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Communicate or Die – A Model for HPV+ and HPV– CSCs and Their Interactions with SDF-1α

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Abstract. *Background/Aim: Cancer stem cells (CSCs) are suspected of being a reason for limited therapy in head and neck squamous cell carcinoma (HNSCC). Stromal cell-derived factor-1α (SDF-1α) plays a critical role in the communication between CSCs and their microenvironment. We investigated the influence of SDF-1α on HPV+/HPV– SCC cell lines to find an approach of explanation for the superior prognosis of HPV+ HNSCCs. Materials and Methods: We evaluated the expression of CD44/CXCR4 on HPV+/HPV– SCC cell lines and monitored the influence of SDF-1α on proliferation, morphology and migration of HPV+/HPV– SCCs. Results: HPV– SCCs showed a significant increase of podia formation and an intensified migration towards SDF-1α. HPV+ SCCs rested nearly unaffected by SDF-1α. Conclusion: Weakened reaction to SDF-1α in HPV+ SCC could lead to an impaired communication between CSCs and their niche, that would result in an increased exposure of CSCs to the harming influence of e.g. chemotherapeutic agents.*

Squamous cell carcinoma of the head and neck (HNSCC) is an epithelial cancer, which shows rising incidence. It is the most common malignancy in the upper aerodigestive tract and the sixth most common cancer worldwide (1). Most of these malignancies, approximately 80%, correlate to the consumption and exposure to alcohol and tobacco (2). In the last decades, respectable advances in surgery, chemotherapy, radiation and antibody therapies have been established. However, the prognosis in most of the cases remains poor. The 5-year survival rate for patients with HNSCC could not be improved, also because the tumor is often diagnosed at an advanced stage (3). After a primary reduction of the bulk of the tumor by the therapeutic options mentioned above, local recurrences and metastatic settlements occur in a large part of patients (4).

The human papilloma virus (HPV) is a ds-DNA holding virus of the papillomaviridae family, that can infect both cornified squamous and anogenital mucosa cells and arises in different subtypes. While some of these, the so called low-risk-types, are known to cause condylomas, the so-called high-risk types like HPV16 and HPV18 are able to give rise to squamous carcinomas (5).

The incidence of HNSCC which are caused by the HPV is rising, especially for the high-risk HPV types 16 and 18, demonstrating the impact of viral etiology besides carcinogens such as ethylalcohol and nicotine (6). While in cervical carcinomas HPV has already been proven as necessary if not sufficient carcinogen (7), its importance in HNSCC is growing yet not fully understood. The viral oncogenes *E6* and *E7* are known to cause the degradation of the tumor suppressor gene *p53 via* the ubiquitin-proteasome-pathway causing an uncontrolled and aggressive proliferation of the infected cells (8). Besides, many other proteins are known to be frequently mutated in HPV+ carcinoma cells (9), including important intracellular messengers like JAK, Raf and Ras (10).

Nevertheless, the clinical outcome for patients with HPV+ HNSCC is significantly better than for HPV– HNSCC.

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HPV+ HNSCCs seem to be more sensitive to radiation and chemotherapy leading to a better survival rate compared to patients with HPV– HNSCC (11).

In the last years, a subpopulation of cancer cells has become a focus of cancer research. These cells called cancer stem cells (CSCs), show an increased resistance against chemotherapeutic agents and radiation therapy (12). In addition, CSCs seem to possess abilities of somatic stem cells such as self-renewal, differentiation and proliferation. By that they are able to promote initiation of neoplastic growth as well as maintenance of the tumor bulk and local recurrences. The high after-treatment recurrence-rate in patients with HNSCC might indicate the significant role of CSCs in the pathophysiology of HNSCC. While the bulk of tumor cells is exposed to the antineoplastic attack, the CSCs remain under the protection from their microenvironment, designated as cancer stem cell niche, which provides antiapoptotic stimuli for the CSCs, enabling them to survive treatment (13). Thereby, CSCs are able to continue malignant renewal.

Identifying these cells is a crucial step to further research for targeted and specific therapy. Prince *et al*. (14) succeeded in separating a certain subpopulation of HNSCC cells, which showed a CD44+ expression and represented <10% of the cells in a HNSCC tumor. These CD44+ cells, but not CD44- cells, were able to give rise to new tumors after being transplanted *in vivo*. Enabling the identification of these cancer cells with stem cell characteristics, CD44 is referred as a CSC marker in HNSCC.

Until today over 20 different isoforms of CD44 are known. They arise by alternative splicing of 10 exons (6-8). Some of these subtypes are also expressed in healthy tissues and are, therefore, not specific for malignant cells (15). It has been shown that the expression of CD44 goes along with an increased metastatic potential of different entities of carcinoma, a higher chance of recurrences and an impaired prognosis of affected patients (16-18).

