Neurosurg Rev (2014) 37:79–88
DOI 10.1007/s10143-013-0495-5

ORIGINAL ARTICLE

Expression of vascular endothelial growth factor receptor 2 (VEGFR-2), inducible nitric oxide synthase (iNOS), and Ki-M1P in skull base chordoma: a series of 145 tumors

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Received: 30 June 2012 / Revised: 17 January 2013 / Accepted: 21 January 2013 / Published online: 3 September 2013

Abstract Chordomas are locally invasive tumors that have a tendency to relapse despite optimal treatment. Specific biological markers might be used to describe their behavior. There is currently no agreement regarding the best way to manage intracranial chordomas. We studied the expression of vascular endothelial growth factor receptor 2 (VEGFR-2), inducible nitric oxide synthase (iNOS), and Ki-M1P in 145 paraffin-embedded tumors. The purpose of our study was to determine: (a) the role of potent angiogenic factors VEGFR-2 and iNOS and their relationship to each other in skull base chordoma and (b) the role of monocytes/macrophages as a potential iNOS source in the angiogenic process. A series of 74 chordoma patients for a total of 145 lesions (including 71 recurrent lesions) and 10 specimens from embryonic notochord were investigated for the expression of iNOS, VEGFR-2, Ki-M1P, and CD-34 using immunohistochemistry. In the majority of the chordomas, correlations were found between iNOS and the immunoreactivity of Ki-M1P ($r=0.5303$, $P<0.0001$). Furthermore, the expressions of Ki-M1P was correlated with VEGFR-2 ($r=0.4181$, $P<0.0001$). Our results indicate that chordomas may respond to receptor tyrosine kinase inhibitors such as VEGFR-2 or modulators of other downstream signaling molecules. The future of VEGFR-2 and iNOS inhibitors as therapeutic agents in the treatment of chordoma will be clearer over the next years as results of the current clinical trials become available and as the factors regulating angiogenesis and the interactions between these factors are elucidated. However, appropriate functional experiments remain to be conducted to prove such a hypothesis.

Keywords Chordoma · VEGFR · iNOS · Monocytes/macrophages marker · Skull base

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Introduction

Chordoma is a rare malignant bone tumor that originates from the remnants of the embryonic notochord, which normally forms and dissolves during early fetal development [10]. Although it is a slow-growing tumor, it displays locally invasive growth. These tumors can develop anywhere in the axial skeleton but are most frequently located at end, that is, the clivus or the sacrum. Cranial base chordomas are difficult to treat because of their proximity to vital neurovascular structures and their propensity to diffusely invade the cranial base and structures that pass through this region [32]. The prognosis is poor in these cases, and many patients develop fatal local recurrences. There is currently no agreement regarding the best way to manage intracranial chordomas.

The chordomas showed strands and clusters of polygonal cells surrounded by pools of mucoid material. Some tumor cells had a large vacuolated cytoplasm (so-called physaliphorous cells). Chordoma shows cords of polygonal cells, extracellular mucin pools, and vacuolar spaces (Fig. 1).

Interactions among growth factors and their receptors are important for almost every biological function of neoplastic tissue [16]. Determination of the activity of a number of signal transduction pathways might contribute to our understanding of the variable nature of chordomas.

A relation between tumor angiogenesis, progression, and macrophages has been shown in a systematic literature review [25]. This occurs by stimulation of tumor vascularization, cell migration, invasion, and metastasis [3, 17]. Furthermore, macrophages promote tumor chemoresistance. It was reported that tumor-associated macrophages (TAM) depletion in some tumors increased the antitumor efficacy of the chemotherapeutic agent paclitaxel [5].

In turn, Ki-M1P is a selective monoclonal antibody, which reacts with physiologic function forms (macrophages of the lymphatic and non-lymphatic tissue), pathological reaction forms (epithelioid cells and foreign body giant cells) and neoplastic variants (tissue infiltration of monocytic leukemia) of the monocyte–macrophage system [26].

A relation between the activated macrophages and the chordoma angiogenesis has not been shown in the previous studies. Vascular endothelial growth factor (VEGF) plays a crucial role in the angiogenesis of numerous solid tumors [19]. Other study states that VEGF is the active angiogenic stimulator in the chondrosarcomas [8]. The VEGF expression is effected directly by macrophages and also via an IL-1β-secretion [14].

The VEGF-A expression of the chondrosarcomas correlates directly with the tumor grade [14, 24]. In turn, the effect of the VEGF proteins is mediated by membrane-bound receptors of the tyrosine kinase family vascular endothelial growth factor receptor (VEGFR)-1, VEGFR-2, and VEGFR-3. VEGFR-2 is the most important mediator for mitogenesis, survival, angiogenesis, and microvascular permeability of the vascular endothelial cells [6, 13].

In general, inducible nitric oxide synthase (iNOS) is not expressed constitutively, but induced in macrophages and many other cells by bacterial endotoxins like lipopolysaccharide or cytokines [10]. It has been shown that an increased iNOS expression of the TAMs promotes the neovascularization and elevates the blood flow [1]. We hypothesize that iNOS has the same impact on chordoma. On the other hand, nitric oxide (NO) seems involved substantially in the antitumor activity of the macrophages [12]. A strong overexpression of iNOS has been verified by oral squamous cell carcinomas [33]. Moreover, there is a correlation between the cervical lymph node metastasis and tumor staging but not with the tumor grading [29]. Thus, iNOS is a therapeutic target for the medical treatment of tumors [7]. A correlation between chordoma growth and iNOS expression is possible, however, not yet proven.

