Pathology – Research and Practice 211 (2015) 641–645

Contents lists available at ScienceDirect

Pathology – Research and Practice

journal homepage: www.elsevier.com/locate/prp

Original Article

A switch from epithelial to mesenchymal properties correlates with lymphovascular invasion in squamous cell carcinoma of the penis

Matthias May[a,*], Sabine Brookman-May[b], Maximilian Burger[c], Stefan Koch[d], Wolfgang Otto[c], Johannes Bründl[c], Knut Albrecht[e], Stefan Denzinger[c]

[a] Department of Urology, St. Elisabeth Clinic Straubing, Straubing, Germany
[b] Department of Urology, Ludwig-Maximilians University, Munich, Germany
[c] Departments of Urology, Regensburg University, St. Josef Medical Centre, Regensburg, Germany
[d] Institute of Pathology at the HELIOS Clinic Bad Saarow, Charité-University Medicine Academic Teaching Hospital, Bad Saarow, Germany
[e] Department of Pathology, Forensic Medicine and Genetics, Hannover Medical School, Hannover, Germany

ARTICLE INFO

Article history:
Received 11 April 2015
Accepted 18 May 2015

Keywords:
Squamous cell carcinoma of the penis
Lymphovascular invasion
Epithelial-to-mesenchymal transition
Immunohistochemistry
Cadherin switch
Targeted therapies

ABSTRACT

The purpose of the study was to assess the incidence and prognostic role of epithelial-to-mesenchymal-transition (EMT) in squamous cell carcinoma of the penis (SCCP).

Sixty tumor specimens of surgically treated SCCP patients characterized by a central histopathologic review were stained with antibodies against E-cadherin, N-cadherin, β-catenin, and vimentin. Staining profiles were scored by two independent raters, and correlated with pertinent clinical and pathological parameters and cancer-specific mortality (CSM; median follow-up: 34 months, interquartile range: 6–60 months).

Correlation statistics proved good interobserver agreement in staining evaluation (*K*-values between 0.62 and 1.00, all $p < 0.001$). Based on consensus decision between both raters, 36 study cases (60%) showed a switch from E-cadherin to N-cadherin (as a hallmark of EMT), which correlated with the presence of lymphovascular invasion ($\rho = 0.287$, $p = 0.026$) while failing to interfere with CSM. Although cadherin switch was correlated with a loss of membranous β-catenin expression ($\rho = 0.629$, $p < 0.001$), none of the study cases showed nuclear β-catenin expression, and only three EMT cases (8.3%) had tumor buds revealing vimentin expression.

Our data suggest that roughly half of surgically treated SCCP cases exhibit EMT, a parameter correlating with lymphovascular invasion. However, further studies are clearly needed to validate the so far unresolved possible role of cadherin switch in terms of predicting nodal spread in patients with SCCP. Moreover, the apparently complex mechanisms driving EMT in SCCP should be explored by future studies, as knowledge about these might provide a so far unexploited basis for the development of novel targeted therapies against SCCP with metastatic dissemination.

1. Introduction

In several cancer entities, the loss of epithelial properties has been reported to coincide with increased invasiveness [1]. Epithelial-to-mesenchymal transition (EMT) represents a process by which epithelial cells undergo a change in appearance and acquire mesenchymal characteristics. In particular, cancer-related EMT has been linked to invasion and metastatic spread, and accordant mediators pathogenically involved in cancer-related EMT have therefore become attractive candidates for the development of oncological targeted therapies [2–4]. However, to our knowledge, the phenomenon of EMT has not been investigated in squamous cell carcinomas of the penis (SCCP) yet.

In the present study, we evaluated the incidence and prognostic implications of EMT in surgically treated patients with SCCP. To this aim, tumor buds at the advancing edge were scored with respect to the expression patterns of a selected marker panel to monitor EMT by immunohistochemistry, and staining profiles were subsequently correlated with pertinent clinical and pathological variables and cancer-specific mortality (CSM) [5–8].

* Corresponding author at: Department of Urology, St. Elisabeth Clinic Straubing, St. Elisabeth Street 23, 94315 Straubing, Germany. Tel.: +49 9421 7101701; fax: +49 9421 7101717.
E-mail address: matthias.may@klinikum-straubing.de (M. May).

