Angiogenic Profile of Uveal Melanoma

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Abstract

Uveal melanoma develops in one of the most capillary-rich tissues of the body and is disseminated haematogenously. Knowledge of the nature and the spatiotemporal expression of angiogenic factors in uveal melanoma is essential to the development of new treatment strategies, especially with regard to improving survival. In this study, we measured the angiogenic potential of several angiogenic factors in different uveal melanoma cell lines, in an in vivo model, and in primary tumour material from patients with melanoma. Most uveal melanoma cell lines expressed vascular endothelial growth factor (VEGF)-A (isoforms 121,165,189), VEGF-B, VEGF-C, VEGF-D, and basic fibroblastic growth factor (b-FGF) to various extents. The expression of VEGF-A 121 was always higher than that of the other VEGF-A isoforms, suggesting that VEGF-A 121 is the most abundant VEGF-A isoform. All experimentally induced tumours expressed VEGF-A, VEGF-B, VEGF-C, VEGF-D, and b-FGF. Similarly, significant amounts of mRNA for VEGF-B, VEGF-C, VEGF-D, and b-FGF were detected in uveal melanoma material from patients. In contrast, VEGF-A mRNA (121,165,189) was low (9/28) or not detectable in the tumour samples. The synthesis of VEGF-A 165 and b-FGF protein by various cell lines was measured by enzyme-linked immunosorbent assay (ELISA). Most uveal melanoma cell lines, but not normal melanocytes, strongly synthesized and secreted VEGF-A 165 and b-FGF during cell culture. Our data suggest that the expression of (lymph)-angiogenic factors may play a causal role in the angiogenesis and progression of uveal melanoma and distant metastasis.
Introduction

Uveal melanoma is the most common primary malignant intraocular tumour in adults, with an annual incidence of 6–8 per million in Caucasians. Uveal melanoma develops in one of the most capillary-rich tissues of the body and has a purely haematogenous dissemination. The mortality rate is high because of the frequent occurrence of metastases, mainly in the liver. The rate-limiting step for tumour growth and haematological dissemination is the development of a tumour microcirculation. Therefore, knowledge of the nature and the spatiotemporal expression of angiogenic factors in uveal melanoma are of major importance for the development of new treatment strategies, especially with a view to improving survival. Studying the expression of the factors involved in neovascularization at a molecular level can provide insight into the mechanisms by which uveal melanomas grow and develop metastatic potential.

Although primary tumour masses can be eradicated by surgical intervention or radiation, an attractive alternative strategy is to treat invasive growth and metastasis with anti-angiogenic agents. Tumours and metastases can grow autonomously because mechanisms regulating cell growth are perturbed and deregulated. For example, the expression of members of the vascular endothelial growth factor (VEGF) family is altered. VEGF-A is a potent angiogenic factor for many solid tumours and a high expression of VEGF-A is associated with a poor prognosis. VEGF-A comprises four isoforms that arise from alternative splicing of a single gene. The 121- and 165-amino acid polypeptides are soluble, whereas the 189- and 206-amino acid polypeptides are bound to heparan sulphate proteoglycans at the cell surface. VEGF-A 165 is the most abundant and VEGF-A 206 the least abundant isoform. Protein levels of VEGF-A 165 in the aqueous humor of eyes with a uveal melanoma are correlated with patient survival, basal tumour diameter and tumour height. One report suggests that uveal melanomas express VEGF-A. In most ciliochoroidal melanomas VEGF-immunoreactivity is correlated with necrosis, but not with the occurrence of systemic metastasis or tumour angiogenesis. VEGF-B and VEGF-C have been found in a variety of aggressive solid tumours. VEGF-C and VEGF-D are potent inducers of lymphangiogenesis, and the strong expression of VEGF-D on the endothelial cells of blood vessels in malignant cutaneous melanoma is consistent with a role in tumour neovascularization. Basic fibroblastic growth factor (b-FGF) is an autocrine and a paracrine growth factor that is mitogenic for endothelial cells. It is expressed by a wide variety of normal cells but not by cutaneous melanocytes. Although mRNA for basic-FGF is expressed in uveal melanomas, exogenous b-FGF does not have a consistent effect on uveal melanoma growth in culture.