In this study, we examined the expression of vascular endothelial growth factor receptor 2, Ki-M1P, CD-34, and iNOS in a cohort of 145 lesions from 74 chordoma and 10 specimens from embryonic notochord and their correlation to each other. The notochord staining was used to demonstrate the possible protein expression of iNOS, Ki-M1P, VEGFR-2, and CD-34 in the spinal notochordal rests that are the presumed precursors of the chordoma.

Methods

Chordomas

A series of 74 chordoma patients for a total of 145 lesions (including 71 recurrent lesions) and 10 specimens from embryonic notochord were treated at the Neurosurgical Departments of the Nordstadt Hospital and of the Hannover Medical School between 1986 and 2007. Tumor recurrence was defined as a return of symptoms and signs, with verification of tumor regrowth radiologically (Table 1).

All patients were included; we had no exclusion criteria other than lack of available material for immunohistochemical analysis.

Cohort demographics

The patients (32 women and 42 men) ranged in age from 16 to 88 years (median age, 58 years). Twenty-four patients experienced recurrence and subsequent resection. Locations of the chordomas were skull base (72) and spinal (2). The two spinal chordoma served as a comparison group.

Multitumor tissue microarray construction

A multitumor tissue microarray (TMA) was assembled and used for comparison of molecular markers expression of chordoma.

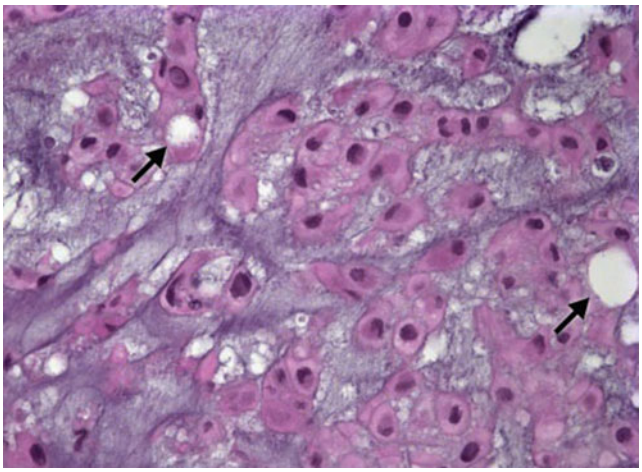

Fig. 1 Photomicrograph (original magnification, ×400; hematoxylin–eosin stain) shows vacuolated cells with intracytoplasmic mucus droplets (physaliphorous appearance) (*arrows*), a finding that is typical of chordoma

Following institutional review board approval, we constructed the tissue microarray as previously described [27].

The TMAs were constructed using a tissue arraying instrument (Beecher Instruments, Hackensack, NJ, USA). Tissue cylinders with a diameter of 0.6 mm were punched from each donor paraffin block in targeted areas corresponding to previously demarcated neoplastic areas on the parallel slide. These tissue cores were then deposited into a recipient "master" paraffin block. The punches were placed 1 mm apart on the x-axis and 1.5 mm apart on the y-axis. Two microarray blocks, respectively, contained 40 and 50 punches. Sections 5 μm thick were cut from the master block, stained with H&E, and reviewed to ensure the presence of morphologically pure cores of chordoma for each case. Morphologic features of each core were confirmed by reviewing the corresponding whole tissue sections stained with H&E. We obtained tissue cores from paraffin-embedded formalin-fixed tissue blocks from the archives at the Department of Pathology, Nordstadt Medical Center, Klinikum Hannover. A pathologist (H.O.) reviewed slides from all blocks to select representative areas of invasive tumor or normal tissue to be scored.

Immunohistochemistry

All slides were processed simultaneously under identical condition using standard methods. Immunohistochemistry was performed on the 145 lesions for the following antibodies: iNOS (1:100, Rabbit Polyclonal Antibody, Dunn, Asbach, Germany), vascular endothelial growth factor receptor 2, Flk-1/KDR/VEGFR2 (1:100, Rabbit Polyclonal Antibody, Dunn, Asbach, Germany), pan-macrophage marker Ki-M1P (1:5,000, kind gift from Prof. Parwaresch, Department of Pathology, University of Kiel, Germany), and CD-34 (1:30, Mouse Monoclonal Antibody, Abcam PlC, Cambridge, UK).

The sections were treated with antigen retrieval. Then they were treated with a primary antibody, followed by staining with an avidin–biotin–peroxidase complex (Immunotech, Marseille, France) or an alkaline phosphatase detection kit (Vector, Burlingame, CA, USA), according to standard immunohistochemical techniques [11]. All slides were run simultaneously under identical conditions and included negative control slides.

Positive and negative control sections were included for each antibody and slide pretreatment, respectively. TMA slides in which incubation with primary antibody was omitted served as the negative controls for each antigen retrieval regimen. TMAs were independently evaluated using the following criteria for specific staining: membranous and/or coarse cytoplasmic staining.

