Polyamines in Regulation of Prostatic Cell Growth

Raymond G. Schipper, Vincent Cuijpers, Johannes C. Romijn, and Albert A. J. Verhofstad

1. Introduction

The prostate and prostate secretions have played an important role in the initial identification of polyamines. One of the polyamines, spermine, was reported in human semen as early as the 17th century by Antoni Van Leeuwenhoek. After the elucidation of the chemical structure of polyamines, about 200 yr later, the biochemical mechanism of action of polyamines in the prostate gland and semen has been studied extensively. Apart from this historical perspective, the prostate has one of the highest polyamine concentrations of any tissue. In rats, the amount of polyamines, especially spermidine, is highest in the ventral prostate followed by the dorsal and lateral prostate, whereas the coagulating glands and seminal vesicles show low values (1). The human prostate gland synthesizes exceptionally high levels of spermine on an average of 130 mg/100 g per day (2).

Growth and function of the prostate gland is controlled by androgens that are responsible for the development of male characteristics, such as hair and beard growth. The most important androgen in men is testosterone. Testosterone, produced primarily in the testis, can diffuse freely into prostate cells, where it is rapidly and irreversibly converted to its metabolically more active form, dihydrotestosterone (DHT), through the activity of the 5-α reductase. Increased DHT levels are suspected to promote DNA synthesis and replication of prostate cells.

One of the effects of testosterone stimulation on prostatic cells is the production of polyamines (3–5). The activities of the polyamine biosynthetic enzymes ornithine decarboxylase (ODC), S-adenosylmethionine decarboxylase (AdoMetDC), and spermidine synthase (SpdS) are induced by androgens in a coordinated way, and expression of these enzymes is primarily localized to the glandular epithelial cells of the prostate (5–7).

Studies on the murine ODC gene revealed that the ODC promoter contains an androgen-responsive element-like sequence that can bind to the androgen receptor in vitro (6). Differential display studies in LNCaP cells showed that androgen regulation
of ODC is directly mediated through the androgen receptor with kinetics of induction similar to that for prostate-specific antigen (PSA) (7). Inhibition of ODC activity with difluoromethylornithine reduces the size of prostatic acini in the developing prostate, retards testosterone-induced regrowth of the prostate in castrated rats, and inhibits insulin-like growth factor 1-induced growth of the prostate (8).

It is apparent that functions of ODC and polyamines in the prostate are related to cellular proliferation and secretory activities. The exact role of polyamines in prostate biology is not yet known, however, and needs further investigation. It has been postulated that spermine is involved in the maintenance of the functional secretory state of prostatic epithelium (9, and references herein). Interestingly, the (rat) prostate contains an androgen-regulated protein that has high affinity toward spermine (i.e., spermine-binding protein) (10). Spermine-binding protein is phosphorylated by cyclic-adenosine monophosphate-independent protein kinases (11).

The functional significance of seminal polyamines is a matter of debate. Studies of human prostatic fluids indicate that spermine is linked to citrate, also present in remarkably high concentrations in the prostate (12). Complex formation with the negatively charged acid citrate would enable polyamines to penetrate in spermatozoa. Spermine has been localized in the middle and top parts of the acrosome, and possibly modulates sperm fertilization competence and the acrosomal reaction (13). However, spermine present in sperm cells may also originate from endogenous polyamine biosynthesis because ODC has been shown to be involved in spermatogenesis (14). Seminal polyamines may have other functions in regulation of seminal clotting or prevention of bacterial growth in the urinary tract (15).

One might expect that prostatic cells compared with other cell types exhibit different mechanisms for regulation of their polyamines because they produce high levels of polyamines. Indeed, polyamine uptake and accumulation is significantly higher in prostate cancer cells compared with nonprostate cancer cell lines (16) because of a unique insensitivity of polyamine transport to regulation by polyamines or polyamine analogs in prostatic (cancer) cells (17). A possible intriguing therapeutic implication of these findings is that exposure to polyamine antimetabolites (e.g., polyamine analogs) would induce downregulation of drug uptake in normal tissues, whereas at the same time, drug uptake is ensured to growth inhibitory levels in the prostate tumor or tissue.

