Cytokine Changes in Response to Radio-/chemotherapeutic Treatment in Head and Neck Cancer

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Abstract. *Background: Radiation and systemic chemotherapy are standard treatment strategies for advanced or metastatic head and neck cancer. However, little is known about the implications and changes in the tumor microenvironment, including the T-helper $(TH)_1/TH_2$ balance in response to these treatment regimens. The aim of the current study was to unravel the effects of chemotherapeutic drugs and radiation on cytokine changes. Materials and Methods: In this study, the effect of radiation and chemotherapeutic treatment (5-fluorouracil and cisplatin) on eight cell lines was determined. Before and after exposure, cytokine levels in culture supernatants of cell lines were evaluated using the Bio-Plex Assay (Bio-Rad) and the Human TH_1/TH_2 Cytometric Bead Array (Becton Dickinson). Results were correlated with parallel measurements for cellular proliferation assessed by cytotoxicity assay. Results: Seven out of eight cell lines of primary tumors or metastases demonstrated an enhanced level of the cytokines interleukin (IL)-1β, IL-6, IL-8, granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage-colony stimulating factor (GM-CSF) and tumor necrosis factor-α (TNF-α), after sub-lethal radiation doses. Under treatment with low concentrations of 5-fluorouracil and cisplatin, all examined cell lines showed an increasing secretion of the cytokines IL-6 and G-CSF. In contrast, sub-lethal doses of both cytostatic drugs revealed a dose-dependent decrease in secretion IL-1β. Regarding GM-CSF and TNF-α, we demonstrated an increase in secretion by the primary tumors under low doses of 5-fluorouracil and cisplatin, whereas the metastases showed a sharp drop of GM-CSF and TNF-α secretion. Chemotherapeutic treatment led to no changes of the IL-8 cytokine profile. Conclusion: The results suggest complex cytokine changes of the tumor microenvironment and more aberrant expression profiles under treatment with radiation and the chemotherapeutic drugs 5-fluorouracil and cisplatin.*

Squamous cell cancer of the head and neck (HNSCC) is still one of the ten most frequent neoplasms worldwide (1). High failure rates, development of recurrence and distant metastases are often observed in patients with advanced HNSCC undergoing conventional radiochemotherapy (2, 3). In order to improve the therapeutic options and patient survival rates, it is necessary to understand the molecular mechanisms and the changes occurring under radiochemotherapy. The microenvironment in HNSCC extensively affects immune functions on distinct levels due to tumor-induced production of numerous immunosuppressive mediators (4). Investigations with primary cultures of human HNSCC cells indicated high secretion of numerous cytokines involved in indirect modulation of immune responses and pro-angiogenic processes (5, 6). Prominent HNSCC-derived cytokines are interleukin (IL)-4, IL-6, IL-8, IL-10, granulocyte macrophage colony-stimulating factor (GM-CSF), vascular endothelial growth factor (VEGF), prostaglandin E_2 (PGE_2), as well as basic fibroblast growth factor (bFGF) (5, 7-10). Table I summarizes HNSCC-relevant cytokines and their attributed cellular functions.

In relation to the immune response, there are two major subtypes of cytokines: T-helper $(TH)_1$ and TH_2. The activation of TH_1 cells is associated with a secretion of interferon-γ (IFN-γ), IL-2 and IL-12, initiating a cellular-dominated immune response. TH_2-corresponding cytokines are IL-4, IL6, and IL-10, leading to a humoral dominated immune response. In healthy individuals, there is a balance between these two kinds of immune responses (31).

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Key Words: HNSCC, cytokines, radio-/chemotherapeutic treatment head and neck cancer.

Table I. *Head and neck squamous cell cancer-relevant cytokines and their attributed functions.*

Cytokine	Attributed cellular function	Reference
Basic fibroblast growth factor (bFGF)	Angiogenesis, metastasis	(11, 12)
Granulocyte colony-stimulating factor (G-CSF)	Angiogenesis, proliferation, migration, inflammatory cell recruitment	(11-13)
Granulocyte macrophage colony-stimulating factor (GM-CSF)	Angiogenesis, proliferation, migration, inflammatory cell recruitment, $CD34^+$ cell mobilization, immunosuppression	(11-14)
Hepatocyte growth factor (HGF)	Angiogenesis	(11, 15)
Interleukin-1 (IL-1)	Cytokine secretion (IL-4, IL-6, GM-CSF)	(5)
Interleukin-1α (IL-1α)	Angiogenesis, proliferation, cell survival, gelatin production	(7, 16, 17)
Interleukin-1β (IL-1β)	Tumor progression, resistance to NK cells, metastasis, gelatin production, regulation of SNAIL and E-cadherin	(8, 17-20)
Interleukin-4 (IL-4)	Immunosuppression	(6)
Interleukin-6 (IL-6)	Inflammation regulation, anti-apoptosis, proliferation, invasion, tumorigenesis	(6, 10, 21-23)
Interleukin-8 (IL-8)	Angiogenesis, tumor growth	(7, 10, 24)
Interleukin-10 (IL-10)	Immunosuppression	(6, 10)
Macrophage migration inhibitory factor (MIF)	Growth regulation	(25)
Platelet-derived growth factor (PDGF)	Angiogenesis	(11, 12)
Prostaglandin-E2 (PGE2)	Immunosuppression	(26, 27)
Transforming growth factor-β (TGF-β)	Immunosuppression	(26)
Tumor necrosis factor-α (TNF-α)	Tumor development, gelatin production	(17, 28, 29)
Vascular endothelial growth factor (VEGF)	Angiogenesis, proliferation, metastasis chemoattraction of $CD34^+$ cells	(11, 12, 30)

