

# Endocan expression is correlated with poor progression-free survival in patients with pancreatic neuroendocrine tumors

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## Abstract

Endocan expression has been reported to be associated with aggressive tumor progression and poor outcomes in various cancers, such as breast cancer, renal cell cancer, lung cancer, gastric cancer, and pituitary adenomas. However, the prognostic significance of endocan in neuroendocrine tumors remains unknown. Thus, the aim of this study was to determine the correlation between endocan expression in pancreatic neuroendocrine tumor (PNET) tissues and progression-free survival. This study included 73 patients with confirmed PNETs who were treated in a single tertiary center in north Taiwan between 1992 and 2015. Immunohistochemical endocan expression and microvessel density (MVD) were examined, and the relationships between these parameters and other clinicopathological characteristics were analyzed. The abovementioned patients were divided into groups according to their endocan expression levels ( $\geq 1\%$  or  $< 1\%$ ) and median MVDs. Negative endocan expression ( $P = .002$ ) and a high MVD ( $P < .001$ ) were significant and favorable prognostic factors for progression-free survival. However, positive endocan expression was significantly associated with a low MVD ( $P = .037$ ) and tumor mitosis (Ki-67 index) ( $P = .028$ ). Multivariate Cox regression analysis showed that positive endocan expression (hazard ratio: 4.778,  $P = .018$ ) and lymph node involvement (hazard ratio: 5.121,  $P = .005$ ) were independent prognostic factors for tumor recurrence.

In conclusion, endocan expression was correlated with poor clinical outcomes in PNETs. Our data indicated that endocan expression may be a reliable marker for predicting tumor recurrence in patients with PNETs.

**Abbreviations:** BMI = body mass index, MVD = microvessel density, PNET = pancreatic neuroendocrine tumor.

**Keywords:** endocan (endothelial specific molecule-1), microvessel density, pancreatic neuroendocrine tumor, progression-free survival

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## 1. Introduction

Pancreatic neuroendocrine tumors (PNETs), which arise from the endocrine cells of the pancreas and exhibit unique genetic, biological, and prognostic characteristics compared with those of pancreatic adenocarcinomas, have become an important clinical problem.<sup>[1,2]</sup> PNETs constitute 1% to 3% of all pancreatic neoplasms and have one of the lowest 5-year survival rates of all gastroenteropancreatic neuroendocrine tumors. Specifically, these tumors are associated with 5-year-survival rates ranging from 42% to 71%, and nonfunctioning PNETs and advanced tumor stages are associated with worse prognoses than functioning PNETs and less advanced tumor stages.<sup>[3–5]</sup> PNETs and neuroendocrine tumors from other sites share common histologic features; however, it has become increasingly apparent that PNETs and other neuroendocrine tumors differ with respect to their molecular pathogenesis, clinical behavior, and responses to certain therapies.<sup>[6,7]</sup> The incidence and prevalence of PNETs have steadily increased over the past 30 years.<sup>[2]</sup> However, much about PNET progression and prognosis remains unknown.<sup>[8]</sup>

The evolution of PNET staging and grading systems are still continuously progressing with neuroendocrine tumors of the gastro-enteropancreatic tract (GEP-NETs).<sup>[9]</sup> According to the WHO 2010 classification, PNET can be classified into 3 groups: neuroendocrine tumor 1 (NET G1), NET G2, and neuroendocrine carcinoma (NEC G3) based on their mitotic counts, which are determined by hematoxylin-eosin staining and Ki-67 indices of tumor tissues. However, another staging systems based on the TNM staging system have been recommended by the European Neuroendocrine Tumor Society (ENETS) and the American Joint

Committee on Cancer (AJCC) used for ductal adenocarcinoma of the pancreas and divided PNETs into 4 groups.<sup>[10,11]</sup> These 2 different staging systems are widely accepted for PNETs approaching by physicians; however, they have not proved their impact on the prognosis in large follow-up series of patients with PNET. As a result, the clinic-therapeutic management of PNETs patients suffered the lack of universally accepted standards for both classifications and staging of the disease.

