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Emanuela Maderna, Andrea Salmaggi, Chiara Calatozzolo, Lucia Limido & Bianca Pollo
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Clinical Study

Nestin, PDGFRβ, CXCL12 and VEGF in Glioma Patients

Different Profiles of (Pro-Angiogenic) Molecule Expression Are Related with Tumor Grade and May Provide Prognostic Information

Emanuela Maderna[1]
Andrea Salmaggi[2]
Chiara Calatozzolo[2]
Lucia Limido[1]
Bianca Pollo[1,*]

Departments of [1]Neuropathology and [2]Neuro-oncology; Fonoazione IRCCS; Istituto Nazionale Neurologico "Carlo Besta"; Milano, Italy

*Correspondence to: Bianca Pollo; Dipartimento di Neuropatologia e Neurologia 5; Istituto Nazionale Neurologico "Carlo Besta"; Via Celoria 11; Milano 20133, Italy; Tel.: +39.02 23942260; Fax: +39.02.23942101; Email pollo.b@istituto-besta.it

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KEY WORDS

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ABSTRACT

Angiogenesis is a key event in the natural progression of gliomas. Nestin, a marker for multipotential neuroepithelial stem cells, is detected in neuroepithelial tumors and in proliferating endothelial cells (ECs) and is involved in the early stages of lineage commitment, proliferation and differentiation. Nestin expression is correlated with proangiogenic chemokines (CXCL12 and its receptor CXCR4) and growth factors (VEGF, PDGF-B and its receptor PDGFRβ).

VEGF expression upregulates CXCR4 on endothelial cells, binding the chemokine SDF1/CXCL12 (Stromal Derived Factor) that has a role on angiogenesis and chemotaxis of endothelial cells; PDGF (platelet-derived growth factor) and PDGFRβ are also crucial by increasing the expression of VEGF.

We performed a retrospective study on the presence and role of nestin-expressing cells in 102 patients with glioma, relating the findings to VEGF, CXCL12, PDGFRβ expression and to clinical outcome (time to tumor progression-TTP and survival time-ST).

Our results suggest that in gliomas the detection of proliferating ECs expressing nestin correlates to histological malignancy grade and clinical outcome. Also, the expression of CXCL12 in low-grade gliomas was the only factor associated with a significantly shorter TTP, suggesting a role of this chemokine in angiogenic shift and/or disease progression.

INTRODUCTION

The so-called "angiogenic" switch,[1] i.e., the breaking down of the physiological balance between endogenous anti- and pro-angiogenic factors, is thought to parallel increasingly malignant tumor behaviour in terms of proliferation and invasion in a variety of human cancers, including glioma; in the most malignant glioma subtype (glioblastoma), new vessel formation, triggered also by hypoxia-activated genes, leads to formation of bizarre vascular elements such as garlands and glomeruli.[2,3]

Some vascular endothelial cells in gliomas express nestin, a marker of neuroepithelial precursor cells and endothelial cells in active proliferation.[4] Nestin is a class VI intermediate filament (IF), highly expressed in multipotential stem cells and progenitor cells, involved in the early stages of lineage commitment, in proliferation and in differentiation, which is downregulated in the differentiated cells composing human adult brain.[5-8] Moreover, neuroepithelial precursor cells expressing nestin may differentiate into endothelial and perivascular cells (pericytes) in response to pro-angiogenic factors.[9] In neuroepithelial tumors,[4,10] nestin may be expressed[11] both in tumor cells and in proliferating endothelial cells.[12,13]

Endothelial cell proliferation in gliomas, leading to the formation of new blood vessels, involves the angiogenic growth factor VEGF,[14,15] a hypoxia inducible angiogenic factor secreted by tumor cells[16] that increases vascular permeability and the expression of which is up-regulated by trascription factors, cytokines and growth factors.[17]

VEGF induces endothelial cell migration and proliferation also through upregulation of CXCR4,[18] the receptor of the pro-angiogenic chemokine SDF1/CXCL12 (Stromal Derived Factor) on endothelial cells;[19] CXCL12 is a lymphocyte chemoattractant;[20] moreover, it acts as a potent chemoattractant for endothelial cells and is a survival factor for glioma cells inducing an activation of the survival kinase that protects them from apoptosis.[21] The molecule and its receptor are overexpressed in malignant gliomas[20,22] and have been related to prognosis.[23]

Although implicated, the role of PDGF in angiogenesis is not as fully understood as that of VEGF. Recently, PDGF has been found to modulate angiogenesis and have a role in pericyte recruitment, throughout chemotactic signals recruiting them during the formation of new vessels. Interaction with PDGFRβ, also expressed by endothelial cells, promotes tube formation and increases the expression of VEGF and also has a role in glial tumorigenesis and growth stimulation of tumor cells.[22] PDGFRβ is overexpressed in glioma endothelial cells and it is involved in vessel maturation through the recruitment of pericytes and smooth muscular cells.[24]

In the aim to better define the profile of expression of nestin, VEGF, CXCL12, PDGFRβ and to assess whether it may have some prognostic relevance in the clinical outcome of patients with glioma, we performed a retrospective study relating nestin,[25] VEGF, CXCL12 and PDGFRβ expression as investigated by immunohistochemistry, to time to tumor progression and survival in 102 patients, all affected by grade II, III or IV gliomas and followed at our Institution after surgery.

