Ultraviolet exposure of melanoma cells induces fibroblast activation protein-α in fibroblasts: Implications for melanoma invasion

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Abstract. Fibroblast activation protein-α (FAP-α) promotes tumor growth and cell invasiveness through extracellular matrix degradation. How ultraviolet radiation (UVR), the major risk factor for malignant melanoma, influences the expression of FAP-α is unknown. We examined the effect of UVR on FAP-α expression in melanocytes, keratinocytes and fibroblasts from the skin and in melanoma cells. UVR induces upregulation of FAP-α in fibroblasts, melanocytes and primary melanoma cells (PM) whereas keratinocytes and metastatic melanoma cells remained FAP-α negative. UVA and UVB stimulated FAP-α-driven migration and invasion in fibroblasts, melanocytes and PM. In co-culture systems UVR of melanocytes, PM and cells from regional metastases upregulated FAP-α in fibroblasts but only supernatants from non-irradiated PM were able to induce FAP-α in fibroblasts. Further, UV-radiated melanocytes and PM significantly increased FAP-α expression in fibroblasts through secretory crosstalk via Wnt5a, PDGF-BB and TGF-β1. Moreover, UV radiated melanocytes and PM increased collagen I invasion and migration of fibroblasts. The FAP-α/DPPIV inhibitor Gly-ProP(OPh)$_2$ significantly decreased this response implicating FAP-α/DPPIV as an important protein complex in cell migration and invasion. These experiments suggest a functional association between UVR and FAP-α expression in fibroblasts, melanocytes and melanoma cells implicating that UVR of malignant melanoma converts fibroblasts into FAP-α expressing and ECM degrading fibroblasts thus facilitating invasion and migration. The secretory crosstalk between melanoma and tumor surrounding fibroblasts is mediated via PDGF-BB, TGF-β1 and Wnt5a and these factors should be evaluated as targets to reduce FAP-α activity and prevent early melanoma dissemination.

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Abbreviations: ECM, extracellular matrix; FAP-α, fibroblast activation protein-α; MMPs, matrix metalloproteinases; PDGF-BB, platelet derived growth factor BB; SDF-1α, stromal cell-derived factor-1α; TGF-β1, transforming growth factor-β1; UVA, ultraviolet radiation A (320-400 nm); UVB, ultraviolet radiation B (290-320 nm); UVR, ultraviolet radiation; uPA, urokinase-type plasminogen activator; WM55P, PM, primary melanoma cells; WM55M1, matched regional metastatic melanoma cells; WM55M2, matched systemic metastatic melanoma cells

Key words: FAP-α, UV irradiation, melanoma, fibroblast, invasion

Introduction

Invasion of malignant tumor cells depends on changes in the microenvironment including activation of extracellular proteases and modification of the tumor stromal tissue by communication between tumor cells and surrounding stromal cells. There is increasing evidence that tumor cells activate stromal fibroblasts to degrade extracellular matrix (ECM) thereby playing a major role in tumor spreading (1). Fibroblast activation protein-α (FAP-α), a serine protease located at the plasma membrane, exhibits when active as a dimer both protease and collagenase activity important for ECM degradation (2,3). Further, a restrictive expression of FAP-α has been demonstrated in reactive fibroblasts in wound healing and in tumor-associated fibroblasts but normal adult tissues are generally FAP-α negative (3,4). A previous study described the expression of FAP-α on the protrusions of malignant melanoma cells (5) and FAP-α has been detected in the reactive stroma of melanocytic nevi and melanoma (6). These results implicate a possible role of FAP-α in the progression of malignant melanoma.

Numerous studies have demonstrated that ultraviolet radiation (UVR) initiates cutaneous melanoma by causing oxidative stress/DNA damage with attendant effects on oncogenes and tumor suppressor genes (7), but the role of UVR on melanoma progression is not studied in detail. However, UVR has been shown to enhance tumorigenicity in primary melanoma by generation of interleukin 8 and up-regulation of matrix-metalloproteinase-2 activity (8,9). Further, the inhibitor of apoptosis protein survivin has been reported to stimulate melanoma growth as well as metastatic spread in UV-induced melanoma in HGF-transgenic mice (10). At present there are

no studies on the effect of UVR on FAP-α expression and nothing is known so far about the crosstalk between melanoma cells and surrounding fibroblasts regarding FAP-α expression related to UVR.

This study was designed to analyze the effects of UVA (320-400 nm) and UVB (290-320 nm) radiation on FAP-α activity in fibroblasts and malignant melanoma and to investigate whether melanoma cells can activate FAP-α on fibroblasts to facilitate melanoma migration and invasion. The function of FAP-α on invasiveness and migratory capability was investigated in a co-culture system of fibroblasts and UV irradiated melanocytes, primary melanoma cells and metastatic melanoma cells. Further, a number of factors described to be involved in secretory communication and tumor spreading; the platelet derived growth factor-BB (PDGF-BB), transforming growth factor-β1 (TGF-β1), the stromal cell-derived factor-1α (SDF-1α), the urokinase-type plasminogen activator (uPA) and signaling protein Wnt5a (11-14) were studied to identify candidates triggering FAP-α expression.

Materials and methods

Cell cultures. All experiments were performed according to the ethical principles of the Helsinki declaration and approved by the Ethics Committee at Linköping University, Sweden. Primary melanocytes, keratinocytes and fibroblasts were obtained from Caucasian donors (0-3 years of age) by means of foreskin circumcisions and pure cultures were established as described previously (15). We have chosen cells from normal skin as substitutes in all experiments as tumor associated fibroblasts are phenotypic and functionally a heterogeneous population. Further, there is no model to secure the properties procure by the tumor microenvironment. The experiments were performed between passage 2-7 and no cells were cultured for more than three weeks in total. Four matched melanoma cell lines were used; the primary melanoma WM164, WM793, WM278, WM55P and respective matched secondary melanoma WM451Lu, WM1205Lu, WM1617, regional WM55M1 and systemic WM55M2 (from the Wistar Institute, Philadelphia, USA). The fibroblasts and melanoma cells were cultivated in RPMI-1640 medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin. Prior to experiments, cells were trypsinized and seeded at $2.5x10^4$ cells/cm^2. Cells were starved in serum-free media 24 h prior to assay. Non-irradiated controls (sham) were analyzed in each study point and the UV dosages, time points and intervals were carefully titrated to achieve optimal peak response.

