ANALYSIS OF MOLECULAR ALTERATIONS OF *FBXL7* IN ESOPHAGEAL AND HEAD AND NECK SQUAMOUS CELL CARCINOMAS

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Introduction

Esophageal and head and neck squamous cells carcinomas (ESCC and HNSCC, respectively) represent over 90% of the tumors originated at these sites.[1, 2]. Besides the same stratified squamous epithelium of origin, these tumors also present a similar etiology (i.e., tobacco smoking and alcohol drinking)[3].

Therefore, considering their histological and etiological similarities, the search for a common molecular mechanism can be useful to clarify the biologic process across these tumors and, perhaps, be helpful in identifying a universal biomarker. In this context, DNA methylation is showing an intimate correlation with risk factor exposure and biological behavior of these tumors[4, 5]. Based on this, our group has performed a methylome analysis of ESCC and HNSCC, including oral (OSCC), laryngeal (LSCC) and oropharyngeal (OPSC) tumors. Our results showed that the methylation profile of one gene, *FBXL7*, can discriminate the histologically normal surrounding tissue from tumor, with high sensitivity and specificity. *FBXL7* protein belongs to the E3 ubiquitin ligase complex, catalyzing the ubiquitination of target proteins for proteasome degradation[6]. Its targets include Aurora A[7] and Survivin[8], both oncogenic proteins responsible for proliferation and survival, respectively, that have already been shown to be overexpressed in ESCC and HNSCC[9, 10, 11, 12].

Objective

The characterization of the molecular alterations of *FBXL7* in ESCC and HNSCC samples.

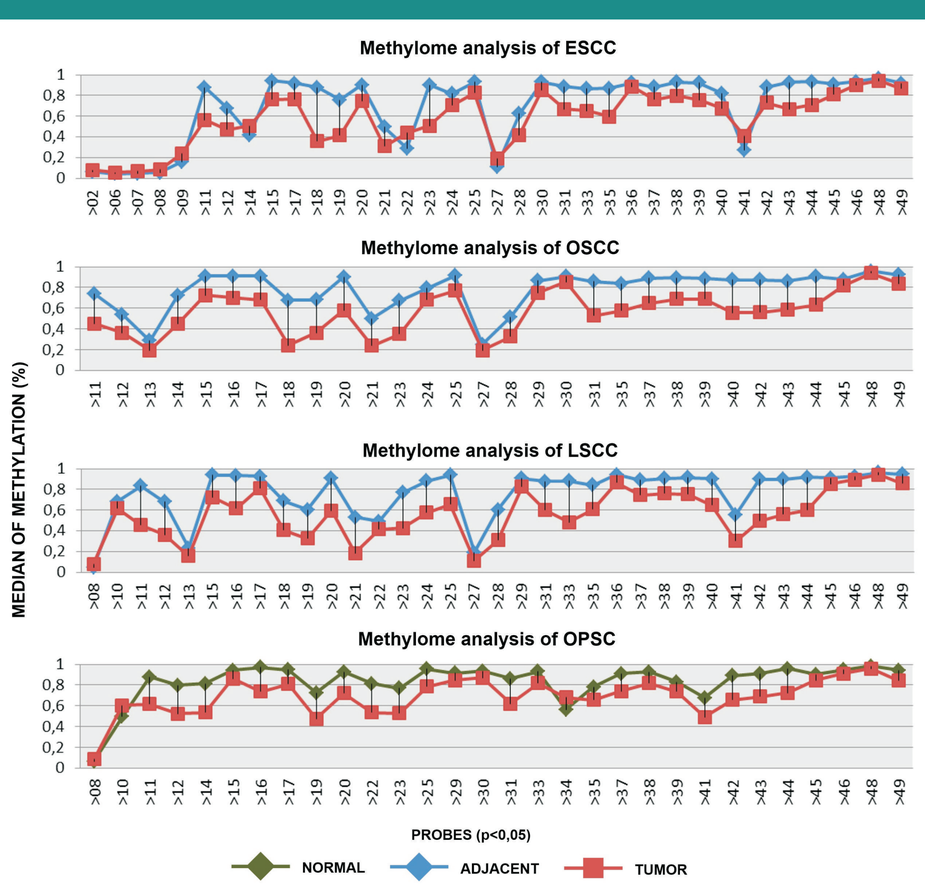
Methodology

- Methylation of *FBXL7* gene body in tumor and surrounding non-tumor tissue from ESCC and HNSCC patients by methylome (Illumina Infinium 450k) and pyrosequencing (Pyromark Q96 ID);
- Expression of *FBXL7* in tumor and surrounding non-tumor tissue from patients by RT-qPCR;
- Statistical analysis were performed in GraphPad Prism 4. All statistical analyses were two sided, and a 0.05 significance level was used;
- All biopsy samples were obtained from BNT/INCA. This project was approved by the CEP-INCA, number 116/11.

