ANTICANCER RESEARCH 24: 325-332 (2004)

The Impact of Stromal Cell Contamination on Chemosensitivity Testing of Head and Neck Carcinoma

RALPH DOLLNER[1], CHRISTOF GRANZOW[2], BURKHARD M. HELMKE[3], ALEXANDRA RUESS[1], ARNO SCHAD[4] and ANDREAS DIETZ[1]

[1]*Department of Otorhinolaryngology, Head and Neck Surgery,* [3]*Department of Pathology and* [4]*Department of Medical Cell Biology, Institute of Anatomy and Cell Biology, University of Heidelberg, Heidelberg;* [2]*German Cancer Research Center, Department of Molecular Pharmacology, Heidelberg, Germany*

Abstract. *Background: Reliable chemosensitivity testing of head and neck squamous cell carcinoma (HNSCC) still faces methodical limitations. Since stromal cell contamination has been found to preclude reliable radiosensitivity testing of HNSCC as well as chemosensitivity testing of lung tumors, the present study investigates the impact of stromal cell contamination on chemosensitivity testing of HNSCC. Patients and Methods: Seventeen biopsies from HNSCC were analyzed. The specimens were investigated using an ex vivo colony formation assay which allows for the quantitative and separate determination of the overall, as well as the epithelial, and stromal response to carboplatin, 5-fluorouracil and docetaxel. Results: The overall chemoresponse was dominated by stromal cell multidrug resistance. However, by selective evaluation of the epithelial chemoresponse, individual chemosensitivity patterns could be identified. Conclusion: Multidrug-resistant stromal cells preclude the reliable assessment of the chemoresponse of HNSCC specimens. Careful correction for stromal cell effects is a prerequisite for the generation of therapeutically useful information.*

In vitro chemosensitivity testing of human malignancies for the prediction of individual chemoresponse has been the focus of many studies over the past decades (1-3). Recently, the role of chemotherapy in the first-line treatment of head and neck squamous cell cancer (HNSCC) has changed due to the introduction of primary chemoradiation as an alternative to primary surgery (4-6). The underlying concept of functional organ preservation through primary chemoradiation prompted secondary research on predictors to identify individual responders (7-11). The attempt to predict the individual response to chemoradiation has also renewed the discussion on a possible role of *in vitro* chemosensitivity assays in head and neck cancer. However, *in vitro* chemosensitivity testing of solid tumors still faces crucial methodical limitations (1,3). One of the most challenging aspects is the architecture of solid tumors *per se*: malignant epithelial and accompanying non-malignant stromal cells in different ratios and patterns.

Stromal cell contamination has been shown to preclude reliable radiosensitivity determination in HNSCC specimens using the clonogenic assay (12). Furthermore, high levels of multidrug resistance were observed in stromal cell colonies present in primary cultures from bronchoscopic lung tumor explants (13). The impact of such stromal contamination on chemosensitivity testing of HNSCC has not been addressed so far.

The present study addresses the impact of stromal cell contamination on the quantitative determination of HNSCC biopsies *ex vivo*. Therefore, an *ex vivo* colony formation assay was established which allows for parallel determination of the overall drug response, as well as the specific drug response of epithelial and stromal elements from HNSCC biopsies. Using this assay, the present study investigates whether stromal cell contamination influences the *ex vivo* identification of individual cytostatic drug response profiles in HNSCC specimens.

Presented in part at the 5th International Conference on Head and Neck Cancer, July 29th-August 2nd, 2000, San Francisco, California, U.S.A. and the 74th Annual Meeting of the German Society of Otorhinolaryngology, Head and Neck Surgery, May 28th-June 1st, 2003, Dresden, Germany.

Correspondence to: Ralph Dollner, MD, Dept. Otorhinolaryngology, Head and Neck Surgery, University of Heidelberg, INF 400, D-69120 Heidelberg, Germany. Tel: ++49-6221-566996, Fax: ++49-6221-564641, e-mail: ralph_dollner@med.uni-heidelberg.de

Key Words: Head and neck carcinoma, HNSCC, chemosensitivity testing, tumor stroma, *ex vivo* culture.

