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ANATOMICAL PATHOLOGY

Higher IL-6 peri-tumoural expression is associated with gastro-intestinal neuroendocrine tumour progression

Sofia S. Pereira[1,2,3,*], Rúben Pereira[1,*], Ana P. Santos[4], Madalena M. Costa[1], Tiago Morais[1], Paula Sampaio[2,5], Bianca Machado[6], Luís Pedro Afonso[6], Rui Henrique[6,7,8], Mariana P. Monteiro[1]

[1]*Endocrine, Cardiovascular and Metabolic Research, Multidisciplinary Unit for Biomedical Research (UMIB), Department of Anatomy of Instituto de Ciências Biomédicas Abel Salazar, University of Porto (ICBAS/UP), Porto, Portugal;* [2]*Institute for Research and Innovation in Health (I3S), University of Porto, Portugal;* [3]*Institute of Molecular Pathology and Immunology of the University of Porto (IPATIMUP), Porto, Portugal;* [4]*Endocrinology Service/Clinical Research Unit, Portuguese Institute of Oncology of Porto Research Center (CI-IPOP), Portuguese Institute of Oncology of Porto (IPO-Porto), Portugal;* [5]*Institute for Molecular and Cellular Biology (IBMC), University of Porto, Portugal;* [6]*Department of Pathology, Portuguese Oncology Institute of Porto (IPO Porto), Portugal;* [7]*Cancer Biology and Epigenetics Group, Research Center of Portuguese Oncology Institute of Porto (CI-IPOP), Portuguese Oncology Institute of Porto (IPO Porto), Portugal;* [8]*Department of Pathology and Molecular Immunology, ICBAS, University of Porto, Portugal;* [*]*these authors contributed equally.*

Summary

An association of well-differentiated gastroenteropancreatic neuroendocrine tumours (WD GEP NETs) with metabolic syndrome (MetS) was recently described. Yet no molecular mechanisms linking the two conditions are known. This study's aim was to identify putative molecular signatures linking WD GEP NETs and MetS to gain further insight into potential mechanisms for this association.

Patients with WD GEP NETs (n=39), pancreatic (panNET) and gastro-intestinal (GI-NET), were clinically evaluated for presence of MetS. WD GEP NETs immunohistochemistry staining for Forkhead box protein M1 (FOXM1), insulin growth factor 1 receptor (IGF1R), Ki-67 and interleukin 6 (IL-6) was performed and quantified by computerised morphometric analysis.

FOXM1, Ki-67, IGF1R or IL-6 expression in WD GEP NETs was not influenced by the presence of MetS. IL-6 peri-tumoural expression was higher in GI-NETs of patients with low HDL cholesterol (0.018±0.005% vs 0.030±0.005%, p=0.02). In GI-NETs, a higher IL-6 expression was also associated with disease progression (0.026±0.004% vs 0.016±0.002%, p=0.03).

In WD GEP-NETs, MetS did not influence FOXM1, IGF1R and IL-6 expression. In GI-NETs, IL-6 expression was influenced by the MetS feature low HDL, and positively associated with disease progression. These data suggest that local and systemic inflammatory status can potentially modulate GI-NET behaviour.

Key words: Gastrointestinal neuroendocrine tumours; pancreatic neuroendocrine tumours; gastroenteropancreatic neuroendocrine tumours; metabolic syndrome; inflammation.

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INTRODUCTION

Gastroenteropancreatic neuroendocrine tumours (GEP NETs) comprise a group of rare and heterogeneous neoplasms that emerge from enterochromaffin epithelial cells of the diffuse endocrine system sparsely throughout the gastrointestinal tract and pancreas.[1,2] GEP NETs were previously considered rare neoplastic diseases. However, epidemiological data have shown an increase in the incidence and prevalence of GEP NETs over the last decades, which was attributed to increased disease awareness and diagnosis driven by the technical improvements observed in imaging and endoscopic techniques employed.[3,4] Indeed, GEP NETs are currently the second most common gastrointestinal malignancy after colorectal cancer.[5]

The prevalence of obesity, metabolic syndrome (MetS) and type 2 diabetes is also escalating worldwide.[6–8] Indeed, links between MetS or MetS individual components and cancer were recently demonstrated for several different malignancies, including endometrial cancer, colorectal cancer and hepatocarcinoma.[9–11]

The relationship between MetS and GEP NET is not as well established, although our group has recently reported that MetS and some MetS individual components, including visceral obesity, dyslipidaemia and high fasting glucose were associated with an increased risk for well-differentiated (WD) GEP NET.[12] Insulin resistance is known to play a key role in the aetiology of MetS.[13,14] Insulin resistance states are responsible for an adaptive increase in circulating insulin levels as a counter regulatory response to overcome the resistance.