Endocan (endothelial cell specific molecule- 1; ESM-1) was originally cloned from a human endothelial cell cDNA library in 1996,<sup>[12]</sup> and is expressed by the vascular endothelium and circulates freely in the bloodstream of healthy subjects. Experimental evidence indicates that endocan plays a key role in regulating major physiologic and pathophysiologic processes, such as cell adhesion, inflammation, and tumor progression.<sup>[13]</sup> Endocan may play different roles in different types of tumors. Endocan expression was upregulated in cancers derived from the lung, kidney, brain, astrocytes, and liver compared with normal control tissues.<sup>[14–16]</sup> This upregulation was correlated with poor survival in patients with the indicated diseases.<sup>[17,18]</sup> Moreover, *in vitro* data, as well as *in vivo* data, have demonstrated that intracellular human endocan plays functional roles in regulating cell growth and facilitating tumor growth.<sup>[19]</sup> However, the specific biological role of endocan in PNETs has not yet been determined.

Thus, the aim of this study was to examine endocan expression in patients with PNETs and to determine the associations between endocan expression and clinicopathological characteristics and progression-free survival.

## 2. Methods

### 2.1. Patient clinicopathological data

Patients with PNETs, as diagnosed by pathologic examination at Taipei Veterans General Hospital between 1992 and 2015, were included in the study. Patients who were unable to provide informed consent were excluded from the study, which was approved by the Ethics Committee of Taipei Veterans General Hospital (2013-11-014CC) and was conducted in accordance with the principles of the Declaration of Helsinki and Title 45, U. S. Code of Federal Regulations, Part 46, Protection of Human Subjects, revised November 13, 2001, effective December 13, 2001. Clinical data, including data regarding disease recurrence and progression-free survival, and pathologic data were obtained through detailed reviews of the medical records of 73 patients with PNETs who had undergone initial surgical or diagnostic tissue sampling procedures. The median age of these patients was 55 years (range 19–86, mean 52.8 years), as summarized in Table S1, <http://links.lww.com/MD/B900>. Follow-up data were available for all patients, and the length of the follow-up period ranged from 0.7 to 263 months (mean 87.5 months). During the follow-up period, 14 patients presented with evidence of disease progression, and 11 patients died. However, only 5 patients were found to have tumor-related causes of death. Clinical data were most recently collected on December 31, 2015.

### 2.2. Tissue specimens

The pathologic specimens used herein were obtained via surgical resections performed in Taipei Veterans General Hospital. After surgery, these specimens were fixed in 10% formalin and embedded in paraffin before being stored for later use. The formalin-fixed paraffin-embedded specimens were eventually retrieved from the archives of the Department of Pathology,

Taipei Veterans General Hospital, for use in this study. Thirty-three tissue samples were obtained from our previous tissue microarrays, which were constructed by obtaining three 1-mm-diameter cores from each tumor tissue specimen and paired adjacent normal pancreatic islet cell tissue specimen.<sup>[20]</sup>

### 2.3. Immunohistochemical staining for endocan and CD34

Immunohistochemical staining was performed using monoclonal anti-human antibodies to endocan (cat#:MEP08, dilution 1:100; Lunginnov, Lille, France) and anti-CD34 antibodies (clone QBEnd-10, dilution 1:75; Dako, Glostrup, Denmark), which were used to measure microvessel density (MVD), on tissue slides. We used archived specimens, which had been fixed in formalin and embedded in paraffin before being archived, for immunohistochemical staining, which was performed with a Bond-Max autostainer (Leica Microsystems, Wetzlar, Germany).