http://dx.doi.org/10.1016/j.prp.2015.05.007
0344-0338/

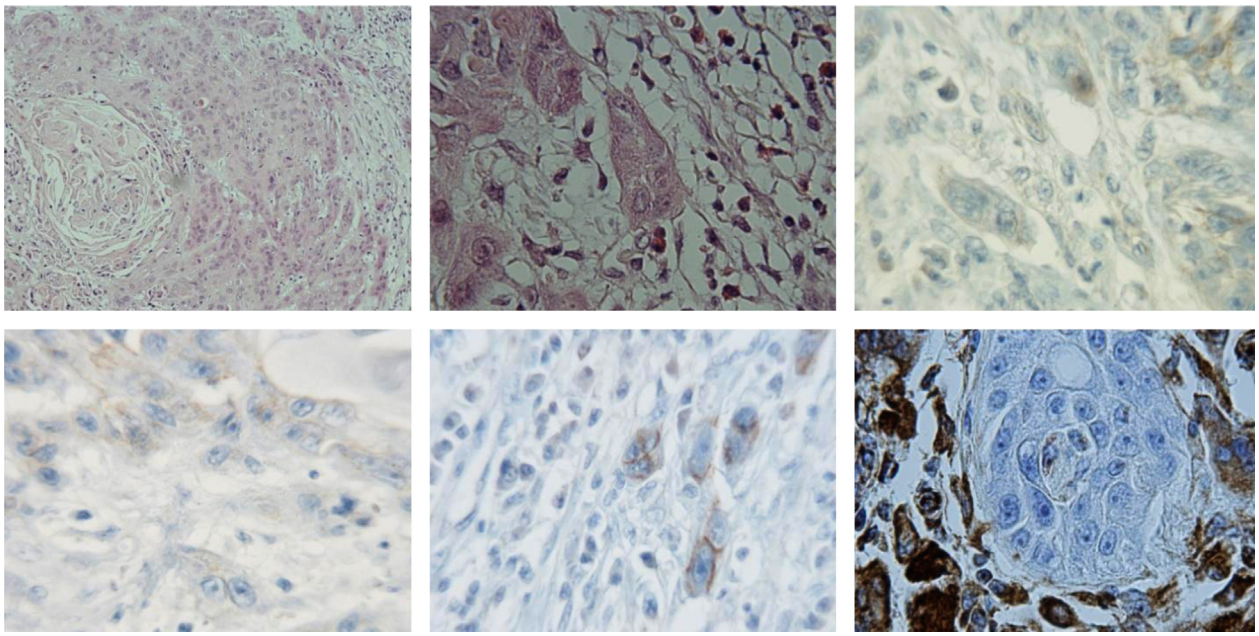

Fig. 1. Squamous cell carcinoma of the penis (SCCP) exhibiting epithelial-to-mesenchymal transition (EMT) by immunohistochemistry. *Top, left:* low power view illustrating the advancing edge of a study SCCP. H&E, 10× objective. *Top, middle*: stronger magnification of the advancing edge shows tumor buds comprising only a few cohesive squamous carcinoma cells. H&E, 40× objective. *Top, right:* tumor buds show loss of membranous β-catenin expression, which is retained at the leading edge. Anti-β-catenin, 40× objective. *Bottom, left*: compared with the leading edge, tumor buds show loss of membranous E-cadherin expression. Anti-E-cadherin, 40× objective. *Bottom, middle*: tumor buds exhibit membranous N-cadherin expression. Anti-N-cadherin, 40× objective. *Bottom, right*: tumor buds but not the leading edge show strong cytoplasmic expression of vimentin. Anti-Vimentin, 40× objective.

2. Materials and methods

2.1. Study cohort

Based upon a retrospective computerized database analysis, a total of 110 consecutive SCCP patients were initially identified. All patients were clinically staged M0 and underwent elective surgery for SCCP in six different German hospitals between January 1993 and December 2010 [5]. None of the study patients received neoadjuvant or adjuvant chemotherapy or radiotherapy. From all patients, American Society of Anesthesiologists (ASA) scores were recorded as a commonly employed physical status classification system for assessing the fitness of patients before surgery [9].

The retrieved archived slides were subjected to a central histopathologic review performed by one clinical pathologist (S.G.) for re-staging according to the recent TNM classification system, as well as to standardize histopathologic classification, lymphovascular invasion (LVI), and Broder's grade [10–14].

After corresponding paraffin-embedded whole-tissue blocks of the study cases were retrieved from the files, one punch (diameter 1.5 mm) was taken from the advancing edge of each study case for tissue microarray (TMA) construction [5]. Subsequently, 4 μm thick serial sections were cut from the same paraffin blocks. Then, conventionally stained whole-tissue serial sections were histopathologically scrutinized for the presence of tumor buds which were defined as single tumor cells or small aggregates comprising five tumor cells or less that have become detached from the invasive front and migrated a short distance into a usually desmoplastic stroma (Fig. 1) [15].

Previous to assessment of patient follow-up and death data, Institutional Review Board approval and approval of Medical Ethics Committee (MEC) of the federal state Brandenburg for evaluating CSM based on retrospective evaluation of death certificates was obtained (LÄKB; MEC.-No.: 34182/13).

2.2. Immunohistochemistry

4 μm thick TMA sections on a region featuring tumor buds by conventional histomorphology were subjected to immunohistochemistry using an automated staining system (Ventana BenchMark). Briefly, following deparaffinization, rehydration, and heat-induced epitope retrieval (CCl mild, Ventana BenchMark), sections were incubated with antibodies directed against E-cadherin (Dako, monoclonal, dilution 1:100), N-cadherin (Abcam, monoclonal, dilution 1:500), vimentin (Dako, monoclonal, dilution 1:100), β-catenin (Santa Cruz, monoclonal, dilution 1:200) and AE1/3 (Dako, monoclonal, dilution 1:200). The incubation time was 12 min for anti-β-catenin and 20 min for the other antibodies employed. Subsequently, the sections were washed with phosphate-buffered saline (PBS), incubated with rabbit anti-mouse IgG (1:50), and then, mouse peroxidase-antiperoxidase conjugate (1:200). Adequate positive and negative controls were run.

Scoring of marker expression using recently published criteria was performed by considering tumor buds at the tumor–host interface (Fig. 1) which represent the micro-anatomic area at which EMT is presumed to take place [6,16–19]. A switch from E-cadherin to N-cadherin expression indicates EMT. For E-cadherin and N-cadherin, only the membranous stain was considered to be a positive immunoreaction. By immunohistochemistry, loss of E-cadherin and/or β-catenin expression was considered if at least 10% of the depicted tumor buds failed to show membranous marker expression [6,16]. Special attention was paid to the presence of ectopic nuclear and/or cytoplasmic marker expression. For a study case to be considered N-cadherin and/or vimentin-positive, marker expression in at least 10% of the depicted tumor buds was required [6,16]. Equivocal study cases were subjected to AE1/3 staining, and co-expression of vimentin and AE1/3 was required in order to classify that study case as being vimentin-positive.

The immunostained TMA sections were read separately by two independent raters (S.G. and K.A.) blinded to clinicopathologic