Patients and Methods

Patients and HNSCC specimens. Thirteen patients with histologically confirmed diagnosis of primary head and neck squamous cell carcinoma (HNSCC) were enrolled in this study (for

Table I. *Patients and tumor characteristics.*

Patient No	Age/ Gender	HNSCC localization	Stage (UICC 1997)	Biopsy site*
1	70 / m	Larynx	$T_4N_{2c}M_0$	P, LN
2	47 / m	Oropharynx	$T_2N2_cM_0$	P, LN
3	61 / m	Hypopharynx	$T_3N_0M_0$	P
4	75 / f	Hypopharynx	$T_4N_{2c}M_0$	P
5	74 / m	Hypopharynx	$T_2N_{2c}M_0$	P, LN
6	52 / m	Oropharynx	$T_3N_{2b}M_0$	P
7	61 / m	Hypopharynx	$T_3N_{2b}M_0$	P, LN
8	75 / f	Oropharynx	$T_3N_{2b}M_0$	P
9	72 / m	Larynx	$T_3N_0M_0$	P
10	53 / m	Larynx	$T_2N_0M_0$	P
11	42 / m	Hypopharynx	T_4N3M0	LN
12	64 / m	Larynx	$T_3N_0M_0$	P
13	59 / m	Oropharynx	$T_4N_0M_0$	P

* Biopsies were taken from the primary tumor (P) or from lymph node metastasis (LN).
The mean wet weight of the harvested specimens was 89.4±35.9 mg (range: 56.7-145.3 mg).

Table II. *Pharmacological indices used the for calibration of cytostatic drug concentration gradients in the ex vivo colony formation assay.*

	CP	5-FU	DTX
IC_{50} (KB cells)§	2.08 µM (±0.07)	1.23 µM (±0.16)	0.28 nM (±0.04)
Clinically tolerated drug plasma levels (Cp)#	24.0 µM (19)	1.5 µM (20)	50.0 nM (21)
Cytostatic drug concentration gradient	2.0-200.0 µM	1.2-307.2 µM	0.28-71.68 nM

§ Mean IC_{50} values (±S.D.) resulted from five independent cytotoxicity tests (n=5) each done in duplicate.
# References are given in parenthesis.

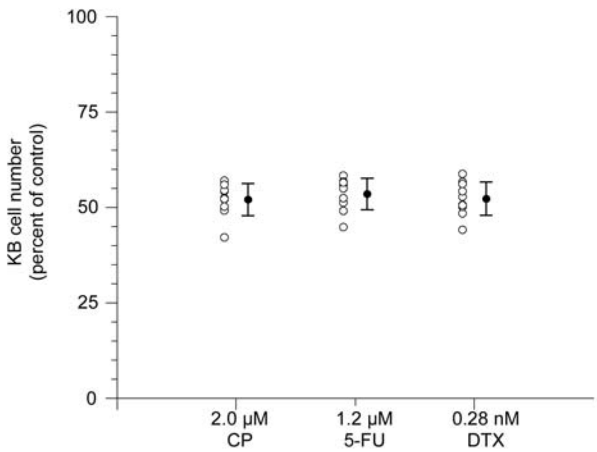

Figure 1. *Results of control experiments with KB cells. The growth inhibitory effect of the IC_{50} of carboplatin (CP), 5-fluorouracil (5-FU) and docetaxel (DTX) is shown (open circles). Filled circles with error bars: Mean±S.D. (n=12).*

details see Table I). After obtaining individual informed consent, a total of 17 biopsies was taken from primary tumors (n=12), or from cervical lymph node metastases (n=5) during surgery at the Department of Otorhinolaryngology, Head and Neck Surgery, University of Heidelberg, Germany. The specimens were kept in ice-cold culture medium for a maximum of 8 hours prior to further processing in the cell culture laboratory.

Drugs, enzymes and reagents. Carboplatin (CP, Bristol, München, Germany), 5-fluorouracil (5-FU, Ribosepharm, München, Germany) and docetaxel (DTX, Rhone-Poulenc Rorer, Köln, Germany) were purchased as pharmaceutical preparations. Nystatin, G-penicillin-Na, gentamycin and streptomycin were purchased from Sigma (München, Germany). Trypsin and collagenase (EC 3.4.24.3, type IV) were obtained from Difco (Detroit, MICH, USA) and Sigma, respectively. All other reagents were of analytical grade.

Cells and cell culture methods. KB cells (ATCC, Bethesda, MD, USA) were propagated, without antibiotics, in flavin-free RPMI 1640 medium (Biochrom, Berlin, Germany) supplemented with 10% (v/v) fetal bovine serum (FBS, Integro, Zaandam, Holland), 1.134 g/l sodium bicarbonate and 1.072g/l HEPES. KB cells were grown at 36.5˚C under 2.5% CO_2 in humidified air (standard conditions) and were routinely subcultivated at alternating intervals of 3 and 4 days, respectively. All experimental steps were carried out under exclusive illumination with sodium-discharge lamps, emitting monochromatically at λ=589 nm (Philips, Marburg, Germany). This specific illumination was used to avoid the known adverse flavin-mediated photooxidative effects in cell culture systems (14-16), especially in chemosensitivity testing (17,18).

