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Curcumin combined with exposure to visible light blocks bladder cancer cell adhesion and migration by an integrin dependent mechanism

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Abstract. – OBJECTIVE: Although the natural compound curcumin exerts antitumor properties *in vitro*, its clinical application is hampered due to rapid metabolism. Light exposure following curcumin application has been demonstrated to improve curcumin's bioavailability. Therefore, this investigation was directed towards evaluating whether light exposure in addition to curcumin application enhances curcumin's efficacy against bladder cancer cell adhesion and migration.

MATERIALS AND METHODS: RT112, UMUC3, and TCCSUP cells were incubated with low curcumin concentrations (0.1-0.4 μg/ml) and then exposed to 1.65 J/cm2 visible light for 5 min. Controls remained untreated or were treated with curcumin or light alone. Cell adhesion to Human umbilical vein endothelial cells (HUVECs), to immobilized collagen or fibronectin and chemotactic behavior, integrin α and β receptor expression with functional relevance, as well as focal adhesion kinase (total and phosphorylated FAK) were evaluated.

RESULTS: Curcumin plus light, but neither curcumin nor light alone, significantly altered tumor cell adhesion and suppressed chemotaxis. Integrin α and β subtypes were dissimilarly modified, depending on the cell line. Suppression of pFAK was noted in RT112 and UMUC3, but not in TCCSUP cells. The integrins α3, α5, and β1 were involved in curcumin's regulation of adhesion and migration. Blocking studies revealed α3, α5, and β1 to be associated with TCCSUP adhesion and migration, whereas α5 and β1, but not α3 contributed to UMUC3 adhesion and migration. Integrin α5 and β1 controlled RT112 chemotaxis as well, but only α5 was involved in the RT112 adhesion process.

CONCLUSIONS: Combining curcumin with light exposure enhances curcumin's anti-tumor potential.

Key Words:
Curcumin, Light irradiation, Bladder cancer, Adhesion, Migration, Integrins.

Introduction

Bladder cancer is associated with high mortality, even though a large number of patients with bladder cancer initially present with nonmuscle-invasive disease[1]. However, disease recurrence is observed between 30 and 56% of patients undergoing transurethral local surgery that is often combined with intravesical chemo or immunotherapy[2]. Radical cystectomy with urinary diversion is the gold standard for those patients with refractory non muscle invasive and muscle-invasive bladder cancer[3]. Although there has been recent progress in the development of systemic treatments with immune checkpoint inhibitors[4], the prognosis for patients with advanced bladder cancer is generally poor[5]. Therefore, improved therapeutic options for both early localized and advanced cancer would increase life expectancy and the quality of life for bladder cancer patients.

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Curcumin is the major constituent and pharmacologically active component of turmeric, an herbal powder isolated from the rhizome of the plant *Curcuma longa* lin. Curcumin exhibits anti-inflammatory, anti-oxidative, and anti-cancer effects *in vitro* and in animal models. Molecular studies have shown that curcumin modulates cell signaling pathways involved in tumor cell proliferation, apoptosis, and invasion. These findings indicate that curcumin could play a decisive role in cancer treatment.

The clinical application of curcumin is restricted due to its low water solubility, poor oral absorption, and rapid metabolism[6]. Improving curcumin's efficacy is therefore necessary before introducing curcumin to cancer treatment. Recent investigations regarding epithelial tumor cells *in vitro*, as well as in a xenograft tumor model, has shown that exposure to visible light elevates curcumin's potential to induce apoptosis and block proliferation[7,8].

Based on these findings, it is postulated that curcumin plus light exposure might become an innovative tool to complement intravesical instillation treatment for bladder cancer. Data about the influence of the curcumin-light combination on the motile behavior of bladder cancer cells are currently not available. The goal of this investigation was to evaluate urothelial cancer cell adhesion and migration *in vitro* in the presence of curcumin and light. Since adhesion receptors of the integrin α and β family play a primary role in the process of tumor cell binding and transendothelial penetration, integrin family members were evaluated as well.

Materials and Methods

Tumor Cells

RT112 and UMUC-3 bladder carcinoma cells were provided by ATCC/LGC Promochem GmbH, Wesel, Germany. TCCSUP cells were from DSMZ, Braunschweig, Germany. All cell lines were cultivated in Roswell Park Memorial Institute-1640 (RPMI-1640), 10% fetal calf serum (FCS), 20 mM HEPES-buffer, 1% glutamax and 1% penicillin/streptomycin (all: Gibco; Karlsruhe, Germany). Subcultures from passages 7-24 were used for the experiments. RT112 is an invasive (pathological stage T2) moderately differentiated (grade 2/3) model of human bladder cancer, whereas TCCSUP is a transitional cell carcinoma, grade 4. UMUC-3 represents a high grade 3, invasive bladder cancer.

Human umbilical vein endothelial cells (HUVECs) were isolated from human umbilical veins and harvested by enzymatic treatment with dispase (Gibco; Karlsruhe, Germany). HUVECs were grown in Medium 199 (M199; Biozol, Munich, Germany), 10% FCS, 10% pooled human serum, 20 µg/ml endothelial cell growth factor (Boehringer, Mannheim, Germany), 0.1% heparin, 100 ng/ml gentamycin and 20 mM HEPES-buffer (pH 7.4). Subcultures from passages 26 were selected for experimental application. The Institutional Ethics Committee of the Goethe-University Hospital, Frankfurt, Germany, approved the investigation and waived the need for consent, since HUVECs were anonymously used for *in vitro* assays with no link to patient data.

Curcumin Treatment

Curcumin (Sigma Aldrich, Taufkirchen, Germany) was stored at –20°C. Prior to use, curcumin was diluted in cell culture medium to the final concentration of 0.1, 0.2, or 0.4 µg/ml (0.27, 0.54, 1.08 µM). Following 1 h curcumin incubation, cell culture medium based on RPMI-1640 was discarded and replaced by phenol red free phosphate-buffered saline (PBS) medium with Ca^{2+}/Mg^{2+} (Gibco/Invitrogen; Karlsruhe, Germany). Thereafter, cell cultures were subjected to visible light exposure (5500 lx, 1.65 J/cm^2; Waldmann UV 801AL, Villingen-Schwenningen, Germany) for 5 min. Cell cultures were also exposed to visible light without curcumin application, or they were treated with curcumin without subsequent light exposure. Cell cultures treated with PBS alone served as controls. Finally, the PBS medium was replaced by the complete cell culture medium indicated above. Tumor cells were then subjected to the assays listed below.

