Epithelial-mesenchymal transition in penile squamous cell carcinomas

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Introduction and objectives

Penile squamous cell carcinoma (PSCC) is a rare tumor associated with high capacity to invade and metastasize, and with both Human Papilomavirus (HPV) -related and HPV-unrelated etiologies. The epithelial to mesenchymal transition (EMT) is a phenomenon fostered by many epithelial tumors that allows dissemination of malignant neoplastic cells and involves loss of intercellular adhesion (E-cadherin decrease), mesenchymal phenotype acquisition (Vimentin increase), and an enhanced migratory potential. We have investigated the presence of EMT markers in PSCCs and their correlation with high-risk HPV infection, metastatic risk and mortality.

Materials and methods

We retrospectively evaluated the presence of EMT markers in a series of 64 PSCCs and their correlation with high-risk HPV infection, metastatic risk and mortality. Expression of E-cadherin, Vimentin and the transcription factors Twist, Zeb1 and Snail were assessed by immunohistochemical staining, and HPV presence was detected by PCR amplification.

Results

Different E-cadherin expression patterns were observed in the distinct histopathological subsets of PSCC (Fig 1). Loss of membranous E-cadherin was observed in 45 out of 64 cases (70.3%) (Table 1). Nuclear or cytoplasmic E-cadherin expression could be observed in some of the cases that had lost membranous expression: nuclear expression was detected in 12 out of 45 cases (26,6%) and cytoplasmic expression in 5 cases out of 45 cases (11,1%). Nuclear E-cadherin expression was significantly associated with a greater mortality: 41,6% of the patients expressing nuclear E-cadherin died due to the disease versus 15,3% of other groups ($p<0.05$). Furthermore, the time of survival was lower in the group with nuclear E-cadherin (Figure 2a). We next analyzed Vimentin expression in the TMAs, and we determined the simultaneous loss of membranous E-cadherin expression and Vimentin over-expression, as a mean to quantify bona fide EMT. Using these two markers, an EMT was shown in 27 out of 62 cases (43,5%) (Figure 3) (two cases showing membranous E-cadherin were lost for Vimentin expression evaluation). Mortality was higher among cases showing EMT than in those without EMT (33% vs 11,4%; p=0,035), as also shown by the Kaplan-Meier analysis (p=0.05 –Figure 2b-).
The presence of HPV was associated with EMT (Figure 4), but no correlation was found between Zeb1, Twist or Snail expression and EMT or mortality.

Table 1: Different expression patterns of E-cadherin and Vimentin depending on the histopathological types of PSCC and the presence of HPV.

Histophatological type	HPV+	Membranous E-cadherin	Nuclear E-cadherin expression	Cytoplasmic E-cadherin expression	Expression of Vimentin
Usual (67%)	23%	1/10 (10%)	3/10 (30%)	2/10 (20%)	4/9 (44%)
	-77%	15/33 (45%)	3/33 (9%)	0	13/33 (39%)
Warty (6%)	100%	0/4 (0%)	0	0	3/4 (75%)
Verrucous (7%)	0%	3/5 (60%)	0	0	1/4 (25%)
Basaloid (6%)	100%	0/4 (0%)	2/4* (50%)	2/4* (50%)	1/4 (25%)
Sarcomatoid (4%)	0%	0/3 (0%)	3/3 (100%)	0	3/3 (100%)
Mixed basaloid/ usual-type (7%)	100%	0/5 (0%)	1/5 (20%)	1/5 (20%)	2/5 (40%)

** These cases shown simultaniously cytoplasmic and nuclear E-cadherin expression.*

Figure 1: A: Verrucous penile squamous cell carcinoma showing a normal membranous E-cadherin pattern; B: Warty PSCC showing a membranous E-cadherin loss; C: Usual type PSCC showing membranous E-cadherin loss; D: Basaloid PSCC showing nuclear and cytoplasmic E-cadherin expression; E: Mixed tumor showing membranous E-cadherin expression in the usual type component and cytoplasmic expression pattern in the basaloid component; F: Sarcomatoid PSCC showing nuclear E-cadherin. Original magnification x 100.

