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Expression and potential role of the peptide orexin-A in prostate cancer

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ABSTRACT

The peptides orexin-A and orexin-B and their G protein-coupled OX1 and OX2 receptors are involved in multiple physiological processes in the central nervous system and peripheral organs. Altered expression or signaling dysregulation of orexins and their receptors have been associated with a wide range of human diseases including narcolepsy, obesity, drug addiction, and cancer. Although orexin-A, its precursor molecule prepro-orexin and OX1 receptor have been detected in the human normal and hyperplastic prostate tissues, their expression and function in the prostate cancer (PCa) remains to be addressed. Here, we demonstrate for the first time the immunohistochemical localization of orexin-A in human PCa specimens, and the expression of prepro-orexin and OX1 receptor at both protein and mRNA levels in these tissues. Orexin-A administration to the human androgen-dependent prostate carcinoma cells LNCaP up-regulates OX1 receptor expression resulting in a decrease of cell survival. Noteworthy, nanomolar concentrations of the peptide counteract the testosterone-induced nuclear translocation of the androgen receptor in the cells: the orexin-A action is prevented by the addition of the OX1 receptor antagonist SB-408124 to the test system. These findings indicate that orexin-A/OX1 receptor interaction interferes with the activity of the androgen receptor which regulates PCa onset and progression, thus suggesting that orexin-A and its receptor might represent novel therapeutic targets to challenge this aggressive cancer.

1. Introduction

The neuropeptides orexin-A and orexin-B, both deriving from the proteolytic cleavage of the precursor molecule prepro-orexin, bind with different affinity two G protein-coupled receptors, namely OX1 and OX2 receptors [1,2]. First discovered in rat hypothalamic neurons projecting their axons towards multiple cerebral areas, orexins and their receptors regulate many functions mediated by the central nervous system such as sleep and wakefulness, appetite/metabolism, addiction and reward, blood pressure and heart rate, sexual behavior, and neuroendocrine homeostasis [1–4]. Orexins and their receptors have been also localized in many peripheral organs where they exert autocrine, paracrine and endocrine activities [5,6]. Dysregulation of multiple physiological processes has been linked to altered expression or functioning of either central or peripheral orexinergic system [7], and orexin receptors have been suggested as therapeutic targets for a variety of human diseases such as insomnia, obesity, drug addiction, cancer and others [8].

In particular, OX1 receptor has been identified as a potential target for colon cancer therapy [9] and, more recently, for prostate cancer (PCa) therapy [10]. However, while orexin-A and OX1 receptor activity in HT29-D4, Caco-2, SW480, and LoVo colon cancer

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cell lines [9,11] and in human colon tumor xenografts in a nude mice model [9] has been well established, the expression and role of orexin-A and OX1 receptor in both normal and cancer prostate cell lines and tissues requires further investigations. Indeed, despite a wide detection of orexin-A and OX1 receptor in the male genital tract of mammals [12–16], conflicting data on their localization in the human prostate have been reported. Nakabayashi and co-workers [17] failed to detect orexin-A immunohistochemical localization and prepro-orexin mRNA expression in the prostate of healthy subjects, whereas Malendovicz and co-workers [18] demonstrated the presence of orexin-A and OX2 receptor but not prepro-orexin and OX1 receptor expression in normal and hyperplastic prostate. More recent studies demonstrated the immunohistochemical localization of both orexin-A and OX1 receptor and the expression of prepro-orexin and OX1 receptor at protein and mRNA levels in normal and hyperplastic prostate tissues [19]. Furthermore, while OX1 receptor expression was demonstrated in cancerous foci of high grade PCa tissues, no orexin-A localization was found in the same tissues as well as in normal prostate tissues [10].

In order to achieve a better understanding of the expression and the potential role of orexin-A and OX1 receptor in the PCa, here, we investigated the immunohistochemical localization of orexin-A and OX1 receptor and the expression of mRNAs coding for prepro-orexin and OX1 receptor and the relative proteins in PCa surgical specimens. *In vitro* studies using the human androgen-dependent prostate carcinoma cell line LNCaP were also performed to evaluate the capability of orexin-A to modulate the activity of the androgen receptor which is functionally active in advanced and castration-resistant PCa [20].

