Wound fluid enhances cancer cell proliferation via activation of STAT3 signal pathway *in vitro*

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Abstract. Wound healing begins immediately after surgery with a modification of the microenvironment via a well-orchestrated interaction between cells, cytokines and growth factors. Some of these growth factors and cytokines have mitogenic effects on cancer cells, which may lead to enhanced cancer cell proliferation and early metastatic events. The present study aimed to investigate the effects of wound fluid (WF) on the head and neck squamous carcinoma cell lines FaDu and HLaC78 *in vitro*. WF was harvested from 7 patients who had undergone a planned neck dissection. The presence of cytokines and growth factors was evaluated with the dot blot assay. Proliferation and cell viability were investigated via MTT assay and Ki-67 staining. Cell invasion was measured via tree-dimensional invasion assay. Western blotting was used to investigate STAT 3 activation. WF contained several cytokines and growth factors responsible for pro- and anti-inflammation, chemotaxis, proliferation and angiogenesis. The proliferation effect of WF on FaDu and HLaC78 was concentration dependent. Media with 40% WF resulted in the highest proliferation effect. FaDu and HLaC78 exhibited enhanced motility after cultivation with 40% WF compared with cultivation with expansion medium. Cultivating cancer cells with WF had no advantageous effect on cell viability after the paclitaxel treatment. Western blot analysis revealed enhanced activation of the STAT3 signaling pathway by WF in both FaDu and HLaC78. In conclusion, surgery leads to excessive release of mitogenic factors. The contact of non-resected cancer cells and these factors may have a negative impact on patient outcome. Future investigations should specifically focus on the inhibition of mitogenic factors following cancer surgery in order to prevent early metastasis and cancer recurrence.

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Introduction

In 2017, 1,688,780 new cancer cases and 600,920 cancer deaths were projected to occur in the US (1). Therapy varies depending on the type of cancer, origin and localization. Surgery is one major option for treating malignant disease. After surgery, wound healing commences immediately. During this process, a complex inflammatory response is triggered, which induces the recruitment, proliferation and activation of cells such as neutrophils, macrophages, natural killer cells, fibroblasts and mesenchymal stem cells (2,3). The process of wound healing is orchestrated via the interaction between different cells, cytokines, and the extracellular matrix. Surgical wound fluid (WF) contains blood cells, immune cells, lymph and paracrine-released factors (4). The composition of factors and cell components in WF differ in a time-dependent manner, and there are ample interindividual differences (5). From an oncological point of view, the mechanisms of wound healing are quite interesting, as non-resected cancer cells may be exposed to mitogenic factors in the wound microenvironment after surgical cancer therapy (6). In a previous study, the presence of a variety of different cytokine and growth factors in the WF of patients who underwent a planned neck dissection was demonstrated (7). The cultivation of mesenchymal stem cells (MSCs) with WF induced enhancement of cell proliferation and cell migration. Most of the cytokines contained in WF are known to be pro-tumorigenic, for example, interleukin (IL)-6. IL-6 is a pleiotrophic cytokine, which is secreted by cells from the immune system or fibroblasts. Cancer cells, for example, from the breast, lung, or prostate also secrete IL-6 (8). Furthermore, IL-6 is an important pro-inflammatory cytokine and a mediator of the immune system, and it stimulates the differentiation of B-cells. Conversely, IL-6 serves an important role in cancer biology by inducing tumor growth via the activation of Ras/Raf/MEK/extracellular signal-regulated kinase 1/2 (9,10). Increased serum levels of IL-6 seem to be associated with severity of disease and poor outcome (11).

Suchi *et al* (12) demonstrated a suppressed cisplatin-induced cytotoxicity in esophageal cancer cells via overexpression of IL-6. Additionally, IL-6 has been linked to enhanced cancer cell migration and metastasis (13).

In the process of cancer progression, the activation of signal transducers and activators of transcription (STAT)3 is important. STAT3 transcription factors are activated by cytokines, growth factors, and hormones (14). The activation of STAT3 is achieved by phosphorylation of its tyrosine and serine residues (15). STAT3 is activated particularly by IL-6 family cytokines, which includes IL-6, IL-8, IL-11 and Oncostatin (16). Cancer cells and cancer surrounding stroma are able to activate STAT3 via autocrine and paracrine production of IL-6 family cytokines (17).

The aim of the present study was to investigate the effects of WF on the head and neck squamous carcinoma cell lines FaDu and HLaC78 in terms of cell viability, proliferation, migration and induction of chemoresistance. Furthermore, the cytokine pattern of WF and possible activation of the STAT3 signaling pathway were also investigated.

