REVIEW PAPER

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α–blockade, apoptosis, and prostate shrinkage: how are they related?

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Purpose. The α1–adrenoreceptor antagonists, such as terazosin and doxazosin, induce prostate programmed cell death (apoptosis) within prostate epithelial and stromal cells in vitro. This treatment should cause prostate volume decrease, However, this has never been observed in clinical conditions. The aim of this paper is to review the disconnect between these two processes.

Methods. PubMed and DOAJ were searched for papers related to prostate, apoptosis, and stem cell death. The following key words were used: prostate, benign prostate hyperplasia, programmed cell death, apoptosis, cell death, α1–adrenoreceptor antagonist, α–blockade, prostate epithelium, prostate stroma, stem cells, progenitors, and in vitro models.

Results. We have shown how discoveries related to stem cells can influence our understanding of α–blockade treatment for BPH patients. Prostate epithelial and mesenchymal compartments have stem (progenitors) and differentiating cells. These compartments are described in relation to experimental in vitro and in vivo settings.

Conclusions. Apoptosis is observed within prostate tissue, but this effect has no clinical significance and cannot lead to prostate shrinkage. In part, this is due to stem cells that are responsible for prostate tissue regeneration and are resistant to apoptosis triggered by α1–receptor antagonists.

Key Words: α1–receptor antagonists ‹› benign prostate hyperplasia ‹› apoptosis ‹› stem/progenitors cells

INTRODUCTION

α_1–adrenoreceptor antagonists, such as terazosin and doxazosin are able to induce programmed cell death (apoptosis) within prostate epithelial and mesenchymal cells [1, 2]. In theory, α_1–adrenoreceptor antagonist treatment with ensuing apoptosis of prostate epithelial and mesenchymal cells should lead to prostate shrinkage. However, clinically, α_1–adrenoreceptor antagonists treatment does not result in prostate volume reduction. This review is designed to 1) analyze the possible pitfalls related to this discrepancy and 2) to demonstrate how stem cell differentiation could influence in vitro and in vivo results presented in this field.

METHODS

PubMed and DOAJ were searched for papers related to prostate, apoptosis, and stem cells. The following key words were used; prostate, benign prostate hyperplasia, programmed cell death, apoptosis, cell death, α_1–adrenoreceptor antagonist, α–blockade, prostate epithelium, prostate stroma, stem cells, progenitors, and in vitro models.

Stem cell classification

Stem cells are classified according to their mitotic and differentiating potential. This potential can be described as the possibility to regenerate tissue, or-

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gans, or even a whole new organism. These properties can be described as unipotential, multipotential, pluripotential, or totipotential. Totipotential stem cells possess the ability to build a whole new organism. Pluripotential stem cells isolated from an embryo inner cell mass can differentiate into all cells, but cannot form a new organism. Epithelial, endothelial, and mesenchymal stem cells are referred as multipotential. Progenitors or unipotential stem cells can regenerate only one defined cell population. Multipotential and unipotential cells are isolated from tissues of adult organisms, so they are usually called „Adult Stem Cells" (ASC). On the other hand many authors suggested that cells with pluripotential characteristics can be isolated from adults, so this classification will probably be changed in the near future [3, 4].

Stem cells and progenitors regenerate prostate epithelium

Adult stem cells (ASC) are able to self renew and maintain the structural and functional integrity of their original tissue. Transit amplifying cells (TAC) are committed progenitors within ASC and their terminally differentiated daughter cells. ASC and TAC are protected and controlled in their self–renewing capacity and differentiation. ASC and TAC occurrence have been considered in many human tissues including prostate epithelium [5, 6]. The prostatic epithelium is composed of five cell type compartments: stem cells, basal epithelial cells, TAC, neuroendocrine cells, and secretory epithelial cells [7]. Basal cells form a single layer on the basement membrane. The stem cells are localized within the basal layer. Prostate epithelial stem cells provide progenitors that differentiate down either a neuroendocrine or exocrine pathway. The maturation along the exocrine pathway initially involves TAC, which differentiate into intermediate cells. These intermediate cells migrate into the luminal layer where they terminally differentiate into non–proliferative secretory luminal cells [8].

Generally two populations of cells capable of regenerating prostatic ducts can be distinguished. The first population (with considerable huge growth potential) resides in the proximal region of ducts and in the urethra, and the survival of these cells does not require the presence of androgens. The second population (with more limited growth potential) is found in the remaining ductal regions and requires androgens for survival [9]. The prostate epithelial basal cells express high levels of integrin $\alpha2\beta1$ and this population can be subdivided into basal stem ($\alpha2\beta1$(hi) CD133+) and TAC ($\alpha2\beta1$(hi) CD133–) [10]. The behavior of CD133+ cells in vitro culture resembled progenitors properties. The viability of these cells after detachment reached 100% in some cases, but the proliferation rate was lower when comparing to CD133(+)/CD133(–) co–cultures. CD133(–) cultures are probably composed of differentiated cells without clonogenic potential. CD133(–) cells had no potential for in vitro proliferation, even in the serum conditioned medium. It can be speculated that CD133(–) cells probably have no ability to regenerate the prostate ducts. These cells were not able to anchor and form monolayer [11]. Some of them can be alive in culture, but the most of them enter an apoptotic pathway. The apoptotic inductions among these cells were probably due to the lack of anchor growth [2, 12].

