Insights in tumorigenesis and metastasis of uveal melanoma

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Bone Morphogenetic Protein 7 Inhibits Tumor Growth of Human Uveal Melanoma *in vivo*

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Abstract

Purpose: Bone morphogenetic protein-7 (BMP7), a member of the TGF-β super family, is essential for early ocular morphogenesis, and lack of BMP7 causes epithelial development disturbances in the eye.

In the present study the association of tumorigenicity and malignant behavior of human uveal melanoma with BMP7 expression and the possibility that overexpression of BMP7 in uveal melanoma affects intra-ocular tumor growth *in vivo* were investigated.

Methods: To establish the role of BMP7 in uveal melanoma progression, the human OCM-1 cell line was stably transfected to overexpress BMP7 (OCM-1 FRT/BMP7) using targeted homologous recombination.

Results: Transcriptional profiling revealed low or no detectable expression of BMP7 in primary tumor tissue from patients with uveal melanoma. In line with these clinical observations, BMP7 mRNA levels were low or not detectable in cultured human uveal melanoma cell lines, when compared with normal cultured melanocytes. Inoculation of OCM-1 FRT/BMP7 cells into the anterior chamber of the eye of nude mice inhibited tumor progression significantly compared to the control cell line (no BMP7 expression).

Conclusion: Collectively, our data provide novel evidence that decreased BMP7 expression contributes to uveal melanoma progression. Furthermore, BMP7 may represent a novel therapeutic molecule for repression of tumor growth in uveal melanoma.

Introduction

Uveal melanoma is the most common primary malignant intraocular tumor in the adult, with a varying annual incidence of 6 to 12 per million in Caucasians [1, 2] . It 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 frequent occurrence of metastases, mainly in the liver [1, 2] . Knowledge of the nature of growth and metastatic behavior in uveal melanoma is essential for the development of new treatment strategies, especially with regard to improvement of survival.

The 35-kDa homodimeric protein bone morphogenic protein (BMP7) -7 is a member of the TGF-β superfamily. Knockout studies have shown that BMP7 (and BMP4) is essential for early morphogenesis of the eye and kidney [1, 3-5] . BMP7 knockout mice revealed deficient ocular growth, due to severe disturbances in epithelial development [5]. Recent evidence suggests that BMP7 plays a role in a functional system in the eye, modulating and balancing the expression of extra-cellular matrix (ECM) proteins (collagen IV, laminin, fibronectin) by meshwork cells of the trabecular system [6] . Disturbances of this balance may result in primary open-angle glaucoma (POAG)[6] .

In addition to ocular development, BMP7 is a prerequisite for induction of condensation and epithelization of metanephric mesenchyme in the kidney [3, 7-9] . In mesangial cells of the kidney, BMP7 counteracts TGF-β induced fibrosis, reversing the process of chronic renal injury and maintaining an epithelial phenotype. Moreover, BMP7 experimental therapy halts progression and reverses the effects of chronic progressive kidney disease . For instance, BMP7 counteracts the increased expression of several (ECM) proteins and connective tissue growth factor (CTGF) in chronic renal fibrosis [7, 10] .

This study was designed to investigate whether tumorigenicity and invasive growth behaviour are associated with modulated BMP7 expression in primary uveal melanoma and in human uveal melanoma cancer cell lines. Our results suggest that BMP7 inhibits growth of human uveal melanoma xenografted into the eye. BMP7 protein may therefore represent a novel therapeutic molecule for repression of tumor growth of uveal melanoma.

Materials and methods

Uveal Melanoma cell line and uveal melanocytes

Seven cell lines (92-1, Mel-202,-285,-290, OCM-1,-3,-8) were obtained from primary uveal melanomas [11-13] . Cell lines OMM-1.3 and -1.5 were obtained from liver metastases. Cell line 92-1 and normal uveal melanocytes (1, 2) were established in our laboratory [14] . 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 (Invitrogen-Gibco, Grand Island, NY), 10% heat-inactivated fetal calf serum (FCS) (Hyclone, Logan, Utah, USA), and 100 IU/ml penicillin (Invitrogen-Gibco). Cell lines OCM-1 and OMM-1 were grown as monolayers in 10 ml/dish Dubecco's modified Eagle's

medium (Invitrogen-Gibco) supplemented with 10% FCS (Hyclone), 100 IU/ml penicillin (Invitrogen-Gibco), and 100 µg/ml streptomycin (Invitrogen-Gibco). Normal melanocytes were grown as monolayer in 10 ml/dish HAM/F12 (invitrogen-Gibco) medium as described by Hu et al [15] . All cells were incubated at 37°C in a humidified atmosphere with 5% CO_2.