The molecular links between insulin resistance and cancer are far from being entirely disclosed. Among the potential candidates are tyrosine kinase receptors (TKRs) signalling pathways, since these are most frequently found to be altered in human cancers. Indeed, insulin can activate MAPK and

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PI3K/AKT/mTOR pathways through IGF1R signalling.[15,16] In addition, IGF1R is highly expressed in WD GEP-NETs and is considered a potential molecular target for a variety of cancer therapies.[17–19] FOXM1 is an essential transcription factor that cross-talks with MAPK and PI3K/AKT/mTOR pathways activation and consequently plays a major role in cell differentiation, cell cycle progression, cell proliferation and tumourigenesis among other biological processes.[20] FOXM1 overexpression is observed in the majority of human solid cancers, including in WD GEP-NETs.[21–23]

Besides that, obesity and MetS are often accompanied by a systemic chronic inflammatory state, in which pro-inflammatory cytokines such as IL-6 are involved.[24,25] Therefore, IL-6 is often expressed in tumour surrounding tissues while being responsible for shaping the tumour microenvironment.[26]

In order to gain further insight into potential mechanisms underlying the association of MetS and WD GEP NET, the purpose of this study was to evaluate the influence of MetS criteria and MetS individual components in the expression of different molecular markers that participate in inflammatory (IL-6) and WD GEP NET metabolic pathways (IGF1-R and FOXM1).

MATERIAL AND METHODS

Patients

Patients diagnosed with WD GEP NETs (n=39) attending a single tertiary referral centre for endocrine tumours were enrolled in this study. Patients were divided into two main groups according to the location of the primary tumour: gastrointestinal (GI-NET) (n=29), and pancreatic (panNET) (n=10) (Table 1).

Presence of MetS was established according to the Joint Interim Statement (JIS) of NHLBI/AHA/WHF/IAS/IASO criteria,[27] which defines the diagnosis of MetS in the presence of at least three of the five risk factors: fasting plasma glucose >100 mg/dL or ongoing glucose-lowering drug treatments; waist circumference >88 cm (female) or >102 cm (male); systolic blood pressure ≥130 mm Hg or diastolic blood pressure ≥85 mm Hg or under blood pressure lowering medications; HDL-cholesterol (HDL-c) <40 mg/dL (male) or <50 mg/dL (female) or drug treatment for reduced HDL-c; triglycerides (TG) >150 mg/dL or under triglyceride lowering drugs (Table 1). All clinical parameters were assessed before surgical intervention for tumour removal.

Immunohistochemistry

Tissue sections (3 µm thick) were dewaxed in xylene and progressively hydrated in a decreasing scale of alcohols (100%, 95% and 70%) until water. Antigen retrieval was performed by incubation in a 10 mM citrate buffer (pH 6.0) with Tween 20 at 0.05% in a microwave at 900 W for 20 min for interleukin 6 (IL-6); by incubation in a 10 mM citrate buffer (pH 6.0) with Tween 20 at 0.05%, in a microwave at 900 W for 25 min after boiling for Forkhead box protein M1 (FOXM1); and by incubation in a 10 mM citrate buffer (pH 6.0) with Tween 20 at 0.05%, in a pressure cooker for 4 min after boiling for insulin growth factor 1 receptor (IGF1R). All the washes required throughout the process were performed in a phosphate buffered saline solution with Tween 20 at 0.05% (pH 7.4). Endogenous peroxidase was inhibited with the incubation of the sections in a solution of hydrogen peroxide and methanol at 3% for 20 min. Incubation with the respective primary antibody, was performed overnight at 4ºC: anti-IGF1R (1:100, ab39675; Abcam, UK), anti-FOXM1 (1:500, sc-502; Santa Cruz Biotechnology, USA), and anti-IL-6 (1:500, ab9324; Abcam). Subsequently, the sections were incubated with the proper secondary antibody [1:200, polyclonal rabbit anti-mouse biotinylated (E0354; Dako, USA), or 1:200, polyclonal swine anti-rabbit biotinylated (E0353; Dako)], for 30 min at room temperature. After that, sections were incubated for 30 min with an avidin-biotin complex (Vector Laboratories, UK) and then revealed with the DAB substrate (3,3′-diaminobenzidine tetrahydrochloride; Dako, USA). All the sections were counterstained with Harris haematoxylin.

Tissue slides immunohistochemically stained for Ki-67, performed as part of routine practice to determine the tumour grade, were retrieved from the pathology department archives and used for morphological analysis.

Immunohistochemical data analysis

Haematoxylin and eosin (H&E) stained slides were used for tumour area delimitation based on morphological criteria by experienced pathologists with no access to patients' clinical information. This area delimitation was then transferred to the immunohistochemistry stained slides.

