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# VEGF-A/VEGF-B/VEGF-C expressions in non-hereditary, non-metastatic phaeochromocytoma

Short title. VEGF-A/VEGF-B/VEGF-C IN PHAEOCHROMOCYTOMA

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**Keywords** phaeochromocytoma; VEGF; vascular endothelial growth factor; mouse double minute 2; mdm2

**Abstract**

Vascular endothelial growth factor (VEGF) is important in pathogenesis of different cancers. The aim of this study is to investigate the relationships between different VEGFs and clinicopathological factors in patients with phaeochromocytomas. Twenty patients (10 men; 10 women) with non-hereditary, non-metastatic phaeochromocytomas were examined for *VEGF* mRNA expressions by polymerase chain reaction. The expressions were correlated with the clinical and pathological factors of the patients. In addition, mouse double minute 2 (MDM2) expression in these tumours were studied by immunohistochemistry. High expressions of *VEGF-A, VEGF-B,* and *VEGF-C* mRNA were detected in 11 (55%), 9 (45%), and 9 (45%) of the tumours respectively. High expression of *VEGF-A* in phaeochromocytomas was significantly correlated with the tumour size ($p$ = 0.025) but did not correlate with patients' age, gender, and tumour laterality. Besides, there was a trend of *VEGF-A* expression correlated with MDM2 expression ($p$ = 0.064). On the other hand, expressions of *VEGF-B* and *VEGF-C* were not significantly correlated with tumour size, patients' age, gender, tumour laterality, and MDM2 expression. In addition, high expressions of *VEGF-B* and *VEGF-A* were associated with increase of tumour size ($p$ = 0.042). Co-expression of different *VEGFs* did not correlate with MDM2 expression. To conclude, there is a role for VEGF-A/VEGF-B/VEGF-C in the pathogenesis of non-hereditary, non-metastatic phaeochromocytomas.

## Introduction

Phaeochromocytoma is a neuroendocrine tumour derived from chromaffin cells in adrenal medulla (Lam, 2015). In the current classification of endocrine tumours by World Health Organisation (WHO), the term "metastatic phaeochromocytoma" is used instead of "malignant phaeochromocytoma" as phaeochromocytoma could have metastatic potential and there is no unequivocal histological system to assess the biological aggressiveness of the tumour (Lam, 2017; Lloyd et al., 2017). Phaeochromocytoma usually produces excess catecholamines with clinical symptoms related to hypertension and hyperglycaemia (Bravo, 1994; Tevosian and Ghayee, 2019; Abe et al., 2019). Eisenhofer and co-workers showed the amount of metanephrines released by the tumour were positively correlated with tumour size (Eisenhofer et al., 2005). Moreover, phaeochromocytoma could lead to phaeochromocytoma crisis, which is a life-threatening endocrine emergency with reported mortality as high as 85% (Newell, 1998; Abe et al., 2012). Riester and co-workers showed the development of phaeochromocytoma crisis was significantly associated with increase of size of the tumour (Riester, et al, 2015). Considering these findings, management of phaeochromocytoma, particularly for big phaeochromocytomas, is very important.

Recent advances in genomic medicine reveal many new genetic mutations in phaeochromocytoma (Papathomas et al., 2021). Approximately 40% of phaeochromocytomas were associated with germline mutation and 60% were sporadic (Dahia, 2014; Pillai et al., 2016). Meanwhile, the mechanism of tumorigenesis, particularly that of tumour growth, in phaeochromocytoma has been controversial. One of the mechanisms of tumour growth and progression is angiogenesis. Phaeochromocytoma is a highly vascular tumour. The vascular endothelial growth factor

(VEGF)-A, the main component of the VEGF family, promotes the growth of vascular endothelial cells derived from blood vessels (Ferrara et al., 2003). VEGF-A plays a key regulatory role in angiogenesis and tumour progression, which initiates two major cascades of tumorigenesis, the RAS-BRAF-MEK pathway and PI3K-AKT-mTOR pathway, in various tumours as well as in phaeochromocytoma (Carmeliet, 2005; McCubrey et al., 2007; Dahia, 2014; Molinaro, et al., 2017). In addition, the roles of VEGF-B and VEGF-C, which are components of the VEGF family, have not been fully revealed in phaeochromocytoma. In the literature, some studies showed the roles of VEGF-B and VEGF-C in the pathogenesis in several cancers (Salven et al., 1998; Skobe et al., 2001; Weekes et al., 2009; Tammela and Alitalo et al., 2010; Salajegheh et al. 2013; Yang et al., 2015; Pan, et al., 2018). Furthermore, some reports indicated that VEGF-B/VEGF-C could facilitate the action of VEGF-A (Cleaver and Melton, 2003; Ferrara et al., 2003).