The stem cell niche is a network of tissue cells and extracellular matrix (ECM) like collagen, proteoglycans and glycoproteins. It is able to harbor one or more stem cells and to control their self-renewing and progenitor function (19).

It is assumed that there is also a pathological pendant of stem cell niches for CSCs, the so-called CSC niche. These microenvironments are thought to exert protective and regulative influence on the CSCs and also to receive reciprocal angiogenetic signals from the CSCs, forming a symbiotic union (20). Understanding the pathways of communication between CSCs and their niche is of high importance for future options of therapy in HNSCC.

Stromal cell-derived factor-1α (SDF-1α) is a cytokine, which is expressed and secreted by several types of tissues such as endothelium and stromal cells (21-23). It is known to be an important messenger in the communication between the CSCs and their CSC niche. SDF-1 arises in two isoforms, which result from alternative splicing - SDF-1α and SDF-1β. SDF-1α consists of 89 amino acids and is encoded by three exons (24). The fact that it is well preserved between different species underlines its evolutionary importance (25). The docking of SDF-1α to its receptor, the 7-transmembrane-receptor CXCR4, results in an intracellular signal cascade, which is not yet fully understood. Probably the stimulus of SDF-1α leads to a complex interaction of different pathways rather than a single signal (22, 26, 27).

Among others, phosphoinositide 3-kinase (PI3K), protein kinase B (Akt) and *phospholipase C* (PLC) seem to play a role in the intracellular signal cascade following the SDF-1α/CXCR4-axis (28). Also, an increased production and secretion of mediators such as the vascular endothelial growth factor (VEGF) and the matrix metallopeptidase 9 (MMP9) is described (29), underlining the role of SDF-1α in the communication between its target cells and their microenvironment.

Faber *et al*. and others were able to demonstrate the polarization and formation of podia of haematopoetic progenitor cells (HPCs) (22) under the stimulus of SDF-1α which both can be understood as requirements for directional migration. This behaviour had also been shown in leukemic cells (30).

The SDF-1α/CXCR4-axis holds a crucial position for hematopoietic stem cells in the regulation of cell homing and adhesion to their supportive cellular microenvironment (31). Establishing a gradient of concentration of SDF-1α starting low at the invasive front of the tumor and rising up to the preferential organs of metastases seems also to be an effective method for targeted metastasis (32). This demonstrates the abuse of pre-existing physiological structures by neoplastic cells for their own pathological needs. However, the influence of SDF-1α on cell proliferation and its effect on cell survival or apoptosis remains controversial (22, 33-35).

Herein, we monitored the expression of CD44 as a cancer stem cell marker and of CXCR4 as a potential target of interaction between CSCs and their supportive cells in the cancer stem cell niche in human SCC cell lines. Furthermore, we investigated the intensity of proliferation, changing in morphology and chemotactic migration of HPV+ and HPV– SCC cells under the influence of different concentrations of SDF-1α.

Materials and Methods

Cell culture. The HPV– HNSCC cell lines UM-SCC 11A (11A) and UM-SCC14C (14C) were obtained from Dr. T.E. Carey (University of Michigan, Ann Arbor, MI, USA) and originated from a human squamous cell carcinoma (SCC) of the oropharynx and the larynx. The HPV+ CERV196 (Cerv) cell line came from a human SCC of the uterine cervix (CLS, Eppelheim, Germany). 14C and 11A were kept in Dulbecco's modified essential medium (DMEM) (Fisher

Scientific and Co., Pittsburgh, PA, USA) supplemented with 10% fetal calf serum (FCS) and antibiotics (Life Technologies Inc., Gaithersburg, MD, USA). Cerv cells were kept in Eagle's minimum essential medium with 2 mM L-glutamine, 10% FCS and Earle's balanced salt solution with 1.5 g/l sodium bicarbonate, 0.1 mM amino acids and 1.0 mM sodium pyruvate. Cell cultures were carried out at 37˚C in a fully humidified atmosphere with 5% CO_2.

Immunofluorescence labelling. To detect the expression of CD44 and CXCR4 in UM-SCC 11A, UM-SCC 14C and CERV196, cells were incubated with CD44–/CXCR4– antibody (mouse monoclonal, 1:100; Abcam, Cambridge, UK) overnight at room temperature followed by incubation with a second biotinylated antibody (anti-mouse, 1:100) for 30 min. After further washing steps with phosphate buffered saline (PBS), cells were treated with streptavidin-Alexa 568 (1:100, red)/ streptavidin-Alexa 488 (1:100, green) (Life Technologies Inc., Gaithersburg, MD, USA) for 30 min at room temperature. Finally, cells were covered in FluorSave™ (Merck Millipore, Billerica, MA, USA) reagent and dried to be evaluated by fluorescence microscopy (Axiophot, Fa. Zeiss, Oberkochen, Germany). Cell nuclei were stained with 4',6-Diamidin-2-phenylindol (DAPI).