Primary uveal melanoma tumors

Twenty-eight enucleated eyes, containing uveal melanoma were included in this study as described previously [16]. The protocol conformed to the requirements of the Declaration of Helsinki. Two melanomas were treated because of incomplete tumor regression after combined treatment with ruthenium-106 plaque radiotherapy and transpupillary thermotherapy. The remaining 26 eyes were enucleated primarily because of a large uveal melanoma. The median age of the patients, 14 women and 14 men, at time of enucleation was 61.5 years (range 42-84 years). The maximal follow-up was 115 months. Tumor diameter varied from 7 to 18 mm (mean 12.5 mm, SD 2.9). After histopathological analysis, 10 tumors were classified as epithelioid, 12 as spindle cell, and 6 as mixed histology. There were 24 choroidal tumors, 2 ciliary body tumors, and 2 ciliary body melanomas with iris involvement. Tumor fragments were snap-frozen in nitrogen and stored at -80°C or paraffin embedded for immunohistochemistry and real-time PCR, respectively.

Establishment of stable transfectants expressing the BMP7 gene

For the establishment of OCM-1 cell line with BMP7 overexpression, we used the OCM-1 FRT cell line [17] . The BMP7 coding sequence (1µg construct CMV-BMP7 FRT [BMP7 cDNA]) was intergrated by targeted homologous recombination using the single FRT site as described previously [17] . Stable transfectants were selected with hygromycine (400 µg/ml, Invitrogen).

Enzyme-linked immunosorbent assay (ELISA) for human BMP7

Levels of human BMP7 were measured with a commercially available specific ELISA kit, with sandwich enzyme immunoassay technique (R&D systems, Abingdon, United Kingdom). A normalisation of number of cells in conditioned medium were analyzed by ELISA for the BMP7 production after 1, 3 and 8 days of culture. Fresh culture medium was used as the negative control. All samples were performed in duplicate.

Cell proliferation assay

OCM-1 FRT and OCM-1 FRT/BMP7 cells were cultured for 1 and 4 days after normalization of 2000 cells/200µl. An MTS assay was performed according to the manufacturer's protocol (Promega, Leiden, The Netherlands). All samples were performed six times.

Isolation of cellular RNA and real- time polymerase chain reaction (RT-PCR)

RNA was isolated from cells in culture and tissue from experimentally induced tumors, as described earlier [17, 18] . RNA from 28 human melanoma tissue samples was isolated with a kit (RNeasy Mini Kit, Qiagen, Venlo, The Netherlands) and proteinase K (20 mg/ml, Qiagen). Reverse transcription was performed with random primers in the presence of an RNase inhibitor (Roche Diagnostics, Rotkreuz, Switzerland). Quantitative real-time PCR (qPCR) was performed with the commercially obtained exon-specific primers for BMP7, vimentin and β-actin (primer catalog numbers numbers: Hs 002333477 m1 [BMP7], Hs 00185584 m 1 [vimentin], and Hs 99999903 m1 [β-actin]: Applied Biosystems, Rotkreuz, Switzerland) on an sequence detection system (ABI Prism 7700, Applied Biosystems). Values were normalized with the housekeeping gene β-actin according to the comparative method of Livak and Schmittgen [19] . All experiments were performed in triplicate.

Transient transfections and transcription reporter assays

OCM-1 FRT and OCM-1 FRT/BMP7 cells were seeded at a density 7.500 cells/cm^2 in DMEM with 10% FCS in 24-well plates. On subsequent days, cells were transiently transfected with 1µg of the indicated constructs with transfection reagent FuGENE 6 (Roche, Mannheim, Germany) following manufacturer's protocol. To correct for transfection efficacy, 100 ng of *Renilla* luciferase (pRL-CMV; Promega, Madison, WI, USA or pRL-CAGCS, Promega) was cotransfected. On day 3, cells were serum starved for 24 hours before to stimulation with TGF-β and /or BMP7 for a duration of 30 hours. On day 5, luciferase activities were quantified (Dual Luciferase Assay, Promega) [20]. Firefly luciferase activity was corrected for *Renilla* luciferase activity. The experiments were performed in triplicate and repeated at least twice. Results are expressed as means ± SEM.