2. Materials and methods

2.1. Antibodies and reagents

Rabbit polyclonal anti-orexin-A antibody supplied with its control peptide (PC362) was obtained from Millipore (Billerica, MA, USA); rabbit polyclonal anti-OX1 receptor antibody (PAB8017) from Abnova (Taipan, Taiwan) and the synthetic blocking peptide (ab188501) from Abcam (Cambridge, UK); rabbit polyclonal anti-prepro-orexin antibody (AB3096), its blocking peptide (AG774), and monoclonal anti-γ-tubulin (MAB1637) antibody from Chemicon International Inc. (Temecula, CA, USA); biotinylated goat anti-rabbit (BA-1000) secondary antibody from Vector Laboratories (Burlingame, CA, USA); horseradish peroxidase goat anti-rabbit IgG (A-0545) from Sigma Chemical Co. (St. Louis, MO, USA). The peptide orexin-A (003-30) was purchased from Phoenix Pharmaceuticals (Karlsruhe, Germany); 3-[4,5-dimethylthiazol-2-yl]-3,5 diphenyl tetrazolium bromide (MTT) kit from Cayman Chemicals (Ann Arbor, MI, USA); OX1 receptor inhibitor SB-408124 (1963) from Tocris Bioscience (Bristol, UK); the primers for human prepro-orexin, OX1 receptor, hypoxanthine ribosyltransferase (HPRT1), and β-actin from Primm (Milan, Italy).

2.2. Surgical specimens

Human PCa specimens were collected from 15 patients (52–75 year old) hospitalized at the Department of Urology of the "A. Cardarelli" Hospital (Naples, Italy) who underwent radical prostatectomy. The procedure followed the rules described in the Declaration of Helsinki, and all patients were previously informed about their contribution. The samples were fixed either in 10% formaldehyde in phosphate buffer or in Bouin's fluid for 48 h, dehydrated in ascending alcohols, clarified in xylene, stored in a methacrylate solution, and embedded in Paraplast for immunohistochemistry. Portions of the collected samples were frozen in liquid nitrogen, and stored at −80 °C for biochemical analyses. Some sections (5–7 μm) were stained with hematoxylin and eosin (H&E) to determine the Gleason's score. Five subject samples were low grade (Gleason's score 3 + 3), five samples intermediated grade (Gleason's score 4 + 3), and five samples high grade PCa (Gleason's score 4 + 5).

2.3. Immunohistochemistry

Paraplast prostate sections were deparaffinized in xylene, hydrated in descending alcohols and incubated in a 3% H_2O_2 solution for 30 min to inhibit endogenous peroxidases. Sections were treated with citrate buffer, pH 6.0, and heated in a microwave oven two times (5 min each) at 750 w to reveal masked antigens. After washing in phosphate buffered saline (PBS) and treatment with normal serum, sections were incubated with anti-orexin-A or anti-OX1 receptor primary antibody overnight at 4 °C. After washing in PBS, the sections were incubated with biotinylated secondary antibodies, and then with ABC reagent in two separate steps of 30 min each at room temperature. 3-3′diaminobenzidine (DAB) was used as a final stain. The preparations were visualized by a Nikon Eclipse E600 light microscope and micrographs were taken using a Nikon Coolpix 800 Digital Camera. Control sections were obtained either substituting the primary antibody with buffer or pre-absorbing the antibody with an excess (10^{-6} M) of its blocking peptide. These controls always resulted to be negative (data not shown). The specificity of the anti-orexin-A antibody had been previously tested in hypothalamus samples from prepro-orexin null mice [21].