Immunohistochemistry. Cells of UM-SCC11A, UM-SCC14C and CERV196 were additionally treated with a peroxidase-block (Fa. Dako, Glostrup, Denmark) for 30 min in a humidified chamber. After washing steps with PBS they were incubated with 10% calf serum for 30 min. Afterwards the cells were incubated with a primary CD44/CXCR4-antibody overnight. Following washing steps with PBS they were treated with a secondary, biotinylated antibody (anti-mouse, 1:100, Fa. GE Healthcare, Chalfont St Giles, Buckinghamshire, UK) for 45 min at room temperature. They were further incubated with a horseradish peroxidase complex (1:100, Fa. GE Healthcare, Chalfont St Giles, Buckinghamshire, UK) for 45 min and afterwards with the substrate solution aminoethylcarbazole (AEC). Additional washing steps were performed followed by coloring *via* Harris hämatoxilin for 30 sec and a final cover with Crystal Mount for microscopic analysis.

Proliferation assay. Proliferation of HPV+ and HPV– SCC cells was measured by the Alamar Blue® (Invitrogen, Darmstadt, Germany) proliferation assay. Proliferation was measured on Days 1, 2, 3 and 4 by measurement of fluorescence at a wavelength of 540 nm (exitation) and 590 nm (emission). Absorbance was monitored at 590 nm. Three independent experiments were performed (n=3).

Micoscropy. Analysis of cell morphology under the influence of SDF-1α was carried out as follows. CD44+ HPV+ and CD44+ HPV– SCC cells were seeded in DMEM (Fisher Scientific and Co., Pittsburgh, PA, USA) supplemented with 10% FCS and antibiotics and then incubated with SDF-1α (0, 10, 100 and 500 ng/ml; Fa. Peprotech, Rocky Hill, NJ, USA) for 24 h. Cell morphology was assessed *via* light microscopy (Axiophot, Fa. Zeiss, Oberkochen, Germany). At least 5 fields of view in each well were evaluated in each of 5 (n=minimum 5) independent experiments.

The ratio of polarized cells with filopodia or a prominent uropod compared to round cells was determined for the different concentrations of SDF-1α (1, 10, 100 and 500 ng/ml) and set in ratio to the negative control.

Migration assay. Chemotaxis was assessed by an *in vitro* 2-chamber Transwell-assay. Different concentrations of SDF-1α (0, 10, 100 and 500 ng/ml) were added to the lower section of a Transwell chamber (8.0-μm pore size, 6.5-mm diameter inserts; Costar®, Corning Inc., Corning, NY, USA). Equal cell numbers of UM-SCC 11A, UM-SCC 14C and CERV196 were seeded in the upper chamber in medium without SDF-1α. After 24 h, the Transwells were removed, and the number of cells that had migrated through the micropores was calculated. As the cell lines used are adherent, cells turned out to migrate through the pores and stick to the bottom of the Transwell membrane. The width of the cell ring measured in nm served as a dimension for the number of cells that migrated (n=6) and was set in ratio to the negative control.

Statistical analysis. All results were plotted as means±standard deviation. To estimate the probability of differences, we adopted the Student's *t*-test (two-tailed distribution, 2-sample equal variance). Probability value of $p<0.05$ was denoted statistically significant and $p<0.01$ statistically highly significant.

Results

Expression of CD44 and CXCR4 in SCC cell lines. Immunofluorescence labelling of HPV+ CERV196, HPV– UM-SCC 11A and HPV– UM-SCC 14C was performed. The CSC marker CD44 was visualized in red color by immunofluorescence labelling *via* Alexa 568. The SDF-1α-receptor CXCR4 was detected in green color *via* Alexa 488. In all three cell lines an intense red fluorescence signal of all cells was detectable, showing the expression of CD44. Here, CD44 was mainly expressed on the cell surface (Figure 1). The majority of cells were also CXCR4+, while CXCR4 showed a predominant cytoplasmic staining pattern (Figure 2). Additionally, the staining of both CD44 and CXCR4 *via* immunochemistry could be detected in all three cell lines (Figures 3 and 4).

Effects on cell proliferation. HPV+ CERV196, HPV– UM-SCC 11A and UM-SCC 14C cells were cultured at 37˚C in a 5% CO_2 fully humidified atmosphere using DMEM (Fisher Scientific and Co., Pittsburgh, PA, USA) supplemented with 10% FCS and antibiotics supplemented with SDF-1α in concentrations of 0, 1, 10, 100 or 500 ng/ml. Proliferation of HNSCC cell lines and CERV196 under SDF-1α was measured by the Alamar Blue® proliferation assay on Days 1, 2, 3 and 4, as described above. The addition of SDF-1α did not have any significant impact on the proliferation or viability of HNSCC cells (Figure 5).