Evaluation of cytostatic drug response in KB cells. Cytotoxicity tests with CP, 5-FU and DTX were performed in 24-well plates (Greiner, Frickhausen, Germany). After inoculation of freshly harvested and resuspended KB cells (3.5 x 10^4 /well), diluted gradients from frozen (-20˚C) stock solution of either CP, 5-FU or DTX were added, except for the control wells. The test plates were

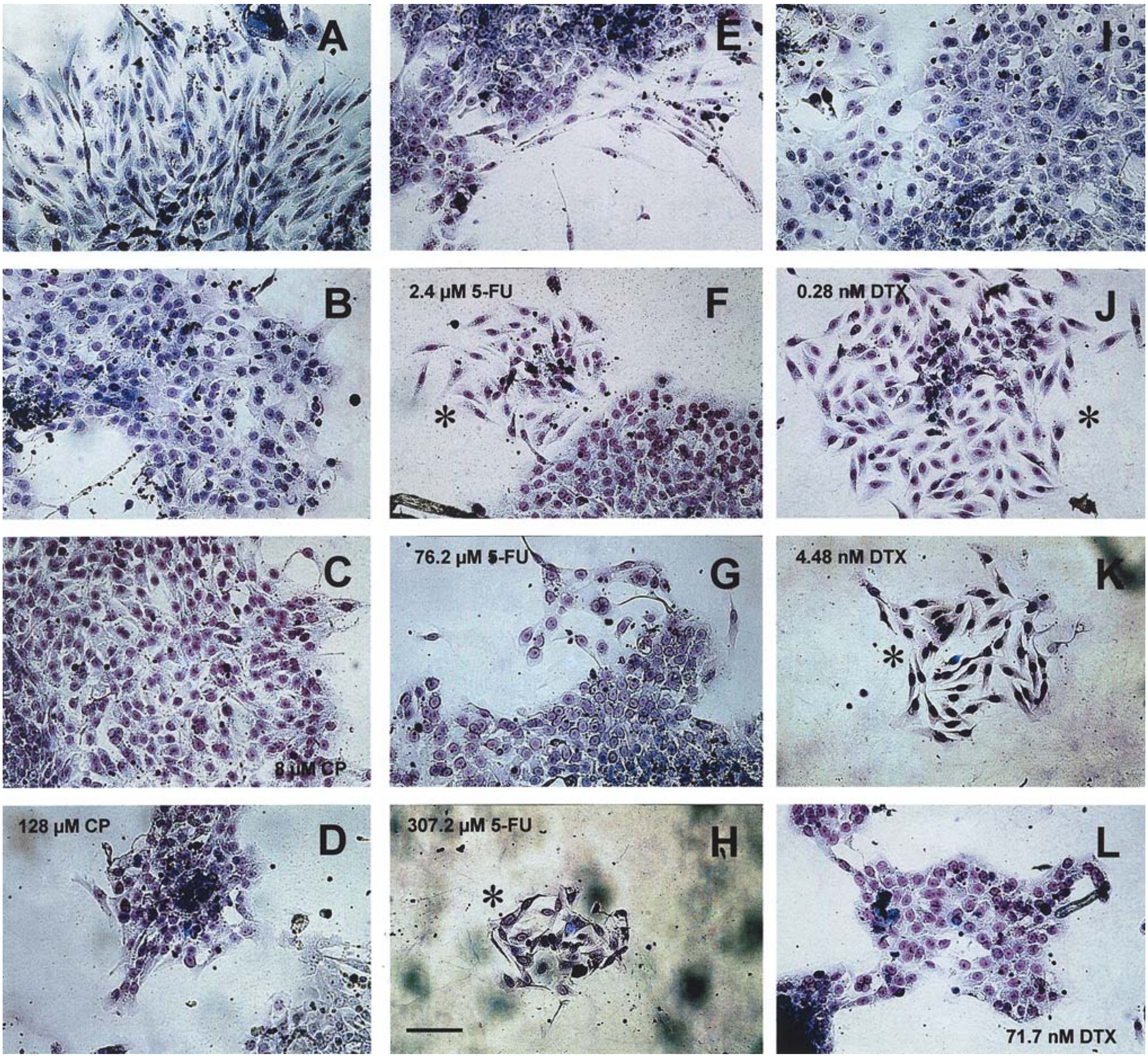

Figure 2. *Microscopic aspects of Giemsa-stained epithelial and stromal cell colonies from HNSCC specimen#7. Figures A,B,E and I: control wells showing epithelial as wells as stromal cell colonies. Figures C and D: epithelial colonies grown in the presence of CP. Figures F, G and H: epithelial and stromal (*) colonies grown in the presence of 5-FU. Figures J, K and L: stromal (*) and epithelial colonies grown in the presence of DTX. The drug concentrations are indicated. Magnification: approx. 100x, scale bar: 100 µM.*