Cytokine secretion profiles of patients with HNSCC show a bias from TH_1 to TH_2 and more aberrant expression profiles with more advanced disease resulting in inhibition of cellular immune response (32, 33). This TH_2-mediated immunity has prognostic implications, as demonstrated in other carcinoma, increased expression of TH_1 cytokines are associated with a favorable prognosis (34). Furthermore, a dendritic cell-based cancer-specific immunotherapy, derived from donors with advanced malignant disease exhibiting TH_2-dominant immunity, as a new potent strategy for various types of carcinomas, showed an impairment of antitumor immune response (35). In this study, we analyzed the influence of radio- and chemotherapeutic treatment on the progression and cytokine secretion characteristics in permanent HNSCC cell lines generated from primary HNSCC and corresponding metastases.

Materials and Methods

Cell culture. The HNSCC cell lines used here were generated on the one hand from primary tumors: a hypopharyngeal carcinoma (*PCI-I*; Pittsburgh Cancer Institute, PA, USA) and an oropharyngeal carcinoma (BHY; Deutsche Sammlung für Mikroorganismen und Zellkulturen, Braunschweig, Germany). On the other hand, the tumor lines designated 'UT-SCC', all established at the Department of Otorhinolaryngology, Head and Neck Surgery (University of Turku; Turku, Finland), were paired and generated from the primary (A) and metastatic lymph nodes (B) of the same patients: a cutis nasi tumor (UT-SCC 12A/B), a left tonsillar carcinoma (UT-SCC 60A/B) and a tongue carcinoma (UT-SCC 74A/B). The cells were cultured in serum-free high-glucose (DMEM; PAA, Pasching, Austria) supplemented with 2.5% HEPES buffer, 1% sodium pyruvate, 1% L-glutamine, 1% nonessential amino acids. All the compounds were endotoxin tested. Incubation was carried out in a humidified atmosphere of 5% CO_2 at 37˚C until there was a sub-confluent layer of cells before sub-culturing. Cells were detached from culture flasks by trypsination, centrifuged (200xg, for 8 min at 30˚C) and resuspended in culture medium as mentioned above.

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide-based cell assay (MTT). Cell proliferation was determined by a quantitative colorimetric MTT assay. This assay determines viable cell numbers based on the mitochondrial conversion of MTT. 5,000 cells were dispersed into each well of a 96-well plate. Twenty-four hours after culture, four different concentrations of 5-flourouracil or cisplatin were added to the cultures, or the cells were radiated at three doses. For comparison, untreated cell cells from each cell line were cultured simultaneously.

After 144 h 10 μl of MTT dye (five mg/ml) was added to each well. After two hours of incubation with MTT, crystals were solubilized and gently shaken for twenty-four hours at room temperature. The absorbance of the reduced formazan product in control and experimental wells was read using a multi-well ELISA reader at a wavelength of 570 nm and 690 nm.

Expression profiles of cytokine and growth factors in cell culture supernatants. Investigating cytokines and growth-factors in supernatants collected from the cell-lines mentioned above, we measured the concentrations of the prominent TH_1-like and the TH_2-like cytokines, as well as the growth-factors G-CSF and GM-CSF using two commonly used bead-specific assays: the Bio-Rad Bio-Plex Assay (Bio-Rad-Laboratories) and the flow cytometry-based Human TH_1/TH_2 Becton Dickinson Cytometric Bead Array (CBA; BD Biosciences). The principle of these methods are similar to a capture sandwich immunoassay. 250,000 cells were seeded into cell culture flasks (25 mm^2) and quadruplicate aliquots

Table II. *Detection of cytokines in the supernatant of eight head and neck cancer cell lines.*

	Cytokines								
Bio-Plex system™	GM-CSF	IFN-γ	IL-2	IL-4	IL-5	IL-10	IL-12	IL-13	TNF-α
Detection	+	–	–	–	–	–	–	–	+
Flex-Set system™	G-CSF	GM-CSF	IL-1β	IL-6	IL-8				
Detection	+	+	+	+	+				