2.4. Western blotting

Human PCa tissue samples were homogenized by an Ultraturrax L-407 at 4 °C with 5 ml/1.5 g tissue of buffer containing 50 mM Tris–HCl (pH 7.4), 150 mM NaCl, 1 mM EDTA, 10 mM NaF, 0.5 g/100 ml deoxycholic acid, 0.1 g/100 ml sodium dodecyl sulphate (SDS), 1% (v/v) Nonidet P-40, 1 mM phenylmethyl-sulphonyl fluoride, 10 μg/ml aprotinin, 10 μg/ml leupeptin, and 1 mM Na_3VO_4. Homogenates were centrifuged at 15,000 × g for 10 min at 4 °C. The total protein amount of supernatants was determined by the Bio-Rad protein assay. Samples containing equal amount of proteins (100 μg) were boiled for 5 min in SDS buffer [50 mM Tris–HCl (pH 6.8), 2 g/100 ml SDS, 10% (v/v) glycerol, 0.1 g/100 ml bromophenol blue and 5% (v/v) beta mercaptoethanol], run on a 12.5% SDS/polyacrylamide gel, and transferred to nitrocellulose using a Mini trans-blot apparatus (Bio-Rad Laboratories). Membranes were blocked for 1 h at room temperature with TBS-T buffer [150 mM NaCl, 20 mM Tris–HCl (pH 7.4), 0.1% Tween 20] containing 5 g/100 ml milk. The blots were incubated overnight with anti-prepro-orexin or anti-OX1 receptor antibody diluted 1:1000 in TBS-T containing 2.5 g/100 ml milk. After washing with TBS-T, the blots were incubated for 1 h with horseradish peroxidase conjugated anti-rabbit IgG diluted 1:3000 in TBS-T containing 2.5 g/100 ml milk. The proteins were visualized by enhanced chemiluminescence. To monitor equal loading of gel lanes, the blots were stripped and re-probed using an anti-γ-tubulin monoclonal antibody.

2.5. Cell cultures

The LNCaP cells (ATCC® CRL-1740™) were cultured in RPMI 1640 (Euroclone, Pero, MI, Italy) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 2 mM L-glutamine, 2 mM sodium pyruvate, 1 × non essential amino acids, 100 U/ml penicillin, 100 mg/ml streptomycin, and 10 μg/ml gentamicin. Cells were

maintained at 37 °C in an humified incubator with 5% CO_2. The confluent cells were detached by trypsin-EDTA. For quantitative analysis of OX1 receptor expression levels, freshly re-suspended cells (3×10^6) were incubated for 3 h with 10^{-7} M orexin-A diluted in serum free culture medium, whereas control cells were treated with serum free culture medium alone. To evaluate orexin-A effect on cell survival, we carried out the MTT assay as previously described [19]. Briefly, 2.5×10^5 LNCaP cells were seeded in 96-well plates for 24 h. Appropriate concentrations of orexin-A, in the absence or in the presence of OX1 receptor antagonist SB-408124, were added to the wells for a 48 h lasting incubation, while control cells were treated with medium alone. Successively, 10 μl of a MTT solution were added to each well for 4 h at 37 °C and 5% CO_2. Finally, the culture medium was gently aspirated and replaced by 100 μl of crystal dissolving solution in order to dissolve the formazan crystals. The absorbance of the solubilized dye, which correlates with the number of living cells, was measured by a Synergy 2 (BioTek) microplate reader at 570 nm equipped with a Gen 5 software.

2.6. RNA extraction, reverse transcription (RT) PCR and real time PCR

Extraction of total RNA from PCa tissues, synthesis of cDNAs for the detection of human prepro-orexin and OX1 receptor, and RT-PCR were performed as described previously [15]. For quantitative real time PCR, total RNA from LNCaP cells was extracted using the PureLink® RNA Mini Kit (Ambion Life Technologies, Monza, Italy). Contaminating genomic DNA was removed by treatment with TURBO DNA-free™ Kit (Ambion Life Technologies) and the total amount of RNA was quantified with a Nanodrop spectrometer. cDNAs were synthesized from 1 to 5 μg RNA using the SuperScript® II Reverse Transcriptase (Invitrogen Life Technologies) and quantitative PCR was performed by using the 7500 Real-Time PCR System and SYBR® Select Master Mix 2X assay (Applied Biosystem, Abbiategrasso, MI, Italy). OX1 receptor and HPRT1 primers were designed by using Primer Express software version 3.0: OX1 receptor forward primer 5′-CCCCACTGGGCCTCATG-3′ and reverse primer 5′-CCCCAGAGCTTGCGGAATA-3′; HPTR1 forward primer 5′-CGTCTTGCTCGAGATGTGATG-3′ and reverse primer 5′-GCACACAGAGGGCTACAATGTG-3′. The amount of target cDNA was calculated by comparative threshold (Ct) method and expressed by means of the $2^{-\Delta\Delta Ct}$ method [22] using HPRT1 as internal control.