Materials and methods

Culture of human carcinoma cell lines FaDu and HLaC78. The head and neck squamous carcinoma cell lines FaDu and HLaC78 were used (18,19). Cells were grown in RPMI-1640 medium (Biochrom, Ltd., Cambridge, UK) with 10% fetal calf serum (FCS) (Linaris Blue Wertheim-Bettingen, Germany), 100 U/ml penicillin, 100 μg/ml streptomycin, 1% sodium pyruvate (100 mM; Biochrom, Ltd.), and 1% non-essential amino acids [100-fold concentration; Biochrom AG; RPMI-expansion medium (RPMI-EM)]. Cells were cultured in flasks at 37˚C with 5% CO_2. The replacement of the medium was carried out every other day, and passaging was performed after reaching 70-80% confluence by trypsinization (0.25% trypsin; Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA). Experiments were performed using cells in the exponential growth phase.

Collection of WF. The WF of 7 male patients (age, 51-88 years; the exclusion criteria was prior administration of radiation therapy) who underwent a planned neck dissection at the Department of Otorhinolaryngology, Plastic, Aesthetic and Reconstructive Head and Neck Surgery at Julius Maximilian University of Wuerzburg (Würzburg, Germany), was collected in 2009 and 2017 from a vacuum drain 72 h after surgery. Written, informed consent was provided by all patients. The study was approved by the Ethics Committee of the Medical Faculty of the University of Wuerzburg. After harvesting WF, centrifugation at 340 x g for 10 min at 4˚C was conducted immediately in order to reduce cell debris. To remove immune cells, a second centrifugation at 340 x g for 10 min at 4˚C in Leucosep medium (GE Healthcare, Chicago, IL, USA) followed. Next, the WF was filtered using a 0.45-μm syringe filter (Sarstedt, Inc., Newton, NC, USA). To avoid bacterial infection, 100 U/ml penicillin and 100 mg/ml streptomycin (1% penicillin/streptomycin) were added.

Cytokine analysis of WF. The cytokine pattern of WF was detected with the dot blot assay. All reagents and materials used, including the C-Series Human Cytokine Antibody Array 3 kit (cat. no. AAH-CyT-3-4) were supplied by RayBiotech Inc. (Norcross, GA, USA). The supplier provided all supplements. After harvesting WF, the presence of cytokines was investigated according to the manufacturer's protocol. First, the WF was added to the membrane for 30 min at room temperature. After several washing steps, incubation for 2 h at room temperature with 1 ml biotin-conjugated antibodies (prefabricated solution) and horseradish peroxidase (HRP)-conjugated streptavidin (1:1,000) was conducted. The labeled proteins were detected via chemiluminescence using detection buffer and exposure to X-ray film. The cytokines were represented as dots with different intensities and diameters. The quantification of the different cytokines was achieved by densitometric methods using ImageJ software (version 1.43u; National Institutes of Health, Bethesda, MD, USA).

Cell viability analysis. The mitochondrial activity of HLaC78 and FaDu cultivated with WF at different concentrations was investigated via MTT assay (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) as described previously (20). FCS was not added to the medium containing WF. First, cells were seeded at a density of $1x10^4$ cells/well in a 96-well round bottom plate. The cultivation medium consisted of RPMI containing WF at various concentrations (10, 20, 30, 40, 50, 60, 70, 80, 90 and 100%). Cells were cultivated for 24 h. After a washing step with PBS, all plates were incubated with 100 μl MTT solution (1 mg/ml) for 5 h at 37˚C. The MTT solution was removed and 100 μl isopropanol was added for a further 1 h at 37˚C. The multi-plate reader (Titertek Multiskan PLUS MK II; Thermo Labsystems, Helsinki, Finland) was used to measure the color conversion at a wavelength of 570 nm. Further experiments were conducted with WF at a concentration of 40%.

The enhanced proliferation activity of cancer cells after cultivation with 40% WF was confirmed through Ki-67 staining. First, the cells were plated on specimen slides. After cultivation with WF for 48 h, fixation was performed using 4% paraformaldehyde in PBS at 4˚C for 30 min. Then, a further 5 min of fixation with 100% acetone at room temperature was performed. Next, cells were incubated with 10% bovine serum albumin (BSA; Carl Roth GmbH and Co., KG, Karlsruhe, Germany) in Tris-buffered saline [200 mM Tris-base, pH 8; 8% NaCl; and 1% Tween-20 (TBS-T); Sigma-Aldrich; Merck KGaA). Incubation at 4˚C of cells in TBS-T containing 1% BSA and a rabbit polyclonal antibody against Ki-67 (1:500; Abcam, Cambridge, UK; cat. no. Ab15580) was assessed overnight. After 3 washing steps with TBS-T, the cells were treated for 1 h in 1% BSA at room temperature with Alexa 555-conjugted goat anti-rabbit secondary antibody (1:500; Invitrogen; Thermo Fisher Scientific, Inc.; cat. no. A21428) and 5 mg/ml DAPI (Sigma-Aldrich; Merck KGaA). Cancer cell lines cultivated in RPMI-EM served as a control. A fluorescence microscope (Leica DMI 4000B Inverted Microscope; Leica Microsystems GmbH, Wetzlar, Germany) was used for cell examination at x100 magnification.