The importance of polyamines in prostatic growth and differentiation has prompted studies to evaluate the clinical relevance of the ODC/polyamine system in prostate cancer, as will be discussed later in this chapter.

2. Polyamines and Cell Kinetic Behavior (Apoptosis, Proliferation) of Prostatic Cells

Prostate growth depends on the balance between death (apoptosis) and proliferation of prostatic cells. To investigate the role of polyamine metabolism in prostatic growth, we have monitored the levels of polyamines, as well as the activities of the polyamine biosynthetic enzyme, ODC, and the polyamine-catabolizing enzyme, Spd/Spm acetyltransferase (SSAT), in castration-induced regression and testosterone-stimulated regrowth of the rat prostate.
Before castration, both proliferation rate and apoptotic rate in the glandular prostatic tissue were less than 2%. In the first hours after castration, the apoptotic rate increased, reaching a maximum level of 15 to 20% after 2 d (Fig. 1A). Concomitantly, ODC activity (Fig. 1B) and polyamine levels (Fig. 1C) dramatically decreased, whereas SSAT activity progressively increased in the prostatic tissue (Fig. 1B). Regeneration of the prostate after suppletion of androgens coincided with a marked increase in ODC activity and recovery of polyamine concentrations to normal limits (Fig. 1A,C, right side). The effect of androgen/suppletion on prostate cell kinetics and polyamine parameters was notably less dramatic in the dorsolateral lobe of the prostate compared with the ventral lobe (results not shown).

Much ambiguity exists on the role of ODC in apoptosis because ODC activity is reported to be increased, decreased, or unchanged depending on the apoptotic stimulus or cell system used (see refs. 18 and 19, and references herein). Remarkably, in most of these studies, ODC increases early during apoptosis but eventually polyamine levels mostly decreased. Several studies imply, however, that the increase in ODC activity is merely a trophic response to stress or is even just an epiphenomenon (see ref. 18 and references herein). The inability of ODC to maintain elevated polyamine levels may be owing to a precocious degradation of ODC during the cell death process. Proteolytic degradation of ODC is regulated by a unique protein, the so-called antizyme (AZ), which binds to ODC and facilitates its breakdown by the cytosolic 26S proteosome. In our recent study on the localization of ODC and AZ, we found that treatment of prostatic cancer cells with 1 mM putrescine resulted in nuclear induction of AZ that colocalized with a fusion protein of ODC conjugated with green fluorescent protein (GFP) (20). Consistent with this is that the proteolytic breakdown observed during apoptosis mostly affects proteins needed for cell cycle and survival. Because polyamines are critically involved in cell-cycle control, ODC may be one of the proteins that are degraded by apoptosis-related proteases. No evidence has yet been found for the proteolysis of ODC by caspases. However, a potential cleavage site (YVAD) for caspases is present in ODC in most animals. Interestingly, the amino acid sequence of the very stable ODC of Trypanosoma brucei contains HVAD, a site less vulnerable to caspase-mediated proteolysis. It is therefore tempting to speculate that ODC is a potential caspase target.

Concomitant to castration-induced apoptosis, SSAT activity progressively increased in the prostatic tissue (Fig. 1B). We observed a similar, relatively late induction of SSAT in calcium-ionophore-induced apoptosis of prostate cancer cells (21). These results suggest that polyamine catabolism is associated with the later stages of apoptosis.

Increased expression of SSAT during apoptosis has also been reported in other studies (see ref. 19 and references herein) suggesting that stimulation of polyamine catabolism is a general cellular response in cell death. Apoptosis induced by polyamine analogs has been associated with highly increased SSAT levels (see Chapters 12 and 15).