2.7. Androgen receptor nuclear translocation assay

LNCaP cells were seeded in T25 flasks at a density of 1.2×10^6 cells and incubated for 2 h with increasing concentrations of orexin-A (from 10^{-8} to 10^{-6} M). Some samples, before adding orexin-A, were treated with 0.4 nM testosterone for 30 min. Some others were previously treated for 30 min with OX1 receptor antagonist before adding testosterone. All substances were diluted in serum free medium which was used also for control cell incubation. Nuclear extract protein concentration was determined using the colorimetric Pierce method (Thermo Scientific) loading 8 μg of nuclear protein extract for each sample in the provided plate. Androgen receptor nuclear translocation was evaluated by a sandwich ELISA method (Abcam, Cambridge, UK) reading the absorbance at 450 nm by a Multiscan Ascent microplate reader (Thermo Scientific).

2.8. Statistical analysis

Statistical analysis was performed using a GraphPad Prism Software (version 5.0). The one-way ANOVA and Bonferroni's Multiple Comparison tests were used to assess the statistical significance of the results obtained from androgen receptor translocation assay and real time PCR experiments. P values were considered significant when <0.05 (*), <0.01 (**) and <0.001 (***).

3. Results and discussion

3.1. Immunohistochemical localization of orexin-A and OX1 receptor in PCa tissues

The results obtained by using the avidin-biotin immunohistochemical method showed orexin-A-immunoreactivity (IR) in all analyzed PCa sections (Fig. 1). Orexin-A-IR appeared as fine granules present in the cytoplasm of adjacent cells grouped in small areas, with large zones lacking of positivity. Such granules tended to cluster in coarse inclusions which sometimes entirely filled the cytoplasm, thus becoming easily visible also at low magnification (Fig. 1a,c). Intensely stained cells appeared to be isolated or grouped in small clusters (Fig. 1b) or aligned along the circular profile of structures recognizable as glandular follicles (Fig. 1a,d). Sometimes the whole epithelium lining a follicle showed a mono- or bi-stratified sequence of orexin-A immunoreactive cells. OX1 receptor-IR was also detected in all examined PCa sections (Fig. 2). Its shape and distribution were closely similar to those described for orexin-A (Fig. 2a–d). Although the data reported are representative of orexin-A- and OX1 receptor-IR detected in high grade PCa tissues, positivity for both orexin-A and OX1 receptor was also found in PCa samples of low and intermediate grade (data not shown). These results, while confirm OX1 receptor immunolabeling in PCa tissues [10], demonstrate for the first time orexin-A localization in the tissues whatever the grade of PCa. The discrepancy between our findings and those reported by Alexandre et al. [10], who failed to detect orexin-A immunostaining in PCa specimens and OX1 receptor in LNCaP cells, may be ascribed either to the different methodological procedures (i.e., different primers and protocols for PCR) or to the high turnover of internalization and/or cellular production of endocrine substances such as orexins and their receptors.

3.2. Expression of prepro-orexin and OX1 receptor mRNAs and proteins in PCa tissues

In order to confirm at molecular level the local presence of orexin-A and its receptor in the tissues, biochemical analyses were carried out on frozen PCa specimens. The expression of mRNAs coding for orexin-A precursor molecule prepro-orexin and OX1 receptor in PCa tissues was analyzed by RT-PCR. This analysis resulted in the amplification of specific DNA fragments of 393 bp for prepro-orexin and 356 bp for OX1 receptor in all samples (Fig. 3A, lns. 3). A 469 bp transcript was obtained from the amplification of β-actin cDNA (Fig. 3A, bottom, lns. 2, 3). No amplification products were obtained when distilled water was used in place of cDNA (negative control) (Fig. 3A, lns. 4), whereas DNA fragments for prepro-orexin and OX1 receptor were detected in a whole rat brain homogenate which was used as positive control (Fig. 3A, lns. 2).