Immunohistochemical scoring for iNOS, VEGFR-2, Ki-M1P, and CD-34

Immunoreactivity was evaluated independently by two pathologists who had no prior knowledge of the clinical data or other histologic findings. Immunoreactivity for the proteases was scored as described previously [18, 34].

Every tumor was given a score according to the intensity of the nucleic or cytoplasmic staining (no staining=0, weak staining=1, moderate staining=2, strong staining=3) and the extent of stained cells (0=none, 1=less than 25 %, 2=25–50 %, and 3=more than 50 %). We determined the sum of these two parameters to evaluate the expression of proteases, from 0 to 6. The cells were graded as negative when there was complete absence of staining (score 0), weak staining (score 1), and moderate staining (score 2), originating from baseline expression. The scores 3–6 were graded as strong positive.

Structures were only counted as microvessels if they stained positively with the vascular marker and morphologically appeared vascular. Expression of CD34 was used to detect microvessel.

In the present study, a new monoclonal antibody termed Ki-M1P is used. Because the Ki-M1P antigen is not destroyed

Table 1 There were 42 lesions as first, 15 as second, 8 as third, and 4 as fourth recurrence

Primary tumor							N=74
Recurrences	1. N=42	2. N=15	3. N=8	4. N=4	5. N=1	6. N=1	N=71
Total							N=145

One patient had a fifth and one patient a sixth recurrence

or masked during routine fixation and paraffin embedding of biopsy tissue samples, Ki-M1P represents a useful diagnostic reagent for the identification of physiological functional and pathologic reaction forms as well as neoplastic variants of the human monocyte/macrophage system even in retrospective studies [26].

Statistical analysis

We evaluated correlation of marker expression by Spearman rank correlation test. All calculations and analyses were performed with SPSS 14.0 for Windows. Significance was considered to be $P<0.05$.

Results

Expression of VEGFR-2 and iNOS showed a cytoplasmic staining pattern with diverse intensity. Tumor cells as well as endothelial cells were stained. VEGFR2 displayed intracytoplasmic and membrane located staining. Immunoreactivity tended to concentrate at the tumor periphery but in several cases also appeared homogeneous over a certain tumor area, whereas CD34 immunoreactivity was confined to endothelial cells and scattered single positive cells in 40 % of the tumor stroma. Over 15 % of lesions had a high immunoreactivity for CD34.

In our study, tumor infiltrating macrophages as well as the tumor cells displayed a strong immunoreactivity for Ki-M1P. In many areas of the tumor, KiM1P demonstrates a cytoplasmic pattern in tumor cells. Ki-M1P proved to be a pan macrophage marker being expressed not only on normal physiologic functional forms of the monocyte/macrophage system but also on all known representatives of pathological reaction forms, for example, under inflammatory conditions of various etiology and within tumors of various origin [26].

Immunohistochemical analysis for Ki-M1P, VEGFR-2, iNOS, and CD-34 expression

Ki-M1P

Chordomas displayed a spectrum of intensities ranging from no (0) expression to very strong (3) expression of Ki-M1P. Among primary chordomas, there were two tumors with no expression (0), 19 with mild (1), 41 with moderate (2), and 83 with strong (3) expression. Four of 10 specimens from notochord had no expression (0), five with mild (1), and one with moderate (2) expression of Ki-M1P.

A representative micrograph of strong (3) Ki-M1P expression (Fig. 2) in chordomas and no expression in notochord (Fig. 3) is shown. Expression pattern of notochord is summarized in (Fig. 4).

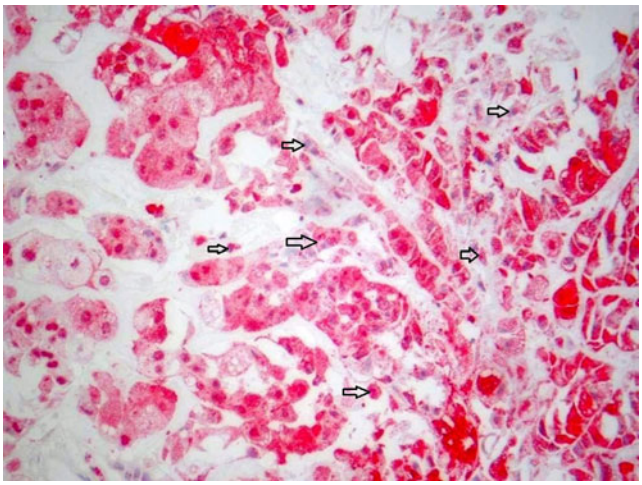

Fig. 2 Representative immunocytochemical stains with anti-macrophage antibodies Ki-M1P. The lesion is filled with numerous macrophages. The distribution of macrophages inside the chordoma is shown (*arrows*), strong staining (3)

VEGFR-2

Chordomas displayed predominately no strong expression of VEGFR-2. There were 114 tumors with no expression (0), 27 with mild (1), 3 with moderate (2), and 1 with strong expression. The low expression of VEGFR-2 has been shown exclusively by the clivus chordomas. A moderate or strong expression was found by the two sacral chordomas as comparison group. Nine of 10 specimens from notochord had no expression (0) and one with mild (1) expression of VEGFR-2. The recurrent cranial base chordoma tumors displayed 34 cases with no expression (0), two with mild (1), and only one with moderate (2) expression of VEGFR-2. The VEGFR-2 expression was diminished than those of the primary tumors; the difference, however, was not significant ($P=0.101$,

