Folia Neuropathologica

Clinicopathological considerations on angiogenic potential in neuroblastoma Schwannian stroma – poor tumours

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Folia Neuropathol 2007; 45 (1): 1-8

Abstract

The aim of the study was to determine microscopic angiogenic parameters of neuroblastoma (NB) Schwannian stroma-poor tumours. Furthermore the associations between vascular parameters and clinicopathological features of tumours and basic prognostic factors were analysed. Examined tissue samples from 62 NB came from 39 untreated and 23 chemotherapy pretreated tumours. The clinicopathological data comprised: patients' age, gender, survival, tumour site and stage, tumour histology and MYCN status.

The morphological analysis of the angiogenic potential concentrated on examination of vascular patterns – classical type or pathological angiogenesis with mural microvascular proliferation (MVP). The quantitative study included semi-automatic assessment of vascular density (VD) in CD34 stained tumour sections.

Pathologic angiogenesis with MVP, including simple and/or glomeruloid type, was encountered in 25 cases and was more frequent in differentiating histology subtype and extraadrenal tumours. VD value ranged from 56 to 385 vessels/mm^2 (median 149). Higher VD was connected with younger patient's age. In untreated tumours VD was significantly higher in infants than in children over one year of age. Pathologic type angiogenesis and lower VD were found to be associated with shorter survival. Our study confirmed high vascularization of NB and revealed common occurrence of vascular pattern with MVP. Angiogenic potential in the analysed group showed diversity related to some clinicopathological tumour features. This points toward heterogeneity of NB tumours in vascular aspects, possibly affecting tumours' reactivity to antiangiogenic therapy.

Key words: *angiogenesis, microvascular proliferation, neuroblastoma, predictive factors, vascular density, vascular pattern*

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Introduction

Neuroblastic tumours, including neuroblastoma (NB), ganglioneuroblastoma and ganglioneuroma, are the most common extracranial solid tumours of infancy and childhood [4,26]. They comprise a complex group of embryonal neoplasms of the sympathetic nervous system. Histological evaluation of NB is focused on the stage of neuroblastic differentiation and Schwannian stroma development [1,18,19,29]. The main prognostic classification system of NB is age-linked histological classification by the International Neuroblastoma Pathology Committee (INPC), adopting the Shimada system [18,29]. The other accepted prognostic factors include cellular DNA content and some molecular alterations with the most important N-myc gene (*MYCN*) amplification [1,3,4]. The above factors allow risk groups to be established and risk-related therapy of NB to be developed [1,4,29]. The prognosis of patients with unfavourable histology, advanced disease stage and with *MYCN* amplification is bad. There is a need for development of new therapeutic strategies and one of them is promising antiangiogenic treatment [16,23].

In many types of cancer the intensity of intratumoural angiogenesis has become one of the markers of biologic aggressiveness and a potential prognostic factor for patients [28,30]. Morphological studies on angiogenesis in NB are few and clinicopathological correlations of angiogenic parameters and their prognostic relevance are not finally established [5,21,24]. Moreover, research on angiogenesis in NB can give possible predictive information for antiangiogenic strategies introduced as an adjunct to traditional therapy [6,23].

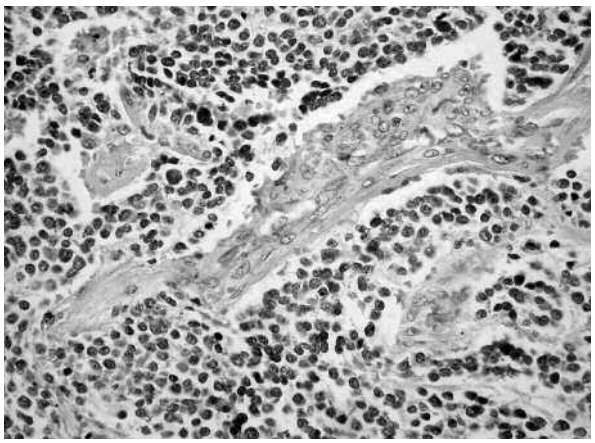

Fig. 1. Microvascular proliferation (MVP) of simple grade – vessels with thickened wall (HE, x400)

The aim of the present study was to describe the angiogenic potential in NB evaluated morphologically according to the pattern of microvascular proliferation and quantitatively with assessment of the intratumoural vascular density. The relationship of vascular parameters to the clinicopathological data and prognostic factors was analysed.