Paclitaxel treatment. In order to evaluate whether WF induces resistance toward chemotherapeutics, cells were cultivated in 40% WF and treated with 10 nM paclitaxel (University of Wuerzburg) for 24 h at 37˚C. Previously, half maximal inhibitory concentration (IC_{50}) was calculated (21). The MTT assay

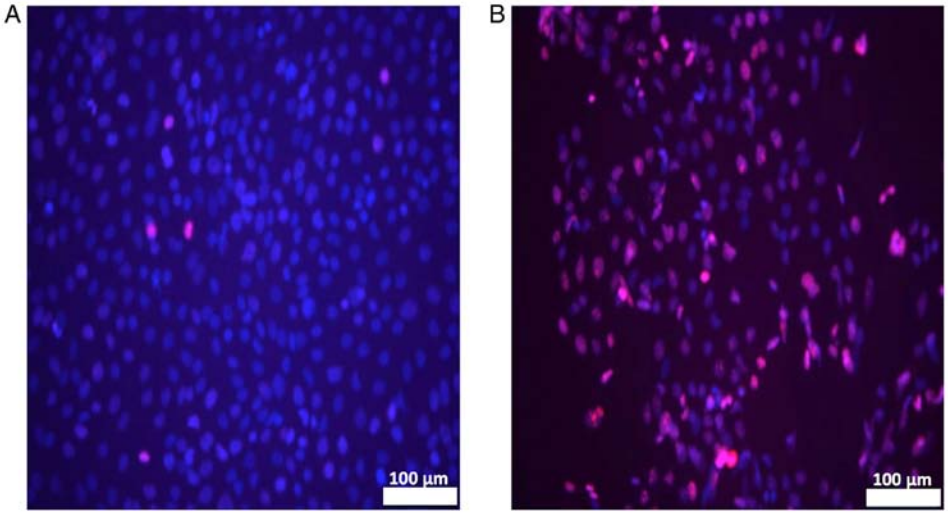


Figure 1. Ki-67 staining of FaDu. FaDu cells were cultured with (A) RPMI-EM and (B) 40% WF. After 48 h, Ki-67 staining was performed. The cell nuclei were stained with DAPI. Cell proliferation was significantly enhanced following cultivation with 40% WF compared with RPMI-EM. RPMI-EM, RPMI-expansion medium; WF, wound fluid.