MATERIALS AND METHODS

One-hundred and two patients surgically treated for glioma in our Institute in the years 1992–2004 were included in the study. The patients were 61 male and 42 female, age ranged from 21 to 68 years (median age 45.5). Tumor location was supratentorial.

All cases from our institution were retrospectively reviewed by a single neuropathologist (B.P.).

Histological diagnosis and grade of tumors according to WHO grading system classification[26]:

- 10 astrocytoma
- 10 oligodendroglioma
- 29 oligoastrocytoma
- 18 anaplastic astrocytoma
- 3 anaplastic oligodendroglioma
- 10 anaplastic oligoastrocytoma
- 22 glioblastoma

Immunohistochemistry. Surgical specimens were fixed in Carnoy, paraffin-embedded and sectioned at 4 μm. Sections were mounted on slides, deparaffinized in xylene, blocked in 10% H_2O_2 and processed for antigen retrieval by MW heating in citrate buffer pH 6.0. The sections were rinsed in TRIS buffer, incubated with normal goat serum for 20', incubated overnight in a humified chamber with the following primary antibodies diluted in TRIS buffer:

Mouse monoclonal anti-nestin (R&D System; Minneapolis, USA 100 μg/ml, 1:100);

Mouse monoclonal anti-CXCL12 (R&D System; 500 μg/ml, 1:10);

Mouse monoclonal anti-VEGF (Neo Markers; Fremont, CA, USA 200 μg/ml, prediluted);

Rabbit polyclonal anti-PDGFβ (Santa Cruz Biotechnology Inc; Santa Cruz, CA, USA 100 μg/ml, 1:100);

Rabbit polyclonal anti-PDGFRβ (Santa Cruz Biotechnology, Inc 200 μg/ml, 1:400).

After three washes in buffer, sections were incubated for one hour with envision peroxidase conjugated. Finally, sections were rinsed and reacted with diaminobenzidine (Liquid DAB Substrate Chromogen System, DakoCytomation Carpinteria, CA, USA), counterstained and mounted. Negative controls (sections in which the primary antibody was substituted by non immune serum) were also stained each run.

Tumors were categorized as positive when they exhibited a moderate to strong plasma membrane and/or cytoplasmic staining in the majority of tumor and endothelial cells which was easily visible with a low-power objective. Staining intensity was evaluated semi-quantitatively using the following scale: -, negative; ±, weak; +, moderate; ++, strong.

Western blot. To evaluate the specificity of the various antisera, few samples of each tumor histotype were assessed by Western Blot.

Sample aliquots of approximately 100 μg of 5 micrometers thick paraffin sections from blocks of astrocytoma, anaplastic astrocytoma, oligodendroglioma, oligoastrocytoma, anaplastic oligoastrocytoma and glioblastoma were deparaffinized and homogenized in cold lysis buffer (100 mM NaCl, 10 mM EDTA, 0,5% Nonidet P-40, 0,5% sodium deoxycholate, 10 mM Tris, pH 7,4). The protein concentration was determined by bicinchoninic acid assay (Pierce, Rockford, IL). Sample aliquots equivalent to 50 μg protein were fractioned on SDS-PAGE and electrotransferred onto nitrocellulose membrane (ImmobilonPVDF; Millipore, Bedford, MA). Blots were blocked in 5% non fat milk in TBST and probed with antibodies: mouse monoclonal anti-nestin (R&D System; 1:1000); mouse monoclonal anti-CXCL12 (R&D System; 1:200); mouse monoclonal anti-VEGF (Neo Markers; prediluted), rabbit polyclonal anti-PDGFRβ (Santa Cruz Biotechnology, Inc 1:400), mouse monoclonal anti-CXCR4 (R&D System; 1:5000). The immunoblot was incubated with appropriate horseradish peroxidase-labelled secondary antibody (anti-mouse and anti-rabbit-conjugated). Primary and secondary antibodies were diluted in non fat milk (for anti-rabbit) or in TBST (for anti-mouse). Signal was visualized using ECL (Amersham Biosciences Arlington Heights, IL) and quantified by densitometry.

Statistical analysis. For statistical analysis, scores were condensed to positive or negative: moderate and strong expression were considered as positive, negative and weak expression were considered as negative.