Material and methods

The examined cases came from the Department of Pathomorphology, Medical University of Gdansk, and the Department of Pathology, Memorial Health Institute in Warsaw. Cases with insufficient tissue material and diagnosed as ganglioneuroblastoma and ganglioneuroma (to make the examined group more homogeneous morphologically) were excluded from this study. Final analysis was performed on 62 cases of neuroblastoma Schwannian stroma-poor tumours from patients treated in the period 1997-2005 in the Department of Paediatrics, Haematology, Oncology and Endocrinology, Medical University of Gdansk, and in the Department of Oncology, Memorial Health Institute in Warsaw. Clinical data included: patients' gender and age, location of the primary tumour, clinical stage of the disease, type of treatment before tumour resection or sampling and survival (from diagnosis to mid-March 2006). The data concerning patients' clinical course and survival were analysed in 33 children from the untreated tumours group; in five the follow-up was too short. Patients from the untreated group were divided into favourable and unfavourable histology subgroups based on Shimada's criteria.

Formalin-fixed, paraffin-embedded biopsy specimens of the tumours were cut into slides 4 µm thick and stained with H-E. The histopathological diagnosis in each case was reassessed by two independent pathologists (EIS, WG) according to modified Shimada classification with INPC criteria [27,29].

Histological examination of vascular stroma included morphological analysis of vascular patterns. There were two types of angiogenesis: classic (made of thin-walled vessels) and pathological angiogenesis with mural microvascular proliferation (MVP). MVP intensity was divided into two types: simple and glomeruloid [17]. Simple type proliferation was defined as signs of cytologic activation of endothelial and perithelial cells with their hyperplasia within a vessel with a single lumen (Fig. 1). Glomeruloid

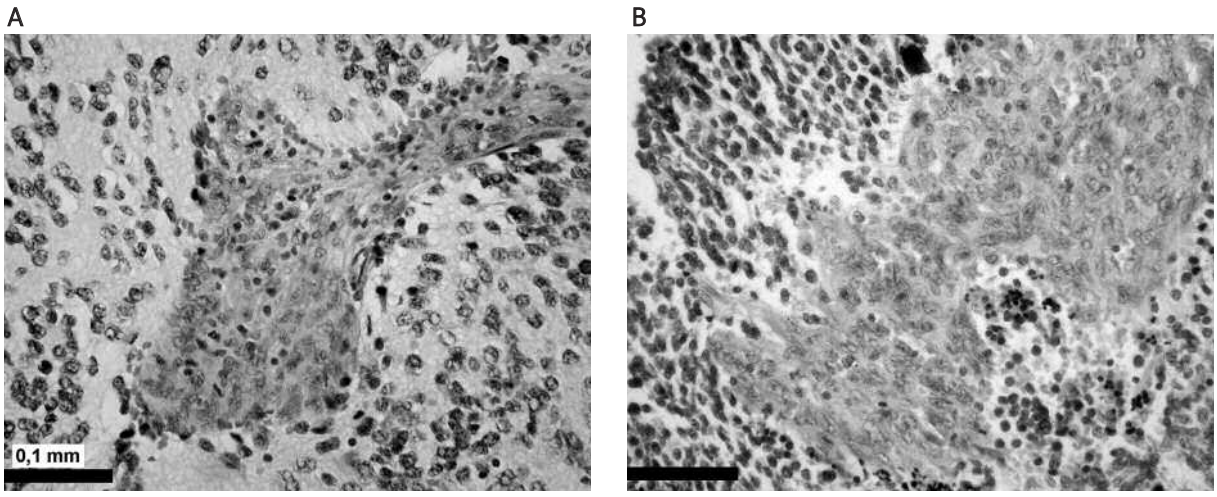

Fig. 2A-B. Microvascular glomeruloid proliferation – multiluminal vascular tufts (HE, x400)

proliferation was defined as hypertrophy and hyperplasia of the cells within the vascular wall with multiple lumens, forming vascular conglomerations in the most intense form (Fig. 2A, B). In every examined case the type of maximal MVP intensity was marked.