In this study, we analysed the expression of five angiogenic factors, VEGF-A, VEGF-B, VEGF-C, VEGF-D, and b-FGF, by semi-quantitative PCR in vitro, in experimentally induced uveal melanoma tumours, and in tissue from uveal melanomas. ELISA monitored the synthesis of VEGF-A 165 and b-FGF proteins by uveal cell lines. The angiogenic effect of several uveal melanoma cell lines and normal melanocytes was tested in an in vitro
angiogenesis assay. Our data indicate that uveal melanoma cell lines and human tumours express high levels of VEGF-B, VEGF-C, and b-FGF, which strongly suggests that certain (lymph)angiogenic factors have a causal role in the pathogenesis of uveal melanoma and distant metastasis.

**Materials and methods**

**Melanoma cell lines and normal melanocytes**

Seven cell lines (92-1, Mel-202,-285,-290, OCM-1,-3,-8) were obtained from primary uveal melanomas. Cell lines OMM-1.3 and -1.5 were obtained from liver metastases. Cell line 92-1 and normal uveal melanocytes (1A, B, 2) were established in our laboratory. Cell lines OCM-3, OCM-8, Mel-202, OMM-1.5, 92-1, Mel-285, OMM-1.3, and Mel-290 were cultured in RPMI 1640 (Gibco, Invitrogen, Breda, The Netherlands), 10% heat-inactivated fetal calf serum (FCS) (Hyclone, Logan, UT, USA), and 100 IU/ml penicillin (Gibco). Cell lines OCM-1 and OMM-1 were grown as monolayers in 10 ml/dish Dubecco’s modified Eagle’s medium (Gibco) supplemented with 10% FCS (Hyclone), 100 IU/ml penicillin (Gibco), and 100 µg/ml streptomycin (Gibco). Normal melanocytes were grown as monolayer in 10 ml/dish HAM/F12 (Gibco) medium as described by Hu et al. All cells were incubated at 37°C in a humidified atmosphere with 5% CO₂.

**Primary uveal melanoma tumours**

The tumours of 28 enucleated eyes with uveal melanoma were included in this study, which conformed to the requirements of the Declaration of Helsinki. Three melanomas were treated before enucleation (ruthenium-106 plaque radiotherapy and transpupillary thermotherapy). The median age of the patients, 14 females and 14 males, at time of enucleation was 61.5 years (range 42-84 years). Tumour diameter varied from 7 to 18 mm (mean 12.5 mm, STD 2.9). After histopathological analysis, 10 tumours were classified as epithelioid, 12 as spindle cell, and 6 as mixed histology. There were 24 choroidal tumours, 2 ciliary body tumours, and 2 ciliary body melanomas with iris involvement. Tumour fragments were snap-frozen in nitrogen and stored at -80°C.

Paraffin-embedded tissue blocks of primary uveal melanoma were used for CD34 staining. For micrometer tissue sections were cut from each paraffin block, prepared on aminopropylethoxysilane-coated slides, and dried overnight at 37°C. Sections were deparaffinised in xylol. Endogenous peroxidase was blocked in 0.3% hydrogen peroxide methanol at room temperature for 20 minutes. Subsequently, the sections were rehydrated. After washing in water, antigen retrieval treatment was done by incubating the sections in a 0.1% trypsin, calcium chloride (0.1%) solution during 20 minutes at 37°C.

After antigen retrieval the slides were rinsed in phosphate-buffered saline (PBS), and the primary antibody, CD34 (Novocastra Laboratories, Newcastle-upon-Tyne, UK, diluted 1:25).
As negative control omission of the primary antibody was used. The reaction was developed with 3-amino-9-ethyl carbazole (AEC) or 3,3’-diaminobenzidine-tetrahydrochloride (DAB). For CD34 evaluation, all slides were bleached with 3.0% (vol/vol) hydrogen peroxide and 1.0% (wt/vol) disodium hydrogen phosphate (Professor Kivela, Helsinki, Finland). As positive control, all cell lines were stained with HLA w6/32 (DAKO, The Netherlands).

Mouse model of human uveal melanoma in the anterior part of the eye

Tumour cells were inoculated into the anterior chamber of mouse eyes as described by Niederkorn et al. 24. OCM-1 cells, which show a high malignancy with metastatic potential to the liver 25 were used in this study. With a glass needle, OCM-1 cells (10⁵/μl) were injected into the anterior chamber of the eye of 8-week-old female Balb-c nu/nu mice. After 4 weeks the mice were killed, and the eyes with tumour were enucleated and used for RNA isolation. All animal experiments were performed in accordance with the ARVO Resolution on the Use of Animals in Ophthalmic and Vision Research.

