INVESTIGATION OF THE FUNCTIONAL ROLE OF *SPRR3* GENE IN ESOPHAGEAL SQUAMOUS CELL CARCINOMA

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INTRODUCTION

Esophageal cancer (EC) is the eighth most frequent cancer and the sixth leading cause of cancer-related deaths worldwide, with squamous cell carcinoma (ESCC) corresponding to 80% of the cases[1,2];

Recently, our group studied the transcriptome of ESCC, which showed changes in the expression profile of different genes associated with the epithelial differentiation process, such as *SPRR3, SPRR2, INV, FLG, LOR;*

SPRR3 is a differentiation-associated gene that belongs to SPRR (small proline rich) family [3] that showed a gradual loss of expression in malignant transformation of the healthy esophagus into ESCC (Fig.1)[4].

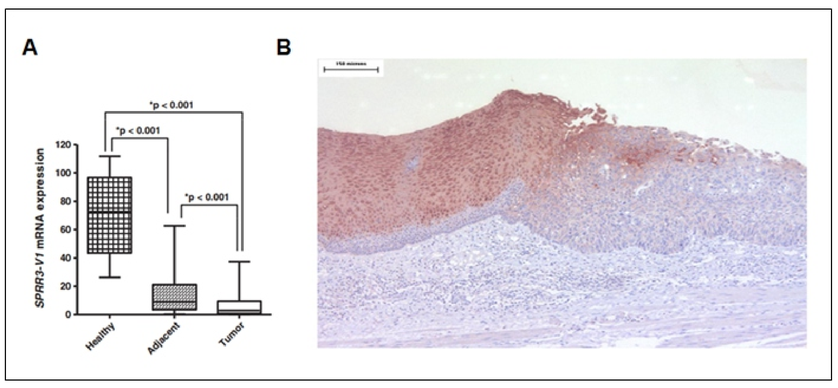

Figure 1: SPRR3 expression profile in CEE. A) Expression *SPRR3* variant 1 in CEE. B) Immunohistochemistry for SPRR3 in tumor tissue (right) with border free of neoplasia (left). De A Simão

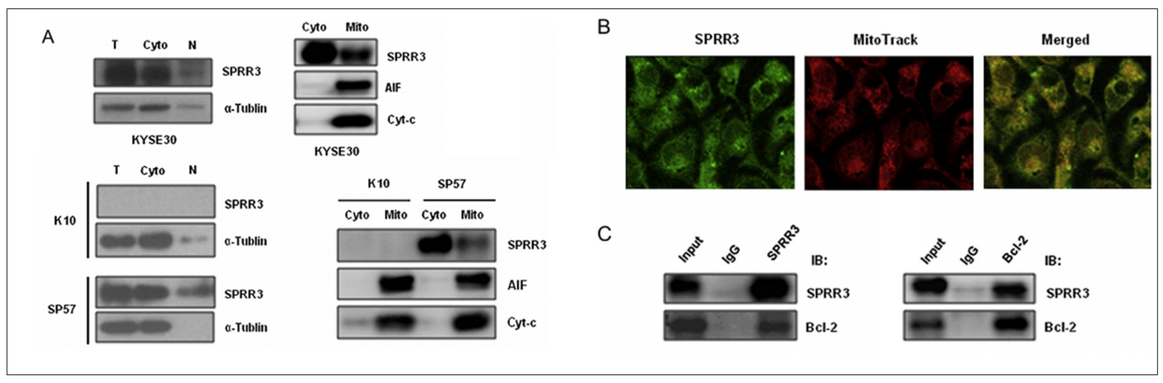

Figure 2: SPRR3 localizes in mitochondria and interacted with Bcl-2 in vivo. A) Analysis of subcellular localization of SPRR3 by subcellular fractionation. Cells were subjected to subcellular fractionation (T, total extract; N, nuclear), and Western blot was performed with cytoplasm (Cyto) and mitochondrial (Mito) fractions. a-Tubulin and AIF were used as cytosolic and mitochondrial marker proteins, respectively. B) Subcellular localization of SPRR3 protein. KYSE30 cells were incubated with 20 nM MitoTracker CMX-Ros or anti-SPRR3, and analyzed by confocal microscope images. C) Co-immunoprecipitation of SPRR3 and Bcl-2. Whole cell lysates from KYSE30 cells were prepared and immunoprecipitation was performed with anti-SPRR3 followed by Western blot with antibodies against Bcl-2 (left panel), or immunoprecipitated with antibodies against Bcl-2 followed by Western blot with anti-SPRR3 (right panel). Anti-Mouse IgG or anti-Rabbit IgG antibody was used as a negative control. Luo et al., 2013.

Recently, it has been shown that the SPRR3 induced expression in the CEE seems to be involved in the decrease of tumorigenicity and in the induction of apoptosis and that may be located in different subcellular compartments (Fig.2)[5].

Therefore, these findings suggest new roles for esophagin that not only its structural function and reiterate the need for new studies that explore the function and distribution of this protein.

OBJECTIVE

This study aims to explore the functional role of *SPRR3* in ESCC.

METHODS

SPRR3 expression will be abrogated or induced in ESCC cell lines (TE1 and TE13), by transfecting specific siRNA or expression vector, respectively, with lipofectamine;

Then, cellular endpoints, such as proliferation, apoptosis, migration and invasion will be checked by crystal violet assay, flow cytometry and transwell migration and invasion assay;

Additionally, global gene expression profile will be investigated upon *SPRR3* expression modulation, by using Affymetrix microarray platform, and the altered signaling pathways will be identified using the Gene Ontology (GO) and KEGG (Kyoto Encyclopedia of Genes and Genomes) platforms;

Finally, the validation of the differentially expressed genes will be performed by RT- qPCR using Tumor and histologically normal adjacent mucosa from ESCC patients.

EXPECTED RESULTS

We intend to contribute to the better understanding of the biology of this tumor in the sense of trying to elucidate the interactions established by *SPRR3* that favors the development of this tumor.

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