### 2.4. Evaluation of endocan expression

Endocan-positive cancer cells exhibited brown cytoplasmic staining. The cell populations were classified into 2 groups as follows: cell populations in which <1% of neoplastic cells discretely expressed endocan were classified into the negative (-) endocan expression group, and cell populations in which  $\geq 1\%$  of morphologically unequivocal neoplastic cells discretely expressed endocan were classified into the positive (+) endocan expression group.<sup>[21]</sup>

### 2.5. Evaluation of intratumoral MVD

All independent CD34-positive vascular structures were included in this analysis irrespective of whether they possessed an identifiable lumen. The numbers of CD34-positive structures were counted in 5 consecutive high-power fields at a magnification of 400X (0.238 mm<sup>2</sup> per field), and the MVD of each tumor was calculated as the number of CD34-positive vascular structures per square millimeter. The PNETs were divided into the following 2 groups on the basis of the median MVD: a low MVD group, in which the actual MVD was lower than the median MVD, and a high MVD group, in which the actual MVD was equal to or higher than the median MVD.<sup>[20,22,23]</sup>

### 2.6. Statistical analysis

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) software (Version 22.0; SPSS Inc., Chicago, IL). All data were expressed as the mean  $\pm$  standard deviation (SD) or as frequencies (percentages). Parametric continuous data were compared between groups using unpaired Student *t* tests, and nonparametric data were compared between groups using the Mann-Whitney *U* test. Categorical data were compared between groups using Chi-square tests with Yates' correction or Fisher exact test, where appropriate. The associations between clinicopathological factors and tumor recurrence were assessed using Cox proportional hazards regression models. A 2-sided *P* value < .05 was indicative of statistical significance.

## 3. Results

### 3.1. Endocan expression in PNETs is associated with a poor prognosis

The prognostic significance of endocan expression was determined by assessing the cytoplasmic staining patterns of 61 human

**Table 1**

**The relationships between endocan expression and PNET clinicopathologic characteristics.**

	Endocan (-) (n=43)	Endocan (+) (n=18)	P
Age, y	52.5 ± 16.2	52.7 ± 16.6	.968
Male, n (%)	25 (58.1%)	12 (66.7%)	.534
BMI, kg/m <sup>2</sup>	23.0 ± 7.6	23.3 ± 3.0	.876
Glucose, mg/dL	116.0 ± 55.9	103.9 ± 49.4	.450
Total cholesterol, mg/dL	162.6 ± 36.8	178.9 ± 46.6	.206
Triglyceride, mg/dL	101.6 ± 86.5	106.4 ± 42.4	.837
Functional status			
Present, n (%)	19 (44.2%)	7 (38.9%)	.926
Insulinoma	16	6	
Gastrinoma	3	1	
Classification according to WHO 2010			
Neuroendocrine tumor Grade 1	27	4	.012
Neuroendocrine tumor Grade 2	14	11	
Neuroendocrine cancer Grade 3	2	3	
Tumor size status			
T1	16	5	.051
T2	17	5	
T3	4	7	
T4	6	1	
Lymph node status			
N0	35	11	.093
N+	8	7	
Distant metastasis			
M0	37	15	.785
M1	6	3	

BMI=body mass index.

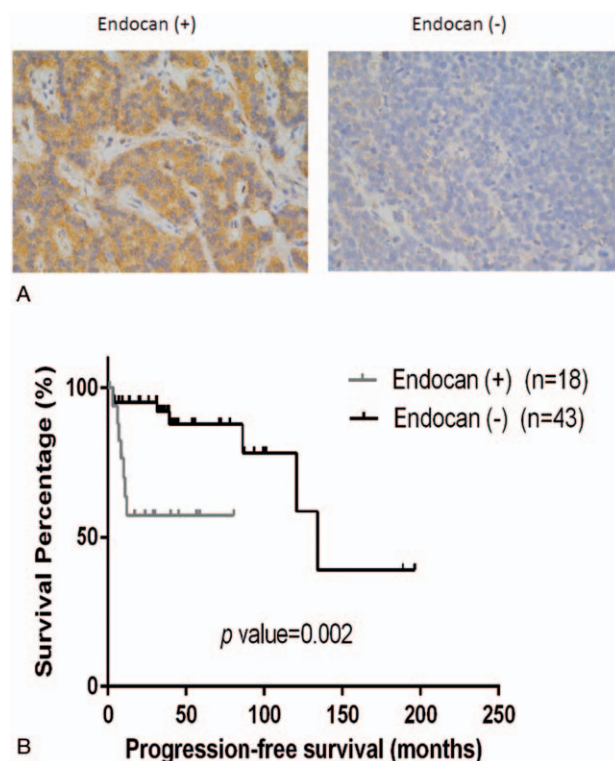
PNET specimens and the same number of adjacent normal tissue specimens. All normal islets of Langerhans in the noncancerous tissue specimens demonstrated negative endocan expression. The relationships between endocan expression levels and PNET clinicopathologic characteristics are summarized in Table 1, and Fig. 1A shows representative examples of tissue specimens exhibiting positive endocan expression. Kaplan–Meier survival analysis showed that positive endocan expression was strongly correlated with reduced progression-free survival compared with negative endocan expression, as shown in Fig. 1B ( $P=.002$ ). Moreover, our data indicate that endocan expression was predictive of a poor prognosis in PNETs.

