Apoptosis induction by quinazoline-derived \(\alpha_1\)-blockers in prostate cancer cells: biomolecular implications and clinical relevance

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Abstract. – In patients with androgen-sensitive prostate cancer androgen-deprivation therapy leads to apoptosis of hormone-dependent cells but it may select out androgen-refractory clones. The apoptosis of prostate cancer cells induced by quinazoline-derived \(\alpha_1\)-adrenoceptor antagonists (doxazosin, terazosin, prazosin) counteracts cell proliferation and may have the potential of reversing or delaying prostate cancer growth. Elucidation of the biomolecular mechanisms whereby quinazoline-\(\alpha_1\)-adrenoceptor-antagonists are able to induce anoikis and apoptosis in androgen-refractory prostate cancer cells would be favorable for designing effective therapeutic regimens in the management of advanced prostate cancer.

Key Words: Prostate cancer, Anoikis, Apoptosis, \(\alpha_1\)-adrenoceptor antagonists, Urology.

Introduction

Prostate cancer is the second commonest malignancy in men, with an estimated incidence in the USA of 232,090, in 2005\(^1\) and it is the third leading cause of death in men worldwide (9% of all male cancer deaths in European Union\(^2\)). The incidence of prostate cancer is rising rapidly in most countries and it is expected to increase substantially in the next future because of worldwide growth of the older population together with widespread prostate specific antigen testing\(^3\).

When prostate carcinoma is diagnosed, therapy can be deferred until symptoms appear – watchful waiting – or straight-away carried out with radical prostatectomy, external beam radiotherapy or brachytherapy, hormones (testosterone-lowering therapies, antiandrogen treatment, combination therapy\(^4\)). Hormone therapy is the main measure for metastatic prostate carcinoma but, after an average of 18 to 24 months\(^5,6\), the patients relapse with androgen-refractory tumor that also become resistant to cytotoxic chemotherapeutic agents, cancer cell apoptotic evasion being a molecular force driving the tumoral progression. Therefore the urgency of developing more effective therapies for prostate carcinoma is caused not only by the projected increase in its incidence but also by the temporary sensitivity of hormone-refractory prostate cancer to currently used cytotoxic agents. Quinazoline-derived \(\alpha_1\)-adrenoceptor antagonists (doxazosin, terazosin, prazosin), by counteracting cancer cell proliferation via apoptosis induction, may have the potential of reversing prostate cancer growth\(^7,9\).