The link between SSAT induction and apoptosis may actually be associated with the action of another polyamine catabolic enzyme, polyamine oxidase, which oxidizes acetylated polyamines generated by SSAT and releases toxic byproducts, such as hydrogen peroxide and aldehydes (see Chapter 12), which in turn, could trigger the apoptotic process.
Fig. 1. Apoptosis and proliferation index, and parameters of polyamine homeostasis in castration-induced involution and testosterone-induced regrowth of the rat ventral prostate. (A) The apoptosis index was expressed as the fraction of in situ nick translation (ISNT)-positive glandular epithelial cells, as well as the proliferation index as the fraction of BrdU positive cells. (B) Activities of the polyamine biosynthetic enzyme ornithine decarboxylase (ODC) and the polyamine catabolic enzyme Spd/Spm N₁-acetyltransferases (SSAT). (C) Concentrations of putrescine (Put), spermidine (Spd), and spermine (Spm).
3. Polyamine Metabolism is Disturbed in Prostate Cancer

The recent development of the microarray technology has allowed analysis of expression levels of thousands of genes in one experiment. In a study to identify genes and pathways that are differentially expressed between androgen-dependent and androgen-independent cancer cells, Jenster et al. from the Department of Urology of the Erasmus MC in Rotterdam performed microarray experiments, using GeneChips that harbored approx 4000 known genes and 3000 expressed sequence tags. On each chip, two batches of RNA were compared by competitive hybridization with the glass-spotted complimentary DNAs. Comparing the expression of ODC, AdoMetDC, and SSAT genes in androgen-responsive LNCaP cells cultured in the absence or presence of 1 nM dihydrotestosterone (DHT) for 16 h, it was observed that androgen stimulated the expression of these enzymes twofold to fourfold (Jenster et al., unpublished).

In a recent study, Rhodes et al. (22) conducted a meta-analysis of four independent microarray datasets to identify consistently significant candidate genes for prostate cancer. This study revealed a consistent and significant deregulation of polyamine biosynthesis in prostate cancer. Specifically, they found that enzymes linked to polyamine synthesis (aspartate transaminase, aminoacylase, ODC, and spermidine synthase) were overexpressed in prostate cancer, whereas ornithine aminotransferase was underexpressed. The net effect is an increased biosynthesis of polyamines. Biologically this makes sense because polyamines have been implicated in cancer cell proliferation, protection from apoptosis, and DNA–protein binding.

To investigate if ODC is involved in the cell kinetic behavior of human prostatic cancer cells, we examined ODC in cultured cells or xenografts of human prostatic cell lines. ODC protein was determined by Western blotting according to Schipper et al. (23). Activity of ODC was measured, as described earlier (24), in 48-h cultures and in xenografts of the prostatic cancer cell lines.

Results, as summarized in Table 1, indicate that ODC protein, as well as ODC activity, correlate with the growth rate of prostatic cancer cells (i.e., the amount of ODC protein is highest in the relatively fast growing tumor lines PC-324, PC-374, and PC-346). This is in agreement with studies in rat prostate-derived Dunning tumors reporting a substantially higher ODC activity in the faster growing sublines (25). Immunoblot analysis of human prostate cancer tissue specimens showed a significantly elevated ODC protein expression in the cancerous tissues as compared with the benign tissues (26). Moreover, studies on the expression levels of the ODC gene in a series of 23 human prostate cancers dissected from radical prostatectomy specimens revealed significantly higher ODC mRNA levels in tumors compared with the benign tissue (27). With a 5-yr follow-up study performed on the same cohort of patients, ODC gene profiling was proven to be an effective method for predicting the recurrence of prostate cancer, especially when combined with clinical–pathological parameters (28).

