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PDGFR α expression as a novel therapeutic marker in well-differentiated neuroendocrine tumors

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ABSTRACT

Aims: To evaluate the biological significance of dense vascular networks associated with low-grade NENs, we assessed the impact of PDGFR α tissue expression in 77 GEP/NEN patients, associating PDGFR α expression with the morphological characterization in low-grade tumors.

Methods and results: Paraffin-embedded specimens of 77 GEP- NEN tissues, collected from January 2006 to March 2018, were evaluated for PDGFR α tissue expression and correlations with clinicopathological characteristics. PDGFR α tissue expression was significantly correlated with grade and the NEN growth pattern ($p < 0.001$) but not with gender, primary site or lymph nodes metastatic status. PDGFR α staining was mainly localized in the vascular pole of the neuroendocrine cells and in Enterochromaffin (EC) cells. In particular PDGFR α tissue expression was significantly more expressed in G2 ($p < 0.001$) than G1 and G3 cases ($p < 0.004$; $p < 0.0002$;) and correlated with an insular growth pattern. PDGFR α tissue expression was associated with the Ki67 index and we found a significant negative trend of association with the Ki67 proliferation index ($P < 0.001$): thus PDGFR α expression is referred to morphological and not to proliferative data.

Conclusions: PDGFR α represents an effective target for new anti-angiogenic treatment in WD- GEP-NENs, in particular in G2 cases, and in G3 cases only when there is a mixed insular-acinar pattern. In this context, it is important to carefully delineate those tumors that might better respond to this type of treatment alone or in combination. Further investigation of the relationship between PD-L1 and PDGFR α is warranted, and may contribute to optimize the therapeutic approach in patients with GEP-NENs.

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Introduction

Unlike traditional chemotherapy, biological therapy and immunotherapy represent a turning point and a revolutionary strategy in new oncology. In particular, rare tumors that are difficult to diagnose, such as gastroenteropancreatic neuroendocrine tumors (GEP-NEN), often defined as occult tumors, lack targeted markers and precise therapies.¹ Despite their new classification (2010/2017 WHO), the fact remains that the clinical behavior of NEN is highly unpredictable, so low-grade cases can unexpectedly often present with advanced disease at diagnosis.^{2,3} At present it is not yet possible to define which biological, pathological or clinical characteristics could be able to characterize this ‘low risk’ subset of patients within the G1-G2 group. Current treatments for G1-2 well-differentiated (WD-NEN) tumors include somatostatin analogues (octreotide or lanreotide), platinum-based chemotherapy and targeted therapies but there is no precise definition of the best sequence.^{4–6} In most cases, these treatments have had poor responses. Therefore, in this era of personalized medicine, biomarkers predictive of response to therapy are central to treatment decision making. For this reason, differentiation, anaplasia, proliferative capacity and metastatic tendencies, angiogenesis and immunosuppression are hallmarks of cancer that have

become the focus of close study.⁷ There is accumulating evidence that angiogenesis and immunosuppression are interconnected and facilitated by shared regulators not only during normal physiological processes, but also in cancer development.⁸ Notably, the vascular network, in terms of entity and typology, is an important regulator of the immune response because it controls lymphocyte trafficking⁹ and also acts as a mediator of localized immunosuppression within the tumor microenvironment (TME).¹⁰ The highly vascular nature of low-grade NETs, and hypoxia-dependent angiogenesis in high-grade NETs, led to an initial interest in angiogenesis inhibition as a treatment possibility in this disease.^{11–15} Whereas a high microvascular density is an adverse prognostic marker in most carcinomas, it seems to be a favorable parameter in pancreatic NENs: the intratumoral microvascular density is higher in low-grade than in high-grade tumors and is associated with a good prognosis, prolonged survival and a higher VEGF expression, a phenomenon called the “neuroendocrine paradox”.^{16,17} This paradox can be explained by the observation that cancer produces a poor imitation, like a monkey’s, of the tissue from which it derives. With increasing aggressiveness, WD NEN can have more vessels, as in normal tissue, compared to PD NEN. However, differences in the vascular architecture of pNENs and GI-NENs are likely associated with