After immunohistochemistry, slides were scanned using the image acquisition Olympus VS110 virtual slide scanning system (Olympus, Japan) and captured with a magnification of 20× using the image acquisition software VS-ASW (Olympus). Images were analysed using the image processing software FIJI (version for Windows; National Institutes of Health, USA). The tumour area was selected using FIJI freehand tool, for the study of the expression of Ki-67, FOXM1 and IGF1R. A peritumoural area of 5 mm distant from the tumour and from 5 mm until the end of the tissue was delimited using the ROI Manager Tool of FIJI to evaluate IL-6 expression.

Using FIJI colour deconvolution plugin (H Dab), the separation of the stained area from the initial image, based in the RGB (red, green and blue) system was performed. Then, the stained area with the IGF1R, FOXM1 and Ki-67 antibodies in the total tissue area of the tumour and the stained area with the IL-6 antibody in the adjacent tissue, were quantified as previously described.[28]

Statistical analysis

Qualitative variables are expressed as number of cases and percentage (%), and the quantitative variables are expressed as mean ± standard error of the mean. The difference between two independent experimental groups was evaluated using the unpaired Student t test for normally distributed variables, and the Mann–Whitney U test for variables that did not meet normality. To correlate the different groups, a Pearson or a Spearman correlation was used depending on the sample's normality. A p value <0.05 was considered statistically significant. All statistical analyses were performed with the GraphPad Prism software version 7.00 (GraphPad, USA) and IBM SPSS Statistics version 24 (IBM, USA), both for Windows.

RESULTS

Expression of Ki-67, FOXM1, IGF1R and IL-6 markers in patients with and without MetS

All tumours expressed Ki-67, FOXM1 and IGF1R, although the percentage of stained area was highly variable (Fig. 1). FOXM1 and IGF1R positive cells were found to be evenly distributed throughout the tumour tissue.

The percentages of the stained tumour areas, both panNETs and GI-NETs, for all the molecular markers Ki-67, FOXM1 and IGF1R were not significantly different when patients with or without MetS were compared (panNETs: p=0.99 for Ki-67, p=0.61 for FOXM1, and p=0.65 for IGF1R; GI-NETs: p=0.62 for Ki-67, p=0.96 for FOXM1, and p=0.19 for IGF1R) (Fig. 2). Nevertheless, the percentage of IGF1R stained area was higher in panNETs and GI-NETs of patients with MetS (Fig. 2).

The percentage of IL-6 stained in the peritumoural area was assessed within 5 mm from the tumour limit and from this limit until the tissue edge. IL-6 was found to be expressed in the peritumoural pancreatic and intestinal stroma, mainly in endothelial, fibroblasts and immune cells. The results showed that the percentage of IL-6 stained area in peritumoural areas of WD GEP-NETs was not significantly different when patients with or without MetS were compared. No difference in peritumoural IL-6 stained area was observed in panNETs with or without MetS [IL-6 (≤5 mm): 0.024 ± 0.009 (with MetS) vs 0.017 ± 0.011 (without MetS), p=0.56; IL-6 (>5 mm): 0.015 ± 0.007 (with MetS) vs 0.015 ± 0.004

