Analysis of molecular alterations of FBXL7 in Esophagus and Oral Cavity Epidermoid Carcinoma

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INTRODUCTION

The esophageal (ESCC) and head and neck squamous cells carcinomas (HNSCC) represent over 90% of the tumors originated at these sites[1, 2]. HNSCC is represented by a group of tumors e.g., oral cavity (OSCC), larynx (LSCC) and oropharynx (OPSC). 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 a universal biomarker. In this context, the DNA methylation is showing an intimate correlation with prognostic, risk factors and biological behavior of these tumors[4, 5]. Based on these findings, our methylome results of ESCC and HNSCC present the methylation profile of one gene, *FBXL7*, that can distinguish the histologically normal tumor surrounding tissue from tumor, with high specificity and sensibility.

FBXL7 protein belongs to the E3 ubiquitin ligase complex, catalyzing the ubiquitination of target proteins for proteasome degradation[6]. Their targets are the 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.

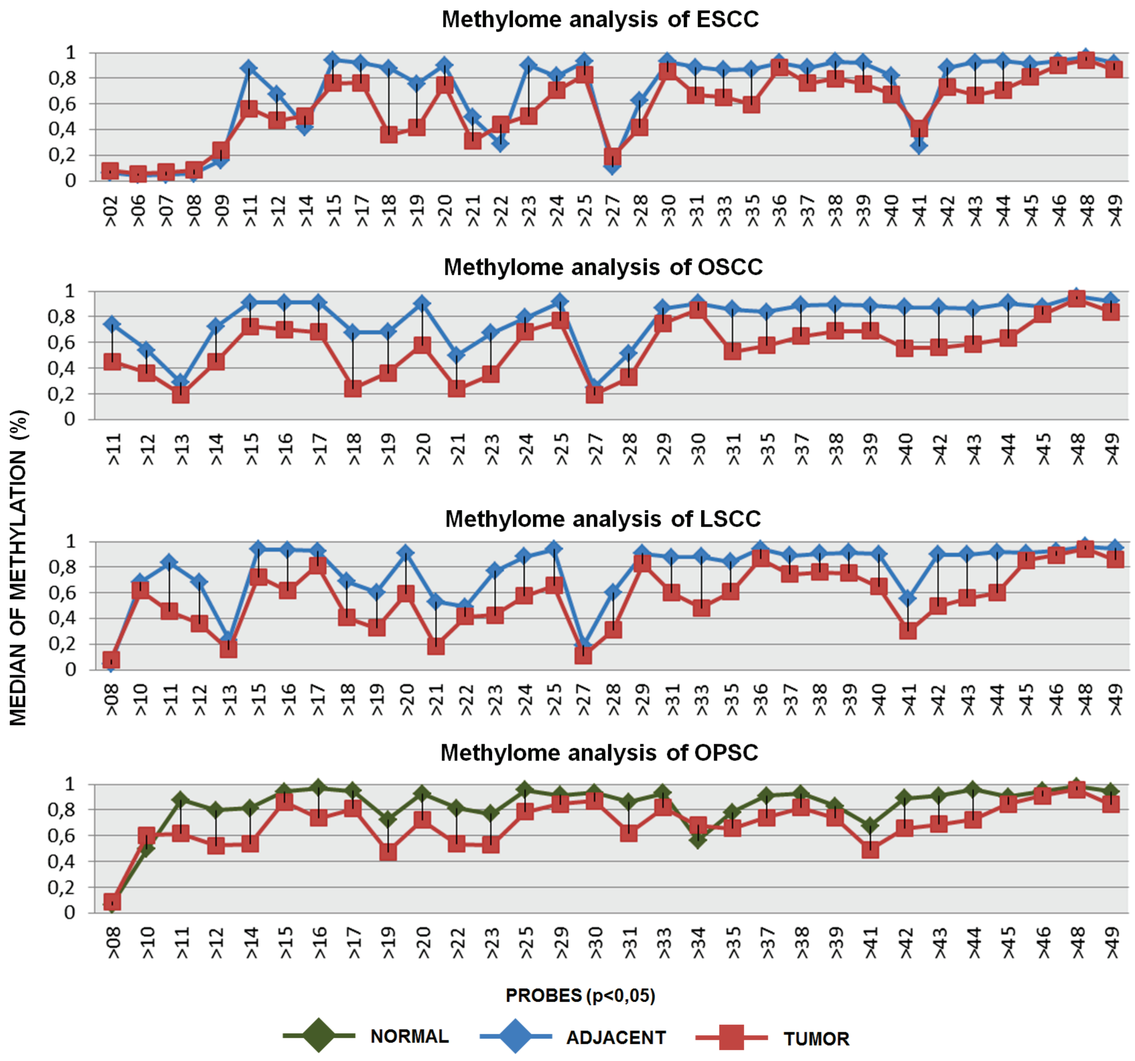
METHODOLOGY

- Methylation of *FBXL7* gene in tumor and surrounding non-tumor tissue from patients with ESCC and HNSCC by methylome (Illumina Infinium 450k) and pyrosequencing (Pyromark Q96 ID);
- Expression of *FBXL7* gene in tumor and surrounding non-tumor tissue from patients with ESCC and LSCC by RT-qPCR;
- Statistical analysis are 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.