significant differences in the structural and functional relationships between tumor cells and vessels.^{18–20} Several pathways for tumor growth and tumor angiogenesis, including the VEGF, fibroblast growth factor (FGF), and platelet-derived growth factor (PDGF) pathways that have recently been identified, have attracted substantial interest due to their potential clinical applications.^{21,22} In particular, the PDGF family is known to be associated with tumor growth in different kinds of tissues, pathological conditions and angiogenesis through two cell-surface tyrosine kinases receptors, PDGFR α and PDGFR β , and to induce angiogenesis by up-regulating VEGF production and modulating the proliferation and recruitment of perivascular cells.²³ Furthermore, PDGFR α is believed to mediate multiple cellular behaviors such as migration, proliferation and cell survival via activation of several downstream pathways including MAPK (Erk1/Erk2), PI3K/Akt and PLC γ /PKC.^{24,25} A number of studies have illustrated the importance of PDGF α and PDGF β in the recruitment of tumor stroma in skin cancer, melanoma, breast cancer, and lung cancer.^{26–28} Several studies highlighted that PDGFs/PDGFRs are often overexpressed in different tumors including colorectal cancer and their expression is associated with diagnosis,^{29,30} tumor growth,³¹ drug resistance,³² invasion, and poor survival.³³ Clearly, GEP-NENs are capable of secreting a wide array of active growth factors which interact with tumour and its surrounding stroma in a complex autocrine and paracrine fashion. By identifying these key members of the growth factor pathway, novel therapeutic targets may be discovered. Many studies have provided that PDGF ligands are expressed by cancer cells, whereas PDGF-Rs are expressed mainly by stromal cells.^{34,35} In particular PDGFR- α has been found on both tumour cell and its surrounding stroma, while PDGFR- β was only found in the stroma.^{36–38} Moreover the distinction between PDGFR-A and PDGFR-B signaling is increasing with the discovery that PDGFR-A, not PDGFR-B, is localized and activated in a specialized structure on the cell membrane, the primary cilium, where PDGFR-A can activate MAPK and Akt signaling.^{39,40} Mutational activation in platelet-derived growth factor receptor alpha (PDGFRA), and stem cell factor receptor (KIT) has been assessed in neuroendocrine tumors to identify biomarkers that could potentially indicate the response of these neoplasms to chemotherapeutics developed against those targets. A good response was obtained in GIST and in other cancers.^{41,42} PDGF and its receptors (PDGFRs) are also expressed in 70% of carcinoid tumors^{37,43} and have been found on both tumor cells and stroma of carcinoid tumors, indicating a possible autocrine loop supporting tumor growth. Given the common neuro-oncogenic nature between GIST and NEN we deemed appropriate to evaluate the expression of PDGFR α in NENs. Moreover, apart from the autocrine mechanism, recent studies revealed a critical role for paracrine PDGF signaling in carcinogenesis through the regulation of epithelial-stromal interactions.⁴⁴ Paracrine PDGF signaling is commonly observed in epithelial cancers, where it triggers stromal recruitment and may be involved in the epithelial–mesenchymal transition phenomenon, thereby affecting tumor growth, angiogenesis, invasion, and

metastasis.^{45–47} Further systematic investigations in this promising field may lead to the discovery of novel therapeutic targets. Here we provide evidence of the impact of PDGFR α tissue expression on the grade and growth pattern of 77 GEP/NEN patients, associating PDGFR α expression with the morphological characterization and with low-grade neuroendocrine tumors and their vasculature pattern. Thus, anti-VEGF/PDGFR α drugs administration may become the pharmacological choice for WD-NENs, using a higher PDGFR α immunostaining pattern as a marker in tumors that show a morphologically bland appearance.

Patients and methods

Patients characteristics

Paraffin-embedded specimens of 77 GEP-NEN tissues were collected from January 2006 to March 2018 at the IRCCS ‘Saverio de Bellis’ of Castellana Grotte (Ba, Italy). The following clinicopathological characteristics were collected for all patients: age, gender, primary site, tumor grade.

Pathological assessment

Histology was assessed in all tumors by two pathologists who reviewed FFPE tissue sections stained with hematoxylin and eosin (H&E), and a representative paraffin block from each specimen was chosen for IHC analysis. On H&E and PAS mucin-stained sections, the cytological characteristics of cells, growth patterns, the presence of ulcerations, perineural infiltration, vascular permeation and the presence of necrosis were evaluated. The specimens were classified according to the WHO 2010 and to the last WHO 2017 only for p-NENs. Morphologically, WD and PD-NENs were also distinguished separately according to the following growth pattern criteria to better analyze differences due to the vascular pattern. In detail, according to Soga and Tazawa,⁴³ the architectural pattern of growth was subclassified as follows: insular solid, trabecular, acinar and poorly-differentiated type.