Table 1 Patients' clinical features and tumour characteristics

	panNET (*n*=10)	GI-NET (*n*=29)
Patients' clinical features		
Sex		
Female (%)	6 (60.00%)	12 (41.38%)
Male (%)	4 (40.00%)	17 (58.62%)
Median age, years (range)	57 (29–75)	64 (41–81)
Metabolic syndrome		
Absent (%)	4 (40.00%)	9 (31.03%)
Present[a] (%)	5 (50.00%)	20 (68.97%)
BMI	26.65 ± 1.76	27.58 ± 0.66
Normal weight (%)	5 (50.00%)	8 (27.59%)
Overweight (%)	2 (20.00%)	12 (41.38%)
Obese (%)	3 (30.00%)	9 (31.03%)
Waist circumference (cm)	93.73 ± 3.18	96.75 ± 2.87
<102 cm in males or <88 cm in females (%)	4 (40.00%)	12 (41.38%)
≥102 cm in males or ≥88 cm in females (%)	5 (50.00%)	16 (55.17%)
Fasting plasma glucose (mg/dL)	103.10 ± 3.64	105.97 ± 4.20
<100 mg/dL (%)	5 (50.00%)	9 (31.03%)
≥100 mg/dL (%)	5 (50.00%)	20 (68.97%)
Type 2 diabetes mellitus		
Absent (%)	7 (70.00%)	21 (72.41%)
Present (%)	2 (20.00%)	8 (27.59%)
HDL (mg/dL)	53.70 ± 4.50	48.48 ± 2.68
≥40 in males or ≥50 mg/dL in females (%)	5 (50.00%)	12 (41.38%)
<40 in males or <50 mg/dL in females (%)	5 (50.00%)	17 (58.62%)
Triglycerides (mg/dL)	122.70 ± 12.87	153.69 ± 15.31
<150 mg/dL (%)	5 (50.00%)	17 (58.62%)
≥150 mg/dL (%)	5 (50.00%)	12 (41.38%)
SBP (mmHg)	136.60 ± 4.41	131.76 ± 2.84
DBP (mmHg)	77.10 ± 2.86	72.83 ± 2.12
SBP <130 mmHg or DBP <85 mmHg (%)	4 (40.00%)	9 (31.03%)
SBP ≥130 mmHg or DBP ≥85 mm Hg (%)	6 (60.00%)	20 (68.97%)
Somatostatin analogues treatment		
No (%)	7 (70.00%)	8 (27.59%)
Yes (%)	3 (30.00%)	21 (72.41%)
Duration (months)	78.00 ± 24.74	67.05 ± 6.29
Tumour characteristics		
WHO grade		
G1 (%)	6 (60.00%)	24 (82.76%)
G2 (%)	4 (40.00%)	5 (17.24%)
Staging		
Local disease (%)	6 (60.00%)	5 (17.24%)
Loco regional disease (%)	1 (10.00%)	2 (13.79%)
Disseminated disease (%)	3 (30.00%)	20 (60.97%)
Functionality		
Functioning (%)	2 (20.00%)	22 (75.86%)
Non-functioning (%)	7 (70.00%)	7 (24.14%)
Disease status		
Stable disease (%)	2 (20.00%)	10 (34.48%)
Disease free (%)	3 (30.00%)	6 (20.69%)
Disease progression[a]	5 (50.00%)	13 (44.83%)
Progression-free survival (months)	83.44 ± 14.22	57.65 ± 5.98
Overall survival (months)	94.27 ± 14.42	77.27 ± 6.28

BMI, body mass index; DBP, diastolic blood pressure; GI-NET, gastrointestinal neuroendocrine tumours; HDL, high-density lipoprotein; MetS, metabolic syndrome; panNET, pancreatic neuroendocrine tumours; SBP, systolic blood pressure.
[a] Defined as tumour progression after the first treatment. The divergence between number of patients and sum of studied parameters translates missing data.

(without MetS), p=0.61]; nor in GI-NETs [IL-6 (≤5 mm): 0.027 ± 0.005 (with MetS) vs 0.021 ± 0.006 (without MetS), p=0.40; IL-6 (>5 mm): 0.019 ± 0.002 (with MetS) vs 0.025 ± 0.006 (without MetS), p=0.85] (Fig. 3).

Expression of Ki-67, FOXM1, IGF1R and IL-6 markers in patients with or without MetS components

The percentages of the stained tumour areas for Ki-67, FOXM1 and IGF1R markers, were not significantly different between patients with or without each individual MetS component, both for panNETs and GI-NETs (Table 2).

The percentage of the peritumoural area stained for the IL-6 marker assessed at two different distances from the tumour was not significantly different between patients with or without several of the MetS individual components, namely high fasting plasma glucose, high BP or raised triglycerides, for both panNETs and GI-NETs (Table 3). However, in GI-NETs the percentage peritumoural area at 5 mm distance or less from the tumour limit stained for IL-6 was significantly higher in the subset of patients with low HDL when compared to patients with normal HDL (0.030 ± 0.0050 vs 0.018 ± 0.0050; p<0.05) (Table 3). In addition, in panNETs the percentage of peritumoural area stained for IL-6 at 5 mm from the tumour limit until the end of the tissue was significantly higher in patients without central obesity when compared with patients with central obesity (0.019 ± 0.0025 vs 0.0078 ± 0.0019; p<0.05) (Table 3).

Ki-67, FOXM1, IGF1R and IL-6 expression and tumour characteristics

The percentages of the stained tumour areas for Ki-67, FOXM1 and IGF1R markers did not differ according to the different tumour's characteristics (Supplementary Table 1, Appendix A).

The percentage of peritumoural area stained for IL-6 was significantly higher at a distance of 5 mm from the tumour until the end of the tissue in GI-NETs that progressed at least once after the initial treatment (Supplementary Table 1, Appendix A; Fig. 4).

No other correlation was observed between characteristics of GEP-NETs and peritumoural IL-6 staining (Supplementary Table 2, Appendix A).

Molecular correlations

Ki-67 and FOXM1 were found to be positively correlated both in panNETs (R=0.648; p<0.05) (Fig. 5A) and in GI-NETs (R=0.606; p<0.001) (Fig. 5B). In addition, a statistically significantly positive correlation between IGF1R and FOXM1 in GI-NETs was found (R=0.608; p<0.001) (Fig. 5C). No other significant correlations were found between the expression pattern of the studied proteins in panNETs or GI-NETs.