Effects on podia formation. Polarization and formation of podia are both requirements for targeted migration of cells. We analyzed podia formation of CD44+ CXCR4+ HPV+/HPV– SCC cells following a 4-h treatment with SDF-1α (0, 1, 10, 100 and 500 ng/ml). Enabling a direct comparison of the HPV+ and HPV– cell lines, we calculated the ratio of polarized cells at different concentrations of SDF-1α and put

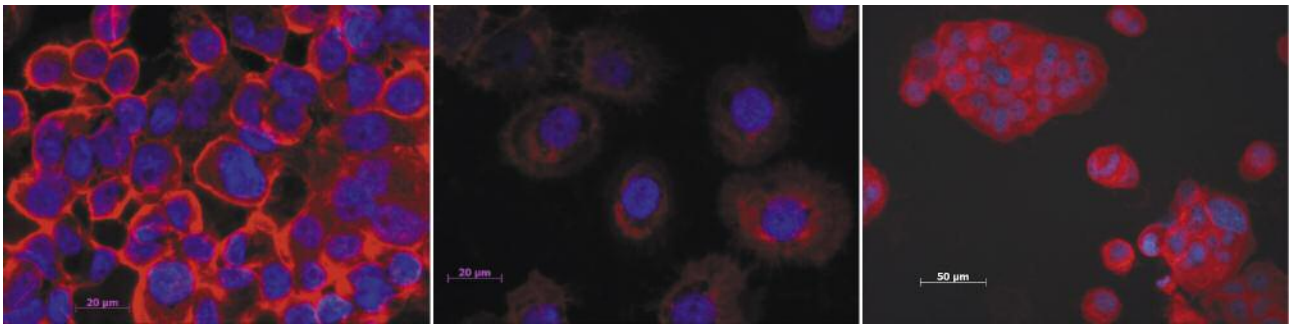

Figure 1. *Immunofluorescence labeling of the HPV– UM-SCC14C (left), the HPV– UM-SCC11A (center) and the HPV+ CERV196 (right) cell lines via Alexa 568. All three cell lines show a red pattern, indicating the presence of the cell surface marker CD44.*

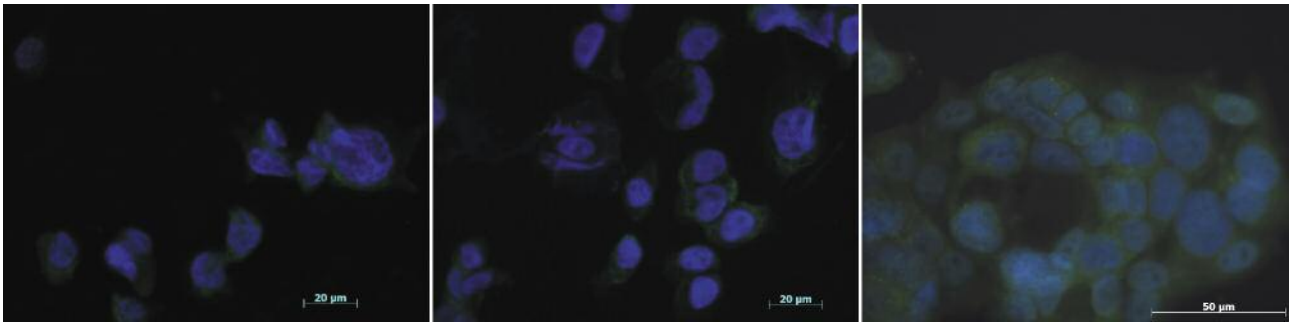

Figure 2. *Immunofluorescence labeling of the HPV– UM-SCC14C (left), the HPV– UM-SCC11A (center) and the HPV+ CERV196 (right) cell lines via Alexa 488. All three cell lines show a green pattern, indicating the presence of the SDF-1α-receptor CXCR4.*

these in ratio to their negative control. For our control experiments (0 ng/ml SDF-1α) the ratio of polarized cells was defined as 1±0. In HPV– UM-SCC 11A and UM-SCC 14C cell lines the ratio of polarized cells increased following treatment with SDF-1α in a concentration-dependent manner (Figure 6). A significant positive correlation between the ratio of polarized cells and the concentration of SDF-1α could be proven (p=0.049, Pearson's R=0.28). The HPV+ CERV196 cell line showed no significant change in ratio of polarized cells at none of the concentrations of SDF-1α. Furthermore, no correlation between the ratio of polarized cells and the concentration of SDF-1α could be found (p=0.924, Pearson's R=0.02). The differences between HPV+ and HPV– cell lines showed a highly significant positive correlation with the concentration of SDF-1α (p=0.00012).

