

The Study and Characterization of Primary Squamous Cell Carcinoma of the Head and Neck and Their Malignant Potential

Simona Dostalova^{1,2}, Martina Raudenska³, Marketa Svobodova³, Michaela Fojtu³, Hana Polanska³, Jan Balvan³, Marek Feith³, Jaromir Gumulec³, Hana Binkova⁴, Zuzana Horakova⁴, Rom Kostrica⁴, Michal Masarik³, Zbynek Heger^{1,2}, Ana Maria Jimenez Jimenez^{1,2}, Ondrej Zitka^{1,2}, Vojtech Adam^{1,2} and Rene Kizek^{1,2}

¹Central European Institute of Technology, Brno University of Technology, Brno, Czech Republic - European Union

²Department of Chemistry and Biochemistry, Laboratory of Metallomics and Nanotechnologies, Mendel University in Brno, Brno, Czech Republic - European Union

³Department of Pathological Physiology, Faculty of Medicine, Masaryk University, Brno, Czech Republic - European Union

⁴Department of Otorhinolaryngology and Head and Neck Surgery, St. Anne's Faculty Hospital, Brno, Czech Republic - European Union

Introduction

Squamous cell carcinomas (HNSCC) form 90% of all head and neck cancers. Even with advanced therapy and operative skills, the 5-year survival rate is no more than 50%¹. HNSCC are not only caused by tobacco and alcohol abuse, there is also a huge involvement of HPV infections². New potentially clinically useful biomarkers are being studied, such as metallothioneins (MT)³ or miRNAs⁴.

Materials/methods

Samples from almost 300 HNSCC positive and healthy patients were tested – biopsy of tumour tissue, adjacent healthy tissue or leucocytes were used in this study and compared with non-cancer individuals. Standard PCR or qRT-PCR methods were employed to detect HPV or expression of certain genes.

Results and conclusion

54% of studied HNSCC patients were HPV-positive, with 21% of HPV 16 subtype, 13% of HPV 18 subtype and 7% of HPV 16 and 18 types co-infection. The expression of commonly studied HNSCC markers *MT2*, *MMP9*, *FLT1*, *VGFA* and *POU5F* was significantly inhibited in HPV-positive HNSCC patients, compared with HPV-negative patients. The HPV-positive patients also showed better disease-specific survival. Moreover, expression of other genes was also studied. *MT2A*, *BAX*, *EGF* and *JUN* showed increased expression in the healthy tissues surrounding the tumour tissue and a significant shift of *BAX/BCL2* ratio was observed. The adjacent healthy tissue was also shown to release several tumour-supporting factors, increasing cancerogenesis. Also, an increase in the expression of the protein metallothionein was observed, in tumour-adjacent tissue and more so in the tumours, evidencing it as a potential HNSCC biomarker. Based on this information, microfluidic label-free bead-based, portable metallothionein immunosensor with electrochemical detection was designed with LOD of 12.5 ng/mL.

The authors gratefully acknowledge financial support from the SPINCANCER NT/14337-3/2013.

- 1 Torre, L. A. *et al.* Global Cancer Statistics, 2012. *CA-Cancer J. Clin.* **65**, 87-108, doi:10.3322/caac.21262 (2015).
- 2 Gillison, M. L., Chaturvedi, A. K., Anderson, W. F. & Fakhry, C. Epidemiology of Human Papillomavirus-Positive Head and Neck Squamous Cell Carcinoma. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **33**, 3235-3242, doi:10.1200/jco.2015.61.6995 (2015).
- 3 Dutsch-Wicherek, M. *et al.* Metallothionein stroma reaction in tumor adjacent healthy tissue in head and neck squamous cell carcinoma and breast adenocarcinoma. *Neuroendocrinol. Lett.* **26**, 567-574 (2005).
- 4 Hauser, B. *et al.* Functions of MiRNA-128 on the Regulation of Head and Neck Squamous Cell Carcinoma Growth and Apoptosis. *PLoS One* **10**, doi:e0116321