DISCUSSION

MetS is a well-established risk factor for different types of cancers.[9–11] More recently, the potential link between MetS and WD GEP-NETs has also been highlighted, since MetS and several individual components of MetS, namely fasting plasma glucose, waist circumference, and dyslipidaemia were found to be more frequent in patients with WD GEP-NETs then in the general population.[12] However, no molecular links for this pathological association that could provide novel information on the mechanisms of disease and lead to the development of targeted interventions were identified. Thus, the main aim of the research herein was to gain further insight into putative molecular links that could provide a

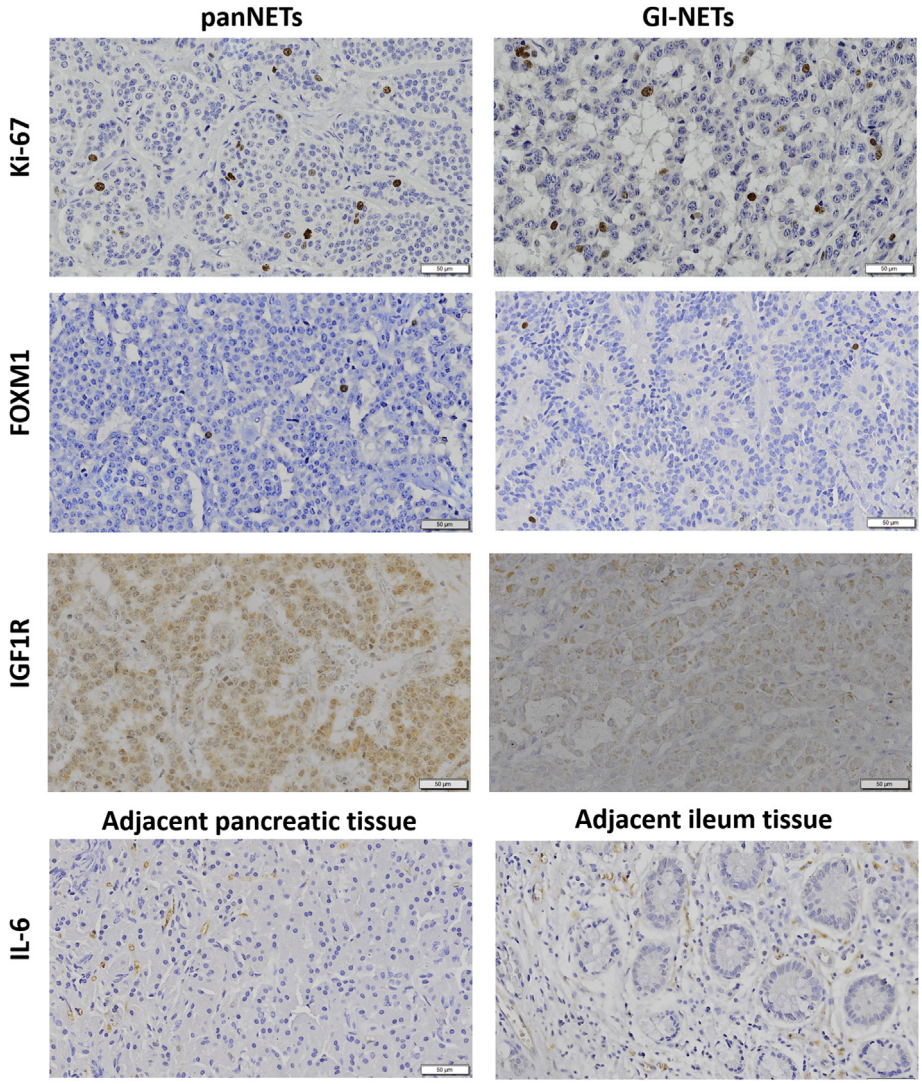

Fig. 1 Immunohistochemistry staining for Ki-67, FOXM1 and IGF1R in panNETs and GI-NETs, and for IL-6 in the pancreatic and ileum tissue adjacent to the panNETs or GI-NETs, respectively.

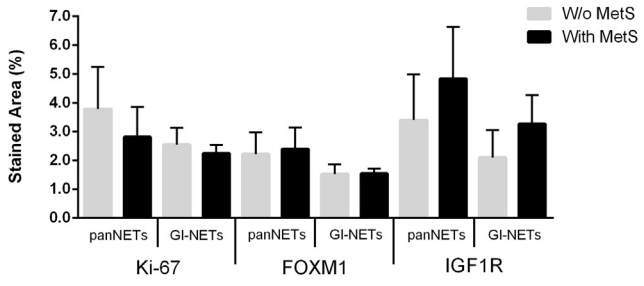

Fig. 2 Percentage of the stained area for Ki-67, FOXM1 and IGF1R, in pancreatic (panNETs) and gastrointestinal neuroendocrine tumours (GI-NETs) of patients, with or without (W/o) metabolic syndrome (MetS).

