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Morphology and MMP-9, AR and IGFR-1 responses of the seminal vesicle in TRAMP mice model

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ABSTRACT

Seminal vesicles are important hormone-dependent accessory sex glands. Transgenic adenocarcinoma of the mouse prostate (TRAMP) model has been used to evaluate malignant diseases in the prostate and in other sexual glands. The aim of this study was to characterize structural and molecular features of the seminal vesicle in different life periods of the TRAMP mice. Groups: Control Group (5 FVB/12 week old mice), TRAMP 12 and 22 Groups (10 TRAMP 12 and 22 week old mice, respectively). Seminal vesicles were evaluated by morphological and immunohistochemical parameters; androgenic receptor (AR), Insulin-like growth factor 1 (IGFR-1) and metalloproteinase 9 (MMP-9). The TRAMP mice showed frequent epithelial proliferation, including cellular stromal invasion, especially in the TRAMP 22 group. Intense AR reactivity was seen in both stroma and epithelial regions in the TRAMP 22 group. Intense IGFR-1 and MMP-9 stromal immunolabeling was identified in both TRAMP groups. Thus, there were structural and molecular changes in the seminal vesicle in TRAMP mice, compromising not only the structure but also the stromal signaling, damaging thus the function and leading to glandular lesions. TRAMP mice could be indicated as a good model to study alterations of the seminal vesicle in association to prostate cancer.

1. Introduction

Seminal vesicles are androgen-dependent accessory sex glands from the male reproductive system, and which secrete important fluid for the reproductive process (Mann and Lutwak-Mann, 1976; Hayward et al., 1996). Also, Bianco et al. (2002) verified that estrogen led to proliferative responses in the seminal vesicles in mice. These responses were organ-specific, considering the different prostatic lobes and seminal vesicles, and were represented by smooth muscle regression, inflammation, basal cell proliferation.

Welsh et al. (2010) verified that smooth muscle cells contribute to stromal-epithelial interaction due to the androgen-dependence of the seminal vesicle.

It is known that primary tumors are rare in seminal vesicles (Takayasu et al., 2015). The prostatic tumor extensions are more frequent in seminal vesicles (Son et al., 2004). According to different authors, seminal vesicle tumors are more related to metastasis than the extraprostatic lesion extension of this gland (Liauw et al., 2003; Katz et al., 2003). In addition, Yeh et al. (2009) described seminal vesicle tumors, which could become malignant in the TRAMP mice model.

According to Gingrich et al. (1999) TRAMP mice have been considered an effective prostate cancer development model, characterizing different lesion grades, which could range from prostatic intraepithelial neoplasia to poorly differentiated glandular adenocarcinoma. The TRAMP mice model expressed SV40 viral oncoproteins in the prostatic epithelial cells under control of the rat probasin gene, which is an androgen responsive promoter (Greenberg et al., 1995; Gingrich et al., 1999; Huss et al., 2001). Also, TRAMP mice develop prostatic intraepithelial neoplasia and/or well-differentiated adenocarcinoma between 10 and 12 weeks of age. And also, 24–30 week old mice develop prostatic adenocarcinoma and a metastatic process in distinct organs such as lungs and lymph nodes (Greenberg et al., 1995; Gingrich et al., 1996).

Insulin-like growth factors (IGF) are a growth factor family which is involved in a combination of binders (IGF-1 and IGF-2) and also two receptors (IGFR-1 and IGFR-2), carrying out an important role in cellular differentiation and proliferation regulation and apoptosis (Jones and Clemmons, 1995; Gennigens et al., 2006). Thus, IGF-1 is a mitogen for a cell variety and exerts this action,

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increasing the DNA synthesis by the expression stimulation of D1 cyclin, which speeds up G1 cell cycle to S phase (Furlanetto et al., 1994).

Cancer IGF involvement has been reported since the late 1990s in various organs such as the prostate (Pollak et al., 1999; Harman et al., 2000; Biernacka et al., 2012), colon (Sandhu et al., 2002), lung (Yu et al., 1999) and also seen in breast cancer in the premenopause (Hankinson et al., 1998; Toniolo et al., 2000). Also, IGFR-1 gene overexpression is a common aspect among the different types of cancer in human beings (Werner and Roberts, 2003).

Also, according to Kaplan et al. (1999) the IGF-1 axis is an important mechanism in the TRAMP mice model, involved in prostate cancer progression, similar to what is found in the human prostatic disease, characterizing IGF-1 as an important factor in the first steps of cancer development in addition to primary prostate cancer progression.

Regarding metalloproteinases (MMPs), these are enzymes involved in the tissue remodeling associated to physiological process such as reproduction, embryonic development and morphogenesis, postnatal organ development and angiogenesis (Bozdagi et al., 2007; Wang et al., 2008), in addition to physiological and pathological processes such as cancer, a period in which these molecules are highly expressed (Ii et al., 2006; Cai et al., 2007).

In addition, MMPs have been suggested to not only be involved in the extracellular matrix degradation but also in making part of stromal and tumor cell interactions (Lynch and Matrisian, 2002). According to Martin and Matrisian (2007), MMPs are known for their pro-tumorigenic capacity. However, there are some studies indicating that the expression of some MMPs, in both primary tumor and metastatic foci, could develop a protective effect in different tumoral progression grades Martin and Matrisian (2007).