In the previous study, mouse double minute 2 (MDM2) was reported to regulate VEGF-A expression in several tumours (Zhou et al., 2011; Xiong et al., 2014; Bradbury et al., 2015). Besides, Lam and co-workers showed mutated p53 protein or expression of MDM2 were poor predictive markers for phaeochromocytoma (Lam et al., 2001). Thus, in the present study, we investigated the expressions of different VEGFs and correlated these with the expressions of p53, MDM2 as well as the clinicopathological features of patients with non-hereditary, non-metastatic phaeochromocytomas.

## Material and Methods

*Materials*

Ethical approval for this study was obtained by Griffith University human research ethics committee (GU ref. nos.: MED/19/08/HREC approved 27 July 2009). The patients (10 men; 10 women) selected for this study had non-hereditary, non-metastatic phaeochromocytoma. They were operated between 1973 and 2015 by surgeons in Hong Kong and Australia (CYL and VL). The non-hereditary base of these phaeochromocytomas were based on the genetic profiles as detailed in our previous studies as well as from clinical data (Pillai et al., 2016). In addition, all the patients had more than 5 year of clinical follow-up to confirm that the tumours were non-metastasizing. All these phaeochromocytomas were prospectively collected and with clinical data and tissue blocks available as well as with pathological diagnoses confirmed by the author (AKL). After reviewing the histological sections of the tumours, a block was chosen from each of the phaeochromocytomas. The selection of block from each patient was based on having adequate portion of tumour tissue (>70% of area occupied by non-necrotic tumour). The characteristics of the patients with phaeochromocytoma used in the study are shown in Table 1. In addition, non-neoplastic adrenal tissues were collected as controls. These non-neoplastic adrenal tissues were from patients with adrenal resected together with renal cell carcinomas during the operation as a part of the procedure. From each of these non-neoplastic adrenal glands, the adrenal medulla region was micro-dissected out for RNA and DNA extractions.

*mRNA extraction and reverse transcription*

Total RNA was extracted from formalin-fixed, paraffin-embedded tissue samples using Qiagen miRNA easy FFPE kits (Qiagen, Hilden, Germany), which were specially designed for purifying total RNA from formalin-fixed, paraffin-embedded tissue sections. Reverse transcription reactions were performed using 1μg total RNA in a final reaction volume of 20μl. RNA was converted to cDNA using miScript Reverse Transcription Kit (Qiagen) according to the manufacturer's instructions. The quality of cDNA was checked by measuring optical density. Each cDNA sample was diluted to 30 ng/μl and was stored at −20 °C until the polymerase chain reaction (PCR) analysis.

*Quantitative real-time PCR*

*VEGF-A*/*VEGF-B*/*VEGF-C* mRNA expressions of each tested sample were examined using quantitative real-time PCR by QuantStudio 6 Flex Real-Time PCR System (ThermoFisher Scientific, Waltham, MA) according to the protocol described previously (Lam et al., 2011; Kasem et al., 2014). Primers for *VEGF-A* were 5′-TCTTCAAGCCATCCTGTGTG-3′ for forward and 5′-TCTGCATGGTGATGTTGGAC-3′ for reverse. The *VEGF-B* primers were 5′-ACCCCCAACCCTGATAAAAG -3′ for forward and 5′- TCCTCATTTCCTCCATCTGC -3′ for reverse. The *VEGF-C* primers were 5′- GGAAAGAAGTTCCACCACCA -3′ for forward and 5′- TGTTAGCATGGACCCACAAG -3′ for reverse. The *GAPDH (glyceraldehyde 3-phophate dehydrogenase)* primers were 5′-TGCACCACCAACTGCTTAGC-3′ for forward and 5′-GCATGCACTGTGGTCATGAG-3′ for reverse. The fold changes in the target genes were calculated for each sample group using the $2^{-\Delta\Delta Ct}$ method with *GAPDH* and control

samples of normal adrenal medulla tissue. The fold changes more than 2 were considered over-expression.

*Investigation of p53 and MDM2 protein expression*

All tissues from patients with phaeochromocytoma were examined for expressions of p53 protein and MDM2 protein using immunohistochemistry as previously described (Lam, et al., 2001). The immunostaining of p53 and MDM was analysed by a pathologist (AKL) under a standard light microscope. Gradings of "0" and "1" were used for this assessment, where "0" represented negative protein staining and "1" represented protein staining.

*Data analysis*

Statistical analyses were performed using STATA® SE version 13.1 (Stata Corporation, College Station, TX, USA). Comparisons between variable groups were analysed using the $\chi^2$ test. Significance of differences was taken as $p < 0.05$.

