THE ROLE OF p53 AND SP1 IN DNA METHYLTRANSFERASES GENE REGULATION

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INTRODUCTION

- DNA methylation is an epigenetic mechanism characterized by the addition of a methyl group to cytosines in CpG dinucleotides and catalyzed by the DNA methyltransferases DNMTs [1, 2].
- 3 DNMTs with catalytic activity have been described in humans *DNMT1, DNMT3A* and *DNMT3B* [2].

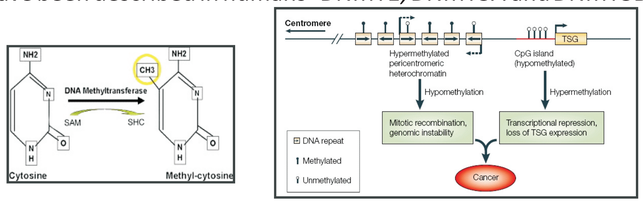

Figure 1: Methylation process catalyzed by DNMTs. Methylation profile in tumor cell.

- DNMTs overexpression has been reported in several different tumors, such as: lung, liver and esophagus tumors[3,4,5].
- Results obtained by our group demonstrated a relationship between *DNMT3B* overexpression and mutations in the tumor supressor gene *TP53* in esophageal squamous cell carcinoma (ESCC)[6].

AIM

To evaluate the role of p53 and SP1 in DNMTs (*DNMT1, DNMT3A* and *DNMT3B*) gene regulation.

Table 1: The association between the presence of *TP53* mutation and the expression levels of *DNMT3B* in the ESCC samples analyzed.

	DNMT3B ≥ 2	*DNMT3B* < 2	TOTAL
Mutated *TP53*	12 (86%)	2 (14%)	14 (100%)
Wild-Type *TP53*	25 (49%)	26 (51%)	51 (100%)

- About half of the human tumors harbors mutations in *TP*53 [7].
- SP1 is a transcription factor overexpressed in a wide range of tumor advanced stage, metastatic potential and poor prognosis [8].
- Interaction between p53 and SP1 in the regulation of different genes has already been reported reduced *DNMT1* gene expression in lung cancer cell line [3].

RESULTS

➢ *DNMT1, DNMT3A, DNMT3B* and *SP1* gene expression in TE-1 and HCT-116 cell lines under basal conditions

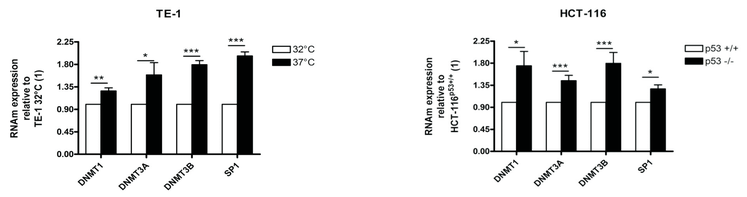

Figure 2: *DNMTs and SP1* basal mRNA expression in the esophageal squamous cell carcinoma harboring wild-type (TE-1 – 32ºC) or mutant (TE-1 – 37ºC) p53 and the colorectal carcinoma expressing (HCT-116$^{+/+}$) or not (HCT-116$^{-/-}$) p53 cell lines by qRT-PCR. *p<0.05, **p<0.01, ***p<0.001

➢ *DNMT1, DNMT3A, DNMT3B* and *SP1* gene expression in TE-1 and HCT-116 cell lines upon *TP53* silencing

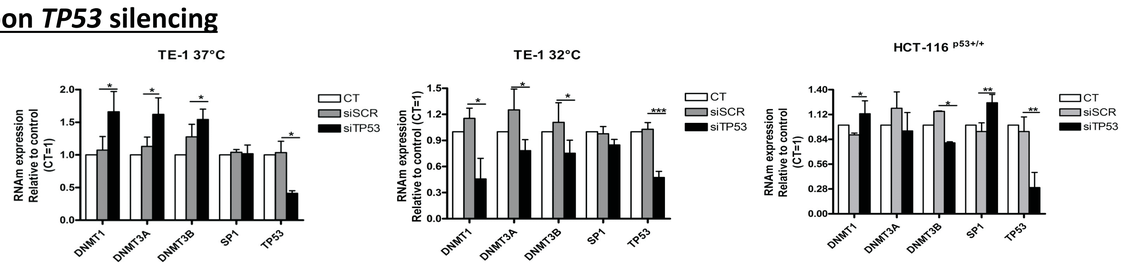

Figure 3: *DNMTs* and *SP1* mRNA expression in the esophageal squamous cell carcinoma harboring wild-type (TE-1 – 32ºC) or mutant (TE-1 – 37ºC) p53 and the colorectal carcinoma (HCT-116) cell lines by qRT-PCR upon *TP53* silencing achieved by transfection of target specif siRNA. *p<0.05, **p<0.01, ***p<0.001