Effects on migration. Chemotaxis of HNSCC-cell lines towards a gradient of SDF-1α was measured using a Transwell migration assay. The HPV+ CERV196 cell line showed no significant alteration in migration rate under the influence of SDF-1α. Although the HPV– UM-SCC 11A and CD44+ CXCR4+ HPV– UM-SCC 14C cell lines showed concurrent formation of podia, they showed a contrary behaviour in migration under the influence of SDF-1α and had to be considered separately. In the HPV– UM-SCC 14A the number of migrating cells increased with increasing concentrations of SDF-1α up to 100 ng/ml but decreased with concentrations higher than that (500 ng/ml). Excluding the values greater than 100 ng/ml, the ratio of migrating cells showed a significantly positive correlation with the concentration of SDF-1α (p=0.05, Pearson's R=0.561). The ratio of migrating cells in the HPV– UM-SCC 11A cell line decreased with increasing concentrations of SDF-1α reaching significant levels of difference at 100 and 500 ng/ml (Figure 7). The differences between the HPV– UM-SCC 14A and HPV+ CERV196 cell lines tended to correlate positively with the concentration of SDF-1α (p=0.053, Pearson's R=0.409).

Discussion

We examined the influence of the cytokine SDF-1α on both HPV+ and HPV– SCC cell lines and monitored proliferation, directed migration and podia formation. We demonstrated the

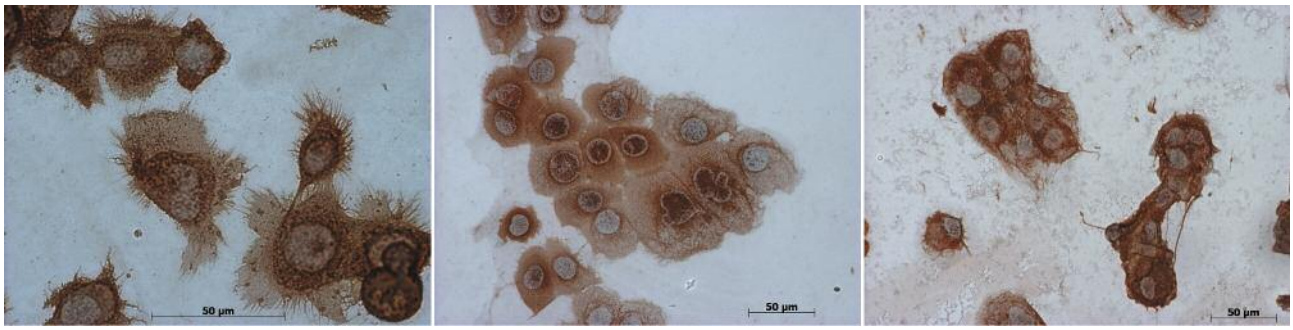

Figure 3. *Immunohistochemistry staining of the HPV– UM-SCC14C (left), the HPV– UM-SCC11A (center) and the HPV+ CERV196 (right) cell lines. All three cell lines show the expression of the cell surface marker CD44.*

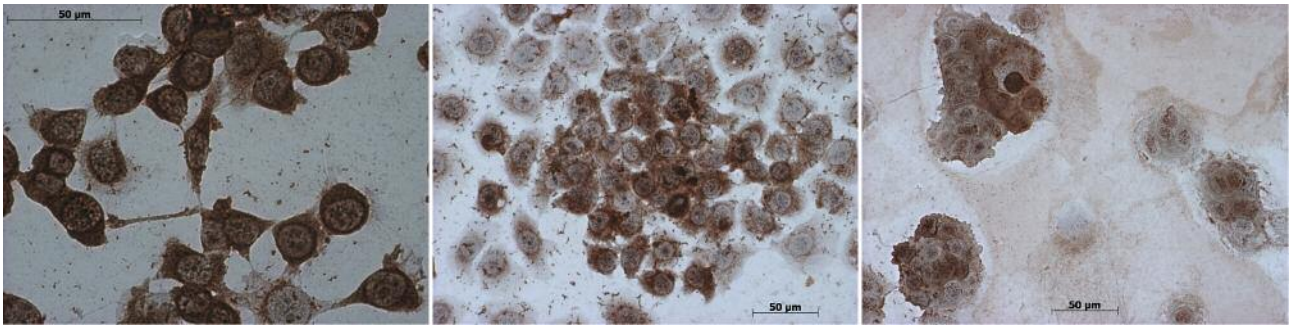

Figure 4. *Immunohistochemistry staining of the HPV– UM-SCC14C (left), the HPV– UM-SCC11A (center) and the HPV+ CERV196 (right) cell lines. All three cell lines show the expression of the SDF-1α-receptor CXCR4.*

expression of the SDF-1α-receptor CXCR4 in all three cell lines, implying them to be a potential target for SDF-1α. Furthermore, we detected the expression of the cell surface marker CD44, which has been shown to allow the enrichment of a subpopulation of tumor cells, which are able to give rise to new tumors after line-transplantations (14). However, CD44 is not an exclusive CSC marker and is also expressed in ordinary tissues (15, 36).

