ZNF281 EXPRESSION EVALUATION AND ITS FUNCTIONAL ROLE IN ESOPHAGEAL SQUAMOUS CELL CARCINOMA

Igor Ribeiro do Nascimento[1], Pedro Nicolau Neto[2], Luis Felipe Ribeiro Pinto[2]

1 Aluno de Aperfeiçoamento 1 - Programa de Carcinogênese Molecular, Instituto Nacional de Câncer (INCA), Rio de Janeiro-RJ, Brasil.

2 Programa de Carcinogênese Molecular - Instituto Nacional de Câncer (INCA), Rio de janeiro-RJ, Brasil.

Abstracts

Introduction: Esophageal cancer is a highly incident and deadly tumor, preferentially affecting males in developing regions, such as Brazil. The most common histological subtype worldwide and in Brazil is the squamous cell carcinoma (ESCC) and its genesis is related to tobacco smoking and alcoholism. Due to late diagnosis and lack of more effective therapeutic modalities, the five-year survival rate is less than 20%. Our group recently studied the ESCC transcriptome of Brazilian patients and identified zinc finger protein 281 (ZNF281) as the transcription factor potentially most associated with the regulation of the differentially expressed genes. This protein has already been associated with the mesenchymal-epithelial transition in other tumors and its expression has been associated with TP53 mutations and mir34a activity. Results: Using the re-analysis of deposited data in The Cancer Genome Atlas and Gene Expression Omnibus, we observed that ZNF281 is overexpressed in ESCC and there is an association between its expression and TP53 mutation in this tumor. Following the analysis of ZNF281 expression in ESCC, using fresh samples and real-time quantitative PCR was observed that ZNF281 is overexpressed in ESCC Brazilian samples. Besides, TP53-mutated ESCC showed higher ZNF281 expression than wild-type tumors. Nevertheless, no association between ZNF281 expression and clinicopathological features of the evaluated patients was observed. In vitro analyzes with a cell model showing a thermosensitive mutation in TP53 (ESCC-derived cell line TE-1) showed an opposite result to that observed in ESCC samples, with higher expression of ZNF281 in cells with wild-type TP53, which reinforces the need to evaluate the expression and role of mir34a in regulating the expression of ZNF281 in ESCC. Furthermore, somatic alterations and transcriptional profile of ZNF281 were similar between ESCC and esophageal adenocarcinoma, indicating that expression of this transcription factor appears to be important for esophageal carcinogenesis, regardless of histological subtype. Conclusion: Therefore, further analysis is needed to understand the ZNF281 expression regulation mechanisms in ESCC, as well as its role in esophageal carcinogenesis through gene expression control and induction of epithelial-mesenchymal transition.

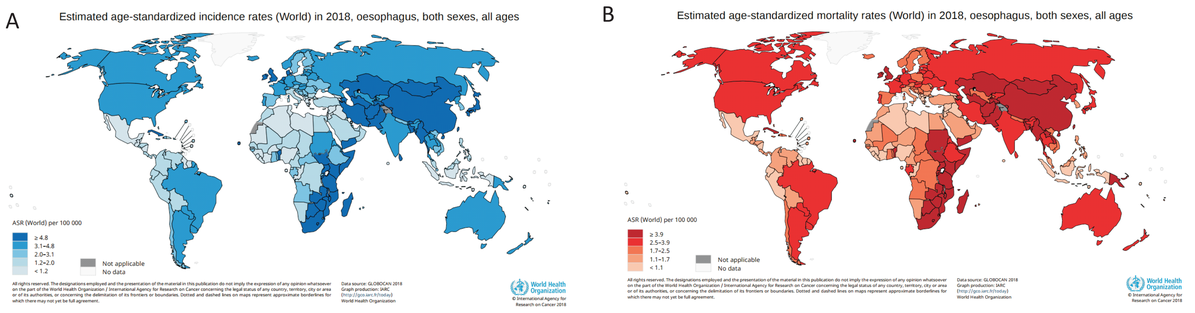


Figure 1: Esophageal cancer incidence and mortality rates worldwide. A - Estimated age-standardized incidence rates (World) in 2018, esophagus, both sexes, all ages. B - Estimated age-standardized mortality rates (World) in 2018, esophagus, both sexes, all ages. Data source: GLOBOCAN 2018. Graph production: IARC