+: ≥5 pg/ml/10^6 cells; –: <5 pg/ml/10^6 cells.

of 200 μl supernatants of control and experimental samples were extracted on ice after 144 h and then stored at –80˚C before analysis. Regular testing of the supernatants was performed to check for bacterial contamination to exclude that the observed effect was not a consequence of microbial contamination. Furthermore, no cytokines were detected neither in cell-free DMEM nor in cytostatic supplements. The cytokine-specific Bio-Plex Human TH_1/TH_2 Assay Kit detecting IL-2, IL-4, IL-5, IL-10, IL-12, IL-13, IFN-γ, GM-CSF and Tumor necrosis factor-α (TNF-α) was purchased from Bio-Rad (Hercules, CA, USA) and used according to the manufacturer's instructions. Fifty microliters of a serial dilution of the standards for the standard curve and 50 μl of the undiluted sample supernatants and control samples after thawing on ice, respectively, were added to a 96-well microtiter plate with beads coated with the corresponding antibodies. Plates were incubated on a plate shaker for 120 min. After unbound cytokines are removed by washing and filtration, biotinylated detection antibodies were added to the reaction. The complexes were detected by the addition of streptavidin-phycoerythrin (streptavidin-PE) by a special microtiter plate reader (Bio-Rad Laboratories GmbH, Munich, Germany). Corresponding data were analysed with the Bio-Plex software manager which automatically calculates the concentration of cytokines from a standard curve. The CBA Human Flex Set (BD Biosciences, San Jose (CA, USA) combines the principles of the sandwich immunoassay with flow cytometry. In our study this array was used for the detection of IL-1β, IL-6, IL-8, GM-CSF and G-CSF. Initially, the bead populations had to be identified in the fluorescence channels APC-Cy7 and APC of the flow cytometer. During the incubation of the beads with a total of 50 μl of cell culture supernatant for 60 min, the cytokines in the supernatant were bound by their corresponding beads. PE-labeled detection antibodies were added to the cytokine-capturing beads to form sandwich complexes. Following incubation and washing, the samples were acquired using a FACSCanto instrument and the results were analyzed with the FCAP Array Software provided by BD Biosciences. For this method, standard curves were also generated for each cytokine and the concentration for each cytokine in samples was also determined by interpolation from the standard curves.

Statistical analysis. The cell viability data were calculated from the density readings at wavelengths of 570 nm and 690 nm. Growth under treatment with cytostatic agents and irradiation was calculated relating to that for control wells (normalized to 100%) and data are represented graphically as the mean±SD in a fitted curve. All growth samples of the MTT assay were carried out in triplicate. When the levels of cytokines were below the limit of detection for each method, the value was considered as zero in the analysis. By MTT assay, the cell number in each culture flask was determined to standardize the concentration of cytokine as that secreted per 10^6 vital tumor cells.

Results

Investigating the patterns of TH_1/TH_2 cytokines in terms of the relative balance between TH_1 and TH_2 profiles and their subsequent polarization toward either TH_1 or TH_2 in response to radio-/chemotherapeutic treatment, we measured 14 cytokines in the supernatants from each cell line. Detection of the tested cytokines in the cell culture supernatants by each method are shown in Table II.

As shown in Table II we detected G-CSF, GM-CSF, IL-1β, IL-6, IL-8 and TNF-α. The supernatant cytokine concentration for IFN-γ, IL-2, IL-4, IL-5, IL-10, IL-12 and IL-13 was below the detection limit (<5 pg/ml/10^6 cells), both in supernatants from cells after cytotoxic exposure and from control cells. For this reason, there is no further mentioning of these cytokines other than in Table II.

Cytokine profiles of cells without treatment. In the supernatant of the eight untreated HNSCC cell lines, we detected GM-CSF and TNF-α using Bio-Plex system™ (Bio-Rad-Laboratories) as well as G-CSF, GM-CSF, IL-1β, IL-6 and IL-8 using the Flex-set-system™ (BD Biosciences), at different concentrations (Table III).

Table III shows a heterogenous profile of cytokines found in HNSCC cell lines, regarding both cytokine type and concentration. Furthermore, small differences were found due to the different methods. The cytokines G-CSF, GM-CSF and IL-1β were detected for all cell lines, except for PCI-I and UT-SCC 74B. While GM-CSF was not found in UT-SCC 74B cells using the Bio-plex-systemTM method, the Flex-set-system™ found a detectable amount of GM-CSF. All cell lines secreted IL-6 and IL-8, whereas TNF-α was only found in the supernatant from PCI-I, UT-SCC 60A, UT-SCC 60B and UT-SCC 74B cells. The cell line UT-SCC 60B secreted a remarkably high concentration of TNF-α (7300.4 pg/ml/10^6 cells), which was much higher than the concentration of the other three cell lines (PCI-I: 2.4 pg/ml/10^6 cells, UT-SCC 60A: 7.2 pg/ml/10^6 cells, UT-SCC 74B: 29.0 pg/ml/10^6 cells).