Tumor Cell Adhesion

To analyze tumor cell adhesion, HUVECs were detached from the culture flask and transferred to 6-well multiplates (Sarstedt, Nümbrecht, Germany). Confluent or sub-confluent RT112, UMUC-3 or TCCSUP cells were also enzymatically detached from the culture flasks using accutase (PAA Laboratories, Cölbe, Germany). Following cell counting, 0.5×10^6 cells were then added to the HUVECs monolayer for 60 min. After this time period, non-adherent tumor cells were removed by repeated washing with warmed (37°C) M199. The remaining cells were fixed with 1% glutaraldehyde. Adherent tu-

mor cells were counted in five different fields of a defined size (5×0.25 mm^2) using a phase contrast microscope and the mean cellular adhesion rate was calculated.

Attachment to Immobilized Collagen or Fibronectin

6-well plates were coated with collagen G which has been extracted from calfskin. Collagen G represents a matrix mixture consisting of 90% collagen type I and 10% collagen type III; Biochrom, Berlin, Germany). Further 6-well plates were coated with fibronectin (derived from human plasma; BD Biosciences, Heidelberg, Germany). Coating was done overnight. Plastic dishes served as background control. Nonspecific cell adhesion was prevented by washing them with 1% bovine serum albumin (BSA) in PBS. 0.5×10^6 tumor cells were then added to each well and left for 60 min incubation. According to the tumor cell adhesion assay, non-adherent tumor cells were washed off, and the remaining adherent cells were fixed with 1% glutaraldehyde. The mean cellular adhesion rate, defined by adherent $cells_{coated\ well}$ − adherent $cells_{background}$, was counted microscopically from five different observation fields.

Chemotaxis

Chemotactic movement was examined as well using six-well transwell chambers (Greiner, Frickenhausen, Germany) with 8-µm pores. 0.5×10^6 tumor cells per ml were placed in the upper chamber in serum-free medium. The lower chamber contained 10% serum as the chemoattractant. After 20 h incubation, the upper surface of the transwell membrane was gently wiped with a cotton swab to remove those cells which didn't migrate underneath the membrane. Cells that had moved to the lower surface of the membrane were stained with hematoxylin and counted microscopically. Five different observation fields (5×0.25 mm^2) were evaluated to calculate the mean chemotaxis rate.

Integrin Surface Expression

Integrin surface expression was compared between cells treated with curcumin plus light, curcumin alone or light alone, and non-treated cells (PBS treatment). The respective cell cultures were washed in blocking solution (PBS, 0.5% BSA) and then incubated for 60 min at 4°C with phycoerythrin (PE)-conjugated monoclonal antibodies directed against the following integrin subtypes: anti-α1 (IgG1; clone SR84, dilution 1:1000), anti-α2 (IgG2a; clone 12F1-H6, dilution 1:250), anti-α3 (IgG1; clone C3II.1, dilution 1:1000), anti-α4 (IgG1; clone 9F10, dilution 1:200), anti-α5 (IgG1; clone IIA1, dilution 1:5000), anti-α 6 (IgG2a; clone GoH3, dilution 1:200), anti-β1 (IgG1; clone MAR4, dilution 1:2500), anti-β3 (IgG1; clone VI-PL2, dilution 1:2500), or anti-β4 (IgG2a; clone 439-9B, dilution 1:250; all BD Biosciences, Heidelberg, Germany). Integrin surface expression was then measured using FACscan (BD Biosciences, Heidelberg, Germany; FL-2H (log) channel histogram analysis; 1×10^4 cells per scan) and expressed as mean fluorescence units (MFU). A mouse IgG1-PE (MOPC-21) or IgG2a-PE (G155-178; BD Biosciences, Heidelberg, Germany) was used as an isotype control.

Integrin Protein Analysis

Western blotting was done to explore the integrin α and β protein content. In this context, tumor cell lysates were applied to a 7% polyacrylamide gel and electrophoresed for 90 min at 100 V. The respective proteins were then transferred to nitrocellulose membranes (1 h, 100 V), which were blocked with non-fat dry milk for 1 h. In a next step, the membranes were incubated overnight with the unconjugated specific monoclonal antibodies provided above. In addition to the integrin α and β subtypes, integrin-related signaling was investigated as well using anti-focal adhesion kinase (FAK; clone 77) and anti-phospho-specific focal adhesion kinase (FAK; pY397; clone 18) antibodies (all: BD Biosciences, Heidelberg, Germany).

Blocking Studies

To determine whether integrin α3, α5, and β1 impact metastatic spread, drug-sensitive or -resistant cells were incubated for 60 min with 10 µg/mL function-blocking anti-integrin α3 (clone P1B5) mouse mAb, anti-integrin α5 (clone P1D6) mouse mAb, or anti-integrin β1 (clone 6S6) mouse mAB (all: from Millipore, Billerica, MA, USA). Controls were incubated with cell culture medium alone. Subsequently, tumor cell adhesion to immobilized collagen, as well as chemotaxis were evaluated as described above.

Statistical Analysis

All experiments were performed 3-6 times. Statistical significance was determined with the Wilcoxon-Mann-Whitney-U-test. Differences were considered statistically significant at a p-value less than 0.05.

Results

Curcumin Plus Exposure to Light Blocks Tumor Cell Adhesion to Immobilized Collagen or Fibronectin

Tumor cell adhesion to immobilized extracellular matrix proteins was evaluated with curcumin concentrations of 0.1, 0.2, and 0.4 µg/ml. Light exposure or application of 0.1 or 0.2 µg/ml curcumin alone did not induce any alteration in the tumor cells' binding behavior. Slight, but significant changes of fibronectin attachment were seen when TCCSUP cells were treated with 0.4 µg/ml curcumin (25% adhesion reduction; Figure 1). Combining curcumin with light exposure distinctly elevated the efficacy of curcumin, since 0.2 and 0.4 µg/ml curcumin then blocked bladder cancer cell attachment to collagen. In RT112 and TCCSUP cells, this effect already became evident at a concentration of 0.1 µg/ml curcumin. Binding to immobilized fibronectin was also suppressed by curcumin plus light exposure with 0.2 and 0.4 µg/ml curcumin (UMUC3) and with 0.4 µg/ml curcumin in the RT112 and TCCSUP cells. Mean adhesion reduction of TCCSUP cells was then >60%. Since this initial investigation showed that employing 0.2 µg/ml curcumin produced significant, but not as great inhibition of cell adhesion, as 0.4 µg/ml curcumin; 0.2 µg/ml curcumin was employed in all further experiments. Possible additive effects of light exposure to effects due to curcumin alone could thus be more clearly discerned.