➢ *DNMT1, DNMT3A, DNMT3B* and *SP1* gene expression in TE-1, TE-13 and HCT-116 cell lines following *TP53* overexpression

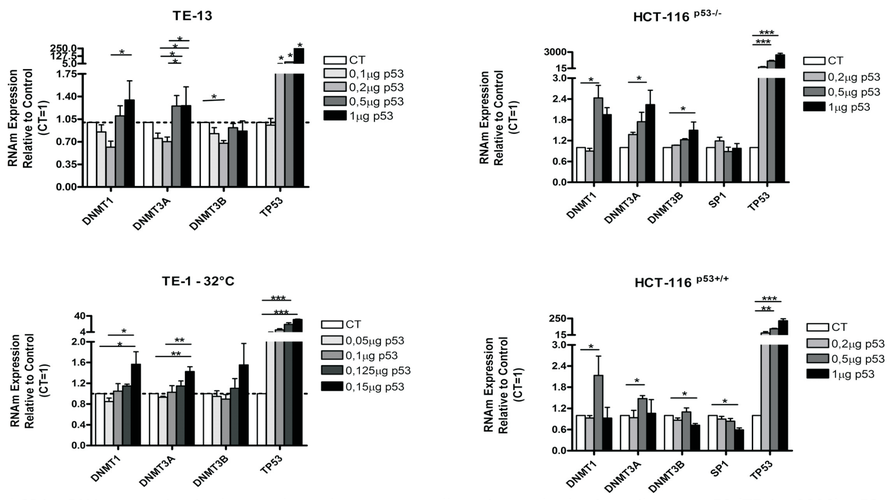

Figure 4: *DNMTs* and *SP1* mRNA expression in the esophageal squamous cell carcinoma harboring wild-type p53 (TE-1 – 32ºC), p53-null (TE-13) and the colorectal carcinoma expressing (HCT-116$^{+/+}$) or not p53 (HCT-116$^{-/-}$) cell lines (HCT-116) cell lines by qRT-PCR following *TP53* overexpression achieved by transfection of p53 expression vector. *p<0.05, **p<0.01, ***p<0.001

➢ *DNMT1, DNMT3A, DNMT3B* and *SP1* gene expression in TE-1 cell line after treatment with MMS 1mM

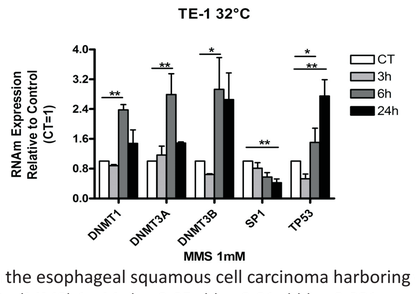

Figure 5: *DNMTs* and *SP1* mRNA expression in the esophageal squamous cell carcinoma harboring wild-type p53 (TE-1 – 32ºC) cell line after treatment with 1mM Methyl Methanesulfonate (MMS) 1mM. *p<0.05, **p<0.01, ***p<0.001

METHODOLOGY

Table 2: Description of the cell lines used in this study.

Cell Line	Tissue of Origin	*TP53* Status
TE-1	Esophageal squamous cell carcinoma	*TP53* temperature sensitive mutant: 32°C - WT p53 and 37°C - mutant p53
TE-13	Esophageal squamous cell carcinoma	Absent
HCT-116	Colorectal carcinoma	WT
HCT-116 $^{p53-/-}$	Colorectal carcinoma	Absent

- **TE-1 cell line was treated with 1mM of Methyl Methanesulfonate (MMS).**
- **TE-1 e TE-13 cell lines were treated with different concentrations of Mithramycin A (50nM, 100nM and 200 nM).**
- ***DNMTs* gene expression was evaluated in cell lines by qRT-PCR.**

➢ *DNMT1, DNMT3A, DNMT3B* and *SP1* gene expression in TE-1 and TE-13 cell lines upon *SP1* silencing

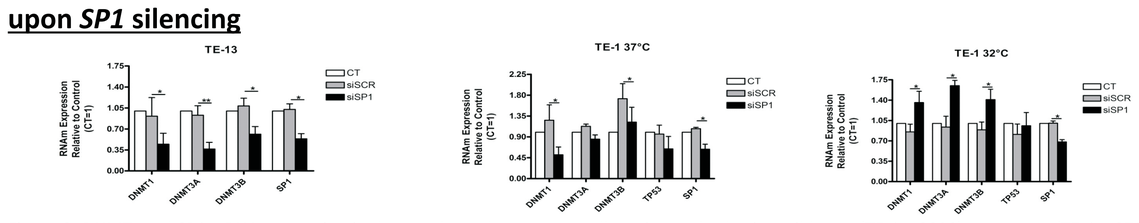

Figure 6: *DNMTs* and *SP1* mRNA expression in the esophageal squamous cell carcinoma harboring wild-type (TE-1 – 32ºC) or mutant (TE-1 – 37ºC) p53 and p53-null (TE-13) cell lines by qRT-PCR upon *SP1* silencing achieved by transfection of target specif siRNA. *p<0.05, **p<0.01, ***p<0.001