Table III. *Secretion of cytokines by HNSCC cell lines after 144 h of culture.*

	Cytokine						
	Bio-Plex system™		Flex-Set system™				
Cell line	GM-CSF	TNF-α	G-CSF	GM-CSF	IL-1β	IL-6	IL-8
BHY	++	–	+++	++	++	+++	++++
PCI-I	-	+	–	–	–	+	+++
UT-SCC 12A	+++	-	+	+++	++	++	++++
UT-SCC 12B	++++	-	+++	+++	+++	++++	++++
UT-SCC 60A	++	+	+++	+++	++	++++	++++
UT-SCC 60B	++++	++++	++++	++++	+++	++++	++++
UT-SCC 74A	+++	–	++++	+++	+++	++++	+++++
UT-SCC 74B	-	++	+	++	+	+++	++++

Cytokine secretion in pg/ml/10^6 cells: –: < 5; +: 5-10; ++: 10-100; +++: 100-1,000; ++++: 1,000-10,000; +++++: 10,000-100,000.

Cytokine profiles of cell lines under treatment with 5-fluorouracil. The eight cell lines were cultured for 144 h with four different concentrations of 5-flurouracil (10, 50, 100, 200 μM). The effect of different concentrations of 5-fluorouracil on cytokine secretion is shown in Figure 1A for the cell line UT-SCC 60A. Highlighting differences between primary tumors and metastases, a combined description of UT-SCC 60A and 60B is shown in Figure 1B and C. As shown in Figure 1, the addition of increasing concentrations of 5-fluorouracil modified the concentration of IL-1β in supernatants, in terms of diminishing values. In contrast, sub-lethal concentrations of the cytostatic drug stimulated the secretion of G-CSF and IL-6 in all screened HNSCC cell lines. Concerning GM-CSF and TNF-α, we observed differentially-modulated cytokine patterns after cytostatic exposure: sub-lethal concentrations of 5-fluorouracil induced an increase in GM-CSF and TNF-α in the primary tumor cell line (Figure 1B and C, UT-SCC 60A), whereas the metastastic cell line reacted with a dose-dependent decrease. The same cytokine response towards 5-fluorouracil was observable in the cell line pair UT-SCC 12A and B, but not in UT-SCC 74A and B (data not shown). Unlike the other cell line pairs, both UT-SCC 74A and B reacted to sub-lethal concentrations of 5-fluorouracil with increasing secretion of GM-CSF (data not shown). UT-SCC 60A/B is the only pair for which both cell lines secrete TNF-α. Interestingly, the primary tumor cell line PCI-I also exhibited a similar expression pattern to that of UT-SCC 60A, and the metastastatic cell line UT-SCC 74B demonstrated a pattern similar to that of UT-SCC 60B (Figure 2A). There was no response after exposure to 5-fluorouracil for the cytokine IL-8 (Figure 1A).

Cytokine profiles of cell lines under treatment with cisplatin. The eight cell lines were cultured for 144 h with four different concentrations of cisplatin (1, 10, 20, 50 μM). Figure 1 demonstrates the dose-dependent cytotoxicity in combination with the correlating cytokine secretion. All screened HNSCC cell lines showed a dose-dependent decrease of IL-1β under cisplatin treatment, whereas a sub-lethal dose induced an increased secretion of G-CSF and IL-6 (Figure 1D). Furthermore, we observed a difference between the cytokine secretion of the primary tumor cell lines and metastatic cell lines for GM-CSF and TNF-α. Low concentrations of cisplatin caused an increase of GM-CSF and TNF-α by the primary tumor cell lines, while the metastatic cell lines reacted with a concentration-dependent decrease of these cytokines (Figure 1E and F). This expression response was found in the cell lines UT-SCC 12A/B, UT-SCC 60A/B, and UT-SCC 74A/B (data not shown), similarly. Apart from the cell lines UT-SCC 60A and B, the cytokine TNF-α was only measurable in supernatants of two other cell lines: PCI-I and UT-SCC 74B. The primary tumor cell line PCI-I reacted to treatment with cisplatin by secreting rising amounts of TNF-α, similarly to UT-SCC 60A, whereas the metastatic cell lines UT-SCC 60B and 74B showed a dose-dependent decrease of TNF-α (Figure 2B). Under treatment with cisplatin, IL-8 was also measurable. However, similarly to the treatment with 5-fluorouracil, the concentration of IL-8 was no altered by exposure to cisplatin (Figure 1D).