incubated for 72 hours under standard conditions. At harvest, trypsinized KB cells were resuspended in culture medium. Cell densities were determined using a Casy I cell analyzer equipped with Casystat software (Schärfe System, Reutlingen, Germany). Growth inhibition values (N) were expressed as a percentage as follows:

(1) $N (\%) = 100 (t-i)/c-i$

where t, c and i represent test, control and inoculum KB cell densities, respectively. The fifty percent growth inhibitory concentration (IC_{50}) was determined from semilogarithmic drug concentration *vs.* growth inhibition plots.

Control experiments with KB cells. Control experiments with KB cells were performed as part of each colony formation assay. Using the same drug solutions and culture media as in the colony formation assays, the control experiments were necessary to verify the cytostatic potency of each drug in the colony formation assay. Control experiments with KB cells were performed as described for cytotoxicity tests in KB cells, although only one concentration (IC_{50} value) was tested per drug. The percent growth inhibition value (N) was determined according to equation (1) and should give a fifty percent growth inhibition (+/- 10%) to ensure adequate pharmacological assay conditions.

Handling of HNSCC specimens. The handling of the specimens as well as the colony formation assay are adaptations to NHSCC of procedures developed for lung tumor explants (13). After harvesting, specimens were minced generating tumor fragments of about 3 mm^3 in size. After dissection, the fragments were transferred into 30 ml of prewarmed, flavin-free RPMI 1640 medium containing 10% (v/v) FBS, nystatin, G-penicillin, gentamycin and streptomycin. For enzymatic disintegration, 300 IU/ml collagenase type IV were added and the suspension was incubated for 24 hours under standard conditions. After centrifugation (100 x g, 5 min), the pellets were carefully resuspended in 30 ml culture medium supplemented as described above (w/o collagenase).

Colony formation assay. Three hundred µl aliquots of the suspension were transferred to microwells coated with extracellular matrix (Pesasel & Lorey, Hanau, Germany). After a short sedimentation period, diluted cytostatic drug solutions (3 µl/well) were added to establish drug concentration gradients (see below) for CP, 5-FU and DTX. Each test plate included a minimum of eight drug-free control wells. After 72 hours incubation time under standard conditions, the cells were washed twice (phosphate-buffered saline, pH 7.8) and the adherent cells and cell colonies were fixed with methanol before Giemsa staining.

Drug concentration gradients. The cytostatic drug concentration gradients in the colony formation assay were calibrated using two pharmacological indices: i) the respective IC_{50} values for KB cells (see Figure 1), and ii) the clinically achievable plasma concentration of the cytostatic drugs (19-21). Accordingly, for each drug a six-step concentration gradient was defined starting with the IC_{50} for KB cells. The gradients covered, as well as exceeded, the clinically achievable plasma drug levels. The exact concentration gradients are given in Table II.

Microscopic evaluation of the drug response. Giemsa staining allowed for microscopical identification of stromal and epithelial cell colonies (> 16 cells) in all specimens tested. To determine the *overall drug response* (OR) of a specimen to a certain drug, the drug concentration which caused a complete suppression of any *ex vivo* colony formation (C_{100}) was determined. Considering the clinically achievable drug concentration (Cp; Table II), the specimens were classified as sensitive, if the C_{100} was below or equal to the respective Cp and as resistant, if the C_{100} exceeded the respective Cp (Table II).

Analogously to the microscopic identification of the C_{100}, those drug concentrations were identified which caused a complete suppression of epithelial (Ce_{100}), or stromal (Cs_{100}) colony formation *ex vivo*. The specimens' *epithelial drug response* (ER) and the *stromal drug response* (SR) were classified in the same manner as described for the *overall drug response* (OR).

Results

Evaluability rate. To consider a HNSCC specimen *evaluable* in the colony formation assay, the following criteria were applied: i) all control wells contained epithelial, as well as stromal colonies, and ii) the IC_{50} values for the tested cytostatic drugs were reproduced (+/- 10%) in the control experiments with KB cells. Accordingly, 16 out of 17 specimens were evaluable (evaluability rate: 94.1%). One specimen (patient #13, Table I) was excluded due to fungal contamination of the *ex vivo* culture. The results from control experiments are shown in Figure 1.