HUVECs-Tumor Cell Interaction

Exposing the tumor cells to light did not alter tumor cell attachment to HUVECs. Adhesion of UMUC3 and TCCSUP was not altered following incubation with 0.2 µg/ml curcumin, and adhesion of RT112 cells was only moderately diminished. However, a strong loss of UMUC3 and RT112 adhesion was noted when 0.2 µg/ml curcumin was combined with exposure to light. Curcumin plus light evoked a significant increase of TCCSUP binding to HUVECs (Figure 2).

Curcumin Combined with Light Exposure Suppresses Tumor Cell Motility

All three cell lines displayed significant suppression of tumor cell chemotaxis after 24 h when exposed to 0.2 µg/ml curcumin and light (Figure 3). Exposure to light alone had no effect on tumor cell motility, and 0.2 µg/ml curcumin alone

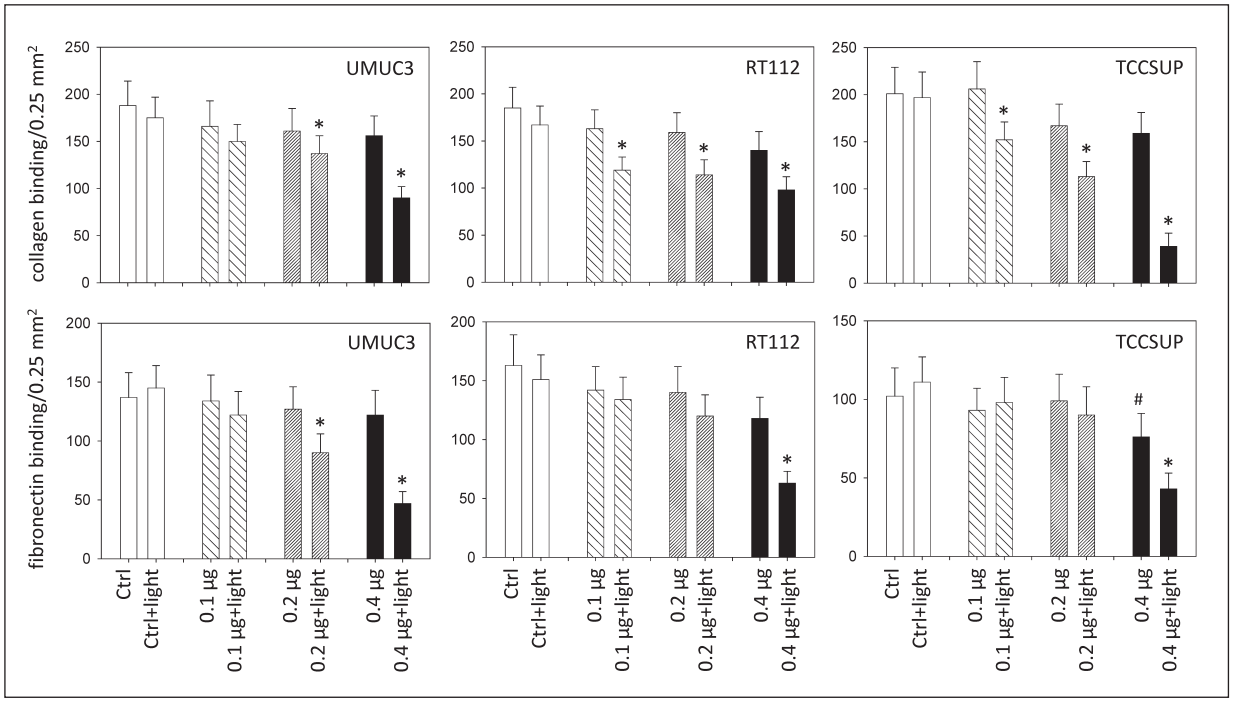

Figure 1. Influence of curcumin with and without exposure to light on binding of UMUC3, RT112, and TCCSUP cells to immobilized collagen or fibronectin. Tumor cells were treated with cell culture medium alone (ctrl), with visible light alone (ctrl+light), with curcumin alone (0.1, 0.2, 0.4 µg/ml) or with curcumin plus light. Bars indicate standard deviation (SD). *indicates significant difference between curcumin alone and curcumin+light. #indicates significant difference between cells exposed to curcumin alone and the untreated control.

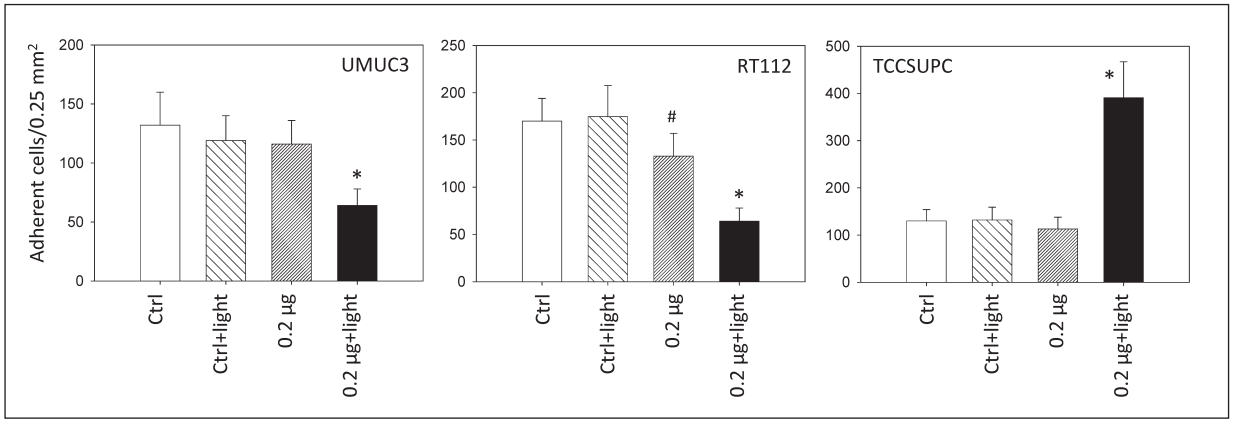

Figure 2. Influence of curcumin with and without exposure to light on adhesion of UMUC3, RT112, and TCCSUP cells to human umbilical vein endothelial cells (HUVECs). Tumor cells were treated with cell culture medium alone (ctrl), with visible light alone (ctrl+light), with 0.2 µg/ml curcumin alone or with 0.2 µg/ml curcumin plus light. Bars indicate standard deviation (SD). *indicates significant difference between curcumin alone and curcumin+light. #indicates significant difference between cells exposed to curcumin alone and the untreated control.

did not (UMUC3, TCCSUP) or only moderately (RT112) influence chemotactic crawling.