The most representative sections with the highest vessel number and without diffuse necrosis were selected for immunohistochemical staining with monoclonal antibody against CD34 (DAKO, 1:50) in 60 cases. We used CD34 based on own experience that this antibody is reliable for angiogenesis studies. The designation of antigen was performed routinely with LSAB 2 method (DAKO) with appropriate positive and negative controls (Fig. 3).

Quantitative analysis was performed with a computed image analyzing system (microscope Olympus BX50, camera CCD-FS-2012P (Bischke), software MultiScan v.5.10 (CSS)). Tumour fields with the highest number of microvessels (hot spots) were identified at 40x. Under 100x magnification, 10 fields from these areas were recorded to the computer memory with total examined area of 3.1 mm^2. Any immunopositive structure (round, oval, ring or irregular) clearly separated from adjacent profiles and other tissue elements were considered as a single countable vessel. In analysis of complicated structures such as glomeruloids and vascular conglomerations, every separate lumen surrounded with chromogen was counted as a single vessel, irrespectively of type of vessel cuts. Vascular profiles were manually pointed and counted automatically on computer images. Mean vessel number in every 10 fields per examined area was counted as the vascular density (VD) (vessel/mm^2). To test reproducibility of assessment of VD it was performed twice in eight randomly selected cases. Correlation of first and second assessment of VD was high ($r= 0.902$; $p<0.002$).

MYCN copy number was determined in the Department of Biology and Genetics of the Medical University in Gdansk. Cytogenetic fluorescence in situ hybridization (*FISH*) studies with *MYCN* probe were performed in 48 cases. In 15 cases *FISH* was carried out on the cells from tumour tissue cultures and in 33 on tumour imprints. The signal number ≥10 was considered as amplification.

All calculated results were collected in a database for statistical evaluation. Chi-square test, Kruskal-Wallis test, U Mann-Whitney test, and Spearman's

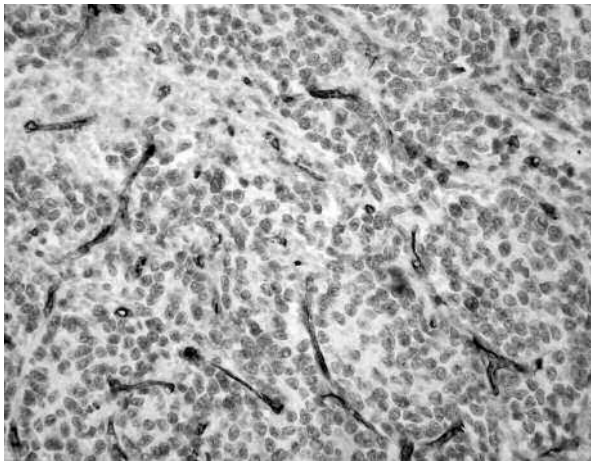

Fig. 3. Numerous microvessels in vascular-rich tumour with classic-type angiogenesis (CD34, x100)

coefficient of correlation test were used as appropriate. A p value of ≤0.05 was considered statistically significant. All calculations were performed using the Statistica for Windows (v 5.1) program (StatSoft Inc. Tulsa, Ok, USA).

Statistical survival analysis (Kaplan-Meyer) of patients stratified by MVP and VD of NB tumours was performed. Overall survival (OS), which is time from the onset of treatment to the death of progression (DOP), was examined after at least 12 months' follow-up. In relations between VD and survival, VD was divided into low and high values, with borderline value of 149 vessels/mm^2 – the median of the untreated group. According to angiogenic pattern and survival correlations, two groups were analysed – with and without MVP. The differences between the groups were verified using log-rank test.