**Results**

*Expression of VEGF-A/VEGF-B/VEGF-C mRNA and MDM2 protein in patients with sporadic, non-metastatic phaeochromocytomas* (Table 1)

High expressions of *VEGF-A*, *VEGF-B*, and *VEGF-C* mRNA were detected in 55% (11/20), 45% (9/20), and 45% (9/20) of the tumours, respectively. MDM2 protein was positive in 45% (9/20) (Figure 1) whereas p53 protein was negative in all phaeochromocytomas (Figure 2).

*Correlation between VEGF-A/VEGF-B/VEGF-C mRNA expressions and clinicopathological factors* (Table 2)

*VEGF-A* expression was significantly correlated with larger tumour size ($p$ = 0.025). Overall, 73% of tumours of diameter ≥ 50mm had high *VEGF-A* expression. Meanwhile, high expression of *VEGF-B* or *VEGF-C* did not correlate with tumour size ($p$ = 0.178). *VEGF-A*/*VEGF-B*/*VEGF-C* expressions did not correlate with age, gender of the patients or tumour laterality ($p$ > 0.1). Besides, *VEGF-A* expression correlated with MDM2 protein expression with near statistical significance ($p$ = 0.064). In summary, 64% of tumours with high *VEGF-A* expression were positive for MDM2, while 22% of tumours with low *VEGF-A* expression were positive for MDM2 expression. High expressions of *VEGF-B* or *VEGF-C* was not correlated with MDM2 expression ($p$ > 0.3).

*Correlation upon tumour growth between VEGF-A mRNA expression and VEGF-B/VEGF-C mRNA expression* (Table 3)

High expression of *VEGF-B* with high expression of *VEGF-A* is associated with larger tumour ($p$ = 0.042). In summary, 86% of phaeochromocytomas with high

expression of both *VEGF-B* and *VEGF-A* were of diameter ≥ 50mm (mean diameter = 76 mm). On the other hand, 50 % of phaeochromocytomas with high expression of *VEGF-A* and low expression of *VEGF-B* had diameter ≥ 50mm (mean diameter = 60mm). In addition, only 22% of phaeochromocytomas with low expression of both *VEGF-A* and *VEGF-B* had diameter ≥ 50mm (mean diameter = 58mm). Moreover, 75% of phaeochromocytomas with high expressions of *VEGF-C* and *VEGF-A* had diameter ≥ 50mm (mean diameter = 74mm), whereas 67 % of phaeochromocytomas with high expression of *VEGF-A* and low expression of *VEGF-C* had tumour with diameter l≥ 50mm (mean diameter = 61mm). Furthermore, phaeochromocytomas having high expressions of *VEGF-C* and *VEGF-A* were associated with larger tumour size, but not significantly ($p = 0.078$).

*Correlations of MDM2 protein expression between VEGF-A mRNA expression and VEGF-B/VEGF-C mRNA expression* (Table 4)

Overall, 71% of phaeochromocytomas with high expressions of *VEGF-B* and *VEGF-A* were positive for MDM2. In addition, 50% of phaeochromocytomas with high expression of *VEGF-A* and low expression of *VEGF-B* were positive for MDM2, whereas 22% of phaeochromocytomas with low expression of *VEGF-A* expression were positive for MDM2. However, high expressions of *VEGF-B* and *VEGF-A* did not significantly correlate with MDM2 expression ($p = 0.142$). Furthermore, 63% of phaeochromocytomas with high expression of *VEGF-C* and *VEGF-A* were positive for MDM2 whereas 67% of phaeochromocytomas with high *VEGF-A* expression and low *VEGF-C* expression were positive for MDM2. High expression of *VEGF-C* and *VEGF-A* did not significantly correlate with MDM2 expression ($p = 0.179$).

**Discussion**

This study is the first pilot study on the roles of VEGF-B/VEGF-C in additional to VEGF-A in non-hereditary, non-metastatic phaeochromocytoma. Besides, this study is also the first to analyse the correlation between VEGF-A/VEGF-B/VEGF-C and MDM2, which plays a crucial role in tumourigenesis of phaeochromocytomas.