Apoptosis and Anoikis: Morphological Features and Biochemical Pathways

Apoptosis or programmed cell death – cell suicide – is an active energy-requiring and gene-directed mechanism, that results in removing unwanted or damaged cells. This process is characterized by sequential morphological features such as chromatin condensation, nuclear fragmentation, cytoplasmic blebbing and budding, loss of cell junctions, cell shrinkage and convolution, which finally lead to cell breaking-up into membrane-bound fragments, known as “apoptotic bodies”, that are ingested by macrophages. Whereas the apoptosis of reticulo-endothelial cells is dominated by nuclear changes, the apoptotic process of glandular epithelial cells, such as those of the prostate gland, requires the aforesaid alterations in cytoplasmic morphology and in cell-cell and cell-extracellular matrix (ECM) interactions. Although apoptosis is an evolutionary conserved process, genome comparison shows that the complexity of apoptotic machinery is vastly increased in vertebrates compared with *Drosophila melanogaster* and *Caenorhabditis elegans*\(^10,11\).
At the molecular level, apoptosis is a series of well-regulated events involving various subcellular systems and it is mainly mediated by the activation of the caspases (interleukin-converting enzyme, ICE-like cysteine proteases). There are two main pathways leading to caspase activation: the first – intrinsic or receptor independent pathway – includes Bax (Bcl-2 associated x-protein) translocation from cytosol into mitochondria, Bak (Bcl-2 antagonist killer) mitochondrial protein activation, release of several mitochondrial apoptogenic proteins such as cytochrome C, Ca++- and Mg++-dependent endonuclease G, SMAC (second mitochondria-derived activator of caspase), which activate caspase 9, that, in turn, activates caspase 3, whereas the second – extrinsic or receptor-dependent pathway – depends on the activation of death-receptor Fas/APO1 (apoptosis inducing protein 1) and underlying Fas-associated death domain protein, FADD, by a specific ligand FasL. Fas is expressed in several cells while FasL is present only in a more restricted number of tissues; Fas-FasL system is activated by some conditions and drugs, including several cytotoxic agents. Besides Fas/APO1, the most well studied death receptor is p55 TNF-R1 (tumor necrosis factor-receptor 1) that is activated when crosslinked by TNF-α. At first, as far as extrinsic pathway is concerned, caspase 8 is activated and, in turn, it may, either directly or through the caspase 7 involvement, activate procaspase 3 → caspase 3 downstream. Caspase 3, as a crucial key mediator of both pathways, induces, through proteolytic cleavage of 85 kDa poly (ADP-ribose) polymerase-1 (PARP-1), DNA fragmentation. Induction of apoptosis has been recognized as the main mechanism of anticancer cytotoxic treatments including chemotherapy and radiotherapy, particularly through p53-dependent apoptotic process. In addition to p53, other apoptosis regulatory genes, such as Bax and Bak, have been found to play crucial roles in determining sensitivity to anticancer agents. Mutations in genes promoting apoptosis or overexpression of Bcl-2 have been found to be associated with poor response to several cytotoxic drugs such as cisplatin, vincristine, cytarabine, paclitaxel as well as to radiations. Insensitivity to apoptotic signals allows neoplastic cells to sustain growth under hypoxia and oxidative stress conditions and promote tumor angiogenesis by HIF, hypoxia inducible factor.

As far as prostate gland is concerned, a complex balance between cell proliferation and apoptosis is fundamental to normal prostate growth. Disruption of this homeostasis, because of apoptotic evasion and/or uncontrolled proliferation, inevitably leads to tumorigenesis, the aptitude of a cancer cell population to grow exponentially representing an imbalance between cell proliferation and cell death. In fact, with regard to tumor pattern, malignant cells may either acquire stem cell-like properties or may themselves be malignant stem cell clones.

The prostate secretory epithelial cells are critically dependent on androgen for survival and they begin to regress about 12 h after androgen-ablation, when the levels of 5-α-reductase/DTH (dihydrotestosterone) fall below that needed to inhibit the involution. TGF-β1 plays a key role in inducing apoptosis of androgen-sensitive prostate epithelial cells, whereas an overexpression of clusterin, gene, also designated testosterone repressed prostate message-2 (TRPM-2), protects them from apoptotic process. Overexpression of Bcl-2 have been found to be apoptotic process sensitizing clusterin with specific antisense oligonucleotides sensitizes androgen-independent prostate cancer cells to cytotoxic agents.

Testosterone-lowering therapies and antiandrogen treatments are known to induce apoptosis in both normal and neoplastic androgen-dependent prostate cells. Luteinizing hormone releasing hormone (LHRH) antagonists
cetrorelix and abarelix have been found to promote apoptosis in prostate cancer cell lines more rapidly than the LHRH agonists. In patients with androgen-dependent prostate cancer, hormone therapy leads to apoptosis of hormone-sensitive cell and to tumor regression but it may actually select out androgen-independent clones.

Apoptosis following loss of cell anchorage – anoikis – occurs in normal epithelial cells when integrin-mediated EMC contacts are lost, thus preventing the spread elsewhere of epithelial cells that are detached from matrix. Integrins regulate interactions between cells and ECM by converting mechanical forces arising from matrix to biochemical intracellular signals. The ECM acts as regulator of the adjacent epithelial cells and the basement membrane plays a stabilizing role on nuclear function by plasma-membrane, cytoskeletal actin polymers and nuclear matrix interactions. In glandular epithelial cells, such as those of the prostate gland, the disruption of the interactions between the cell membrane integrins and ECM is a critical step in the apoptotic pathway. Whereas the normal epithelial cells are anchorage-dependent and the loss of their adhesion to ECM affects intracellular signaling pathways that lead to apoptosis, malignant tumor cell instead survive and proliferate without anchorage by evading anoikis.