➢ *DNMT1, DNMT3A, DNMT3B* expression in TE-1 and TE-13 cell line after treatment with Mithramycin A

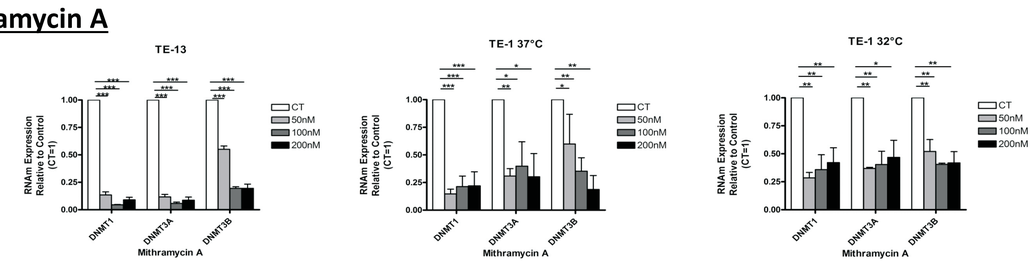

Figure 7: *DNMTs* mRNA expression in the esophageal squamous cell carcinoma harboring wild-type p53 (TE-1 – 32ºC) and p53-null (TE-13) cell lines after treatment with Mithramycin A. Mithramycin A is a G-C specific DNA binding drug, and prevents, subsequently, SP1 DNA binding. *p<0.05, **p<0.01, ***p<0.001

➢ *DNMT1, DNMT3A, DNMT3B* and *SP1* gene expression in TE-1, TE-13 and HCT-116 cell lines following SP1 overexpression

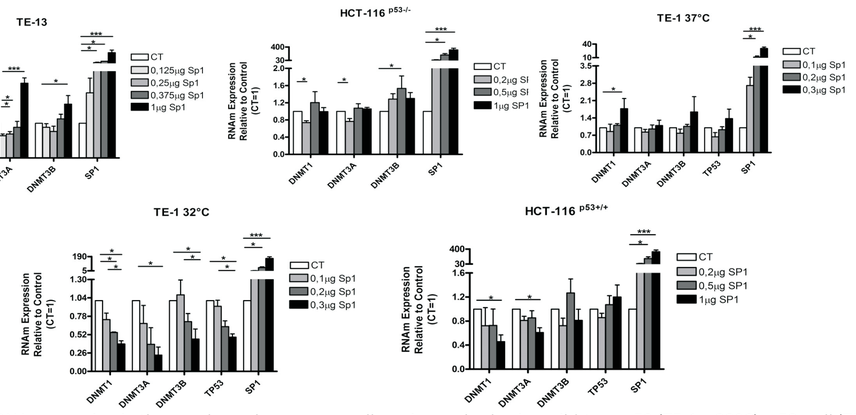

Figure 8: *DNMTs* and *SP1* mRNA expression in the esophageal squamous cell carcinoma harboring wild-type p53 (TE-1 – 32ºC), p53-null (TE-13) and the colorectal carcinoma expressing (HCT-116$^{+/+}$) or not p53 (HCT-116$^{-/-}$) cell lines by qRT-PCR following *SP1* overexpression achieved by transfection of p53 expression vector. *p<0.05, **p<0.01, ***p<0.001

➢ Evaluation of the presence of the p53 and SP1 responsive elements in *DNMTs* and *TP53* promoter regions

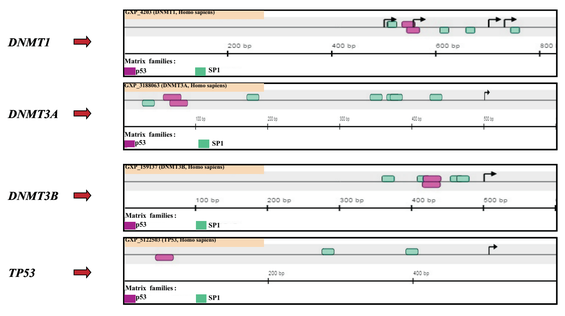

Figure 9: Evaluation of the presence of the p53 and SP1 responsive elements in *DNMTs* and *TP53* promoter regions. The analysis was performed using the software MatInspector.

CONCLUSION

These data show that p53 and SP1 play a role in DNMTs gene regulation, in a dose-dependent manner, and further experiments are needed to understand the mechanisms by which this regulation occurs.

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