Actually, the definitive identification of CSCs might only be possible using a combination of both including and excluding cell surface markers, of which a wide part is not yet discovered. Currently, a set of including CSC markers such as CD44 (14), ALDH1 (37, 38), CD133 (39), CD271 (40), Nanog and Oct4 (41) and excluding markers such as CD3, CD10, CD18, CD31, CD64 and CD140 (36, 42) is under discussion. Additionally, it seems that the molecular phenotype of CSCs is dependent on the tumor entity (14, 43, 44).

Regarding the influence of SDF-1α on the proliferation of HPV+ and HPV– SCC cell lines, our results showed no significant changes between the negative control and the incubation with different concentrations of SDF-1α. These findings are in line with previous studies (22, 32, 35). On the other hand, there are also studies to suggest an influence of SDF-1α on proliferation and partially survival of cells (33). Overall, the effect of SDF-1α on the proliferation rate of cancer cells seems controversial.

Furthermore SDF-1α is considered a significant intercellular messenger for chemotaxis. It seems that neoplastic malignancies establish a gradient of concentration of SDF-1α starting low at the invasive front of the tumor and rising up to the preferential organs of metastases and therefore are able to spread metastatic settlements (21).

This model is in line with findings, that CXCR4 is mostly expressed in the invasive front of the tumor, providing the ability to adopt SDF-1α and by this to migrate directedly (45).

Our results demonstrate a weaker response of HPV+ SCC cell lines to the stimulus of SDF-1α compared to HPV– SCC cell lines. HPV– SCC cell lines showed a significant increased amount of podia formation in a dose-dependent manner and an intensified migration under the influence of SDF-1α, while HPV+ SCC cell lines rested nearly unaffected by SDF-1α.

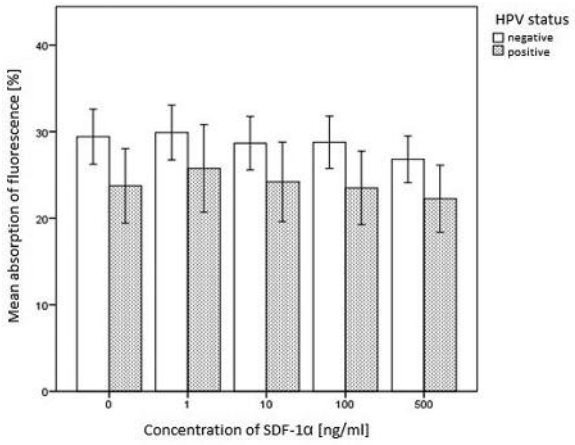

Figure 5. *Effect of SDF-1α on the proliferation of CD44+ CXCR4+ HPV+/HPV– cells. Influence of SDF-1α at increasing concentrations on the proliferation of CD44+ CXCR4+ HPV+/HPV– cells was evaluated via the Alamar BlueR proliferation assay. The addition of SDF-1α did not have a significant impact on the mean proliferation of neither HPV+ nor HPV– cells, even on increased concentrations of SDF-1α (0, 1, 10, 100 and 500 ng/ml SDF-1α).*

