Immunohistochemical Expression of Mdm2 and p53 in Penile Verrucous Carcinoma

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Abstract. Verrucous carcinoma (VC) of the penis is an uncommon squamous tumor that pursues a biologically indolent course. Unlike conventional squamous cell carcinoma (SCC) of the penis, pathogenic roles for human papillomavirus (HPV) infection and p53 mutation have not been reported in VC. We compared the immunohistochemical expression of Mdm2 and p53 in 7 cases of VC and 7 cases of SCC. The Mdm2 gene product preferentially labeled the perinuclear membrane in the granular layer of VC tumor cells, whereas SCC cases showed weak, focal, cytoplasmic staining for Mdm2. The mean labeling index for Mdm2 was higher in VC compared to SCC [79.3 (SE ±7.2) in VC vs 18.3 (SE ±2.4) in SCC, p <0.001]. In SCC cases, the normal surrounding skin showed mild granular-layer staining and dysplastic foci that failed to stain with Mdm2 antibody. Weak p53 immunolabeling was seen within nuclei of scattered tumor cells in the cases of VC, whereas the SCC cases showed strong nuclear staining of p53 throughout the tumors. The mean labeling index for p53 was lower in VC compared to SCC [24.8 (SE ±3.9) in VC vs 64.7 (SE ±9.0) in SCC, p <0.01]. In SCC cases, the normal surrounding skin showed moderate staining for p53, preferentially confined to the basal layer. Dysplastic foci in the cases of SCC showed increased p53 labeling. In summary, immunohistochemical analysis showed significantly different levels of expression of Mdm2 and p53 in penile VC vs SCC. Overexpression of the Mdm2 gene product may be important in the pathogenesis of VC. Since Mdm2 is a negative regulator of p53, overexpression of Mdm2 may explain why p53 is down-regulated and, therefore, permissive to oncogenic transformation. (received 2 July 2002; accepted 8 July 2002)

Keywords: Mdm2, p53, verrucous carcinoma, squamous cell carcinoma, penis

Introduction

The reported prevalence of HPV in penile carcinoma is highly variable, from 15 to 71 % depending on the tumor type and the sensitivity of the detection method [1-2]. Penile carcinomas include several histological subtypes. The most common tumors are well-differentiated, keratinizing squamous cell carcinomas that resemble the SCCs that arise in non-genital skin [3,4]. The second most common tumor subtype is verrucous carcinoma [4]. Few studies have analyzed the different histological types separately [1,3-7]. Of particular importance is a study by Rubin et al [5], in which the relationship of HPV to PC was examined. Great differences were observed in HPV prevalence, depending on the histological subtypes of PC. While basaloid and warty carcinomas were consistently associated with HPV, only minorities of keratinizing and verrucous PCs were positive for HPV DNA. Verrucous carcinoma of the penis analyzed in their study was HPV-negative. Of a total of 26 cases reported in additional studies, only 3 cases were positive for low-risk HPV DNA (serotypes 6 and 11) [7-9].

The HPV genome encodes 2 proto-oncogenes that interfere with the functions of retinoblastoma protein and p53 tumor suppressor protein [10-11]. Fidelity of cellular DNA replication is maintained by p53. Alteration of p53 expression by HPV renders
cellular DNA susceptible to the carcinogenic effects of mutagens and may induce malignant transformation of the cell due to accumulation and propagation of DNA error.

Mutations in the p53 tumor suppressor gene occur in approximately 50% of human tumors [12]. A key regulator of p53 is the Mdm2 protein [13]. Absence of functional Mdm2 protein leads to deregulation of p53 and eventually to cell death. Excessive Mdm2 expression may lead to constitutive inhibition of p53 and promote unrestricted cell cycling (Fig. 1). Mdm2 exhibits a dual relationship with p53 [13-15]. Binding of Mdm2 to p53 can repress the transcriptional function of p53 and may lead to complete elimination of p53 through proteolysis. On the other hand, binding of p53 to the Mdm2 gene stimulates p53 transcription. This negative feedback mechanism keeps control of p53.

Mdm2 proto-oncogene is amplified in human sarcomas and is overexpressed in a wide variety of other human cancers [15-16]. In animal models, the incidence of sarcomas observed in Mdm2-transgenic mice in the presence or absence of functional p53 demonstrates that, in addition to Mdm2-mediated activation of p53, Mdm2 plays a p53-independent role in oncogenesis [15-16]. In addition to an amino-terminal p53 domain, the primary structure of Mdm2 contains other putative functional domains, including a nuclear localization signal, an acidic transcription activation domain, a central zinc finger element, and a carboxy-terminal zinc finger element [16]. The presence of these domains suggests that Mdm2 may bind directly to DNA and affect gene transcription. Mdm2 has been reported to form a complex with the retinoblastoma tumor suppressor protein and E2F1 and DP1 transcription factors, which elevate the expression of E2F-responsive genes and lead to cellular progression from the G1 to S phases of the cell cycle [17].

We investigated the following hypotheses: (a) Mdm2 expression is increased in penile verrucous carcinoma compared to conventional squamous cell carcinoma and to normal skin, and (b) p53 expression is decreased in penile verrucous carcinoma compared to conventional squamous cell carcinoma and to normal skin.

