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CLINICAL INVESTIGATION **Genitourinary Cancer**

CASTRATION THERAPY OF PROSTATE CANCER RESULTS IN DOWNREGULATION OF HIF-1α LEVELS

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Background and Purpose: Neoadjuvant androgen deprivation in combination with radiotherapy of prostate cancer is used to improve radioresponsiveness and local tumor control. Currently, the underlying mechanism is not well understood. Because hypoxia causes resistance to radiotherapy, we wanted to test whether castration affects the degree of hypoxia in prostate cancer.

Methods and Materials: In 14 patients with locally advanced prostate cancer, six to 12 prostatic needle core biopsy specimens were taken prior to castration therapy. Bilateral orchidectomy was performed in 7 patients, and 7 were treated with a GnRH-agonist (leuprorelin). After castrationm two to four prostatic core biopsy specimens were taken, and the level of hypoxia-inducible factor-1α (HIF-1α) in cancer was determined by immunofluorescence.

Results: Among biopsy specimens taken before castration, strong HIF-1α expression (mean intensity above 30) was shown in 5 patients, weak expression (mean intensity 10–30) in 3 patients, and background levels of HIF-1α (mean intensity 0–10) in 6 patients. Downregulation of HIF-1α expression after castration was observed in all 5 patients with strong HIF-1α precastration expression. HIF-1α expression was also reduced in 2 of 3 patients with weak HIF-1α precastration expression.

Conclusions: Our data suggest that neoadjuvant castration decreases tumor cell hypoxia in prostate cancer, which may explain increased radiosensitivity after castration. © **2012 Elsevier Inc.**

Hypoxia-inducible factor, HIF-1α, Prostate cancer.

INTRODUCTION

Prostate cancer is the most common cancer in the western countries (1). Most patients with localized prostate cancer (*ie*, the tumor is confined to the prostate gland), who have relatively long life expectancy and low comorbidity, may be offered curative treatment with either radical prostatectomy or radiotherapy. In the case of poorly differentiated high-risk tumors, the latter treatment is currently combined with neoadjuvant hormone therapy with the use of a gonadotropin-releasing hormone (GnRH) agonist. Clinical studies have demonstrated synergism between androgen ablation and radiotherapy (2), which improves tumor control and patient survival, although the biologic explanation of the mechanism is not yet fully defined.

Growth of solid tumors such as prostate cancer is characterized by neovascularization and increased glycolysis as a result of the hypoxic microenvironment of the tumor. The hypoxia-inducible factor 1 (HIF-1) complex is an important transcription factor that regulates cellular adaptation to hypoxia and transcription of genes involved in angiogenesis, cell survival, glucose metabolism, and tumor invasion (3).

HIF-1 is a member of the basic helix-loop-helix (bHLH)-PER-ARNT-Sim (PAS) family of transcription factors. HIF-1 is a heterodimer composed of the HIF-1α and HIF-1β subunits (4). The β subunit is ubiquitously expressed in most cells, and the α subunit, which is the critical determinant of HIF-1 activity, is regulated posttranslationally in response to hypoxia. In normoxic conditions, HIF-1α is ubiquitinated by the von Hippel-Lindau tumor suppressor protein (pVHL) and degraded by the proteasome. However, in response to hypoxia, HIF-1α is stabilized and can thus bind to the β subunit. Upon dimerization of the two subunits. the HIF-1 complex translocates to the nucleus, where it

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binds to DNA at hypoxia-responsive elements (HREs), which are common to HIF-1–responsive genes, thus modifying transcription. Resulting gene expression increases metabolic resistance of the cell to hypoxia and apoptosis and stimulates angiogenesis by subsequent production of vascular endothelial growth factor (VEGF) (5). HIF-1α is upregulated in most cancers, including prostate cancer (6).

Immunohistochemical studies show upregulation of HIF-1α in prostate cancer compared with normal prostate and benign prostatic hyperplasia (7). Moreover, upregulation of HIF-1α is likely to be an early event in the development of prostate cancer, given that increased levels were observed in high-grade intraepithelial neoplasia, which is considered the precursor of prostatic adenocarcinoma. Furthermore, prostate tumor tissue adjacent to the intraepithelial neoplasia showed an even more pronounced upregulation of HIF-1α expression (8).