VEGF-A is the key mediator of angiogenesis in cancers, which is essential for cancer development and growth (Ferrara and Davis-Smyth, 1997; Ferrara et al., 2003; Carmeliet, 2005). It could promote the growth of vascular endothelial cells derived from blood vessels and lymphatics (Ferrara and Davis-Smyth, 1997; Ferrara et al., 2003). Tumour vasculature, which is affected by VEGF-A, was reported to be structurally and functionally abnormal due to pathological angiogenesis associated with tumour growth (Ferrara and Davis-Smyth, 1997; Ferrara et al., 2003). Besides, VEGF-A expression correlated with lymph node metastases in malignancies, such as in papillary thyroid carcinoma (Salajegheh et al., 2013). VEGF-A induces angiogenesis and proliferation mainly through vascular endothelial growth factor receptor (VEGFR)-2. In addition, VEGF-A affect angiogenesis through VEGFR-1 by promoting induction of matrix metalloproteinase (MMP)-9, urokinase-type plasminogen activator (uPA), and tissue-type plasminogen activator (tPA) (Bergers et al., 2000; Ferrara et al., 2003; Carmeliet and Jain, 2011).

In phaeochromocytoma, the function of VEGF-A has been investigated (Feng et al., 2011; Ferreira et al., 2014). Feng and co-workers showed the rate of positive VEGF-A protein expression in patients with metastatic phaeochromocytoma was higher than that in patients with non-metastatic phaeochromocytoma (Feng et al., 2011). Ferreira and co-workers showed positive VEGF-A protein expression on immunostaining in patients with

metastatic phaeochromocytoma was more common than that in patients with non-metastatic phaeochromocytoma (Ferreira et al., 2014). In addition, there was no significant difference of VEGF-A expression between patients with sporadic, non-metastatic phaeochromocytoma and those with multiple endocrine neoplasia type 2 (MEN2) associated phaeochromocytoma. In the study, VEGF-A protein expression did not significantly associate with tumour size in all patients with phaeochromocytoma, which included metastatic or MEN2 associated phaeochromocytomas (Ferreira et al., 2014). In the present study, *VEGF-A* expression significantly correlated with tumour size ($p = 0.025$). This discrepancy might be due to the different subjects being studied. In this study, we exam a more unique group of patients who have non-hereditary, non-metastatic phaeochromocytomas.

Recent studies revealed that VEGF-B or VEGF-C had roles for tumorigenesis in some cancers in addition to VEGF-A. VEGF-B was initially revealed to stimulate endothelial cell activity and promotes angiogenesis (Olofsson et al., 1996). VEGF-B expression was detected in several malignancies and associated with tumour angiogenesis (Salven, et al., 1998). VEGF-B promotes induction of MMP-9 through VEGFR1. Yang and co-workers reported that expression of VEGF-B impaired primary tumour growth of melanoma and fibrosarcoma as well as induced metastases in vivo and in vitro study. Besides, expression of VEGF-B in squamous cell carcinoma of lung and melanoma correlated with poor patients' survival (Yang et al., 2015). Meanwhile, Albrecht and co-workers reported VEGF-B inhibited angiogenesis in pancreatic endocrine tumour (Albrecht et al., 2010). Thus, VEGF-B function in tumour progression remains controversial. The expression and function of VEGF-B have not been investigated in phaeochromocytoma.

VEGF-C promotes angiogenesis of various tumours (Salven et al., 1998; Kodama et al., 2008). In addition, VEGF-C promotes lymphangiogenesis and increases lymph node metastases of several malignancies (Skobe et al, 2001; Tammela and Alitalo, et al., 2010; Salajegheh et al., 2013; Pan et al., 2018). The protein induces angiogenesis through VEGFR2 and lymphangiogenesis through VEGFR3 (Ferrara et al., 2003; Matsumoto et al., 2013; Kasem et al., 2014). In phaeochromocytoma, there is correlation noted between *VEGF-C* expression and *VEGF-A* expression (Isobe et al., 2006), but no studies have demonstrated the function of VEGF-C in the tumour.

In the present study, *VEGF-B* expression is first being analysed in phaeochromocytoma. Besides, we investigated the association between *VEGF-B*/*VEGF-C* expression and clinicopathological factors. The findings showed that *VEGF-B*/*VEGF-C* expression did not correlate all these factors. On the other hand, we demonstrated the facilitative function of VEGF-B/VEGF-C with VEGF-A in tumour growth. VEGF-B could assist VEGF-A function of tumour growth by two possible mechanism; 1) more VEGF-A binds to VEGFR-2 compared to VEGFR-1 due to competitive binding of VEGF-B to VEGFR-1; 2) induction of MMP9, uPA, and tPA by VEGF-B promotes tumour angiogenesis in addition to the function of angiogenesis by VEGF-A. Furthermore, VEGF-C might assist VEGF-A function of tumour growth through VEGFR-2 together and promote angiogenesis. The results in this study showing high expression of *VEGF-B* and *VEGF-A* are associated with significant increase of tumour size. On the contrary, high expression of *VEGF-C* and *VEGF-A* is related to increase of tumour size though the effect was not of significant. The results indicate that *VEGF-B* has more facilitative roles in the function of *VEGF-A* in promoting tumour growth than *VEGF-C* in non-hereditary, non-metastatic phaeochromocytoma.