### 3.2. A high MVD in PNETs is associated with a good prognosis

The prognostic significance of MVD was determined using 55 human PNET specimens obtained from patients with clinical follow-up data. Figure 2A shows representative examples of specimens with different MVDs. The PNETs were divided into 2 groups on the basis of the median MVD. The high MVD group showed longer progression-free survival than the low MVD group (Fig. 2B,  $P<.001$ ).

### 3.3. Endocan expression levels were correlated with a low MVD and the Ki-67 index

We next examined the potential associations between endocan expression levels and MVD. We found that 42.9% of samples with positive endocan expression exhibited a low MVD, whereas 85.2% of samples with negative endocan expression exhibited a



**Figure 1.** Endocan is expressed in tumors and is correlated with a poor prognosis. (A) Endocan expression in a representative PNET tissue specimen, as demonstrated by immunohistochemical staining (400X). (B) Kaplan–Meier plot of the progression-free survival of 61 patients with PNETs stratified according to their endocan expression levels. The patients were divided into 2 groups according to their endocan expression levels.

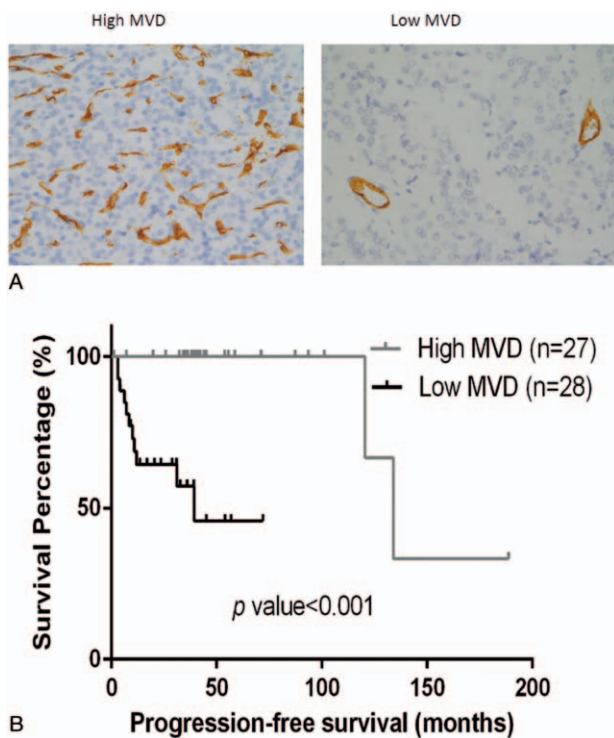
high MVD (Table 2;  $P=.037$ ). We also found that endocan expression was positively correlated with the Ki-67 index (Table 3;  $P=.028$ ). The Ki-67 index is a scoring system that measures cell growth and proliferation<sup>[24]</sup> and has been shown to predict biological behavior, chemotherapy responses, and survival in patients with different types of tumors, including neuroendocrine tumors.<sup>[25]</sup> Taken together, these findings indicate that endocan expression in PNETs is associated with a lower MVD and increased tumor mitosis; thus, positive endocan expression is associated with greater malignant potential.

### 3.4. Endocan expression was an independent risk factor for PNET recurrence

We subsequently investigated the associations between clinicopathological characteristics, tumor endocan expression levels and MVD, and the risk of tumor recurrence. Univariate analysis showed that positive endocan expression, lymph node involvement, and tumor metastasis displayed increased hazard ratios for tumor recurrence, and Cox regression analysis showed that positive endocan expression is an independent risk factor for PNET recurrence [hazard ratio: 4.778 (95% confidence interval: 1.307–17.457,  $P=.018$ ) (Table 4)].

