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Phenotypic and metabolic aspects of prostatic epithelial cells in aged gerbils after antisteroidal therapy: Turnover in the state of chromatin condensation and androgen-independent cell replacement

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ABSTRACT

The gerbil is a rodent considered a good model for studies of prostatic morphophysiology under different experimental conditions. Studies involving castration and steroidal blockers of aged gerbils showed that the glandular epithelium persists after long-term therapy, preventing the organ atrophy. Thus, the objective of this study was to evaluate the phenotypic characteristics and behavior of prostatic epithelial cells that remained after different periods of hormone ablation in aged gerbils. The identification of elements that influenced the survival of this cell type was performed by morphometric, nuclear phenotypes, ultrastructural and immune histochemical analysis. The most significant responses to treatment, by analyzing morphometric features, were observed during the first three time points (day 1, day 3, and day 7), after which there appeared to be an adjustment of the gland to the hormone ablation. All treatments led to changes in the state of chromatin condensation, DNA methylation pattern and phenotypic changes indicated cell senescence. Additionally, an increase in the basal cells seemed to guarantee self-renewal properties to the epithelium. These data indicate that changes occur at many levels, including gene expression and nuclear architecture in the epithelial cells, when aging and steroidal blockade are associated. These aspects are important when considering castration-resistant prostate cancer, a malignant tumor posing difficult therapeutic intervention.

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Introduction

Aging associated with androgen imbalance is considered the most important risk factor in the development of prostate cancer (Abate-Shen and Shen, 2000; Raghow et al., 2000). Inhibition of hormonal pathways remains the first measure to prevent this injury (Lee et al., 2002). Testosterone and dihydrotestosterone suppression in the serum can be performed by orchiectomy (surgical castration) and/or antiandrogen administration (Ruijter et al., 1999). The gland regression occurs due to activation of apoptotic pathways, which primarily affect the secretory epithelial cells. The basal cells, another epithelial cell subtype, and stromal cells are less sensitive to androgen suppression and are maintained (Bruyninx et al., 1999; Campos et al., 2010, 2011).

In castrated mice, prostatic mass reduction involves mainly the loss of distal ducts, while atrophic alterations are minimal in the proximal ducts (Sugimura et al., 1986). In Brown Norway rats, castration-induced apoptosis is lobe-specific and the apoptotic index decreases with aging (Banerjee et al., 1995, 2000). These events indicate that there is a variation in androgen dependency among different portions of the prostate, as well as among the epithelial cells, and there may be other factors that allow the survival of groups of cells independent of hormone (Banerjee et al., 1998).

Although there is a direct correlation between prostate cancer and aging, there is still lacking a complete model, for example, in rodent species that promises a better understanding of the molecular pathways that are hyper-regulated or down-regulated during prostate cancer development in the elderly. In gerbils, a rodent considered a good model for studies of prostatic morphophysiology

Abbreviations: ANOVA, analysis of variance; CpG-rich DNA regions, phosphodiester bond between cystosine and guanine; DNA, deoxyribonucleic acid; ECEA, Ethics Committee Experimentation Animal; p63, transformation-related protein 63; SD, standard deviation; SE, standard error.

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under different experimental conditions (Corradi et al., 2004; Cordeiro et al., 2008; Scarano et al., 2008), the development of spontaneous premalignant and malignant lesions in old animals is frequent (Pegorin de Campos et al., 2006; Campos et al., 2008, 2010, 2011; Gonçalves et al., 2010, 2013). In an experiment involving castration and steroidal blockers, 18-month-old gerbils had high apoptotic death levels identified on the first day of therapy, indicating an immediate response by the secretory epithelial cells. However, the acinar units that prevailed after the long-term therapy exhibited characteristics of a functional gland and prevented organ atrophy (Campos et al., 2010). The characterization of elements that influence the survival of epithelial cells under these conditions has not been exploited and these aspects are important when considering castration-resistant prostate cancer, a malignant tumor posing difficult therapeutic intervention.

An important field of investigation in the search for treatment of human disease, including prostate cancer, is the modulation of chromatin modifications and karyometry of neoplasic and non-neoplasic cell nuclei (Montironi et al., 2000, 2003; Dimauro and David, 2009). Chromatin modifications impact all biological processes related to DNA, including gene expression, genomic stability and nuclear high order organization (Dimauro and David, 2009). Additionally, aging is also associated with changes in patterns of gene expression and analysis of DNA methylation suggests that mammalian aging is associated with an overall decrease in heterochromatin, but an increase at specific sites in the genome (Villeponteau, 1997; Larson et al., 2012; Tsurumi and Li, 2012). These aspects are relevant and appear promising to better understand the development of prostate cancer, a disease directly associated with aging. In this study we present the aged gerbil as a feasible model to evaluate the phenotypic characteristics and behavior of prostatic epithelial cells remaining after different periods of surgical castration associated with antisteroidal therapy. As there are few studies evaluating the behavior of the nucleus of epithelial cells remaining after these therapies, better detailing of this organelle, i.e. size, shape, and morphology was also performed. Measurement of condensed chromatin clusters was also used as indicative of variability in the state of chromatin condensation and change in gene expression after treatments. Eighteen-month-old animals tolerated treatment well and provided a systematic prostate microenvironment similar to that found in elderly humans, the time of life during which the highest incidence of malignant tumors occur in this gland. Phenotypic alterations in the cell, in the structure and ultrastructure of the chromatin and DNA methylation pattern, indicated that the treatments promoted activation of mechanisms associated with cellular senescence and gene silencing. An increase in the basal cells seemed to guarantee self-renewal properties to the epithelium with an adjustment of the gland to the steroidal ablation condition at the end of the treatment.