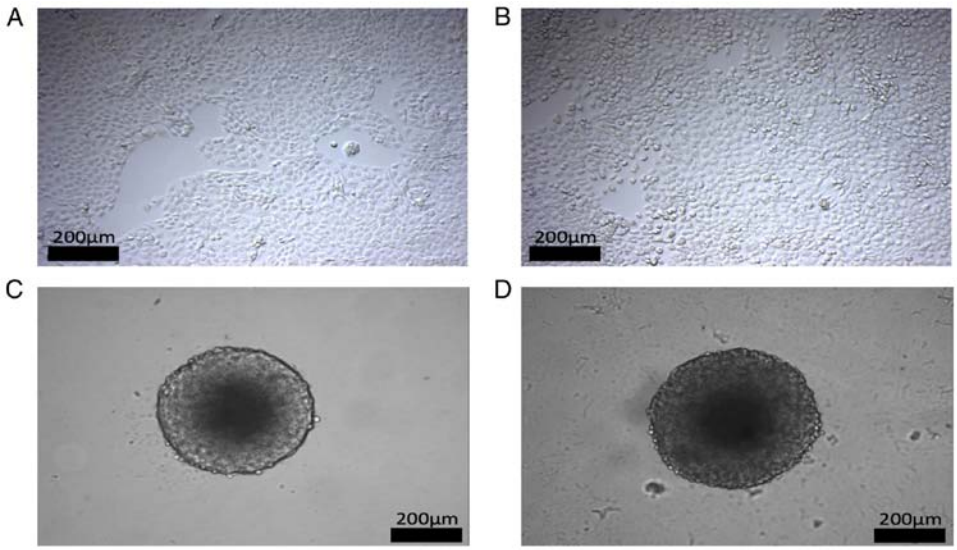


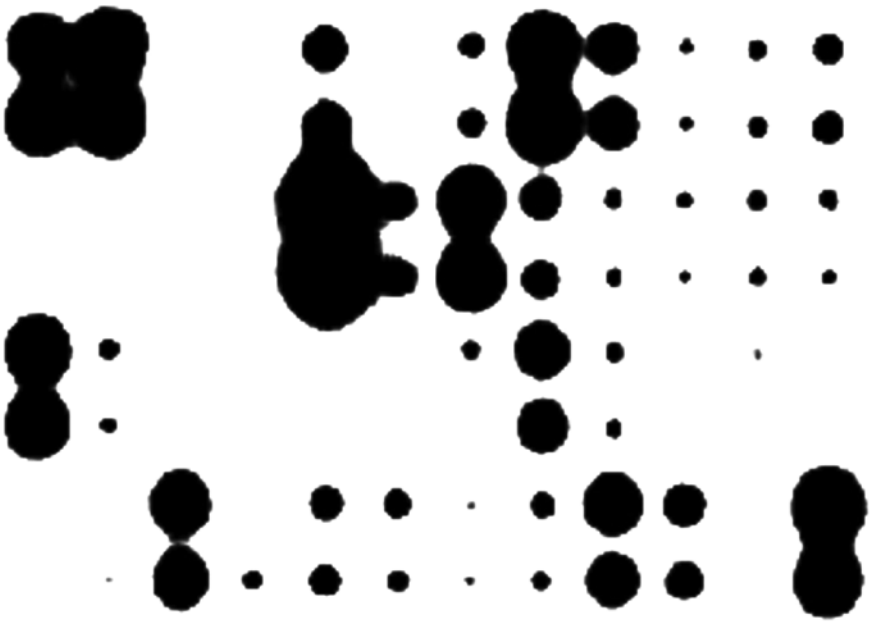
Figure 2. Microscopic analysis of cancer cells. Microscopic analysis of (A) FaDu and (B) HLaC78 in monolayer and (C) FaDu and (D) HLaC78 in spheroid assay with 40% wound fluid was conducted.

was used to determine cell viability as described above. Cancer cells cultivated with RPMI-EM and treated with paclitaxel served as a control.

Three-dimensional invasion assay. A possible alteration in the cell invasion activity was investigated using the three-dimensional invasion assay. First, a 96-multiwell plate was coated with 0.1% agar (Sigma-Aldrich; Merck KGaA). Next, spheroids were generated from $6x10^3$ cells (FaDu or HLaC78). After 72 h, spheroids were transferred to well plates coated with extracellular matrix (1:80; Sigma-Aldrich; Merck KGaA). The cells were able to spread out from the spheroids in the well plate. To determine the migration area, the cells were imaged directly after being transferred and after 24 h of culture using an inverted microscope (magnification, x50; Leica Microsystems GmbH). The migration area was calculated using ImageJ software (version 1.43u).

Analysis of STAT3 activation via western blotting. Western blotting was performed as previously described (22). Cells (FaDu and HLaC78) were harvested by trypsinization and dissolved in radioimmunoprecipitation assay buffer (PBS containing 1% NP40, 0.5% sodium deoxycholate, and 0.1% SDS); then were supplemented with 10 μg/ml phenylmethanesulfonyl fluoride (PMSF). Protein concentration was then determined according to the method detailed previously by Lowry *et al* (23).

Equal amounts (20 μg/lane) of total protein lysates were loaded onto 10% SDS-PAGE and transferred by electroblotting to a polyvinylidene difluoride membrane. The blots were blocked for 1 h at room temperature with TBS-T (10 mM Tris, 150 mM NaCl, 0.05% Tween-20, pH 8.0) containing 5% non-fat dry milk. Subsequently, the membrane was incubated with primary antibody against STAT3 (1:500; Cell Signaling Technology, Inc., Danvers, MA, USA; cat. 9145) overnight



+	+	–	–	ENA-78	GCSF	GM-CSF	GRO	GRO-a	I-309	IL-1a	IL-1β
+	+	–	–	ENA-78	GCSF	GM-CSF	GRO	GRO-a	I-309	IL-1a	IL-1β
II-2	II-3	II-4	II-5	II-6	II-7	II-8	II-10	II-12	II-13	II-15	IFN-g
II-2	II-3	II-4	II-5	II-6	II-7	II-8	II-10	II-12	II-13	II-15	IFN-g
MCP-1	MCP-2	MCP-3	MCSF	MCD	MIG	MIP-1d	RANTES	SCF	SDF-1TA	RC	TGF-β1
MCP-1	MCP-2	MCP-3	MCSF	MCD	MIG	MIP-1d	RANTES	SCF	SDF-1TA	RC	TGF-β1
TNF-α	TNF-β	EGF	IGF-1	Angogenin	OncostatinM	Thrombopoietin	VEGF	PDGF-BB	Leptin	–	+
TNF-α	TNF-β	EGF	IGF-1	Angogenin	OncostatinM	Thrombopoietin	VEGF	PDGF-BB	Leptin	–	+