## 4. Discussion

In this study, we demonstrated that endocan may have potential as a new prognostic marker for PNETs. We observed a strong correlation between endocan expression in tumor tissues and



**Figure 2.** A high MVD is correlated with a good prognosis. (A) MVDs of representative PNET tissue specimens (400X). (B) Kaplan–Meier plot of the progression-free survival of 55 patients with PNETs stratified according to the median MVD.

unfavorable prognoses in PNETs. Endocan is a tumor growth factor; therefore,<sup>[19]</sup> we subsequently measured intratumoral MVDs in PNETs. We found that a high MVD was a favorable prognostic factor for progression-free survival and that endocan expression levels were negatively correlated with MVD. Finally, we performed Cox regression analysis to identify the risk factors for tumor recurrence and found that endocan expression and tumor lymph node involvement are independent risk factors for recurrence.

To understand the role of endocan in cell proliferation, Scherpereel et al<sup>[19]</sup> overexpressed endocan in human embryonic kidney cells (HEK 293) in severe combined immunodeficiency mice. These authors found that endocan overexpression increased HEK 293 cell proliferation, as the glycan moiety and protein core of endocan facilitated tumor growth. Consistent with this finding, small interfering RNA-mediated endocan inhibition significantly inhibited gastric cancer cell proliferation.<sup>[21]</sup> Moreover, Chen et al<sup>[26]</sup> reported that shRNA-mediated

**Table 2**  
**Relationship between endocan and MVD.**

MVD	Endocan		P
	Negative	Positive	
Low	16 (57.1%)	12 (42.9%)	.037
High	23 (85.2%)	4 (14.8%)	

Patients were divided into 2 groups based on the median MVD. Patients were also divided into 2 groups according to their endocan expression levels. The endocan-positive group had a lower MVD than the endocan-negative group.

MVD = intratumoral microvessel density.

**Table 3**  
**Relationship endocan expression and tumor mitosis (Ki-67 index; Ki-67).**

Ki-67	Endocan		P
	Negative	Positive	
<3%	25 (89.3%)	3 (10.7%)	.028
3–20%	13 (59.1%)	9 (40.9%)	
>20%	2 (50.0%)	2 (50.0%)	

Patients were divided into 2 groups according to their endocan expression levels. High endocan expression correlated more strongly with tumor mitosis than low endocan expression.

endocan knockdown in oral squamous cell carcinoma cells overexpressing nerve growth factor receptors abrogated tumor growth kinetics, as well as tumor invasion and metastasis capabilities. In this study, we demonstrated the existence of an association between endocan and an indicator of aggressiveness, namely, the mitotic count (the Ki-67 index). These observations suggest that endocan plays an important role in tumor growth.

We found that endocan expression was strongly associated with progression-free survival in PNETs in our series, whose follow-up period was long. We tested the associations between clinicopathological parameters and tumor recurrence using a Cox proportional hazards model, which showed that only endocan expression and lymph node involvement were independently associated with PNET recurrence. Endocan was previously found to be a predictor of disease recurrence only in cardiometabolic diseases—including myocardial infarction—polycystic ovarian disease and obstructive sleep apnea syndrome.<sup>[27–29]</sup> This is first study to provide evidence indicating that endocan is a predictor of disease recurrence in PNETs.

Interestingly, endocan is expressed normal endocrine tissues characterized by a high vascular density. However, all the normal islets of Langerhans analyzed herein displayed negative endocan expression, suggesting that endocan is involved only in tumor mitosis in PNETs. A similar finding was also reported by El Behery et al,<sup>[30]</sup> who observed strong endocan immunoreactivity in the endothelium of ovarian cancer tumor tissues but not in the endothelium of normal ovarian tissues.