Results

The analysed NB Schwannian stroma-poor cases were diagnosed in 19 girls and 43 boys. Material from 39 tumours was examined before (untreated) and in 23 cases after the induction chemotherapy (treated). Patients' age at surgery ranged from 2 weeks to 10 years, with median age of 10 months (mean 25.6 months; SD 29.6); 34 patients were infants (54%). Five patients were in stage I of the disease, five in stage II, 19 in III, 25 in IV and 8 patients were in stage IVs. Most of the patients were in III and IV stage of the disease (84%). The sites of primary tumours were: adrenal glands in 36, abdominal extraadrenal in 18, mediastinal in 6 and unestablished in 2 patients. The period of survival in 33 analysed patients from the untreated group ranged from 6 weeks to 142 weeks.

Histopathological examination revealed 5 undifferentiated, 35 poorly differentiated and 22 differentiating tumours. MKI was low in 13, intermediate in 17 and high in 9 untreated cases. There were 22 cases in the favourable and 17 in the unfavourable histology group.

The distribution of blood vessels and angioarchitecture within and among the tumours were heterogeneous. 37 tumours presented classic angiogenesis pattern with small thin- walled vessels. The vessels formed vascular-rich areas within tumour lobules and/or areas with low vascularization sometimes limited to the tissue surrounding neoplastic lobules and nests. In 25 cases (40.3% of all tumours) pathological angiogenesis pattern with signs of microvascular proliferation (MVP) was revealed. MVP was unevenly distributed especially within the interlobular septa. It was encountered focally or as multiple dispersed thickened microvessels, forming glomeruloid structures, which were sparse or created vascular garlands. Isolated simple type MVP was present in 9 cases (14.5%) and in 16 the glomeruloid MVP type was also encountered (25.8%).

MVP occurred more frequently in NB differentiating subtype (14/22) than in the other subtypes (chi-square test; p=0.015). Moreover, MVP was a more frequent finding in the extraadrenal tumours (15/26) than in adrenal sites (12/36), with a significant difference in untreated tumours: 12/19 extraadrenal, 6/20 adrenal tumours (chi square test p=0.03).

Vascular density was measured in 60 cases and its values ranged between 56 and 385 vessels/mm^2, with a mean value of 166.6 vessels/mm^2 (median 149, SD – 89.33). A negative correlation was found between patients' age and VD, so younger age was related to higher VD (r=-0.35; p=0.019). In untreated tumours the age-linked difference of VD values was stronger (r=-0.47; p=0.003). In infantile untreated tumours mean VD was higher (mean 195.4; SD 95; median 174) than in children over one year (mean 130.5; SD 74; median 122) and this difference was statistically significant (U-Mann Whitney p=0.02).

A tendency for higher VD in tumours without MVP was observed (Chi square test; p=0.055).

Amplification of the *MYCN* gene was found in 14/48 examined cases (29%); additionally, in a further 6 tumours (12.4%) *MYCN* low copy number gains (3-9) were detected.

The analysis of other relationships (VD vs patients' gender, chemotherapy, stage, tumour site and MVP presence or type, *MYCN* status; MVP presence or type vs stage, age, patients' gender, risk group and *MYCN* amplification) did not reveal any significant correlations. There were no significant differences between the treated and untreated group in MVP frequency and intensity or VD value.

The analysis of relations between angiogenesis pattern, measured by MVP presence, and survival showed that patients with MVP have shorter survival than patients with classical pattern of angiogenesis, but this difference was not statistically significant (log rank p=0.15) (Fig. 4). The group of patients with higher VD showed a relation,

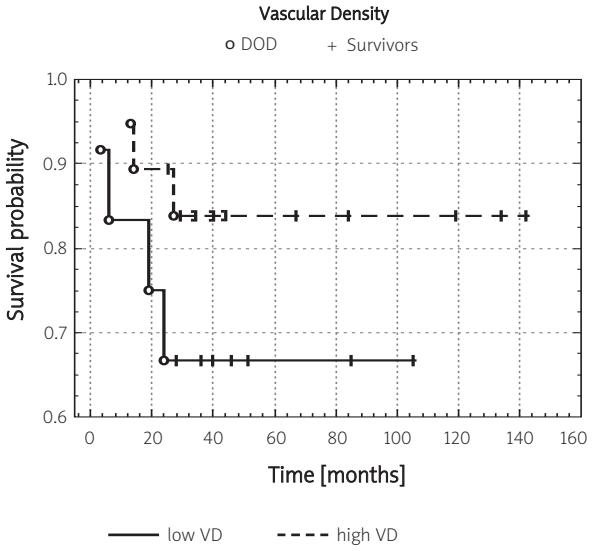

Fig. 4. Overall survival curves for patients with low vascularity of NB versus patients with high vascularity of NB (log rank p=0.12)