Isolation of cellular RNA and reverse transcription-polymerase chain reaction (RT-PCR)

RNA was isolated from cells in culture and tissue from experimentally induced tumours as described by Chomczynski and Sacchi 26. RNA from human melanoma tissue was isolated using the RNeasy Mini Kit (Qiagen) and proteinase K (20 mg/ml) (Qiagen, Venlo, The Netherlands). Denatured RNA 10 μl (0.1 μg/ml; 10 minutes at 70°C and quickly chilled on ice) was reverse transcribed into complementary DNA (cDNA) in a 100-μl reaction volume containing 4 μl first strand buffer (75 mM KCl, 3 mM MgCl₂ and 50 mM Tris-HCl [pH 8.3]), 1 μl 0.1 mM dithiothreitol (DTT), 1 μl 7.5 mM deoxynucleoside triphosphate (dNTP), 0.1 μl random primers (2000 ng/μl), 0.25 μl murine myeloid leukaemia virus (M-MLV) (Life Technologies-Gibco, Breda, The Netherlands), 0.5 μl Rnasin (Promega, Leiden, the Netherlands), and 3.15 μl H₂O. Reverse transcription was performed at 37°C for 60 minutes and 70°C for 10 minutes, followed by quick chilling on ice. All cell lines were corrected for any variation in cDNA content on the basis of their β2-microglobulin content, according to the method by Van der Pluijm et al. 27. Human-specific primer sets were used to identify angiogenic factors (table 1). cDNA was co-amplified with human specific primers over 32-38 cycles in a 25-μl reaction mixture containing reaction buffer (75 mM Tris-HCl, pH 9.0, 20 mM (NH₄)₂SO₄, 0.01% (wt/vol) Tween 20), 2.0 mM MgCl₂, 200 μM dNTPs, 0.25 μM sense (S) and antisense (AS) primer (Eurogentec, Seraing, Belgium) and 0.125 U Goldstar DNA polymerase (Eurogentec). Negative controls were run in parallel in each experiment. PCR conditions were set at 94°C (denaturation) and 56°C (annealing) and 72°C (extension). Aliquots of 15 μl of each amplified sample with a 100-base pair (bp) DNA ladder (Life Technologies-Gibco BRL) were subjected to electrophoresis on 1% agarose gels containing 0.5 μg EtBr/ml and photographed. Computerized densitometry
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(Scion Image for Windows) was used to measure the intensity of each band. All tests were performed in duplicate.

<table>
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<th>Human transcript</th>
<th>Forward primer type (5'- 3')</th>
<th>Reverse primer type (3'-5')</th>
<th>Tm (C)</th>
<th>Expected amplicon size (bp)</th>
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<tr>
<td>b-FGF</td>
<td>ATGGCAGCCGGAGCATCACC</td>
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<td>234</td>
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<td>VEGF-A 189/165/121</td>
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<td>558/497/366</td>
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<td>VEGF-D</td>
<td>CAGTGAAGCGATCATCTCAGTC</td>
<td>TAAGGCTCTCCTCATGCAACA</td>
<td>56</td>
<td>372</td>
</tr>
</tbody>
</table>

Table 1: Human-specific sense (S) and anti-sense (AS) primers.

Enzyme-linked immunosorbent assay (ELISA) for human VEGF-A 165 and b-FGF

Levels of VEGF-A 165 (Biosource, Etten-Leur, The Netherlands) and b-FGF (R&D, Oxon, UK) were measured with commercially available specific ELISA kits using the sandwich enzyme immunoassay technique. Supernatant levels of b-FGF were not measured in the experiments in which melanocytes in culture were stimulated with b-FGF protein (see above). For negative controls culture medium was used.

In vitro angiogenesis assay

Conditioned medium from several uveal melanoma cell lines was tested for induction of in vitro angiogenesis by the method described by Deckers et al. 28. Conditioned media from OCM-1, OMM-1.3, OMM-1.5, Mel-202, and melanocytes 1A were collected after 48 hours of culture. Fetuses were removed on gestation day 17 from pregnant Swiss albino mice and the metatarsals were dissected. Cultures were performed in sextuple with a positive control (VEGF-A 1 μg/ml, Oncogene, Breda, The Netherlands) and each experiment was repeated at least twice. Metatarsals were cultured for 14 days and medium was replaced every 7 days. At the end of the culture period (14 days), the cultures were fixed in ZnMF fixative for 15 minutes at room temperature and subsequently stained for PECAM-1. After staining, the formation of tube-like structures was quantified by computerized image analysis using Image Pro Plus 3.0 for Windows 95/NT (Media Cybernetics, Silver-Springs, MD, USA).