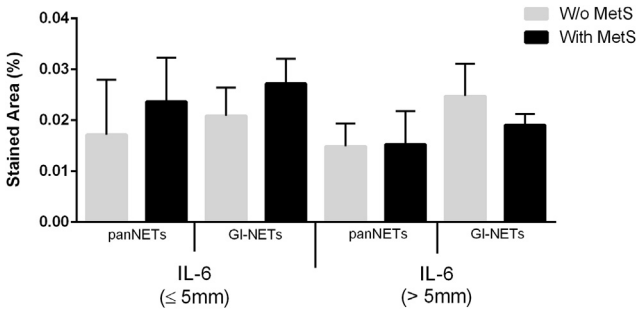

Fig. 3 Percentage of the peritumoural areas (≤5 and >5 mm) stained for IL-6 in pancreatic (panNETs) and gastrointestinal neuroendocrine tumours (GI-NETs) of patients with or without (W/o) metabolic syndrome (MetS).

pathological rationale for the association of WD GEP-NETs and MetS or any individual component of MetS. To achieve this goal, FOXM1 and IGF1R were selected as markers of the molecular pathways involved in GEP-NETs biology and IL-6 was chosen as to evaluate the inflammatory environment in the periphery of the tumour.

Despite the rationale for the potential involvement of these molecular pathways in the likelihood of MetS being associated with WD GEP-NETs, our results were not able to demonstrate any significant association between IGF1R and FOXM1 expression in WD GEP-NETs and MetS or any of the MetS individual components, which could suggest that expression of these molecules is not influenced by the presence of MetS. Nevertheless, it should be noted that IGF1R expression in WD GEP-NETs, both in panNETs and GI-

Table 2 Percentage of the tumour area stained by the immunohistochemical markers, Ki-67, FOXM1 and IGF1R, in pancreatic and gastrointestinal neuroendocrine tumours

MetS components	Ki-67	FOXM1	IGF1R
Pancreatic neuroendocrine tumours			
BP, normal : raised[a]	2.74±1.28 : 3.73±1.27 *p*=0.61	2.06±0.77 : 2.68±0.85 *p*=0.62	3.18±1.67 : 5.51±1.98 *p*=0.47
Fasting plasma glucose, normal : raised[b]	3.78±1.46 : 3.04±1.20 *p*=0.91	2.67±0.67 : 2.28±0.89 *p*=0.47	6.18±4.34 : 4.01±0.87 *p*=0.51
Triglycerides, normal : raised[c]	4.06±1.30 : 2.85±1.24 *p*=0.48	2.35±0.69 : 2.49±0.89 *p*=0.76	2.88±1.10 : 6.22±2.30 *p*=0.27
HDL, normal : low[d]	3.37±1.20 : 3.29±1.43 *p*=0.84	2.02±0.61 : 2.85±1.01 *p*=0.50	4.08±1.31 : 5.26±2.47 *p*=0.71
Central obesity, absent : present[e]	3.02±1.76 : 2.55±0.73 *p*=0.90	2.21±1.06 : 1.83±0.26 *p*=0.64	3.90±2.60 : 4.98±2.08 *p*=0.80
Gastrointestinal neuroendocrine tumours			
BP, normal : raised[a]	2.42±0.57 : 2.30±0.30 *p*=0.84	1.71±0.37 : 1.46±0.15 *p*=0.77	2.83±1.15 : 2.94±0.96 *p*=0.51
Fasting plasma glucose, normal : raised[b]	2.21±0.47 : 2.39±0.33 *p*=0.99	1.38±0.32 : 1.61±0.17 *p*=0.49	3.25±1.13 : 2.75±0.96 *p*=0.77
Triglycerides, normal : raised[c]	2.48±0.48 : 2.16±0.38 *p*=0.56	1.70±0.22 : 1.34±0.21 *p*=0.23	3.70±1.25 : 1.93 : 0.56 *p*=0.11
HDL, normal : low[d]	1.89±0.34 : 2.70±0.39 *p*=0.13	1.27±0.18 : 1.76±0.22 *p*=0.11	2.12±0.68 : 3.55±1.23 *p*=0.28
Central obesity, absent : present[e]	2.39±0.42 : 2.30±0.36 *p*=0.87	1.51±0.26 : 1.56±0.19 *p*=0.86	2.12±0.68 : 3.55±1.23 *p*=0.28

BMI, body mass index; BP, blood pressure; HDL, high-density lipoprotein; MetS, metabolic syndrome.
[a] Systolic BP ≥130 mmHg or diastolic BP ≥85 mmHg.
[b] Fasting plasma glucose ≥100 mg/d.
[c] Triglycerides ≥150 mg/dL.
[d] HDL <40 mg/dL (male) or <50 mg/dL (female).
[e] Waist circumference ≥102 cm (male) or ≥88 cm (female).