FAP inhibition. The cell permeable H_2N-Gly-Pro diphenyl-phosphonate (Gly-ProP$(OPh)_2$, 100 μM, stock in DMSO), verified to block FAP-α and dipeptidylpeptidase IV (DPPIV) activity was used 24 h prior to experiments (16). Controls for DMSO effects showed no interference with the experiments.

SiRNA transfection against FAP-α was performed with 1 μg FAP-α siRNA (CGGAATTTAATGATACGGATA, Qiagen, Germantown, MD, USA) and 6 μl RNAiFect Transfection Reagent (Qiagen). Optimal transfection conditions for the cells were determined by titration, using Alexa Fluor 555 labeled non-silencing siRNA, with a scrambled sequence without homology to mammalian genes (AATTCTCCGAACGTGTC ACGT, Qiagen). This siRNA was used as negative control and siRNA targeting Lamin A/C (AACTGGACTTCCAGAAGA ACA, Qiagen) served as positive control, as recommended by the manufacturer.

In order to balance specificity, concentration and time axis we confirm corresponding effects of the two inhibitors used in all experiments (not shown). The time axis for optimal blockage was best compatible with Gly-ProP$(OPh)_2$ therefore chosen for all experiments.

Co-culture system. Co-cultures were established with fibroblasts cultured in the bottom wells and melanocytes, keratinocytes, fibroblasts, or primary (WM55P) and matched metastatic (regional WM55M1; systemic WM55M2) melanoma cells in inserts, with a pore size of 0.2 μm to avoid cell passage. The inserts were submerged into the wells after UVA, UVB or sham exposure and analyzed at optimal time interval for peak response as titrated.

UV radiation. The UVB source was Philips TL20W/12 tubes (Philips, Eindhoven, The Netherlands) emitting in the spectral range 280-370 nm with a main output between 305-320 nm. For UVA a Medisun 2000-L tube (Dr Gröbel UV-Elektronik GmbH, Ettlingen, Germany; 340-400 nm) was used. The output was 1.44 mW/cm^2 for UVB and 80 mW/cm^2 for UVA. A Schott WG 305 cut-off filter (Mainz, Germany) was used. The measurements were done with an RM-12 (Dr Gröbel UV-Elektronik GmbH) and a PUVA Combi Light dosimeter (Leuven, Belgium). Exposure was performed in phosphate buffered saline (w/o sodium bicarbonate). The radiation doses (UVA 6 J/cm^2, UVB 60 mJ/cm^2) were titrated to achieve minimum apoptotic or necrotic cell contamination.

Immunocytochemistry and nuclear morphology. Directly after UVR fresh culture medium was added and when FAP-α expression peaked the cells were fixed in 4% paraformaldehyde for 20 min at 4˚C and processed for immunocytochemistry. The cells were permeabilized with 0.1% saponin/5% fetal bovine serum solved in phosphate buffered saline (w/o sodium bicarbonate) and incubated overnight at 4˚C with the monoclonal anti-mouse primary antibody FAP-α (Santa Cruz Biotechnology, Santa Cruz, CA, USA) followed by incubation with a secondary Alexa Fluor 488 conjugate antibody (Molecular Probes, Eugene, OR, USA) for 1 h at room temperature. The samples were mounted in Vectashield® Mounting Media supplemented with 4',6-diamidino-2-phenylindole (DAPI) (1.5 μg/ml, Vector Laboratories, Burlingame, CA, USA) and inspected in a Nikon Eclipse E600W fluorescence confocal microscope. In each culture dish 200 cells were randomly selected and the fraction of FAP-α positive cells were counted. Negative controls incubated without primary antibody showed no staining.

Western blot analysis. The protein samples were separated on a Ready gel (Bio-Rad Laboratories) and transferred to a Hybond™-P blotting membrane (Amersham Biosciences, Buckinghamshire, UK). Subsequently, the blots were saturated with 5% non-fat dry milk (Bio-Rad Laboratories) in phosphate buffered saline supplemented with 0.05% Tween 20 at 4˚C overnight. The immunodetection was performed by incubating

