FBXL7 GENE BODY HYPOMETHYLATION IS A COMMON FEATURE IN ESOPHAGEAL AND HEAD AND NECK SQUAMOUS CELL CARCINOMAS

DIEGO CAMUZI CASSIANO[1]; LUIS FELIPE RIBEIRO PINTO[1],[2]; SHEILA COELHO SOARES LIMA[1]

[1]Programa de Carcinogênese Molecular– CPQ – Instituto Nacional de Câncer; [2]Departamento de Bioquímica – IBRAG – Universidade do Estado do Rio de Janeiro

Introduction and Aims

Esophageal and head and neck squamous cells carcinomas (ESCC and HNSCC) are highly incident and lethal in Brazil, and usually diagnosed at late stages[1]. Besides the same squamous epithelium of origin, these tumors also present similar etiology[2]. Therefore, the search for common molecular mechanisms can be useful to clarify the biologic processes involved in their development and in identifying a universal biomarker. In this context, DNA methylation shows an intimate correlation with environmental exposure and biological behavior in cancer[3].

Objective

Based on this, our group has performed a global methylation analysis to identify common biomarkers in ESCC and HNSCC.

Methods

FBXL7 methylation analysis was performed by methylome (Illumina Infinium 450K) and pyrosequencing. *FBXL7* mRNA expression was evaluated by RT-qPCR and protein levels were assessed by immunohistochemistry.

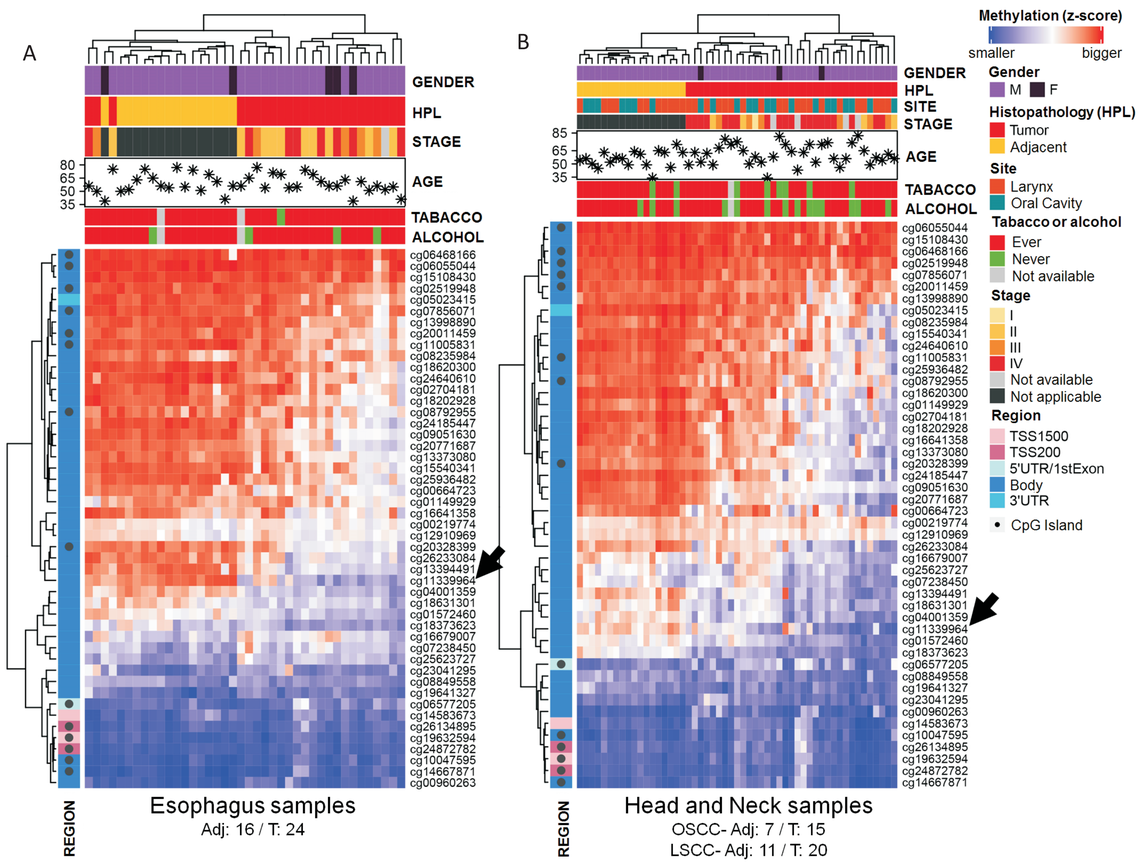

Figure 1: FBXL7 gene body is hypomethylated in ESCC and HNSCC. A. Methylation profile of ESCC patients. B. Methylation profile of LSCC and OCSCC patients. Arrow: Probe selected for pyrosequencing validation and further analyses (cg11339964).