Luciferase reporter gene constructs

For intracellular signaling of TGF-β the CAGA-luciferase construct (kindly provided by Dr. P ten Dijke, Dept. Molecular Cell Biology, Leiden University Medical Center, The Netherlands), consisting of 12 Smad3/Smad4 binding sequences (CAGA boxes) and the luciferase-coding sequences was used. The CAGA boxes confer TGF-β stimulation to a heterologous promoter reporter construct, whose activity depends on binding of activated Smad3/Smad4 transcription factor complexes [20]. The BRE-luciferase construct (kindly provided by Dr. P. ten Dijke), that is based on the mouse Id1 promoter, was used to study the presence and functionality of BMP-receptors [21].

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

Female Balb C *nu/nu* mice were purchased from Charles River (Charles River, Maastricht, The Netherlands). They were housed in ventilated cages under sterile conditions according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Sterile food

and water were provided at libitum. Mice were 8 weeks old at the time of the intra-ocular injection of tumor cells.

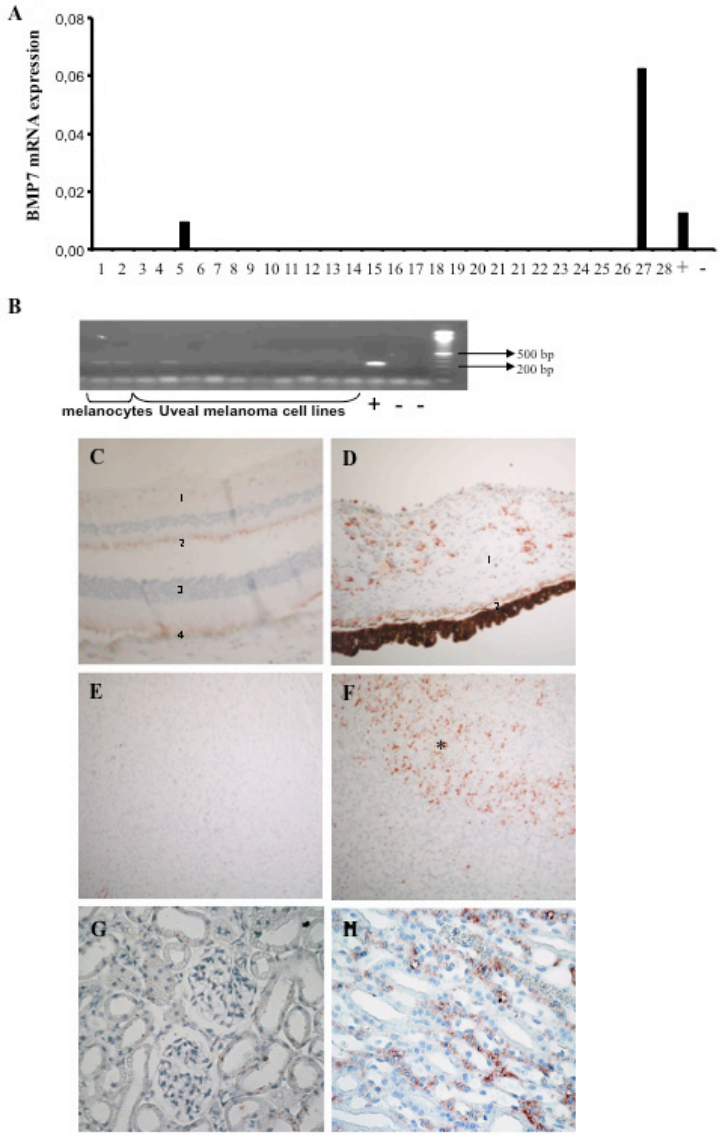

Figure 1: *BMP7 expression in primary uveal melanoma.* **(A)** *Real-time analyse of BMP7 gene expression in 28 tissue samples of uveal melanoma showed 26 with no expression for BMP7 and two tumors were found to be positive for BMP7. (***B***) RT-PCR analysis of BMP7 expression in normal uveal melanocytes and 10 uveal melanoma cell lines. Nine uveal melanoma cell lines were BMP7 negative and only normal melanocytes and OCM-3 showed detectable BMP7 expression. (****C****): BMP7 staining on paraffin-embedded tissue blocks of the primary uveal melanoma samples used for real-time PCR (1A and B) was performed. Positive staining was detectable in non-cancerous tissue,*