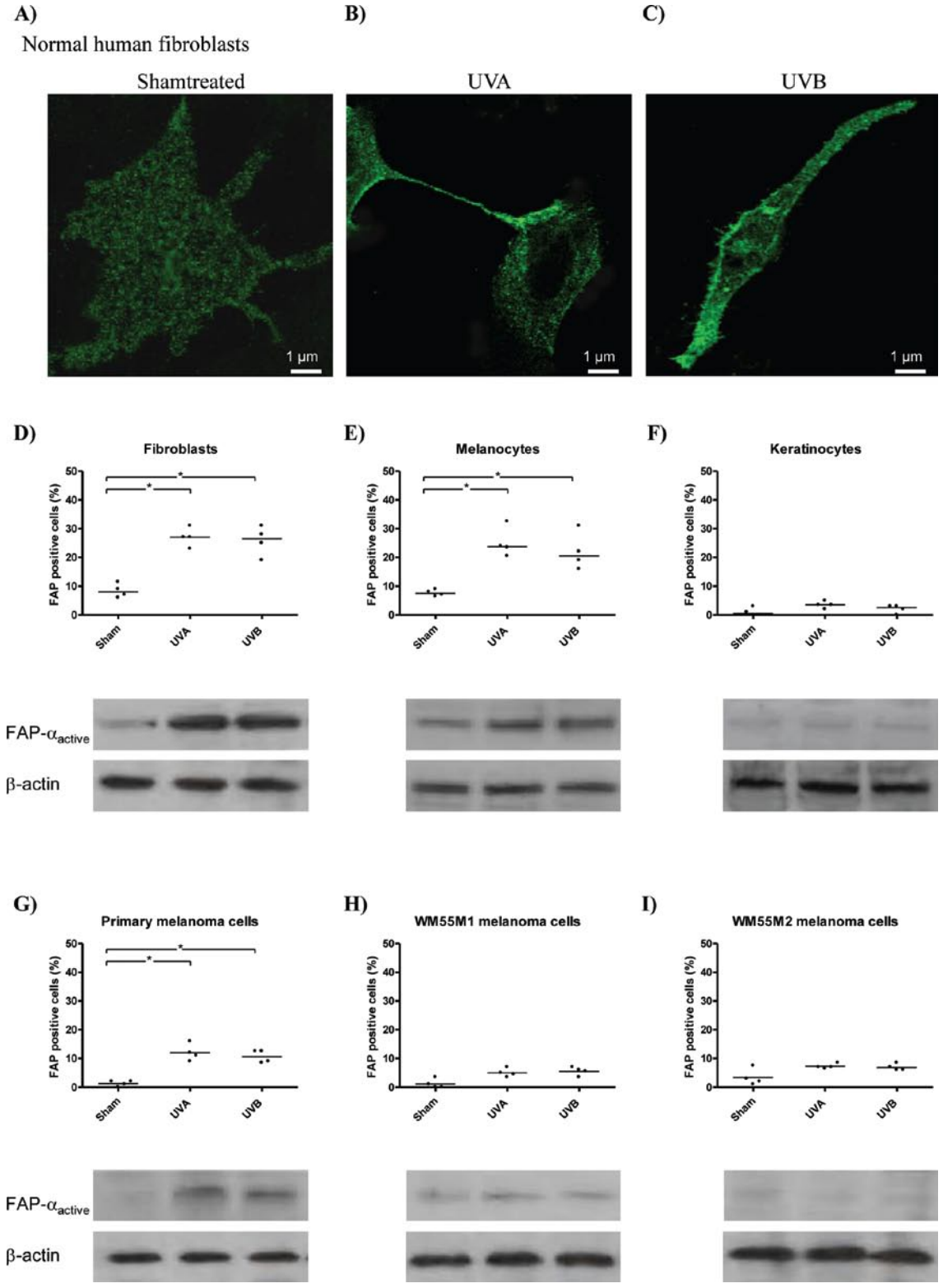

Figure 1. UVA and UVB induce FAP-α in fibroblasts, melanocytes and primary melanoma cells. Expression of FAP-α in normal human fibroblasts analyzed by immunocytochemistry 4 h after (A) shamtreatment, (B) UVA (6 J/cm^2) and (C) UVB (60 mJ/cm^2) radiation. Percentage of FAP-α positive cells analyzed by immunocytochemistry (n=4) and protein expression in a representative Western blot in (D) fibroblasts, (E) melanocytes, (F) keratinocytes, (G) primary WM55P, (H) regional metastatic WM55M1 and (I) systemic metastatic WM55M2 melanoma cells 4 h after shamtreatment, UVA and UVB radiation. Horizontal line indicate median of four experiments, $^*p<0.05$.

for 2 h at room temperature with the polyclonal primary antibody uPA, Wnt-5a and PDGF-BB (all from Santa Cruz Biotechnology), or with one of the following monoclonal primary antibodies: FAP-α, SDF-1α and TGF-β1 (all from Santa Cruz Biotechnology). Then horse radish peroxidase [mouse-, rabbit- (Dako) or goat- (Santa Cruz)] conjugated