Results

The methylome analysis comparing tumor and histologically normal surrounding tissue (adjacent) revealed a hypomethylation profile along 19 probes located in *FBXL7* gene body in ESCC (Figure 1A), laryngeal squamous cell carcinoma (LSCC) and oral squamous cell carcinoma (OCSCC) (Figure 1B). The methylation profile of the probe with the highest accuracy to discriminate tumor and adjacent tissues was validated by pyrosequencing (Figure 2A), confirming its high accuracy (Figure 2A) and evidencing its association with survival (Figure 2C) in ESCC. *FBXL7* gene expression did not differ between tumor and adjacent samples (Figure 2D), but protein staining showed lower nuclear positivity in ESCC and LSCC relative to their respective adjacent tissues (Figures 3A-D). In oropharyngeal squamous cell carcinomas, *FBXL7* methylation varied according to HPV status, indicating an association with risk factor (Figures 4A-B). A deeper analysis of *FBXL7* genomic region revealed it comprises an endogenous retrovirus (MER4A1) and is associated with an enhancer region (Figure 5A), which might have an impact on the expression of other genes. Correlation between *FBXL7* methylation and transcriptomic data in LSCC revealed an inverse correlation with two genes, which were also overexpressed (Figure 5B-C). Methylation profile of 450k probes in repetitive elements was evaluated and only cg11339964 *(FBXL7)* showed a hypomethylation profile for all tumors (Figure 5D).

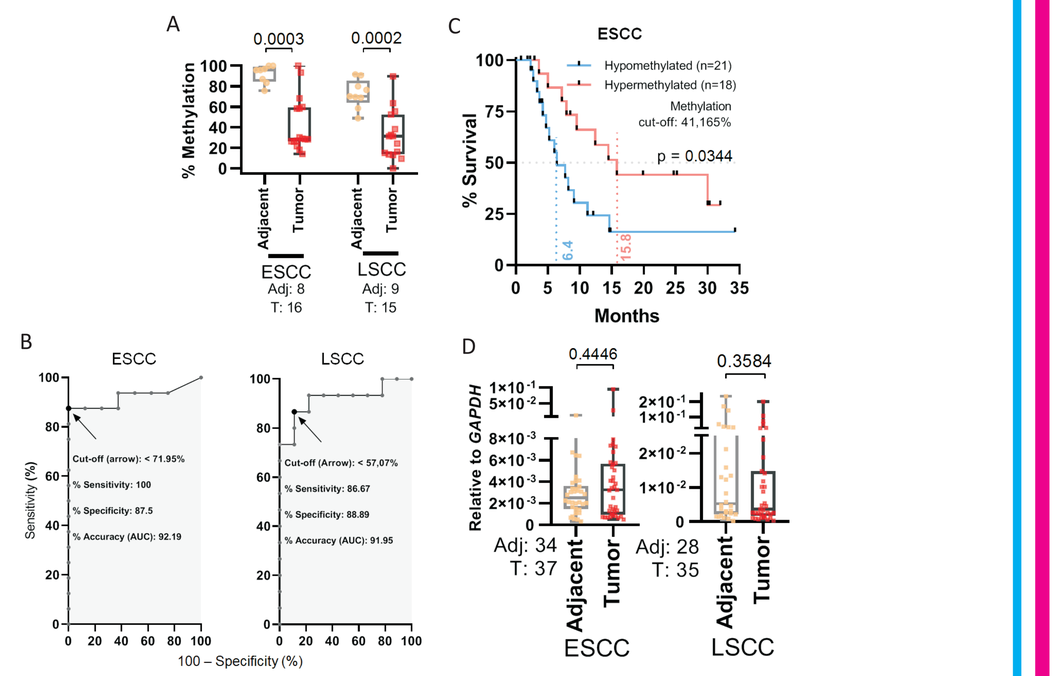

Figure 2: *FBXL7* methylation profile is associated with survival but not with its mRNA expression. A. Validation of methylome data by pirosequencing in ESCC and LSCC. B. ROC curves for discriminating tumors and adjacent tissues according to FBXL7 methylation. C. Kaplan-Meier curve showing the impact of FBXL7 methylation on overall survival of ESCC patients. D. FBXL7 gene expression in ESCC and LSCC.

