Influence of disruption of HPV E1/E2 genes on the expression of Vascular Endothelial Growth Factor gene in Cervical Cancers positive for HPV18

Diogo Lisbôa Basto[1,2], Shayany Pinto Felix[2], Luís Felipe Leite Martins[3], Flávia de Miranda Correa[3], Cláudia Bessa Pereira Chaves[4], Liz Maria de Almeida[3], Miguel Ângelo Martins Moreira[2].

1-Genetics Department, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil 2-Present Address: Genetics Program, Instituto Nacional de Câncer, Rio de Janeiro, Brazil
3-Population Research Department, Instituto Nacional de Câncer (INCA), Rio de Janeiro, Brazil 4-Gynecologic Section and Molecular Carcinogenesis Program Instituto Nacional de Câncer, Rio de Janeiro, Brazil

INTRODUCTION

The E5 oncoprotein is a small HPV protein associated with V-ATPase, inhibiting acidification of endosomes, increasing the amount of Epidermal Growth Factor Receptor (EGFR) in the cell membrane (Dimaio & Petti., 2013; Virology, 445,99-114). EGFR activation induce cell proliferation and the transcription of Vascular Endothelial Growth Factor (VEGF), responsible for angiogenesis (Kim et al., 2006; Cell Mol Life Sci, 63, 7-8, 930-8) (*Figure 1*). On the other hand, expression of E5 could be lost after HPV integration into the host genome. The association between E5 with EGFR turnover was established studies with cell lines, oropharynx carcinoma and cervical lesion associated with HPV16.

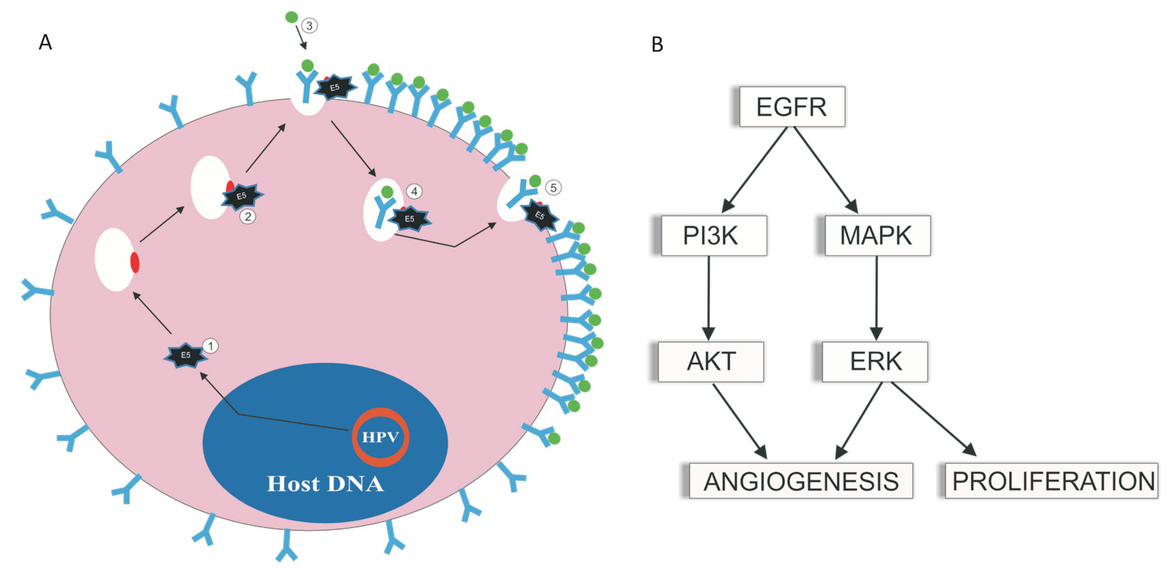

Figure 1. **A)** Illustration of EGFR turnover in cell expressing E5: 1- HPV produce the E5 protein; 2- E5 protein bind to V-ATPase of the endosome and inhibits its acidification; 3- The ligand binds to EGFR ; 4- EGFR is endocited and is not degraded because E5 is blocking the passage H^+ through the endosome membrane; 5- EGFR is recycled to the cell surface. B) These mechanism promotes a superexpression of EGF-Receptor in cell surface and can promote angiogenesis and cell proliferation.

AIM

In this study, we investigated changes in gene expression of *EGFR* or *VEGFA* associated to the presence of E5 transcripts and the disruption of E1/E2 viral genes for integration status of the viral genome in cervical cancer infected by HPV18.

METHODS

A total of 62 with cervical cancer samples had the DNA isolated, 48 of these had the total RNA obtained. The disruption status was verified by PCR using 8 pairs of primers covering *E1* and *E2* genes, RNA were converted to cDNA by RT-PCR then tested for the presence of *E5* transcript. The *EGFR* and *VEGF* mRNA were quantified by qPCR using as reference gene *GAPDH,* the analysis method used to quantify the gene expression was the ΔCT. *Table 1* shows general information of the patients.