ST and TTP ware analyzed by Kaplan-Meier survival curves and differences in this parameter between subgroups of patients by log-rank test; this test was used for equality of survival distributions. The variables considered for the univariate analysis consisted of patient-related and tumor-related features (i.e., age, tumor grade, immunohistochemical expression of VEGF, CXCL12, Nestin and PDGFR). The analysis were carried out using StatView 5.0.1 computer software (SAS Institute, Cary, NC) for Kaplan-Meier survival curves. For all statistical analyses $p < 0.05$ was considered significant.

RESULTS

Molecule expression. Immunohistochemical study. In our immunohistochemical study, nestin was variably expressed in neoplastic cells and in endothelial proliferations, with a correlation to malignancy grade, TTP and survival time (ST).

In low grade gliomas the expression of nestin (Fig. 1A–F) and CXCL12 (Fig. 2B and C) in endothelial cells can be used as a marker of undifferentiation, suggesting a critical role in early phases of neovascularization and malignant progression. CXCL12 was expressed in endothelial cells and/or some tumor cells. The staining was localized in the inner lining of the lumen and rarely in the cytoplasm of tumor cells with variable intensity. The negative staining of oligodendrocyte tumor cells (Fig. 1D and E) indicates that nestin is typical, although not exclusive to the astrocytic commitment.[25]