Tumor hypoxia is associated with poor prognosis and increased resistance to radiotherapy. HIF-1α has an important role in regulating tumor radiosensitivity through its impact on apoptosis, metabolism, proliferation, and angiogenesis (9). Tumor cells with upregulated HIF-1α levels are more radioresistant than HIF-1α–deficient counterparts (10). For localized prostate cancer, it has been shown that increased expression of HIF-1α identifies patients at high risk for biochemical failure (11). Studies in prostate cancer cell lines have found that acute hypoxia promotes a more aggressive metastatic phenotype (12). Here, we hypothesize that androgen ablation downregulates hypoxia, as measured by the expression of HIF-1α in human prostate cancer.

METHODS AND MATERIALS

Patients and biopsy specimen collection

After approval from the regional ethics committee at Uppsala University (Dnr 2007/170), 20 patients with newly diagnosed prostate cancer were enrolled. At diagnosis, six to 12 prostatic needle core biopsy specimens were taken from each patient. All patients were treated with castration. After castration (*ie*, approximately 1 month after surgery or 2 months after initiation of pharmacologic castration, respectively), another two to four core biopsy specimens were taken. At diagnosis, the biopsy specimens were taken randomly. After castration, we took fewer samples and focused on palpable nodules to increase the likelihood of finding cancer cells.

Both before and after castration, we chose biopsy specimens rich in cancer cells and used them for HIF-1 staining. Thus, there was a high probability that the same tumor areas were represented at both biopsy occasions. In 6 patients the postcastration biopsy specimens did not include representative cancer areas and hence were excluded from this study. The median age of the 14 patients included in this study was 78 years (range, 59–87 years). Thirteen patients had locally advanced cT3-4 tumors, and only 1 patient had an organ-confined cT2 tumor. The median serum prostate-specific antigen was 98 ng/mL (range, 3–1021 ng/mL). The median serum testosterone level was 11.0 nmol/L (range, 6.6–23.0 nmol/L). The median prostate volume measured with transrectal ultrasound was 52 mL (range, 20–100 mL).

Bilateral orchidectomy was performed in half of the patients, and the other half was treated with a GnRH agonist (leuprorelin) (Table 1). The mean time and standard deviation from orchidectomy to repeated biopsy was 26 $\pm$ 19 days and from administration of GnRH-agonist to repeated biopsy 54 $\pm$ 14 days. At the time of postcastration biopsy, the serum testosterone levels varied from 0.3 to 0.9 nmol/L in surgically castrated patients and 0.3 to 1.7 nmol/L in patients receiving GnRH agonist therapy.

Histologic and immunofluorescence evaluation

Two biopsy specimens before and two after castration were analyzed in each patient. The biopsy speciens were embedded in paraffin and sectioned. One section from each biopsy specimen was stained with hematoxylin and eosin (HE) and graded according to the Gleason system (13). Sections adjacent to the HE-stained sections were used for immunofluorescence studies. These sections were deparaffinized and rehydrated before antigen retrieval in Tris/EDTA pH 9.0 in a pressure cooker. After blocking in 3% BSA, the sections were incubated with the primary antibody HIF-1α (1:500, H-206 St. Cruz) at 4°C overnight. After extensive rinsing, the sections were incubated with the secondary antibody (donkey

Table 1. Characteristics of patients and tumors in the study

Patient number	Patient age (y)	Prostate volume, mL	cT	GS	PSA, ng/mL		Testosterone, nmol/L		Days between biopsies	Castration method
					Before castration	After castration	Before castration	After castration		
6	70	71	4	8	1021	21	12	0.9	53	GnRH agonist
9	83	20	3	7	8	0.3	14	0.8	70	GnRH agonist
10	87	59	3	7	34	1.9	15	0.7	60	GnRH agonist
12	61	26	2	7	17	6.4	ND	1.7	42	GnRH agonist
14	78	90	3	7	326	55	7	0.3	88	GnRH agonist
18	69	50	3	10	380	5.4	11	0.3	105	GnRH agonist
19	77	34	3	7	107	2.6	11	1.0	55	GnRH agonist
2	67	65	3	10	2.9	3.7	23	0.3	16	Orchidectomy
5	86	52	3	9	70	53	7	1.0	8	Orchidectomy
8	63	44	3	8	650	134	13	0.9	83	Orchidectomy
15	59	90	4	9	736	29	8	0.9	46	Orchidectomy
16	82	ND	3	7	16	1.1	16	0.6	49	Orchidectomy
17	84	46	4	8	438	138	9	0.6	29	Orchidectomy
20	83	100	3	9	89	23	10	0.3	40	Orchidectomy

Abbreviations: ND = not done; cT = clinical tumor stage; GG = Gleason grade; GS = Gleason score.