DNA methylation associated with *DNMT1* overexpression as a probable cause of esophagin loss in esophageal cancer

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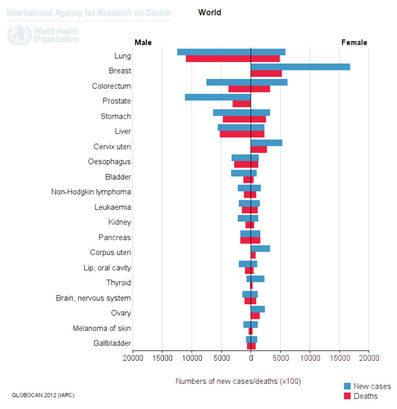
INTRODUCTION

- Esophageal cancer (EC) is the sixth most frequent cancer and is the sixth leading cause of cancer-related deaths worldwide and squamous cell carcinoma (ESCC) corresponds to 80% of the cases worldwide and in Brazil [1,2].
- Esophagin (SPRR3), a member of the SPRR family of cornified envelope precursor proteins, is strictly linked to keratinocyte terminal differentiation [3].
- A previous study from our group showed a gradual loss of esophagin expression in malignant transformation of the healthy esophagus into ESCC[4]. However, the molecular mechanisms involved in *SPRR3* silencing are unknown.



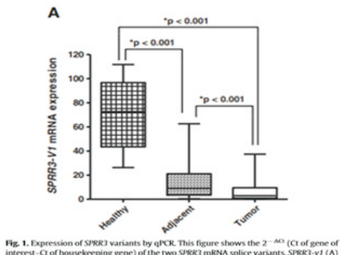


Fig. 1. Expression of *SPRR3* variants by qPCR. This figure shows the $2^{-\Delta CT}$ (Ct of gene of interest –Ct of housekeeping gene) of the two *SPRR3* mRNA splice variants, *SPRR3-v1* (A) *Statistically significant.

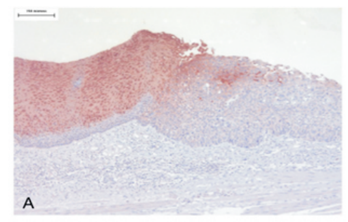


Fig. 3. Immunohistochemical staining of *SPRR3*. A–C. Images from the same section in which a surgical margin was present. A – This image presents the transition between normal esophageal epithelia (left) and in situ carcinoma (right). A clear reduction in *SPRR3* expression is seen in tumoral areas (scale bar – 150 μm).

- DNA methylation is the one of the most important and the best studied epigenetic mechanisms[5].
- The methylation pattern is copied and maintained during DNA replication in a process catalyzed by DNA methyltransferases (DNMTs) [5] .

OBJECTIVE

Examine DNA methylation as a regulatory mechanism of esophagin expression in ESCC.

METHODOLOGY

- Three CpG sites (Site a, Site b and Sitec) of esophagin gene were analyzed by pyrosequencing in esophageal tumor and matched surrounding non-tumor tissue from 15 patients with ESCC;
- A receiver operating characteristic (ROC) curve was plotted for the use of *SPRR3* methylation as a marker to distinguish tumoral esophagus from normal-appearing ESCC surrounding mucosa;
- RT-qPCR was performed to evaluated esophagin and *DNMT1* expression in the same samples.
- A correlation curve was plotted to examine the association between the studied markers.

RESULTS

Gradual decrease of esophagin expression levels in esophageal tissues

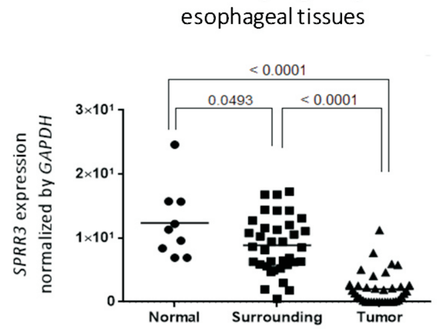


Figure 1: Comparison of *SPRR3* expression in healthy volunteers and ESCC patients.

The methylation levels of the evaluated CpG sites were significantly higher in tumors in comparison with the adjacent tissue

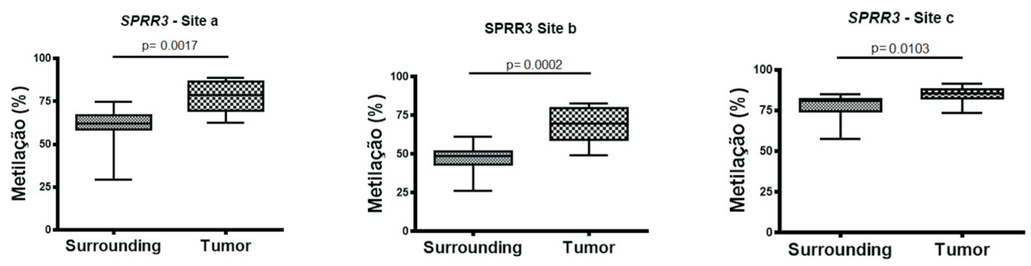


Figure 2:Comparison of three sites of *SPRR3* methylation in ESCC patients.