Types of colonies formed from HNSCC specimens. Giemsa staining of the *ex vivo* formed colonies allowed for microscopical identification of epithelial as well as stromal colony formation (> 16 cells) in each well of the microtiter plate. In control wells, colonies of either epithelial or of stromal cells (Figures 2A, 2E and 2I) were found in explants from primary tumors and from lymph node metastases. The proportion of epithelial and stromal colonies in the control wells varied markedly between the specimens. A microscopical comparison of the stromal proportion in the *ex vivo* cultures with hematoxylin/eosin-stained histology from the same tumors was done independently by three investigators. No correlations between the original tumor (histology) and the *ex vivo* cultures were found (data not shown). Examples of epithelial and stromal cell colonies grown from a HNSCC specimen are shown in Figure 2.

Overall drug response of HNSCC specimens. The complete suppression of any *ex vivo* colony formation was investigated in 16 HNSCC specimens by graded exposure to CP, 5-FU and DTX. The overall response (OR) to each single drug is shown in Table III. Only specimen #13 (Table III) showed a sensitive OR to DTX. Apart from this selective sensitivity, the overall response of the remaining HNSCC specimens revealed resistant response patterns to DTX. All specimens were resistant to CP and 5-FU.

Cell type-specific drug response in HNSCC specimens. To identify the cell type-specific drug response, the suppression of epithelial as well as stromal *ex vivo* colony formation were determined separately. The results are shown in Table III.

Epithelial cytostatic drug response (ER). One specimen showed a sensitive ER to carboplatin (specimen #9, Table III). The remaining 15 specimens were found to be resistant according to the ER definition. For 5-FU, none of the 16 investigated specimens showed a sensitive ER. Four specimens (specimen #4, #9, #13 and #14) were found to be sensitive to DTX. In specimen #9 the sensitivity to DTX was combined with a sensitivity to carboplatin.

Stromal cytostatic drug response (SR). The drug response patterns of the stromal elements were different from those of the epithelial elements from HNSCC specimens. In all specimens stromal cells were found to be resistant to CP. Moreover, the comparison of the corresponding Cs_{100} and Ce_{100} for CP showed in 13 out of 16 specimens (81%) that

Table III. *Overall, epithelial and stromal cytostatic drug response patterns of HNSCC specimens ex vivo.*

Specimen No. / Patient#	Site of biopsy§	Overall suppression of colony formation			Specific suppression of epithelial colony formation						Specific suppression of stromal colony formation					
		OR			Ce_{100}			ER			Cs_{100}			SR		
		CP	5-FU	DTX	CP (μM)	5-FU (μM)	DTX (nM)	CP	5-FU	DTX	CP (μM)	5-FU (μM)	DTX (nM)	CP	5-FU	DTX
01 / 1	P	●	●	●	>200	>307	>71.7	●	●	●	128.0	>307	71.7	●	●	●
02 / 1	LN	●	●	●	>200	>307	>71.7	●	●	●	>200	>307	>71.7	●	●	●
03 / 2	P	●	●	●	128.0	>307	>71.7	●	●	●	128.0	>307	>71.7	●	●	●
04 / 2	LN	●	●	●	>200	>307	17.9	●	●	○	32.0	19.2	71.7	●	●	●
05 / 3	P	●	●	●	32.0	307	>71.7	●	●	●	200.0	>307	>71.7	●	●	●
06 / 4	P	●	●	●	200.0	307	>71.7	●	●	●	200.0	>307	>71.7	●	●	●
07 / 5	P	●	●	●	200.0	307	>71.7	●	●	●	200.0	>307	17.9	●	●	○
08 / 5	LN	●	●	●	>200	>307	>71.7	●	●	●	>200	>307	>71.7	●	●	●
09 / 6	P	●	●	●	8.0	>307	17.9	○	●	○	200.0	307	71.7	●	●	●
10 / 7	P	●	●	●	200.0	>307	>71.7	●	●	●	200.0	307	71.7	●	●	●
11 / 7	LN	●	●	●	200.0	76.8	>71.7	●	●	●	>200	>307	>71.7	●	●	●
12 / 8	P	●	●	●	>200	>307	71.7	●	●	●	>200	>307	17.9	●	●	○
13 / 9	P	●	●	○	128.0	307	17.9	●	●	○	128.0	>307	17.9	●	●	○
14 / 10	P	●	●	●	>200	>307	17.9	●	●	○	>200	>307	71.7	●	●	●
15 / 11	LN	●	●	●	128.0	307	>71.7	●	●	●	128.0	307	1.12	●	●	○
16 / 12	P	●	●	●	200.0	307	>71.7	●	●	●	128.0	>307	4.48	●	●	○