*including retina, cillairy body and iris. The retina showed positive staining of (1) the nerve fiber layer, (2) the inner nuclear layer, (3) the outer segments with external limiting membrane and diffuse staining of the choroid. (**D**) BMP7 immunolocalisation in (1) the iris stroma and (2) non-pigmented epithelium of the iris of a normal eye. (**E**) Representative example of a primary uveal melanoma that lacks BMP7 expression. (**F**) Only two cases showed significant BMP7 staining (*) of parts of the tumor, which corresponded with the real-time analyses. (**G, H**) Medullary rays of the kidney were used as a positive control (**G**), kidney tubular epithelium) and glomeruli (**H**) as negative control [24, 25] . Original magnification (**C, F**) 20X, (**G, H**) 40X.*

To analyze the effect of BMP7 *in vivo*, we used the model for induction of tumor growth in the anterior chamber of the eye [17]. Tumor cells were orthotopically inoculated into the anterior chamber of mouse eyes (only right eyes, each group n = 10) as described earlier by Niederkorn et al. [22]. With a glass needle OCM-1 FRT cells or OCM-1 FRT/BMP7 cells at a concentration of 10^5/4µl were injected into the anterior chamber of the eye of an 8-week-old female Balb C *nu/nu* mice. After 3 weeks all eyes, were enucleated and killed for histomorphometric analyses and for immunohistochemistry.

Immunohistochemistry

After enucleation, the eyes were immediately fixed in 5 ml of 4% para-formaldehyde (Lommerse Pharma, Oss, The Netherlands). After 24 hours the eyes were dehydrated for 2 hours in 70% ethanol, 1 hour in 90% and 30 minutes in 99% and processed for paraffin embedding. Hematoxylin-eosin (H&E) and Periodic acid-Schiff (PAS) staining were performed on 5-µm serial sections of tumorous eyes. Histomorphometric measurements of tumor burden were performed on central sections through the tumor (largest tumor area). Total tumor area (expressed in square millimeters), as an estimate of total tumor burden, was measured by image analysis (NIH-Image 1.62b7 images analysis software; availble by ftp at zippy nimh.nih.gov/orat http://rsb.info.nih.gov/nih-image; developed by Wayne Rasband, National Institutes of Health, Bethesda, MD) [23]. Antibodies against human BMP7 (rabbit polyclonal α-BMP7, 2854ab, Vukicevic; 10µg/ml) and vimentin (Abcam Ltd, Cambridge, United Kingdom) were used for immunohistochemistry on serial sections of tumorous eyes, OCM-1 FRT/BMP7 cells and OCM-1 Wt cells (cultured on chamber slides). Polyclonal BMP7 antibody was used in a dilution of 1:100 and vimentin in a dilution of 1:50. For the BMP7 staining, medullary rays of the kidney were used as a positive and glomeruli as negative control [24, 25] .

Sections were incubated with primary antibodies overnight, followed by incubations with biotin-labeled rabbit-anti-mouse IgG and a preformed complex of biotin labeled horseradish peroxidase and streptavidin (DAKO, Glostrup, Denmark). Immune complexes were visualized with 0,05% diaminobenzidine and 0,0015% hydrogen peroxide. Slides were counterstained in Mayer's hematoxylin (Merck, Amsterdam, The Netherlands). OCM-1 FRT/BMP7 cells and OCM-1 FRT cells cultured on chamber slides were also stained with vimentin antibody. The level of vimentin staining was quantified by two independent observers (scale 0-3; 0=

absent, 1= low, 2= moderate and 3= high).

Statistical analysis

Statistical analysis was performed with ANOVA (SPPS for Windows; version 13.0). Results are expressed as mean ± standard error of the mean (s.e.m.) of at least two experiments, unless indicated otherwise. $P < 0,05$ was considered significant.

Results

BMP7 expression primary uveal melanoma tissue and in vitro

Real-time PCR analyses revealed that BMP7 expression was low or undetectable in most of the tested uveal melanoma tumor tissues (in 26 of the 28 patients tested, figure 1A). In line with these clinical observations, BMP7 expression in most human uveal melanoma cell lines was low or undetectable, whereas normal melanocytes express relatively high amounts of BMP7 mRNA (figure 1B). Immunolocalization of BMP7 revealed positive staining in the retina and ciliary body of the normal eye (figure 1C, 1D). Our observations, in line with several studies, showed BMP7 expression in all retina layers, with high levels of expression being presented in the inner and outer nuclear layers [3, 26, 27] . BMP7 was expressed in the nerve fiber layer, inner nuclear layer, outer segments and external limiting membrane of the retina and diffuse staining of the choroid (figure 1C). BMP7 immunolocalization was not detected in 93 % of the tested tumors with primary uveal melanoma (figure 1E, 1F).