ECM anchorage protects cells from Fas death receptor-induced apoptosis, while ECM detachment induces Fas-mediated cell death by increasing the expression level of Fas and by decreasing, on the other hand, the expression level of c-Flip, an endogenous antagonist of caspase 8. Therefore detachment-induced apoptosis results from activation of the Fas by its specific ligand FasL, hence Fas-FADD complex formation and, in sequence, caspase 8 → 3 activation. Bim (Bcl-2 interacting mediator of apoptosis) is a critical mediator of anoikis in epithelial cells; its expression is strongly induced after cell detachment while its downregulation, caused by interfering RNA (RNAi), blocks anoikis. Disanchorage-induced expression of Bim requires lack of β1-integrin attachment, downregulation of EGF (epithelial growth factor) receptor expression and inhibition of Erk pathway. Dap3 (death-associated protein 3) is a pro-anoikis GTP-binding protein: its overexpression increases caspase activation that is induced by cell detachment whereas its down-regulation by antisense oligonucleotide inhibits anoikis.

On the contrary, Fak (focal adhesion kinase), a non receptor protein tyrosine kinase, when activated by integrins, can suppress anoikis while caspase 3, that is able to cleave Fak, allows the apoptosis. Src expression and activity contribute to tumorigenicity by inducing resistance to anoikis via an increased phosphorylation of Akt, a mediator of cellular survival pathway, whereas decreased Src expression by transfection with anti-sense Src vectors rises the susceptibility to anoikis. Phosphatidylinositol-3-kinase (PI3-K)/Akt and mitogen-activated protein kinases (MAPK) induce anoikis-suppressing effects and promote cell proliferation by phosphorylation of certain transcription factors such as c-fos, c-jun and c-myc, which then induce gene expression, leading to DNA synthesis.

**Apoptosis Induction by Quinazoline based α₁-blockers in prostate cancer**

In addition to improvement in lower urinary tract symptoms due to dynamic component – smooth muscle tone – of the benign prostatic hyperplasia, the quinazoline derived α₁-adrenoceptor antagonists (prazosin, doxazosin, terazosin), but not sulfonamide based tamsulosin, have been shown to induce apoptosis in benign and malignant prostate cells.

Quinazoline, C₈H₆N₂, is a compound made-up of two fused six-membered simple aromatic rings (benzene ring + pyrimidine ring) while sulfonamide, -SO₂NH₂, is a functional group made-up of sulfone group connected to amine group. Intriguingly, even Tarceva and Iressa, two HER1 (human epidermal growth factor receptor 1)-TK (tyrosine kinase) reversible inhibitors, and GW-572016, a dual HER1/HER2-TK reversible inhibitor, are quinazoline-based compounds; instead EKB-659, an irreversible HER1-TK inhibitor, as well as linomide, is quinoline (benzopyridine, C₉H₇N)-derived substance. Besides to promote the apoptosis and inhibit the angiogenesis, HER-TK inhibitors, unlike quinazoline-α₁-blockers, are also able to induce cell-cycle arrest.

Induction of apoptosis in prostate cancer cells by quinazoline-α₁-blockers in associated with activation of specific receptor dependent or extrinsic apoptotic pathway, that involves caspase downstream until the nuclear fragmentation. In fact, doxazosin induces FAAD recruitment and, in sequence, caspase 8 → 3 activation, thus implicating Fas death receptor-mediated apoptosis;
on the other hand, suppression of doxazosin-induced apoptosis by caspase 8 inhibitors, supports the involvement of caspase 8 in this apoptotic process.

Quinazoline α₁-blockers may be stimulating anoikis by influencing cell adhesion since they are able to reduce Fak levels either by decreasing its expression at transcriptional level or by promoting its caspase 3-mediated cleavage. Maspin, a mammary serine protease inhibitor with tumor suppressive activity, "enhances", in the meaning that improves, the "apoptotic threshold" of prostate cancer cells in response to quinazoline-derived α₁-adrenoceptor antagonist doxazosin. On the other hand, Bcl-2 overexpression in prostate cancer cells significantly inhibits doxazosin-induced anoikis.