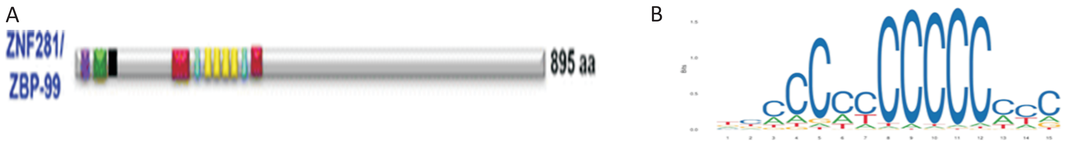
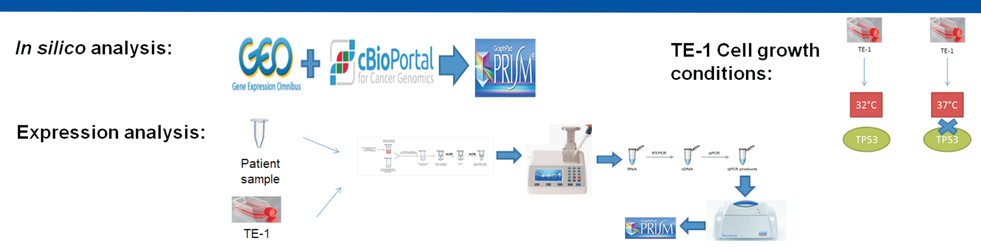
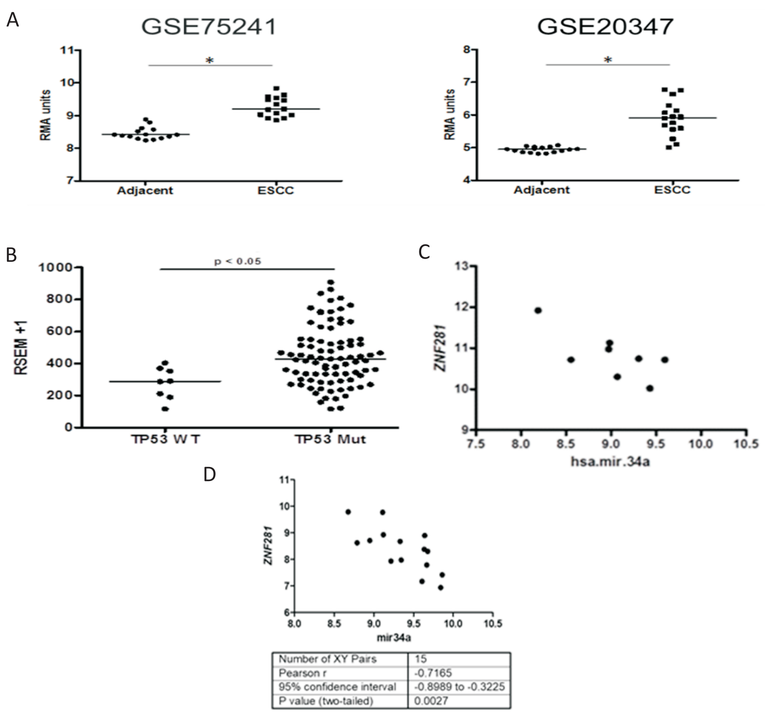


Figure 2: Characteristics of the transcriptional factor ZNF281. A- The ZNF281 protein displays characteristics of a transcription factor as it contains four C2H2 zinc-finger domains (residues 263 to 368). Modified from Wang *et al*., 2008. B - ZNF281 DNA binding sequence. JASPAR 2018.

Methods



Results



Number of XY Pairs	15
Pearson r	-0.7165
95% confidence interval	-0.8989 to -0.3225
P value (two-tailed)	0.0027

Figure 3: *ZNF281* is overexpressed in ESCC and its expression may be associated with *TP53* mutations and mir34a activity. A - *ZNF281* was overexpressed in two GEO datasets comparing the expression profile of ESCC and adjacent non-tumoral mucosa. B - *ZNF281* expression was significantly higher in *TP53*-mutated ESCC samples in the TCGA data. C - *ZNF281* expression was inversely correlated with *mir34a* expression in wild-type *TP53* ESCC samples in the *TCGA* data. D - Reviewing data from the transcriptome published by our group using both wild-type and mutant *TP53* samples, it was observed that *ZNF281* expression correlated inversely with *mir34a* expression.