The presence of the two proteins prepro-orexin and OX1 receptor in the PCa tissues was confirmed by Western blotting, using a rabbit polyclonal antibody raised against a 17 amino acid peptide mapping near the C-terminus of human prepro-orexin or a rabbit polyclonal antibody raised against a peptide mapping near the C-terminus of human OX1 receptor, respectively. The detected proteins showed a molecular mass of 16 kDa as expected for prepro-orexin (Fig. 3B, upper blot, lns. 2), and 50 kDa as expected for OX1 receptor (Fig. 3B, middle blot, lns. 2). The specificity of the response was confirmed by pre-incubation of the prepro-orexin or

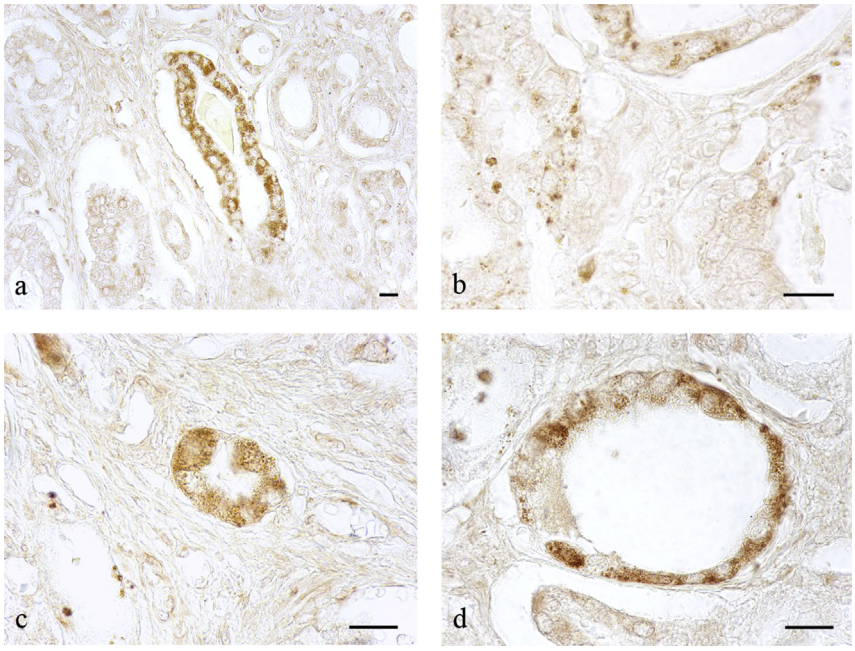

Fig. 1. Orexin-A-immunoreactivity in high grade PCa tissues. a–d: positive cells are scattered in the neoplastic tissue (b) or aligned along the circular profile of glandular follicles (a,c,d). c–d: the granular shape of OxA immunoreactive material in the cytoplasm is clearly visible at higher microscopic magnification of follicular cells. Avidin-biotin immunohistochemical method. Bars: 20 μm.

OX1 receptor antibody with their respective blocking peptides. There was no expression of prepro-orexin and OX1 receptor in these preparations (Fig. 3B, lns 3), whereas the presence of the proteins was detected in the whole rat brain homogenate used as positive control (Fig. 3B, lns 1). The stripping of the upper blots and their re-probing with monoclonal anti-γ-tubulin antibody demonstrated equal loading of proteins in all lanes (Fig. 3B, bottom).

These results definitely demonstrate the local presence of orexin-A in the cancerous prostate epithelium. Indeed, we not only detected orexin-A immunostaining in all analyzed PCa tissues, but we also found the expression of the orexin-A precursor molecule prepro-orexin both at mRNA and protein levels, thus suggesting that OX1 receptor expressed by PCa cells is probably activated by endogenous orexin-A *in vivo*.