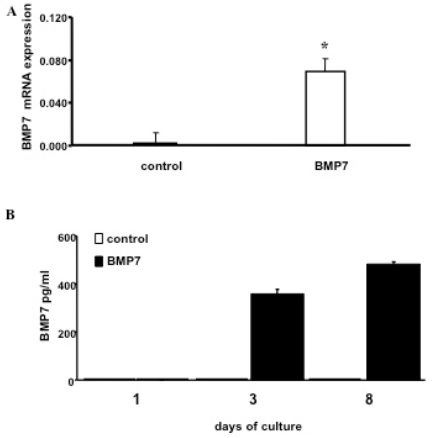

Figure 2: *BMP7 overexpression in OCM-1 human uveal melanoma cell line.* **(A)** *Relative mRNA expression levels of BMP7 in OCM-1 FRT cells and OCM-1 FRT/BMP7 cells. BMP7 mRNA was expressed at significantly higher level in OCM-1 FRT/BMP7 cells. Data are the mean expression ± SD. Results are from two single experiments in triplicate. Values were normalized against β-2M.* **(B)** *BMP7 protein expression and secretion into the culture medium*

by OCM-1 FRT cells and OCM-1 FRT/BMP7 cells (ELISA). Results are analysed from two single experiments in duplicate.

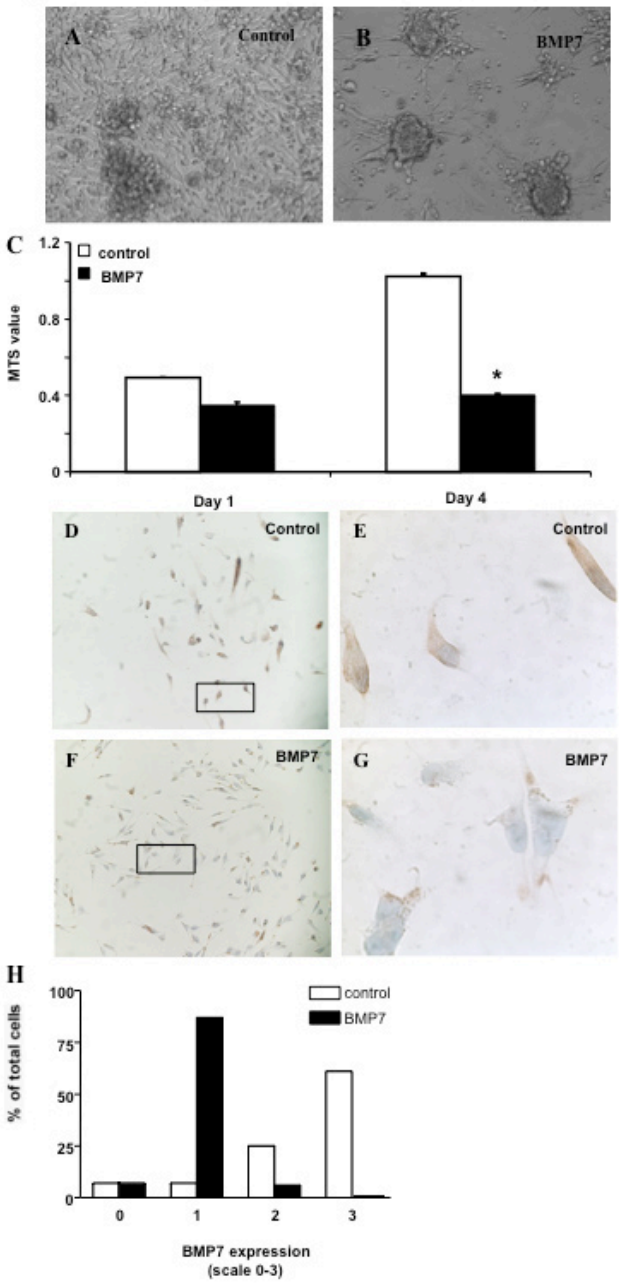

Figure 3: *Growth and morphologic characteristics of OCM-1 in the absence or presence of BMP7 . OCM-1 FRT (A) and OCM-1 FRT/BMP7 (B) cells after 5 days of in cell culture. (C): BMP7 expression affected cellular proliferation of OCM-1 cells. (n=6). Vimentin immunolocalisation in OCM-1 FRT cells (D, E) and OCM-1 FRT/BMP7 cells*