Resistant (●) and sensitive (○)drug response in the colony formation assay is indicated for the overall drug response (OR), the epithelial drug response (ER) and the stromal drug response (SR).
Patient number. § Biopsies from the primary tumor (P) or from lymph node metastasis (LN).

complete suppression of stromal colonies required similar or higher drug concentrations than required for suppression of epithelial colony formation (Table III). As for CP, the stromal response to 5-FU revealed resistance to 5-FU for all specimens. Again, the comparison of the corresponding Cs_{100} and Ce_{100} for 5-FU showed in 87.5 percent of the tested specimens similar, or higher resistance levels in stromal colonies compared to the epithelial cell colonies. Distinct stromal sensitivities were found only for docetaxel. In 5 HNSCC specimens, stromal colony formation was suppressed by docetaxel in lower concentrations than the clinically achievable plasma concentration (specimen #7, #12, #13, #15 and #16, Table III). In the remaining eleven specimens, colonies from the tumor stroma were resistant to docetaxel.

Discussion

Stromal cell contamination is an extensively discussed methodical limitation of most *ex vivo* procedures for chemosensitivity testing of solid tumors (1-3). For radiosensitivity testing using the clonogenic assay, this contamination has been described to preclude reliable test results for HNSCC specimens (12). Up to now, the impact of stromal cell contamination to chemosensitivity testing of HNSCC specimens has not been systematically investigated.

The present study was designed to investigate whether stromal cell contamination has an impact on chemosensitivity testing of HNSCC specimens. Furthermore, we tried to estimate the relevance of a distinction between epithelial and stromal elements in such test procedures.

The *ex vivo* colony formation assay for HNSCC specimens used in this study aims to determine the quantitative chemosensitivity of a given specimen corrected for stromal cell contamination. This was achieved by unselected *ex vivo* cultivation and chemosensitivity testing of all cellular elements from each specimen. The later fixation and staining of the colonies allows for a morphological identification of epithelial and stromal colonies, and thereby the determination of the quantitative epithelial and stromal *ex vivo* drug response.

Since cellular cytostatic drug resistance in HNSCC has been commonly attributed to malignant cells (22),one would expect that the formation of non-epithelial colonies would be suppressed by lower drug concentrations than epithelial colonies. In parallel to the findings concerning the radioresistance of tumor fibroblasts (12), our results indicate that stromal HNSCC tumor cells are also highly resistant to cytostatic drugs. Consistently, we found surprisingly low chemosensitivities of non-epithelial cellular elements in the tested HNSCC specimens. In fact, stromal *ex vivo* colony formation was suppressed in only 10 out of 48 tests by a lower cytostatic drug concentration than that necessary to suppress the formation of epithelial colonies (Table III).

It is essential for *in vitro* chemosensitivity tests to attribute the results to the malignant cell population from a given tumor. Our results indicate that the unselective determination of the "overall response" leads to crucial misinterpretations of test results. In the tests reported here, the overall drug response of the examined HNSCC specimens showed for 15 out of 16 specimens resistant drug response patterns to all drugs investigated. If the overall drug response were to be taken as the individual drug response, 4 out of 5 (80%) sensitive ER patterns would not have been identified due to stromal cell drug contamination (specimen #4, #9, #13 and #14; Table III). These findings are in agreement with the previously reported influence of stromal cell contamination in radiosensitivity testing of HNSCC specimens using the clonogenic assay (12).

The present study has focused on the impact of stromal cell contamination on chemosensitivity detection in HNSCC. The underlying mechanisms of the surprisingly high chemoresistance in stromal tumor cells was not investigated in detail. However, the finding was somewhat surprising since normal dividing cells of tumor patients are generally considered chemosensitive (23). Nevertheless, several authors have described P-glycoprotein expression in non-malignant tumor cells (24-26) and it appears that additional factors from the tumor stroma do contribute to the chemotherapeutic response of malignant cells (27, 28). It can be assumed, that multidrug-resistant stromal cells distort the assessment of the cytostatic drug response of HNSCC specimens, regardless of the procedures used (*e.g.* microarray technology (29)). Therefore, further investigations on the role of fibroblast-tumor interaction in HNSCC chemosensitivity are needed. Recently, Ballo and coworkers have established an experimental model which allows the study of this particular issue in established HNSCC cell lines (30). Further studies on the nature of stromal cell chemoresistance and pharmacological fibroblast-tumor interactions are required to clarify its relevance for cancer therapy.