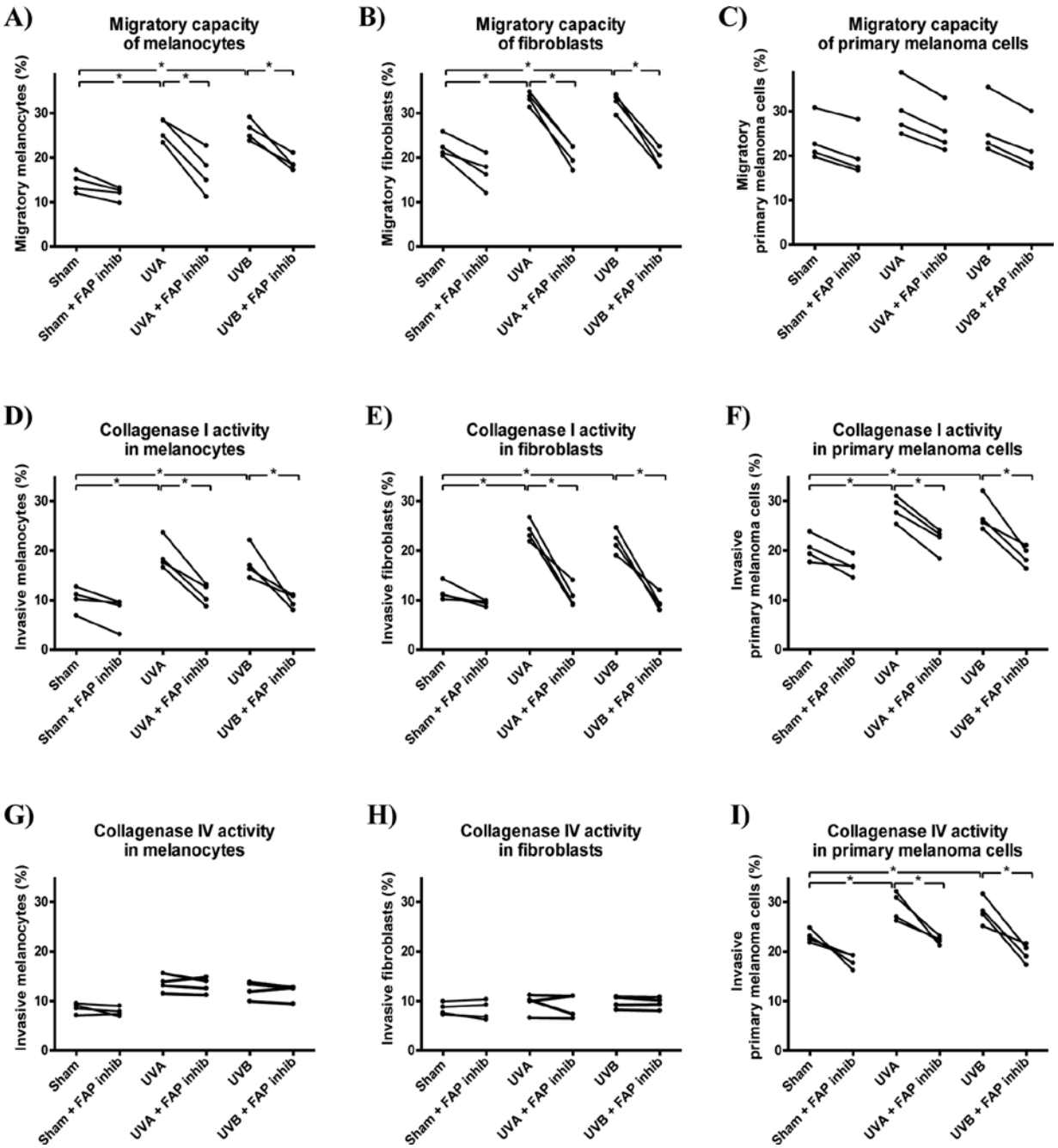

Figure 2. FAP-α regulates migration and invasion. Percentage of migrated (A) melanocytes, (B) fibroblasts and (C) primary melanoma cells analyzed by Cultrex® Migration Assay. Percentage of invasive (D, G) melanocytes, (E, H) fibroblasts and (F, I) primary melanoma cells with collagenase I or collagenase IV activity analyzed by Cultrex Collagen I Cell Invasion Assay and Cultrex Collagen IV Cell Invasion Assay. Cells were shamtreated or exposed to UVA (6 J/cm^2) or UVB (60 mJ/cm^2) prior to assay and then incubated for 24 h. For FAP-α/DPPIV inhibition Gly-ProP(OPh)$_2$ (100 μM, 24 h) was supplemented to the cell culture. (n=4, $^*p<0.05$).

corresponding secondary antibodies were added for 1 h. As internal loading control the membranes were reprobed with β-actin antibodies (Santa Cruz Biotechnology) for total cell fractions. Densiometric quantification of the bands was performed with Gel-Pro Analyzer 3.1 (MediaCybernetics, Silver Spring, MD, USA).

Cell migration/invasion assay. Cultrex® 96 well Migration Assay, Collagen I Cell Invasion Assay or Collagen IV Cell Invasion Assay were used (Trevigen Inc., Helgerman Ct. Gaithersburg, MD, USA). For migration assay top invasion chambers (8 μm pore size) were uncoated. For invasion assays chambers were coated with either 0.7% collagen I solution or 0.5% collagen IV solution (titrated to achieve optimal invasion for each cell type used). Cells were grown (5x10^4 cells/well) in the top chamber and either shamtreated or exposed to UVR. Further, media were supplemented with stimulators (10 ng/ml of recombinant PDGF-BB, SDF-1α, TGF-β1, uPA, Wnt5a) or the cell permeable FAP-α/DPPIV inhibitor Gly-ProP(OPh)$_2$. Migrated or invaded cells were then dissociated and treated

Table I. Secretory communication from melanocyte, primary and matched metastatic melanoma cell cultures.

	FAP-α	PDGF-BB	TGF-β1	uPA	Wnt5a	SDF-1α
Melanocyte						
Shamtreated	-	-	-	-	+	-
UVA	-	+	+	-	+	-
UVB	-	+	+	-	+	-
Primary (WM55P) melanoma						
Shamtreated	-	+	+	+	+	-
UVA	-	+	+	+	+	-
UVB	-	+	+	+	+	-
Regional (WM55M1) melanoma						
Shamtreated	-	+	+	-	+	-
UVA	-	+	+	-	+	-
UVB	-	+	+	-	+	-
Systemic (WM55M2) melanoma						
Shamtreated	-	+	+	-	+	-
UVA	-	+	+	-	+	-
UVB	-	+	+	-	+	-

Analysis of supernatants from melanocyte, primary and matched metastatic melanoma cell cultures irradiated with UVA (6 J/cm^2), UVB (60 mJ/cm^2) or shamtreated. Relative amount of released substance (+) is compared to respective shamtreated cells (supernatants from three experiments were pooled before analysis).

with Calcein-AM whereafter the assay chamber was read at 485 nm excitation, 520 nm emission. Data were compared to standard curves to determine the percentage of cells that have migrated (no coating), or invaded (through collagen I or IV).

Analysis of secreted factors by Western blotting. Cells were grown for 24 h whereafter the supernatants were collected, centrifuged (300 x g, 5 min) and concentrated using Amicon Ultra-4 Centrifugal filter (3 kDa; Millipore Corporation, Billerica, MA, USA) at 4,000 x g for 30 min at 4˚C. The supernatants from respective cell cultures with or without radiation or with supplements of 10 ng/ml recombinant SDF-1α, TGF-β1, PDGF-BB, Wnt5a and uPA were supplemented to fibroblasts.

Statistical analysis. Statistical evaluation was performed by Kruskal Wallis multiple comparison test as pre-test, followed by Mann-Whitney for comparison between groups. All $p<0.05$ were considered significant.

Results

The expression of FAP-α was investigated in cultured human fibroblasts, keratinocytes, melanocytes, primary melanoma cells and matched metastatic melanoma cells. Fibroblasts and melanocytes showed a low but recognizable FAP-α expression, but the other cell types did not prove to be FAP-α positive (Fig. 1A, D-I). However, 4 h after UVA or UVB radiation FAP-α expression was induced at the plasma membrane in fibroblasts, melanocytes and in primary melanoma cells (Fig. 1B-E, G), whereas keratinocytes and metastatic melanoma cells remained FAP-α negative (Fig. 1F,H,I).