4. Polyamines as Biomarkers for Prostate Cancer

The observation that not only a number of enzymatic steps in polyamine synthesis, but also enzymes directing substrates toward polyamines, are overexpressed in
<table>
<thead>
<tr>
<th>Cell line</th>
<th>Origin</th>
<th>Androgen dependent</th>
<th>AR pathway</th>
<th>Diff. grade</th>
<th>Td (d)</th>
<th>ODC protein</th>
<th>ODC activity (pmol/min/mg)</th>
<th>Spd (nm/mg)</th>
<th>Spm (nm/mg)</th>
<th>Spd/Spm ratio</th>
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<tbody>
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<td>PC-295</td>
<td>LN</td>
<td>Yes</td>
<td>Intact</td>
<td>++</td>
<td>18</td>
<td>+</td>
<td>0.64</td>
<td>1.41</td>
<td>0.32</td>
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<tr>
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<td>PC</td>
<td>Yes</td>
<td>Intact</td>
<td>++</td>
<td>13</td>
<td>+</td>
<td>0.32</td>
<td>1.45</td>
<td>0.22</td>
<td></td>
</tr>
<tr>
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<td>TUR</td>
<td>No</td>
<td>No AR</td>
<td>–</td>
<td>10</td>
<td>++</td>
<td>0.61</td>
<td>0.57</td>
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</tr>
<tr>
<td>PC-339</td>
<td>TUR</td>
<td>No</td>
<td>No AR</td>
<td>–</td>
<td>20</td>
<td>+</td>
<td>0.81</td>
<td>0.49</td>
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<tr>
<td>PC-374</td>
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<td>Sensitive</td>
<td>Intact</td>
<td>+</td>
<td>10</td>
<td>++</td>
<td>0.30</td>
<td>1.41</td>
<td>0.21</td>
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<tr>
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<td>Intact</td>
<td>++</td>
<td>7</td>
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<td>1.81</td>
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<tr>
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<td>No AR</td>
<td>–</td>
<td>12</td>
<td></td>
<td>4.8</td>
<td>4.1</td>
<td>1.17</td>
<td></td>
</tr>
<tr>
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<td>PC-3</td>
<td>No</td>
<td>No AR</td>
<td>1.3</td>
<td>18.4</td>
<td>19.8</td>
<td>12.5</td>
<td>1.58</td>
<td></td>
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</tr>
<tr>
<td>Du-145</td>
<td>Brain</td>
<td>No</td>
<td>No AR</td>
<td>±</td>
<td>15</td>
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<td>1.6</td>
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</tr>
<tr>
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<td>No AR</td>
<td>2.5</td>
<td>7.7</td>
<td>5.2</td>
<td>4.3</td>
<td>1.20</td>
<td></td>
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</tr>
</tbody>
</table>

Results are the mean of at least two different experiments. Variability between duplicate determinations was within 10% for the data shown here. PC, primary prostate tumor; LN, lymph node; TUR, transurethral resection; AR, androgen receptor; Td, tumor doubling time; -C, in vitro cell line; Diff., differentiation; Spd, spermidine; Spm, spermine.
prostatic cancer implies that polyamines may be suitable markers for prostate cancer. In the following sections, studies on the use of polyamines in body fluids and tissues as biomarkers for prostatic cancer are discussed.

4.1. Circulating Polyamines as Markers and Tracers for Prostatic Malignancies

Polyamines or their acetylated forms can also be secreted by cells and released into the circulation. Subsequently, circulating polyamines and their acetylated products can be taken up and reused by polyamine-demanding cells. Transport of polyamines from one cell to another via body fluids is believed to be carrier-mediated by either proteins (e.g., anti-polyamine antibodies, lipoproteins) or by cells (peripheral blood cells). Moulinoux et al. showed that more than 95% of circulating spermidine and spermine are transported by red blood cells (RBCs) and that RBC polyamine levels correlate with tumor progression in tumor-grafted animals. Results from these animal studies and investigations in patients suggest that RBC polyamine levels reflect the proliferation status of normal tissues and tumors including prostate carcinomas (29,30).

As demonstrated by these studies, interorgan polyamine transport is realized by means of circulating polyamines, which participate in supporting normal and malignant cell proliferation. Because neoplastic growth requires high concentrations of polyamines, radioactively labeled polyamines have been used as tracers for imaging and characterization of cancers. Clark and Fair (31) reported that 14C-putrescine, when injected in rats, preferably accumulated in the prostate. In vitro and in vivo uptake of radiolabeled 14C-polyamines by rat prostate-derived Dunning tumor lines was investigated by Heston et al. (32,33). These studies indicated that both labeled putrescine and spermidine are imported in cultured cells whereas, in tumor-bearing animals, labeled putrescine and cadaverine, but not spermidine and spermine were taken up by the tumor. Uptake of 14C-putrescine by the rat prostatic tumor was higher than in the dorsolateral prostate. In contrast, in the ventral prostate uptake equals the amount taken up by the tumor. Pretreatment with difluoromethylornithine and androgen stimulation enhanced the uptake of 14C-putrescine in the tumor but not in other tissues.