Results

Expression profile of angiogenic factors in uveal melanoma cell lines and cell lines of normal melanocytes

We used semiquantitative RT-PCR analysis to examine the expression of five angiogenic
Figure 1: Semi-quantitative PCR analysis of mRNA levels of different VEGF types (VEGF-A, B, C, D) in normal melanocyte cell lines (1A, 1B, 2) and uveal melanoma cell lines (OCM-3, OCM-8, Mel 202, OCM-1, OMM-1.5, 92-1, Mel 285, OMM-1.3, Mel 290). Expected sizes: VEGF-A198/165/121= 558/497/366 bp, respectively; VEGF-B=222bp; VEGF-C=293 bp; VEGF-D= 372; - = negative control.

factors in different cell lines. VEGF-A was expressed in relatively high amounts in OCM-8
and OCM-1 cells but in very low or undetectable amounts in other cell lines (melanocytes 1A, 1B, 2, Mel-202, 92-1). The expression of VEGF-A 121 was always higher than that of VEGF-A 165 or 189, suggesting that VEGF-A 121 is the most abundant VEGF isoform (figure 1A). Messenger RNA for VEGF-B and VEGF-C was detected in all uveal melanoma cell lines tested and in normal melanocytes.

**Figure 2:** Semi-quantitative PCR analysis of levels of mRNA for b-FGF in normal melanocyte cell lines (1A, 1B, 2) and uveal melanoma cell lines (OCM-3, OCM-8, Mel-202, OCM-1, OMM-1.5, 92-1, Mel-285, OMM-1.3, Mel-290). Expected size: 234bp. - = negative control.

The expression of VEGF-B and VEGF-C was higher than that of VEGF-A (figure 1 B, C). In contrast, VEGF-D was expressed only in cell lines OCM-1, OCM-8, and OMM-1.3 (figure 1 D). Basic FGF was expressed in high amounts in Mel-285, Mel-290, and in lower amounts in OCM-8 but was not expressed in the other cell lines (figure 2).

**VEGF A 165 and b-FGF in supernatants of normal melanocytes and uveal melanoma cell lines**

ELISA measured VEGF-A 165 and b-FGF in the supernatants of 10 uveal melanoma cell lines and three cell lines of normal melanocytes. Human uveal melanoma cell lines secreted VEGF-A 165 under basal conditions whereas normal melanocytes did not (Figure 3). OCM-1
cells produced high amounts of VEGF-A 165. The ELISA results were in line with the PCR results for VEGF-A expression. Only a limited number of the tested uveal melanoma cell lines (92-1, OCM-1) produced detectable amounts of b-FGF protein in vitro (Figure 3). The expression of b-FGF mRNA in uveal melanoma cell lines did not always reflect the synthesis and secretion of b-FGF protein.

**Figure 3:** Protein levels of VEGF-A 165 (Biosource) (A) and b-FGF (R&D) (B) in supernatant of uveal melanoma cell lines (OCM-3, OCM-8, Mel-202, OCM-1, OMM-1.5, 92-1, Mel-285, OMM-1.3, Mel-290) were determined by ELISA. In each case, $1 \times 10^5$ cells/ml were plated out. Supernatant of normal melanocytes (1A, 1B, 2) was tested only for VEGF-A 165.

**In vitro angiogenesis assay**
Conditioned media from OCM-1, OMM-1.3, OMM-1.5, Mel-202 cells, and from normal melanocytes 1A were tested for their ability to stimulate angiogenesis using an in vitro assay of capillary outgrowth from fetal mouse metatarsal explants (figure 4a). Of all cell lines tested, OCM-1 most strongly stimulated in vitro angiogenesis (figure 4b). Only those uveal melanoma cell lines that produced high amounts of VEGF-A 165 in culture were able to stimulate in vitro angiogenesis significantly. Normal melanocytes were unable to stimulate angiogenesis. The production of b-FGF was unrelated to angiogenesis in vitro.

**Expression profile of angiogenic factors in mouse model of uveal melanoma**
Semiquantitative species-specific PCR analysis was performed for tumour-derived angiogenic factors (VEGF-A, VEGF-B, VEGF-C, VEGF-D, and b-FGF) isolated from tumours growing in the eyes of Balb-c nu/nu mice following injection of human OCM-1 uveal melanoma cells into the anterior chamber of the eye. These tumours expressed VEGF-A (121,165,189), VEGF-B, VEGF-C, VEGF-D, and b-FGF (figure 5). Interestingly, OCM-1 cells in vitro did not express b-FGF.