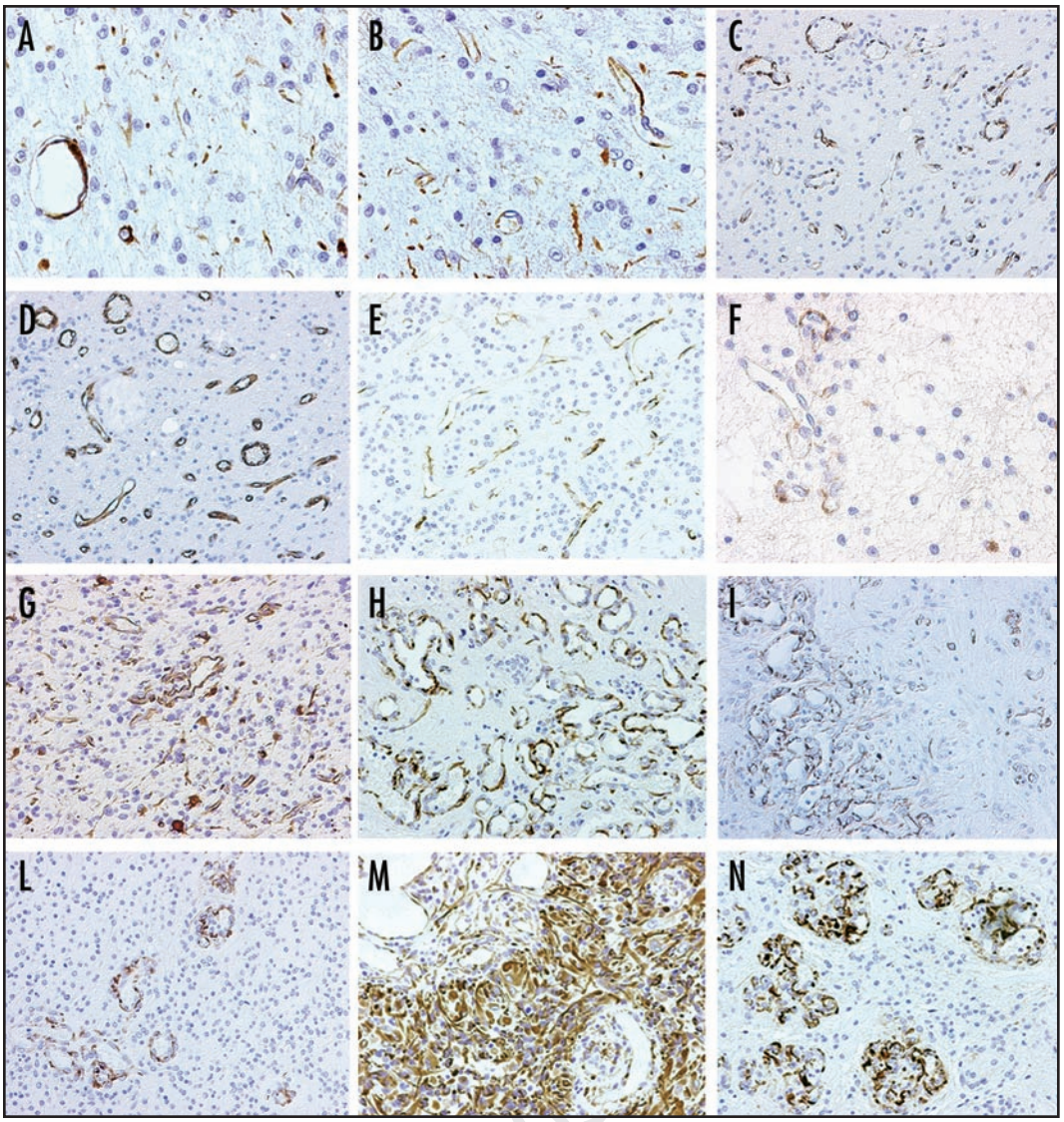

Figure 1. Nestin immunohistochemistry expression on paraffin sections of human gliomas: astrocytoma (A and B), oligodendroglioma (C–E), oligoastrocytoma (F), anaplastic astrocytoma (G), anaplastic oligodendroglioma (H and I), anaplastic oligoastrocytoma (L) and glioblastoma (M,N).

In low-grade (II) astrocytoma, oligodendroglioma and mixed glioma, nestin has a weakly positive staining in the newly formed vessels and only rarely a delicate cytoplasmic nestin staining of tumor cells (Fig. 1A–F). This pattern of immunostaining correlated with CXCL12 expression (Fig. 2A–C), while we did not observe positivity for VEGF.

Among low grade gliomas, the lowest levels of CXCL12 were observed in astrocytoma (Fig. 2A) and oligodendroglioma (Fig. 2B) while in oligoastrocytoma the positive staining for CXCL12 was higher than in the other histotypes (Fig. 2C).

In grade III and IV glioma nestin staining was more expressed in proliferating endothelium and in tumor cells in correlation to the grade of malignancy (Fig. 1G–I and L).

In glioblastoma the typical nestin staining had a fibrillar distribution along the processes and the cytoplasm of tumor cells; moreover, nestin was expressed in endothelial proliferations, that usually formed a typical pattern of glomeruloid structures (Fig. 1M and N). In these high grade gliomas nestin expression was overlapping the expression of CXCL12 in endothelial cells (Fig. 2D–G) and also showed a correlation with VEGF (Fig. 3G).

In GBM, almost half of the cases were positive for CXCL12 staining in endothelial or tumor cells, in particular immunoreactivity for CXCL12 was localized in tumor cells near areas of necrosis and in proliferating endothelial cells (Fig. 2H and I).

All glioma tissue specimens expressed CXCR4 in tumor and endothelial cells but the positive and diffuse nuclear staining was not unequivocally interpreted[27] and only few cases showed cytoplasmic positivity, in most cases the nuclear staining also involved healthy surrounding tissue.

PDGFRβ was expressed in the vasculature of low and high grade gliomas, with a correlation with malignancy grade; the intensity of the expression differed significantly among tumors (Fig. 3A–E) and we observed strong immunostaining in endothelial cell proliferations of glioblastoma (Fig. 3F). The expression of the receptor in tumor vessels was confirmed by our immunohistochemical and molecular results while in neoplastic cells it was scanty regardless of tumor grade. On the other hand, PDGF immunoreactivity was scanty or negative in almost all tissue specimens.

No expression of VEGF was detected in LGG, while we observed that anaplastic oligodendroglioma (Fig. 3G) and glioblastoma (Fig. 3H) showed an intense immunoreactivity to VEGF, that can be correlated to the expression of nestin. In glioblastoma VEGF was expressed mainly in tumor and endothelial cells around necrotic areas, suggesting that the upregulation of this growth factor was induced by hypoxia. A correlation was also found between expression of VEGF and PDGFRβ.

Analysing the different percentages of positivity in expression (Table 1) between LGG and HGG we can note that nestin (Fig. 4) and CXCL12 (Fig. 5) show a similar overall trend, with increasing immunopositivity in relationship to malignancy grade, while PDGFR (Fig. 6) shows higher expression than nestin and CXCL12 in HGG and similar percents in low grade gliomas.

In parallel, in order to asses the specificity of antisera, we performed a Western Blot analysis in a few samples. The antibodies recognized only the band corresponding to the protein against which they had been generated, without any cross-reactivity with other proteins. The expression levels of proteins were different among the tumors: PDGFR, nestin and CXCL12 revealed similar patterns with a more

