Oral Oncology (2008) 44, 78–85

available at www.sciencedirect.com

ScienceDirect

journal homepage: http://intl.elsevierhealth.com/journals/oron/

ORAL ONCOLOGY

DNA ploidy, proliferative capacity and intratumoral heterogeneity in primary and recurrent head and neck squamous cell carcinomas (HNSCC) — Potential implications for clinical management and treatment decisions

Holger G. Hass [a,*], Andreas Schmidt [b], Oliver Nehls [c], Stephan Kaiser [d]

[a] *Department of Oncology, Haematology and Palliative Care, Marienhospital (Teaching Hospital University of Tuebingen), Boeheimstr. 37, 70199 Stuttgart, Germany*
[b] *Bundeswehrkrankenhaus (BWK) Koblenz, Germany*
[c] *Department of Haematology, Oncology, Pulmology and Rheumatic Diseases, University of Tuebingen, Germany*
[d] *Department of Gastroenterology and Hepatology, Medical School, University of Tuebingen, Germany*

Received 5 November 2006; received in revised form 19 December 2006; accepted 20 December 2006
Available online 9 March 2007

KEYWORDS
HNSCC;
BrdU labeling index;
DNA ploidy;
Intratumoral heterogeneity

Summary Despite new diagnostic and therapeutic strategies (combined radiochemotherapy, EGFR antibody Cetuximab), the prognosis of head and neck squamous cell carcinoma (HNSCC) is still poor and more information regarding prognosis is essential to establish earlier and better treatment options. To elucidate the role of DNA ploidy and cellular proliferation, resected tumors of 48 patients with primary or recurrent HNSCC were analyzed by flow cytometry and *in vitro*-5-bromodeoxyuridine incorporation (BrdU). The results were compared with histopathological findings such as tumor size, lymph node involvement and tumor differentiation. To assess the influence of intratumoral heterogeneity of these biological parameters, multiple biopsies (>3) were analyzed by flow cytometry and BrdU-incorporation in 12 larger (>4 cm diameter) tumors. BrdU-labeling index (LI%) was significantly higher in aneuploid HNSCC and correlated significantly with poor histologic differentiation of the analyzed tumor tissues ($P < 0.001$). Furthermore, a trend for higher LI% in nodal positive tumors was observed. Aneuploid HNSCC showed significantly more often tissue dedifferentiation ($P = 0.049$) and in most cases an advanced tumor stage, especially in tumors with biclonal cell lines. Lymph node involvement was also seen more often in aneuploid and undifferentiated tumors. As in aneuploid tumors

* Corresponding author. Tel.: +49 711 6489 8101; fax: +49 711 6489 8102.
E-mail address: holgerhass@vinzenz.de (H.G. Hass).

1368-8375/$ - see front matter
doi:10.1016/j.oraloncology.2006.12.013

recurrent HNSCC showed in most cases a higher LI% and poor tissue differentiation, but as a result of the small collection of samples there was no correlation between aneuploidy and tumor recurrence. To proof the robustness of the acquired data and to estimate the influence of intratumoral heterogeneity to ploidy and LI% multiple biopsies were analyzed in larger tumors. Using a specific statistical algorithm a secure estimation of ploidy and LI% was possible by a single biopsy in these tumors. These findings indicate aneuploidy and proliferative activity as important findings for malignant progression in HNSCC. An estimation of these biological parameters may be useful for identification of patients with high risk for lymph node involvement or tumor recurrence and pre-treatment can be performed by a single biopsy. As a conclusion, these patients may benefit from more aggressive treatment.

Introduction

In 2002, more than 600000 new cases of head and neck squamous cell carcinoma (HNSCC) were diagnosed worldwide[1] and in 2005 HNSCC were the fifth most common cancer in the United States.[2] HNSCC includes squamous cell carcinomas of the oral and oropharyngeal mucosa, together with those of the lip, tongue and the laryngopharynx. Main risk factors for the development of HNSCC are nicotine use and alcohol consumption, which are the main reasons for the overall male-to-female gender ratio from 3:1. Very often a lower socio—economic status in patients with HNSCC is seen and several studies have shown an increasing incidence of HNSCC in European countries in the last decade.[3]

Although substantial advances in treatment especially in reconstructive and plastic surgery or radiotherapy for early-stage disease (stage I or II) have been made over the last 15 years and new therapeutic strategies for advanced or metastatic tumor stages (neoadjuvant radiochemotherapy, EGFR antibody cetuximab) have been developed actually, the overall prognosis of HNSCC remains poor and until now many important clinical problems in treatment of HNSCC are still not resolved. Actually, there is still no ''gold standard'' in surgical treatment for patients with extensive neck disease (N2 or N3) established and the lack of suitable criteria to predict the response to chemo- and or radiotherapy for individual patients remains still a major problem. Therefore, more prognostic information and an earlier identification of patients with a high risk for disease or recurrence after prior treatment is extremely important to obtain better local disease control and to minimize the risk for metastasis or local tumor recurrence.

The analysis of cellular proliferation and DNA ploidy with flow cytometry has been used in an attempt to identify new prognostic factors to optimize treatment decisions in HNSCC.

An abnormal (aneuploid) DNA content is one of the most common biological characteristic in human solid tumors and may contribute to tumor formation and often to acquired resistance to some chemotherapeutic agents. The tumor cells become aneuploid as a result of aberrant mitotic divisions caused by previous defects in cytokinesis or centrosome amplification or impaired mitotic checkpoint response. As seen in other human cancers, aneuploidy is a multi-step process in carcinogenesis and correlates with tumor progression.[4—6] These observations are in line with earlier results in patients with HNSCC, whereas a strong correlation between aneuploid DNA content and advanced tumor stage or poor histological differentiation as a sign for malignant progression were noted.[7—10]

Another attempt to characterize biological behavior and malignant progression in HNSCC is the estimation of proliferative activity. One of the most established technique to analyze cellular proliferation is the detection of incorporated 5′-Bromodeoxyuridine (BrdU) in tumor cells. However, the value of this biological parameter for clinical outcome in patients with squamous cell cancer is yet to be confirmed. Some authors have shown a relation between elevated BrdU labeling indices (LI) and poor histological differentiation or advanced tumor stages in HNSCC with increased risk of lymph node involvement,[11,12] whereas other studies were unable to confirm these findings.[13]

Despite these observations in primary HNSCC, the importance of DNA ploidy and proliferative activity regarding the risk for tumor recurrence is still unclear and controversially discussed, although some studies had shown a correlation between DNA aneuploidy and recurring tumor development.[14,15]

Another unresolved question is the clinical importance of intratumoral heterogeneity in patients with HNSCC. As for other human cancers occurrence of genetic heterogeneity in HNSCC is a well-known observation,[16] but the importance for biological behavior and tumor progression is currently being debated. In some medical centers the determination of proliferative capacity and DNA ploidy is performed pretreatment or operatively in most cases by a single biopsy and as a result, the predictive value of these molecular findings is limited by intratumoral heterogeneity of the malignant cell clones.[17]

The aim of this study was primarily to analyze primary and recurring HNSCCs with regard to DNA ploidy, cellular proliferation and histopathological findings such as tumor size, histologic differentiation (grading) and lymph node status. Secondary points were to illuminate the role of DNA ploidy and proliferative activity (BrdU LI%) as potential prognostic factors for tumor recurrence and lymph node involvement, and further to analyze intratumoral heterogeneity of these parameters to evaluate the matter of how representative a single diagnostic biopsy with regard to DNA ploidy and proliferative activity for clinical routine is.