Thus, considering the seminal vesicle importance in male reproduction and also the unclear aspects about the biology of seminal vesicle lesions, the aim of the study herein was to characterize structural and AR, IGFR-1 and MMP-9 features of the seminal vesicle in different life periods from the TRAMP mice model, identifying possible pre-neoplastic and neoplastic lesions in this gland, simultaneously or not with prostate cancer.

2. Materials and methods

2.1. Animals and experimental procedures

A total of 5 male FVB mice (12 weeks old) and 10 male transgenic TRAMP mice (12 and 22 weeks old, respectively, 5 mice *per group*) were provided by the Multidisciplinary Center for Biological Investigation on Laboratory Animal Science (CEMIB) at the University of Campinas and divided into 3 groups: FVB Control Group; TRAMP 12 Group (5 TRAMP/12 week-old mice) and TRAMP 22 Group (5 TRAMP/22 week-old mice). All animals were kept individually in plastic cages receiving water and solid feed diet *ad libitum* (NUVILAB, Colombo, PR, Brazil). At the end of experimental period, the mice were anesthetized with Xylazine hydrochloride 2% (5 mg/kg im; König, São Paulo, Brazil) and Ketamine hydrochloride 10% (60 mg/kg im, Fort Dodge, Iowa, USA) (Ethical approval: Committee for Ethics in Animal Research—University of Campinas, protocol n°: 3136-1) and seminal vesicle samples were collected for morphological and immunohistochemical analyses.

2.2. Light microscopy analysis

Seminal vesicle samples from all experimental groups were collected and fixed in Bouin solution (picric acid aqueous solution + formaldehyde + acetic acid) for 10 h. After that, the tissues were washed in 70% ethanol, with subsequent dehydration in increasing alcohol bath series. Then, the fragments were diaphanized with xylene for 2 h and embedded in a plastic polymer (Paraplast Plus, ST. Louis, MO, USA). Then, the materials were cut in a microtome (Hyrax M60, Zeiss, Munich, Germany) into 5 μm thick sections and stained with hematoxylin-eosin and Masson's trichrome (Junqueira et al., 1979) and photographed in a light microscope (Nikon Eclipse E-400).

2.3. Androgen receptor (AR), insulin-like growth fator 1 receptor (IGFR-1) and metalloproteinase-9 (MMP-9) immunohistochemical analyses

Seminal vesicle samples were collected from 5 animals in each experimental group, the same used for light microscopy analyses. Antigen retrieval was achieved by boiling the sections in a 10 mM citrate buffer (pH 6.0) three times for 5 min in a microwave oven. In order to block endogenous peroxidase, 0.3% H_2O_2, diluted in methanol, was applied to the sections for 20 min, while non-specific binding was prevented by incubation in bovine serum albumin (A4503, Sigma–Aldrich, St. Louis, MO, USA) (3% BSA in TBS-T buffer) for 1 h at room temperature. The AR, IGFR-1 and MMP-9 antigens were detected using rabbit polyclonal anti AR N-20 (sc-816) (Santa Cruz Biotchenollogy, California, USA), rabbit polyclonal anti IGFR-1α N-20 (sc-712) (Santa Cruz Biotchenollogy, California, USA) and goat polyclonal anti MMP-9C-20 (sc-6840) (Santa Cruz Biotchenollogy, California, USA) antibodies, diluted (1:25–1:50) in 1% BSA and applied to the sections overnight at 4 °C. After being washed with TBS-T, the sections were incubated in Horsehadish peroxidase (HRP)-conjugated secondary antibodies for 2 h at room temperature. The secondary antibodies used were donkey anti-goat IgG (sc-3851) (Santa Cruz Biotechnology, California, USA) and goat anti-rabbit IgG (W4018) (Promega Corporation, Madison, WI, USA). Diaminobenzidine (DAB) (458619, Sigma–Aldrich, St. Louis, MO, USA) chromogen solution with the substrate H_2O_2 was applied to the sections for 10 min to detect peroxidase activity. Seminal vesicle tissue was then lightly counterstained with Harris Hematoxylin and photographed in a Nikon Eclipse E-400 photomicroscope (Nikon, Tokyo, Japan). Positive antibody reaction, as shown by the occurrence of DAB precipitate, was evaluated in the seminal vesicle sections of 5 animals per group. The immunoreactivity intensity was graded 0 for negative staining (0%), 1 for weak staining (33%), 2 for moderate staining (33–66%), and 3 for intense staining (greater than 66%) (modified Tomas and Kruslin, 2004; Tuxhorn et al., 2002a,b), considering 7 fields in each animal from different experimental groups.

3. Results

3.1. Light microscopy

3.1.1. FVB control group

The seminal vesicle secretory epithelium was simple with folded mucosa, showing columnar cells and basal nuclei in addition to basal cells (Fig. 1a–d). The glandular stroma showed smooth muscle cells and collagen fibers around the glandular structure (Fig. 1a–d).

3.1.2. TRAMP 12 group

This group showed not only healthy glandular regions, which demonstrated simple epithelium with columnar cells with basal nuclei, but also structural alterations in the seminal vesicle. The seminal vesicle epithelium showed regions which cellular proliferation, characterizing glandular intraepithelial neoplasia. The cells from intraepithelial neoplasia showed enlarged nuclei and condensed chromatin. And also, some glandular regions presented basal membrane discontinuity and some epithelial cells had invaded the stroma (Fig. 2a–d).