Figure 3. Cytokine assay of WF. The dot blot assay was used to analyze the presence of different cytokines in WF. According to the manufacturer, a table was used to assign the different dots to cytokines. Various types of cytokines responsible for pro- and anti-inflammation, chemotaxis, proliferation and angiogenesis were present in WF. WF, wound fluid; IL, interleukin; MCP, monocyte chemoattractant protein; TNF, tumor necrosis factor; EGF, epidermal growth factor; MCSF, macrophage colony-stimulating factor; IGF-1, insulin-like growth factor 1; ENA-78, epithelial-derived neutrophil-activating protein 78; MCD, DDHDHD; GCSF, granulocyte colony stimulating factor; MIG, monokine induced by γ-interferon; GM-CSF, granulocyte-macrophage colony-stimulating factor; MIP, macrophage inflammatory protein; GRO, growth related oncogene; RANTES, regulated on activation normal t expressed and secreted; VEGF, vascular endothelial growth factor; SCF, stem cell factor; PDGF, platelet-derived growth factor; I-309, chemokine (C-C motif) ligand 1; SDF, stromal cell-derived factor; TARC, thymus- and activation-regulated chemokine; IFN, interferon; TGF, transforming growth factor.

at 4˚C. Subsequently, the membrane was washed and incubated with a species-specific secondary antibody (1:10,000; anti-rabbit immunoglobulin G; HRP-linked antibody, Cell Signaling Technology, Inc.; cat. no. 7074) for 1 h at room temperature to visualize the specific bindings. Protein expression was detected with an enhanced chemiluminescence system (GE Healthcare), according to the manufacturer's protocol. Jurkat cells (STAT3 control extracts; Cell Signaling Technology, Inc.; cat. no. 9133) were used as a positive control. α-tubulin (1:2,000; Sigma-Aldrich; Merck KGaA; cat. no. T5168) was used as control. The DNA-ladder was purchased from Thermo Fisher Scientific, Inc. (cat. no. 26616).

Statistical analysis. All data were transferred to standard spreadsheets and analyzed using GraphPad Prism 6.0 (GraphPad Software, Inc., La Jolla, CA, USA). First, whether the distribution was Gaussian was analyzed. In the case of Gaussian distribution, unpaired Student's t-test was used; otherwise, the Kruskal-Wallis test was performed. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Culture of human carcinoma cell lines FaDu and HLaC 87 with WF. The cultivation of FaDu and HLaC78 with WF was possible. The cancer cells were cultivated with WF at different concentrations. WF at a concentration of 40% induced the highest proliferation in FaDu and HLaC78. Higher and lower concentrations exhibited reduced cell proliferation (data not shown). Therefore, further experiments were conducted with 40% WF. Cancer cell proliferation was confirmed using Ki-67 staining, as the expression of Ki-67 is associated with cell division (24). Ki-67 staining revealed an enhanced number of Ki-67 positive cells cultured with WF compared with RPMI-EM (Fig. 1). Microscopy also revealed vital cells in a monolayer as well as in spheroid configuration (Fig. 2).

Cytokine analysis of WF. The dot blot assay demonstrated that WF is comprised of a variety of different cytokines and growth factors. A table was used in order to assign the different dots to corresponding cytokines (Fig. 3).

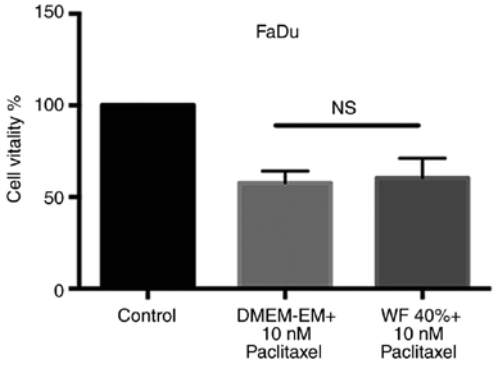


Figure 4. Evaluation of chemoresistance. FaDu cells were cultivated with WF and treated with 10 nM paclitaxel for 24 h. In order to identify differences in cell viability, an MTT assay was performed. This test revealed no significant differences in cell viability following paclitaxel treatment between the cultivation of FaDu with 40% WF compared with the control group. WF, wound fluid.