**Table 4**  
**Results of the univariate analysis of and multivariate Cox proportional hazard model for the association between clinicopathological parameters and tumor recurrence.**

Univariate	Hazard ratio	Recurrence		P
		95% CI		
Endocan expression	5.587	1.610–19.231		.007
MVD expression	0.009	0.000–1.397		.067
Age	1.026	0.993–1.060		.129
Gender (male)	1.655	0.944–2.902		.078
BMI	0.929	0.822–1.049		.235
Tumor size	1.420	0.936–2.155		.100
LN involvement	4.202	1.672–10.526		.002
Metastasis	4.219	1.672–10.638		.002
<b>Multivariate</b>				
Endocan expression	4.778	1.307–17.457		.018
LN involvement	5.121	1.636–16.209		.005

The multivariate analysis was adjusted for endocan expression, gender, lymph node involvement, and metastasis.

BMI = body mass index, LN = lymph node, MVD = intratumoral microvessel density.

The main limitations of this study were its small sample size and low event rate in the overall survival analysis. PNETs are rare malignancies, and most patients affected by the disease are diagnosed with distant metastasis, which makes sample collection difficult. All the cases in this study were confirmed to be PNETs with good prognoses by histologic analysis. This finding accounts for the abovementioned low event rate and the minimal effects of the event rate on progression-free survival and overall survival. Only 5 patients died of the disease; therefore, it was difficult to perform a comprehensive evaluation of the significance of endocan in the overall survival analysis. The role of endocan in PNETs requires further evaluation in future studies. The other limitation was hard to reproduce the scoring system of IHC staining, including interobserver concordance. Therefore, computerized morphometric tools of staining analysis might be used in the future studies.

## 5. Conclusion

Endocan expression was correlated with poor clinical outcomes in patients with PNETs. Our results indicated that endocan expression may be a reliable marker for predicting tumor recurrence in patients with PNETs. Further investigation of the role of endocan on tumor growth in PNETs is warranted.