All four melanoma cell lines showed corresponding level of FAP-α expression as well as migration and invasion when UVA/B irradiated or sham treated (data not shown). The primary melanoma cells WM55P and melanoma cells from matched regional WM55M1 and systemic WM55M2 were chosen as representative cell line.

We further investigated whether UV-induced FAP-α expression stimulates cell migration and invasion through collagenase I and IV activity. Migration increased following UVA and UVB radiation in melanocytes, fibroblasts and primary melanoma cells (Fig. 2A-C). The FAP-α/DPPIV inhibitor Gly-ProP(OPh)$_2$ significantly decreased this response suggesting FAP-α as an important protein in cell migration. The UV-induced invasive capacity of melanocytes, fibroblasts and primary melanoma cells measured as an effect of collagen I degradation was also FAP-α dependent (Fig. 2D-F). Only the primary melanoma cells showed FAP-α regulated collagenase IV activity i.e. ability to cross basement membrane (Fig. 2G-I).

Crosstalk between cells was investigated in a co-culture system with fibroblasts and inserts with either melanocytes, keratinocytes or matched primary and metastatic melanoma cells. Expression of FAP-α protein in fibroblasts was unaffected when in co-culture with shamtreated melanocytes, whereas a significant increase in FAP-α expression and number of FAP-α positive fibroblasts were detected when cultured with melanocytes pre-irradiated with UVA or UVB (Fig. 3A). Interestingly, co-culture of fibroblasts with cells from shamtreated primary melanoma (WM55P) and from regional metastatic melanoma

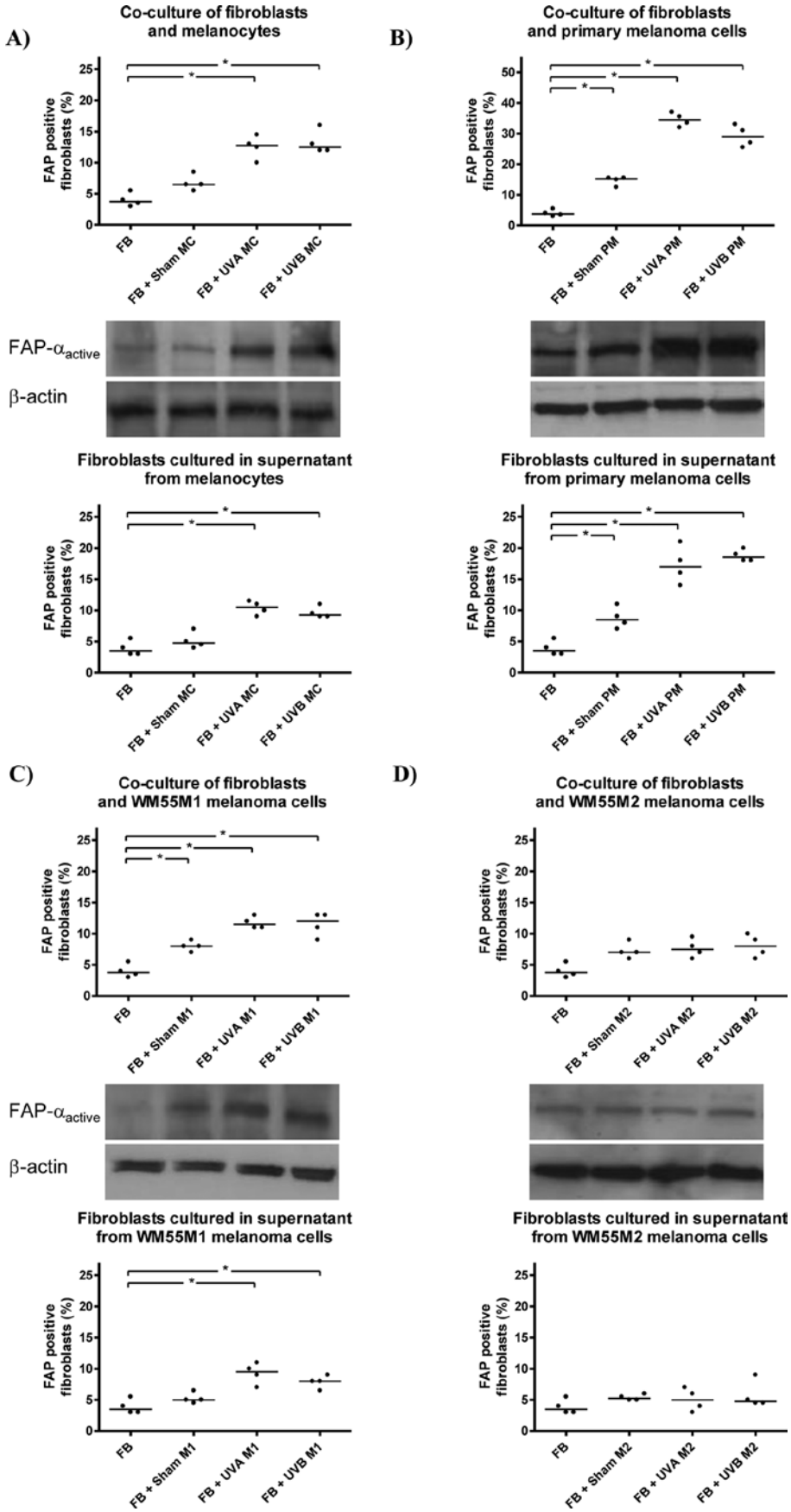

Figure 3. Melanocytes and melanoma cells induce FAP-α expression in fibroblasts. Percentage of FAP-α positive fibroblasts analyzed by immunocytochemistry (n=4) and protein expression of the co-cultures in a representative Western blot. Fibroblasts (FB) were either co-cultured with (A) melanocytes (MC), (B) primary WM55P (PM), (C) regional metastatic WM55M1 (M1) and (D) systemic metastatic WM55M2 (M2) melanoma cells or cultured with supernatants of these cells. The cells in (A-D) were shamtreated or exposed to UVA (6 J/cm^2) or UVB (60 mJ/cm^2) prior to 4 h of co-culture. Horizontal line indicates median of four experiments, $^*p<0.05$.