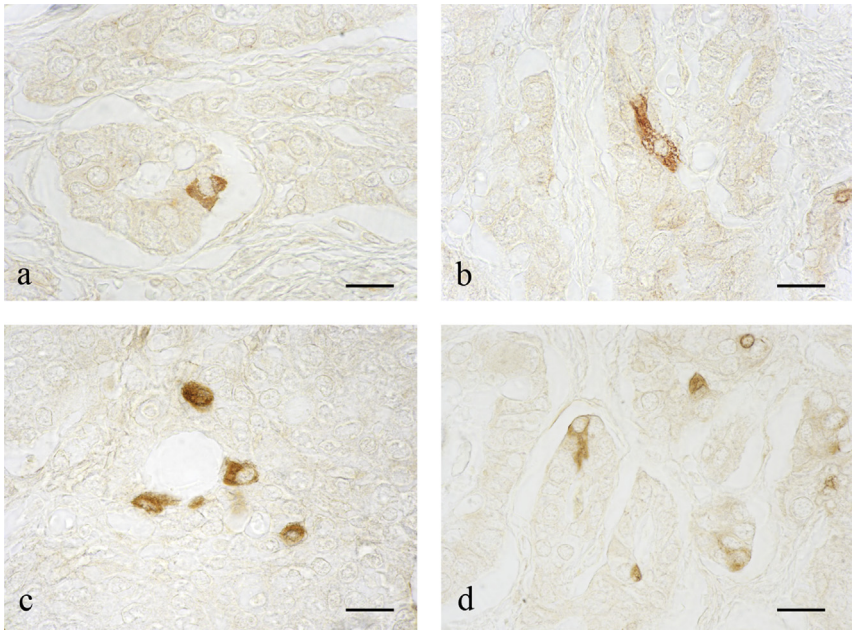

Fig. 2. OX1 receptor-immunoreactivity in high grade PCa tissues. Differently shaped positive cells are isolated in the neoplastic tissue (a,b) or organized in small clusters of elements (c,d) whose cytoplasm shows various staining intensity. The epithelial shape of all positive cells is clear although the original organization of the tissue is lost. Avidin-biotin immunohistochemical method. Bars: 20 μm.

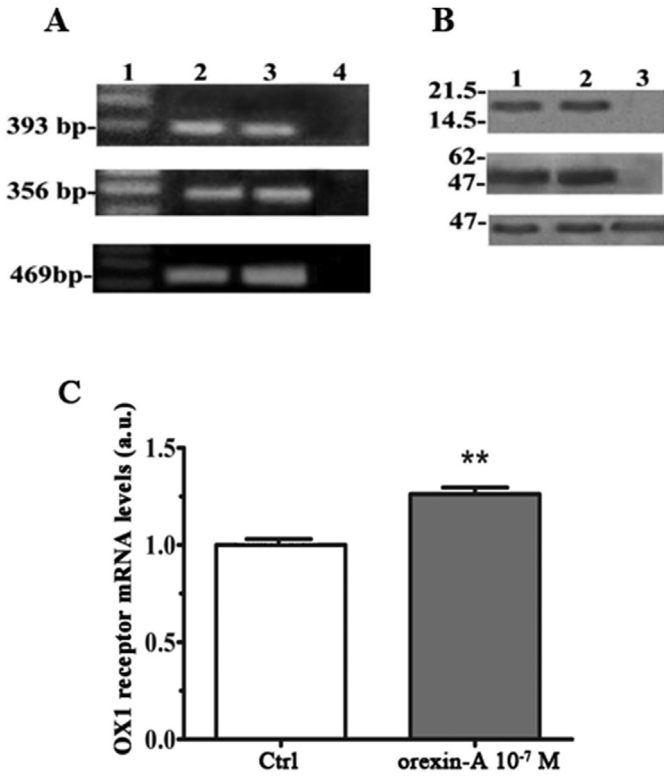

Fig. 3. Expression of prepro-orexin and OX1 receptor in PCa tissues and OX1 receptor up-regulation by orexin-A in LNCaP cells. A. RT-PCR. Lane 1, DNA ladder; lane 2, prepro-orexin (upper) or OX1 receptor mRNA transcripts in whole rat brain sample (positive control); lane 3, prepro-orexin or OX1 receptor mRNA transcripts in PCa tissue samples; lane 4, negative control (no cDNA input). The bottom reports the β-actin mRNA transcripts in all samples (internal control). B. Western blotting. Lane 1, homogenate from whole rat brain (positive control); lane 2, homogenate from PCa tissues; lane 3, prostate homogenate treated with the antisera directed against prepro-orexin (upper blot) or OX1 receptor pre-absorbed with their respective control peptides (negative control). The blots were stripped and re-probed with an anti-γ-tubulin monoclonal antibody to ensure equal loading of proteins in all lanes (lower blot). Molecular mass markers are indicated on the left. C. Expression of OX1 receptor mRNA in control (Ctrl) or orexin-A (10^{-7} M) treated LNCaP cells as measured by real time PCR. OX1 receptor mRNA levels are expressed in arbitrary units (a.u.). **P < 0.01. Similar results of those reported in A, B, and C were obtained from five separate experiments of identical design.