IHC and IHC evaluation

IHC analysis for PDGFR α was performed in the FFPE of 77 patients with NENs. Tumor sections of 4 μ m were freshly cut and dried at 60 °C for 30 min. IHC analysis was carried out in sections after deparaffinization for 30 min and then rehydration in grades of alcohol. Antigen retrieval was performed at 90°C for 20 min with CitratoBuffer. To assess the PDGFR α staining employed for the present study, antibodies (clone C-20 Santa Cruz Biotechnology, Inc. CA, at 1:100 dilution) were evaluated on the NENs, using an automated autostainer (cat. K5007, Dako, Glostrup, Denmark). The Real Envision DAB Substrate Kit (DAKO) was used according to the manufacturer’s instructions. The staining pattern of PDGFR α antibodies was as expected: cellular membranous/submembranous and cytoplasmic. PDGFR α expression was scored for all staining patterns, according to both the staining intensity and the percentage of positively stained cells, by two independent, blinded pathologists; discrepancies in the interpretation of scoring were resolved by

consensus. PDGFR α expression was scored: 0: (no staining) negative; 1: weak expression, but weaker than the positive control, staining in <5 % of tumor cells; 2: moderate expression in \geq 5 % of tumor cells; and 3: strong; more than control membrane staining in >5 % of the tumor cells. For the data assessment, our cases were considered positive for PDGFR α expression only if they had scores of 2+ or 3+.

Statistical analysis

Correlations between PDGFR α expression and clinicopathological parameters were determined by Chi-square test. A p-value less than 0.05(*) was considered statistically significant. Associations of the PDGFR α score and Ki67 index were analyzed with the Kruskal-Wallis rank test; Wilcoxon rank-sum (Mann-Whitney) test. All evaluations were performed using StataCorp. 2007 Stata Statistical Software: release 10 (StataCorp LP, College Station, TX, USA).

Results

Patient population

The characteristics of the 77 cases of NENs are summarized in Table 1. Patients median age was 68 years (range: 24–96): there were 37 females (48.05%) and 40 males (51.95%). Primary sites included the stomach (27.27%), small intestine (29.87%), liver (12.99%), gallbladder (2.60%), colon rectum (7.79%) appendix (6.49%) and pancreas (12.99%). The cases included are classified, according to 2010/2017 WHO, as follows: 50 grade 1 (64.94%), 12 grade 2 (15.58%) and 15 grade 3 (19.48%).

The architectural growth pattern was subclassified as follows: 31 insular solid type (40.26%), 14 trabecular type (18.18%), 17 acinar type (22.08 %) and 13 poorly-differentiated type (16.88%). Among PD- NEN, we highlighted 2 (2.60%) patterns with a mixed insular-acinar form (Table 1). Although different architectural types may be related to the different site of origin, we

Table 1. Clinicopathologic features of 77 patients with NEN.

	n = 77
Gender (M) (%)	40 (51.95)
Age (yrs) (M \pm SD)	65.31 \pm 14.69
Median, (min-max)	68 (24–96)
Localization	
Stomach	21 (27,27)
Small Intestinal	23 (29,87)
Colon- rectum	6 (7,79)
Appendix vermiforme	5 (6,49)
Pancreas	10 (12,99)
Liver	10 (12,99)
Gallbladder	2 (2,60)
Grade (%)	
G1	50 (64,94)
G2	12 (15,58)
G3	15 (19,48)
Pattern Growth (%)	
Acinar	17 (22,08)
Insular	31 (40,26)
Trabecular	14 (18,18)
Poorly differentiated	13 (16,88)
Angioinvasion	
Absent	66 (86,00)
Present	11 (14,00)
Lymphocytic infiltration	
Absent	66 (86,00)
Present	11 (14,00)

Table 2. Different architected types related to site of origine.