In conclusion, we want to emphasize that methods to detect individual drug response profiles in HNSCC should allow for stroma cell correction. Besides the colony formation assay reported here, the histoculture drug response assay (HDRA) seems to fulfil this requirement (1,31,32). Using this assay, Singh and coworkers have recently shown that survival in head and neck cancer patients is predictable by chemosensitivity determined by the HDRA (33).

Acknowledgements

The authors wish to thank Dr. Jutta Heix for helpful discussions and critically reading the manuscript. The perfect technical assistance of Barbara Liebetrau is gratefully acknowledged.

References

1 Hoffman RM: *In vitro* sensitivity assays in cancer: a review, analysis, and prognosis. J Clin Lab Anal *5*: 133-143, 1991.

2 Cortazar P and Johnson BE: Review of the efficacy of individualized chemotherapy selected by *in vitro* drug sensitivity testing for patients with cancer. J Clin Oncol *17*: 1625-1631, 1999.

3 Cramer AB and Woltering EA: Chemosensitivity testing: a critical review. Crit Rev Clin Lab Sci *28*: 405-413, 1991.

4 Le Febvre JL, Chevalier D, Luoinski B, Kirkpatrick A, Colette L and Sahmoud T: Larynx preservation in pyriform sinus cancer: preliminary results of a European Organization for Reasearch and Treatment of Cancer phase III trail. EORTC Head and Neck Cooperative Group. J Natl Cancer Inst *88*: 890-899, 1996.

5 Dietz A, Nollert J, Eckel H, Volling P, Schroeder M, Staar S, Conradt C, Helmke B, Dollner R, Mueller RP, Wannenmacher M, Weidauer H and Rudat V: Organerhalt beim fortgeschrittenen Larynx- bzw. Hypopharynxkarzinom durch primäre Radiochemotherapie. HNO *50*: 146-154, 2002.

6 The Department of Veterans Affairs Laryngeal Cancer Study Group: Induction chemotherapy plus radiation compared with surgery plus radiation in patients with advanced laryngeal cancer. The Department of Veterans Affairs Laryngeal Cancer Study Group. N Engl J Med *324*: 1685-1690, 1991.

7 Bradford CR, Zhu S, Wolf GT, Poore J, Fsher SG, Beals T, McClatchey KD and Carey TE: Overexpression of p53 predicts organ preservation using induction chemotherapy and radiation in patients with advanced laryngeal cancer. Department of Veterans Affairs Laryngeal Cancer Study Group. Otolaryngol Head Neck Surg *113*: 408-412, 1995.

8 Rudat V, Stadler P, Becker A, Vanselow B, Dietz A, Wannenmacher M, Molls MDJ and Feldmann HJ: Predictive value of the tumor oxygenation by means of pO2 histography in patients with advanced head and neck cancer. Strahlenther Onkol *177*: 462-468, 2001.

9 Dietz A, Vanselow B, Rudat V, Conradt C, Weidauer H, Kallinowski F and Dollner R: Prognostic impact of reoxygenation in advanced cancer of the head and neck during the initial course of chemoradiation or radiotherapy alone. Head Neck *25*: 50-58, 2003.

10 Doweck I, Denys D and Robbins KT: Tumor volume predicts outcome for advanced head and neck cancer treated with targeted chemoradiotherapy. Laryngoscope *112*: 1742-1749, 2002.

