Abstract. – Challenges in the discovery of more potent agents to treat the castration-resistant prostate carcinoma (CRPC) reflect the frustrating condition due to development of its drug-resistance in addition to hormone-refractoriness. Although among the different CRPC therapy modalities, the chemotherapy regimens might seem conceptually outclassed as exhibiting a scant tumor cell-selectivity if compared with new molecular mechanism-based agents (so-called “smart drugs”), nevertheless, combo-therapies which combine the chemotherapeutic highly killing potential with specific mechanism-targeting products, seem to be effective antitumor measures. Thus, both microtubule (taxanes, epothilones, noscapine, Vinca-derivatives) and actin filament (pertenoxytoxins, cytochalasin D)-targeting agents may supply valuable outcomes in CRPC, either alone or in combination with “smart drugs” such as tyrosine- or multi-kinase receptor blockers, mTOR (mammalian target of rapamycin) inhibitors, monoclonal antibodies against various growth factor signaling receptors. Among the microtubule-inhibiting drugs, taxanes are able, by binding the tubulin, to cause polymerization and stabilization of the microtubules with following suppression of their dynamic properties at the mitotic spindle, that results in cancer cell cycle block at G2/M phase together with apoptosis. Cabazitaxel, a novel taxane-based agent, unlike other taxane compounds, exhibits low propensity for P-glycoprotein (Pgp)-mediated plasmalemmal drug efflux pump, thus, avoiding the development of taxane-resistance. Epothilones are a family of novel microtubule-targeting drugs, like taxane inhibiting microtubule dynamic behaviour at mitotic spindle and, therefore, preventing cancer cells from mitosis. Unlike docetaxel and paclitaxel, epothilones maintain their cytotoxic performance even in cancer overexpressing Pgp. Epothilone B-promoted radiosensitivity enhancement has been shown in radioresistant human prostate cancer cells, because such agent is able to delay DNA-strand break repair together with prolonging cell cycle block. To insightfully understand either microtubule or actin filament meshwork-targeting drug pharmacodynamics, functional cytoskeletal features such as cytoskeleton-related molecule cargo logistics, are preliminary taken into consideration.

Key Words: Nanotechnology, Cytoskeletal logistics, Androgen resistance, Cancer stem cells, Radiation therapy.

Introduction

Prostate carcinoma (PC) is the most common and second most lethal male cancer in USA and European countries, although most recently both diagnostic and therapeutic advances have been made in lowering its mortality⁴. Despite initial organ-confined tumor therapy with surgery or radiation, sometimes PC runs into a recurrence that temporarily may be controlled by androgen-deprivation modalities. Even though such treatment, both reducing blood testosterone levels through Gn-RH pituitary receptor agonists (triptorelin, leuprorelin, goserelin, buserelin) or antagonists (Degarelix, Abarelix), and preventing DHT (dihydrotestosterone)-binding to peripheral nuclear androgen receptor (AR) through anti-androgens (bicalutamide, nicalutamide, flutamide, MDV 3100), the last showing an effectiveness even in bicalutamide-resistant PC, might induce a rapid biochemical/clinical responses, inescapably, after an average of 18 to 24 months, the disease becomes refractory to hormone therapy – castration-resistant prostate carcinoma, CRPC – with subsequently a median survival of 12 to 18 months⁵.⁶. CRPC refers to the condition of prostate cancer growth at castrate androgen levels (serum testosterone less than 50 ng/ml), biochemically shown by a continuous PSA rise, on three consecutive measurements, after antiandrogen withdrawal for at least 28 days or following an additional secondary hormone manipulation.