Certain cytokines are responsible for inflammation, e.g., tumor necrosis factor-α and -β. IL-6 showed the highest density. Several anti-inflammatory cytokines were represented as well. These cytokines are IL-6, IL-10, IL-13 and transforming growth factor-β. Factors that induce chemotaxis (25), such as monocyte chemotactic protein (MCP)-1, MCP-2, MCP-3 and IL-8, and factors responsible for angiogenesis such as vascular endothelial growth factor, angiogenin, insulin-like growth factor-1, IL-7, growth-regulated oncogene (GRO), GRO-α and platelet-derived growth factor-BB wee also identified (Fig. 3).

Analysis of chemoresistance. FaDu and HLaC78 exhibited enhanced proliferation following cultivation with 40% WF compared with RPMI-EM. Due to the high number of cytokines and growth factors, the investigation of a resistance induction toward chemotherapeutic substances seemed worthwhile. Therefore, FaDu were cultivated with WF for 24 h. After 24 h, the cells were treated with 10 nM paclitaxel. Previously, the IC_{50} (10 nM) of paclitaxel was investigated in FaDu (data not shown). The MTT assay revealed no significant differences between WF compared with control after paclitaxel treatment (Fig. 4).

Three-dimensional invasion assay. A possible alteration in the cell invasion activity was investigated using the three-dimensional invasion assay. RPMI-EM served as a control. To determine the migration area, spheroids were imaged directly after being transferred (Fig. 5A and B). In this condition, cells were able to spread out from the spheroids. After 24 h, the cells were photographed again (Fig. 5C and D). WF induced an enhancement in cell motility. The invasion area of cells cultivated with WF was significantly higher compared with the control (Fig. 5E).

Analysis of STAT 3 activation via western blotting. The highest IL-6 signal was the observed in the WF. In order to investigate the activation of STAT3 by WF, a western blot analysis was performed. The western blotting revealed an enhanced phosphorylation of STAT3 in FaDu and HLaC78 following cultivation with WF compared with cultivation with RPMI-EM (Fig. 6). α-tubulin was used as control.

Discussion

Wound healing begins directly after surgery with a modification of the microenvironment via a well-orchestrated interaction between cells, cytokines and growth factors. The process of normal wound healing is dynamic and divided into 4 overlapping phases: Hemostasis, inflammation, proliferation, and remodeling (26,27). The first phase is activated by the endothelial vasoconstriction and clotting cascade. Furthermore, the secretion of pro-inflammatory cytokines and growth factors is induced (28). The inflammatory phase starts immediately, and the migration of cells such as neutrophil granulocytes, monocytes and MSCs is induced (28,29). This is followed by the proliferation phase and the remodeling phase.

Notably, growth factors involved in wound healing can also promote cancer progression and metastasis. Platelet-derived growth factor (PDGF), for example, has an important role in each stage of wound healing (30). Solid cancers express PDGF-receptors, and the stimulation of these receptors may promote carcinogenesis (31). In cases of incomplete tumor-removal during surgery, PDFG potentially comes into contact with non-resected cancer cells, which may lead to the enhancement of cancer cell proliferation (32).

Additionally, GRO has an important role in wound healing by modulating cell migration and angiogenesis as well. In particular, GRO-α seems to promote cancer proliferation, angiogenesis and metastasis (33,34). The dot blot assay revealed that wound fluid (WF) contains several factors that have mitogenic effects on cancer cells. However, it revealed the presence of granulocyte-macrophage colony-stimulating factor (GM-CSF) as well. GM-CSF tends to induce apoptosis and drug sensitization in cancer cells. A previous study demonstrated that GM-CSF induced drug sensitization in breast cancer cells (35). Increasing GM-CSF in the cancer milieu may be a suitable therapeutic regime in cancer treatment.

One of the most important cytokines identified in WF is IL-6. IL-6 is a pleiotrophic cytokine and has an important role in inflammation, immune response hematopoesis and oncogenesis (36). There is an association between inflammatory diseases, e.g. Crohn's disease and malignant neoplasia, and particularly cancer of the head and neck, and high levels of IL-6 (37,38). The signaling cascades induced by IL-6 depend on targeted cell receptors. One of the most important signals activated by IL-6 is the janus kinase/STAT pathway. STAT proteins are involved in several signaling pathways. There are 7 different STAT family members (STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, and STAT6) (39). IL-6 is the most potent activator of STAT3 (40). As WF contains IL-6 in high concentrations, it seemed worthwhile to investigate whether WF induces enhanced activation of STAT3 in cancer cells. The western blot assay revealed a strong activation of STAT3 by WF compared with DMEM-EM. Several other factors such as IL-10, epidermal growth factor (EGF) and PDGF are also potential activators of STAT3 (41-43). WF contains a variety of different growth factors for the activation of STAT3. There may be synergistic effects between these factors with respect to the induction of STAT3 activation, which in turn leads to an enhanced proliferation of cancer cells.