More recently, the potential of labeled putrescine as tracer for cancers has been studied using positron emission tomography (PET). PET-scan imaging of the Dunning R3327H prostatic carcinoma demonstrated a high tumor uptake of the labeled polyamine N-(3-[18F]fluoro-propyl)putrescine (34,35). Uptake of 11C-putrescine using PET-scan imaging has also been examined in brain tumors showing similar results (36). However, PET studies in humans showed that the uptake of 11C-putrescine in the prostates of normal controls exceeds uptake in prostates of patients with localized prostatic cancer (37). These results suggest that 11C-putrescine is not useful to trace human prostate adenocarcinoma.

4.2. Spermine as a Biomarker for Malignant Behavior of Prostatic Cancer

Spermine is present in the prostate in high concentrations (10–20 mM). Several studies suggest that a reduction of spermine levels may result from shifts in cellular behavior of prostatic (cancer) cells (38–40).
Polyamine measurements in our panel of cultured cells or xenografts of human prostatic cell lines showed that the less differentiated cell lines (e.g., PC-324, PC-339) contained clearly less spermine than the tumor cell lines with differentiation characteristics resembling (androgen dependence, androgen receptor content, specific marker expression) more normal prostate epithelium (e.g., PC-295, PC-310, PC-346, and PC-374) (see Table 1).

These observations prompted us to search for a nondestructive tool to evaluate polyamine levels in biopsies and in vivo in the prostate (38). Such a tool would be very useful to determine the predictive value of the polyamine content in prostate cancer as a marker for its biological behavior. Proton magnetic resonance spectroscopy has been applied to metabolic studies of prostate cancer in animal models and in humans (39). This technique is particularly attractive as it is possible to obtain metabolic information noninvasively from multiple, distinct regions of prostatic tissue in situ and from intact biopsy material. In vitro proton nuclear magnetic resonance (NMR) of extracts of prostatic tissue and cells gives an immediate overview of numerous compounds present in these tissues or cells. We are now able to assign signals in 1H NMR spectra from extracts of the human prostate to spermine. Moreover, in 2D 1H NMR studies of human prostate biopsy tissue, we obtained evidence for the presence of large amounts of spermine (38).

Recently, we explored the applicability of a newly developed NMR technology (i.e., high-resolution magical angle spinning [HRMAS]) to investigate intact ex vivo prostatic tissue material (40). These studies show that with HRMAS a far higher sensitivity and resolution can be achieved as compared with conventional ex vivo NMR methods. The high sensitivity of HRMAS even allows the detection of polyamines and other metabolites in fine needle biopsy material in a relatively short measuring time, enlarging the clinical potential of magnetic resonance spectroscopy. Moreover, improved analysis by adding J-resolved NMR becomes feasible in routine use since this analysis requires only a little time.

Biochemical and NMR studies of polyamine levels in biopsies from prostates of patients showed that normal and benign hyperplastic prostatic tissues have a high spermine content, whereas in tumor tissue, especially in prostatic carcinoma with metastases, spermine levels were reduced (38–40).

Interestingly, the NMR studies show that spermine reduction is accompanied by a reduction in the levels of another abundant metabolite in prostatic fluid, namely citrate. Both spermine and citrate are produced in the epithelial tissue and excreted in the lumen of the prostate, where they appear to form a complex (11).

It is not yet clear whether the reduction of spermine levels is actively involved in the process of prostate carcinogenesis. During prostatic cancer growth the epithelial tissue structure is slowly destroyed, accompanied by a reduction of the luminal space. Hence the reduction of spermine (and citrate) could merely reflect the changes in ductal morphology as a result of a conversion of prostatic tissue from a benign to a malignant phenotype. On the other hand, studies of Zetter et al. (41–43) suggest that spermine is actively involved in the regulation of prostate cancer cell growth. High spermine levels present within the prostatic microenvironment suppress prostatic growth by upregulation of AZ. Escape from this regulation may emerge from altered inducibility of AZ.
and/or an acquired insensitivity towards spermine. Koike et al. (42) reported that rat prostatic cancer cell lines showed a differential sensitivity toward spermine. In the spermine-insensitive cells, AZ levels were not upregulated. AZ possibly acts as an important tumor suppressor by degrading growth-promoting factors, such as ODC and, as was shown recently, cyclin D1 (43).