Feature		n	*ZNF281* mRNA median expression	p-value
Sex	Male	25	0,0086	0,9
	Female	6	0,0078	
Age	<60	15	0,0086	0,6
	=60	16	0,0091	
Tumor site	Upper	4	0,0068	0,59
	Medium	24	0,0086	
	Lower	3	0,0087	
Tumor Grade	2	21	0,0089	0,14
	3	9	0,0075	
	NA	1		
Tumor Stage	Early	6	0,017	0,13
	Late	23	0,008	
	NA	2		

Table 1: Clinicopathological characteristics of patients with ESCC included in the study and association analysis with *ZNF281* expression. No association was observed with the evaluated information.

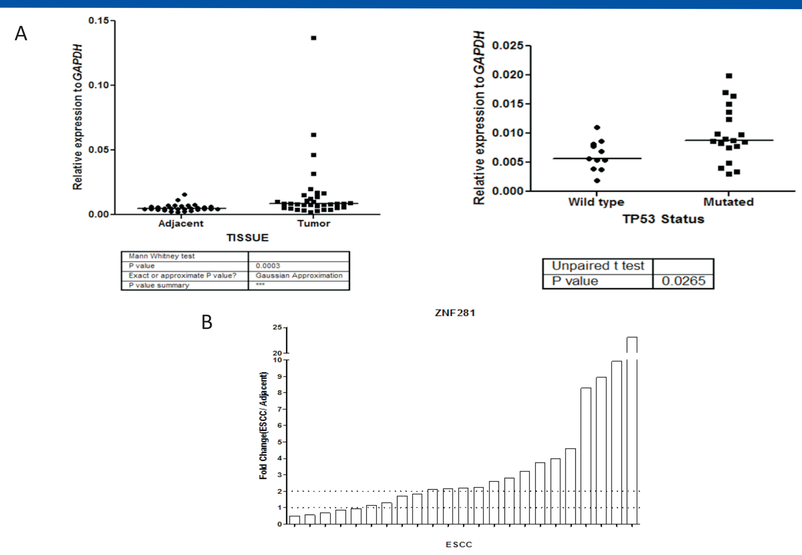


Figure 4: The *ZNF281* gene expression validation in ESCC. A - Through RT-qPCR, the *ZNF281* overexpression in ESCC was validated and *ZNF281* was once again higher in *TP53*-mutated samples (p = 0.02). B - The *ZNF281* increased expression in ESCC in relation to adjacent mucosa was a common phenomenon in 27 of 31 analyzed samples.

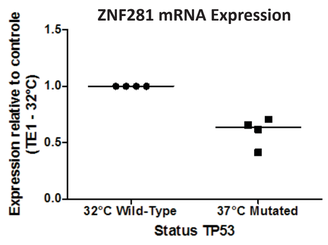


Figure 5: Evaluation of ZNF281 expression in ESCC TE-1 derived cell line possessing TP53 wild-type (32°C) and mutated (37°C). In disagreement with our hypothesis, TE-1 cells with *TP53* wild-type showed higher *ZNF281* expression than TE-1 cells with TP53 mutated, but without statistical significance (n = 4; p = 0.125).

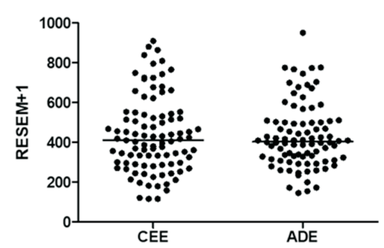


Figure 6: *ZNF281* expression in the two major histological subtypes of esophageal cancer. It was not observed differences in *ZNF281* expression between ESCC (96) and EAC (89) using TCGA data (p = 0.98).

Conclusion

Therefore, further analysis is needed to understand the ZNF281 expression regulation mechanisms in ESCC, as well as its role in esophageal carcinogenesis through gene expression control and induction of epithelial-mesenchymal transition.

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

Financial support

FAPERJ · INCA · SUS · MINISTÉRIO DA SAÚDE · PÁTRIA AMADA BRASIL GOVERNO FEDERAL