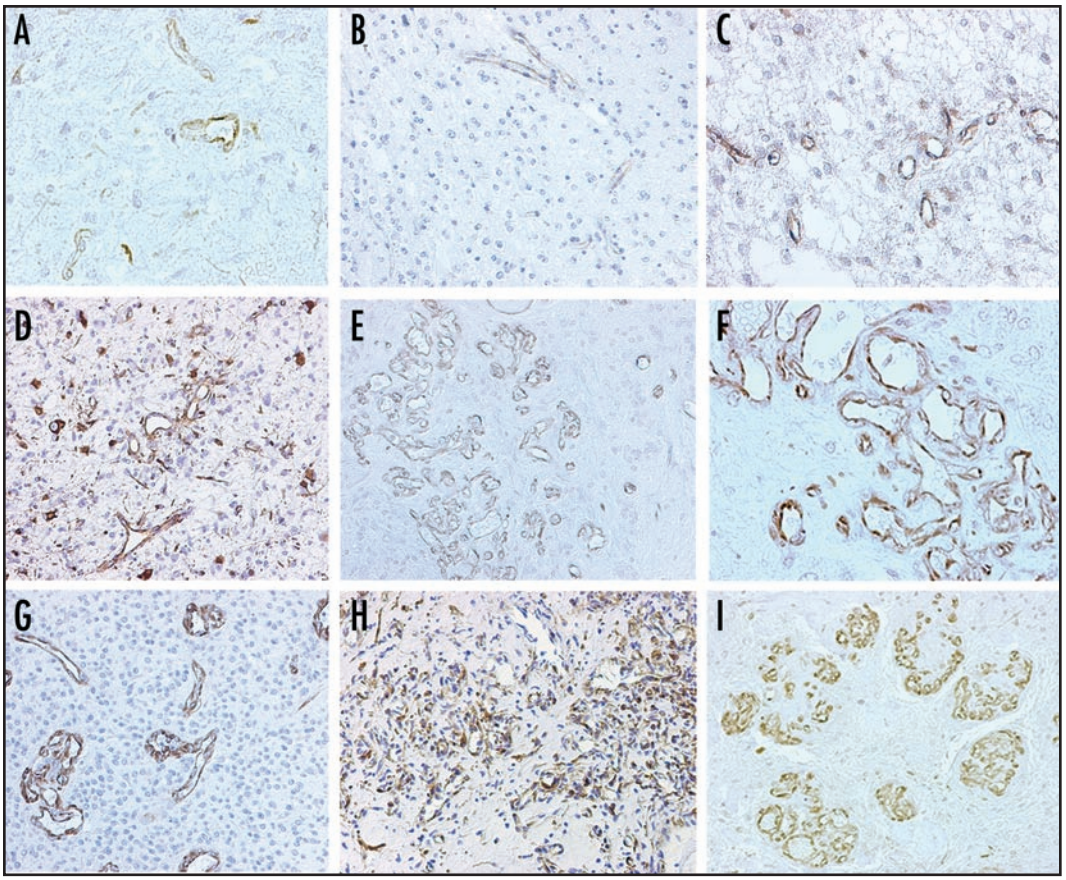

Figure 2. CXCL12 immunohistochemistry expression in gliomas: astrocytoma (A), oligodendroglioma (B), oligoastrocytoma (C), anaplastic astrocytoma (D), anaplastic oligodendroglioma (E and F), anaplastic oligoastrocytoma (G) and glioblastoma (H and I).

intense signal in anaplastic astrocytoma and glioblastoma, weak bands in oligoastrocytoma and variable signal in oligodendroglioma. For VEGF we observed bands with similar intensity in all tumors (unshown data).

Clinical features, molecule expression and clinical outcome. In our study, differences in age, histotype and tumor grade were associated with differences in TTP and in ST; after a follow up ranging from 3 to 167 months (median 52), 54 patients are dead. Among low grade gliomas (LGG), patients with oligodendroglioma had a more prolonged TTP (median 100) and ST (estimated median 117) than patients with astrocytoma and mixed glioma. At the end of the follow up only 18 patients are dead.

In HGG patients, 36 out of 53 patients are dead; median TTP was 29 in anaplastic glioma and 8 in glioblastoma patients, while median ST was 45 in anaplastic glioma and 12 in glioblastoma patients.

The curves in Figure 7 show overall survival in grade II, III and IV glioma patients.

In LGG, age at onset younger than the median age of the group (32 years) was not statistically related to a longer ST and TTP, while in HGG patients age at onset younger than median age (49 years) was associated to a more prolonged TTP ($p = 0.0075$) and ST ($p = 0.012$).

We analyzed the statistical correlations between molecule expression, time to tumor progression and survival time. Results of univariate analysis are reported in Table 2.

In all grade gliomas positivity for nestin, PDGFR and VEGF on tumor and endothelial cells was associated to a significantly shorter TTP and ST. No significant correlation was found between immunoreactivity for CXCL12 and TTP/ST.

In LG gliomas we have not found statistically significant impact of molecule expression on TTP, with the exception of CXCL12 ($p = 0.04$).

In HGG patients, nestin and VEGF showed a correlation with a shorter TTP. As far as ST is concerned, positivity for nestin and PDGFR expression was associated to a shorter survival time, while CXCL12 showed an opposite trend.