Constant efforts are in progress to identify new cancer cell, either molecular or structural, targets towards which direct more effective therapeutic measures⁶.⁷.
Overall View on Both Current and Emerging Novel Therapies in CRPC

First and foremost, it has been pointed out, in several clinical experiences, that the progression from hormone-dependent to hormone-independent PC may be delayed by intermittent androgen-deprivation therapy and, on the other hand, as soon as a biochemical relapse arises, the antian- drogen (flutamide) withdrawal may sometimes induce a temporary decline in blood PSA levels. But the unreliability and the transience of such effects suggest a timely carrying out of more suitable current approaches mean-while looking at pipeline measures, all together including:

- Combination of estrogens (ethinyl-estradiol) and somatostatin analogs (lanreotide, pasireotide) as neuroendocrine targeting therapy, to affect, besides the tumor cells, also their microenvironment that can play an antiapoptotic role;
- Inhibition over 17α-hydroxylase/17-20 lyase (Cyp17)-aromatase through abiraterone, a selective blocker of adrenal androgen generation;
- Chemotherapy with cytotoxic drugs, among which cytoskeleton-targeted agents such as Vinca alkaloids (vinblastine, vinorelbine, vinflumine), nesacapine, taxanes and epothilones, pertenotoxins;
- Molecular mechanism-based strategy with “smart drugs” capable of either blocking cell proliferation signaling pathways or inducing apoptosis. To the former group belong receptor tyrosine-kinase or multikinase inhibitors among which those interfering with HER-1 (human epidermal growth factor receptor-1, EGFR), HER-2 (human epidermal growth factor receptor-2, also called c-erbB-2/neu as a homolog of the rat neu oncogene), PDGFR (platelet-derived growth factor receptor), VEGFR (vascular endothelial growth factor receptor) whereas to the latter group, recombinant TRAIL (tumor necrosis factor-related apoptosis inducing ligand) and poly (ADP-ribose) polymerase (PARP) inhibitors.

Death cell receptor-mediated anoikis/apoptosis seems to be also achieved, according to recent studies, by quinazoline-derived α1-adrenoceptor antagonists. Also the inhibition of IGF-1R signaling (Insulin-like growth factor-1 receptor activation of both MAPK, mitogen-activated protein kinases, and PI3K, phosphatidil-inositol 3-kinase/AKT) can exert cancer cell growth decrease. Regarding it, anti-IGF-1R antibodies may provide tumor growth inhibitory effects. VEGFR multikinase inhibitors (Sorafenib, Sunitinib) as well as anti-VEGF monoclonal antibodies (Bevacizumab) or VEGF-decoy receptor Afiblercept, by playing an antiangiogenic role, may induce indirect antitumoral effects.

- Bone (metastasis)-targeted therapy through either RANKL (receptor activator of NF-kB ligand) antagonist Denosumab or endothelin-A receptor antagonist Atrasentan; also bone-seeking α-emitter Ra-223 seems to be useful, intravenously administered, in bone metastatic CRPC;
- Immunotherapy: anti-prostate cancer autologous dendritic cell-based vaccine (e.g., Sipuleucel-T, made-up of autologous dendritic cells loaded ex vivo with PAP, prostatic acid phosphatase, tumor-associated antigen), anti-cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) monoclonal antibody Ipilimumab to overcome chemotherapy-resistance;
- Epigen-therapy through hystone-deacetylase inhibitors, such as suberoylanilide, or DNA-methyltransferase blockers;
- Targeting the pluripotent stem cell (PSC)-like cancer cells as expressing PSC-transcription factors such as OCT3/4 and SOX2, that can start the prostate tumorigenesis.

Cytoskeletal Molecule Cargo Logistics

The cytoskeletal complex includes cell interior microtubules and cell cortex actin microfilaments together with wide microtrabecular reticulum. The microtubules are hollow cylindrical structures, whose wall is made up of a series of small protein heterodimeric subunits, a negatively charged α-tubulin and a positively charged β-tubulin, thus, such dimer sets acting as dipoles. Showing a dynamic plastic behaviour, they are capable of self-polymerization or, instead, self-depolymerization, depending on the various cell conditions. The energy needed for their polymerization is supplied by GTP (guanosine triphosphate) hydrolysis to GDP. Actin microfilaments are helicoidal temporary structures – dissipative structures – of G-actin globular protein, which rapidly develop, by polymerization, from a cytosolic stock of their subunits, and, just as rapidly
dissolve as soon as they are no longer necessary. The energy required for their polymerization is provided by ATP (adenosine triphosphate) hydrolysis to ADP. Some drugs are able to specifically enhance or inhibit both microtubule- and actin microfilament-polymerization. The microtrabecular reticulum consists of 3-D intertwined actin filament network, that interconnects all cell structures including the nucleus cytoskeleton and chromosomes, with following feed-back control system between nucleus and cytoplasm, moreover contributing to intra-cell dynamic organization of organelles.