5. Polyamine Metabolism as a Target for Treatment of Prostate Cancer

Depletion of intracellular polyamines invariably results in growth inhibition or induction of cell death. Therefore, the polyamine metabolic pathway is an attractive target for chemotherapy of prostate cancer.

As shown by our group and others, polyamine inhibitors and polyamine analogs are effective inhibitors of different phenotypes of human prostatic cancer cells and, furthermore, are able to inhibit the in vivo growth of prostatic tumors (reviewed in 44). Compared with polyamine inhibitors, polyamine analogs may have more clinical value because these compounds can be cytotoxic.

Unfortunately, only a limited number of (biologically different) human prostate cancer cell lines are available. Widely used cell lines, such as DU-145 and PC-3, though undisputed with respect to their origin, lack typical prostate-specific characteristics such as PSA secretion. In contrast, the PC-346C cell line has retained several properties that are characteristic for prostate (cancer) cells, including androgen responsiveness and production of prostate-specific markers (45). PC-346C should therefore be considered a better representative for prostate cancer than the previously studied cell lines. An additional possibility offered by the PC-346C cell line is the option to examine the effect of androgens on the response to polyamine analog treatment.

We have recently tested newly developed polyamine analogs on the in vitro growth of the PC-346C cell line. These compounds are based on the tetramine N₁,N₁₂ bis(ethyl) spermine (BE-4-4-4) and pentamine 1, 19-bis(ethylamine)-5,10, 15-triazonadecane (BE-4-4-4-4), but are restricted in their chain flexibility (developed and manufactured by Prof. Dr. L. J. Marton, Dr. H. Basu, and Dr. B. Frydman, SLIL Biomed Inc., Madison, WI) (46–49). All six polyamine analogs tested had a similar dose-dependent inhibitory effect on the growth of the cell lines DU-145, PC-3, LNCaP, and PC-346C, with activities in the micromolar range (Table 2). The inhibition of growth was from an increased rate of cell death rate because an increase of the proportion of apoptotic cells was seen after polyamine analog treatment (52).

PC-346C cells produce and secrete relatively high levels of PSA in a hormone-dependent fashion. Treatment of PC-346C cultures with polyamine analogs dose dependently suppressed the PSA concentration in culture medium (52).

The growth inhibitory effects of polyamine analogs are likely to depend on the intracellular accumulation of the analog or on the consequent ability to alter the pools of the natural polyamines. Intracellular concentrations of putrescine, spermidine, and spermine, as well as of SL-11098, were determined in PC-346C cells after exposure to the analog for 5 d (Table 3). The results indicated that treatment of PC-346C cells with SL-11098 resulted in a marked decrease of intracellular putrescine, an incomplete reduction of spermidine levels, and increased spermine concentrations. Notably, the
accumulation of SL-11098 in the cells was four to five times higher in the absence of androgen (Table 3). The levels of all three polyamines in SL-11098–treated cells were lower in the absence than in the presence of androgen.

Polyamine analogs have been shown to be effective inducers of AZ (50, 51). Our recent studies using GFP-conjugated ODC (20) showed that treatment of PC-346C cells with the polyamine analog SL-11093 resulted in an induction of AZ in the nucleus, similar to the effects of putrescine stimulation. AZ colocalized with ODC-GFP also in PC-346C nuclei. These preliminary data indicate that, at least partly, polyamine analogs may act on cell growth by inducing AZ.

Table 2
Growth Inhibitory Effects of Polyamine Analogs on Human Prostate Cancer Cells

<table>
<thead>
<tr>
<th>Analog</th>
<th>Structure</th>
<th>IC50-values (μM)</th>
<th>DU-145</th>
<th>PC-3</th>
<th>LNCaP</th>
<th>PC-346C</th>
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<tbody>
<tr>
<td>SL-11093</td>
<td>[Image]</td>
<td>0.26</td>
<td>0.31</td>
<td>2.1</td>
<td>5.6</td>
<td></td>
</tr>
<tr>
<td>SL-11098</td>
<td>[Image]</td>
<td>0.28</td>
<td>0.20</td>
<td>3.5</td>
<td>5.0</td>
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<tr>
<td>SL-11199</td>
<td>[Image]</td>
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<td>0.19</td>
<td>3.2</td>
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</tr>
<tr>
<td>SL-11118</td>
<td>[Image]</td>
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<td>0.25</td>
<td>2.1</td>
<td>7.6</td>
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</tr>
<tr>
<td>SL-11121</td>
<td>[Image]</td>
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<tr>
<td>SL-11128</td>
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<td>0.09</td>
<td>1.3</td>
<td>6.5</td>
<td></td>
</tr>
</tbody>
</table>