Table 1. Characterization of tumor samples. Number of samples, mean age (± SD) of patients, median age of patients and tumor histological type. SCC: squamous cell carcinoma; ADC: adenocarcinoma

		Type of cancer cells								
		ADC (n=19)				SCC (n=42)				Other (n=1)
Mean age	Median age	Grade 1	Grade 2	Grade 3	without Grade	Grade 1	Grade 2	Grade 3	without Grade	
47,45 (±11,54)	45	9	6	3	1	2	25	15	1	1

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RESULTS

Disruption in *E1/E2* genes were detected in 47 of 62 samples (75,8%). The presence of E5 transcripts were more frequent in samples without disruption in *E1/E2* (12/14 vs. 15/35) (*Table 2*). Tumors that present *E5* transcripts had a lower expression of *VEGF* (-36.45 vs -33.56, p=0.50017), and those without disruption in E1/E2 had a lower expression of *VEGF* (-43.56 vs. -33.65, p=0.7542) (*Figure 2*).

Table 2. Results of disruption status and E5 mRNA expression in all samples studied

	E1/E2 Status	
	With Disruption	Without Disruption
With E5 Expression	15	20
Without E5 Expression	13	1
ND	12	1
	40	22

*ND- Not detected, samples without RNA

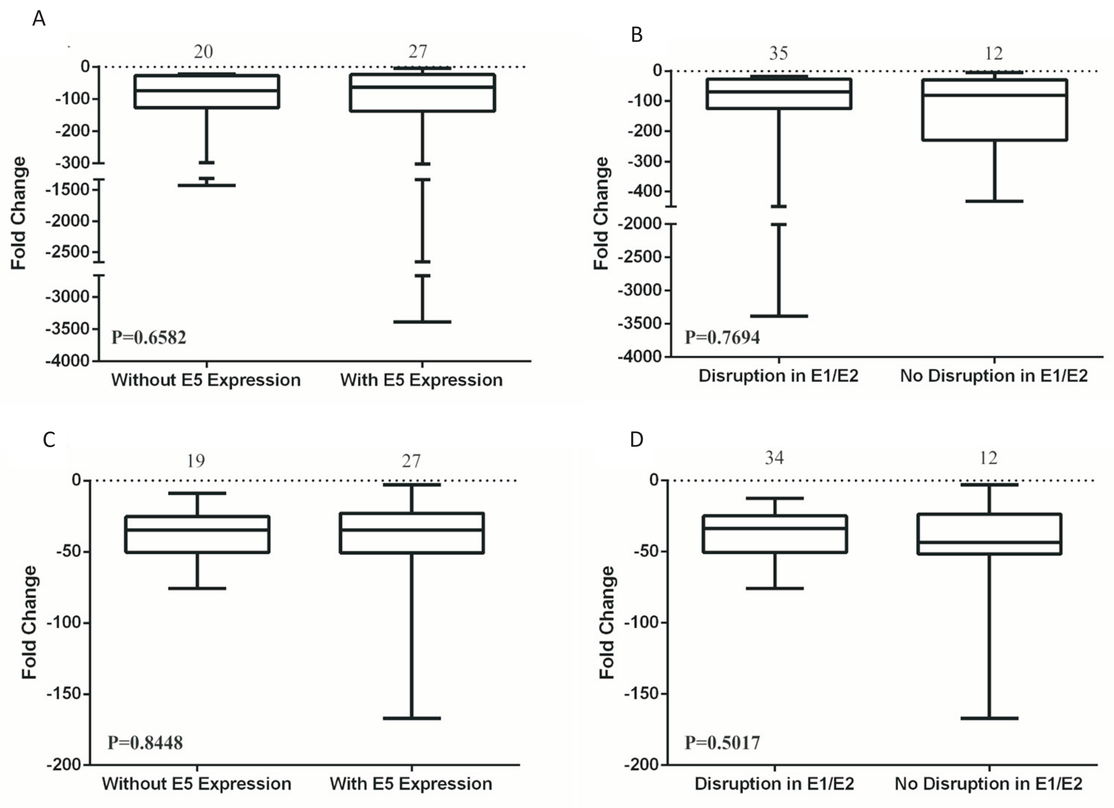

Figure 2. Mann-Whitney test showing the differences in gene expression related to integration status or expression of E5 mRNA for both genes *EGFR* and *VEGFA*. **A)** *EGFR* fold change in samples with E5 or without E5 transcript; **B)** *EGFR* fold change in samples with or without disruption in E1 or E2 genes; **C)** *VEGFA* fold change in samples with E5 or without E5 transcript; D**)** *VEGFA* fold change in samples with or without disruption in E1 or E2 genes. The fold change was estimated in respect to transcripts of *GAPDH.*

CONCLUSION

Although the expression of *E5* mRNA was more frequent in cells with intact *E1/E2* ORFs, it was not detected a significant differences between the expression of *E5* and the level of expression of *EGFR* or *VEGF* . The same was observed for the presence of intact or disrupted *E1/E2* ORFs. Our data did not allowed to establish a association between the expression of *E5* and the expression of *EGFR* and *VEGF* at transcriptional level.

Projeto Gráfico: Setor de Edição e Informação Técnico-Científica / INCA

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