

Changes in Androgen Receptor Expression as a Molecular Marker of Progression from Normal Epithelium to Invasive Cancer in Elderly Patients with Penile Squamous Cell Carcinoma

Valerio Gaetano VELLONE^{1,2#}, Emanuele COZZANI^{3#}, Leonardo PEÑUELA^{5#}, Roberto RUSSO³, Bruno SPINA², Carlo TONCINI², Carlo TERRONE^{4#} and Aurora PARODI^{3#}

¹Pathology, Department of Integrated Surgical and Diagnostic Sciences, University of Genoa, ²Pathology Unit, San Martino Polyclinic Hospital, ³Section of Dermatology, DISSAL, University of Genoa, San Martino Polyclinic Hospital, Via A. Pastore, 1, IT-16132, Genoa, ⁴Department of Urology, University of Genoa, IRCCS San Martino-IST, and ⁵Fetal and Perinatal Pathology Unit, Istituto Giannina Gaslini, Genoa, Italy. E-mail: emanuele.cozzani@unige.it

[#]These authors equally contributed to this paper.

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Penile squamous cell carcinoma (SCC) is uncommon (1, 2). Two distinct pathways lead to penile SCC: one is linked to human papillomavirus (HPV) infection and is associated with undifferentiated penile intraepithelial neoplasia (PeIN); the other is linked to non-neoplastic epithelial disorders, such as epithelial hyperplasia or lichen sclerosus (LS) and is associated with differentiated PeIN (3–7). Little is known about molecular changes in the genesis of penile cancer. Only one study has reported the expression of hormonal receptors, such as oestrogen receptors (ER α), and androgen receptor (AR) in normal penile epithelium, with no sign of progesterone receptor (PR) expression (8). Thus far, however, no studies have investigated the possible role of these receptors in the transition from normal epithelium to male genital LS or penile SCC.

The aims of the present study were to describe histological and immunohistochemical features of SCCs, to examine the modulation of hormonal receptors ER α , AR, PR and molecular proliferation markers (p53 and Ki-67) in normal penile epithelium and in SCC, and to extend this evaluation to penile lesions adjacent to SCC.

MATERIALS AND METHODS

This retrospective study was conducted on 21 consecutive patients who had undergone surgical resection of penile cancer in San Martino Polyclinic Hospital, Genoa, Italy, in the period 2006 to 2015. All the curative-intended surgical specimens (wide excisional biopsies, partial and total penectomies) were fixed in 10% buffered formalin, processed and embedded in paraffin to obtain haematoxylin-eosin-stained slides. In each case, a representative block containing both normal (NE) and tumour tissue (K), as well as the SCC-associated penile lesion (PRE), was chosen in order to obtain further sections. Squamous multi-layered epithelium devoid of major inflammation, and displaying normally orientated cellular maturation, was considered normal epithelium (NE). Squamous cell hyperplasia was defined as epithelial hyperplasia and hyperkeratosis, increased mitotic figures in basal and prickle cell layers and mild dermal chronic inflammation, with no atypia. Lichen sclerosus was defined as severe hyperkeratosis, thin epidermis and presence of a homogenized band of dense fibrosis affecting the papillary dermis. Differentiated PeIN was defined as hyperkeratosis, hypergranulosis, acanthosis, with cytological atypia. SCC was defined as variable cytological atypia, high mitotic index, infiltrating pattern and presence of keratin pearls. The following antibodies were used for immunohistochemistry testing: Androgen Receptor (Cell Marque, Rocklin, USA, Clone

SP107, pre-diluted), Estrogen Receptor α (Ventana, Innovation Park Dr. Tucson, USA, Clone SP1, pre-diluted), Ki67 (Ventana, Clone 30-9, prediluted), Progesterone Receptor (Ventana, Clone 1E2, pre-diluted), p53 (Ventana, Clone DO-7, prediluted). Appropriate negative and positive controls were implemented according to the manufacturer's data-sheets. Expression was evaluated by considering the percentage of cells exhibiting immunoreactivity as well as intracellular localization. In normal tissue and in SCC-associated penile lesions, analysis was restricted to the epithelial component, where the number of positive (brown-stained) cells in a random field of 300 cells was counted; the reactivity pattern of cell positivity was also recorded (i.e. basal, lower, middle and upper thirds of the epithelium, or diffuse). In tumours, the number of positive cells was counted in 5 separate fields of 100 cells each (total number 500 tumour cells). The results were expressed as the mean percentages of positively stained nuclei in relation to the total number of epithelial- or tumour-cell nuclei considered. The staining intensity was not evaluated.

RESULTS

Clinical-pathological features of the study population are shown in Table S1¹.

Immunohistochemistry

Androgen receptor. In NE, SCC-associated lesions and SCCs, AR expression displayed mean \pm standard deviation (SD) values of $52.0 \pm 32.7\%$, $28.1 \pm 28.0\%$ and $0.9 \pm 3.0\%$ labelled cells, respectively. Moreover, positivity in SCCs was confined to the lower third of the epithelium; in SCC-associated lesions, the distribution of positivity was variable; in NE, positivity was diffuse. Significant differences ($p < 0.001$) in staining indexes was observed between NE and K and between PRE and K. Although the AR staining index proved lower in PRE than in NE, the difference failed to reach statistical significance (Fig. S1¹).