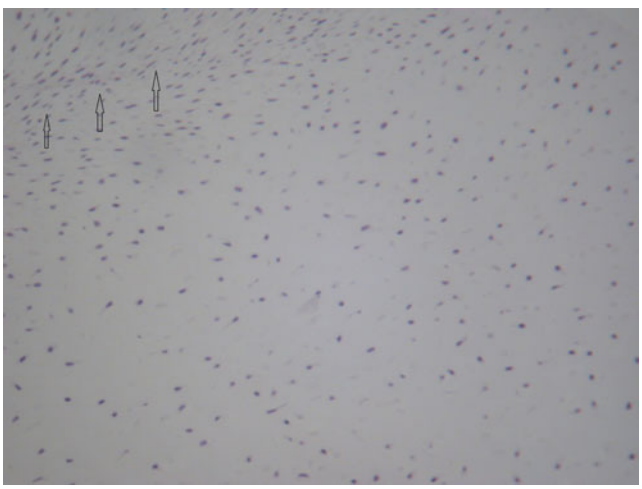

Fig. 3 Notochord without (0) expression of Ki-M1P. This inner layer of notochord sheath was found to have many cell nuclei (*arrows*). There is a lacking of macrophages infiltration (KiM-1P), no staining (0)

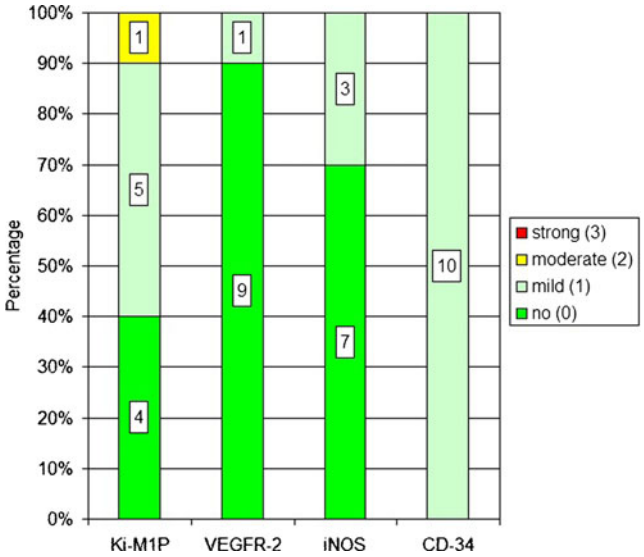

Fig. 4 Expression of Ki-M1P, VEGFR-2, iNOS, and CD-34 in notochord ($n=10$). The expression of Ki-M1P and CD-34 was primarily mild. VEGFR-2 and iNOS showed predominantly no expression

χ^2 test). A representative micrograph of mild (1) VEGFR-2 expression in chordoma is shown in (Fig. 5).

iNOS

Chordomas displayed predominately moderate expression of iNOS. There were 26 tumors with no expression (0), 47 with mild (1), 39 with moderate (2), and 33 with strong expression. Three of 10 specimens from notochord had mild expression (1) and seven with no expression (0) of iNOS. The recurrent chordoma tumors displayed moderate (2) and strong (3) expression of iNOS. A representative micrograph of strong (3) iNOS expression in chordoma is shown in (Fig. 6).

CD-34

Chordomas displayed predominately mild expression of CD-34, 58 mild with (1), 62 with moderate (2), and 25 with strong expression. All specimens from notochord had mild expression (1) of CD-34.

Correlation of marker expression in chordoma tumors

For chordomas, there was a relationship between VEGFR-2 expression and iNOS expression and Ki-M1P expression. There was a significant correlation between VEGFR-2 expression and iNOS expression level ($P=0.026$, Spearman $\rho=0.178$).

Chordomas that had high iNOS expression were also likely to have high Ki-M1P expression. We could find out a significant correlation between iNOS expression and Ki-M1P expression level ($P=0.001$, Spearman $\rho=0.521$). Expression of VEGFR-2 and Ki-M1P showed also positive correlation ($P=0.003$, Spearman $\rho=0.237$). Also there is a significant correlation between CD-34 expression and VEGFR-2 expression level ($P=0.010$, Spearman $\rho=0.067$). Expression pattern of primary and recurrent chordomas is summarized in (Figs. 7 and 8a–d). A significant correlation between the chordoma localization (in the brain stem, suprasellar region, and cavernous sinus) as well as its recurrence and the VEGFR-2 expression is found (Table 2). Patient survival data are summarized in Fig. 9. Follow-up information was available for 29 patients; follow-up ranged from 1 to 5 years. The 5-year survival rate was 77.9 % in patients with lower VEGFR-2 expression and was 43.4 % in those with higher VEGFR-2 expression (Fig. 10); there was a significant difference noted with regard to their survival rate ($P=0.035$). Future studies with a larger