In this study, MDM2 expression was detected in 45% of patients with non-hereditary, non-metastatic phaeochromocytoma. p53 is a well-known tumour suppressor and mutant forms of p53 are positive for immunostaining because of having longer half-lives. Considering *p53* mutations existing in metastatic phaeochromocytoma more than non-metastatic phaeochromocytoma (Yoshimoto, et al., 1998), it could explain that all patients were negative for p53 in our study cohorts. On the other hand, MDM2 expression was detected in approximately half of the patients. MDM2 is well-known as a suppressor of p53, and expression of MDM2 leads to promote tumour progression in the p53-dependent pathway. Furthermore, MDM2 regulates cellular proliferation, cell migration and invasion, apoptosis, and angiogenesis in p53-independent pathway in cancers (Zhang et al., 2003; Huang et al., 2016). Zhou and co-workers showed MDM2 could bind to the *VEGF-A* mRNA directly and regulate *VEGF-A* stability in human neuroblastoma cells (Zhou, et al., 2011). In addition, Xiong and colleagues revealed MDM2 regulated angiogenesis and tumour growth *via* VEGF-A in breast carcinoma, and the role of MDM2 in VEGF-A could be through p53-independent pathway in vivo and in vitro (Xiong et al., 2014). This finding consistent with our results on phaeochromocytoma in which MDM2 positivity could associate with *VEGF-A* expression without p53 mutation.

In phaeochromocytoma, detection of high expression of VEGF-A was reported previously but its regulation and mechanism has been unknown. The results in this study indicated VEGF-A expression could be regulated by MDM2 expression. Besides, this study revealed not only *VEGF-B/VEGF-C* but also co-expression of *VEGF-B*/*VEGF-C* and *VEGF-A* did not associate with MDM2 expression. Regarding the association

between expressions of MDM2 and VEGF-B/VEGF-C, there were no previous reports in phaeochromocytoma or other human tumours. Our results indicated that in phaeochromocytoma, over-expression of *VEGF-B*/*VEGF-C* might not be affected by MDM2. Thus, the effect on tumour growth by co-over-expression of *VEGF-B*/*VEGF-C* and *VEGF-A* in phaeochromocytoma might not depend on MDM2.

This study had several limitations. First, the study cohort of this study was small because phaeochromocytoma is a rare tumour, and we investigated only non-hereditary, non-metastatic phaeochromocytomas. Second, we did not perform the investigations about somatic mutations. Thirdly, *VEGF* mRNA expression, instead of protein expression, was used to correlate with expression of p53 protein and MDM2 protein. On the other hand, in our previous studies using tissue blocks, we noted that correlations of *VEGF* mRNA expression could be better studied (with respect to technical aspects) by mRNA expression and could show the interactions with protein expression such as p53 (Salajegheh et al., 2013; Maroof et al., 2019). It also worth noting that expression of VEGF mRNA and protein expression correlates with each other (Salajegheh et al., 2013). Thus, future studies are required to confirm the findings of this study.

To conclude, this study revealed a role of VEGF-A as well as VEGF-B/VEGF-C in the pathogenesis of non-hereditary, non-metastatic phaeochromocytoma.

Furthermore, the possible correlation between expressions of MDM2 and VEGF-A is demonstrated in non-hereditary, non-metastatic phaeochromocytomas. These results might lead to new knowledge about tumour growth of phaeochromocytoma. The consideration of interactions of VEGF-A/VEGF-B/VEGF-C in phaeochromocytoma should be recommended. Future studies are required to confirm our results and investigate the mechanisms of VEGFs in pheochromocytomas.

**Figure Legends**

Figure 1. Positive nuclear expression of MDM protein (3,3′-diaminobenzidine/ haematoxylin, x 40, scale bar =50μm).

Figure 2. (A). Positive nuclear expression of p53 protein in a familial paraganglioma (control) and (B). negative nuclear expression of p53 in non-familial, no metastasising phaeochromocytoma (3,3′-diaminobenzidine/ haematoxylin x 20, scale bar = 100μm).

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**Availability of data and materials:** On request.

**Authors' contribution**

Ichiro Abe: running of experiments; drafting of manuscript.

Farhadul Islam: supervision on the experimental works

Chung Yau Lo: clinical data and specimen collection

Victor Liew: clinical data and specimen collection

Suja Pillai: contribution to experimental works

Alfred K. Lam: overall supervision; revision of manuscript.