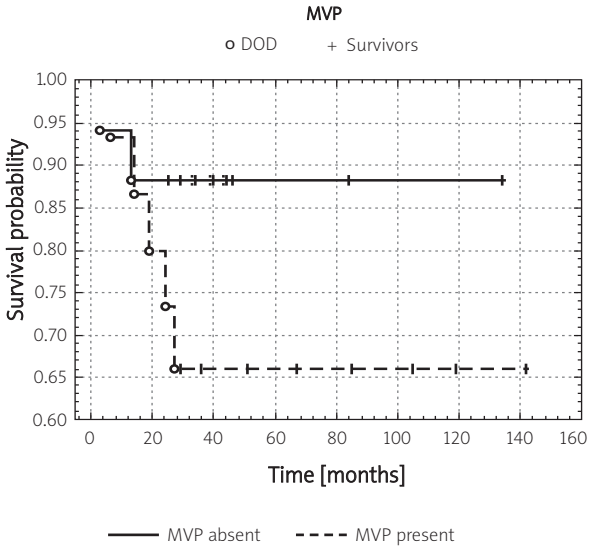

Fig. 5. Overall survival curves for patients with NB with MVP features versus patients with NB without MVP features (log rank 0.15)

although insignificant, with longer survival (log rank p=0.12) (Fig. 5).

Discussion

Neuroblastoma Schwannian stroma-poor tumours comprise the most common group of neuroblastic tumours [27,29]. NB can be recognised as small localised tumours, successfully treated with surgical resection alone (stages I, II) or locally invasive stage III and metastatic tumours (stage IV) associated with a serious clinical outcome [4,26]. In most cases, however, the disease is already locally advanced or disseminated at diagnosis [3,4]. Infants under one year of age have a relatively good prognosis, whereas older patients are prognostically worse, even when aggressively treated [3,4,26].

A knowledge of clinicopathological aspects of angiogenesis in NB can provide an insight into this tumour biology and is a prerequisite for successful introduction of promising antiangiogenic therapy into the clinics [16,23]. The angiogenic process is regulated by complex interactions among growth factors and inhibitors and is also closely associated with cancer stromatogenesis [7,9,14]. Some angiogenic factors contribute to aggressive tumour biology by additional direct influence on neoplastic cells [7,9]. It is accepted that in tumour biopsies the extent of angiogenesis is sufficiently reflected by the number of microvascular profiles, assessed in hot spots [28,30]. The majority of malignant tumours prove the association of high vascular density with worse prognosis (breast, colon, urothelial cancer); however, in some types of cancer the correlations are inverse, reflecting differences in angiogenic mechanisms [6,28,30]. Some of these differences may be based as well on non-angiogenesis dependent pathways for tumour vascularization [20,25]. Moreover, in some tumours angiogenic potential is expressed not only by numerical increase but also by vascular patterns or angioarchitecture, especially pathological angiogenesis with morphological changes called microvascular proliferation (MVP) [2,28]. The type of vascular patterns, classical versus pathological, seems to have prognostic significance in some types of cancer [2,28]. Nowadays some antiangiogenic regimens are introduced to the clinics, and information about angiogenic potential of the tumour can serve as a predictive factor.