In the present study, the cancer cells exhibited enhanced motility following cultivation with WF. The reason for this enhancement may be the presence of chemokines such as

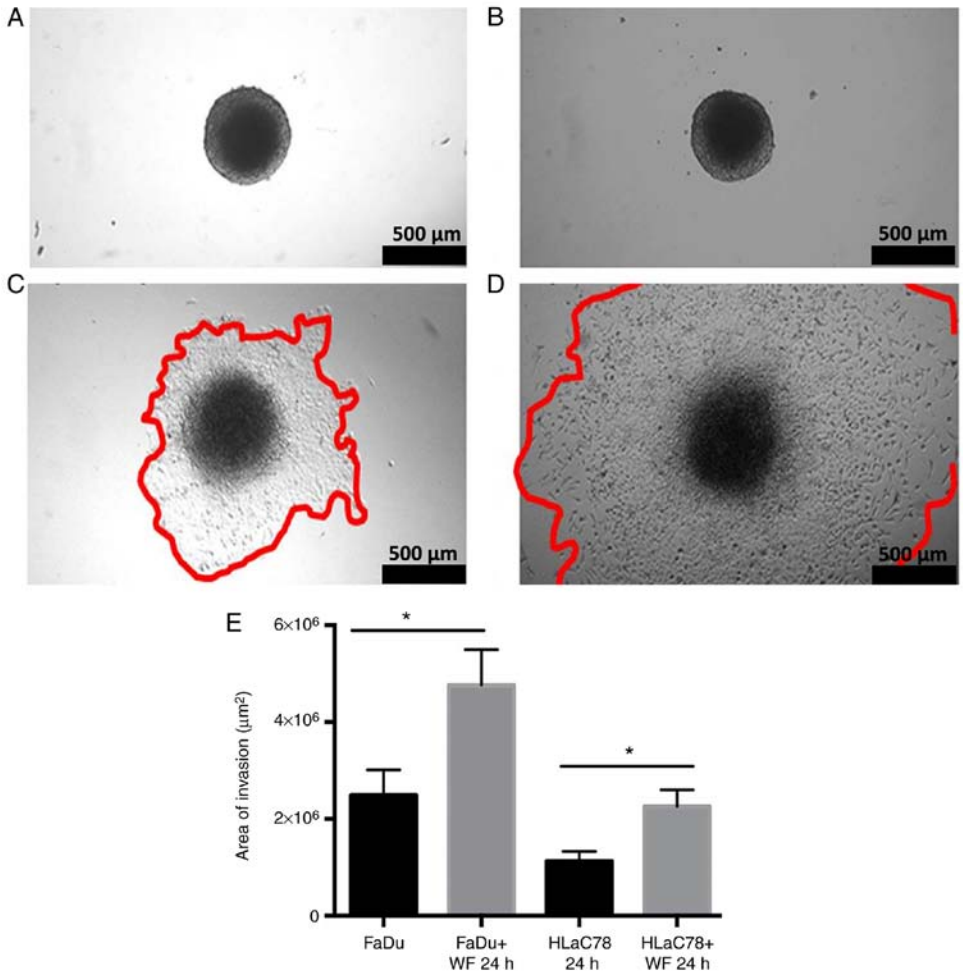


Figure 5. Cancer cell motility. Spheroids made of cancer cell lines were cultivated in (A) RPMI-EM and (B) 40% WF. Then, they were transferred to well plates without coating in an adherent condition. After 24 h, the migration area of (C) Fadu cultivated in RPMI-EM and (D) Fadu cultivated in 40% WF was measured using ImageJ software. (E) The migration capability of FaDu and HLaC78 was enhanced significantly after cultivation with 40% WF compared with cultivation with RPMI-EM. RPMI-EM, RPMI-expansion medium; WF, wound fluid.