Integrin α and β Expression Profile

Surface expression of integrin α and β subtypes was investigated, since these receptors are involved in adhesion and migration regulation. UMUC3 cells were characterized by strong expression of the integrin family members α3, α5, and β1. The integrins α2, α6, and β3 were also distinctly expressed. α4 was moderately expressed, whereas α1 and β4 were not detectable (Figure 4). In RT112, highest expression levels were noted for α2, α3, α6, β1, and β4. The integrins α1, α4, and β3 were not present on the cell surface membrane, and α5 was only moderately detectable. The integrins α1, α4, and β3 were also not detectable on TCCSUP cells. Strongest expression was noted for α3, α5, α6, and β1. The subtypes α2 and β4 were detected as well.

Curcumin Plus Light Exposure Alters Integrin α and β Expression

Light exposure or curcumin alone did not induce significant alterations in the integrin expression level. However, significant, though differing alterations in the three examined cell lines were seen when curcumin was combined with exposure to light. All integrin subtypes, expressed on UMUC3 (α2, α3, α5, α6, β1, and β3), were di-

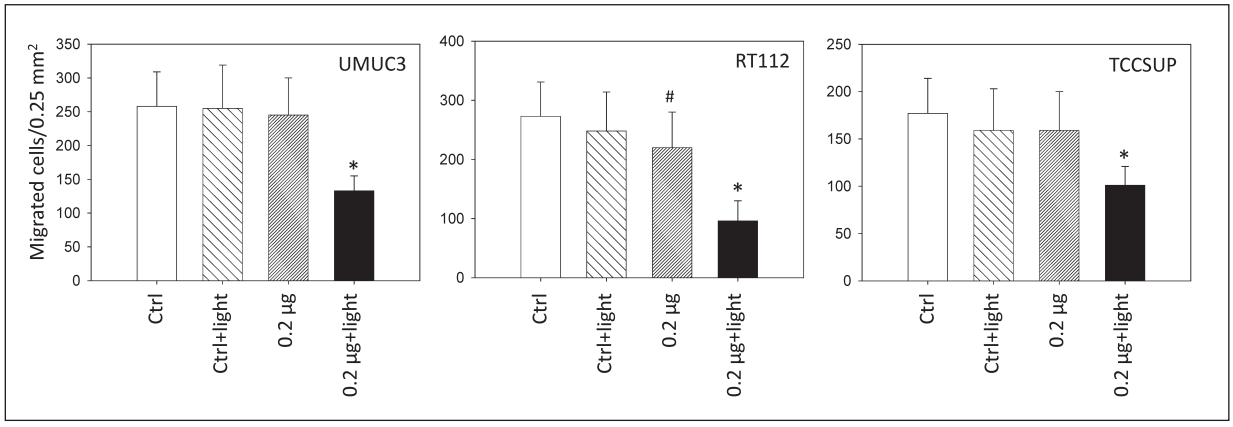

Figure 3. Motility analysis of UMUC3, RT112, and TCCSUP cells exposed to visible light alone (ctrl+light), to 0.2 µg/ml curcumin alone or to 0.2 µg/ml curcumin plus light. Controls (ctrl) remained untreated. *indicates significant difference between curcumin alone and curcumin+light. #indicates significant difference between cells exposed to curcumin alone and the untreated control.

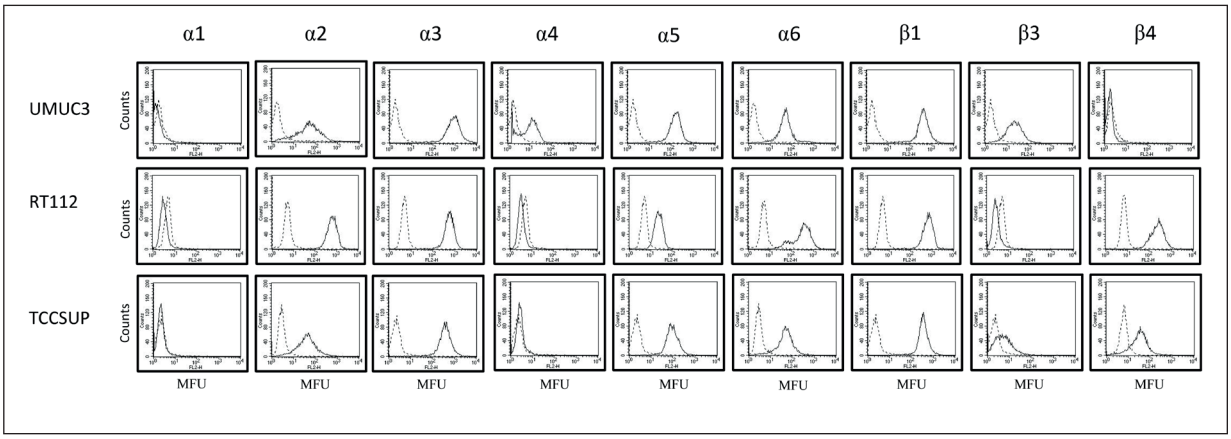

Figure 4. Flow activated cell sorting (FACS) analysis of integrin α and β subtype expression in UMUC3, RT112, and TCCSUP cells. Mean fluorescence value [MFU] is shown. Solid line indicates fluorescence produced by the specific antibody, dashed line shows fluorescence produced by the isotype controls. One of three independent experiments.

minished by curcumin plus light. In RT112 only the subtypes α3, α6, and β1 (but not α2, α5, β4) were reduced by curcumin plus light, whereas TCCSUP responded by down-regulating all integrins, except α6 (Figures 5 and 6).

Total integrin content was evaluated by Western blotting. Curcumin plus light caused a decrease in α3, α5, α6, and β1 in UMUC3. The integrins α6 (moderately), β1 and β4 were lost in RT112, whereas α3 increased, compared to controls (Figure 7). Down-regulation of α2, α5, α6, and β3 but up-regulation of α3, β1 and β4 was seen in TCCSUP cells. FAK was not modified, but pFAK was suppressed by the curcumin-light combination in UMUC3 and RT112.

Blocking Studies

The physiologic relevance of the integrin subtypes α3, α5, and β1 was analyzed using function associated monoclonal antibodies. Blocking α5 and β1 diminished UMUC3 adhesion and chemotaxis, with effects on adhesion being stronger than on chemotaxis (Figure 8). Blocking α5 also suppressed RT112 adhesion and chemotaxis, but blocking β1 only acted on the motile behavior of this cell line. In TCCSUP blocking α3, α5, or β1 led to a significant reduction in the number of adherent and motile cells, compared to controls.

Discussion

Curcumin is considered a complementary tool in cancer treatment. However, poor bioavailability due to poor aqueous solubility and rapid metabolism remains a major problem with curcumin application. Recent publications indicate that water soluble drug carriers such as liposomes, nanoparticles, or nano-emulsions improve efficacy. Still, these methods require further optimization. The strategy applied in the present investigation to increase curcumin efficacy was carried over from studies on oral squamous cell carcinoma and melanoma cells, where combining curcumin with visible light exposure increased curcumin's efficacy[9,10].