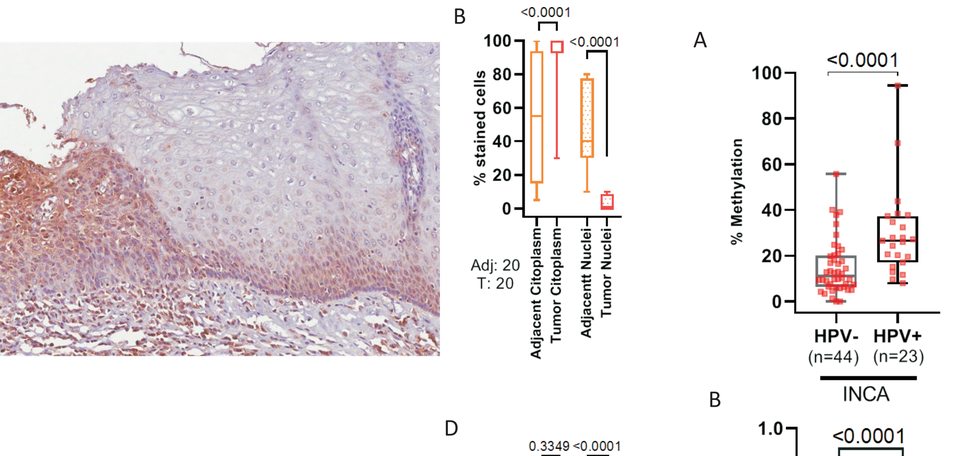

Figure 3: FBXL7 protein levels are altered in both ESCC and LSCC. A. Representative slide of FBXL7 immunostaining in ESCC. B. Quantification of FBXL7 positivity in ESCC according to subcellular location. C. Representative slide of FBXL7 immunostaining in LSCC. D. Quantification of FBXL7 positivity in LSCC according to subcellular location.

Figure 4: *FBXL7* methylation might be associated with risk fator exposure in oropharyngeal squamous cell carcinoma. FBXL7 methylation profile in tumor samples from Brazil (A) and from London (B).

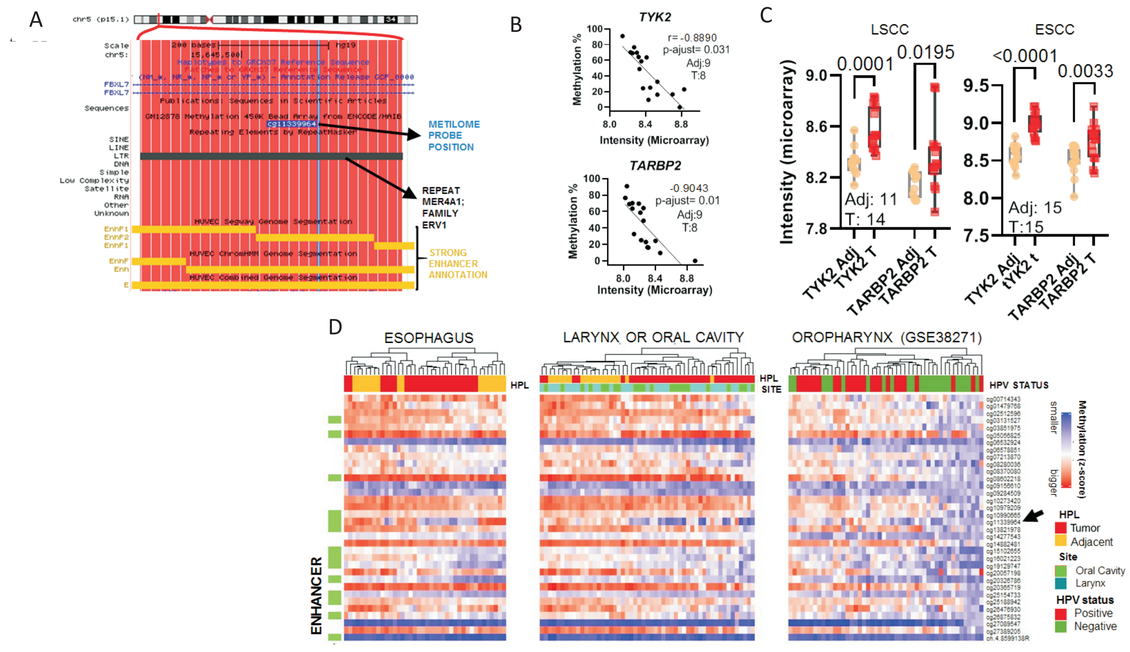

Figure 5: *FBXL7* contains an endogenous retrovirus and enhancer. A. Co-localization of MER4A1/ERV1, enhancer and methylome probe whitin FBXL7. B. Significant correlations between cg11339964 methylation and transcriptomic data in LSCC. C. mRNA expression of genes inversely correlated with cg11339964 methylation in LSCC. D. Heatmap showing the methylation profile of MER4A1-associated probes in ESCC, LSCC, OCSCC and OSCC

Conclusion

FBXL7 gene body is commonly hypomethylated in ESCC and HNSCC, suggesting its potential as a biomarker, but the impact of this alteration requires further investigation. FBXL7 is part of an E3 ubiquitin ligase complex and its biological and oncologic functions are poorly understood.

Projeto Gráfico: Área de Edição e Produção de Materiais Técnico-Científicos / INCA

References

1- CA Cancer J Clin. 2018 Nov;68(6):394-424
2-Chung et al.. BMC Gastroenterology 2013; 13:154.
3- Cancer Prev Res (Phila). 2011 Dec;4(12):1982-92.