3.3. Orexin-A treatment of LNCaP cells up-regulates OX1 receptor gene expression, and inhibits cell survival

In order to get more insights into the role of orexin-A and its receptor in the physiopathology of the prostate, we performed *in vitro* experiments using the LNCaP cell line. We first measured by real-time PCR the expression levels of mRNA coding for OX1 receptor in both untreated LNCaP cells and cells exposed to orexin-A (10^{-7} M) for 3 h. This quantitative analysis revealed that OX1 receptor gene is expressed in LNCaP, even though at low extent (mean C_t value 34,23917), and that orexin-A cell treatment up-regulates the receptor gene expression (Fig. 3C). Particularly, cell treatment with a physiological concentration of orexin-A increased by 25% OX1 receptor gene expression.

The finding that the expression of OX1 receptor is up-regulated by LNCaP cell exposure to orexin-A is consistent with previous data showing that orexin-A treatment stimulates the expression of OX1 receptor gene in luteal and follicular cells of the rat ovary [23], cultured rat calvarial osteoblast-like (ROB) cells [24], rat insulin-secreting beta-cell line (INS-1 cells) [25] and in rat hepatocytes [26]. In these latter cell types, orexin-A mediated OX1 receptor up-regulation results in cell proliferation increase and protection of cells from apoptosis [25,26], whereas it exerts a notable inhibitory effect on the proliferative activity of ROB cells [24]. Thus, we evaluated by MTT assay whether orexin-A induced up-regulation of OX1 receptor in LNCaP cells would affect cell survival. The results showed that 10^{-7} M orexin-A causes a statistically significant decrease of LNCaP cell survival with respect to control cells (Fig. 4A). The addition to the test system of OX1 receptor antagonist SB-408124 prevented the orexin-A inhibitory effect on cell survival. Further investigations are in progress to establish whether the orexin-A/OX1 receptor interaction mediates apoptosis in LNCaP cells.

3.4. Orexin-A counteracts testosterone-induced nuclear translocation of androgen receptor in LNCaP cells

The prostate is an androgen-dependent organ which synthesises the androgen receptor, a hormone activated transcription