NETs, although not reaching statistical significance was numerically higher in tumours of patients with MetS when compared to patients without MetS, suggesting that higher insulin levels in patients with MetS are likely to be involved in the upregulation of IGF1R and subsequent pathway activation.

Ki-67 is a well-known cell proliferation marker routinely used in clinical practice for pathological staging of several tumours including GEP-NETs. In our study, MetS or any of the MetS individual components were not found to be associated with a higher Ki-67 expression in WD GEP-NET to suggest that MetS could influence cell proliferation rate and eventually influence the tumour biological and clinical behaviour. Nonetheless, a positive correlation between Ki-67 and FOXM1 expression in WD GEP-NETs, both panNETs and GI-NETs, was similar to a previous report involving GEP-NETs.[21] In GI-NETs, FOXM1 and IGF1R expression were found to be positively correlated. This correlation further supports that FOXM1 expression is stimulated and activated by IGF1R in GI-NETs.

Chronic inflammatory conditions are well recognised risks of cancer. Inflammatory bowel disease (IBD) is associated with increased risk of GEP-NETs,[29–32] further suggesting that chronic inflammation within the gastrointestinal tract could promote hyperplasia and neoplastic transformation of neuroendocrine cells.[30] In addition to the widely accepted role of inflammation in tumourigenesis, it has become evident that the inflammatory microenvironment is a key component in tumour biology.[33] Since IL-6 is a cytokine often expressed in tumour surrounding tissues, and its systemic levels have been found to be increased in patients with obesity and MS,[24–26] IL-6 expression surrounding the tumour was elected as a means to assess inflammatory activity in WD GEP-NETs of patients with potentially different systemic inflammatory profiles. The area that surrounds a tumour with the ability to influence tumour environment has not yet been strictly defined and is potentially variable depending on the type and location of cancer. Thus, it is not surprising that previous studies have used a wide range of distances from the tumour limit, spanning from a few millimeters to 1 cm wide, that were defined as adjacent tumour tissue.[34–37] In our study, whenever available a maximum distance of 5 mm from the tumour limit was selected as the definition of peritumoural tissue to assess IL-6 expression in the tumour microenvironment.[34,35]

IL-6 expression in WD GEP NETs peritumoural tissue of patients with and without MetS was not found to be significantly different, both within the 5 mm perimeter from the tumour limit or 5 mm or higher until the tissue limit. Since MetS is a known chronic low-grade inflammatory state,[24] IL-6 expression surrounding WD GEP NETs of patients with MetS was expected to be higher, which was not corroborated by our findings. Nonetheless, it should be noted that IL-6 expression in WD GEP-NETs, both in panNETs and GI-NETs, despite not reaching statistical significance was numerically higher in the area within 5 mm of the limit of tumours of patients with MetS when compared to patients without MetS. In addition, in patients with panNETs and central obesity, a lower peritumoural IL-6 expression was noticed in contrast to what was previously described in the literature for tumours other than GEP-NETs.[24]

However, IL-6 expression in the peritumoural area of GI-NETs was significantly higher in patients with low HDL when compared to tumours in patients with normal HDL, suggesting that HDL could be an important modulator of the inflammatory environment in GI-NETs, unsurprisingly given

Table 3 Percentage of the peritumoural area immunohistochemically stained with IL-6 at two different distances from pancreatic and gastrointestinal neuroendocrine tumour margins

MetS components	IL-6 (≤5 mm)	IL-6 (>5 mm)
Pancreatic neuroendocrine tumours		
BP, normal : raised[a]	0.019±0.010 : 0.022±0.0091 *p*=0.81	0.015±0.0036 : 0.014±0.0067 *p*=0.35
Fasting plasma glucose, normal : raised[b]	0.019±0.010 : 0.022±0.0090 *p*=0.91	0.014±0.0044 : 0.015±0.0065 *p*=0.89
Triglycerides, normal : raised[c]	0.019±0.012 : 0.022±0.0081 *p*=0.84	0.010±0.0047 : 0.018±0.0060 *p*=0.35
HDL, normal : low[d]	0.024±0.011 : 0.017±0.0072 *p*=0.84	0.013±0.0038 : 0.016±0.0077 *p*=0.69
Central obesity, absent : present[e]	0.022±0.013 : 0.016±0.0079 *p*=0.71	**0.019±0.0025 : 0.0078±0.0019** ***p*=0.01**
Gastrointestinal neuroendocrine tumours		
BP, normal : raised[a]	0.022±0.0055 : 0.026±0.0049 *p*=0.68	0.025±0.0062 : 0.018±0.0022 *p*=0.60
Fasting plasma glucose, normal : raised[b]	0.025±0.0053 : 0.024±0.0050 *p*=0.47	0.023±0.0058 : 0.019±0.0022 *p*=0.77
Triglycerides, normal : raised[c]	0.024±0.0050 : 0.026±0.0058 *p*=0.75	0.020±0.0026 : 0.021±0.0044 *p*=0.70
HDL, normal : low[d]	**0.018±0.0050 : 0.030±0.0050** ***p*=0.02**	0.017±0.0027 : 0.024±0.0040 *p*=0.17
Central obesity, absent : present[e]	0.019±0.0047 : 0.029±0.0052 *p*=0.13	0.022±0.0047 : 0.020±0.0028 *p*=0.96