11 Rudat V, Dietz A, Schramm O, Conradt C, Maier H, Flentje M and Wannenmacher M: Prognostic impact of total tumor volume and hemoglobin concentration on the outcome of patients with advanced head and neck cancer after concomitatant boost radiochemotherapy. Radiother Oncol *53*: 119-125, 1999.
12 Stausbøl-Grøn B, Nielsen OS, Beten SM and Overgaard J: Selective assessment of *in vitro* radiosensitivity of tumour cells and fibrobas from single tumour biopsies using immunocytochemical identification of colonies in the soft agar clonogenic assay. Radiother Oncol *37*: 87-99, 1995.
13 Granzow C, Kopun M, Heuser M, Herth F and Becker HD: Photochemical precautions and stromal cell evaluation required for chemosensitivity testing of lung tumor explants. submitted.
14 Griffin FM, Ashland G and Capizzi RL: Kinetics of phototoxicity of Fischer's medium for L5178Y leukemic cells. Cancer Res *41*: 2241-2248, 1981.
15 Grzelak A, Rychlik B and Bartosz G: Light-dependent generation of reactive oxygen species in cell culture media. Free Rad Biol Med *30*: 1418-1425, 2001.
16 Yamamoto F, Nishimura S and Kasai H: Photosensitized formation of 8-hydroxydeoxyguanosine in cellular DNA by riboflavin. Biochem Biophys Res Commun *187*: 809-813, 1992.
17 Dollner R, Granzow C and Dietz A: Improved chemosensitivity-profiling of HNSCC: preliminary results. *In*: Metastases in Head and Neck Cancer,(Eds. Lippert BM and Werner JA), 459-462. Tectum, Marburg, 2001.
18 Granzow C, Kopun M and Kröber T: Riboflavin-mediated photosensitization of vinca alkloids distorts drug sensitivity assays. Cancer Res *55*: 4837-4843, 1995.
19 Oguri S, Sakakibara T, Mase H, Shimizu T, Ishikawa, Kimura K and Smyth RD: Clinical pharmacokinetics of carboplatin. J Clin Pharmacol *28*: 208-215, 1988.
20 Au JL, Rustum YM, Ledesma EJ, Mittelman A and Creaven PJ: Clinical pharmacological studies of concurrent infusion of 5-fluorouracil and thymidine in treatment of colorectal carcinoma. Cancer Res *42*: 2930-2937, 1982.
21 Extra JM, Rousseau F, Bruno R, Clavel M, Le Bail N and Marty M: Phase I and pharmacokinetic study of Taxotere (RP 56976; NSC 628503) given as a short intravenous infusion. Cancer Res *53*: 1037-1042, 1993.
22 Bier H: Chemotherapeutic drug resistance in the management of head and neck cancer. Eur Arch Otorhinolaryngol *250*: 200-208, 1993.
23 Kerbel RS: A cancer chemotherapy resistant to resistance. Nature *390*: 335-336, 1997.
24 Schlaifer DBP, Attal M, Voigt JJ, Laurent G and Delsol G: Immunohistochemical detection of multidrug resistance associated p-glycoprotein in stromal cells of malignant lymphomas. Nouv Rev Fr Hematol *32*: 365-367, 1990.
25 Schlaifer D, Laurent G, Chittal S, Tsuruo T, Soues S and Muller C: Immunohistochemical detection of multidrug resistance associated P-glycoprotein in tumor and stromal cells of human cancer. Brit J Cancer *62*: 182, 1990.
26 Iwahana M, Utoguchi N, Mayumi T, Goryo M and Okada K: Drug resistance and P-glycoprotein expression in endothelial cells of newly formed capillaries induced by tumors. Anticancer Res *18*: 2977-2980, 1998.
27 Song S, Wientjes MG, Gan Y and Au JLS: Fibroblast growth factors: an epigenetic mechanism of broad spectrum resistance to anticancer drugs. Proc Natl Acad Sci *97*: 8658-8663, 2000.
28 Panayitidis P, Jones D, Ganeshaguru K, Foroni L and Hoffbrand AV: Human bone marrow stromal cells prevent apoptosis and support the survival of chronic lymphocytic leukaemia cell *in vitro*. Br J Haematol *92*: 97-103, 1996.
29 Staunton JE, Slonim DK, Coller HA, Tamayo P, Angelo MJ, Park J, Scherf U, Lee JK, Reinhold WO, Weinstein JN, Mesirov JP, Lander ES and Golub TR: Chemosensitivity prediction by transcriptional profiling. Proc Natl Acad Sci *98*: 10787-10792, 2001.
30 Balló H, Koldovsky P, Hoffmann T, Balz V, Hilebrandt B, Gerharz C-D and Bier H: Establishment and characterization of four cell lines derived from human head and neck squamous cell carcinomas for an autologous tumor-fibroblast *in vitro* model. Anticancer Res *19*: 3827-3836, 1999.
31 Robbins KT, Connors KM, Storniolo AM, Hanchett C and Hoffman RM: Sponge-gel-supported histoculture drug-response assay for head and neck cancer. Arch Otolaryngol Head Neck Surg *120*: 288-292, 1994.
32 Robbins KT, Varki NM, Storniolo AM, Hoffman H and Hoffman RM: Drug response of head and neck tumors in native-state histoculture. Arch Otolaryngol Head Neck Surg *117*: 83-86, 1991.
33 Singh B, Li R, Xu L, Poluri A, Patel S, Shaha AR, Pfister D, Sherman E, Goberdhan A, Hoffman RM and Shah J: Prediction of survival in patients with head and neck cancer using the histoculture drug response assay. Head Neck *24*: 437-442, 2002.

Received September 1, 2003
Accepted December 15, 2003