**Gene expression of angiogenic factors in primary uveal melanoma of patients**
Tissue from primary uveal melanomas removed from 28 patients was analysed for the expression of angiogenic factors. VEGF-A mRNA (121,165,189) expression was low (9/28) or not detectable in the removed melanomas (figure 6). In contrast, all melanomas expressed VEGF-B and VEGF-C and some tumours expressed VEGF-D (1/28) and b-FGF (15/28).

**Figure 4:** Conditioned medium (48 hours) from cell lines OCM-1, OMM-1.3, OMM-1.5, Mel-202, and melanocytes 1A was aspirated and stored until further use. Gestation day 17 fetal mouse bone explants were cultured in 24-well plates containing 150 μl alpha MEM with 10% heat-inactivated FBS, and P/S. After 72 hours, medium was replaced by fresh medium containing supernatant of cell lines OCM-1, OMM-1.3, OMM-1.5, Mel-202 and melanocytes 1A after 48 hours and control medium, and as positive control VEGF-A (1 μg/ml). Medium was refreshed after 7 days and cultures were fixed after 14 days of culture. PECAM-1 staining was performed and the area of PECAM-1 positive tubular structures was quantified and expressed as percentage of control (A). Representative images (B) of OCM-1 and Mel-202-treated, control cultures (CO) are depicted. Quantification of vascular outgrowth in presence of the different supernatant and control (VEGF-A 1 μg/ml) was calculated. Cultures were performed in 6-fold. 1:10 = 10 times diluted, co = control.

Western blot analysis and ELISA, respectively, confirmed the specificity of VEGF-A 165 and VEGF-C expression by retina and tumour material. VEGF-C levels were higher in melanoma than in retina, whereas the opposite was found for VEGF-A 165.
Figure 5: (A, B, C, D, E) Semiquantitative PCR analysis of levels of mRNA for tumour-derived VEGF types (A, B, C, D) and b-FGF in eyes of nude mice inoculated with 2.10^6 human OCM-1 uveal melanoma cells into the anterior chamber of the eye. After normalization of the cDNA samples to human β2-microglobulin (0.9.10^2 copies/reaction), expected sizes: VEGF-A 189/165/121=558/497/366 bp, respectively; VEGF-B= 222 bp; VEGF-C= 293 bp; VEGF-D=372 bp; b-FGF=234 bp - = negative control, + = positive control.
No significant correlation was found between VEGF-B, VEGF-C, VEGF-D, or b-FGF expression and tumour characteristics, such as tumour size or location, and survival data, probably because of the small number of melanomas investigated.

**Discussion**

In our investigation of the expression of VEGF and b-FGF in uveal melanoma we found that all tested cell lines, in vivo experimentally induced tumours and specimens of uveal melanoma patients expressed a wide range of angiogenic factors that are known, individually or in combination, to play a direct role in uveal melanoma growth. Most uveal melanoma cell lines expressed VEGF-A (121,165,189), VEGF-B, VEGF-C, VEGF-D, and b-FGF to various extents. VEGF-A 121 appeared to be the most abundant of the VEGF A isoforms. All experimentally induced tumours expressed VEGF-A, VEGF-B, VEGF-C, VEGF-D, and b-FGF. Significant amounts of VEGF-B, VEGF-C, VEGF-D, and b-FGF were detectable in uveal melanoma material from patients, whereas VEGF-A (121,165,189) was not. Most uveal melanoma cell lines, but not normal melanocytes, synthesized and secreted VEGF-A 165 and b-FGF during cell culture. Only those uveal melanoma cell lines that produced high amounts of VEGF-A 165 in culture were able to stimulate significantly angiogenesis in vitro.