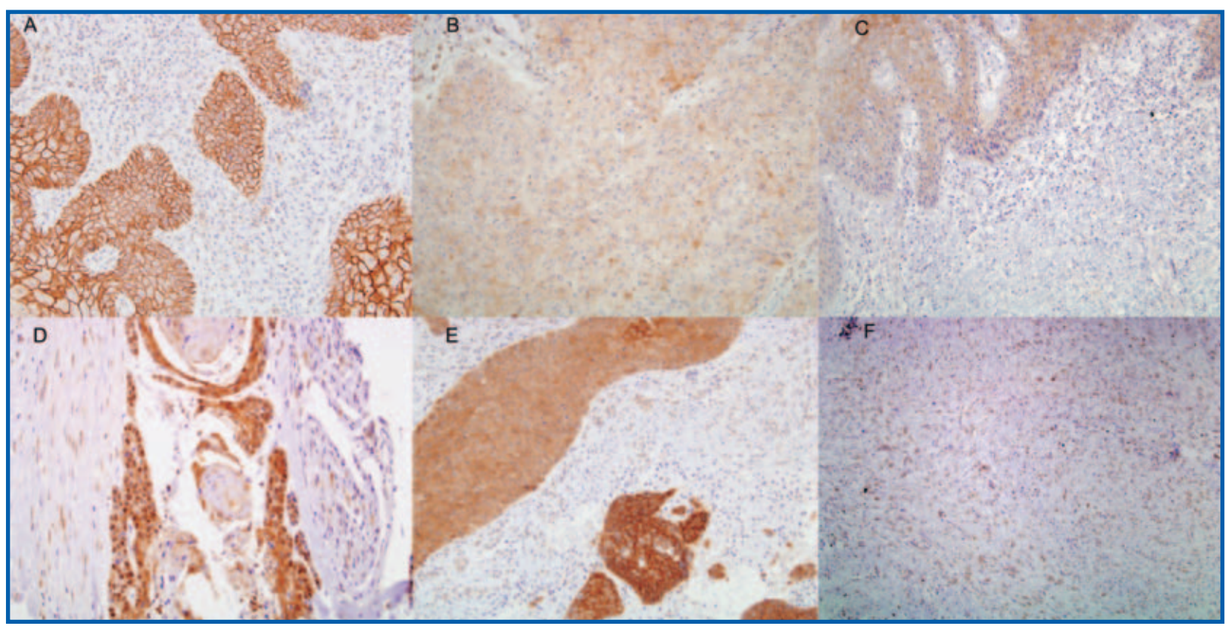


Figure 2: Kaplan-Meier analyses showing that a) nuclear E-cadherin expression is significantly associated with mortality (p<0,05) and, b) Epithelial to mesenchymal transition markers are also significantly associated with mortality (p=0.05).

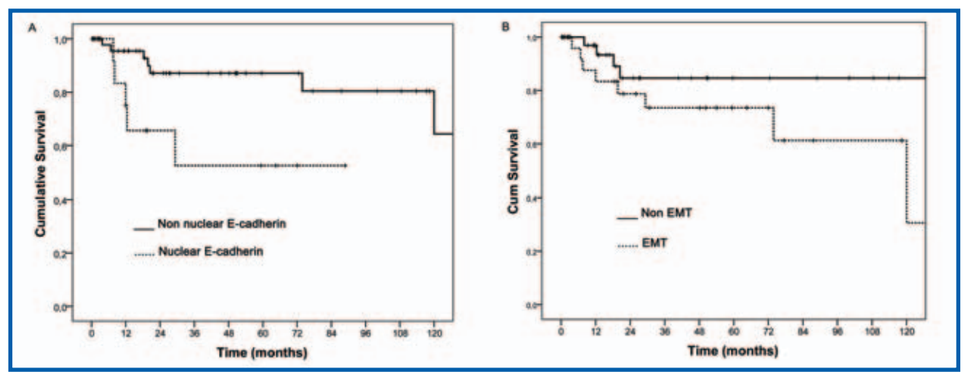


Figure 3: (A) Haematoxylin and eosin staining showing a verrucous PSCC with (B) membranous E-cadherin; (C) negative Vimentin expression (D) no TWIST expression and (E) no ZEB1 expression. (F) hematoxylin and eosin staining showing a sercomatoid PSCC with (G) nuclear E-cadherin expression; (H) Vimentin expression; (I) nuclear Twist expression and (J) nuclear ZEB1 expression. Original magnification x 100.

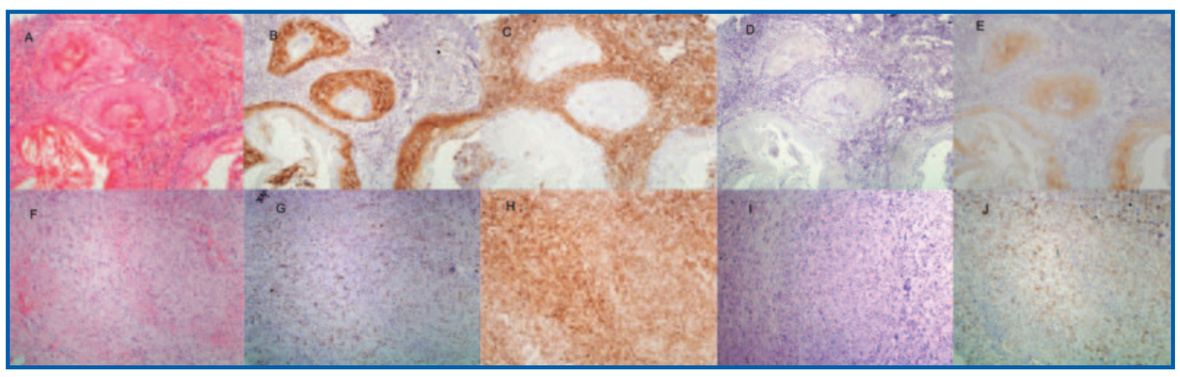
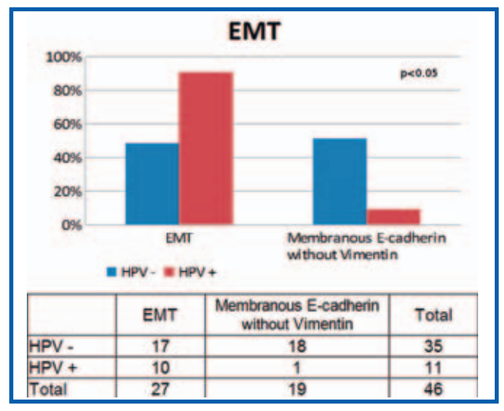


Figure 4: Epithelial to mesenchymal transition positivity is associated with Human papillomavirus infection status.



	EMT	Membranous E-cadherin without Vimentin	Total
HPV -	17	18	35
HPV +	10	1	11
Total	27	19	46

Conclusions

Our study suggests that EMT markers are associated with an increased mortality rate and aggressive PSCC subtypes. In addition, our findings suggest that HPV infection may be an important factor in promoting or allowing the activation of an EMT program in this malignant neoplasm.

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