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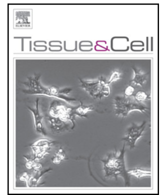

Prolactin, EGFR, vimentin and α-actin profiles in elderly rat prostate subjected to steroid hormonal imbalance

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The aim of this study was to characterize and relate the prolactin (PR), epidermal growth factor receptor (EGFR), α-actin and vimentin immunoreactivity in the prostate of elderly rats subjected to steroid hormonal imbalance. Senile and young rats were divided into the young group (YNG), the senile group (SE), the castrated group (CAS), the estrogen-deficient group (ED), the castrated + estrogen group (CASE), and the estrogen-deficient + androgen group (EDTEST). PR and EGFR increased in the estrogen and androgen ablation groups. In addition, EGFR influenced the immunolocalization by changing it from the prostatic stroma to the epithelium in elderly rats. Hormone ablation in elderly rats, not only related to androgen but also estrogen, led to increased stromal EGFR immunolocalization. The α-actin pattern decreased in the groups with estrogenic imbalance. Moreover, vimentin increased in the senile and estrogen deficient group. To conclude, we can suggest that EGFR contributed towards the proliferative process in the prostate, by means however, of different mechanisms, considering the androgenic and estrogenic pathways. Also, our results indicated that prolactin could be activated not only in an androgen-independent pathway but also in an estrogen independent pathway. Finally, PR and vimentin immunolocalization increase, in the prostatic stroma in the group showing estrogenic ablation, could be one of the factors which contribute to the reactive stroma formation.

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1. Introduction

Morphogenesis, proliferation and differentiation of prostatic cells are regulated by androgens (Imamov et al., 2005). Hormone imbalance is a general factor, which leads to morphological and functional changes in the prostate in different species, including human beings (Roy-Burman et al., 2004).

Androgen deficiency can cause prostatic regression, apoptosis activation and extracellular matrix remodeling in this gland (Vilamaior et al., 2000). In addition, it is known that the prostate of rodents can suffer successive stages of epithelial regeneration after androgen ablation, followed by hormone replacement (Taylor and Risbridger, 2008).

Aging has been considered one of the main predisposing factors for the development of prostate malignancies, considering the development of pro-angiogenic microenvironment during this life period (Montico et al., 2015a). The occurrence of proliferative lesions, inflammation and increased mitogenic factor levels and tissue remodeling factors are involved in tumorigenic processes in the prostate of elderly mice (Montico et al., 2014). In addition, we showed correlation between steroid hormone and signaling via fibroblast growth factors in the prostatic microenvironment of aging rats, highlighting hormone ablation (Hetzl et al., 2013). Our previous study showed that not only estrogens but also androgens are proliferative elements in the prostate, however, by means of different pathways during aging (Hetzl et al., 2013). Also, we concluded that the hormone steroid endogenous changes during senescence could contribute substantially to glandular imbalance (Hetzl et al., 2013).

According to Cunha et al. (2002) estrogens act synergistically with testosterone, influencing not only the normal prostatic functions, but also the glandular pathogenesis. Estrogen biosynthesis occurs from an androgen substrate by means of estrogen aromatization using the aromatase enzyme (Risbridger et al., 2003). The estrogenic effects in the prostate result from the binding between hormone and specific estrogenic receptors (ERα, ERβ), which are expressed in the stroma and epithelium, respectively (Bonkhoff and Berges, 2009; Ho and Habib, 2011). Moreover, it is a known fact that

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estrogen action could be verified in different pathways considering the estrogenic receptors (Ellem and Risbridger 2009, 2010). ERα is involved in gland carcinogenesis, leading to anormal proliferation, inflammation and even development of pre-malignant lesions. On the other hand, ERβ could supress the tumour growth through anti-proliferative, anti-inflammatory, and anti-carcinogenic pathways in the prostate (Ellem and Risbridger, 2009, 2010; Hartman et al., 2012). Also, according to Nellemann et al. (2005), the androgen receptor (AR) expression in the prostate could be under regulation of both estrogen and androgen, considering that testosterone administration associated with anti-estrogen, or estrogen administration associated with anti-testosterone reduced these sexual hormone effects in this gland. Furthermore, it is possible that elevated estrogens and also endocrine disrupting chemical exposure, by means of ER signaling pathways in the adult prostate progenitor cells, may directly reprogram or transform these cells and lead to tumor initiating capacity cells (Hu et al., 2012).

The prolactin (PRL)–prolactin receptor (PRLR) signaling complex has been involved in breast lesions and prostate carcinoma (Damiano and Wasserman, 2013), suggesting involvement in breast and prostate cancer onset or progression. Different authors verified that excessive prolactin stimulation could induce hypertrophic changes in the prostatic stroma and epithelium in vitro and in vivo studies (Nevalainen et al., 1991, 1997).

The epithelial-stromal interaction has a relevant role in the development and maintenance of the mature prostate phenotype (Hayward et al., 1996). Tuxhorn et al. (2002) verified that the replacement of the normal stroma for a reactive stroma could modify the epithelial and stromal interaction, affecting cancer progression. These same authors showed that the prostatic reactive stroma is formed by fibroblast and more than 50% myofibroblasts, a stroma cell with activated phenotype and which is not in the normal prostatic stroma (Tuxhorn et al., 2002). The myofibroblast is described as an intermediate cellular type between fibroblast and smooth muscle cell, considering the cytoskeleton protein expression and structural aspects (Gabbiani et al., 1972). Thus, according to Tuxhorn et al. (2001), a fibroblast changes its phenotype to myofibroblast in the damaged tissue, which could be characterized by vimentin and α-actin expressions.