Cytokine profiles of cell lines under radiation. The eight cell lines were irradiated at three different doses (6, 12, 18 Gy) and cultured for 144 h. As previously, the trend of the cytokine concentrations in the supernatants are exemplarily shown by the cell line pair UT-SCC 60A and B (Figure 1J, K, L). Following radiation, we observed different cytokine modulations to these findings under chemotherapy. All examined cytokines were increased under sub-lethal radiation doses. Only the UT-SCC 60B cell line was an exception, with decreasing secretion of GM-CSF and TNF-α.

Discussion

Recent data confirmed that patients with HNSCC had a partial TH_2 cytokine bias and a more aberrant cytokine expression with more advanced disease (32). This TH_2 phenotype included enhanced levels of the TH_2 cytokines IL-4, IL-6 and IL-10, as well as diminished levels of the TH_1 cytokine IFN-γ. Since levels of the TH_1 cytokines IL-2 and GM-CSF are also elevated, this bias towards the TH_2 phenotype is incomplete (32). Individual characteristics and cytokine expression levels of patients with HNSCC are critical factors for prognosis and efficiency of anti-tumor therapy. The differences are most likely due to specific half-life kinetics, metabolism, or binding protein modulation parameters (4, 36, 37). The rare early tumor stages I and II are treated primarily with radiation and surgery, whereas patients with advanced cancer (stage III and IV) often receive a multi-modal therapy consisting of surgery, radiation and chemotherapy. As most carcinomas are diagnosed in advanced stages, radiochemotherapy represents an essential part of most treatments (38). Radiochemotherapy leads to various changes, not only in the radiated area, but also for the whole body. The systemic cytokine changes have been partly described in a few studies. However, there have been no investigation of cytokine behavior under chemotherapy without radiation. Most studies included the cytokines IL-6 and IL-8, which showed an increasing blood concentration under radiochemotherapy (39, 40). Silver observed an increase in concentration of IL-1β and IL-10 four weeks after radiochemotherapy with paclitaxel and carboplatin (40). Meirovitz also IL-1, IL-10 and TNF-α examined in addition to IL-6 and IL-8. Two weeks after a radiochemotherapy regimen consisting of cisplatin, carboplatin, 5-fluorouracil and docetaxel, concentrations of IL-6 and IL-8 increased, but not these of IL-1 and IL-10. Both cytokines showed no changes after the combined treatment. In contrast to IL-6 and IL-8, serum concentrations of TNF-α decreased under radiochemotherapy (39). These investigations illustrate that the cytokine profiles of patients with HNSCC under radiochemotherapy are more aberrant and the bias from TH_1 to TH_2 more intensified. *Ex vivo* studies are even rarer: only one publication showed that cisplatin induced IL-6 expression and tumorigenicity (41). The behavior of the other cytokines and the local changes of the micromilieu are still unknown. Hence, we investigated the expression patterns of 14 cytokines in eight HNSCC cell lines under treatment with radiation and chemotherapeutic drugs. As described in the literature we also observed an increase in some cytokine secretions in response to radio- or chemotherapeutic treatment. A decrease of secretion of IL-1β was measurable under chemotherapy with both cytostatic drugs used here. In contrast to the serum cytokine data of Silver, we demonstrated enhanced expression of IL-1β under radiation. In HNSCC, increased secretion of IL-1β has been implicated in tumor progression, resistance to natural killer cells and up-regulation of SNAIL (19, 20). The transcription factor SNAIL, in turn, suppressed E-cadherin expression and enabled a complete epithelial mesenchymal transition in HNSCC (42, 43). IL-6, which is a multi-functional regulator of immune response and highly expressed in HNSCC, was secreted in rising amounts under radiation and treatment with 5-fluorouracil and cisplatin (21). The same observation was described by Meirovitz *et al*. (39). As an exception to our measurements, only the metastatic cell line UT-SCC 60B reacted to radiation with decreased secretion of IL-6. A modulated expression of this cytokine has implications on its suggested function in HNSCC, such as cell proliferation, tumorigenesis and invasive potential as the first step of tumor metastasis (22, 23). In addition to IL-6, we also detected IL-8, another prominent HNSCC-derived cytokine. IL-8 showed no response to exposure to 5-fluorouracil and cisplatin. In contrast, radiation induced increasing expression of this cytokine. IL-8 is known to promote angiogenesis and tumor growth activity and is elevated in the serum of patients with HNSCC after combined radiochemotherapy (7, 39). The secretion of G-CSF showed a relatively uniform behavior towards anticancer therapy: all investigated therapies, radiation as well as a treatment with 5-fluorouracil and cisplatin at sublethal doses, resulted in stimulation of cytokine secretion. It has been shown that G-CSF, as well as GM-CSF, promote tumor progression, angiogenesis and the recruitment of inflammatory cells in HNSCC (13). Recently, studies in other tumors have revealed G-CSF to be an efficient agent for promoting cycling of dormant hematopoietic stem cells and cancer stem cells (44, 45). For the G-CSF-related cytokine GM-CSF, we observed an interesting progression in most studied cell lines: low concentrations of 5-fluorouracil and cisplatin caused increased secretion of GM-CSF in primary tumor cell lines, whereas the metastatic cell lines reacted with a concentration-dependent decrease of this cytokine. Similarly, all examined cell lines showed enhanced secretion of GM-CSF under sub-lethal radiation with one exception, a concentration-dependent decrease was measurable by the metastatic cell line UT-SCC 60B. We are unaware of any studies on HNSCC that included G-CSF or CM-CSF and their changes in response to radiochemotherapy. The cytokine-modulating effect that we measured, has an impact on the functions of GM-CSF, such as in angiogenesis, proliferation, migration, and inflammatory cell recruitment, as well as $CD34^+$ cell mobilization (13, 14). Another analyzed cytokine was TNF-α, which revealed changes of secretion under radio-/chemotherapy. Only in the supernatants of four cell lines did we detect the pro-inflammatory cytokine TNF-α. Under treatment of radiation and chemotherapy with 5-fluorouracil and cisplatin, we measured enhanced levels of TNF-α in the two primary tumor cell lines, whereas the two