Irreversible inhibition of α₁-adrenoceptors with phenoxybenzamine does not abolish the apoptotic effect of quinazoline α₁-blockers on human prostate cancer cells, because their apoptotic activity is independent of antagonistic action against α₁-adrenoceptors. On the other hand, doxazosin-induced activation of caspase 3 is not abolished by norepinephrine and by other α₁-adrenoceptor agonists, thus confirming the non-adrenergic nature of this process. Moreover, it is highly unlikely that quinazoline α₁-blockers may be involved in controlling prostate cancer cell growth by inhibiting α₁-adrenoceptor-mediated mitogenic responses via PI3-K/PKC (protein kinase C) – MAPK, pathway leading to DNA synthesis. Instead quinazoline α₁-adrenoceptor antagonists may induce apoptosis by involving activation of TGF-β signaling.

In addition to anoikis induction, quinazoline α₁-blockers are able to suppress prostate cancer vascularity by inhibiting the growth of vascular endothelial cells trough modulation of VEGF (vascular endothelial growth factor)-mediated angiogenesis and by interfering with FGF-2 (fibroblast growth factor-2) activity.

Moreover, combinations of doxazosin with either etoposide or adriamycin have synergistic cytotoxic effects on androgen-refractory human prostate DU 145 and PC-3 cancer lines. The synergistic activity of doxazosin and adriamycin can be explained by cross-talking mechanisms between doxazosin-induced apoptotic signaling and adriamycin related IP3-K/PGK/MAPK, pathway. The antagonistic effects of doxazosin and paclitaxel may refer to interfering action of taxanes, as well as epothilone-macrolides, with dynamic equilibrium of tubulin polymerization/depolymerization, that is a different mechanism from adriamycin.

Conclusions

Hormone therapy is the treatment of choice for locally advanced and metastatic prostate cancer but, after a short period, the patients relapse with an androgen-refractory tumor that, what’s more, becomes resistant to second-line hormone manipulations (estrogens, corticosteroids, adrenolytics) and to several cytotoxic drugs like vinblastine, cisplatin, taxanes, mitoxantrone, etoposide, usually in combination therapy with estramustine phosphate. Overexpression of Bcl-2 or expression of mutant p53 have been associated with resistance to these agents as well as radiations. The challenges in the implementation of effective therapeutic modalities, including gene therapy and vaccines, for advanced prostate carcinoma, reflect the dramatic condition of cancer cell apoptosis evasion in the development of drug-resistance.

A tremendous amount of knowledge, over the past years, on genetics and molecular pathways of tumorigenesis has provided the opportunity for the discovery of new agents specifically designed to target critical molecular steps involved in cancer progression; as far as apoptosis is concerned, the challenge remains how these informations may be used to kill selectively tumor cells while sparing normal cells.

Efforts have been made to restore apoptotic process in cancer cells by using either antagonists of antiapoptotic proteins (Bcl-2 and Bcl-х₁) or, on the contrary, death receptor activators such as tumor necrosis factor-related apoptosis inducing ligand (TRAIL) or, even more, agonists of proapoptotic proteins such as caspases (for a perspective approach, cancer cell-targeted caspase-releasing nanoparticles).

The apoptotic effect elicited by quinazoline-derived α₁-adrenoceptor antagonists in androgen refractory prostate cancer cell lines, together with their antiangiogenic effects, may have clinical relevance in therapeutic management of advanced prostate cancer. An interesting point, on this subject, will be the combined use of these drugs with other cytotoxic agents targeting both horizontally and vertically at certain steps in the molecular pathways of anoikis-apoptosis.
Moreover it is likely that doxazosin might inhibit growth and/or induce apoptosis in prostate cancer cells even by interfering with their 1A- and 1B-receptor targets of serotonin, that is released by prostate neuroendocrine cells and has an important growth factor role, together with several neuropeptides, in hormone refractory prostate cancer cell proliferation.

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