The results presented here indicate that combining curcumin with light may also be of benefit in treating bladder carcinoma. Whereas low concentrations of curcumin exerted only slight effects on the adhesion and migration capabilities of bladder cancer cells, significant alterations were noted when curcumin treated cells were exposed to light. The chemotactic activity and motility of the three bladder cancer cell lines examined in the present investigation were down-regulated by the light-curcumin combination. However, the decrease in motility was due to a suppressed attachment rate to HUVECs by UMUC3 and RT112 cells, but enhanced attachment in TCCSUP cells. The diminished chemotaxis of UMUC3 and RT112 may, at least in part, be ascribed to the reduced number of cells binding to endothelium, allowing fewer cells to transmigrate. TCCSUP cells, on the other hand, may establish a sticky contact to HUVECs, hindering their migration. This remains speculative, since the motile behavior of the tumor cells through an endothelial cell monolayer was not investigated.

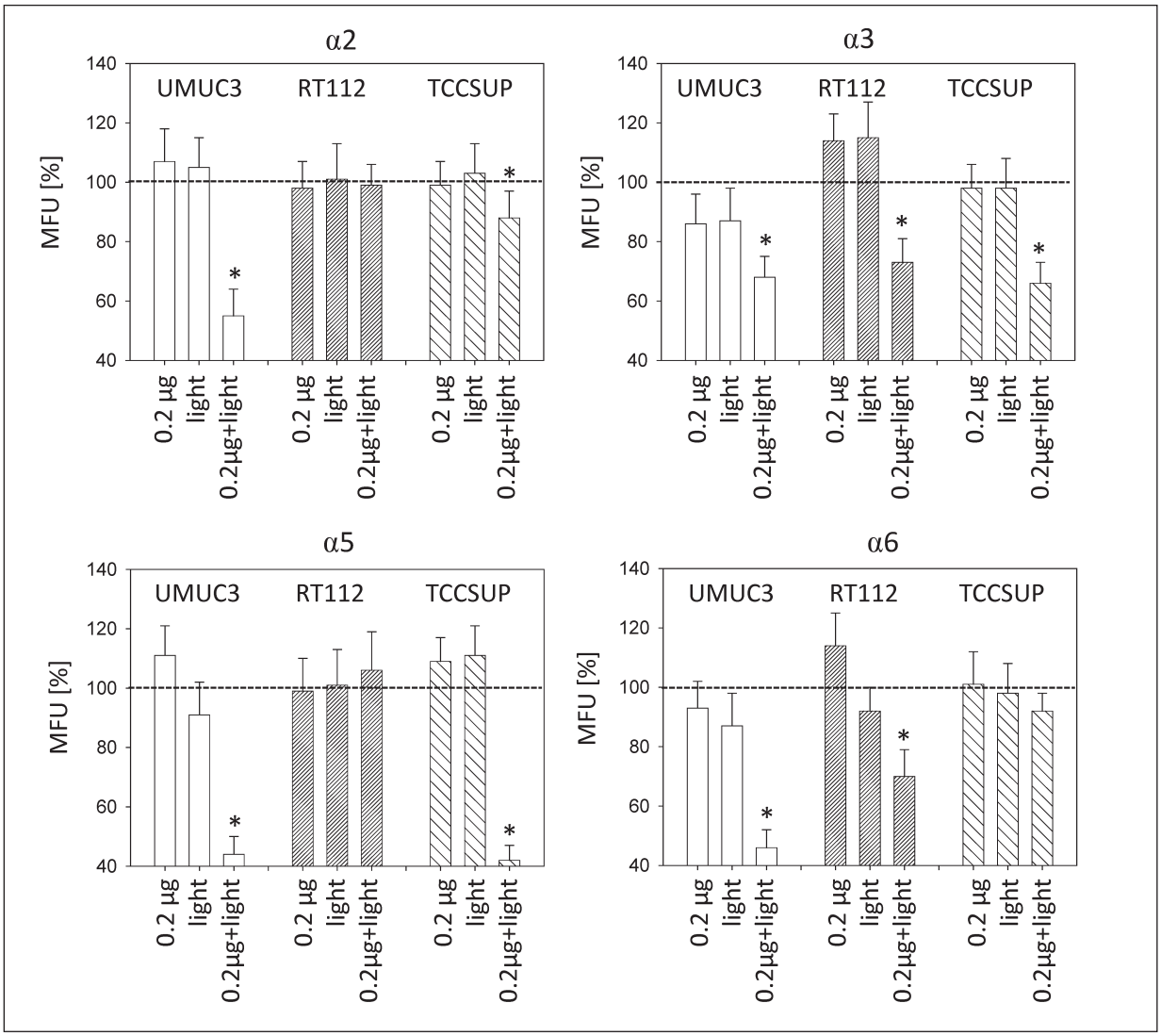

Figure 5. Integrin α subtype expression on UMUC3, RT112, and TCCSUP cells after exposing the cells to 0.2 µg/ml curcumin alone, to light alone or to 0.2 µg/ml curcumin plus light. Integrins were evaluated by flow activated cell sorting (FACS). Mean fluorescence values (MFU) shown as percentage, compared to untreated controls set at 100%. *indicates significant difference to controls.

The integrin expression pattern of the three bladder cancer cell lines was disparately influenced by curcumin plus light. Differing influence on integrins in diverse cell lines has also been noted with the histone deacetylase (HDAC) – inhibitor, valproic acid, which suppresses adhesion in a broad panel of bladder cancer cell lines[11]. Each cell line possesses a characteristic receptor set and a non-homogenous integrin pattern was detected on the UMUC3, RT112, and TCCSUP controls before curcumin application in the present investigation. Consequently, it may be expected that drug treatment influences integrin subfamilies in disparate cell lines differently. Indeed, differing integrin guided adhesive behavior in several tumor sublines has previously been reported. Blocking the α3 integrin subunit inhibited HCV29 bladder cancer cell attachment to the matrix proteins laminin and fibronectin but had an opposite effect on T24 and Hu456 cell adhesion. Similarly, blocking α5 integrin has been shown to down-regulate HCV29 and BC3726 cell-matrix interaction, whereas binding of the bladder cancer cell lines T24 and Hu456 was enhanced[12].