DISCUSSION

In the natural progression of glioma, the formation of new blood vessels from preexisting capillaries is a key event.[28] The high angiogenic activity in tumors is correlated with aggressiveness and poor patient survival in high grade glioma, while in low grade glioma the role of angiogenesis is still under investigation.[29]

During progression from low-grade glioma to high grade (grade III and IV) glioma, remarkable changes in vascular phenotype and in molecular features occur: malignant glioma vessels are characterized by high endothelial cell proliferation, which leads to formation of glomeruloid, garland-like, clustered bizzarre vascular formations or to a "classic" vascular pattern; molecular changes occurring in malignant glioma include loss of tumor-suppressor genes and gain of oncogenes[30] and overexpression of angiogenesis-related factors,[16,17] among which SDF1/CXCL12, PDGFRβ and VEGF. These factors are all involved also in autocrine-paracrine pathways potentially able to enhance tumor growth/survival.[31]

The relationship between undifferentiated tumor cells and the vascular supply has recently been emphasized also for GBM-derived cancer stem cells;[32] these cells are mostly nestin positive and may account both for glioblastoma recurrence/progression and for glioblastoma-associated angiogenesis; however, nestin is not per se a marker of stemness and it is also expressed on other progenitor cells among which endothelial progenitors. In this study, in order to investigate the amount and the meaning of nestin expression in relationship with the presence of SDF1/CXCL12, PDGFRβ and VEGF, we performed an immunohistochemical and molecular study on 102 patients with gliomas of different histotype and malignancy grade.

Nestin expression in glioma showed a correlation with tumor grade and clinical outcome in other works.[11,25] In our work, in agreement with literature data, nestin was expressed more frequently in HGG, both by tumor and by endothelial cells, being predictive of a

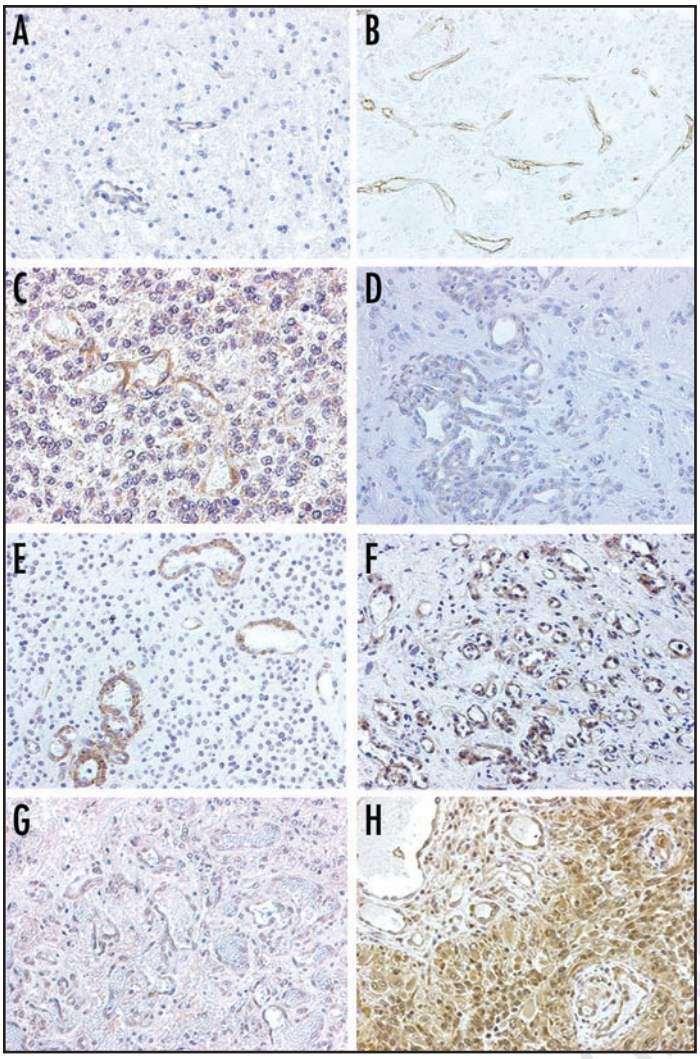

Figure 3. PDGFR expression in: astrocytoma (A), oligodendroglioma (B), anaplastic astrocytoma (C), anaplastic oligodendroglioma (D), anaplastic oligoastrocytoma (E) and glioblastoma (F). VEGF immunoreactivity in anaplastic oligodendroglioma (G) and glioblastoma (H).