Localization	Growth Pattern			Total n (%)	p-value*
	Acinar n (%)	Insular n (%)	Trabecular n (%)		
Stomach	2 (11.76)	9 (29.03)	7 (50.00)	18 (29.03)	0.115
Small Intestinal	5 (29.41)	13 (41.94)	3 (21.43)	21 (33.87)	
Colo-rectum	0 (-)	3 (9.68)	1 (7.14)	4 (6.45)	
Appendix	2 (11.76)	1 (3.23)	2 (14.29)	5 (8.06)	
Pancreas	4 (23.53)	4 (12.90)	1 (7.14)	9 (14.52)	
Liver	2 (11.76)	1 (3.23)	0 (-)	3 (4.84)	
Gallbladder	2 (11.76)	0 (-)	0 (-)	2 (3.23)	

highlighted a low association between type and origin (p 0.115; Table 2). In particular, in our cases, stomach neoplasms are mostly of trabecular type (50.00%), small intestine and colon rectum are frequently of insular type (41.94%; 9.68 %), whereas pancreas, liver and gallbladder neoplasms are mostly of acinic type (23.53%; 11.76%; 11.76 %) while the appendix has a mixed pattern between acinic (11.76%) and trabecular type (14.29%) (Table 2).

PDGFR α expression in nens

To evaluate the biological significance of dense vascular networks associated with low-grade NENs, we analyzed the PDGFR α tissue expression in our 77 NENs. Among these, 18 cases resulted negative (23.4%) and 59 cases positive (76.6%). The relationship between PDGFR α expression and gender was not significant (P = 0.47). Our analysis showed that PDGFR α tissue expression was significantly correlated with grade (p < 0.001; Table 3) but not gender, primary site or lymph nodes metastatic status. The PDGFR α staining intensity score on neoplastic cells was different among the three WHO grades. Based on the PDGFR α signal intensity, we created a score (Table 4) from 0 to 3+ (absent to strong). Importantly, PDGFR α expression was absent in 13 G3 patients (86.67%), while in G2 PDGFR α expression was present in all cases, although with a different intensity: medium staining in 3 (25%) cases and strong in 7 (58%) cases (Table 3). These data indicate that PDGFR α tissue expression was significantly stronger in G2 than G1 and G3 cases (p 0.004; p < 0.0002; Table 4). Furthermore, a statistically significant difference in PDGFR α expression was observed between the NEN growth pattern and grade, specifically in insular type G2 and G1 cases. G1 cases with an insular type pattern had a score of 2+ (60.71%) while the rest (6 patients) had a score of 3+ (100%). G2 patients showed a medium (2+) PDGFR α signal in 66.67 % and strong (3+) in 85.71 % of

Table 3. PDGFR α expression on different grade of NEN.

	Categories PDGFR α		p-value*
	Absent (n = 18)	Present (n = 59)	
Gender (M) (%)	8 (44.44)	32 (54.24)	0.47
Grade (%)			<0.001
G1	5 (10.00)	45 (90.00)	
G2	0 (0.00)	12 (100)	
G3	13 (86.67)	2 (13.33)	

*Chisquare-test.

Table 4. PDGFR α expression on different grade of NEN.

	Grade			p-value*	Comparisons (p-value) [§]		
	G1	G2	G3		(b)vs(a)	(c)vs(a)	(c)vs(b)
	(a)	(b)	(c)				
Score PDGFR α (%)				<0.001			
Absent	5 (10.00)	0 (0.00)	13 (86.67)		0.02	<0.0001	<0.0001
Weak	11 (22.00)	2 (16.67)	0 (0.00)		0.67	0.0004	0.14
Medium	28 (56.00)	3 (25.00)	2 (13.33)		0.04	0.0004	0.46
Strong	6 (12.00)	7 (58.33)	0 (0.00)		0.004	0.01	0.0002

*Chisquare-test

^Fisher's exact test

§Test z for proportions.

