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Extended Abstract

13-Gene DNA Methylation Analysis from Oral Brushing: A Non Invasive Diagnostic Tool in the Follow-Up of Patients Surgically Treated for Oral Cancer †

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1. Introduction

Patients treated for Oral Squamous Cell Carcinoma (OSCC) showed a significant risk to develop a loco-regional relapse during follow-up period. In clinical practice the follow-up strategy for early detection of recurrence or second primary tumors still consists of periodic visual examination and palpation of the oral cavity during the 5-year aftercare period.

Our research group recently developed a non-invasive procedure to identify oral carcinomas at early stage starting from oral brushing, quantitatively measuring the DNA methylation level of a panel of 13 genes [1].

The procedure resulted highly related to the presence of a malignant process (sensitivity 96.6%, specificity 100%). This high accuracy stimulated us to apply our non-invasive diagnostic tool in a cohort of patients previously treated for Oral Squamous Cell Carcinoma. Aim of the study is to evaluate if 13-gene DNA methylation analysis by oral brushing may be a useful procedure to identify patients surgically treated for OSCC at risk of developing a secondary tumor.

2. Materials and Methods

The study population included 49 consecutive surgically treated OSCC. Oral brushing sample collection was performed during patient follow up almost 6 months after OSCC treatment, within the regenerative area after OSCC resection. In all brushing specimens the DNA methylation level of ZAP70, GP1BB, KIF1A, ITGA4, LINC00599, MIR193, MIR296, TERT, LRRTM1, NTM, EPHX3, FLI1 and PARP15 was evaluated by quantitative Bisulfite-Next Generation Sequencing (NGS). Each sample was defined positive or negative in relationship to a specific algorithm and cut-off value [1]. Positive scores in the regenerative area after OSCC resection were analyzed together with other histologic poor prognostic factors for any relationship with appearance of loco-regional relapse.

3. Results

16/49 brushing specimens collected 6 months after surgery from regenerative area after OSCC showed a positive score.

During follow up period 7/49 (14.3%) patients developed a secondary OSCC during follow-up period (mean follow-up: 18.9 months): 6/7 patients showed positive samples from regenerative areas. The presence of a positive score resulted the most powerful variable related to the appearance of locoregional relapse, greater than presence of perineural invasion detected in the surgical OSCC sample.

4. Conclusions

These preliminary results seem to indicate that our novel assay may be proposed as an indicator of disease before appearance of clinical signs and symptoms in surgically treated OSCC patients. Further studies with larger cohort of patients, with adequate follow-up period and brushing sampling collection at different moments are needed to elucidate the prognostic potential of our assay.

Conflicts of Interest: The authors declare no conflict of interest

Reference

1 Morandi, L.; Gissi, D.; Tarsitano, A.; Asioli, S.; Gabusi, A.; Marchetti, C.; Montebugnoli, L.; Foschini, M.P. CpG location and methylation level are crucial factors for the early detection of oral squamous cell carcinoma in brushing samples using bisulfite sequencing of a 13-gene panel. *Clin. Epigenet.* **2017**, *9*, 85.