BMI, body mass index; BP, blood pressure; HDL, high-density lipoprotein; MetS, metabolic syndrome.
Bold figures are statistically significant.
[a] Systolic BP ≥130 mmHg or diastolic BP ≥85 mmHg.
[b] Fasting plasma glucose ≥100 mg/d.
[c] Triglycerides ≥150 mg/dL.
[d] HDL <40 mg/dL (male) or <50 mg/dL (female).
[e] Waist circumference ≥102 cm (male) or ≥88 cm (female).

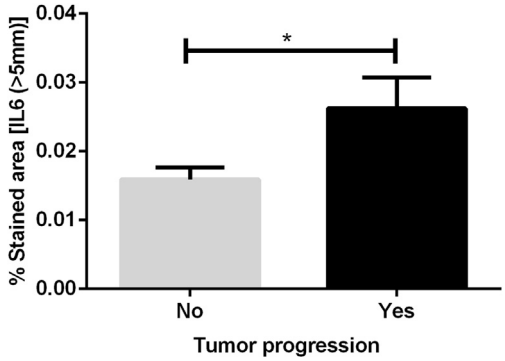

Fig. 4 Percentage of peritumoural area stained for IL-6 at a distance of 5 mm from the tumour until the end of the tissue in GI-NETs, according with tumour progression (Mann–Whitney U test: **p*=0.05).

the extensively described anti-inflammatory properties of HDL.[38,39] Besides that, a higher IL-6 expression in the peritumoural area of GI-NETs was observed in tumours of patients with progressive disease. So, our data further support the role of chronic inflammation in the modulation of GI-NETs behaviour, as previously proposed.[30,40]

Despite the novel findings brought by conducting this study, a few limitations that could impact data interpretation deserve to be considered. Although WD GEP NETs are the second most frequent digestive neoplasia, these tumours are still not very frequent, thus the small number of tumours available for analysis in our series is understandable, in particular given that this originated from a single centre. Indeed, the small numbers in our series represent a limitation to the extent of the conclusions retrieved, as compared to what could be expected if a larger sample multicentre series was available. In the presence of a larger sample size some of the trends observed in this study could eventually reach significance.

Nevertheless, novel pathways of research were unravelled, in particular leading to the need to focus on the detailed characterisation of the role of local and systemic inflammatory status on GI-NETs biology.

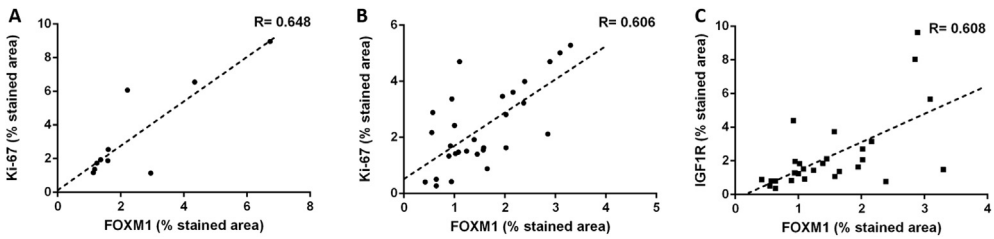

Fig. 5 (A) Correlations between FOXM1 and Ki-67 in pancreatic neuroendocrine tumours. (B) Correlations between FOXM1 and Ki-67 and (C) FOXM1 and IGF1R in gastrointestinal neuroendocrine tumours.

CONCLUSION

The influence of MetS in the molecular inflammatory and metabolic profile of WD GEP-NET was assessed. IL-6 expression in tissues surrounding GI-NETs was influenced by MetS features and positively associated with disease progression. In contrast, FOXM1 and IGF1R expression in WD GEP-NETs were not influenced by MetS. In summary, our findings suggest that the inflammatory status could be a potential mechanism linking MetS and GI-NET in addition to having a putative role in the modulation of GI NET behaviour.

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APPENDIX A. SUPPLEMENTARY DATA

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pathol.2019.07.001.

Address for correspondence: Mariana P. Monteiro, MD, PhD, ICBAS – UP, Building nº1 – 3rd Floor, Rua Jorge Viterbo Ferreira, 228, 4050-313, Porto, Portugal. E-mail: mpmonteiro@icbas.up.pt

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