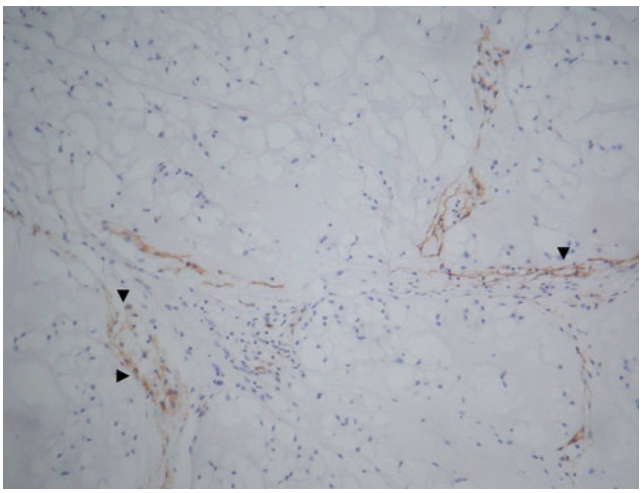

Fig. 5 Chordoma with mild VEGFR-2 staining mainly around the vessels and cytoplasm. The presence of the VEGFR2 receptor in the tissue was assessed by immunohistochemical staining with an anti-VEGFR2 antibody. Endothelial cells serve as an internal positive control for VEGFR-2 (*arrows*). VEGFR-2 staining was documented around the vessels and cytoplasm, mild staining (1)

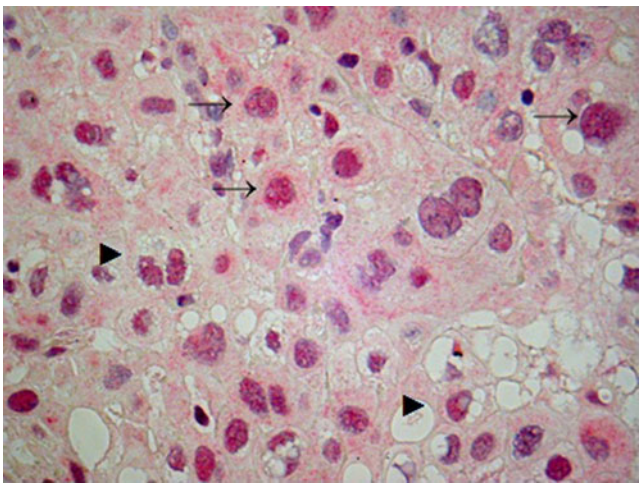

Fig. 6 Chordoma with strong (3) iNOS staining. Physaliferous cells and round to oval nuclei (*arrowhead*) and macrophages (*arrows*)

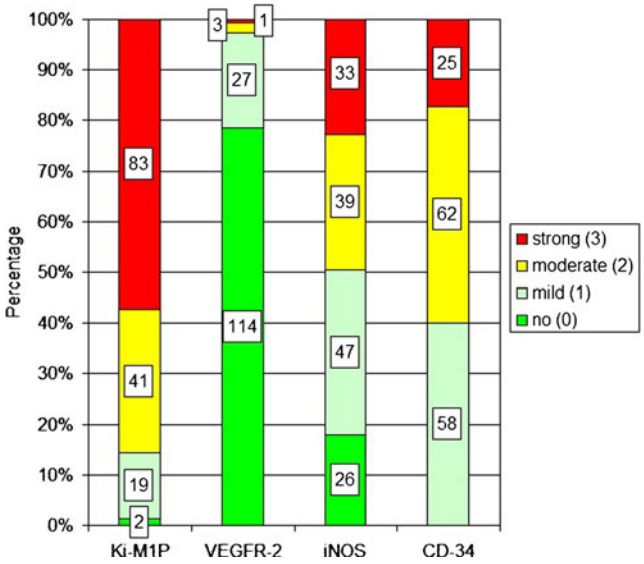

Fig. 7 Expression of Ki-M1P, VEGFR-2, iNOS, and CD-34 in primary chordoma (n=145). (1) Ki-M1P expression was noted to be primarily strong. (2) Vascular endothelial growth factor receptor 2 (*VEGFR-2*) expression showed predominantly no expression. (3) iNOS expression in primary chordoma was variable from no expression to strong expression. (4) Expression of CD-34 in primary chordoma was predominantly mild or moderate

number of patients are needed to be able to interpret these correlations.

Discussion

Chordoma is a slow growing malignant neoplasm that is believed to originate from notochordal remnants located along the craniovertebral axis [28]. Chordomas typically present in adults 40 years of age or older, and males are affected more often than females. A recent study by Deniz et al. [4] found that expression levels of basic fibroblast growth factor, transforming growth factor α, and fibronectin were all correlated with local recurrence and aggressive biological behavior.

In the present study, we decided to focus our investigations on the differential protein expression of Ki-M1P, iNOS, and VEGFR-2 in skull base chordoma. As inhibitors of iNOS and VEGF become more widely used in the clinical setting, a better understanding of the signaling pathway in vivo, their pattern of expression, and activation is of critical importance because it may be helpful in predicting the target tumor population that will benefit from therapies.