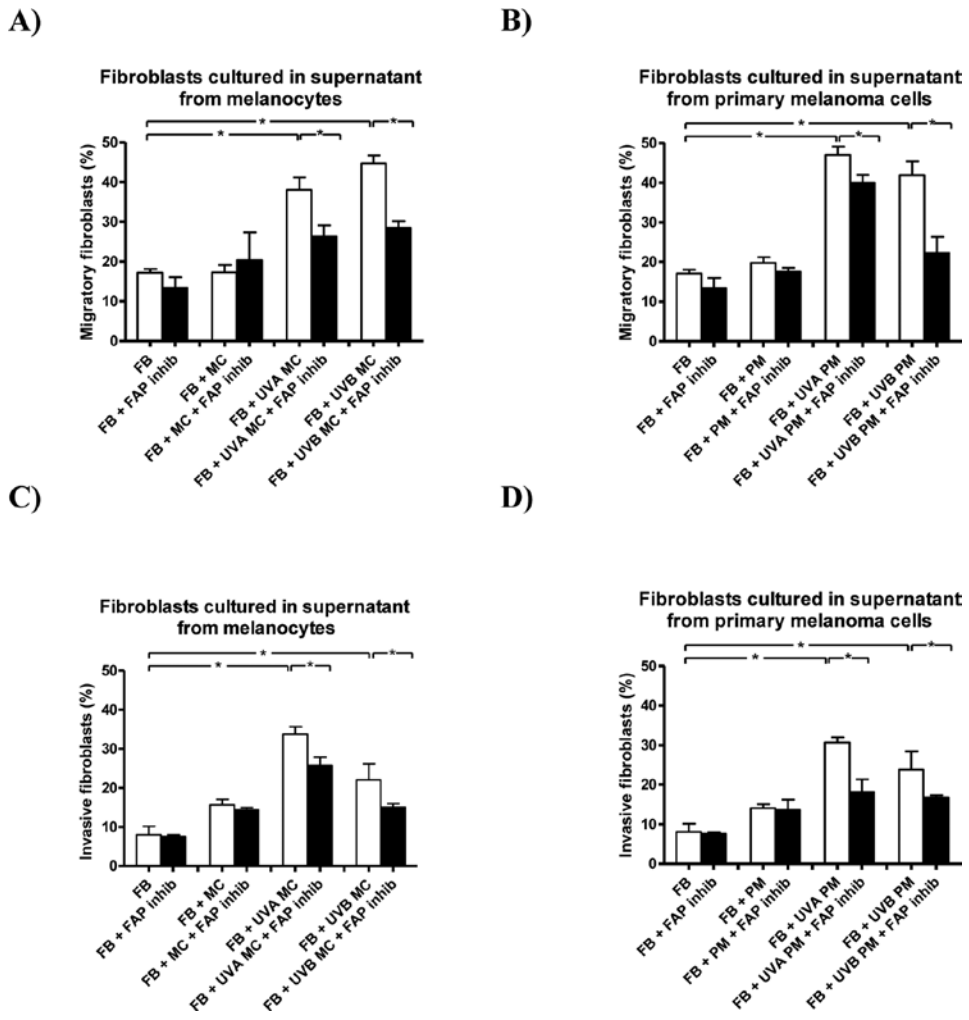

Figure 4. Supernatant from melanocytes and melanoma cells induce FAP-α driven migration and invasion. Percentage of migrated and invasive fibroblasts analyzed by Cultrex Migration Assay and Cultrex Collagen I Cell Invasion Assay. Fibroblasts (FB) were cultured for 24 h in supernatants derived from shamtreated or UVA (6 J/cm^2) or UVB (60 mJ/cm^2) irradiated (A, C) melanocytes (MC) and (B, D) primary WM55P (PM) melanoma cells. For FAP-α/DPPIV inhibition Gly-ProP(OPh)$_2$ (100 μM, 24 h) was supplemented to the cell culture (n=4, $^*p<0.05$).

cells (WM55M1) increased the levels of FAP-α in fibroblasts, which was further increased after UVA or UVB radiation (Fig. 3B and C). The most pronounced FAP-α expression was noted when in co-culture with primary melanoma cells. No significant effect on the FAP-α expression was seen in fibroblasts when in co-culture with systemic metastatic melanoma cells (WM55M2) (Fig. 3D) or with keratinocytes (not shown).

The inserts had a pore size of 0.2 μm which should prevent cell migration, but dendritic penetration cannot be excluded. Therefore, control experiments were performed in which supernatants from the different cell cultures were added to fibroblasts to study whether cell to cell contact is required to induce FAP-α. In agreement with its respective co-culture results indicating secretory crosstalk, supernatants from both UVA and UVB irradiated melanocytes and cells from primary melanoma and regional metastatic melanoma cells induced FAP-α in fibroblasts (Fig. 3A-C). Supernatants from primary melanoma cells without prior UVR induced FAP-α in fibroblasts (Fig. 3B).

To confirm secretory crosstalk we added supernatants from UVA or UVB irradiated cultures of melanocytes and cells from primary melanoma or metastatic melanoma cells to study fibroblast migration and collagenase I and IV activity. Supernatants from irradiated melanocytes and primary melanoma cells induced FAP-α-dependent migration and collagenase I activity in fibroblasts as verified by the FAP/DPPIV inhibitor Gly-ProP(OPh)$_2$ (Fig. 4A-D) whereas supernatants from shamtreated melanocytes, shamtreated primary melanoma cells and the matched metastatic melanoma cells did not (not shown).