SPRR3 methylation is able to distinguish the adjacent mucosa from tumor samples

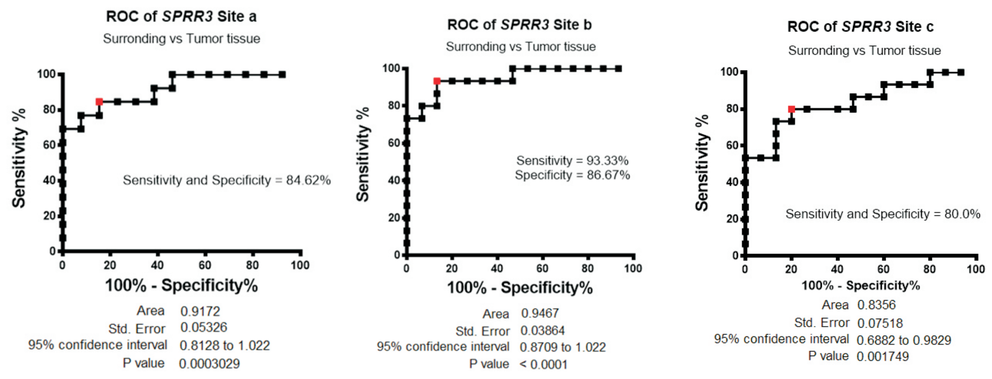


Figure 3:Receiver operating characteristic (ROC) curve for the discrimination of normal-appearing surrounding tissue and tumor tissue of ESCC patients, according to *SPRR3* methylation.

There is an inverse correlation between esophagin expression and DNA methylation for all CpG sites evaluated

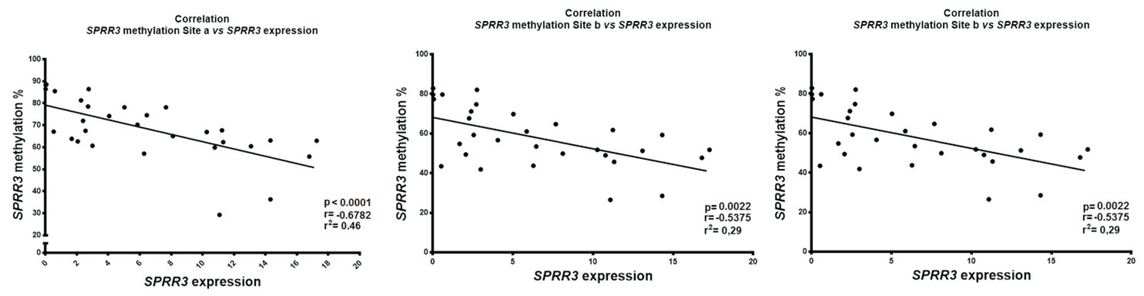


Figure 4: Correlation comparison of three sites of *SPRR3* methylation and *SPRR3* expression in ESCC patients.

There is an inverse correlation between expression of *DNMT1* and *SPRR3*

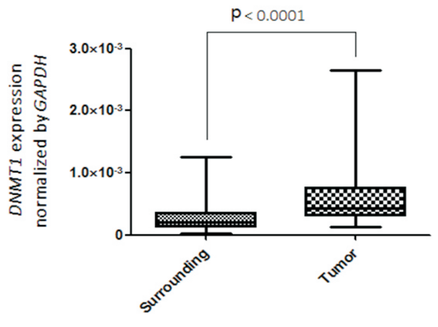


Fig.ure 5: Comparison of *DNMT1* expression ESCC patients.

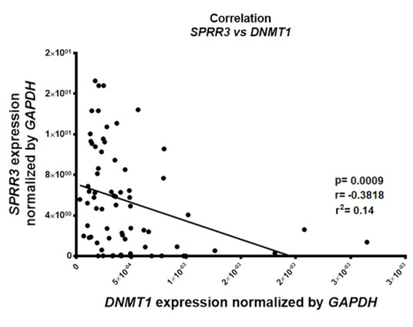


Figure 6: Correlation between *SPRR3* and *DNMT1* expression in ESCC patients.

There is an inverse correlation between *DNMT1* expression and esophagin DNA methylation for all CpG sites evaluated

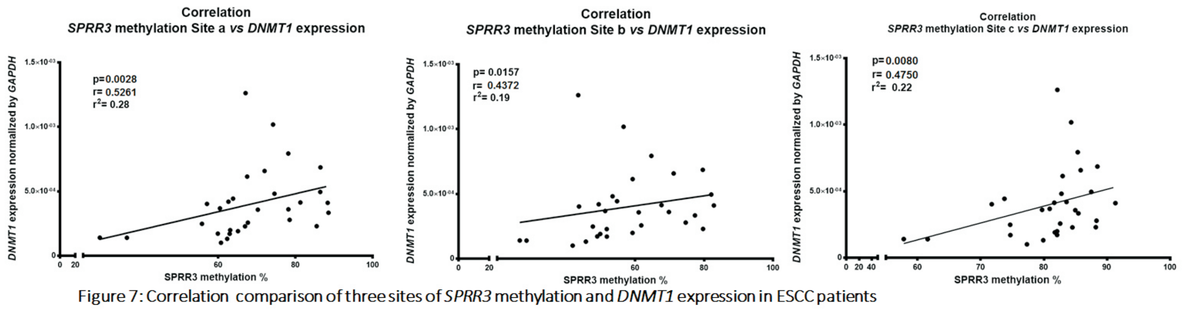


Figure 7: Correlation comparison of three sites of *SPRR3* methylation and *DNMT1* expression in ESCC patients

CONCLUSION

- Our data suggest DNA methylation induced by DNMT1 as a possible mechanism for esophagin silencing in ESCC.

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