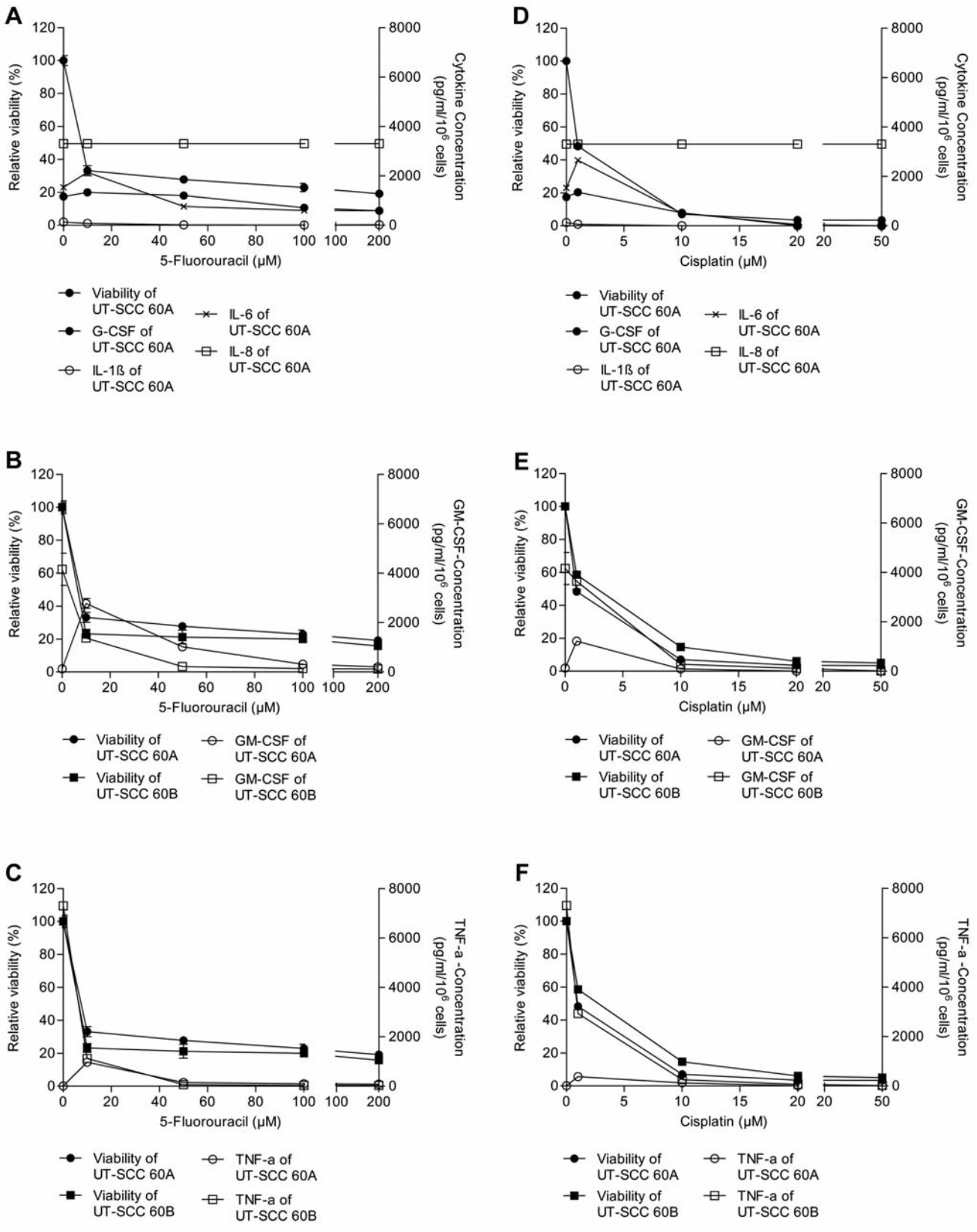

Figure 1. *continued*

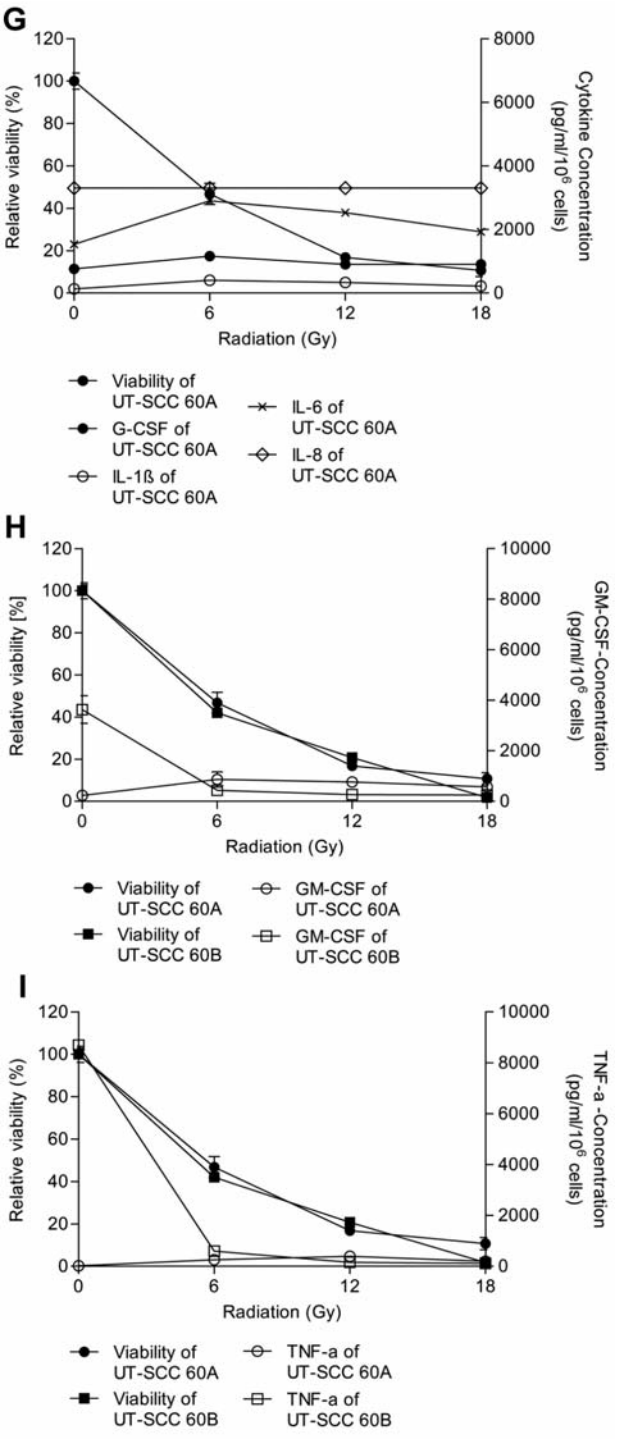

Figure 1. *Dose-dependent cytotoxicity and cytokine secretion of the cell lines UT-SCC 60A and B under treatment with 5-fluorouracil, cisplatin, and radiation. For clear presentation of the low concentration ranges, segmentation of the x-axis was added. A: Cytokines with similar concentration patterns in all cell lines, i.e. there were no differences between primary tumor (UT-SCC 60A) and the corresponding metastatic line (UT-SCC 60B). B and C: Different patterns of GM-CSF and TNF-α expression between primary tumor and metastatic cell lines. Data are means±SD; relative viability: n=3-6, GM-CSF: n=2, other cytokines: n=1.*