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## References

- Jiao Y, Shi C, Edil BH, et al. DAXX/ATRX, MEN1, and mTOR pathway genes are frequently altered in pancreatic neuroendocrine tumors. *Science* 2011;331:1199–203.
- Yao JC, Hassan M, Phan A, et al. One hundred years after “carcinoid”: epidemiology of and prognostic factors for neuroendocrine tumors in 35,825 cases in the United States. *J Clin Oncol* 2008;26:3063–72.
- Fraenkel M, Kim MK, Faggiano A, et al. Epidemiology of gastroenteropancreatic neuroendocrine tumours. *Best Pract Res Clin Gastroenterol* 2012;26:691–703.
- Wei IH, Harmon CM, Arcerito M, et al. Tumor-associated macrophages are a useful biomarker to predict recurrence after surgical resection of nonfunctional pancreatic neuroendocrine tumors. *Ann Surg* 2014;260:1088–94.
- Fraenkel M, Kim M, Faggiano A, et al. Incidence of gastroenteropancreatic neuroendocrine tumours: a systematic review of the literature. *Endocr Relat Cancer* 2014;21:R153–63.
- Rindi G, Bordi C. Endocrine tumours of the gastrointestinal tract: aetiology, molecular pathogenesis and genetics. *Best Pract Res Clin Gastroenterol* 2005;19:519–34.
- Rindi G, Wiedenmann B. Neuroendocrine neoplasms of the gut and pancreas: new insights. *Nat Rev Endocrinol* 2012;8:54–64.
- Furukori M, Imai K, Karasaki H, et al. Clinicopathological features of small nonfunctioning pancreatic neuroendocrine tumors. *World J Gastroenterol* 2014;20:17949–54.
- Liu TC, Hamilton N, Hawkins W, et al. Comparison of WHO Classifications (2004, 2010), the Hochwald grading system, and AJCC and ENETS staging systems in predicting prognosis in locoregional well-differentiated pancreatic neuroendocrine tumors. *Am J Surg Pathol* 2013;37:853–9.
- Rindi G, Falconi M, Klersy C, et al. TNM staging of neoplasms of the endocrine pancreas: results from a large international cohort study. *J Natl Cancer Inst* 2012;104:764–77.
- Yang M, Zeng L, Zhang Y, et al. TNM staging of pancreatic neuroendocrine tumors: an observational analysis and comparison by both AJCC and ENETS systems from 1 single institution. *Medicine (Baltimore)* 2015;94:e660.
- Lassalle P, Molet S, Janin A, et al. ESM-1 is a novel human endothelial cell-specific molecule expressed in lung and regulated by cytokines. *J Biol Chem* 1996;271:20458–64.
- Sarrazin S, Adam E, Lyon M, et al. Endocan or endothelial cell specific molecule-1 (ESM-1): a potential novel endothelial cell marker and a new target for cancer therapy. *Biochim Biophys Acta* 2006;1765:25–37.
- Perey L, Benhattar J, Peters R, et al. High tumour contamination of leukaphereses in patients with small cell carcinoma of the lung: a comparison of immunocytochemistry and RT-PCR. *Br J Cancer* 2001;85:1713–21.
- Leroy X, Aubert S, Zini L, et al. Vascular endocan (ESM-1) is markedly overexpressed in clear cell renal cell carcinoma. *Histopathology* 2010;56:180–7.
- Maurage CA, Adam E, Mineo JF, et al. Endocan expression and localization in human glioblastomas. *J Neuropathol Exp Neurol* 2009;68:633–41.
- Grigoriu BD, Depontieu F, Scherpereel A, et al. Endocan expression and relationship with survival in human non-small cell lung cancer. *Clin Cancer Res* 2006;12:4575–82.
- Huang GW, Tao YM, Ding X. Endocan expression correlated with poor survival in human hepatocellular carcinoma. *Dig Dis Sci* 2009;54:389–94.
- Scherpereel A, Gentina T, Grigoriu B, et al. Overexpression of endocan induces tumor formation. *Cancer Res* 2003;63:6084–9.
- Chen MH, Yeh YC, Shyr YM, et al. Expression of gremlin 1 correlates with increased angiogenesis and progression-free survival in patients with pancreatic neuroendocrine tumors. *J Gastroenterol* 2013;48:101–8.
- Zhao W, Sun M, Li S, et al. Biological and clinical implications of endocan in gastric cancer. *Tumour Biol* 2014;35:10043–9.
- Takahashi Y, Akishima-Fukasawa Y, Kobayashi N, et al. Prognostic value of tumor architecture, tumor-associated vascular characteristics, and expression of angiogenic molecules in pancreatic endocrine tumors. *Clin Cancer Res* 2007;13:187–96.
- Vermeulen PB, Gasparini G, Fox SB, et al. Quantification of angiogenesis in solid human tumours: an international consensus on the methodology and criteria of evaluation. *Eur J Cancer* 1996;32:2474–84.
- Vilar E, Salazar R, Perez-Garcia J, et al. Chemotherapy and role of the proliferation marker Ki-67 in digestive neuroendocrine tumors. *Endocr Relat Cancer* 2007;14:221–32.
- Nadler A, Cukier M, Rowsell C, et al. Ki-67 is a reliable pathological grading marker for neuroendocrine tumors. *Virchows Arch* 2013;462:501–5.
- Chen C, Shin JH, Eggold JT, et al. ESM1 mediates NGFR-induced invasion and metastasis in murine oral squamous cell carcinoma. *Oncotarget* 2016;7:70738–49.
- Kundi H, Balun A, Cicekcioglu H, et al. Admission endocan level may be a useful predictor for in-hospital mortality and coronary severity index in patients with ST-segment elevation myocardial infarction. *Angiology* 2017;68:46–51.
- Bicer M, Guler A, Kocabas GU, et al. Endocan is a predictor of increased cardiovascular risk in women with polycystic ovary syndrome. *Endocr Res* In press 2016.
- Kanbay A, Ceylan E, Inonu Koseoglu H, et al. Endocan: a novel predictor of endothelial dysfunction in obstructive sleep apnea syndrome. *Clin Respir* In press 2016.
- El Behery MM, Seksaka MA, Ibrahim MA, et al. Clinicopathological correlation of endocan expression and survival in epithelial ovarian cancer. *Arch Gynecol Obstet* 2013;288:1371–6.