The α3, α5, and β1 integrins, clearly detected on all three tumor-cell-line surface membranes, were investigated to evaluate their physiologic relevance. Of these, α5 integrin was the only recep-

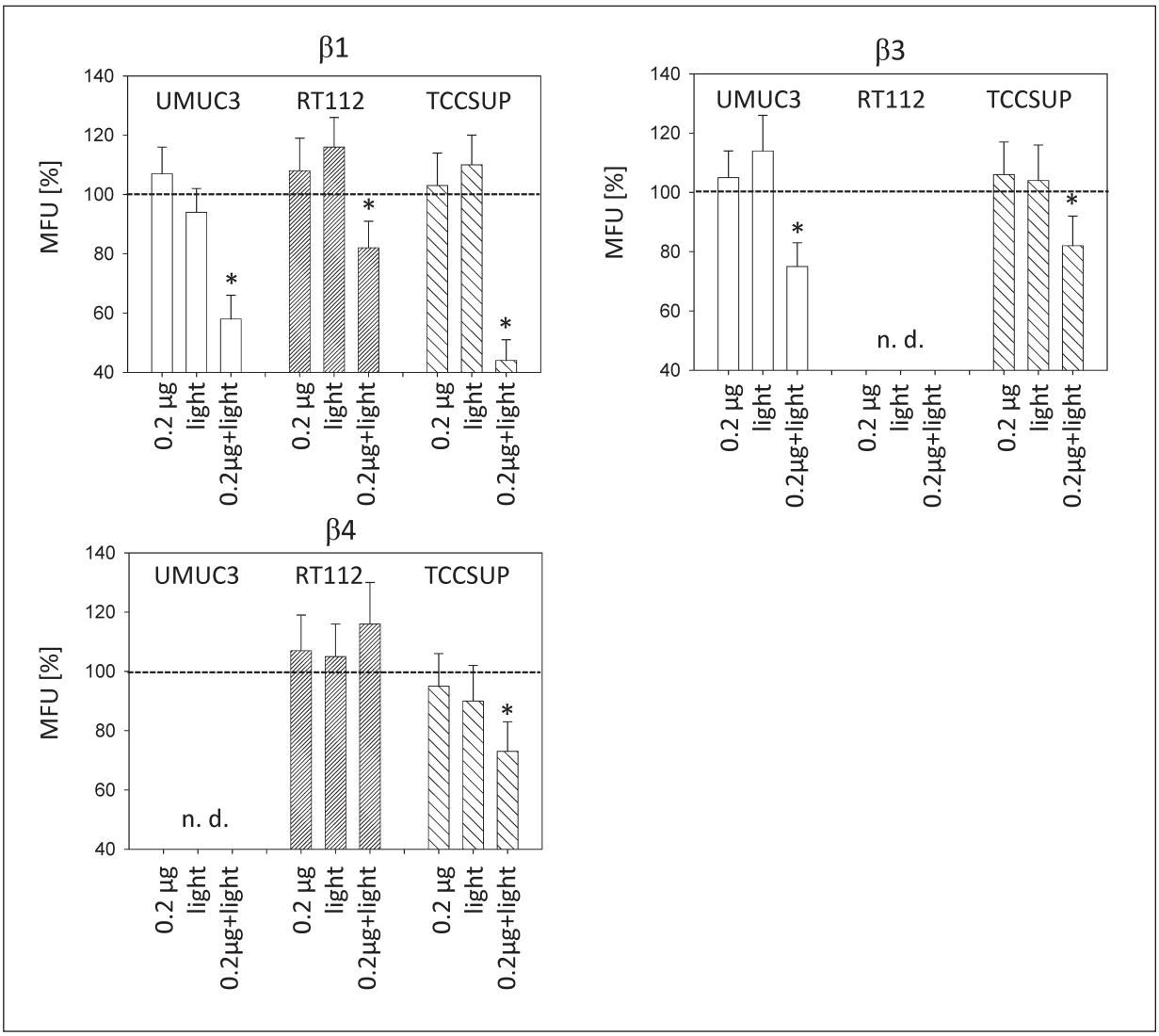

Figure 6. Integrin β subtype expression on UMUC3, RT112, and TCCSUP cells after exposing the cells to 0.2 µg/ml curcumin alone, to light alone or to 0.2 µg/ml curcumin plus light. Integrins were evaluated by flow activated cell sorting (FACS). Mean fluorescence values (MFU) shown as percentage, compared to untreated controls set at 100%. *indicates significant difference to controls.

tor on all three tumor cell lines controlling both adhesion and chemotaxis. Curcumin combined with light exposure strongly reduced the α5 receptor on UMUC3 and TCCSUP cells, and might be one mechanism by which curcumin prevents motile spreading of these cell lines. A strong correlation between integrin α5 and bladder cancer stage has been shown[13]. In a study by Xu et al[14], sensitivity of bladder cancer cells to mitomycin-C was re-established by suppressing integrin α5 and downstream pathways. This finding is important since prophylactic intravesical instillation with mitomycin-C is part of the standard treatment for non-muscle invasive bladder cancer[15]. Whether curcumin prevents or delays mitomycin-C resistance has not yet been explored and deserves evaluation.

Integrin β1 was closely involved in the migratory activity of all bladder cancer cell lines, so it may be assumed that a curcumin induced loss of this integrin contributes to diminished chemotaxis. In good corroboration, recent data on a murine bladder cancer model demonstrated integrin β1 to be involved in metastases formation, and results from The Cancer Genome Atlas (TCGA) project have shown that expression of β1 correlates with a worsened clinical outcome in bladder cancer patients[16]. Since light exposure greatly enhanc-

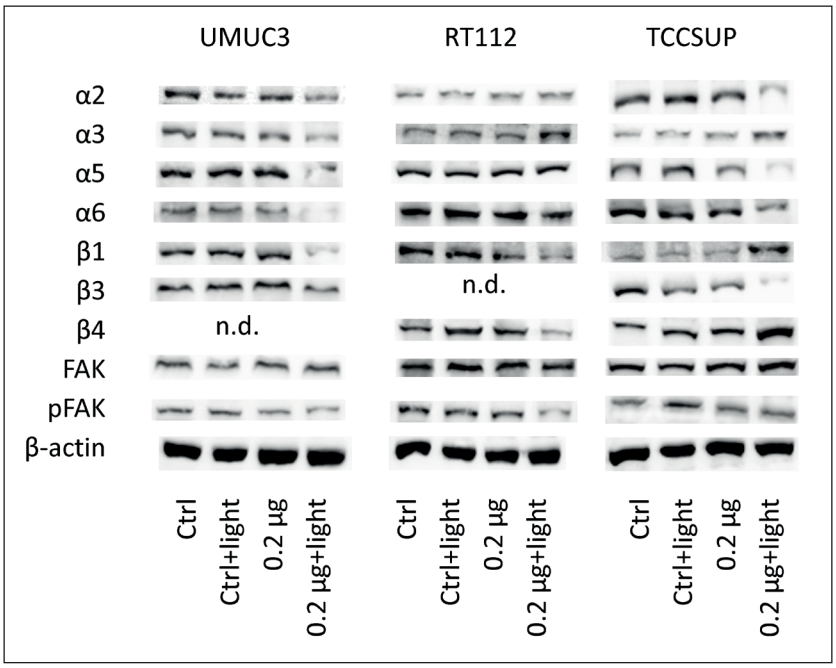

Figure 7. Integrin α and β subtypes, focal adhesion kinase (FAK), and phosphorylated focal adhesion kinase (pFAK) protein level in UMUC3, RT112, and TCCSUP cells. Tumor cells were treated with cell culture medium alone (ctrl), with light alone (ctrl+light), with curcumin alone (0.2 µg/ml) or with curcumin plus visible light. One representative of three Western blots is shown. n.d.= not detectable.