cases. In Insular pattern cases PDGFR α expression was higher than in acinar and trabecular patterns, and the insular type was significantly associated with a medium and strong PDGFR α ($p < 0.001$; Table 5; Figure 1). Among poorly-differentiated NEN (G3), only 2 (2.60%) cases expressed a pattern like that of the mixed insular-acinar form and had a score 2+, while in 97.40 % of G3 cases PDGFR α expression was absent. These data indicate that PDGFR α tissue expression is correlated with an insular growth pattern (Figure 2A–B) and that the PDGFR α signal is lost with the reduction and loss of differentiation. In particular, we highlighted PDGFR α staining mainly localized in the vascular pole of the neuroendocrine cell (Figure 2C–D), suggesting a peculiar functional role, as occurs for hormonal products. In insular type cases we observed emargination due to the polarization of the cells and a more intense positivity at the invasive tumor front than in the center of tumors where necrosis is often present (Figure 2B). Moreover, we detected a ahigh expression of PDGFR α in Enterochromaffin (EC) cells with a finely granular but intense positivity, with peripheral-membranous enhancement at the vascular pole of neuroendocrine cells (Figure 3). PDGFR α tissue expression was associated with Ki67 staining. We found a significant negative trend of

Table 5. PDGFR α intensity score on neoplastic cells.

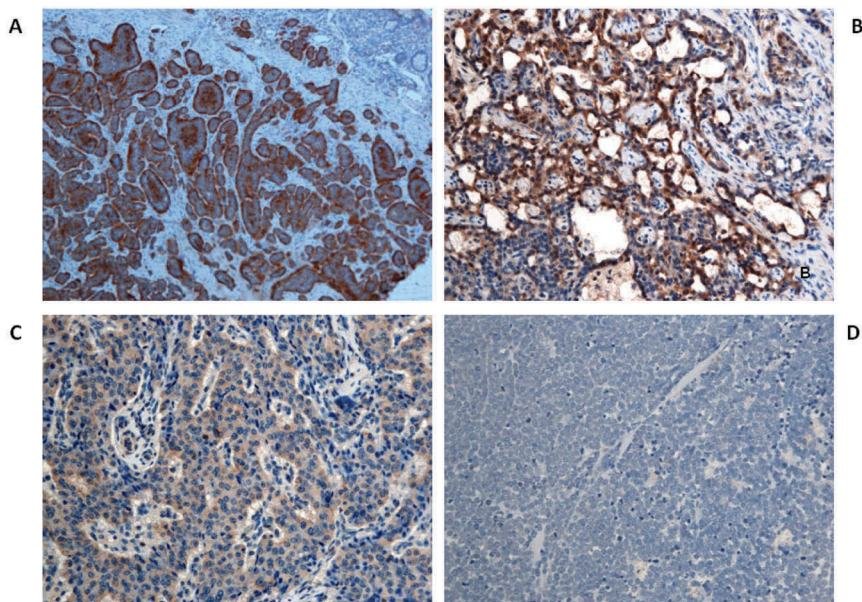
	Score PDGFR α				p-value*
	Absent (0)	Weak (1+)	Medium (2+)	Strong (3+)	
Grade (G1)					
Pattern Growth (%)					<0.001 ^
Acinar	1 (20.00)	3 (27.27)	11 (39.29)	0 (0.00)	
Insular	0 (0.00)	0 (0.00)	17 (60.71)	6 (100)	
Trabecular	4 (80.00)	8 (72.73)	0 (0.00)	0 (0.00)	
Grade (G2)					
Pattern Growth (%)					0.05 ^
Acinar	0 (0.00)	0 (0.00)	1 (33.33)	1 (14.29)	
Insular	0 (0.00)	0 (0.00)	2 (66.67)	6 (85.71)	
Trabecular	0 (0.00)	2 (100)	0 (0.00)	0 (0.00)	
Grade (G3)					
Pattern Growth (%)					0.01 ^
Poorly differentiated	13 (100)	0 (0.00)	0 (0.00)	0 (0.00)	
Pattern like insular-acinar	0 (0.00)	0 (0.00)	2 (100)	0 (0.00)	

*Chisquare-test

^Fisher's exact test

§Test z for proportions.

association with the Ki67 proliferation index ($P < 0.001$): as the PDGFR α score increased, so the Ki67 index decreased, therefore PDGFR α is referred to morphological and non-proliferative data (Figure 4)

**Figure 1.** Representative patterns of PDGFR α in different architectural types: A) insular solid type B) acinar type C) trabecular type D) poorly-differentiated type. (magnification 20 \times).

