NEOPLASIA
www.neoplasia.com

Volume 13 Number 6 June 2011 pp. 526–536 526

# Androgen Depletion Induces Senescence in Prostate Cancer Cells through Down-regulation of Skp2[1,2]

**Zuzana Pernicová*, Eva Slabáková*, Gvantsa Kharaishvili†, Jan Bouchal†, Milan Král‡, Zuzana Kunická§, Miroslav Machala¶, Alois Kozubík*,§ and Karel Souček***

*Department of Cytokinetics, Institute of Biophysics, AS CR, Brno, Czech Republic; †Laboratory of Molecular Pathology and Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University, Olomouc, Czech Republic; ‡Department of Urology, University Hospital, Olomouc, Czech Republic; §Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic; ¶Department of Chemistry and Toxicology, Veterinary Research Institute, Brno, Czech Republic

## Abstract

Although the induction of senescence in cancer cells is a potent mechanism of tumor suppression, senescent cells remain metabolically active and may secrete a broad spectrum of factors that promote tumorigenicity in neighboring malignant cells. Here we show that androgen deprivation therapy (ADT), a widely used treatment for advanced prostate cancer, induces a senescence-associated secretory phenotype in prostate cancer epithelial cells, indicated by increases in senescence-associated β-galactosidase activity, heterochromatin protein 1β foci, and expression of cathepsin B and insulin-like growth factor binding protein 3. Interestingly, ADT also induced high levels of vimentin expression in prostate cancer cell lines *in vitro* and in human prostate tumors *in vivo*. The induction of the senescence-associated secretory phenotype by androgen depletion was mediated, at least in part, by down-regulation of S-phase kinase-associated protein 2, whereas the neuroendocrine differentiation of prostate cancer cells was under separate control. These data demonstrate a previously unrecognized link between inhibition of androgen receptor signaling, down-regulation of S-phase kinase-associated protein 2, and the appearance of secretory, tumor-promoting senescent cells in prostate tumors. We propose that ADT may contribute to the development of androgen-independent prostate cancer through modulation of the tissue microenvironment by senescent cells.

*Neoplasia (2011) 13, 526–536*

Abbreviations: ADT, androgen deprivation therapy; AIPC, androgen-independent prostate cancer; AR, androgen receptor; CS, charcoal-stripped fetal bovine serum; EMT, epithelial-to-mesenchymal transition; HP1β, heterochromatin protein 1β; IL-8, interleukin-8; IGFBP3, insulin-like growth factor binding protein 3; NED, neuroendocrine differentiation; SA-β-gal, senescence-associated β-galactosidase; SASP, senescence-associated secretory phenotype; Skp2, S-phase kinase-associated protein 2

Address all correspondence to: Karel Souček, PhD, Institute of Biophysics, AS CR, Královopolská 135, CZ-612 65 Brno, Czech Republic. E-mail: ksoucek@ibp.cz

[1]This work was supported by grants IGA MZD 9600-4/2008 and 9956-3/2008, and by a grant from the Czech Science Foundation 310/07/0961. Institutional support comes from grant nos. AV0Z50040507 and AV0Z50040702 from the Academy of Sciences of the Czech Republic, grant nos. MSM0021622430 and MSM6198959216 from the Ministry of Education, Youth and Sports of the Czech Republic, and EU infrastructure support CZ.1.05/2.1.00/01.0030. The authors declare no conflict of interest.
[2]This article refers to supplementary materials, which are designated by Table W1 and Figures W1 to W3 and are available online at www.neoplasia.com.
Received 19 January 2011; Revised 9 March 2011; Accepted 10 March 2011


DOI 10.1593/neo.11182

## Introduction

Androgen deprivation therapy (ADT) is an important treatment for advanced stage prostate cancer and is achieved by androgen receptor (AR) blockade and/or medical or surgical castration [1,2]. Although ADT is initially very effective, treated tumors inevitably progress to androgen-independent prostate cancer (AIPC), which is currently incurable and fatal. The mechanism through which ADT causes androgen independence is therefore of considerable clinical importance.

One possible mechanism for the development of AIPC is modulation of the tissue microenvironment by neuroendocrine (NE)-like cancer cells, which emerge after ADT [3,4]. NE-like cancer cells are capable of regulating the proliferation, invasion, and secretory activity of surrounding cells through a paracrine mechanism involving a range of secreted neuropeptides and cytokines (e.g., gastrin-releasing peptide, serotonin, interleukin-8 (IL-8) [5]). The impact of the microenvironment on prostate cancer progression may be further enhanced by tumor heterogeneity characterized by the presence of multiple foci of proliferative inflammatory atrophy, high-grade prostatic intraepithelial neoplasia, and carcinoma in the peripheral zone of prostate [6].