es curcumin's effects, combining curcumin with light might provide a distinct benefit for tumor patients by preventing rapid elevation of integrin β1.

Specimens from 36 bladder cancer patients displayed a high expression level of integrin α3, compared to normal tissue, and it has been proposed that integrin α3 might represent a therapeutic target and prognostic biomarker for bladder cancer[17]. The use of α3 as a biomarker may be questioned since it has been shown in the present investigation that curcumin-light only reduces adhesion and migration in TCCSUP, but not in UMUC3 and RT112. However, of the three cell lines evaluated here, TCCSUP was the most aggressive and integrin α3 might specifically be involved in regulating invasive progression of highly dedifferentiated tumor cells. This might explain why a significant correlation between overall survival and α3 expression could not be demonstrated in a cohort of bladder cancer patients with low pT1

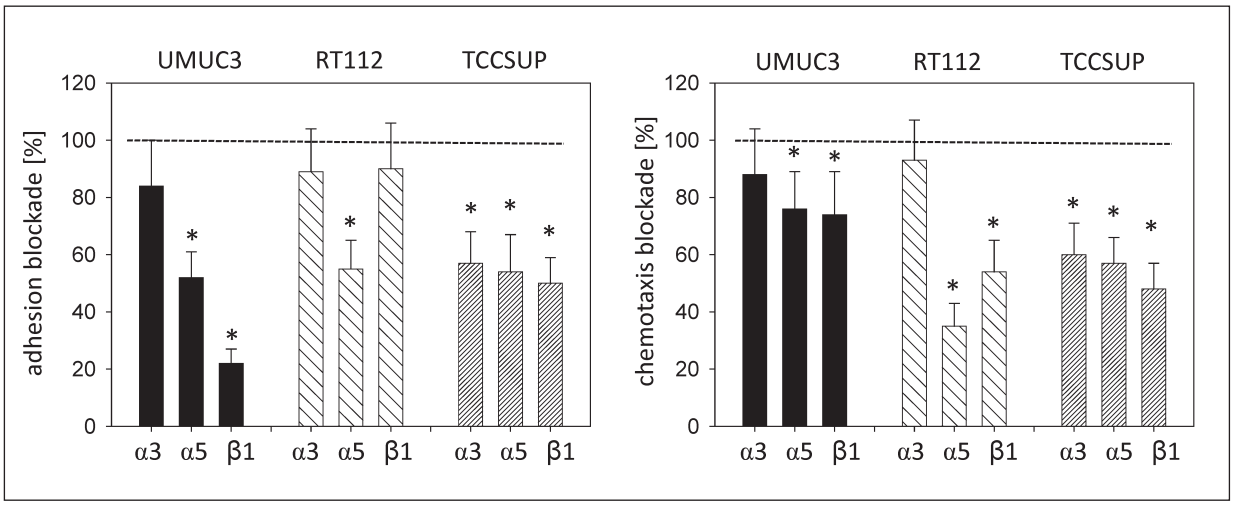

Figure 8. Influence of integrin α3, α5, or β1 blockade on UMUC3, RT112, and TCCSUP cell adhesion to immobilized collagen (left) and on chemotaxis (right). Cells were preincubated for 60 minutes with a function-blocking anti-integrin mAb. Controls were untreated and set to 100%. *indicates significant difference to controls.

and pT2 pathological staging[18]. Still, the relevance of α3 in bladder cancer requires further attention. Loss of UMUC3 and RT112 cell proliferation and induction of UMUC3 apoptosis has recently been documented following curcumin and light application[19]. Speculatively, down-regulation of α3 surface expression by curcumin plus light exposure could influence the cell signaling machinery by inhibiting proliferation (and/or apoptosis) rather than invasion. To verify this, the relevance of α3 in regulating bladder cancer growth requires further investigation.

The integrin-related signaling molecule pFAK was reduced in RT112 and UMUC3 cells by curcumin plus light. This is interesting, since an integrin α3-FAK-cross-communication has already been documented, and targeting this axis suppressed cell migration and invasion in bladder cancer[17]. Blocking FAK by a monoclonal antibody against integrin α3 also inhibited bladder cancer cell proliferation, as demonstrated *in vitro* and *in vivo*[20]. Relevance of the integrin-FAK-axis on tumor growth processes should, therefore, not be ruled out.

It is notable that pFAK was not reduced by the curcumin-light combination in TCCSUP cells. Furthermore, α3 and β1 integrins in TCCSUP cytoplasm increased, pointing to receptor translocation from the outer cell surface, where these integrin subtypes decreased following drug treatment, to the intracellular compartment. Hypothetically, this translocation process could modulate intracellular signaling events, so that adhesion of TCCSUP to HUVECs increases.

The exact mechanism underlying the advantageous effect of light exposure is still not clear. A light-dependent energy transfer during curcumin-protein interaction may enhance the influence of curcumin on protein function and cell regulation[8]. Curcumin may also photo-generate reduced forms of molecular oxygen[21], or both the photo-catalytic effect of curcumin and photo-activation may serve as triggering factors[22].

Clinically, curcumin-light treatment might be of interest when Bacillus Calmette-Guérin (BCG) instillation therapy is employed. Indeed, photodynamic therapy has been recommended as an innovative option to treat urothelial carcinomas refractory to BCG[23]. A trial was recently conducted on 45 subjects with non-muscle-invasive bladder cancer with an optimal 100 J/cm^2 light exposure[24]. Feasibility of curcumin instillation has also been documented in an animal model, where intravesical treatment with a cyclodextrin-curcumin complex with BCG resulted in a lower tumor number, compared to BCG alone or controls[25].

Conclusions

In summary, low dosed curcumin plus light exposure blocks bladder cancer cell adhesion and migration in three different cell lines by an integrin dependent mechanism. The identified integrin changes are, however, not the same for all three cell lines.

