ALTERATIONS OF MET-HGF PATHWAY IN ESOPHAGEAL SQUAMOUS CELL CARCINOMA

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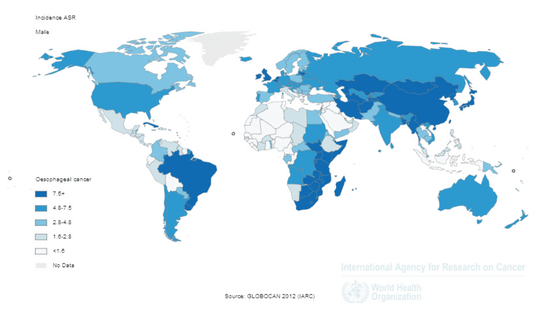
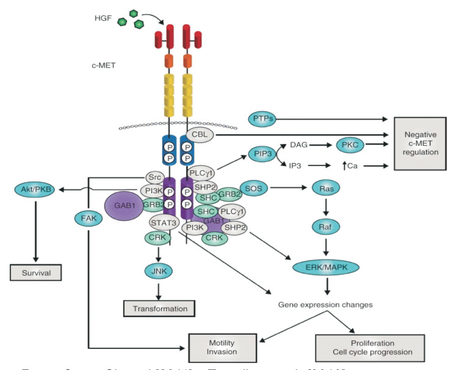
INTRODUCTION

- Esophageal cancer is among the ten most incident and lethal tumors in the world, ranking 6th in incidence and 5th in mortality among men.
- Esophageal squamous cell carcinoma (ESCC) corresponds to more than 80% of esophageal cancer cases in Brazil and worldwide;
- The main risk factors for ESCC development are alcohol and tobacco consumption, similar to head and neck tumors, as laryngeal squamous cell carcinoma (LSCC)
- The high lethality of esophageal cancer is associated with a late diagnosis, leading to ineffective treatment. This demonstrates the need for detection of biomarkers and new therapeutic approaches for this disease.



- Among the most promising signaling pathways in tumors of the gastrointestinal tract, the one activated by the binding of hepatocyte growth factor (HGF) to its receptor MET stands out.



Fonte: Organ SL et al.[2011] e Trusolino et. al. *[2010]*

GOAL

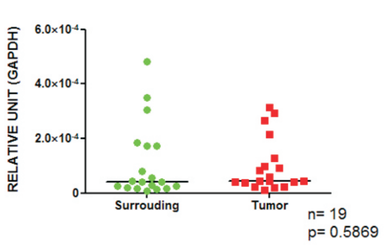
To evaluate the expression of *MET* and *HGF* in ESCC, comparing their expression in tumor and its respective non-tumor surrounding mucosa.

RESULTS

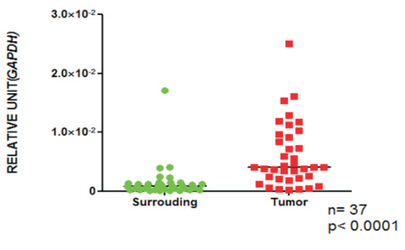
Clinical-pathological data

VARIABLE	n
Gender	
Male	23 (79,0%)
Female	06 (21,0%)
Age (years)	60 (48 – 79)
Tumor Topography	
Upper Third	8 (27,6%)
Upper-middle third	3 (10,3%)
Middle Third	5 (17,4%)
Middle-lower third	7 (24,0%)
Lower third	3 (10,3%)
Not available	3 (10,3%)
Degree of differentiation	
Well	0
Moderately	21 (72,4%)
Poorly	07 (24,1%)
Not Available	01 (3,5%)
T (TNM)	
In situ	0
1	02 (7,0%)
2	0
3	07 (24,0%)
4	02 (7,0%)
Not available	18 (62,0%)
Death	
Yes	17 (58,5%)
No	11 (38,0%)
Not available	01 (3,5%)
Survival (median)	9 months