A number of factors described to be involved in secretory communication and tumor spreading (PDGF-BB, TGF-β1, SDF-1α, uPA, Wnt5a) were analyzed in supernatants from these cell cultures to identify candidates triggering FAP-α expression. We found Wnt5a in the supernatants in both shamtreated and UV exposed melanocytes whereas PDGF-BB and TGF-β1 only in the supernatants from UV exposed melanocytes. In melanoma cells, PDGF-BB, TGF-β1 and Wnt5a were released in the supernatants, whereas uPA was released only from the primary melanoma cells (Table I). FAP-α release was not detected in any of the supernatants, which exclude autocrine FAP-α regulation (Table I). To verify the chain of events between melanocytes or melanoma cells and fibroblasts we supplemented recombinant PDGF-BB, TGF-β1 and Wnt5a to the fibroblast

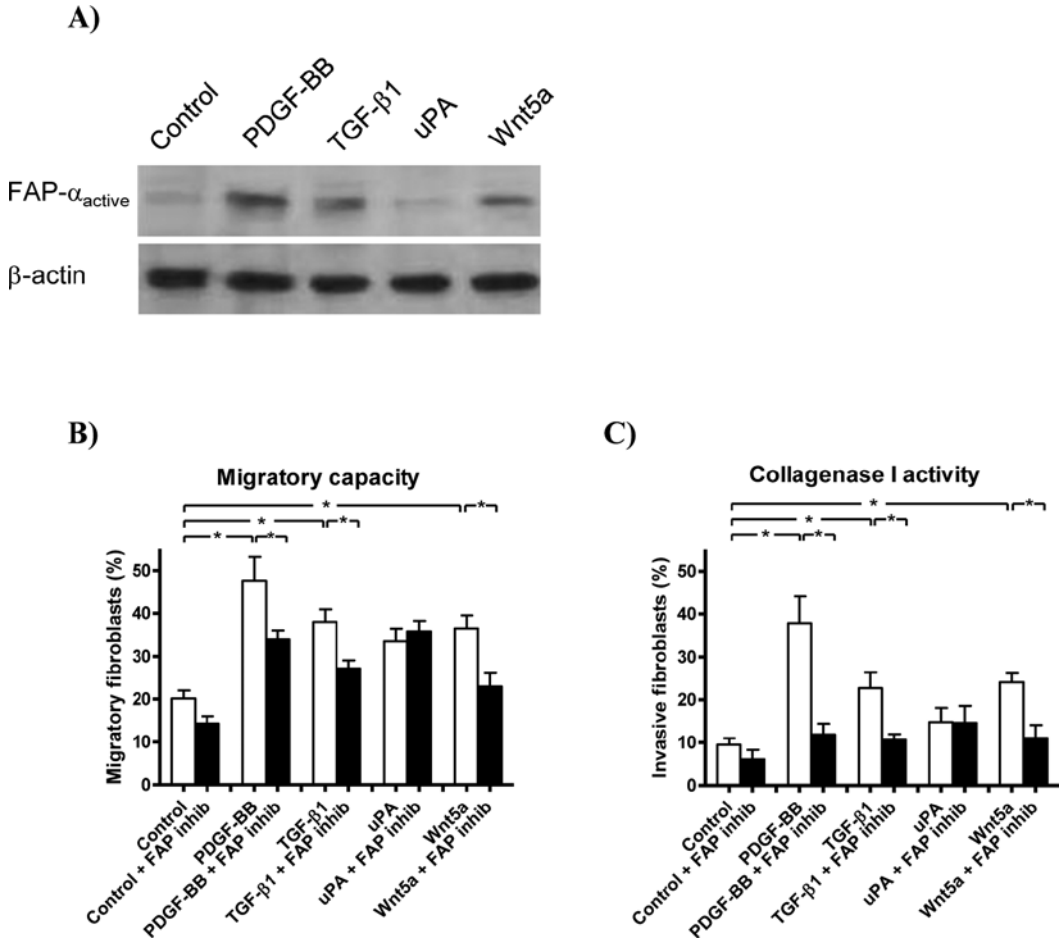

Figure 5. PDGF-BB, TGF-β1 and Wnt5a protein induce FAP-α mediated migration and invasion. (A) Effect of supplementation of 10 ng/ml recombinant platelet derived growth factor BB (PDGF-BB), transforming growth factor-β1 (TGF-β1), urokinase-type plasminogen activator (uPA) and Wnt5a on expression of FAP-α in normal human fibroblasts in one representative blot. β-actin served as a positive control. (B) Percentage of migrated fibroblasts analyzed by Cultrex Migration Assay and (C) percentage of invasive fibroblasts analyzed by Cultrex Collagen I Cell Invasion Assay. Fibroblast cell culture medium was supplemented with recombinant PDGF-BB, TGF-β1, uPA, and Wnt5a for 24h. For FAP-α/DPPIV inhibition Gly-ProP(OPh)$_2$ (100 μM, 24 h) was supplemented to the cell culture (n=4, $^*p<0.05$).

cultures. FAP-α expression in fibroblasts was significantly amplified as well as migration and collagenase I activity (Fig. 5) indicating a possible regulatory role for secretory communication from melanocytes and melanoma cells on FAP-α expression through PDGF-BB, TGF-β1 and Wnt5a.

Discussion

Mechanisms that enable melanoma cells to invade into adjacent tissue have been subject of intense research. One crucial aspect of tumor invasion and metastasis is degradation of ECM. Much research has focused on ECM degrading enzymes e.g. matrix metalloproteinases (MMPs) and cathepsins (17). MMPs have been shown to play a role also in melanoma invasiveness and metastatic behaviour (18) but clinical trials using several generations of MMP inhibitors failed to slow down tumor invasion (19). The widespread expression of many of these enzymes is likely to limit their potential as therapeutic targets. In contrast, the ECM degrading enzyme FAP-α is expressed in tumor stromal fibroblasts in epithelial cancers and not described in normal adult tissue (3,4,20). In addition to its proteolytic activity FAP-α has adhesive, angiogenetic and proliferative capacities (3,21,22).