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Conflict of Interests

The authors declare that they have no conflict of interests.

References

1) Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. CA Cancer J Clin 2014; 64: 9-29.
2) von der Maase H, Sengelov L, Roberts JT, Ricci S, Dogliotti L, Oliver T, Moore MJ, Zimmermann A, Arning M. Long term survival results of a randomized trial comparing gemcitabine plus cisplatin, with methotrexate, vinblastine, doxorubicin, plus cisplatin in patients with bladder cancer. J Clin Oncol 2005; 23: 4602-4608.
3) Shirodkar SP, Lokeshwar VB. Potential new urinary markers in the early detection of bladder cancer. Curr Opin Urol 2009; 19: 488-493.
4) Nadal R, Bellmunt J. Management of metastatic bladder cancer. Cancer Treat Rev 2019; 76: 10-21.
5) Witjes JA, Compérat E, Cowan NC, De Santis M, Gakis G, Lebret T, Ribal MJ, Van der Heijden AG, Sherif A; European Association of Urology. EAU guidelines on muscle invasive and metastatic bladder cancer: summary of the 2013 guidelines. Eur Urol 2014; 65: 778-792.
6) Willenbacher E, Khan SZ, Mujica SCA, Trapani D, Hussain S, Wolf D, Willenbacher W, Spizzo G, Seeber A. Curcumin: new insights into an ancient ingredient against cancer. Int J Mol Sci 2019; 20. pii: E1808.
7) Laubach V, Kaufmann R, Bernd A, Kippenberger S, Zöller N. Extrinsic or intrinsic apoptosis by curcu-min and light: still a mystery. Int J Mol Sci 2019; 20. pii: E905.
8) Bernd A. Visible light and/or UVA offer a strong amplification of the anti-tumor effect of curcumin. Phytochem Rev 2014; 13: 183-189.

9) Beyer K, Nikfarjam F, Butting M, Meissner M, König A, Ramirez Bosca A, Kaufmann R, Heidemann D, Bernd A, Kippenberger S, Zöller N. Photodynamic treatment of oral squamous cell carcinoma cells with low curcumin concentrations. J Cancer 2017; 8: 1271-1283.
10) Buss S, Dobra J, Goerg K, Hoffmann S, Kippenberger S, Kaufmann R, Hofmann M, Bernd A. Visible light is a better co-inducer of apoptosis for curcumin-treated human melanoma cells than UVA. PLoS One 2013; 8: e79748.
11) Juengel E, Meyer dos Santos S, Schneider T, Makarevic J, Hudak L, Bartsch G, Haferkamp A, Wiesner C, Blaheta RA. HDAC inhibition suppresses bladder cancer cell adhesion to collagen under flow condi-tions. Exp Biol Med (Maywood) 2013; 238: 1297-1304.
12) Lityńska A, Przybyło M, Pocheć E, Laidler P. Adhesion properties of human bladder cell lines with extracellular matrix components: the role of integrins and glycosylation. Acta Biochim Pol 2002; 49: 643-650.
13) Saito T, Kimura M, Kawasaki T, Sato S, Tomita Y. Correlation between integrin alpha 5 expression and the malignant phenotype of transitional cell carcinoma. Br J Cancer 1996; 73: 327-331.
14) Xu T, Qin L, Zhu Z, Wang X, Liu Y, Fan Y, Zhong S, Wang X, Zhang X, Xia L, Zhang X, Xu C, Shen Z. MicroRNA-31 functions as a tumor suppressor and increases sensitivity to mitomycin-C in urothelial bladder cancer by targeting integrin α5. Oncotarget 2016; 7: 27445-27457.
15) Hendricksen K. Device-assisted intravesical therapy for non-muscle invasive bladder cancer. Transl Androl Urol 2019; 8: 94-100.
16) Liu YR, Yin PN, Silvers CR, Lee YF. Enhanced metastatic potential in the MB49 urothelial carcinoma model. Sci Rep 2019; 9: 7425.
17) Wang JR, Liu B, Zhou L, Huang YX. MicroRNA-124-3p suppresses cell migration and invasion by targeting ITGA3 signaling in bladder cancer. Cancer Biomark 2019; 24: 159-172.
18) Sakaguchi T, Yoshino H, Yonemori M, Miyamoto K, Sugita S, Matsushita R, Itesako T, Tatarano S, Nakagawa M, Enokida H. Regulation of ITGA3 by the dual-stranded microRNA-199 family as a potential prognostic marker in bladder cancer. Br J Cancer 2018; 118: e7.
19) Roos F, Binder K, Rutz J, Maxeiner S, Bernd A, Kippenberger S, Zöller N, Chun FK, Juengel E, Blaheta RA. The antitumor effect of curcumin in urothelial cancer cells is enhanced by light exposure in vitro. Evid Based Complement Alternat Med 2019; 2019: 6374940.
20) Li C, Yang Z, Du Y, Tang H, Chen J, Hu D, Fan Z. BCMab1, a monoclonal antibody against aberrantly glycosylated integrin α3β1, has potent antitumor activity of bladder cancer in vivo. Clin Cancer Res 2014; 20: 4001-4013.
21) Bruzell EM, Morisbak E, Tønnesen HH. Studies on curcumin and curcuminoids. XXIX. Photoinduced cytotoxicity of curcumin in selected aqueous preparations. Photochem Photobiol Sci 2005; 4: 523-530.
22) Niu T, Tian Y, Cai Q, Ren Q, Wei L. Red light combined with blue light irradiation regulates prolifera-tion and apoptosis in skin keratinocytes in combination with low concentrations of curcumin. PLoS One 2015; 10: e0138754.
23) Karl A, Grimm T, Jokisch F, Gaisa NT, Stief CG. Non-muscle invasive bladder cancer: Current aspects of diagnostics, local therapy options and the update of the 2016 WHO classification. Urologe A 2016; 55: 1247-1258.
24) Filonenko EV, Kaprin AD, Alekseev BY, Apolikhin OI, Slovokhodov EK, Ivanova-Radkevich VI, Urlova AN. 5-Aminolevulinic acid in intraoperative photodynamic therapy of bladder cancer (results of mul-ticenter trial). Photodiagnosis Photodyn Ther 2016; 16: 106-109.
25) Falke J, Parkkinen J, Vaahtera L, Hulsbergen-van de Kaa CA, Oosterwijk E, Witjes JA. Curcumin as treatment for bladder cancer: a preclinical study of cyclodextrin-curcumin complex and BCG as intravesi-cal treatment in an orthotopic bladder cancer rat model. Biomed Res Int 2018; 2018: 9634902.