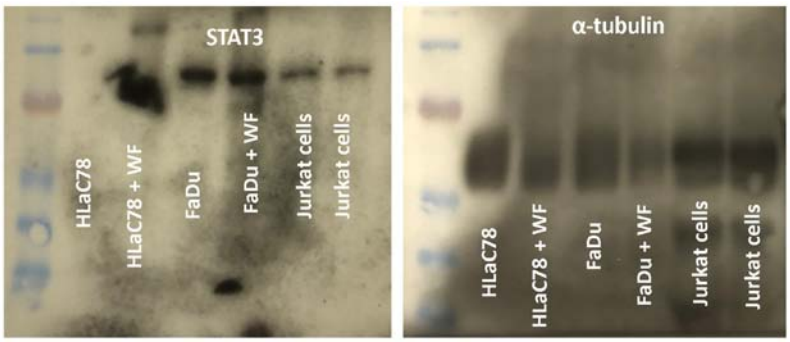


Figure 6. STAT3 expression following WF treatment. The expression levels of STAT3 were examined by western blot analysis in FaDu and HLaC78 treated with WF. The western blotting revealed the expression of STAT3 in FaDu and HLaC78 after cultivation in RPMI-EM and in WF. The expression STAT3 was enhanced in cells cultivated with WF. α-tubulin was used as a loading control. STAT3, signal transducer and activator of transcription factor 3; WF, wound fluid.

chemokine (C-C motif) ligand 5 (CCL5) in WF. Chemokines are produced and secreted by the majority of cell types and induce cell migration and various physiological and pathological processes (44). A variety of chemokines are produced during wound healing. In an experiment conducted by Karnoub *et al*, breast cancer cell motility was enhanced and promoted via secretion of CCL5 from MSC. By adding anti-CCL5, the enhancement of cell motility was counteracted (45). The dot blot assay revealed a strong secretion of CCL5. The contact between residual cancer cells and CCL5 may support cancer cell motility and metastasis. Besides the cytokines and growth factors, lipid acids serve an important role in cancer progression. Surgery may result in microenvironmental stress due to acidosis. The reason for acidosis after surgery are hypovolemia, hypoperfusion and lactic acidosis (46). According to Corbet and Feron (35)

and Menard *et al* (47,48), acidosis and hypoxia result from an accumulation of lipoproteins. This is associated with increased spheroid-formation capacity *in vitro* and enhanced metastatic potential of cancer cells *in vivo*.

Additionally, other groups have conducted the cultivation of breast cancer cells and WF as well. Wang *et al* (49) recently demonstrated an enhanced proliferation and migration capacity of breast cancer cells following cultivation with WF. They revealed the presence of several cytokines and growth factors. However, the evaluation of signaling cascades was not conducted. Licitra *et al* (50) previously investigated the stimulation of EGF receptor (EGFR)-positive residual cancer cells after surgery in the head and neck cancer. They demonstrated an enhanced cell proliferation in EGFR-positive cancer cells, which was inhibited by adding anti-EGFR reagents. The present study demonstrated an enhanced secretion of IL-6 in the WF. The activation of STAT3 via IL-6 may be one of the main reasons for the enhancement of cell proliferation.

The interval between surgery and postoperative radiation therapy is usually 4-6 weeks. During this period, residual cancer cells may recover and form novel tumor manifestations and early metastases. The delayed adjuvant therapy may not target these metastatic cells, which attenuates their survival prognosis significantly. Most cancer cells in the head and neck express epidermal EGFR (51). The EGFR pathway modulates cancer proliferation and metastasis and cancer survival. Sano *et al* (52) previously postulated that the reason for local-regional failure of oral squamous cell carcinoma may be due to the activation of the EGFR pathway in residual cancer cells during wound healing. Hence, the administration of an anti-EGFR monoclonal antibody such as cetuximab may be valuable. Other monoclonal antibodies such as bevacizumab may result in the inhibition of early vasculogenesis. However, bevacizumab is also associated with multiple complications involved in wound healing and wound infection (53), which may delay the administration of planned adjuvant therapy and counteract the survival prognosis.

In conclusion, enhanced cancer cell proliferation after cultivation with WF was demonstrated in the present study; this was achieved via activation of the STAT3 signaling pathway. Furthermore, WF supported cancer cell motility. However, enhanced resistance to paclitaxel, was not observed. Overall, the present findings emphasize the importance of WF in cancer cell proliferation and motility during wound healing. Future studies should focus on the inhibition of mitogenic factors after cancer surgery in order to prevent early metastasis and cancer recurrence.

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Availability of data and materials

The datasets analysed during the present study are available from the corresponding author upon reasonable request.

Authors' contributions

AS and SH designed and conceived the study. AS, RE, SH, TM, PI and MB performed the experiments. The interpretation of the data was made by AS, SH and TG. RH and NK contributed to the writing of the manuscript and were involved in data interpretation. TG, AS and RE collected the samples. All authors read and approved the manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics approval and consent to participate

Informed consent was provided by all patients. The study was approved by the Ethics Committee of the Medical Faculty of the University of Wuerzburg (Würzburg, Germany).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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