**Ethical approval and consent to participate.**

Ethical approval for this study was obtained by Griffith University human research ethics committee (GU ref. nos.: MED/19/08/HREC).

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**Table 1.** Clinical characteristics, *VEGF-A*/*VEGF-B*/*VEGF-C* mRNA expressions, and p53/MDM2 protein expression of the patients with phaeochromocytoma.

| Total number of patients = 20 | |
|---|---|
| Mean age ± standard deviation (years) | 41 ± 16 |
| Gender (Female/Male) | 10/10 |
| Tumour laterality (Right/Left) | 16/4 |
| Mean tumour size ± standard deviation (mm) | 65 ± 25 |
| Morbidity of hypertension | 20 (100 %) |
| Morbidity of diabetes mellitus | 3 (15%) |
| Patients with expression of *VEGF-A* | 11 (55%) |
| Patients with expression of *VEGF-B* | 9 (45%) |
| Patients with expression of *VEGF-C* | 9 (45%) |
| Patients with positive expression of p53 | 0 (0%) |
| Patients with positive expression of MDM2 | 9 (45%) |

**Table 2.** Clinicopathological variables and *VEGF-A*/*VEGF-B*/*VEGF-C* expressions in patients with non-hereditary, non-metastatic phaeochromocytoma.

| | *VEGF-A* | | | *VEGF-B* | | | *VEGF-C* | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Patients with high expression** | **Patients with low expression** | ***P*** | **Patients with high expression** | **Patients with low expression** | ***p*** | **Patients with high expression** | **Patients with low expression** | ***p*** |
| **mour size (mm)** | | | | | | | | | |
| s than 50 | 3 (15%) | 7 (35%) | 0.025* | 3 (15%) | 7 (35%) | 0.178 | 3 (15%) | 7 (35%) | 0.178 |
| er (or = 50) | 8 (40%) | 2 (10%) | | 6 (30%) | 4 (20%) | | 6 (30%) | 4 (20%) | |
| **e (years)** | | | | | | | | | |
| s than 40 | 5 (25%) | 6 (30%) | 0.343 | 5 (25%) | 6 (30%) | 0.582 | 4 (20%) | 7 (35%) | 0.391 |
| er (or =) 40 | 6 (30%) | 3 (15%) | | 4 (20%) | 5 (25%) | | 5 (25%) | 4 (20%) | |
| **nder** | | | | | | | | | |
| nale | 5 (25%) | 5 (25%) | 0.653 | 6 (30%) | 4 (20%) | 0.068 | 5 (25%) | 5 (25%) | 0.653 |
| le | 6 (30%) | 4 (20%) | | 3 (15%) | 7 (35%) | | 4 (20%) | 6 (30%) | |
| **terality** | | | | | | | | | |
| ht | 8 (40%) | 8 (40%) | 0.369 | 6 (30%) | 10 (50%) | 0.110 | 7 (35%) | 9 (45%) | 0.822 |
| t | 3 (15%) | 1 (5%) | | 3 (15%) | 1 (5%) | | 2 (10%) | 2 (10%) | |
| **DM2 expression** | | | | | | | | | |
| itive | 7 (35%) | 2 (10%) | 0.064 | 5 (25%) | 4 (20%) | 0.391 | 5 (25%) | 4 (20%) | 0.391 |
| gative | 4 (20%) | 7 (35%) | | 4 (20%) | 7 (35%) | | 4 (20%) | 7 (35%) | |

<0.05 was considered significant.

**Table 3.** Detailed evaluation of the correlation of tumour growth between *VEGF-A* expression and *VEGF-B/VEGF-C* expressions in patients with non-hereditary, non-metastatic pheochromocytomas.