Receptor status and cell differentiation

Receptor status is supposed to be the most important part of the molecular target in the BPH treatment [13, 14]. It is relevant only with the assumption that prostatic tissue is homogenous. In fact, prostate tissue has two major cell populations: epithelial and mesenchymal. These cells have different origin and function, but interactions between them are well recognized [15, 16]. These interactions are crucial for gland development, tissue hemostasis, and prostate hyperplasia etiology, as well. Moreover, both populations i.e. epithelial and mesenchymal have differentiating cells. The process of differentiation starts from stem cells (or progenitors) to the fully differentiated progenies [6, 9]. The receptor status has to be analyzed in the light of cell origin and its differentiation, as well. This obvious but underestimated issue is often omitted.

How do antagonists of α_1–receptor induce apoptosis within prostate epithelial cell?

It has been previously reported that doxazosin and terazosin pro–apoptotic activity was independent of its capacity to antagonize α_1–adrenoreceptors on prostate cells. Many pathways leading to apoptosis within the prostate have been proposed [17–25]. The recent results show that doxazosin triggers apoptosis within benign and malignant prostate cells via an imprecisely defined receptor mechanism related to the tumor necrosis factor receptor family (TNFRs) [24–28]. TNFRs such as CD95 (FAS), TNFR–1, TNFR–2, and CD40 may be responsible for triggering apoptosis [29, 30]. Oligomerized CD95 forms a death–inducing signaling complex. Two signaling pathways (type I and II) have been identified [31, 32]. TNFR–1 and TNFR–2 have significant homology in their extracel-

lular domains; however, their cytoplasmic domains are different. TNFR–1 and TNFR 2 were able to mediate information for cell survival as well as apoptotic signals. CD40 can mediate both proapoptotic and antiapoptotic signals [33, 34, 35]. Trimerization is an active status of TNF family receptors. Trimerization occurs after ligand binding or spontaneously [36, 37]. The counterbalance of TNFRs plays a crucial role in apoptotic response within prostate epithelial cells. Doxazosin increased TNF receptor expression and probably supports the naturally occurring upregulation of the TNF family of receptors. Doxazosin had an effect on CD40 and CD95 expression on the prostate epithelium [25]. All these findings confirm previous reports that doxazosin triggered apoptosis within the prostate epithelium via the TNF family–related proteins. Initiation of apoptosis was a result of these proteins crosstalk rather than a single receptor–dependent pathway activation. TNF receptor self–assembly process should be recognized as one of the potential mechanisms of triggering apoptosis after doxazosin treatment.

Stem cells can be found within prostate cell lines – implications

Models of primary cell cultures are not popular in investigational medicine. The primary culture implies a culture of cells established immediately after tissue isolation. The goal of the primary culture is their uniqueness, i.e. the patient. Primary cultures offer a unique possibility of performing repeatable tests on the material derived from the patient. It is very difficult to find in laboratory conditions, the model reflecting the process of stem cell or progenitor maturation and differentiation. It is reasonable to extrapolate that primary cultures in vitro can be such a simplified model illustrating the dependencies that exist between stem cells and their differentiated progenies [38, 39]. Immortalized or cancer cell lines are often used for in vitro studies. The results elaborated on established cell lines are burdened with an error arising from the lack of diversity in terms of cell aging and differentiation. Immortalized and tumor cell lines do not undergo the process of replicational aging, which is equivalent to the aging process characteristic for vivo conditions [40, 41]. On the other side primary cultures give the opportunity to study the impact of cell age within growing in vitro colonies [7, 42–45]. The morphology of cells in the primary cultures at the time of their establishment tends to reflect on the importance of stem cells and differentiated in the primary cultures originated from normal or cancer cells. In the clusters of cells that give rise to the primary cultures, the progenitor cells are found [46]. They can be combined, e.g. with fragments of basement membrane and contain the other cells forming a niche of normal stem cells [7, 45]. Stem cells give rise to epithelial cell cultures in vitro, whereas differentiated cells have inferior properties in this regard. Each digested and prepared for in vitro culture tissue must have a certain number of progenitor cells, whose potential determines an appropriate number of divisions. The proliferation of stem cells gives rise to a colony of intensive and long–dividing cells (holoclones). Cells with low ability to proliferate form colonies of paraclones, which probably do not contain stem cells [42, 43, 44].