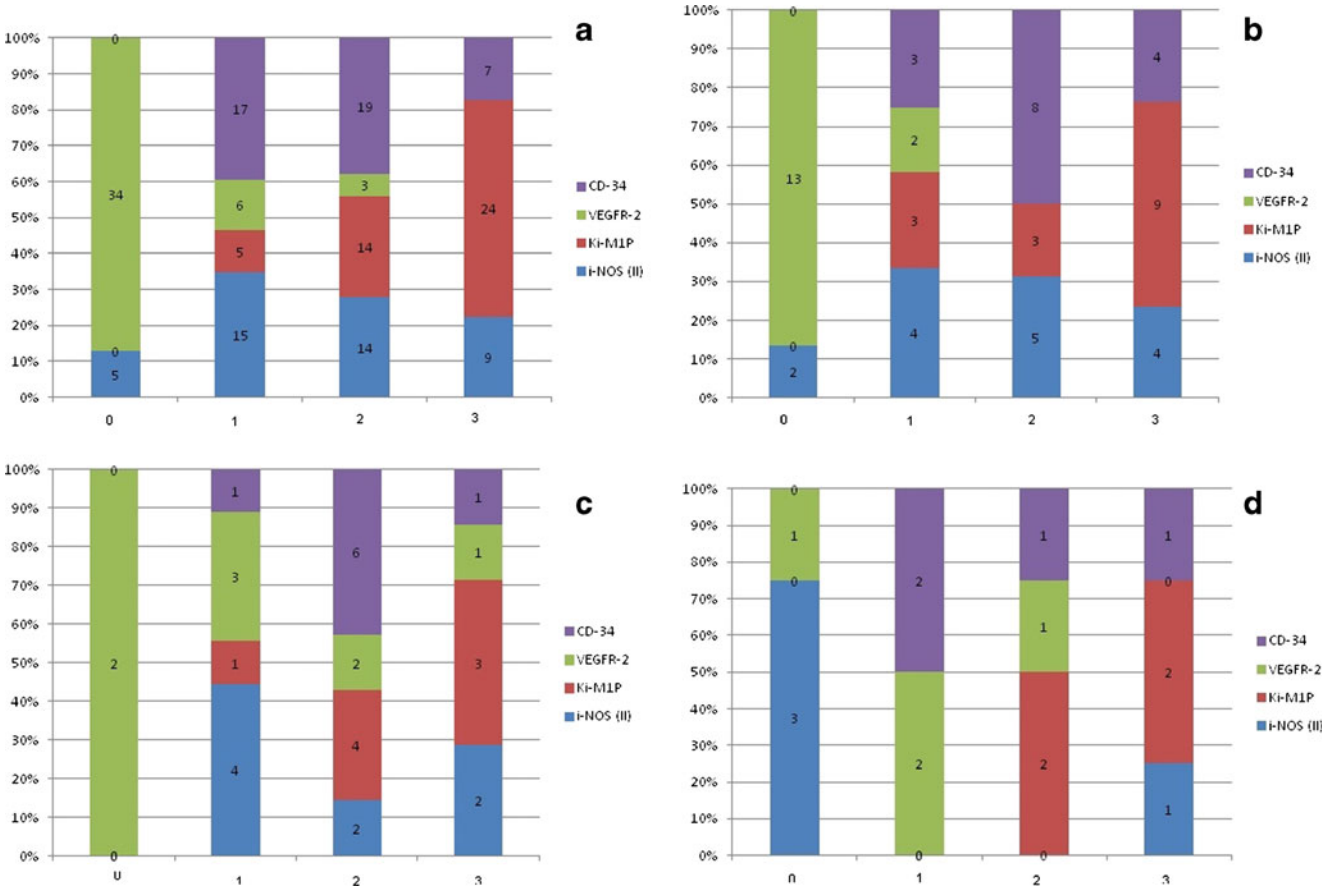

Fig. 8 **a**–**d** Expression pattern of first (N=42), second (N=15), third (N=8), and fourth (N=4) recurrences of chordomas are summarized in **a**–**d**. There was a slight increase in expression of iNOS, VEGFR-2, Ki-M1p, and CD34 among the first to the fourth recurrences. **a** 1. Recurrence (N=42). **b** 2. Recurrence (N=15). **c** 3. Recurrence (N=8). **d** 4. Recurrence (N=4)

Table 2 Correlation between age, gender, tumor recurrence, tumor-localization, and expansion with the immunohistochemical findings ($n=58$); p values (bold for $p<0.1$) (U test)

Characteristic	iNOS	Ki-M1P	VEGFR-2	CD34
Age	0.157	0.288	0.210	0.311
Sex	0.768	0.762	0.836	0.656
Recurrence	0.442	0.174	**0.0503**	0.303
Post. fossa	0.774	0.974	0.213	0.897
Brain stem	0.662	0.113	**0.0129**	0.735
4. Ventricle	0.949	0.439	0.543	0.685
Suprasellar	0.523	0.297	**0.0645**	0.401
3. Ventricle	0.581	0.129	0.247	0.575
Cavernous sinus	0.827	0.686	**0.0290**	0.687

Inducible nitric oxide synthase

Nitric oxide is a highly reactive free-radical compound with a short half-life known to affect many cellular processes, including vasodilation, cytotoxicity in immunological responses, and neurotransmission. Nitric oxide has been shown to exhibit tumoricidal activity by inducing tumor cell cytolysis, but paradoxically may also contribute to tumor growth by promoting neovascularization of tumor masses. The expressions of iNOS and VEGF are closely related to tumor angiogenesis [31]. It is shown that the expression of iNOS in most tumor tissue is higher than that in the normal one [30]. Nitric oxide produced through iNOS induction may increase the vascular permeability and accelerate the nutrient supply of tumor tissue and finally promote the tumor growth [23]. In the literature, there are no comparative studies noted for chordomas. However, it was documented that a strong correlation exists between the activity of the NOS pathway, angiogenesis, and metastatic behavior in head and neck cancer [9]. In this study, we found that the rate of expression of iNOS of primary chordoma was variable from no expression to strong expression, which was strongly related to the Ki-M1P expression ($P=0.0001$, Spearman $\rho=0.5303$).