The range and inter-tumour variability of VD in our group were high and similar to results in some highly vascularised malignant solid tumours [2,13]. In our study the cases selection bias based on availability of material could influence the character and representativity of the patient group. The comparison of our results with the few other studies on NB is difficult due to different methodology, the diversity of the cohorts of patients, as well as the

way of presentation of the results [5,21,24]. Meitar et al. [21] counted vessels manually on HE stained slides on whole 50 NB sections and found that higher vascular index (above 4 vessels/mm^2) correlated with disseminated disease, poor survival and *MYCN* amplification. Canete et al. [5] examined several vascular parameters on CD34 stained sections from 69 NB fully automatically. They received very high VD values and found no correlations of vascular parameters with any prognostic factors. Ribatti et al. [24] in a group of 20 tumours stained with F VIII adopted their own special criteria for counting vessels and reported higher VD in advanced NB stages, especially IVs.

Our study was performed on a group of NB Schwannian stroma-poor tumours. It showed a statistically significant relationship between VD and patients' age, with higher VD values in younger patients. This relationship is found for the first time in NB, although it was noted before in primitive neuroectodermal tumours (PNETs) [13]. Possibly in infantile NB the role of intratumoural angiogenesis is different than in children more than one year of age. Perhaps better vascular supply in NB in infants recalls the conditions during embryological development [14]. In infants tumour regression and involution during apoptosis or tumour differentiation are more common than progression [3,4]. Our results suggest that infantile NB because of high vascular density is more prone to antiangiogenic therapy. The tendency for a significant relation between higher VD and longer survival is possibly connected to the age of patients in our group. High vascularization in infant NB tumours was identified in the earlier study [8].

Some studies show an association of *MYCN* amplification with enhanced angiogenesis of human NB assessed with VD or angiogenic factor expression [9,15,21]. It seems that *MYCN* influences angiogenic factors indirectly by down-regulation of endothelial cell growth inhibitors [15]. In our study relations between vascular parameters and *MYCN* amplification were not found. No difference was found either between treated and untreated tumour groups, suggesting resistance of the tumour vascular bed to first-line classic chemotherapy. Chemotherapy can cause remarkable histological changes in some NB.

VD, such a popular parameter estimating the intensity of angiogenesis, gives only information about the presence of vessels, without insight into their morphological or functional properties [11,28]. In some neoplasms specific features of tumour vascularity are used as basic diagnostic histologic criteria. The best known example is microvascular proliferation in glioblastoma with vascular garlands and glomeruloid structures. In glioblastoma intense MVP has been reported as an independent prognostic factor [2,17]. On the other hand pathological angiogenesis with MVP is common in benign pilocytic astrocytoma [11].

Morphological studies on angiogenesis and vascularization in NB are sparse [9,18,21]. In the few examined cases of NB Gaudin and Rosai [10] showed features of MVP as tufts of proliferating endothelial and perithelial cells and glomeruloid structures. Joshi et al. [18] described MVP in 7 from 53 analysed tumours, confirming this feature as unusual for NB. A study of vascular patterns considered as a measure of intensity of NB angiogenesis has been carried out for the first time and we found MVP in about 40% of cases, including glomeruloid form. Glomeruloid vessels are a form of pathologically overexpressed angiogenesis, which forces the capillary network to transform focally into irregular structures made of proliferation of endothelial cells and pericytes [2,11]. The role of MVP in the context of antiangiogenic therapy is unknown; however, these vessels are thought to be less effective in blood and nutrients circulation [6,30]. Interestingly, in our group MVP was a more frequent finding in the extraadrenal tumours than in adrenal. This fact may suggest the influence of local tissue factors on NB vascularization. Tumour location influences vascularization in PNETs, and it differs between supra- and infratentorial tumours, which are in fact diverse in molecular aspects [12]. Furthermore we found that MVP occurred more frequently in differentiating subtype of NB, usually on the borders of interlobular septa. In this NB subtype Schwann cells are present in the fibrovascular septa and stromal formation may be substantial in some tumours [29]. During maturation of NB, first S-100 positive cells have perivascular location and have stromal, non-neoplastic origin [1,22]. Perhaps vessels with MVP features take part in NB stroma development. On the other hand, analysis of the relation between MVP presence and survival showed a tendency for shorter survival in patients with MVP, similar to the study by Birner et al. on glioblastoma [2]. The authors explain this linkage by the influence on clinical outcome by variable expression of angiogenic proteins.