Oestrogen receptors α and progesterone receptor. ER α immunoreactivity was recognized in 5 cases only, and was very weak. None of the specimens tested showed substantial expression of PR.

p53. In NE, SCC-associated lesions and SCCs, p53 expression displayed mean \pm SD values of $2.8 \pm 5.0\%$,

¹<https://www.medicaljournals.se/acta/content/abstract/10.2340/00015555-3004>

7.5 ± 21.8% and 24.3 ± 31.4% labelled cells, respectively. These differences proved statistically significant ($p < 0.001$) on comparing SCC with NE or with associated lesions, but not on comparing NE with associated lesions. Ki-67. In NE, SCC-associated lesions and SCCs, Ki-67 expression displayed mean ± SD values of 5.6 ± 3.6%, 12.9 ± 9.8% and 30.5 ± 14.6% labelled cells, respectively. Moreover, positivity in NE was confined to the lower third of the epithelium; in SCC-associated lesions, positivity was seen in both the lower and the middle thirds of the epithelium; in SCCs, positivity was widespread. Significant differences in staining indexes were observed between NE and K and between PRE and K ($p < 0.001$). Although the Ki67 staining index was higher in PRE than in NE, the difference failed to reach statistical significance.

DISCUSSION

Twenty-one patients underwent surgical resection of penile cancer in the 10-year period, confirming that this disease is uncommon. LS features were found in more than half (57.1%) of specimens, confirming LS as an SCC-associated lesion, regardless of its clinical diagnosis. Indeed, according to the literature, up to 50% of SCC are associated with LS (9–11); in the present study, however, we found a closer association. Cases of differentiated PeIN not associated with LS as a precursor lesion accounted for only a minority of patients. No cases of HPV-related types of SCC were found, and none of the specimens showed signs of HPV infection; nevertheless, we are aware that, since specific immunohistochemical or molecular investigations were not performed, HPV infection cannot be definitely excluded. In this study, a statistically significant loss of AR expression was found in the transition from normal epithelium to SCC-associated lesion and from this latter to penile malignancy. Distribution of immunopositivity also decreased; in normal epithelium, it was diffuse, whereas it was less extensive in SCC-associated lesions and totally absent in cancer. The more cells that were labelled, the broader the immunopositivity became. The present study is the first to describe this kind of decrease in AR expression in penile cancer. As expected, the increase in Ki-67 and p53 expression in the transition from normal epithelium to SCC-associated lesion, and from this latter to penile malignancy, indicates an increase in proliferative activity. An increase was also found in SCC-associated lesions in comparison with normal epithelium. In this case, however, statistical significance was not reached, although it was expected, at least for p53 in LS cases, on the basis of previous studies (12). With the progression of transition, Ki-67 immunopositivity spread from basal layers

to the upper third of the epithelium, more evidently in less differentiated tumours. ER α and PR did not yield interesting results. ER α was rarely, and weakly, positive in normal epithelium and in pre-neoplastic lesions, and PR was not expressed in NE, PRE or K. In fact, according to the scant literature, ER α should be positive in normal penile epithelium (8).

In conclusion, our preliminary study demonstrates that the transition from normal epithelium to penile cancer is characterized by a significant loss of AR expression. These results, if confirmed by larger studies, may suggest using topical androgen-based products in patients with genital lesions at high-risk for cancer development.

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The authors have no conflicts of interest to declare.

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Table SI. Clinical-pathological features of study population

Feature	
Normal epithelium, <i>n</i>	21
Squamous cell carcinoma, <i>n</i>	21
Usual type	15
Verrucous	3
Sarcomatoid	2
Mixed (with sebaceous areas)	1
with associated squamous cell hyperplasia	7
with associated lichen sclerosis	12
with associated differentiated penile intraepithelial neoplasia	2
Age, years, median (range)	70 (52–87)
T, <i>n</i> (%)	
Tis	2 (9.5)
T1a	5 (23.8)
T2	7 (33.3)
T3	7 (33.3)
Grade, <i>n</i> (%)	
1	7 (33.3)
2	5 (23.8)
3	9 (42.9)
Loco-regional lymph node status, <i>n</i> (%)	
Negative	1 (4.8)
Positive	1 (4.8)
Unknown node status (no lymphadenectomy)	19 (90.5)

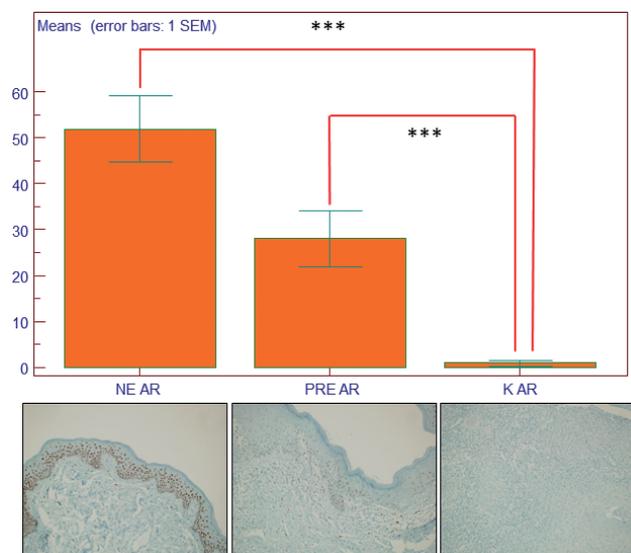


Fig. S1. Penile carcinoma and associated lesions: androgen receptor (AR). Top: the AR-staining indexes of normal epithelium, pre-neoplastic changes and invasive cancer are compared by means of bar plots. Bottom: representative immunohistochemical staining for the AR samples (original magnification 100×). Observe the lower AR molecular expression in the invasive cancer sample.