The high expression of Ki-M1P can be ascribed to the high infiltration of chordoma with monocyte/macrophages. We assume that the high infiltration of chordoma with monocyte/macrophages enhances the tumor growth through the release of iNOS.

Vascular endothelial growth factor receptor-2

VEGFR-2 is exclusively expressed in endothelial cells and appears to play a pivotal role in endothelial cell differentiation and vasculogenesis [21]. Many studies using molecular techniques have provided evidence for the role of VEGFR-2 in tumor vascularization, growth, and metastasis [22]. VEGFR-2 is considered to be the main mitogenic signaling receptor for VEGF [15]. In the present study, we found that the rate of expression of VEGFR-2 was related to the iNOS expression ($P=0.0023$, Spearman $\rho=0.3300$).

VEGF produced by the tumor cell can be bound with the surface acceptor of vascular endothelial cell and promote the production of nitric oxide that can transmit messages between the cells and induce tumor angiogenesis [36]. A study by Song et al. [31] showed that the expressions of iNOS and VEGF were closely related to tumor angiogenesis and might be

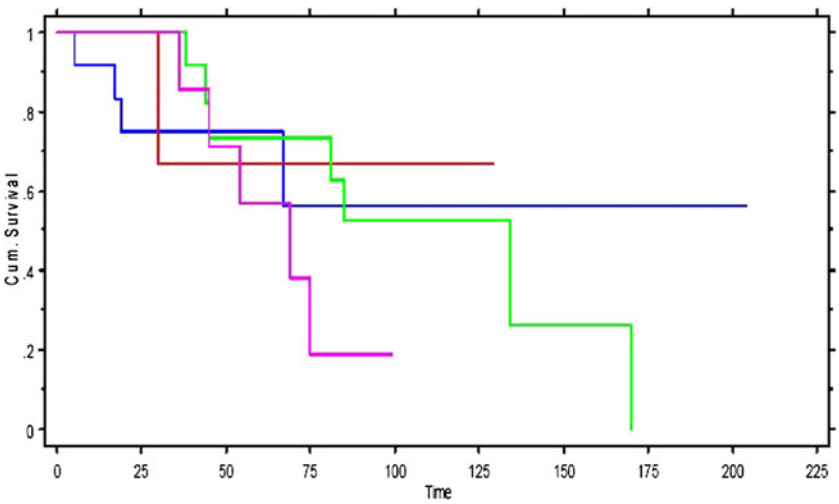

Fig. 9 Kaplan–Meier survival curve of the chordoma patient related to radical resection and radiation therapy. We conclude that the addition of radical surgery to radiation therapy did not improve the survival outcome of patients with a skull base chordoma. Thus, the efficacy of radical surgery plus radiation compared with radiation alone or subtotal resection did not demonstrate a statistically significant survival difference (time in months). — Cum. Survival (radiation therapy/no radical resection). — Cum. Survival (radiation therapy/radical resection). — Cum. Survival (no radiation therapy/no radical resection). — Cum. Survival (no radiation therapy/radical resection)

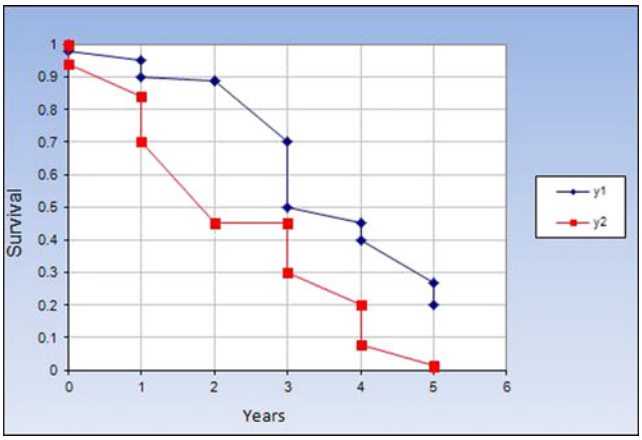

Fig. 10 Survival according to the expression of VEGFR-2 in skull base chordomas. Lesions with a lower level of VEGFR-2 expression were found to have a significantly better prognosis than those with higher VEGFR-2 expression. — *Y1* VEGFR-2 score 0 and 1 ($n=20$). — *Y2* VEGFR-2 scores 2 and 3 ($n=9$)

important factors involved in gastric carcinoma angiogenesis. In the present study, we have shown that the majority of the clivus chordomas have no expression of VEGFR-2.

We suggest that the low to absent expression of VEGFR-2 in primary clivus chordomas may be due to steroid therapy with dexamethasone which was routinely given to these patients. Dexamethasone is a well-known inhibitor of the VEGFR-2 expression [35]. In comparison with the clivus chordoma, we have documented a considerably higher protein expression of VEGFR-2 in spinal chordoma as control group. These patients were not treated with dexamethasone preoperatively. The spinal chordoma was found to express high levels of VEGFR-2 (Fig. 11). Although we cannot provide a definite explanation for the mechanism by which dexamethasone affects the VEGFR-2 expression, there are suggestive observations.