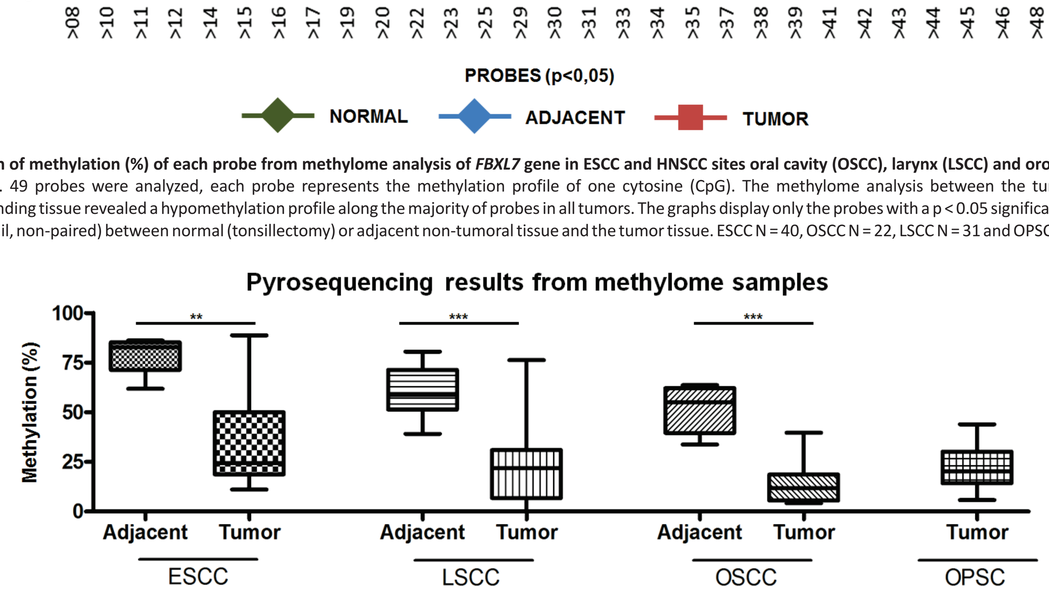
HYPOTHESIS

Alteration on methylation profile of *FBXL7* is a common feature in ESCC and HNSCC in consequence of risk factors or the epithelium transformation.

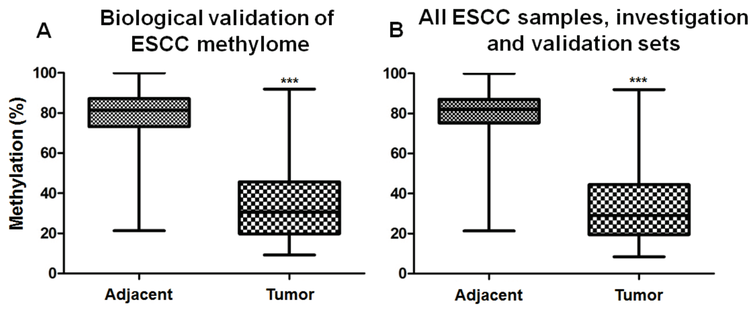
RESULTS



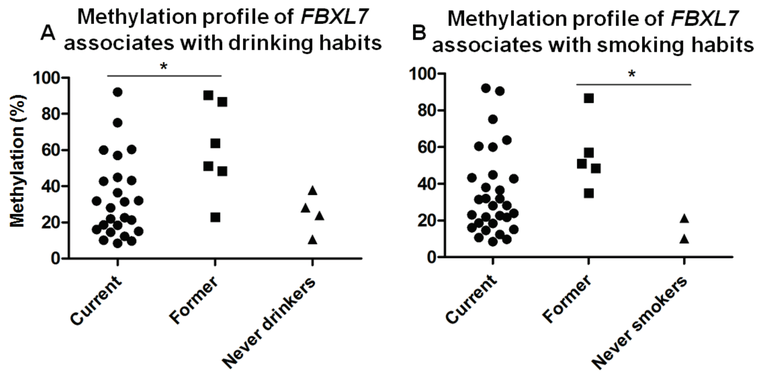
Median of methylation (%) of each probe from methylome analysis of *FBXL7* gene in ESCC and HNSCC sites oral cavity (OSCC), larynx (LSCC) and oropharynx (OPSC). 49 probes were analyzed, each probe represents the methylation profile of one cytosine (CpG). The methylome analysis between the tumor and surrounding 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.



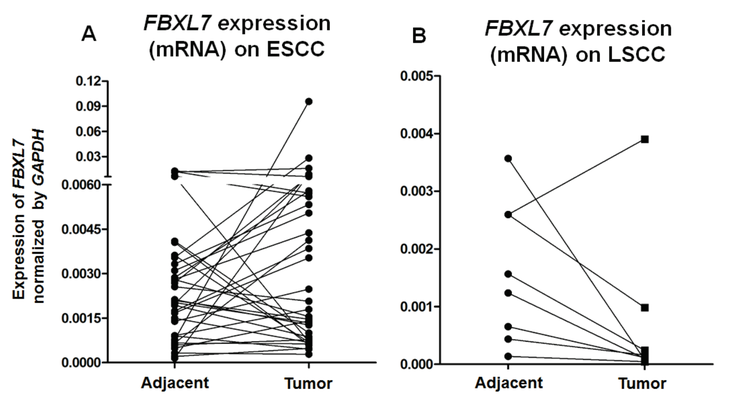
Methylome assay of *FBXL7* was validated with pyrosequencing. Using the same samples, we found hypomethylation profile in ESCC (**p = 0,001, N = 25, non-paired), OSCC (***p = 0,0003, N = 22, non-paired) and LSCC (***p < 0,0001, N = 27, non-paired) samples.



Biological validation of *FBXL7* in ESCC methylation assay. The hipomethylation profile is held in both scenarios. A – 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 clinicopatho-logical data of patients. A - 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. B - smoking habits (current, former and never smokers, p = 0,0345, N = 37, Kruskal-Wallis test).



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

CONCLUSION

FBXL7 is commonly hypomethylated in ESCC and HNSCC, but the impact of this alteration requires further investigation.

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