.

11. Kanda N, Seno H, Konda Y, et al. STAT3 is constitutively activated and supports cell survival in association with survivin expression in gastric cancer cells. Oncogene 2004; 23: 4921-4929.

12. Jenkins BJ, Grail D, Nheu T, et al. Hyperactivation of Stat3 in gp130 mutant mice promotes gastric hyperproliferation and desensitizes TGF-beta signaling. Nat Med 2005; 11: 845-852.

13. Merchant JL. What lurks beneath: IL-11, via Stat3, promotes inflammation-associated gastric tumorigenesis. J Clin Invest 2008; 118: 1628-1631.

14. Jarnicki A, Putoczki T, Ernst M. Stat3: linking inflammation to epithelial cancer - more than a "gut" feeling? Cell Div 2010; 5: 14.

15. Nicholson SE, De Souza D, Fabri LJ, et al. Suppressor of cytokine signaling-3 preferentially binds to the SHP-2- binding site on the shared cytokine receptor subunit gp130. Proc Natl Acad Sci U S A 2000; 97: 6493-6498.

16. Takahashi-Tezuka M, Yoshida Y, Fukada T, et al. Gab1 acts as an adapter molecule linking the cytokine receptor gp130 to ERK mitogen-activated protein kinase. Mol Cell Biol 1998; 18: 4109-4117.

17. Putoczki T, Ernst M. More than a sidekick: the IL-6 family cytokine IL-11 links inflammation to cancer. J Leukoc Biol 2010; 88: 1109-1117.

18. Howlett M, Menheniott TR, Judd LM, Giraud AS. Cytokine signalling via gp130 in gastric cancer. Biochim Biophys Acta 2009; 1793: 1623-1633.

19. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988; 16: 1215.

20. Contal C, O'Quigley J. An application of changepoint methods in studying the effect of age on survival in breast cancer. Computational Statistics & Data Analysis 1999; 30: 253-270.

21. Yasui W, Sentani K, Motoshita J, Nakayama H. Molecular pathobiology of gastric cancer. Scand J Surg 2006; 95: 225-231.

22. Shang J, Peńa AS. Multidisciplinary approach to understand the pathogenesis of gastric cancer. World J Gastroenterol 2005; 11: 4131-4139.

23. Lehmann U, Schmitz J, Weissenbach M, et al. SHP2 and SOCS3 contribute to Tyr-759-dependent attenuation of interleukin-6 signaling through gp130. J Biol Chem 2003; 278: 661-671.

24. Chen J, Huang XF. Interleukin-6 promotes carcinogenesis through multiple signal pathways. Comment on: Clinical significance of interleukin-6 gene polymorphism and IL-6 serum level in pancreatic adenocarcinoma and chronic pancreatitis. Dig Dis Sci 2009; 54: 1373-1374.

25. Lin MT, Lin BR, Chang CC, et al. IL-6 induces AGS gastric cancer cell invasion via activation of the c-Src/RhoA/ROCK signaling pathway. Int J Cancer 2007; 120: 2600-2608.

26. Judd LM, Ulaganathan M, Howlett M, Giraud AS. Cytokine signalling by gp130 regulates gastric mucosal healing after ulceration and, indirectly, antral tumour progression. J Pathol 2009; 217: 552-562.