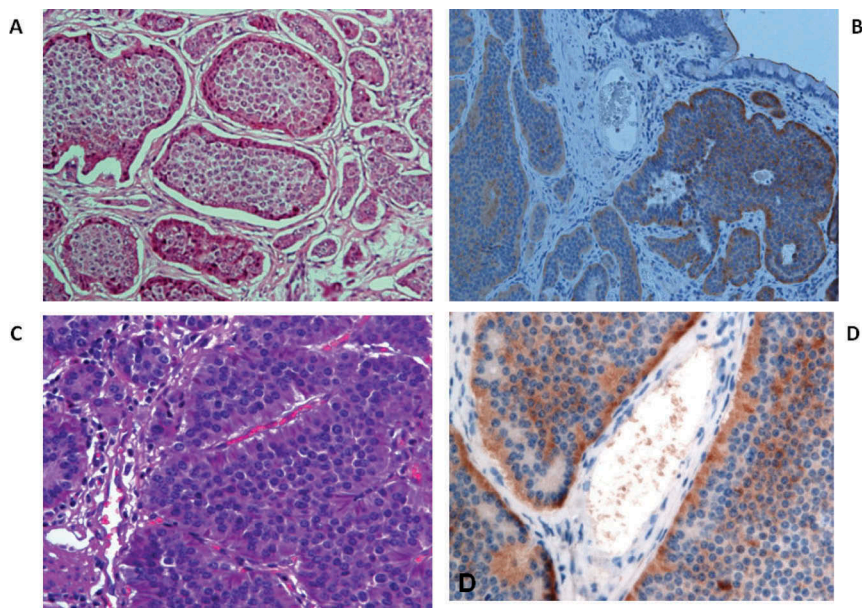


Figure 2. A-B) PDGFR α tissue expression in the insular solid growth pattern C-D) PDGFR α staining localized in the vascular pole of the neuroendocrine cell.

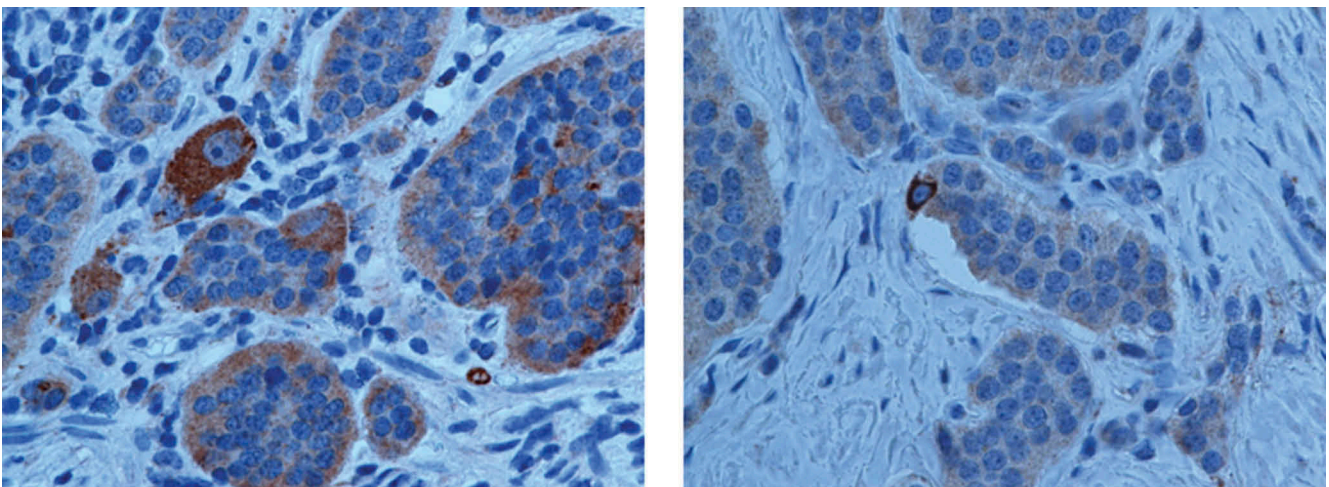


Figure 3. Representative PDGFR α staining in Enterochromaffin (EC) cells with a finely granular positivity with peripheral-membranous enhancement at the vascular pole of neuroendocrine cells.