The proper understanding of in vitro models, used for research purposes, should be linked to the heterogeneity of degree of cell differentiation. Under this assumption, when examining the effect of different substances on an established cell line, one can demonstrate on a heterogenous group of cells with varying degrees of differentiation and proliferative potential, different receptor expression, and resistance to drugs [47]. Experiments based on an accepted model of "homogenous" population representing established, immortalized, or cancerous cell lines may lead to false results. In vitro experiments on such "homogenous" cell lines inform us only about the overall toxicity of the tested substance or drug. Results obtained from "homogenous" or "primary cell culture–heterogenous" in vitro culture models can be different. An example is the influence of doxazosin (an α_1–receptor antagonist), which induces apoptosis in the epithelial cells and prostate's stroma in vitro [2, 17, 23, 25, 26]. The primary culture of the prostate's epithelium is composed of stem cells and differentiated cells [7, 11, 45, 48, 49]. High concentrations of doxazosin lead to massive apoptosis of prostate cells when immortalized or cancer cell lines are tested [2, 26, 32]. On the other hand, the same high concentrations of doxazosin trigger apoptosis within only a small amount of prostate epithelial stem cells [50]. The same complex at the same concentration tested on a whole cell population, stem cells, or on differentiated cells can exert different effects [41]. Stem cells and differentiated cells show different sensitivity to different cytotoxic agents, inducing apoptosis. Therefore, contrasting conclusions can be drawn when analyzing different populations such as stem and differentiated cells.

The proper information from in vitro cytotoxic tests have been obtained only when primary cultures are analyzed with an emphasis on separate analysis for stem and differentiated cells. Primary cultures showed heterogeneity of cell differentiation. It is very difficult to perform separation of stem and dif-

ferentiated cells within established immortalized or cancer cell lines [51]. Although it is believed that models of heterogeneous primary cultures of normal cells have their counterparts in the tumor cell culture. As a result it can be assumed that in the cancer cell lines, heterogeneity in terms of differentiation is also observed [52–56]. Cancer cultures also have a population of stem cells that are responsible for their growth and other characteristics of a tumor's biological properties [55, 57]. The mechanisms responsible for cell resistance against medicines and other substances vary between stem cells and differentiated cells [6, 58, 59, 60]. The analysis of the results of in vitro cytotoxicity tests should be based on separate analysis describing the cytotoxicity for stem cells and separate analysis describing the cytotoxicity for differentiated cells. The model of the heterogeneous cancer cell line is still a simplified model and needs to be developed. This model partially explains discrepancies between the results obtained from in vitro experiments and those from clinical practice. With the rapidly developing techniques of cell isolation it is not just a theoretical model, but it can be used in studying the effects of a substance's in vitro influence, probably helping to predict the real drug effectiveness [41].

Stem cells are resistant to apoptotic stimuli – Is it the key?

Are stem cells important for the treatment of BPH patients? Normal prostate epithelial stem cells and progenitors were identified and found to have a basal cell phenotype together with the expression of CD133 antigen [56, 61]. Several methods of stem cell isolation were reported [48, 62, 63]. It was proven that progenitor cells were responsible for primary prostate epithelial cultures in vitro growth [7, 8, 11, 45]. Is it important for BPH patient treatment to recognize stem cell populations? The stem cell population is very small and located only in the proximal parts of the prostatic ducts. This population has not been regarded as an important part of in vitro experimental methodology. However, more recently, it has been recognized that stem cells may be valuable crucial for drug testing experiments. Stem cells are characterized by low sensitivity to action of proapoptotic agents, which is correlated with high expression of antiapoptotic proteins, high ability to repair damaged DNA, and high expression of ATP–binding cassette drug transporters [50, 58, 59, 60, 64].

It has been demonstrated that α_1–adrenoreceptor antagonists, such as terazosin and doxazosin induce prostate programmed cell death (apoptosis) without affecting cell proliferation in vivo and in vitro via α_1–adrenoreceptor–independent actions [1, 2, 24]. The α_1–adrenoreceptor antagonist treatment leads to apoptosis of prostate epithelial and mesenchymal cells. However, stem cell compartmentalization may preclude effects on prostate volume reduction. Several studies support the concept that a subpopulation of stem cells resides among basal cells, which is capable of giving rise to other stem cells, basal epithelial, luminal epithelial, and neuroendocrine cells [4, 9, 10, 45, 49, 61, 63, 65, 66]. It has been shown that prostatic epithelial tissue contained a side population constituting 1–1.38% of the epithelial cells and exhibiting low cell cycle activity [4, 45, 48, 62]. It was shown that only a few CD133 positive cells can form a prostatic epithelium in animal and human models [11, 48]. Doxazosin decreases cell number within co–cultures of stem and differentiated prostatic epithelial cells, but stem/progenitor cells are generally not sensitive to doxazosin treatment. There is a suspicion that the differential influence of doxazosin on progenitor and differentiated cells can be responsible for lack of prostate volume decrease after α1–antagonist treatment [50].

CONCLUSIONS

1. Prostate epithelial and mesenchymal compartments have stem (progenitors) and differentiating cells.
2. Cell lines used in experimental setting have stem and differentiating cells.
3. α_1–receptor antagonists, doxazosin and terazosin, induce apoptosis with prostate differentiated epithelial cells.
4. Stem cells/progenitors are resistant to α_1–receptor antagonists, doxazosin and terazosin.
5. Apoptosis can be evoked within prostate tissue, but this effect has no clinical significance and cannot lead to prostate shrinkage, since stem cells are still able to regenerate prostate tissue.

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