(F, G) after 2 days in culture. (H): Quantitation of vimentin expression by immunohistochemestry in OCM-1 FRT and OCM-1 FRT/BMP7 cells (scale 0-3, 0= no expression, 1=low, 2=moderate, 3=high expression) Original magnification: (A, B) X 16,;(C, D, E) X20; (F, G) X100.

The BMP7 immunolocalization data are therefore in full agreement with mRNA expression profile of the same tumors.

BMP7 overexpression in uveal melanoma cell line OCM-1

Targeted homologous recombination using the Flp-In system (Invitrogen) was performed to overexpress BMP7 in OCM-1 (OCM-1 FRT/BMP7) human uveal melanoma cells, thus avoiding clonal variability, as described previously by us [17, 28]. Real-time PCR and ELISA analysis revealed the expression of BMP7 both at the transcriptional and protein level in OCM-1 FRT/BMP7 (figure 2A, B respectivily) as expected, whereas the single FRT host control cell line (OCM-1 FRT) did not express detectable amounts of BMP7 protein (≤60 pg/ml).

Striking differences in morphology and inhibition of proliferation were observed between OCM-1 FRT and OCM-1 FRT/BMP7 *in vitro*. After 3 days, OCM-1 FRT/BMP7 cells grow in a multilayer, in contrast to OCM-1 FRT control cells (figure 3A, B). In addition to morphological differences, a significant change in cell proliferation rate was observed between the OCM-1 FRT control cells and OCM-1 FRT/BMP7 (figure 3C).

Furthermore, BMP7 overexpression coincided with diminished expression of vimentin, which was identified previously as a marker of invasiveness for uveal melanoma [29] . Vimentin expression in OCM-1 FRT/BMP7 cells was mainly localized in the cytoplasmatic podosomes of the cells, whereas the control cell line display cytoplasmatic staining (figure 3 D - G). Enforced BMP7 expression strongly diminished protein levels of vimentin (figure 3H).

Smad-mediated TGF-β and BMP signaling in OCM-1 cells

OCM-1 FRT cells showed significant BRE-luciferase activity, indicating the presence of functioning, activated type I BMP-receptor complexes and active Smad-dependent signaling. In OCM-1 FRT/BMP7 cells, which stably overexpressed BMP7, BRE-luciferase activity was significantly increased as expected (figure 4A, B). Next, we tested whether BMP7 expression can antagonize Smad-dependent TGF-β signaling, as recently described by us in breast cancer and prostate cancer [28, 36]. The presence of functionally active TGF-β receptor complexes in the OCM-1 cells was demonstrated by the dose-dependent activation of the CAGA-luciferase reporter, whose activity depends on activated Smad3/4 transcription factor complexes. Addition of BMP7 to TGF-β-stimulated OCM-1 cells, however, did not significantly inhibit the TGF-β-driven CAGA-luciferase activity (figure 4C), which contrasts studies performed in breast cancer and prostate cancer [28, 36].