Materials and Methods

Formalin-fixed, paraffin-embedded, archival tissue from 14 cases of penile carcinoma, including 7 cases of verrucous carcinoma (Fig. 2) and 7 cases of squamous cell carcinoma (Fig. 3), were immunostained with monoclonal antibodies to p53 (clone BP 53-12-1 Biogenex, San Ramon, CA; 1:40 dilution), Mdm2 (clone 1F2 Calbiochem, La Jolla, CA; 1:200 dilution). None of the patients were immunosuppressed. All of the lesions were obtained from sun-protected penile cutaneous sites. Three samples of normal penile skin were obtained from young adults (circumcision specimens).

Histological sections were cut at 5-µm intervals and treated with 0.1 M citrate (pH 6.0) in a microwave oven (800 W, 15 min) for antigen retrieval before immunostaining with an avidin-biotin-peroxidase kit (“Elite” kit, Vector Labs, Burlingame, CA), according to manufacturer’s procedure. Slides were counterstained with hematoxylin for 1 sec. Tonsil tissue (positive for

Fig. 1. Cell cycle diagram that depicts the relationships of p53 and Mdm2.
Fig. 2. Photomicrograph of penile verrucous carcinoma (VC) (H&E, magnification x 100)

Fig. 3. Photomicrograph of penile squamous cell carcinoma (SCC) (H&E, magnification x 100)
Mdm2) and colorectal adenocarcinoma (positive for p53) were used as positive controls. Normal mouse serum was substituted for the primary antibodies as a negative control.

Immunoperoxidase-stained sections were examined by light microscopy. A positive result was defined as a significant increase in intranuclear or cytoplasmic epithelial staining. The number of stained cells was expressed as a percentage. For each case, ~5 high-powered fields at 400 x magnification were counted, yielding a total of ~100 cells/case. The mean labeling index (MLI) for p53 or Mdm2, based on the independent findings of 2 observers, was expressed as mean ± SE.

Results

Mdm2 was preferentially labeled in the perinuclear membrane of tumor cells of the VC cases. There was diffuse staining within the granular layer of each of the VC tumors (Fig. 4). In contrast, the SCC cases showed weak, focal, cytoplasmic staining for Mdm2. The mean labeling index for Mdm2 among cases of VC and SCC was significantly higher in VC cases compared to SCC cases (79.3 ± 7.2 in VC vs 18.3 ± 2.4 in SCC, p <0.001). The dysplastic foci and normal surrounding skin adjacent to the SCC cases failed to stain with the Mdm2 antibody.

Weak p53 immunolabeling was seen within the nuclei of scattered tumor cells in the cases of VC. In contrast, SCC cases showed strong nuclear staining throughout the tumors. The mean labeling index for p53 was lower in cases of VC compared to SCC (24.8 ± 3.9 in VC vs 64.7 ± 9.0 in SCC, p <0.01). Normal surrounding skin showed moderate staining for p53, preferentially confined to the basal layer. Dysplastic foci in the cases of SCC yielded an increased p53 labeling index (45.1±9.7).

Discussion

In the present study, immunohistochemical studies shows increased staining of Mdm2 in the cytoplasm of tumor cells in the VC cases. In contrast, p53
antibodies gave decreased staining of the VC tumor cells. These results may have important pathogenic implications. Excessive Mdm2 expression may lead to constitutive inhibition of p53 and promote unrestricted cell cycling. Binding of Mdm2 to p53 represses the transcriptional function of p53 and may lead to elimination of p53 through proteolysis [14-15]. Since p53 is viewed as a tumor suppressor, Mdm2 may promote cell proliferation by relieving p53-mediated suppression of the cell cycle [14-17].

A precedent exists for Mdm2-mediated tumor promotion [15]. Transfection of Mdm2 into a variety of cells was found to elevate expression of a reporter gene placed under the transcriptional control of an E2F-responsive promoter, suggesting that Mdm2-Rb complex formation and Mdm2-E2F-1/DP1 complex formation stimulate the expression of E2F-responsive genes. Because complex formation was observed both in vitro and in vivo in cells deficient for p53, Mdm2 appeared to play a p53-independent role in promoting progression from G1 to S phase of the cell cycle. Since Mdm2 turnover is normally extremely rapid, factors that prolong its half-life can be expected to alter its steady-state concentration and affect the function of the Mdm2/p53 loop.

The mechanism by which Mdm2 becomes overexpressed is unknown. It is possible that a qualitative defect in the Mdm2 gene-product prolongs Mdm2 expression. On the other hand, over-expression of Mdm2 at the gene level, due to unknown reasons, could explain our findings. Similarly, decreased immunohistochemical staining for p53 may either reflect pathologic down-regulation by Mdm2 or represent an unrelated qualitative defect in the gene product that prevents its antigenic detection.

Interestingly, the accentuated granular layer pattern of Mdm2 staining of VC tumors may be germane to the pathogenesis of these lesions. Other workers have shown that Mdm2 over-expression normally occurs in the differentiated layers of the upper spinous and granular layers, counteracting the effect of p53 that is up-regulated in the less differentiated basilar layers [18,19]. Given conspicuous expression of Mdm2 in the granular layer of VC tumors, compared to both SCC and normal surrounding skin, the excess Mdm2 may lead to pathologic down-regulation of p53 expression. Further studies (eg, Western blot assays for Mdm2 and p53; physiologic assessments of the functional state of Mdm2 and p53) are needed to test these hypotheses.

In summary, Mdm2 expression is increased and p53 expression is decreased in penile VC relative to SCC. These results are consistent with an ability of Mdm2 to repress p53. It remains to be determined how Mdm2 is up-regulated in penile VC and how this over-expression may influence p53 function.

References