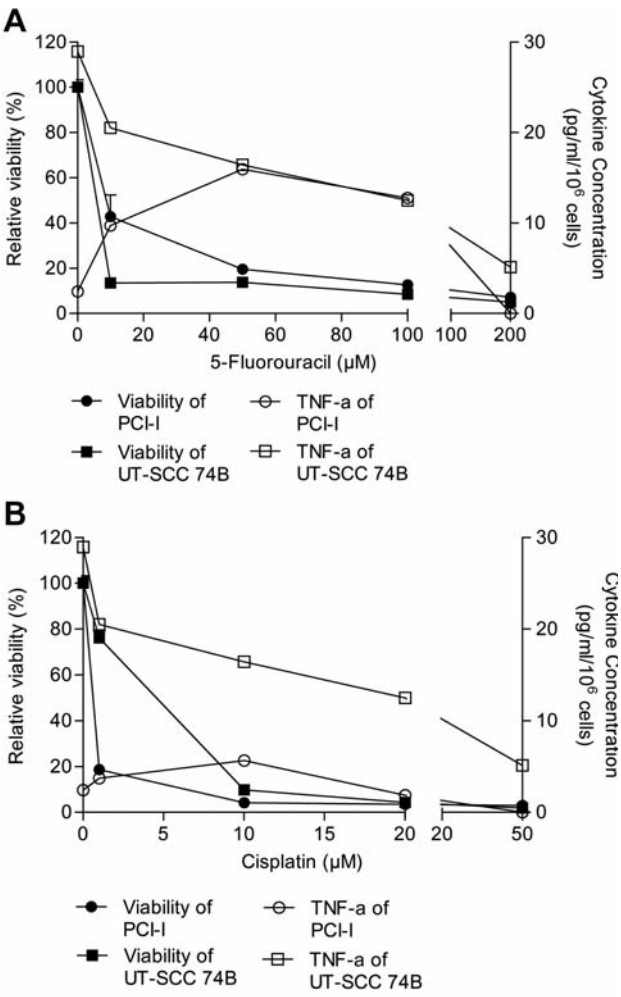

Figure 2. *Dose-dependent cytotoxicity and cytokine secretion of the cell lines PCI-I and UT-SCC 74B under treatment with 5-fluorouracil (A) and cisplatin (B) As shown in Figure 1, increasing concentrations of 5-fluorouracil caused an increased TNF-α level in the primary tumor PCI-I, as far as UT-SCC 60A in Figure 1, and a dose-dependent decrease of TNF-α in the metastatic cell line UTSCC 74B, similar to UT-SCC 60B in Figure 1. Data are means±SD; relative viability: n=3-6, TNF-α: n=1.*

metastatic cell lines showed a dose-dependent decrease of this cytokine. A drop of serum TNF-α in patients with HNSCC after radiochemotherapy has beend described (39). In HNSCC, TNF-α enhanced the enzymatic activities of matrix metalloproteinase-2 and -9, as well as cell invasion (29). These cytokine changes raise questions concerning the genesis of the differences. It is noticeable that only the UT-SCC 60B cell line reacted to radiation with reduced secretion of IL-1β, IL-6, GM-CSF and TNF-α, whereas all other cell lines increased cytokine levels under this kind of treatment. In addition, the cell line UT-SCC 60B also had the highest levels of IL-1β, IL-6, GM-CSF and TNF-α under untreated

conditions. Many studies demonstrated IL-4 and IL-10 to be expressed at low levels in primary HNSCC cultures as well as in permanent cell lines (5, 8, 10). In comparison, we did not measure significant levels (>5 pg/ml) of these cytokines. Summarizing, our results showed enhanced levels of the cytokines IL-6 and G-CSF, as well as decreased levels of IL-1β of primary tumor cell lines and their metastatic cell lines under sub-lethal chemotherapy with 5-fluorouracil and cisplatin. Furthermore, treatment of the primary tumor cell lines with both these cytostatic drugs resulted in increasing levels of GM-CSF and TNF-α, whereas the metastatic lines reacted with a dose-dependent decrease of these cytokines. Expression of IL-8 did not change under therapy with these chemotherapeutic drugs. Moreover, radiation of HNSCC cells led to increasing concentrations of all examined cytokines with only one metastatic cell line as an exception, which revealed a decreasing secretion of IL-1β, IL6, GM-CSF and TNF-α. These data strongly suggest that a radiochemotherapy leads to complex changes of the tumor microenvironment.

Competing Interests

The Authors declare that they have no competing interests.

Acknowledgments

We thank Professor Dr. Mandic, Department of Otolaryngology, Head and Neck Surgery, University of Marburg, Germany, for providing the UT-SCC cell lines and Professor Grénman for establishing these cell lines.

We thank Birgit Hüsing and Brigitte Wollmann for their skillful support in some parts of this work.

We are grateful to all members of the Department of Otorhinolaryngology for helpful discussions and a comfortable atmosphere. This work was supported by grants from the Mildred-Scheel-Stiftung (Deutsche Krebshilfe), the Werner-and-Klara-Kreitz-Stiftung, the Monika-Kutzner-Stiftung, and the Rudolf-Bartling-Stiftung.

We also thank Invitrogen Bio-Rad and Becton Dickinson for their technical support and in providing us with kits to test.

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Received April 5, 2013
Revised April 29, 2013
Accepted April 30, 2013