Materials and methods

Animals and experimental design

One hundred twenty senescent male gerbils (18 months old) were handled in accordance with institutional guidelines and the experiment was approved by the Animal Experimentation Ethics Committee of Sao Paulo State University (protocol number: 004/06 – ECEA). The gerbils were housed in plastic cages, under conventional conditions (25 °C, 40–70% relative humidity, 12 light:12 dark), in pathogen-free conditions, and supplied water and balanced chow ad libitum. Animals were divided into six groups of five each to comprise the control and treated groups. The latter were surgically castrated, while the first control group was composed of intact animals (G1). The other two control groups were formed by castrated animals that received the drug dilution vehicle (G3) and castrated ones that were not administered the vehicle (G2). Additionally, the three experimental groups received subcutaneous injections of flutamide antiandrogen (Sigma–Aldrich, St. Louis, MO, USA; 10 mg/kg/day) (G4), tamoxifenantiestrogen (Sigma–Aldrich; 1 mg/kg/48 h) (G5) and flutamide plus tamoxifen (G6) for 1, 3, 7 or 30 days post-castration. Vegetable oil at the dose of 0.1 ml/application/animal was used as the drug dilution vehicle. The applied therapies aimed to increase the hormonal imbalance in the prostate typical of senescence. Before necropsy, the animals were anesthetized by CO_2 inhalation and then decapitated. The prostate was removed and submitted to light microscopy and ultrastructural procedures.

Histochemistry

For light microscopy, some of the prostatic fragments were fixed for 24 h in 4% paraformaldehyde in phosphate-buffered saline and embedded in paraffin (Histosec™; Merck, Darmstadt, Germany) and the others in Karnovsky fixative (0.1 M Sörensen phosphate buffer, pH 7.2, containing 5% paraformaldehyde and 2.5% glutaraldehyde). The latter fragments were embedded in glycol methacrylate resin (Leica Historesin Embedding Kit™, Nussloch, Germany).

Historesin-embedded prostate were cut into sections of 2 μm and submitted to staining by hematoxylin–eosin and Feulgen reaction. The paraffin sections (5 μm) were submitted to immunohistochemical tests. The tissue sections were analyzed in an Olympus photomicroscope (Olympus, Hamburg, Germany) and the microscopic fields were digitalized using the Image-Pro-Plus software version 4.5 for Windows (Media Cybernetics, Inc., Bethesda, USA).

Immunohistochemistry

The antibodies applied in the present study were: anti-5-methylcytidine (mouse monoclonal, 33D3, Santa Cruz Biotech, Santa Cruz, CA, USA) and anti-p63 (mouse monoclonal, 4A4, Santa Cruz), both at the dilution 1:100. For the analysis, paraffin sections were deparaffinized, rehydrated through graded alcohol, and then subjected to antigen retrieval in 10 mM citrate buffer pH 6.0, at 97 °C for 20–30 min. The blockade of endogenous peroxidase was obtained by covering the slides with hydrogen peroxide (3% in methanol) for 20 min and the blockade of non-specific protein–protein interactions was achieved by incubating sections with 3% bovine serum albumin (BSA, Sigma–Aldrich, St. Louis, MO, USA) also for 20 min. After pretreatment, the sections were incubated overnight at 4 °C with the antibodies diluted in 1% BSA. After that, slides were incubated with Novo Link Max Polymer detection system (Leica Biosystems, Novocastra, Newcastle-on-Tyne, UK) or EnVision™ + Dual Link (Dako North America, Carpinteria, CA, USA) and the positive signals were visualized as brown precipitates utilizing 3,3′-diaminobenzidine tetrahydrochloride solution (DAB liquid, Dako). Negative controls were included in each staining series by omission of the primary antibodies. Hematoxylin was used for counterstaining.

Transmission electron microscopy

Ventral lobe fragments were fixed by immersion with 3% glutaraldehyde (Sigma–Aldrich) plus 0.25% tannic acid (Sigma–Aldrich) solution in Millonig's buffer, pH 7.3 containing 0.54% glucose (Sigma–Aldrich) for 24 h (Cotta-Pereira et al., 1976). After washing with the same buffer, they were post-fixed with 1% osmium tetroxide (Sigma–Aldrich) for 2 h, washed again,

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