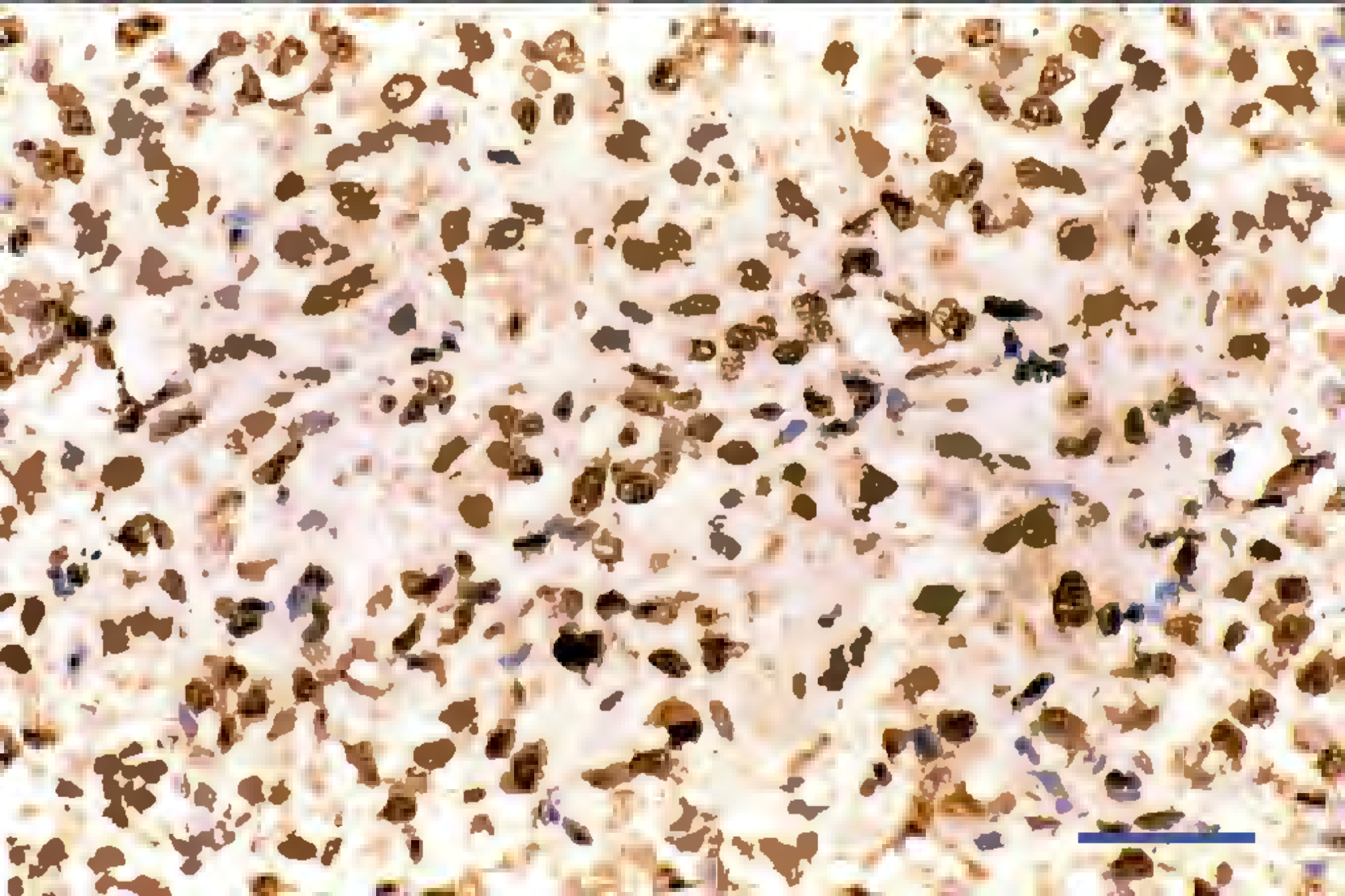
| | ***VEGF-A*: patients with high expression** | | ***VEGF-A*: patients with low expression** | ***p*** |
|---|---|---|---|---|
| | ***VEGF-B*: patients with high expression** | ***VEGF-B*: patients with low expression** | | |
| **Tumour size (mm)** | | | | |
| Less than 50 | 1 (5%) | 2 (10%) | 7 (35%) | 0.042* |
| Over (or =) 50 | 6 (30%) | 2 (10%) | 2 (10%) | |
| Size (mean ± SD) | 76 ± 18 | 60 ± 21 | 58 ± 30 | |
| | ***VEGF-A*: patients with high expression** | | **VEGF-A: patients with low expression** | ***p*** |
| | ***VEGF-C*: patients with high expression** | ***VEGF-C*: patients with low expression** | | |
| **Tumour size (mm)** | | | | |
| Less than 50 | 2 (10%) | 1 (5%) | 7 (35%) | 0.078 |
| Over 50 (or =) | 6 (30%) | 2 (10%) | 2 (10%) | |
| Size (mean ± SD) | 74 ± 19 | 61 ± 23 | 58 ± 30 | |