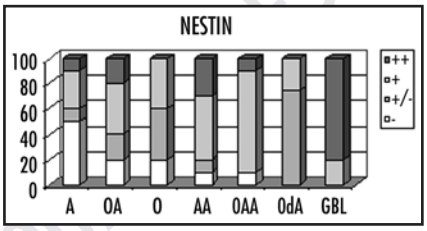

Figure 4. Immunohistochemistry expression of nestin in endothelial and tumor cells evaluated semi-quantitatively (percent value—Y axis) on different grade gliomas (X axis). Staining intensity was evaluated semi-quantitatively using the following scale: -, negative; ±, weak; +, moderate; ++, strong. A, astrocytoma; OA, oligoastrocytoma; O, oligodendroglioma; AA, anaplastic astrocytoma; OAA, anaplastic oligoastrocytoma; OdA, anaplastic oligodendroglioma; GBL, glioblastoma.

significantly shorter TTP and ST. Interestingly, we detected a higher percentage of positivity for nestin expression in HGG but we also observed a positivity for nestin in a proportion of patients with LGG;

Table 1 **Immunopositive percentages of molecule expression in all grade glioma**

	NESTIN	CXCL12	VEGF	PDGFR
Astrocytoma (n = 10)	40%	60%	-	20%
Oligoastrocytoma (n = 29)	52%	62%	-	38%
Oligodendroglioma (n = 10)	40%	40%	-	40%
Anaplastic astrocytoma (n = 8)	75%	62%	12%	38%
Anaplastic oligoastrocytoma (n = 11)	87%	85%	10%	50%
Anaplastic oligodendroglioma (n = 3)	33%	33%	33%	100%
Glioblastoma (n = 22)	100%	58%	61%	100%

it is tempting to speculate that undifferentiated cells may indeed be present also in these tumors.

In high grade gliomas, a consistent overall positive staining for CXCL12 in newly formed vessel endothelial cells and tumor cells in regions adjacent to necrotic areas has been detected.[23] The presence of nestin and CXCL12 in high-grade glioma is probably a sign of immaturity, while in low grade glioma it may be a sign of impending disease progression. Moreover, colocalization of nestin and CXCL12 may suggest that nestin-positive cells may at least partly represent subsets of cells (endothelial progenitors) able to partecipate in angiogenesis via chemotactic CXCL12-mediated signals.

In high grade glioma, CXCL12 has been shown to be expressed together with its receptor CXCR4 and their interaction may contribuite to angiogenesis, proliferation and migration of tumor cells.[21,33]

In this study, CXCR4 colocalized with CXCL12 but the significance of our results was not easy to evaluate because of the diffuse nuclear staining pattern, so we have not considered CXCR4.

Concerning CXCL12 expression, we found a higher percentage of positivity in HGG, both in endothelial and in tumor cells; however, at variance with data obtained for nestin, a negative prognostic impact of CXCL12 positivity was only detected in LGG patients, in whom CXCL12 expression was predictive of a shorter TTP and —with a borderline significance—of a shorter ST. These findings are in line with our previous results.[34,35] On the other hand, CXCL12 expression was associated with a significantly longer ST in HGG patients; this result is not easy to explain; we might speculate that the position of the CXCL12 locus on chromosome 10q could be related to a loss of both *PTEN*[36] and CXCL12 in those glioblastoma patients displaying loss of chromosome 10 or of its portions. In this case, the worse prognosis might be due to the more biologically relevant loss of *PTEN*, and this could explain the unexpected association of CXCL12 expression with a better prognosis.

We have also considered the most important angiogenic growth factor, VEGF, which plays a crucial role in tumor progression;[37] its expression in tumor and endothelial cells is also associated with tumor grade. In low grade glioma, little is known about the role of VEGF, that is weakly expressed, and other angiogenic factors than VEGF may be implicated in neoangiogenesis; recent studies have identified a role of this growth factor as a prognostic marker of survival in patients with low grade astrocytoma,[38,39] although not all authors have obtained analogous results;[40] on the other hand, a prognostic significance of VEGF in oligodendroglioma seems unlikely.[41] Positive staining has been described as more intense in tumor cells and vessels of glioblastoma, in particular around necrotic areas and in glomeruloid proliferations; this suggests that VEGF

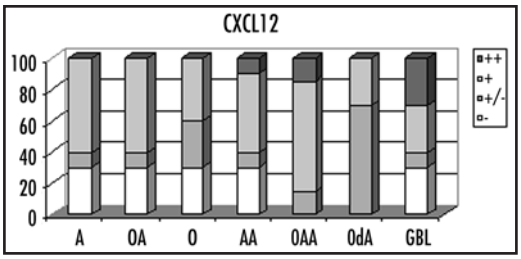

Figure 5. Immunohistochemistry expression of CXCL12 in endothelial and tumor cells evaluated semi-quantitatively (percent value–Y axis) on different grade gliomas (X axis). Staining intensity was evaluated semi-quantitatively using the following scale: -, negative; ±, weak; +, moderate; ++, strong. A, astrocytoma; OA, oligoastrocytoma; O, oligodendroglioma; AA, anaplastic astrocytoma; OAA, anaplastic oligoastrocytoma; OdA, anaplastic oligodendroglioma; GBL, glioblastoma.