Activation of AR signaling by a variety of growth factors and cytokines, such as insulin-like growth factor 1, keratinocyte growth factor, epidermal growth factor [7], IL-6 [8], or IL-8 [9], may also contribute to development of AIPC [10]. Significantly, these factors may also be secreted by senescent epithelial cells [11]. Senescence is a general cell biologic phenomenon that limits the life span of cells and prevents unlimited cell proliferation. Although senescent cells do not proliferate, they are resistant to apoptosis and remain metabolically active [12]. Irreversible cell cycle arrest, the hallmark of senescence, can be triggered by a variety of stimuli including deregulated expression of some oncogenes [13,14] or tumor suppressors [15,16], telomere shortening [17], oxidative stress [18], and chemotherapeutic drugs [19,20]. For these reasons, induction of senescence is a potent defense against tumorigenesis. There is a dark side to this defense mechanism, however: metabolically active senescent cells may promote tumorigenicity of neighboring malignant cells through the secretion of a range of growth factors and cytokines [11,21,22]. Aged fibroblasts with this senescence-associated secretory phenotype (SASP) are not the only cells capable of modulating the prostate microenvironment and promoting carcinogenesis [23]; senescent prostate epithelial cells can also behave in this manner [24].

Senescence can be induced by a variety of signaling pathways, such as p53-p21$^{Cip1/Waf}$, p19$^{Arf}$-p53, and p16$^{INK4a}$-RB [25], which may interact with one another or act independently to arrest cell proliferation [12]. Recently, S-phase kinase-associated protein 2 (Skp2) was shown to play an important role in the promotion of senescence by oncogenic Ras or by inactivation of PTEN [26]. Skp2, a crucial component of the Skp, Cullin, F-box–containing complex, is an E3 ligase involved in cell cycle progression through degradation of p27$^{Kip1}$ and other targets [27]. Elevation of Skp2 occurs in a variety of cancers, including prostate [28,29]. Interestingly, Skp2 is regulated by AR signaling, and inhibition of AR leads to down-regulation of Skp2 and decreased cell proliferation [30,31].

Here we show that ADT induces senescence and neuroendocrine differentiation (NED) of prostate cancer cells. Our results reveal a previously unsuspected relationship between the inhibition of AR signaling, down-regulation of Skp2, and the appearance of highly metabolically active tumor-promoting senescent cells in prostate cancer tissue. We propose that this mechanism plays a significant contribution to prostate tumor progression.

## Materials and Methods

### *Cell Cultures and Androgen Depletion*

LNCaP cells (human prostate carcinoma cells; DSMZ, Braunschweig, Germany) were cultivated in phenol red–free RPMI 1640 media (Invitrogen, Carlsbad, CA) supplemented with $NaHCO_3$ (Sigma Aldrich, St Louis, MO), penicillin/streptomycin, and 5% fetal bovine serum (FBS; both PAA, Pasching, Austria). For androgen depletion studies, LNCaP cells were cultivated in 5% dextran/charcoal-stripped FBS (CS). LAPC-4 cells (xenograft-derived human prostate carcinoma cells [32]) were cultivated in Iscove modified Dulbecco medium (Invitrogen) supplemented with $NaHCO_3$, penicillin/streptomycin, 10% FBS, and 1 nM R1881 (PerkinElmer, Waltham, MA). For androgen depletion studies, cells were cultivated in Iscove modified Dulbecco medium with 10% CS. All cells were grown in a humidified incubator (37°C, 5% $CO_2$).

### *Androgen-Depleted Cell Growth Conditions*

For the induction of senescence and NED, the cells were seeded in appropriate growth medium with FBS, grown for 24 hours, and at day 0, the growth medium was replaced with growth medium supplemented with either complete FBS or CS. Cells were cultivated without reseeding for 2 to 16 days, medium was changed twice a week, and cells were harvested on days 2, 4, 8, and 16.

### *Western Blot Analysis of Cell Extracts*

Protein extract preparation, gel electrophoresis, and Western blot analysis were performed as previously described [33] using antibodies described in Supplementary Materials and Methods. α-Tubulin or β-actin was used as loading controls. Signal densities were analyzed using ImageJ software (National Institutes of Health, Bethesda, MD) and normalized to the appropriate loading control.

### *Detection of Telomerase Activity*

Telomerase activity was detected using TRAPeze XL Telomerase Detection Kit (Millipore, Billerica, MA) according to the manufacturer's recommendation.

### *Immunofluorescence and Confocal Microscopy*

Cells were fixed and stained with appropriate antibodies as described in Supplementary Materials and Methods, and nuclei were visualized by counterstaining with TOPRO-3 (Invitrogen) or DAPI (4′, 6-diamidino-2-phenylindole; AppliChem, Darmstadt, Germany). Fluorescent-stained samples were mounted in Mowiol 4-88/DABCO (Calbiochem, Merck, Darmstadt, Germany) and viewed on a LSM Leica SP5 (Leica Microsystems, Wetzlar, Germany) confocal microscope.

### *Flow Cytometry and Detection of Intracellular Antigens*

Trypsinized cells were fixed in 2% paraformaldehyde at 4°C, permeabilized, and incubated with appropriate antibodies (described in Supplementary Materials and Methods) diluted in PBS with 300 μg/ml digitonin. Stained cells were analyzed on a FACSCalibur or FACS Aria II Sorp (Becton Dickinson, San Jose, CA) cell sorter, and flow cytometry data were analyzed using FlowJo software (TreeStar, Ashland, OR). The median fluorescence index (MFI) was calculated as the ratio of