Discussion

Angiogenesis, like immunosuppression, is a crucial step in the development, invasiveness and malignant behavior of numerous solid tumors, and in many cases angiogenic regulation under the control of VEGF, FGF and PDGF plays a central role that may be strategically exploitable in the therapeutic setting. It has been widely demonstrated that VEGF and PDGF participate in changing the vascular permeability of endothelial cells and hence the tumor malignant potential expressed as metastatic power.⁴⁸ As one of the peculiar features of GEP-NETs is a prominent hypervascularization of tissue, this offers an attractive target for anti-angiogenic treatment. The data available about gastrointestinal NENs are scarce and contradictory,^{18,20} and the relation between angiogenic factors (VEGF, PDGF, FGF), density, typology of the

tumor vascular network and prognosis in GI-NENs cases is not straightforward. In the present study we investigated the impact of PDGFR α tissue expression on the grade and growth pattern of 77 GEP/NEN patients, associating PDGFR α expression with the morphological characterization of low-grade neuroendocrine tumors.

Low grade neuroendocrine tumors (G1-G2) have an extraordinary vascularization which is associated with the expression of many proangiogenic factors mimicking normal endocrine organ products. GI-NENs secrete a number of biologically active vascular endothelial growth factors that interact with the tumor cells and the surrounding stroma in a complex autocrine/paracrine fashion. In NENs, intratumor vessel density is associated with a good prognosis and prolonged survival, unlike in other digestive tumors. Recent

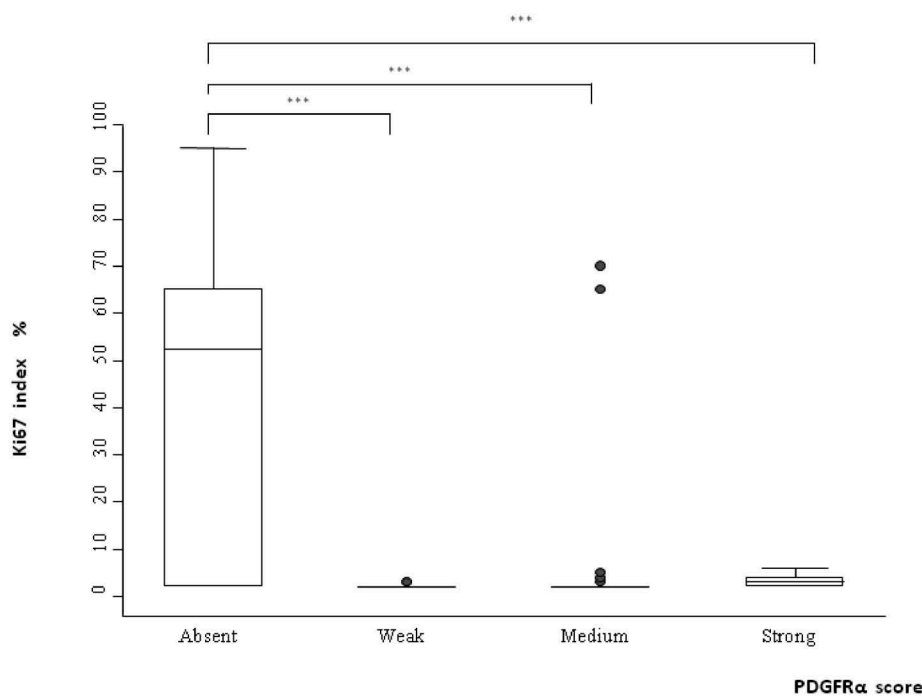


Figure 4. Association between the PDGFR α score and Ki67 index in NENs patients ($p < 0.001$): Box plots of PDGFR α score and Ki67 index (expression %) *** = $p < 0.001$.

studies revealed a critical role for paracrine PDGF signaling in carcinogenesis through the regulation of epithelial-stromal interactions, thereby affecting tumor growth, angiogenesis, invasion, and metastasis. Our results indicate, for the first time, that PDGFR α tissue expression was significantly correlated with grade and, in particular, was more expressed in G2 than G1 and G3 cases ($p = 0.004$; $p < 0.0002$). NENs with stronger PDGFR α staining (G1-G2) were less aggressive than weaker PDGFR α staining NENs, confirming the “neuroendocrine paradox” already observed for pNENs and their vascular pattern.¹⁶ Therefore, PDGFR α tissue expression is correlated with an insular growth pattern and the PDGFR α signal is lost with the reduction and loss of differentiation; in fact, in 97.40 % of our G3 cases PDGFR α expression was absent. The reduction in PDGFR α expression was associated with a reduction in microvessel density and tumor growth. To demonstrate that PDGFR α plays an important mediator role in the epithelial – stromal-interaction we highlighted PDGFR α staining mainly localized at the vascular pole of the neuroendocrine cell with a marked emargination due to the polarization of the cells along the vessels; we also evidenced a more intense positivity at the invasive tumor front than in the center of the tumors, where necrosis is more often present. Considering that currently the marker of choice to better classify these tumors remains the ki67 index, along with the morphological characterization, we evaluated the association between ki67 and PDGFR α . In particular, we found a negative trend of association of Ki67 with the proliferation index ($P < 0.001$). Thus demonstrates that PDGFR α is referred to morphological and not to proliferative data. The functional importance of anti-vascular endothelial growth factor in tumor angiogenesis and in immunosuppression has