Thus, considering the steroid hormone imbalance in late life period, as well as, the role of this imbalance in prostatic lesion development and progression, the aim of the study herein was to characterize and correlate prolactin, EGFR, α-actin and vimentin immunoreactivity in the prostatic epithelium and stroma of elderly rats, following steroid hormone imbalance.

2. Material and methods

2.1. Experimental procedures

Fifty 10-month-old male Sprague–Dawley rats (SE, CAS, ED, EDTEST and CASE) and ten 4-month-old male Sprague–Dawley rats (YNG) were used and divided into 6 groups. This study was approved by the institutional Committee for Ethics in Animal Research (University of Campinas- UNICAMP, protocol number 2412-1).

The *Young Group* (YNG) received subcutaneous peanut oil injections (5 ml/kg body weight) on alternate days for 30 days. The *Senile Group* (SE) received subcutneous peanut oil injections (5 ml/kg body weight) every other day for 30 days. In the *Castrated Group* (CAS), the androgen withdrawal was carried out by chemical and surgical castration. In the surgical castration, the animals were anesthetized with xylazine hydrochloride 2% (im 5 mg/kg; König, São Paulo, Brazil) and ketamine hydrochloride 10% (60 mg/kg, im, Fort Dodge, Iowa, USA), followed by the removal of the testes. In the chemical castration, the animals were subjected to chemical castration treatment with subcutaneous injections of 10 mg/kg of flutamide (Sigma Chemical Co., St. Louis, MO, USA) diluted in 10 ml peanut oil on alternate days for 30 days (modified Shin et al., 2002; Hetzl et al., 2013). The rats in the *Estrogen-deficient Group* (ED) received subcutaneous injections of 1 mg/kg tamoxifen (Sigma Chemical Co., St. Louis, MO, USA) diluted in 1 ml of peanut oil every 48 h for 30 days to block estrogen receptors (ERα and ERβ) (modified Tilley et al., 1987; Hetzl et al., 2014). To block the aromatization of testosterone resulting in estrogen, the animals received subcutaneous injections of 1 mg/kg letrozole (LET-Femara, Novartis-Pharma, Basel, Switzerland) diluted in 1 ml of peanut oil on alternate days for 30 days (modified Tobin and Canny 1998; Hetzl et al., 2014). In the *Castrated + Estrogen Group* (CASE), the removal of androgens was carried out by chemical and surgical castration. After castration, similar to that performed for the castrated group, the animals received subcutaneous 17β-estradiol (Sigma Chemical Co., St. Louis, MO, USA) injections (25 mg/kg body weight) diluted in 25 μl of peanut oil on alternate days for 30 days (modified Prins et al., 2001; Hetzl et al., 2014). *Tamoxifen–letrozole + Androgen Group* (EDTEST); following the treatment similar to the tamoxifen–letrozole group the animals received subcutaneous testosterone cypionate (Deposteron, Novaquímica, São Paulo, Brazil) injections (5 mg/kg body weight) diluted in 5 ml peanut oil every other day for 30 days (modified Sáttolo et al., 2004; Hetzl et al., 2014).

After 30 days of treatment, the animals were anesthetized with 2% xylazine hydrochloride (5 mg/kg i.m.; König, São Paulo, Brazil) and 10% ketamine hydrochloride (60 mg/kg, i.m., Fort Dodge, Iowa, USA) and then sacrificed. All the animals received water and the same solid diet ad libitum (Nuvilab, Colombo, PR, Brazil). Samples were collected from the ventral lobe of the experimental animals in each group and evaluated in terms of light microscopy and immunohistochemistry.

2.2. Morphological analysis

Prostatic samples from five animals (per group) for histological analysis were fixed in Bouin's solution, embedded in paraplast (Paraplast Plus, ST. Louis, MO, EUA), cut into 5-μm thick sections by means of a microtome (Hyrax M60, Zeiss, Alemanha) and submitted to Masson's trichrome staining (Junqueira et al., 1979). The photomicrographs were obtained using a Nikon Eclipse E-400 photomicroscope.

2.3. Immunohistochemical analyses to epidermal growth fator receptor (EGFR), prolactin (PR), alpha actin (α-actin) and vimentin (VIM)

Prostate samples of five senile animals of each group were used for immunostaining. The pattern protocols were similar to those described by Hetzl et al. (2014). After, the EGFR, PR, α-actin and VIM antigens were located using antibodies: *rabbit* polyclonal (sc-03) (*Santa Cruz Biotechnology, EUA*) for EGFR, *goat* polyclonal (sc-7805) (*Santa Cruz Biotechnology, EUA*) for PR, *rabbit* polyclonal (ab5694) *(abcam, EUA)* for α-actin, *mouse* monoclonal ab8069 (*abcam, EUA*) for VIM diluted (1:35–50) in 1% BSA and stored overnight at 4 °C. HRP Envision Kit (Dako Cytomation, Inc., Carpenteria, CA, USA) was used for the detection of antigens. After washing with a TBS-T buffer, the sections were incubated with HRP conjugate secondary antibody from the Envision kit (Dako Cytomation, Inc., Carpenteria, CA, USA) for 40 min and subsequently developed with diaminobenzidine (DAB), counterstained with Methyl Green and Harris hematoxylin and evaluated in a Nikon Eclipse E-400 light microscope (Nikon, Tokyo, Japan). The prostatic cuts of five senile animals from each group were evaluated using DAB brownish

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