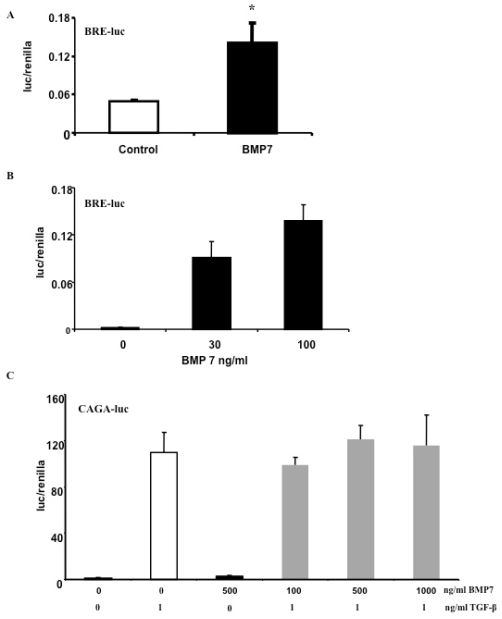

Figure 4: *BMP and TGF-β pathway activity in OCM-1 cells.* **(A)** *Smad-dependent BMP pathway activity in OCM-1 FRT and OCM-1 FRT/BMP7 cells transfected with BMP response element (BRE)-luciferase reporter constructs.* **(B)** *BRE-luciferase activity in OCM-1 FRT cells upon challenge with BMP7 (30 and 100 ng/ml).* **(C)** *TGF-β-induced CAGA-luciferase reporter activity in OCM-1 FRT cells indicate the presence of functionally active TGF-β receptor complexes (open bar). Addition of BMP7 to TGF-β stimulated OCM-1 FRT cells did not significantly inhibit TGF-β driven CAGA-luciferase activity (grey bars). Values were normalized to Renilla (CAGA-luc/pRL-CAGGS, BRE4-luc/pRL-CMV). Results are analysed from two single experiments in triplicate.*

BMP7 overexpression and in vivo tumor progression of human uveal melanoma

Three weeks after intra-ocular inoculation of OCM-1 FRT (control cells) and OCM-1 FRT/BMP7 cells all mice (n=20) were killed and the tumors were enucleated. After 21 days, the control group of mice already had macroscopically detectable tumors in the eye. These tumors developed in the anterior chamber with outgrowth into the back of the eye. When BMP7 expressing uveal melanoma cells were used, histomorphometric analyses revealed a significant reduction in tumor burden (figure 5A). Furthermore the localization of the tumors differed between the two experimental groups (figure 5B, C-E). BMP7 overexpressing tumors were localized in and around the lens and displayed no signs of invasion into the back of the eye (figure 5 C, E).

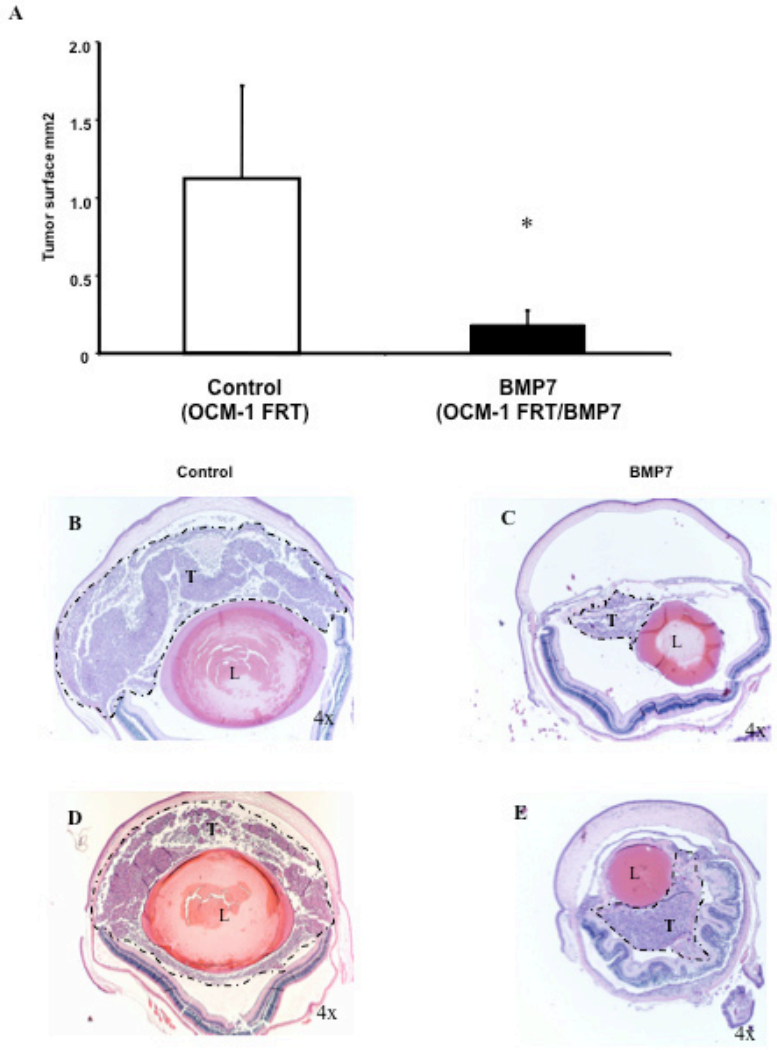

Figure 5: *BMP7 over-expression and uveal melanoma progression in vivo. Three weeks after intraocular inoculation, all eyes were enucleated and immunohistochemical analyses were performed (H&E staining). (C, E) Tumors induced by OCM-1 FRT/BMP7 cells were significantly ($p < 0.05$) smaller than those in control group (B, D) (OCM-1 FRT). Tumors induced by OCM-1 FRT cells were mainly located in the anterior chamber and the posterior pole of the eye, compared with the tumors induced by OCM-1 FRT/BMP7 cells which were located in and around the lens.*