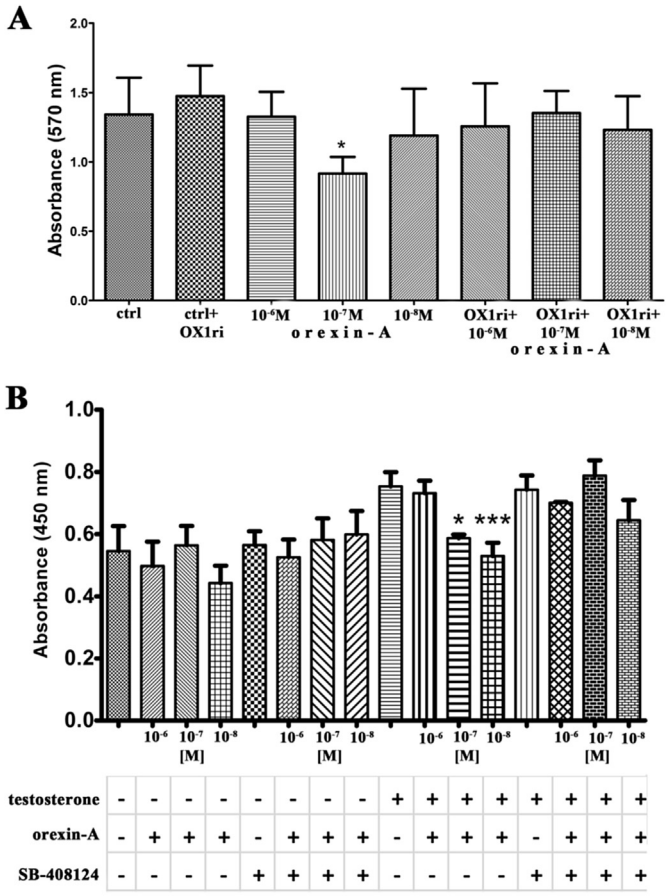

Fig. 4. A. Effect of orexin-A on the LNCaP cell survival. Orexin-A was added to LNCaP cells and after 48 h MTT assay was performed. Orexin-A at the doses of 10^{-7} M caused a decrease of cell survival (P < 0.05) as compared to control (ctrl) cells. The administration of OX1 receptor antagonist SB-408124 (OX1ri) reversed the effect of orexin-A. B. Effect of orexin-A on the nuclear translocation of androgen receptor in LNCaP cells in absence and presence of testosterone. Different concentrations of orexin-A did not induce nuclear translocation of the androgen receptor in native LNCaP cells. Conversely, orexin-A at the doses of 10^{-7} and 10^{-8} M induced a significant reduction of the nuclear translocation of the androgen receptor after testosterone (0.4 nM) cell pre-treatment. Cell pre-treatment with OX1 receptor antagonist SB-408124 resulted in the inhibition of the testosterone-dependent effect of orexin-A on androgen receptor nuclear translocation. The administration of SB-408124 alone to the cells had no effect. The bar diagrams represents the means ± SE. *P < 0.05, ***P < 0.001 (versus the testosterone sample). The data reported in A and B are the means of three independent experiments performed with each sample in replicate of three.

factor. The metabolic functions of androgen receptor in normal prostate are circumvented in PCa to drive tumor growth, and the androgen receptor acquires growth-promoting functions during PCa development and progression through both genetic and epigenetic mechanisms [27]. Thus, the biological pathways promoting proliferation, differentiation and survival of malignant prostate tissues are strictly regulated by androgens and androgen receptor activity. Beside surgery, androgen deprivation therapy represents the primary treatment for PCa at the moment. However, PCas may become resistant to the therapy within two years, and relapse invariably occurs in castration-resistant PCa (CRPC).

Several mechanisms for castration resistance have been proposed, including the reactivation of androgen receptor signaling in patients who have previously undergone castration therapy [20,27]. Indeed, the androgen receptor is highly expressed and functionally active in CRPC due to androgen synthesis from adrenal steroids. Despite very low circulating levels of ligand, androgen receptor signaling is maintained by multiple mechanisms, and a wide array of factors has been implicated in androgen receptor stimulation in the development of CRPC. These factors include an increased expression of the androgen receptor, mutations that confer broader ligand specificity to the receptor, changes in the ratios between the androgen receptor and its co-regulators, and up-regulation of cross-talk signal transduction pathways that can activate the androgen receptor in a ligand-independent manner [27]. Thus, the androgen receptor signaling has been pinpointed as a target for the development of novel therapies for CRPC treatment [20].

In this study, we evaluated whether orexin-A shows the capability to affect androgen receptor activity in LNCaP cells. In particular, the effect of orexin-A on androgen receptor nuclear translocation in LNCaP cells was evaluated both in the absence or in the presence of exogenous testosterone. The results obtained showed that cell exposure to orexin-A alone, in a concentration range from 10^{-8} M to 10^{-6} M, does not affect androgen receptor translocation in LNCaP cells (Fig. 4B). Conversely, in the presence of nanomolar concentrations of testosterone, low doses of orexin-A (10^{-7} and 10^{-8} M) caused a significant reduction of androgen receptor nuclear translocation in LNCaP. The effect of orexin-A was fully inhibited by adding to the test system the OX1 receptor antagonist SB-408124. These data demonstrate that orexin-A/OX1 receptor interaction is able to antagonize testosterone-induced androgen receptor nuclear translocation, thus suggesting that orexin-A might play a role in the regulation of androgen/androgen receptor signaling in PCa. The potential role of orexin-A as a co-regulator of the androgen receptor activity in PCa is an intriguing topic that deserves further in depth investigations for the development of novel therapies for the cure of this cancer.

Conflicts of interest

None.

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Transparency document

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