Our analysis of NB Schwannian stroma-poor tumours disclosed their high angiogenic potential, quite often with the presence of the pathological angiogenesis pattern with MVP. We describe for the first time distinct angiogenic patterns in NB. We found some differences in NB vascularization related to patients' age, tumour pathology and location. Examined angiogenic parameters seem to influence NB patients' survival; however, this finding needs confirmation in a bigger group of patients. Our results point toward the heterogeneity of neuroblastic tumours in vascular aspects, which can influence their response to antiangiogenic therapy.

Acknowledgements

Supported by grant of the Polish State Committee for Scientific Research No. 6PO5E-091-21 and W-951 Medical University of Gdansk.

The authors would like to thank: Prof. Janusz Limon and Dr Iwona Kardas from the Department of Biology and Genetics, Medical University of Gdansk for genetic analyses of *MYCN* amplification.

References

1. Ambros I, Hata J, Joshi V, Roald B, Dehner LP, Tuchler H, Potschger U, Shimada H. Morphologic features of neuroblastoma (Schwannian stroma-poor tumors) in clinically favorable and unfavorable group. Cancer 2002; 94: 1574-1583.
2. Birner P, Piribauer M, Fischer I, Gatterbauer B, Marosi C, Ambros PF, Ambros IM, Bredel M, Oberhuber G, Rossler K, Budka H, Harris AL, Hainfellner JA. Vascular patterns in glioblastoma influence clinical outcome and associate with variable expression of angiogenic proteins: evidence for distinct angiogenic subtypes. Brain Pathol 2003; 13: 133-143.
3. Bown N. Neuroblastoma tumor genetics: clinical and biological aspects. J Clin Pathol 2001; 54: 897-910.
4. Brodeur GM. Neuroblastoma: biological insights into a clinical enigma. Nat Rev Cancer 2003; 3: 203-216.
5. Canete A, Navarro S, Bermudez J, Pellin A, Castel V, Llombart-Bosch A. Angiogenesis in neuroblastoma: relationship to survival and other prognostic factors in a cohort of neuroblastoma patients. J Clin Oncol 2000; 18: 27-34.
6. Carmeliet P, Jain R. Angiogenesis in cancer and other diseases. Nature 2000; 407: 249-257.
7. Chlenski A, Liu S, Cohn S. The regulation of angiogenesis in neuroblastoma. Cancer Lett 2003; 197: 47-52.
8. Drozynska E, Izycka-Swieszewska E, Balcerska A. Angiogenesis in neuroblastoma (abstract). Med Pediatr Oncol 2002; 39: 386.
9. Eggert A, Ikegaki N, Kwiatkowski J, Zhao H, Brodeur GM, Himelstein BP. High-level expression of angiogenic factors is associated with advanced tumour stage in human neuroblastomas. Clin Cancer Res 2000; 6: 1900-1908.
10. Gaudin PB, Rosai J. Florid vascular proliferation associated with neural and neuroendocrine neoplasms. A diagnostic clue and potential pitfall. Am J Surg Pathol 1995; 19: 642-652.
11. Gesundheit B, Klement G, Senger C, Kerbel R, Kieran M, Baruchel S, Becker L. Differences in vasculature between pilocytic and anaplastic astrocytomas of childhood. Med Pediatr Oncol 2003; 41: 516-526.
12. Goldbrunner RH, Pietsch T, Vince GH, Bernstein JJ, Wagner S, Hageman H, Selby DM, Krauss J, Soerensen N, Tonn JC. Different vascular patterns of medulloblastoma and supratentorial primitive neuroectodermal tumors. Int J Dev Neurosci 1999; 17: 593-599.
13. Grotzer MA, Wiewrodt R, Janss AJ, Zhao H, Cnaan A, Sutton L, Rorke LB, Phillips PC. High microvessel density in primitive neuroectodermal brain tumors of childhood. Neuropediatrics 2001; 32: 75-79.
14. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 2000; 100: 57-70.
15. Hatzi E, Breit S, Zoephel A, Ashman K, Tontsch U, Ahorn H, Murphy C, Schweigerer L, Fotsis T. MYCN oncogene and angiogenesis: down-regulation of endothelial growth inhibitors in human neuroblastoma cells. Purification, structural, and functional charcterization. Adv Exp Med Biol 2000; 476: 239-248.
16. Izbicka E, Izbicki T. Therapeutic strategies for the treatment of neuroblastoma. Curr Opin Investig Drugs 2005; 6: 1200-1214.
17. Izycka-Swieszewska E, Rzepko R, Borowska-Lehman J, Stempniewicz M, Sidorowicz M. Angiogenesis in glioblastoma – analysis of intensity and relations to chosen clinical data. Folia Neuropathol 2003; 41: 15-21.
18. Joshi VV. Peripheral neuroblastic tumors: pathologic classification based on recommendations of International Neuroblastoma Pathology Committee (modification of Shimada classification). Pediatr Dev Pathol 2000; 3: 184-199.
19. Joshi VV, Silverman JF, Altshuler G, Cantor AB, Larkin EW, Neill JS, Norris HT, Shuster JJ, Holbrook CT, Hayes FA et al. Systematization of primary histopathologic and fine-needle aspiration cytologic features and description of unusual histopathologic features of neuroblastic tumors: a report from the Pediatric Oncology Group. Hum Pathol 1993; 24: 493-504.
20. Kim E, Moore J, Huang J, Soffer S, Manley CA, O'Toole K, Middlesworth W, Stolar CJ, Kandel JJ, Yamashiro DJ. All angiogenesis is not the same: Distinct patterns of response to antiangiogenic therapy in experimental neuroblastoma and Wilms tumor. J Pediatr Surg 2001; 36: 287-290.
21. Meitar D, Crawford SE, Rademaker AW, Cohn SL. Tumor angiogenesis correlates with metastatic disease, N-myc amplification, and poor outcome in human neuroblastoma. J Clin Oncol 1996; 14: 405-414.
22. Nagoshi M, Tsuneyoshi M, Enjoji M. S-100 positive undifferentiated neuroblastomas with a special reference to the tumor stroma related to favorable prognosis. Pathol Res Pract 1992; 188: 273-283.
23. Ribatti D, Ponzoni M. Antiangiogenic strategies in neuroblastoma. Cancer Treat Rev 2005; 31: 27-34.
24. Ribatti D, Surico G, Vacca A, De Leonardis F, Lastilla G, Montaldo PG, Rigillo N, Ponzoni M. Angiogenesis extent and expression of matrix metalloproteinase-2 and -9 correlate with progression in human neuroblastoma. Life Sci 2001; 68: 1161-1168.