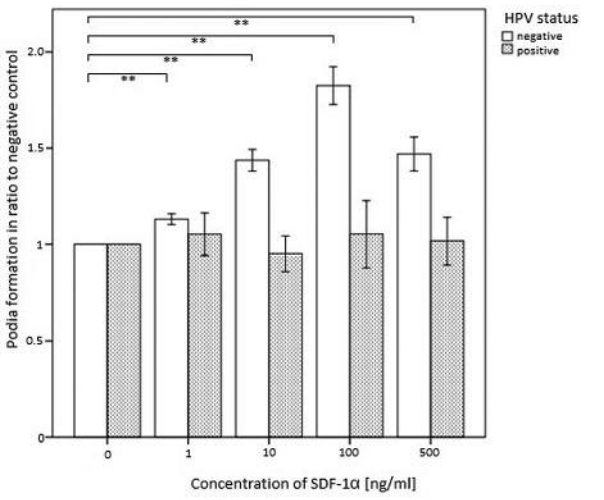

Figure 6. *Effects of SDF-1α on the formation of podia of CD44+ CXCR4+ HPV+/HPV– SCC cells. Formation of filopodia and prominent uropodia was evaluated via microscopy following a 4-h treatment with SDF-1α at increasing concentrations (0, 1, 10, 100 and 500 ng/ml). In HPV– cell lines, the ratio of polarized cells increased under the influence of SDF-1α in a concentration-dependent manner (means: 0 ng/ml SDF-1α 1.0, 1 ng/ml SDF-1α 1.13±0.09 p=0.000221, 10 ng/ml SDF-1α 1.43±0.17 p=0.0000003749, 100 ng/ml SDF-1α 1.82±0.31 p=0.00000011953, 500 ng/ml SDF-1α 1.46±0.27 p=0.000046. **p<0.01). SDF-1α did not show a significant impact on the formation of podia in CD44+ CXCR4+ HPV+ CERV196 cell line (means: 0 ng/ml SDF-1α 1.0, 1 ng/ml SDF-1α 1.05±0.24 p=0.644, 10 ng/ml SDF-1α 0.95±0.20 p=0.61, 100 ng/ml SDF-1α 1.05±0.39 p=0.766, 500 ng/ml SDF-1α 1.01±0.27 p=0.89).*

It has to be mentioned that in our migration experiments the HPV– UM-SCC 11A cell line showed a behaviour similar to the HPV+ CERV196 and contrary to the HPV– UM-SCC 14C line, while its behaviour in podia formation was concurrent with the HPV– UM-SCC 14C line. First this could be due to a recurrent negative feedback mechanism of the SDF-1α-stimulus which could occur at lower concentrations of SDF-1α in the HPV– UM-SCC 11A line than in the HPV– UM-SCC 14C line. On the other hand, there are several studies in which the HPV– UM-SCC 11A cell line was used as a model for HPV– HNSCC cells and in which it has demonstrated a behaviour similar to the HPV– UM-SCC 14C line regarding the reaction to SDF-1α and other stimuli (46-49).

The weakened reaction of the HPV+ CSCs to SDF-1α could be explained by the theory that signal pathway molecules of the SDF-1α/CXCR4-axis might have been mutated by HPV oncogenes. We compared these gene products that are supposed to be a critical part of the SDF-1α/CXCR4-axis with these, that are often mutated in HPV+ cells. Hereby we found several proteins which appear in both groups, *e.g.* the *protein kinase B* (Akt), the nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) and the phosphoinositide 3-kinase (PI3K). First, they are important components of the intracellular pathway which is activated after the docking of SDF-1α to his receptor CXCR4 and required for the continuation of the migration signal. On the other hand, they are also frequently found in studies about genetic changes in HPV+ tumor cells as examples of proteins which differ from their wild type (10, 50-57).

These proteins, which are both crucial parts of the SDF-1α/CXCR4-axis and apparently also a preferential target for mutations in HPV-infected cells, display a potential cause for eventual malfunctions of the SDF-1α/CXCR4-axis in HPV-positive tumor cells.

A mutated form of these signal pathway molecules as they can more likely be expected in HPV+ CSCs may not be able to keep their functional potential and maintain an appropriate reaction to the stimulus of SDF-1α as our results may indicate. This might be either due to changes in the surface of the gene product proteins and a resulting inhibited lock-key mechanism or to an already permanent activated mutation-phenotype which cannot be stimulated any further by SDF-1α.

Either way, a weakened reaction of HPV+ CSCs to SDF-1α could lead to an impaired communication between the CSCs and their cancer stem cell niche which would imply a worsen migration potential for the CSCs. However, the ability to migrate in a chemotactic way or to perform homing if required is an important factor for the survival of tumor cells. As soon as they are under an antineoplastic attack such as chemotherapy, which represents a stress situation for the whole body, the CSCs are suspected to use chemotactic