provided a convincing rationale for the development of inhibitors targeting the VEGF signaling pathway.⁴⁹ Targeted therapy with the vascular endothelial growth factor pathway inhibitor sunitinib and the mTOR inhibitor everolimus has been shown to improve progression-free survival in patients with progressive pancreatic but not midgut NENs,^{50,51} although this success had only a limited impact on overall survival of cancer patients and rarely resulted in durable responses.⁵² WD-GEP-NEN are characterized by an unpredictable clinical course, thus the association between PDGFR α expression and the morphological characterization of low-grade neuroendocrine tumors defines PDGFR α as an active participant in tumorigenesis. This should drive the development of novel therapies aimed at reducing tumor growth, possibly suppressing PDGFR α production. Angiogenic agents as drugs targeting the stroma of different cancers will become very important in the future due to the recent observation and publication of data about a “new organ”, the so-called “interstitium” that represents the ubiquitous stromal scaffold of all human systems, and plays an active role, not only offering passive support in carcinogenesis.⁵³ We recognize that our 77 NENs cases, being rare tumors, are necessarily a small cohort, but the remarkable incidence of PDGFR α positivity obtained in our patients with grade G2 disease warrants larger future studies. Given the recent success of immunotherapies alone or combined in some tumors, antiangiogenic treatment associated with immune checkpoint blockers has become an attractive strategy in GEP- NEN. In our previous work we highlighted the role of programmed cell death ligand 1 (PD-L1) in GEP-NEN:⁵⁴ PD-L1 expression was significantly associated with a high-grade WHO classification (G3), becoming the new gold standard for G3 NEN

discrimination. Furthermore, pharmacological approaches using anti-PD-1 antibodies may become the logical choice for the treatment of G3 cases with a poor prognosis, while for G2 cases anti-angiogenic drugs could be an excellent therapeutic choice. A recent work highlighted the fact that targeted antiangiogenic and immune-based therapies have improved outcomes in advanced kidney cancer, yet novel strategies are needed to extend the duration of these benefits and extend them to more patients and tumor varieties.⁵⁵ Therefore, targeting the tumor vasculature to induce vessel normalization may provide a promising strategy to optimize the efficacy of currently employed immunotherapies as it could reduce the level of immunosuppression in the tumor microenvironment (TME). In this regard, since NEN are notoriously clinically and biologically heterogeneous tumors resulting in unpredictable clinical outcomes, it is clear that to exploit the full potential of the immune system to cure cancer, we will have to act at multiple levels in order to ‘normalize’ the TME. Furthermore, based on these observations, treatment with a simultaneous combination of anti-PDGFR α and anti-PD-L1 antibodies may yield durable responses in all GEP-NEN cases. Moreover, PDGFR α might be a useful prognostic biomarker in WD- GEP-NENs. At present, it seems that G2 tumors might be the most suitable targets. In this context, it is important to carefully delineate those tumors that might better respond to this type of treatment. Further investigation of the relationship between PD-L1 and PDGFR α is warranted, and may contribute to optimize the therapeutic approach in patients with GEP-NENs.

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Conflict of Interest

The authors declare no conflict of interest related to this study. **Compliance** with ethical standards.

Notes on contributor

Elisabetta Cavalcanti: study design, data interpretation, evaluation, writing and editing of the manuscript. A. Ignazzi: assembly of tissue and data, study design and immunohistological analysis. F De Michele: histological evaluation. ML Caruso: evaluation and editing of the manuscript. All authors read the manuscript and approved its submission.

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