Angiogenic factors of the VEGF family are specific endothelial cell mitogens. VEGF-A, -B, -C induce angiogenesis and act as survival factors for the endothelium \(^9, 29\) and VEGF-C and VEGF-D are associated with the development of lymphatic vessels \(^16, 30\). VEGF-A synthesized by melanoma cell lines in vitro promoted angiogenesis in vitro, suggesting that VEGF-A may contribute to tumour angiogenesis. Protein levels of VEGF-A 165 in the aqueous humor of eyes with a uveal melanoma are correlated with patient survival, basal tumour diameter, and tumour height \(^10, 11\). The observation that uveal melanoma tissue from our patients expressed low or not detectable amounts of mRNA for the VEGF-A isoforms is consistent with recent reports showing that VEGF-A 165 is largely absent in uveal melanoma \(^14, 31, 32\). A possible explanation for this apparent discrepancy is that VEGF-A 165 protein is synthesized elsewhere and stored in the tumour and surrounding tissue. Boyd et al. \(^12\) suggested that VEGF-A may contribute to angiogenesis in uveal melanoma, although its expression is not correlated with metastatic potential or angiogenic markers. It should be noted, however, that in vitro cultures not always reflect the clinical situation but should be considered as a model for disease. In addition steady-state mRNA levels of any transcript may differ as well due to the microenvironment (tumor-stroma interactions). It may, therefore, be that mRNA for VEGF-A cannot always be detected due to mRNA instability while bioactive VEGF-A is clearly expressed at the protein level. It appears therefore that tumor cell lines and in vivo models may not always be reflective of the human disease.

The route by which a tumour can grow and metastasize is determined, at least in part, by its
Figure 6: (A) Fragments from 28 uveal melanomas collected from freshly enucleated eyes were snap-frozen in -80°C. Semiquantitative PCR analysis of levels of mRNA for VEGF types (VEGF-A, B, C and D) and b-FGF was done. Expected sizes: VEGF-A 189/165/121=558/497/366 bp, respectively; VEGF-B=222 bp; VEGF-C=293 bp; VEGF-D=372 bp and b-FGF=234 bp. - = negative control, + = positive control. (B, C) Staining of the primary uveal melanoma also revealed the presence of blood vessels visualized by staining with CD34 antibody for vascular endothelial cells. Original magnification x20 (B) and x40 (C).
ability to induce angiogenesis and/or lymphangiogenesis. The expression by tumour cells of soluble growth factors which are (lymph)-angiogenic (e.g. VEGF family factors) could be an important determinant of the route of metastatic spread. However, mRNA for VEGF-A (121,165,189) was not detectable in uveal melanoma material from patients, whereas mRNA for VEGF-B, VEGF-C, and VEGF-D was. VEGF-B is expressed by a variety of benign and malignant tumours 33, 34 and VEGF-C is expressed by melanoma, breast cancer and lymphoma9. In a recent molecular profiling study 35 VEGF-C was significantly upregulated (30-fold) in highly versus poorly invasive uveal melanoma cells. In our study, VEGF-B and VEGF-C were abundantly expressed by uveal melanoma in vitro and in vivo, even though this tumour is disseminated solely by haematogenous route 36. These results indicate that lymphangiogenic spread might be also a possible route of metastasis in uveal melanoma 37. However, Clarijs et al. 14 showed that VEGF-C was not able to induce lymphangiogenesis in the normal eye and in uveal melanoma. Interestingly there were no lymphatic vessels in and around the tumour. VEGF-C has been found to induce the formation of new blood vessels, but only in early development or in certain pathological settings such as tumorigenesis 38. A few studies have reported VEGF-D in tumour tissue, i.e. skin melanoma 16. We found that uveal melanoma cell lines expressed VEGF-D-, in experimentally induced tumours and in a limited number of clinical specimens.

Up-regulation of the expression of members of the VEGF family, especially VEGF-B and -C might be mediated by up-regulation of common transcription factors, or by angiogenic factors acting through a second messenger system by inducing the expression of other angiogenic factors. Indeed, a synergistic reaction between basic-FGF and VEGF has been reported 38-40. In our study, b-FGF protein was detected in the supernatant of almost all uveal melanoma cell lines, suggesting that it is synthesized endogenously. We suggest that b-FGF could be an important target for new anti-angiogenic treatment modalities for uveal melanoma.

In conclusion, we investigated the expression of the four members of the VEGF family and b-FGF in human uveal melanoma cell lines, in experimentally induced uveal melanomas, and in uveal melanoma tissue from patients. Our data support the notion that the ubiquitous expression of (lymph)-angiogenic factors plays a causal role in the pathogenesis of uveal melanoma and distant metastasis. Therefore anti-angiogenesis therapy may be a new approach to the treatment of highly vascularized tumours such as uveal melanoma. Additional studies are warranted to address more precisely the pathogenic role of the different angiogenic genes involved in growth and metastasis of uveal melanoma.

Acknowledgements

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-3, -8 were kindly provided by Dr. J. Kan-Mitchell (University of California, San Diego, CA) and cell line OMM 1 by Dr. G.P.M. Luyten (Rotterdam University Hospital).

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