IC50 values, drug concentrations required for 50% reduction of cell growth as compared with untreated controls, were determined from dose-effect curves. Results are the mean of at least two different experiments. Variability between duplicate determinations was within 10% for the data shown here.

Table 3
Intracellular Concentrations of Polyamines and SL-11098 in PC-346C Cells Exposed for 5 Days to 5 μM SL-11098 in the Absence and Presence of Androgen (R1881)

<table>
<thead>
<tr>
<th>Additions</th>
<th>Put</th>
<th>Spd</th>
<th>Spm</th>
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</thead>
<tbody>
<tr>
<td>None</td>
<td>14.2</td>
<td>9.1</td>
<td>11</td>
<td>ND</td>
</tr>
<tr>
<td>10 μM SL-11098</td>
<td>1.1</td>
<td>5.3</td>
<td>15</td>
<td>23.4 ± 2.7</td>
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<tr>
<td>0.1 nM R1881</td>
<td>17.8</td>
<td>8.7</td>
<td>15.5</td>
<td>ND</td>
</tr>
<tr>
<td>0.1 nM R1881 + 10 μM SL-11098</td>
<td>7.4</td>
<td>7</td>
<td>31.8</td>
<td>5.2 ± 2.0</td>
</tr>
</tbody>
</table>

Values, reported in nmol polyamine/mg total cell protein, are the means ± SD from triplicate determinations.

ND, not detectable.
6. Conclusions

The studies reviewed here clearly show that the polyamines are of essential importance for the regulation of cell proliferation and differentiation of prostatic glandular epithelial cells. There is also evidence that ODC and polyamines are crucial for cellular transformation of prostatic cells. Our results obtained with xenograft models of prostate cancer indicate that the expression of ODC is higher in the more malignant variants.

Our studies and data from the literature show that changes in the activity of ODC and other polyamine metabolic enzymes and levels of polyamines are accompanied by alterations in cell kinetic behavior (cell growth, apoptosis) of prostatic (cancer) cells. In particular, spermine, present in high concentration in the prostate, seems to inhibit growth of prostatic cancer cells as long as they remain within the confines of the prostate gland. This explains why during the initial phase of the disease prostate cancer generally grows slowly. Conversion of prostatic epithelium into carcinoma is accompanied by a decrease of spermine concentrations. From these data, we infer that disturbance of the ODC/polyamine system might be an important determinant of malignant behavior of prostatic cancer cells.

The standard treatment of disseminated prostate cancer (i.e., androgen ablation therapy) is aimed to stimulate apoptosis in prostatic cancer cells. However, in the long term, androgen ablation therapy fails because of hormone resistance and concomitant escape of androgen ablation-induced apoptosis. Research shows that polyamine analogs are effective inhibitors of growth of prostatic cancer cells. Our results show that these six novel polyamine analogs, some of which are entering clinical trials, are potent inhibitors of prostate cancer cell growth in vitro. Our findings indicate that androgen-depleted, hormone-responsive, and androgen-independent prostate cancer cells have retained, or even gained, sensitivity to polyamine analogs. Therefore, combined therapy by hormone ablation and polyamine analog treatment might be considered as a potential new strategy for the treatment of advanced prostate cancer.

Data concerning gene activity related to ODC/polyamine metabolism during tumor progression of prostate carcinoma are hardly available. Information on which genes are activated or switched off during exposure of prostatic carcinoma cells to drugs interfering with components of the ODC/polyamine system would greatly improve our understanding of the roles of polyamines in prostate physiology.

In conclusion, polyamines are critically involved in the regulation of prostatic (cancer) growth and therefore might be suitable targets for the diagnosis, prognosis, and chemotherapeutic intervention of prostate cancer.

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