25. Ribatti D, Vacca A, Dammacco F. New non-angiogenesis dependent pathways for tumor growth. Eur J Cancer 2003; 39: 1835-1841.
26. Schmidt ML, Lukens JN, Seeger RC, Brodeur GM, Shimada H, Gerbing RB, Stram DO, Perez C, Haase GM, Matthay KK. Biologic factors determine prognosis in infants with stage IV neuroblastoma: A prospective Children's Cancer Group Study. J Clin Oncol 2000; 18: 1260-1268.
27. Schwab M, Shimada H, Joshi V, Brodeur GM. Neuroblastic tumors of adrenal and sympathetic nervous system. In: Chapter 9. Peripheral neuroblastic tumors. In: Pathology and Genetics of tumours of the Nervous system. Kleihues P, Cavenee WK (eds.). IARC Press, Lyon 2000; pp. 153-161.
28. Sharma S, Sharma MC, Sarkar C. Morphology of angiogenesis in human cancer: a conceptual overview, histoprognostic perspective and significance of neoangiogenesis. Histopathology 2005; 46: 481-489.
29. Shimada H, Ambros IM, Dehner LP, Hata J, Joshi VV, Roald B, Stram DO, Gerbing RB, Lukens JN, Matthay KK, Castleberry RP. The International Neuroblastoma Pathology Classification (the Shimada system). Cancer 1999; 86: 364-372.
30. Weidner N. Angiogenesis as a predictor of clinical outcome in cancer patients. Hum Pathol 2000; 31: 403-405.