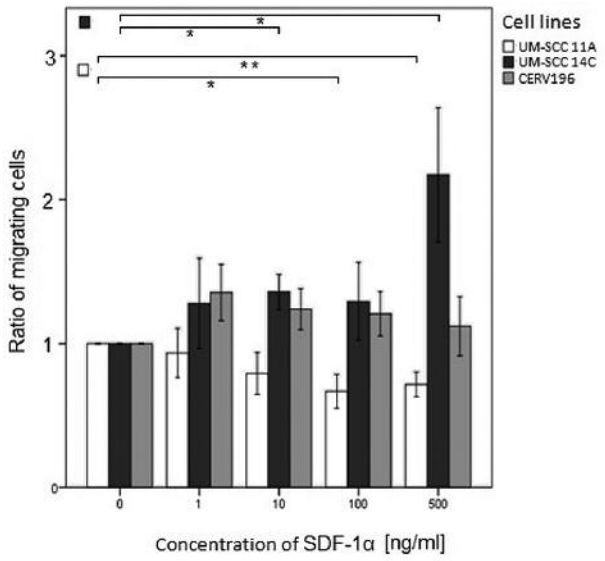

Figure 7. *Effect of SDF-1α on migration of CD44+ CXCR4+ HPV+/HPV– SCC cells. Chemotaxis towards a gradient of SDF-1α was assessed in a Transwell migration assay. While CD44+ CXCR4+ HPV+ CERV196 showed no significant changes in migration (means: 0 ng/ml SDF-1α 1.0, 1 ng/ml SDF-1α 1.35±0.43 p=0.107, 10 ng/ml SDF-1α 1.23±0.31 p=0.131, 100 ng/ml SDF-1α 1.20±0.34 p=0.215, 500 ng/ml SDF-1α 1.12±0.45 p=0.575), CD44+ CXCR4+ HPV– UM-SCC 14C showed increasing migration with increasing concentrations of SDF-1α migration (means: 0 ng/ml SDF-1α 1.0, 1 ng/ml SDF-1α 1.27±0.70 p=0.4, 10 ng/ml SDF-1α 1.35±0.24 p=0.012, 100 ng/ml SDF-1α 1.29±0.54 p=0.256, 500 ng/ml SDF-1α 2.17±1.04 p=0.036) and CD44+ CXCR4+ HPV– UM-SCC 11A showed decreasing migration (means: 0 ng/ml SDF-1α 1.0, 1 ng/ml SDF-1α 0.93±0.37 p=0.681, 10 ng/ml SDF-1α 0.79± 0.35 p=0.185, 100 ng/ml SDF-1α 0.66±0.28 p=0.018, 500 ng/ml SDF-1α 0.71±0.21 p=0.008. *p<0.05 **p<0.01).*

migration *via e.g.* SDF-1α to find the protecting pro-survival stimuli of their CSC niche. Failure of this tactic would ultimately result in an increased exposure of the CSCs to the harming chemotherapeutic agents and thereby in an improved prognosis for the patient.

The immunosurveillance model describes a theory after which all body cells are under permanent observation of the immune system. According to the immunosurveillance model the immune competency of an individual is a critical factor for the development of neoplasia, because immune cells are able to detect malignancies in an early stage and even to eliminate them (58). However, in most of the cases tumor cells originate from the hosts own physiological cells and therefore cause a high rate of immune tolerance (59). The cellular changes caused by HPV are suspected of displaying new target structures for the recognition of cancer cells by immune cells, allowing their early intervention (60, 61). With this explanation, the immunosurveillance theory is until today one of the most accepted theories for the better clinical outcome of HPV+ HNSCCs. Several studies could detect a positive correlation between the degree of lymphocytic invasion of the tumor and the clinical outcome of patients with several malignant etiologies, pointing out the importance of immunoevading mechanisms for a successful and unrestricted growth of tumor (62-66). As long as there is a sufficient communication between the CSCs and their CSC niche, the niche provides an antiapoptotic environment for the CSCs (67), antagonizing the apoptotic stimuli of immune cells. Failure of immunosurveillance by congenital or acquired immunodeficiencies leads to an increased development of tumors (68).

On the other hand, a failure of communication between the CSCs and their CSC niche, by *e.g.* HPV oncogenes, would also weaken the protection of the CSC niche on the CSCs. This would allow a greater attack potential for the immune system and strengthen the prognosis of patients with HNSCC. Thereby these mechanisms would provide a possible and at least partial explanation for the better clinical outcome of HPV+ HNSCC.

Because of the use of antineoplastic treatments with a high potential for side-effects, the ascertainment of prognostic factors plays an important role in clinical routine for risk-benefit calculations of therapy. Since the latest update of the AJCC's cancer staging manual (69), the HPV-status of carcinoma of the oropharynx has found its way into the TNM-classification of HNSCC. However, it is possible that the impact of the HPV status on the prognosis of HNSCC in an individual patient might be dependent on different individual endogenous factors, that are not yet entirely described. Thereby, the HPV-status of a tumor remains an uncertain factor in the risk-benefit calculation of therapy. In our *in vitro* experiments, the differences in morphology between HPV+ and HPV– HNSCC cell lines correlated positively with the concentration of SDF-1α while the differences in migration tended to correlate positively with the concentration of SDF-1α. Therefore, it could be hypothesized that for patients with high endogenous levels of SDF-1α the ascertainment of the HPV status of an HNSCC would play a greater role for a risk-benefit calculation of therapy than for those with low endogenous levels of SDF-1α.

While the ascertainment of HPV in HNSCC has been recommended quite recently, it remains to be seen how predictable the individual prognosis of patients with HNSCC, by factors such as the HPV-status or endogenous levels of SDF-1α, will be in the future.

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