Discussion

In this study we provide novel evidence for the role of BMP7 in uveal melanoma progression. BMP7 was expressed in normal epithelial tissues of the eye, particularly in different retinal cell layers and the iris. Low (or nondetectable) expression of BMP7 was found in nearly

all of primary uveal melanoma tumors, whereas BMP7 expression was observed in normal melanocytes, as expected [3, 24, 26] .

Orthotopic xenografts of BMP7-expressing OCM-1 uveal melanoma cells (vs. control cells) resulted in a significant inhibition of tumor progression. These results suggest that decreased BMP7 expression in normal uveal melanocytes disturbs melanocyte homeostasis and may contribute to tumor progression. In line with these *in vivo* observations, a marker of the aggressiveness and invasiveness of uveal melanoma, vimentin, was strongly downregulated in BMP7-overexpressing uveal melanoma cells [30, 31] .

In a variety of tissues, BMP7 appears to be a mediator of epithelial homeostasis and a prerequisite for maintenance of differentiated epithelial phenotype [7, 8, 32-34] . Furthermore, in mesangial cells of the kidney, BMP7 counteracts TGF-β induced fibrosis, reversing the process of chronic renal injury and maintaining an epithelial phenotype. Moreover, BMP7 experimental therapy has halted progression and reversed the effects of chronic progressive kidney disease [7, 10, 35] . BMP7, like other BMPs, bind to the BMPRII present on the cell membrane and recruit a type I receptor, BMPRIA (ALK-3) or BMPRIB (ALK-6), forming a complex. BMP receptor type I (ALKs) are transmembrane serine/threonine kinase proteins that self phosphorylate after formation of the BMP-receptor II-receptor I complex and acquire the ability to phosphorylate Smad proteins, a family of TGF-β transducers [7] . Recent evidence from our group in breast and prostate cancer supports the notion that BMP7 inhibits tumor progression and metastasis [28,36]. Furthermore, BMP7 antagonized the protumorigenic effects of TGF-β on epithelium-to-mesenchym transition via a Smad-mediated mechanism (BMP7-induced inhibition of CAGA-luciferase and stimulation of E-cadherin expression) [28, 36]. In the present study, OCM-1 cells expressed functionally active BMP receptor complexes. The presence of functionally active TGF-β receptor complexes in the OCM-1 cells was demonstrated by the dose-dependent activation of the CAGA-luciferase reporter, whose activity depends on activated Smad3/4 transcription factor complexes. In the presence of TGF-β, exogenous BMP7 addition did not result in a downregulation of CAGA-luciferase reporter activity. In OCM-1 cells, BMP7, therefore does not able antagonize TGF-β-induced Smad signaling. Our observations seem contradictory to our recent findings in breast and prostate cancer [36]. It should be noted, however, that BMP7 may also interact with other pathways that are critical in tumor progression and metastasis[28, 36]. In line with the latter studies in carcinomas [28,26], in the present study BMP7 expression has decreased in uveal melanoma when compared with normal melanocytes of the eye. BMP7 expression in breast cancer and prostate cancer is inversely related to tumorigenicity, and these findings are in accordance to the observed low BMP7 expression in human uveal melanoma primary tumors and in human melanoma cell lines. In keeping with our observations in uveal melanoma, stable overexpression of BMP7 in human breast cancer cells by targeted homologous recombination also results in a significant decrease in tumor progression and (bone) metastasis [28]. Although speculative at present, decreased BMP7 expression in uveal melanoma development may

contribute to increased invasiveness, as has been suggested for epithelial cancers [28, 36, 37]. Of interest, recent evidence suggests that BMPs can also affect the tumorigenic potential of human tumor –initiating cells with a progenitor/stem cell phenotype [38].

Although much remains to be understood about the complex role of BMP signals in cancer, a decrease of BMP7 expression during uveal melanoma progression may contribute to the acquisition of an invasive phenotype. BMP7 may, therefore, represent a novel therapeutic agent for repression of tumor growth in uveal melanoma.

Acknowledgements

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