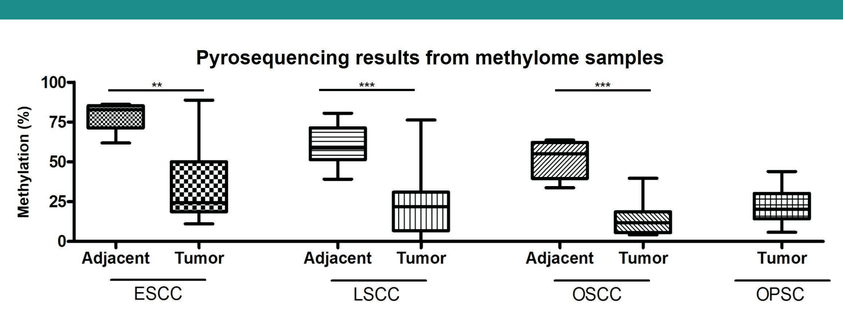
Hypothesis

Alterations of *FBXL7* methylation profile is a common feature in ESCC and HNSCC, as a consequence of risk factor exposure and/or squamous epithelium transformation, and leads to the dysregulation of its expression and of Aurora A and Survivin, its ubiquitination targets.

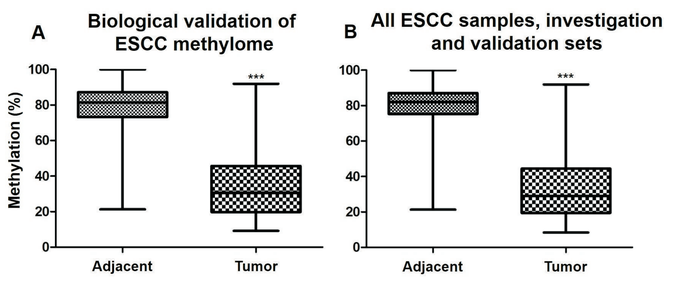
Results



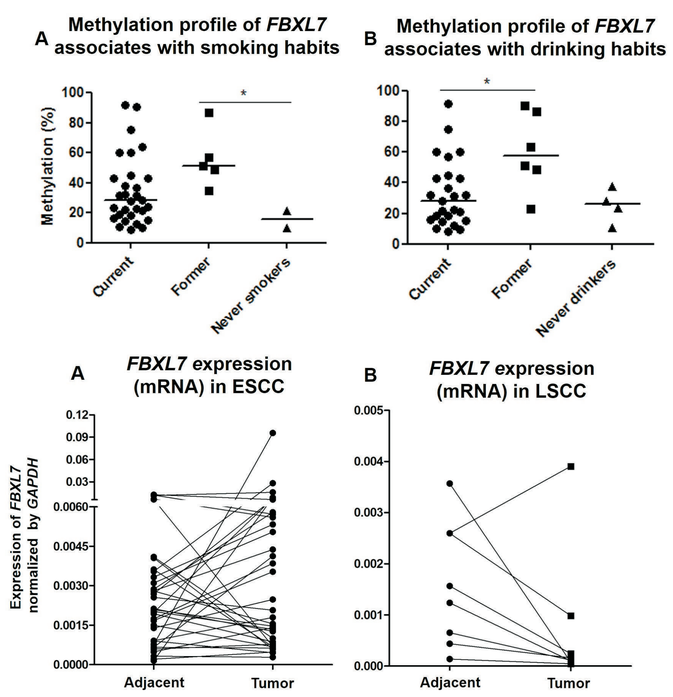
Median of methylation (%) of each probe from methylome analysis of *FBXL7* gene in ESCC and HNSCC sites: oral cavity (OSCC), larynx (LSCC) and oropharynx (OPSC). 49 probes were analyzed, each probe represents the methylation profile of one cytosine (CpG). The methylome analysis between the tumor and surrounding tissue revealed a hypomethylation profile along the majority of probes in all tumors. The graphs display only the probes with a $p < 0.05$ significance level (two-tail, non-paired) between normal (tonsillectomy) or adjacent non-tumoral tissue and the tumor tissue. ESCC N = 40, OSCC N = 22, LSCC N = 31 and OPSC N = 32.



Methylome assay of *FBXL7* was validated with pyrosequencing. Using the same samples, we found a hypomethylation profile in ESCC (**p = 0,001, N = 25, non-paired), OSCC (***p = 0,0003, N = 22, non-paired) and LSCC (***p < 0,0001, N = 27, non-paired) samples.



Biological validation of *FBXL7* methylation in ESCC.
The hipomethylation profile is held in both scenarios. A – Vlaidation set (samples that were not include in the methylome) (***p<0,0001, N = 42, paired). B – Combination of investigation and validation sample sets (***p<0,0001, N = 56, paired).



Association between the methylation profile of *FBXL7* in ESCC and risk factor exposure.
A - smoking habits (current, former and never smokers, p = 0,0345, N = 37, Kruskal-Wallis test). B – Alcohol consumption (current, former and never drinkers, p = 0,0423, N = 35, Kruskal-Wallis test).
*p < 0,05 *post hoc* Dunn's Multiple Comparison Test.

***FBXL7* gene expression in ESCC and LSCC samples.**
A – Expression in ESSC shows no significant difference between adjacent and tumor (p = 0,3463, N = 35, paired). B – Gene expression in LSCC shows no significant difference (p = 0,0781, N = 8, paired).

Conclusion

FBXL7 gene body is commonly hypomethylated in ESCC and HNSCC, suggesting its potential as biomarker, but the impact of this alteration on gene expression requires further investigation.

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