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Original contribution

A clinical, pathologic, and molecular study of p53 and murine double minute 2 in penile carcinogenesis and its relation to prognosis☆,☆☆

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Summary Penile carcinoma constitutes up to 16% of male malignancies in developing countries. Changes in the p53 and murine double minute 2 pathway are important events in various cancers. Associate alterations in murine double minute 2 and p53 expression were evaluated by molecular techniques, with the clinical data of 297 cases of penile carcinoma. Automated immunohistochemistry was performed for murine double minute 2 and p53 using the primary antibodies SPM14 and DO7, respectively. Fluorescent in situ hybridization was performed using the probes murine double minute 2 at 12q15 and *TP53* at 17p13.1. Slides were digitalized, and bright-field and fluorescent images were analyzed. *TP53* was sequenced in 16 cases. The expression of p53 was higher in poorly differentiated, infiltrative border, corpus spongiosum, corpora cavernosa, and invasive urethral carcinomas. Patients who died of disease also expressed higher levels of p53. p53-negative tumors were associated with higher overall survival. Murine double minute 2 showed no difference of expression in any group of tumors, no correlation with p53 expression. No alterations in genes or chromosomes were observed. Mutations in *TP53* were observed in 4 of 16 cases: p.T170M, p.L252P, p.C176Y, and the novel

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c.803_810del8; these changes correlated with p53 expression by immunohistochemistry. Murine double minute 2 is not useful in the prognosis of penile carcinoma by immunohistochemistry. Additional studies on the transcriptional, posttranscriptional, and epigenetic aspects are necessary to understand the interactions between p53 and murine double minute 2 because we did not observe any numeric alterations by fluorescent in situ hybridization. Examining p53 is helpful in identifying patients with more aggressive tumors and may be crucial in selecting the most suitable surgical procedure.

1. Introduction

Penile carcinoma (PC) is rare in industrialized countries but is common in many developing nations [1]. The incidence of PC in Europe and the United States is 0.8 per 100 000 men per year. In Brazil, PC constitutes between 5% and 16% of male malignancies, depending on the geographic region [1]. An unfavorable socioeconomic status is associated with late diagnosis of PC, which has implications for its treatment and outcome, resulting in increased morbidity and mortality [1]. Despite the identification of some risk factors, such as the presence of phimosis and human papillomavirus, much of the molecular pathogenesis of PC remains largely unknown [2].

TP53 is essential for the growth, development, and differentiation of normal cells and ensures genomic stability [3,4]. This tumor suppressor gene is one of the most important genes of tumorigenesis because mutations in this gene exist in more than 50% of all cancers [5,6]. Such mutations often result in the overexpression of p53 protein containing alterations that impede its normal function [3]. In addition, p53 translated from a mutant gene has a longer half-life, in opposition to the wild-type protein. Because both share epitopes, the mutated protein can be detectable by immunohistochemistry (IHC) [7,8]. Lopes et al [9] correlated the overexpression of p53 protein with an unfavorable prognosis in PC. In the same study, p53 immunoreactivity represented also an independent factor of lymph node metastasis, and the simultaneous occurrence of p53 expression and the presence of human papillomavirus–DNA were associated with a worse prognosis [9].

p53 is regulated primarily by its interaction with murine double minute 2 (MDM2), which regulates the stability of p53 by degrading it. Thus, MDM2 inhibits p53-dependent cell cycle arrest and apoptosis. In addition to interacting with MDM2, p53 controls the transcription of MDM2, resulting in a self-regulated feedback loop [10-12]. Imbalances between p53 and MDM2 can result in uncontrolled cell growth and, subsequently, malignant transformation [13]. Hence, the mechanisms by which *TP53* is regulated and by which p53 interacts with MDM2 should be examined closer because they may be key indicators of the prognosis of several cancers and potential targets for its prevention, guiding the development of novel approaches for molecular therapy and surgery.

In contrast to the well-established prognostic significance of alterations in p53 in various carcinomas and sarcomas, the clinical value of anomalies in the *TP53* and *MDM2* pathway has not been sufficiently investigated in PC. Thus, it was the purpose of the present study to examine the immunohistochemical expression of p53 and MDM2 in 297 cases of PC, to determine whether protein alterations were associated to gene and chromosomal numeric alterations or to gene mutations. Such findings were compared with clinicopathologic and outcome parameters to establish additional biologic markers for clinical usage.

2. Materials and methods

2.1. Case selection

A total of 297 cases of PC were selected from the database of the Department of Pathology, Hospital AC Camargo, Brazil, for the construction of a tissue microarray. All cases diagnosed at our institution between 1980 and 2009 were included if there was sufficient archival tissue for the studies to be performed. Clinicopathologic information was obtained from the patients' files. The study was approved by the ethics committee of the institution.

2.2. Immunohistochemistry and fluorescence in situ hybridization

For the IHC, the primary antibodies were anti-MDM2 (clone SMP14) and anti-p53 (clone DO7) (both provided by Dako, Carpinteria, CA). Based on preliminary tests, the antibodies were diluted at 1:25. All IHC procedures were performed on an Autostainer Link 48 (Dako) using the Flex Plus visualization system per the supplier's specifications.

Immunohistochemical stains were evaluated both on a light microscope and on the APERIO automated slide scanner (Aperio, Vista, CA) with an automated image analysis software. For the visual evaluation (performed by J. J. and F. A. S.), each spot was scored as for staining intensity and semiquantification of positive cells (nuclear reactivity for p53 and MDM2).

Fluorescence in situ hybridization (FISH) technique was performed manually, using the probes *MDM2* at 12q15