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A novel panel of biomarkers predicts radioresistance in patients with squamous cell carcinoma of the head and neck

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Abstract ***Purpose:*** Global gene expression analysis was performed on pre-treatment biopsies from patients with squamous cell carcinoma of the head and neck (SCCHN) to discover biomarkers that can predict outcome of radiation based therapy.
Methods: We initially evaluated RNA expression using cDNA microarray analysis of 38 patients that received radiotherapy (RT). The five strongest candidates (*VEGF*, *BCL-2*, *CLAUDIN-4*, *YAP-1* and *c-MET*) were then analysed in pre-treatment biopsies in a second group of 86 patients who received radiation based treatment using immunohistochemical staining (IHC), prepared by tissue microarray.
Results: In the first population, 13 of 38 (34%) had no (NR) or partial response (PR) to RT. cDNA microarrays revealed 60 genes that were linked to response to therapy. In the second series, 12 of 86 patients (14%) experienced NR or PR to CRT. Cause specific survival (CSS) and recurrence free survival (RFS) at 2 years was 85% and 90% and at 3 years 81% and 84%, respectively. Biomarkers predictive for NR/PR were increased expression of vascular endothelial growth factor (VEGF) ($p = 0.02$), Yes-associated protein (YAP-1) ($p < 0.01$), CLAUDIN-4 ($p < 0.01$), c-MET ($p < 0.01$) and BCL-2 ($p = 0.02$). Biomarkers predictive of poor RFS were

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YAP-1 ($p = 0.01$) and BCL-2 ($p < 0.01$). Biomarkers predictive of poor CSS were YAP-1 ($p = 0.04$), VEGF ($p = 0.03$) and CLAUDIN-4 ($p = 0.03$). Furthermore, when YAP-1 and c-MET expression levels were combined the prediction of radio-resistance was increased.
Conclusion: All five biomarkers were predictive of poor response to radiation based therapy. In particular, YAP-1 and c-MET have synergistic power and could be used to make treatment decisions.

1. Introduction

Radiation (RT) or concurrent chemoradiation (CRT) is the predominating primary therapy in patients with squamous cell carcinoma of the head and neck (SCCHN) [1] with the goal of achieving both tumour control and organ preservation. In recent years biological agents have been successfully added to the treatment armamentarium [2]. However, current standard chemoradiation strategies have reached the upper limits of toxicity with complications and side effects including, but not limited to, acute and chronic mucositis, xerostomia, oesophageal strictures and death. In conjunction with the high costs for these treatment modalities and often long and difficult rehabilitation, careful assessments are necessary prior to treatment to make the proper decision for individual cases. Approximately 15% of patients that receive primary radiation based therapy do not have a complete response or a recurrence and will need salvage surgical procedures.

The basis for current treatment decisions are primarily based on the patient's tumour-nodal-metastases (TNM) staging. However, patients with the same TNM stages have heterogeneous responses to therapy. Because these patients are often receiving RT and in more advanced stages, CRT, as the sole therapy it becomes important to determine how each patient could respond to their respective therapy, and to separate those who are at high risk for poor response and loco-regional recurrence. Personalised treatment decisions based on biomarkers that go beyond TNM-classification leading to individualised treatment plans could reduce morbidity and potentially improve survival by avoiding treatment failures. There is reason to believe that a better understanding of the biological properties of these tumours, as measured in patient's pre-treatment biopsies, may lead us to predict response to RT and CRT and thus allow for tailored patient specific therapeutic strategies.

Due to the heterogeneous nature of tumours, it is less likely that any one specific marker will have prognostic or predictive value. Therefore, screening methods, such as gene profiling, would be an initial step to identify groups of target genes, after which validation of specific genes from these profiles should be analysed in detail. To date, more than 60 gene expression profiling studies have been published in the field of head and neck squamous cell carcinoma (SCCHN) with variable objectives, methods and results [3]. We have shown that gene expression profiles can relate to sensitivity to cisplatin in SCCHN cell lines [4]. However, only three studies have addressed the specific issue of correlation between expression profiles and radiation and chemo-sensitivity in SCCHN patients [5–7]. Ganly et al. [5] identified 17 genes correlated with loco-regional response in a series of 35 patients with laryngo-pharyngeal carcinoma treated by platinum-based chemoradiotherapy. Of these genes only *MDM2*, *ERB2*, *H-RAS* and *VCAM-1* were considered valid as potential predictive genes by RTqPCR. In a larger group of patients (92 cases), Pramana and colleagues were not able to find a robust predictive classifier [6]. In a more recent, but pilot study of only 14 cases, Dumur et al. identified six algorithms based on a 142 probe set that could differentiate complete responders to chemotherapy or radiotherapy [7].

In the present study we wanted to expand this knowledge by attempting to identify biomarkers for response to radiation in two independent sets of clinical specimens. The first set of 38 specimens (Group I) was studied for global gene expression analysis to identify informative genes. A second set of 86 patients (Group II provided the material to study the prognostic significance of a limited panel of markers, selected from the cDNA microarray studies utilising immunohistochemistry as a clinically applicable laboratory technique. The purpose of this set of markers would be to help clinicians to assess clinical response to CRT in patients with SCCHN as analysed in their diagnostic biopsies, and by doing so identifying potential non-responders before treatment decisions are being made. This strategy could lead to individualised management of patient based on such biological staging.

2. Materials and methods

2.1. Group I patients and tumour samples

We initially studied pre-treatment biopsies from 65 patients with HNSCC, obtained at Department of Otolaryngology, Lund University Hospital, Sweden, during 1994–2001, as approved by the ethics committee at the University. Pre-treatment biopsies were obtained during diagnostic endoscopies under anaesthesia. Specimens were placed in 10% dimethyl sulfide (DMSO) in RPMI