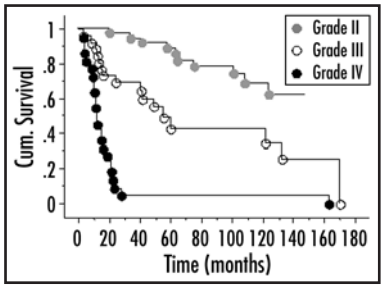

Figure 7. Kaplan-Meier curves show survival time (ST) of the three subgroups of patients with grade II, III and IV glioma.

may be implicated in driving an unusual pattern of endothelial proliferation.[3] The high level of VEGF in GBM (detected also in post-surgical endocavitary fluids) indicates that this protein has a central role in the growth of HGG[42] and its expression can be correlated with the expression of nestin as shown by our data. In our study, VEGF expression was not detected in LGG, while it was frequent (although not matching 100%) in HGG, in which it was related to a significantly shorter TTP.

Finally we found a weak positivity for PDGFRβ in low grade gliomas, that might be used to identify patients with a potentially more aggressive disease; in the literature, a high expression of PDGFRα has been shown to predict a longer survival in low-grade gliomas by Ribom,[43] but the meaning of this finding is unclear (these authors speculate that PDGFRα may indeed be somehow a marker for presence of an oligodendroglial component within the tumor);

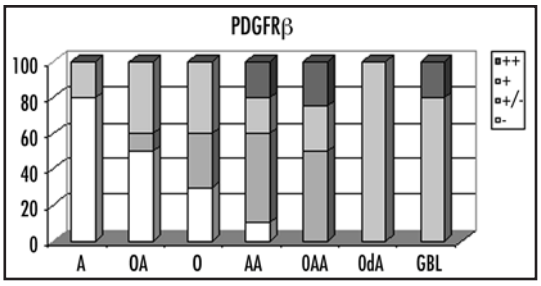

Figure 6. Immunohistochemistry expression of PDGFR in endothelial and tumor cells evaluated semi-quantitatively (percent value–Y axis) on different grade gliomas (X axis). Staining intensity was evaluated semi-quantitatively using the following scale: -, negative; ±, weak; +, moderate; ++, strong. A, astrocytoma; OA, oligoastrocytoma; O, oligodendroglioma; AA, anaplastic astrocytoma; OAA, anaplastic oligoastrocytoma; OdA, anaplastic oligodendroglioma; GBL, glioblastoma.

as a matter of fact, Varela[44] found that overexpression of PDGFRα could be used to identify low grade astrocytoma patients with a more aggressive disease, while it did not have a prognostic value in anaplastic astrocytoma and glioblastoma.

Our study investigated PDGFRβ expression, yielding results similar to those reported by Varela for PDGFRα (namely, a predictive value for a worse outcome in low-grade glioma patients displaying positivity for PDGFR expression); an increased expression of PDGFRβ in endothelial cells of high grade gliomas was also seen while the expression of PDGF was scanty or negative in both endothelial and tumor cells. Previous data suggest that high levels of expression of PDGF and PDGFR in high grade glioma enhance angiogenesis by stimulating VEGF expression on tumor endothelium;[22] we can expect that the malignant features of glial tumors are associated with an upregulation of PDGFR on endothelial cells of tumor vessels.[45]

To assess the correlation of ST and TTP with histopathological and clinical characteristics (age, histology and tumor grade), we performed an univariate statistical analysis, which, as far as clinical parameters are concerned, showed statistically significant impact of age at onset on ST only in HGG. Overall, the restricted number of patients did not allow a multivariate approach.

Despite these limitations, the presence of different expression levels of the investigated proteins in gliomas indicates a possible role as prognostic markers of clinical outcome and provides information about new possible therapeutic targets.

Actually, the molecules investigated in this study are implicated at various levels with the processes of increased proliferation, migration and neovascularization associated with growth/progression of glioma; the integration of the expression profile of these molecules with clinical and molecular biology markers will hopefully lead to a more targeted therapeutic approach in these tumors.

Table 2 **Univariate analysis of statistical correlation between molecule expression, time to tumor progression and survival time in different subgroups of glioma**

	ALL		LGG (49)		HGG (53)	
	TTP	ST	TTP	ST	TTP	ST
Age < median				p = 0.85		p = 0.01*
Nestin	p = 0.001*	p = 0.0005*	p = 0.28	p = 0.64	p = 0.03*	p = 0.04*
CXCL12	p = 0.161	p = 0.51	p = 0.04*	p = 0.06*	p = 0.54	p = 0.02
VEGF	p = 0.004*	p = 0.042*	n.c.	n.c.	p = 0.04*	p = 0.27
PDGFR	p = 0.05*	p = 0.0154*	p = 0.8	p = 0.83	p = 0.09*	p = 0.02*

n.c., not calculable. *Asterisk denotes worse outcome in pts positive for the investigated markers/older than median age in the subgroup.

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