VEGFR2 expression in nonendothelial mesenchymal tissues and tumors is rare. However, this receptor is expressed at least in fetal cartilage, as also reported by others [2] and therefore might also be expected in cartilaginous tumors. Miettinen et al. [20] have observed that chordoma also seems to commonly contain VEGFR2-positive tumor cells, perhaps as a reflection of its distant relationship with cartilage.

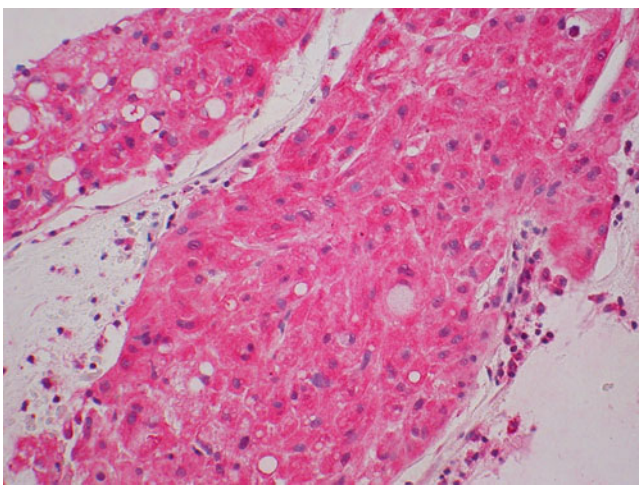

Fig. 11 Spinal chordoma with strong (3) VEGFR-2 staining

We found increased iNOS expression in most chordomas combined with high Ki-M1P expression ($P=0.0001$). Therefore, we speculate that the macrophage infiltration into the chordoma as one of the predominant leukocyte by tumor development [22] may play a significant role in the growth of chordoma producing iNOS. Furthermore, activated macrophages express the iNOS, which is the major source of NO production in these tissues [31]. Zhang et al. [35] noted that because the peak of VEGFR-2 expression after wounding occurs after the peak of macrophage infiltration, it is likely that macrophages play a direct role in inducing VEGFR-2 expression by secreting cytokines, growth factors, and NO. Regulating VEGFR-2 expression seems to have a profound effect on angiogenesis.

In the present study, our analysis did not control for all confounding variables via a multivariate regression model due to the relatively small number of patients. Some biases may be inherent in this type of analysis, and we acknowledge this is a limitation.

The number of cases presented here does not allow for any definitive conclusions. But because chordomas are rare and because they present a very difficult surgical challenge and are often incurable, we try to carry out work that attempts to increase the understanding of the molecular mechanism of chordomas. Future studies should incorporate a larger number of patients.

Conclusion

It is widely believed that angiogenesis, the formation of new blood vessels, plays a key role in malignant tumor development, growth, and invasion.

VEGFR-2 and iNOS might act with a synergistic effect and can positively regulate the angiogenesis in skull base chordoma. Positive expression of VEGFR-2 might indicate the local recurrence of skull base chordoma. The result suggests that some specific drugs which inhibit VEGF or their receptor (VEGFR-2) may have a good therapeutic effect for skull base chordoma. However, angiogenesis involves a variety of molecules other than VEGF and iNOS; further studies are definitely required to determine the appropriate target molecules of this therapeutic strategy.

Our results nonetheless indicate that chordomas may respond to receptor tyrosine kinase inhibitors such as VEGFR-2 or modulators of other downstream signaling molecules. Further study on the mechanism of their regulation will probably offer a new approach to anticancer treatment.

Acknowledgments Special thanks go to Prof. Dr. med. Dr. h. c. mult. M. Samii from the International Neuroscience Institute Hannover/ Germany, Department of Neurosurgery, for providing us with a large amount of data on chordoma patients due to his excellent experience in skull base surgery.

Conflict of interest I, *Dr. Reza Akhavan-Sigari*, declare that none of the authors of the above manuscript has declared any conflict of interest within the last 3 years which may arise from being named as an author on the manuscript.

Funding statement I, *Dr. Reza Akhavan-Sigari*, declare that I have no proprietary, financial, professional, or other personal interest of any nature or kind in any product, service, and/or company that could be construed as influencing the position presented in, or the review of, the manuscript mentioned above.

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Comments

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Tumor growth or progression correlates with angiogenesis in many human malignant neoplasms. Chordoma grows slowly; however, it generally shows locally aggressive behavior and frequently recurs after excision. Vascular formation is poor in chordoid lobules and abundant in intralesional fibrous septum, particularly on the surface, suggesting induction of tumor vessels by chordoma cells [1]. As a consequence, it seems interesting to study angiogenesis in chordoma; however, there were few papers in this field [2, 3]. In the current study, Akhavan-Sigari and colleagues reported the expression of angiogenic factors using tissue microarray in a large series of chordoma and investigated the relationship between the immunoprofile and clinicopathologic parameters. They found a higher VEGF-R positivity in recurrent lesions than in primary lesions. In addition, they revealed that patients with higher VEGF-R expression showed poorer prognosis. It is very significant to show the relationship between angiogenesis and aggressive clinical course of chordoma because angiogenic process is a potential therapeutic target for chordomas.

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