In this study, we describe UV-related crosstalk between melanoma cells and fibroblasts leading to increased expression of FAP-α in fibroblasts and we demonstrate for the first time a significant increased expression of FAP-α in fibroblasts, melanocytes and primary melanoma cells after UVA as well as UVB exposure. Our results suggest that UVR stimulates melanoma migration and invasion through its capacity to up-regulate FAP-α both in melanocytes and fibroblasts from the skin and in melanoma cells and thereby activate ECM degradation. Further, the co-culture studies revealed that this upregulation of FAP-α was due to cellular crosstalk between the melanocyte, melanoma cells and fibroblasts. The UV-mediated ability of tumor cells to activate and regulate surrounding stromal cells is a early step in melanoma conformation and establishment. We are speculating that repetitive UV exposure of an early, undetected malignant melanoma in the skin activate microenvironmental communication between tumor and stromal cells inducing FAP-α in the surrounding fibroblasts thus leading to exaggerated ECM degradation facilitating tumor invasion. We found that primary melanoma cells, but not metastatic cells, stimulate FAP-α expression in fibroblasts even without prior UVR. This unique ability of primary melanoma cells together with their capacity to increase

their own expression level of FAP-α after UV exposure provides additional opportunities to regulate their own proliferation and dissemination emphasizing FAP-α as an early tumor marker.

On the contrary, keratinocytes are FAP-α negative and UV exposure did not induce FAP-α in these cells. Neither did UV exposed keratinocytes induce FAP-α in fibroblasts suggesting that these cells do not contribute to FAP-α mediated melanoma dissemination.

The physiological function of FAP-α expression in normal melanocytes and fibroblasts after UVR is not known. We hypothesize that FAP-α might have a role in melanocyte migration within the dermis during the life cycle of melanocytic nevi developing from junction nevi to compound and intradermal nevi. Dermal fibroblasts expressing FAP-α after UV exposure might facilitate melanoma growth and migration or invasion of other cells within the dermis as for example melanoma cells. This was supported by the present invasion and migration assays, which revealed an increased FAP-α/DPPIV mediated migratory capacity and increased collagenase I activity of fibroblasts after UVA and UVB exposure. We can not exclude a role of DPPIV in migration and invasion as the inhibitor Gly-ProP$(OPh)_2$ used to block FAP-α even inhibits DPPIV. Interestingly, UV exposed melanocytes and primary melanoma cells, but not metastatic melanoma cells, induce FAP-α/DPPIV mediated migration and collagenase I activity in fibroblasts. Metastatic melanoma cells most likely develop UV and FAP-α/DPPIV independent mechanisms for invasion once these cells have left the dermis.

Only the primary melanoma cells showed FAP-α/DPPIV mediated collagenase IV activity, which is essential for early melanoma dissemination when crossing the basement membrane. When active through dimerization FAP-α cluster with DPPIV and with other surface enzymes, thereby FAP-α is an important source of collagenase activity and/or regulate other enzymes involved in modifying ECM (23). The active complex achieves in concert effective ECM degradation and might be an early marker of melanoma progression. To understand the invasion process in detail further studies of other basement membrane proteins (24,25) and the cross-talk with various populations of tumor associated fibroblasts are needed.

The present results describe a novel mechanism how UV exposure through collagenase activity facilitates melanoma cell spreading directly through FAP-α activation of the melanoma cells and via paracrine activation of dermal fibroblasts into FAP-α expressing fibroblasts. Our experiments excluded that this FAP-α induction in fibroblasts was due to soluble FAP-α cleaved from cell membranes of melanocytes and melanoma cells (26). Instead, the cross talk enabling melanoma cells to involve surrounding fibroblasts in a malignant agenda was found to be mediated by the growth factors PDGF-BB, TGF-β1, and signaling protein Wnt5a. We showed that these factors were released from melanocytes and from melanoma cells, while the plasminogen activator uPA was released only from melanoma cells. We found supplementation with recombinant PDGF-BB, TGF-β1 and Wnt5a to increase FAP-α expression, collagenase I activity and migration capacity in fibroblasts. This response decreased significantly following inhibition of FAP-α/DPPIV indicating that PDGF-BB, TGF-β1 and Wnt5a induced invasion and migration of fibroblasts is FAP-α/DPPIV mediated. These factors might be candidates for management of melanoma by regulating FAP-α/DPPIV individually or in various combinations. PDGF-BB has both autocrine and paracrine properties in melanoma and contributes to tumor angiogenesis and stroma formation through chemoattractant activity towards fibroblasts (12). Chen *et al* showed that ovarian cancer cells induce FAP-α in fibroblasts via TGF-β1 leading to increased proliferation, invasion and migration (27). Recent data support the vital role of Wnt5a signaling in melanoma progression and development of melanoma metastasis (13,28,29). PDGF-BB, TGF-β1 and Wnt5a should be evaluated as targets to reduce FAP-α activity and prevent early melanoma dissemination. Our results describe additional ways how these factors may be involved in melanoma progression.

In conclusion, our results demonstrate for the first time that UV exposure induces FAP-α in fibroblasts and melanocytes of the skin and primary melanoma cells thereby activating ECM degradation enabling migration and invasion. Primary melanoma cells showed UV-mediated collagenase IV activity, which is essential for early melanoma dissemination when crossing the basement membrane. We further describe that UVR of melanocytes and melanoma cells stimulate FAP-α/DPPIV mediated collagenase I activity and migration of unexposed fibroblasts via PDGF-BB, TGF-β1 and Wnt5a enabling dermal infiltration of the melanoma. Future studies using RNAi or blocking antibodies against these candidate mediators may reveal which are required for FAP-α action. Thus, UV exposure of melanoma cells might be involved in activating fibroblasts into FAP-α expressing fibroblasts and as a consequence supporting invasion process of melanoma cells emphasizing the importance of UV avoidance. To verify the function of FAP-α expression in melanocytes, melanoma cells and fibroblasts after UVR *in vivo* studies including animal melanoma skin models are required.

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