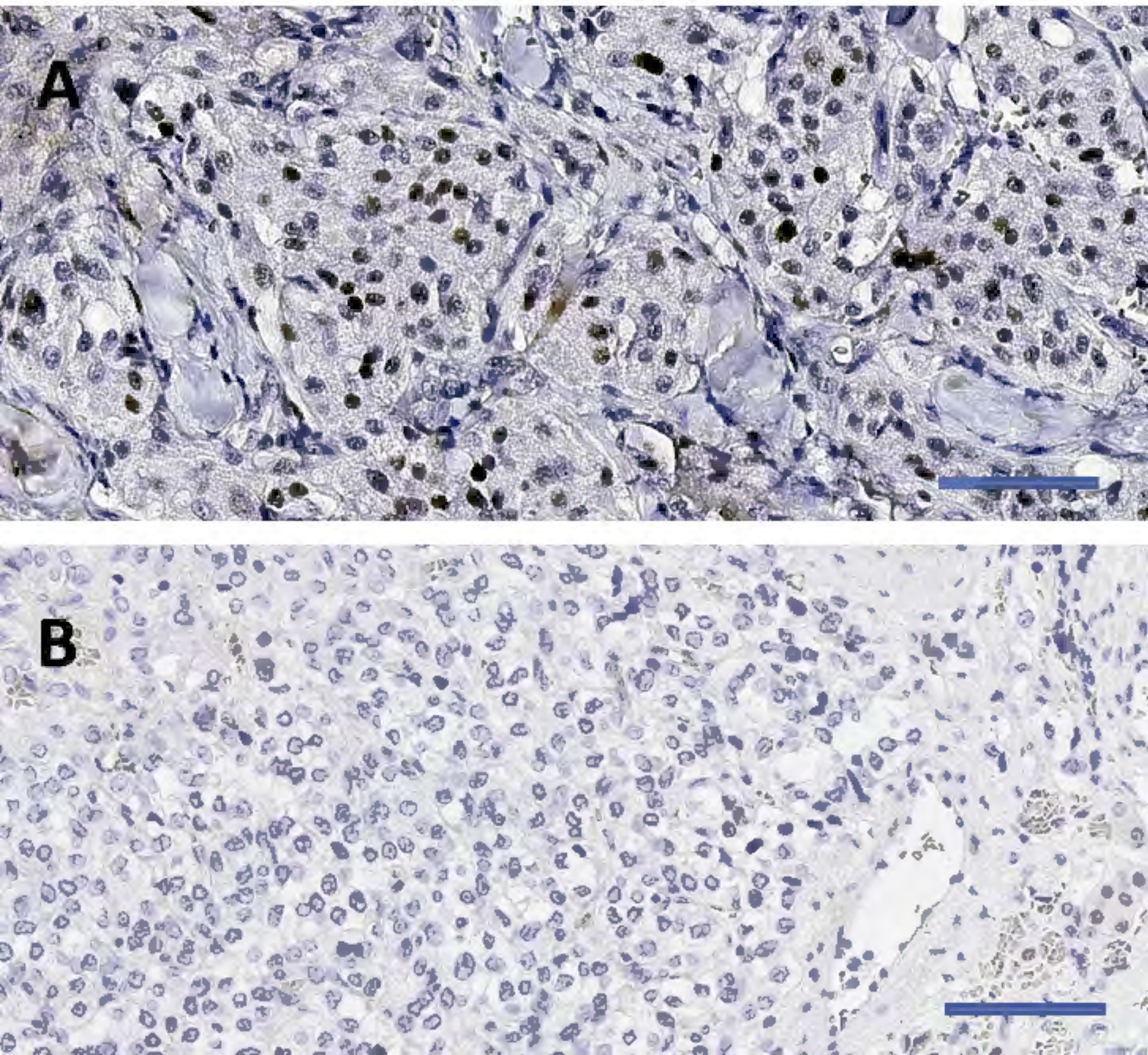
D: standard deviation; *$p<0.05$ was considered significant.

**Table 4.** Detailed evaluation of the correlation of MDM2 protein expression between *VEGF-A* expression and *VEGF-B*/*VEGF-C* expressions in patients with non-hereditary, non-metastatic pheochromocytomas.

| | ***VEGF-A*: patients with high expression** | | ***VEGF-A*: patients with low expression** | ***p*** |
|---|---|---|---|---|
| | ***VEGF-B*: patients with high expression** | ***VEGF-B*: patients with low expression** | | |
| **MDM2 protein** | | | | |
| Positive | 5 (25%) | 2 (10%) | 2 (10%) | 0.14 |
| Negative | 2 (10%) | 2 (10%) | 7 (35%) | |
| | ***VEGF-A*: patients with high expression** | | ***VEGF-A*: patients with low expression** | ***p*** |
| | ***VEGF-C*: patients with high expression** | ***VEGF-C*: patients with low expression** | | |
| **MDM2 protein** | | | | |
| Positive | 5 (25%) | 2 (10%) | 2 (10%) | 0.17 |
| Negative | 3 (15%) | 1 (5%) | 7 (35%) | |

*$p<0.05$ was considered significant.




A
B