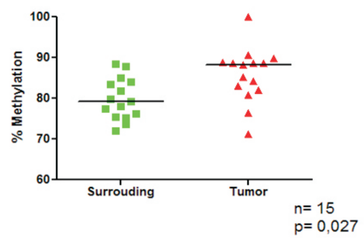
***METv1* expression in ESCC**



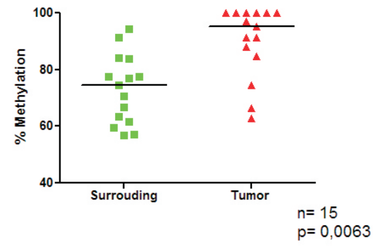
***METv2* expression in ESCC**



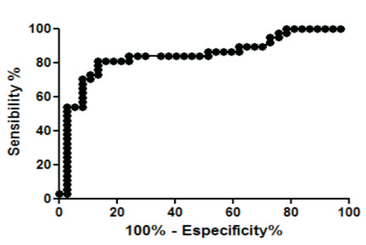
Analysis of *MET* intragenic CpG site 1



Analysis of *MET* intragenic CpG site 2



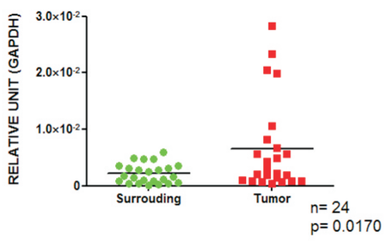
ROC curve of *METv2* expression



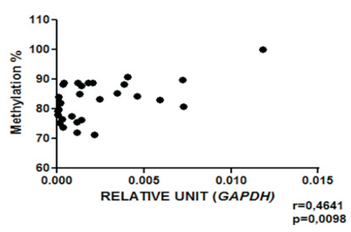
Parameters to discriminate ESCC from Normal

Accuracy: 84.11%
Sensitivity: 86.49%
Specificity: 81.08%

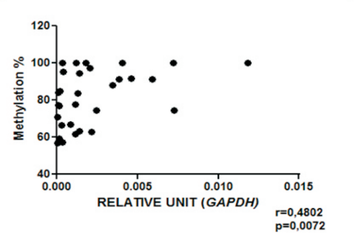
***HGF* expression in ESCC**



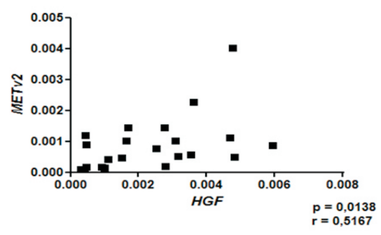
Correlation between CpG site 1 methylation and *MET* expression



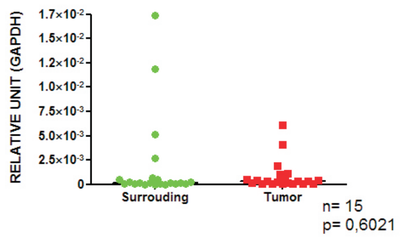
Correlation between CpG site 2 methylation and *MET* expression



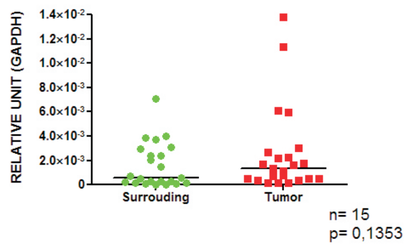
Correlation between *MET* and *HGF* expression



***METv1* expression in LSCC**



***METv2* expression in LSCC**



METHODOLOGY

- ESCC Patients included in this study were admitted in the Brazilian National Cancer Institute between December 2012 and June 2013;
- Samples were subjected to RNA extraction using RNeasy® Mini Kit (Qiagen) followed by reverse transcription reaction for cDNA synthesis;
- The expression of two variants of *MET* gene were evaluated by quantitative PCR (qPCR) using specific primers for each variant;
- The clinical-pathological data of the patients were collected from their medical records.
- Samples were subjected to DNA extraction using DNeasy® Blood and Tissue (Qiagen) followed by DNA modification using EZ DNA Methylation-gold.
- Converted DNA was used for amplification of specific intragenic CpG regions of *MET* by PCR and finally submitted to pyrosequencing by Pyromark Q96.

PERSPECTIVES

- Collect clinicopathological data and correlate with *MET* expression, methylation and patient`s survival;
- Immunohistochemistry for MET;
- *In silico* analysis of *MET* differential splicing.
- Evaluate the methylation profile of LSCC samples.

CONCLUSION

- In ESCC, only *MET* variant 2 is overexpressed in comparison with surrounding tissue and its expression levels could be a good biomarker for ESCC diagnosis;
- HGF is also overepressed in ESCC, but showed no correlation with *MET* expression.
- Expression of *MET* variant 2 was positively correlated with the methylation status of intragenic CpG sites.
- Although ESCC and LSCC share the same risk factors, we observed no changes in the expression of *MET* variants in LSCC.

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