Cytoskeletal functions exceed that of sheer mechanical cell scaffold, because the cytoskeleton plays an important role in cell contractility, cytokinetics and, given its links with the inner part of plasmalemmal receptors, in intra-cell signal transduction. Microtubules, as filamentary oscillating structures (Fröhlich’s coherent vibrations), cooperate with mitochondria in generating cell electromagnetic fields.

Molecular motors that transport molecule cargoes – protein complexes, receptor-ligand sets, secretory vesicles, organelles – along the cytoskeleton, include myosin family compounds, running on cell cortex actin filaments, and kinesin/cytosplasmic dynein, travelling on cell interior microtubules, both paths representing polarized mono-rails for the cargo bidirectional, either endocytic or exocytic, translocation, mean-while the motor proteins converting the ATP-to-ADP-hydrolysis-derived energy into motion.

The endocytosis plays a leading role in plasmalemmal receptor-ligand complex internalization, thus driving extra-cell molecular signals to induce specific changes in gene expression. That’s how occurs also for the stepwise endocytic movements, along actin-to-tubulin pathway, of several signaling receptors, such as HER-1, HER-2 and other growth factor receptors. In both endocytic and exocytic routes, selective logistic mechanisms put the vesicular cargoes with high specificity dependently on their coat protein components while protein adaptors are able to bind cargoes to specific coat pits. Moreover, to allow a selective recruitment of protein cargoes from the cytosol, membrane lipids, acting as specific “rafts”, sort them at sequential steps of both endocytic and exocytic paths. Just like this, the tyrosine kinase receptors, through a specific endocytic lipid rafts, are brought to the endoplasmic reticulum where PI3P (phosphatidylinositol-3, 4, 5-triphosphate), is synthesized, triggering, in turn, the PI3P/AKT-mTOR signaling pathway.

Quite recently, it has been demonstrated that TFEB (transcriptor factor EB), a master gene for lysosomal biogenesis, coordinates the whole logistics of autophagic pathway, from the formation of autophagosomes (cell scavenger organelles) and their intracellular ride to fusion with the lysosomes, where the substrate degradation is performed.

The biochemical pattern of cytoskeletal molecule cargo translocation must be today integrated with a biophysical model, where charged particles (electrons, protons, ions), photons (optical and infraoptical radiations) and quantized vibrational-mechanical waves are looked as signal-messengers travelling on the cytoskeleton. Furthermore, microtubules, as electrical polar structures – oriented dipoles – are able to direct the molecule cargoes towards specific targets, their charge-energy resulting from GTP (guanosine triphosphate) hydrolysis to GDP. What’s more, a fraction of such energy can induce cytoskeleton vibrations with following generation of electromagnetic fields. Just in this regard, some mechanisms of malignancy – anois of cancer cells, local invasion and distant metastasis – may be assumed as dependent on the cell abnormal electromagnetic influences.

Cytoskeleton Targeting Chemotherapy

Until the mid-1990s, CRPC was considered refractory to chemotherapy regimens while afterwards it has been demonstrated that the mitoxantrone-prednisone combined treatment can have a palliative effectiveness in CRPC-diseased men. Seven years ago, taxane (docetaxel)-based chemotherapy showed to carry a significant survival advantage in CRPC, further improved by recent resorting to cabazitaxel. Efforts intended to develop novel agents capable of overcoming drug resistance-related mechanisms have led to try the effectiveness of epothilones in taxane-refractory tumours.

Among the microtubule-inhibiting drugs, taxanes – docetaxel, paclitaxel, cabazitaxel – are able, by binding the tubulin, to cause microtubule polymerization-stabilization with following suppression of their dynamic properties at the mitotic spindle, that induces cancer cell mitotic arrest (cell cycle block at G2/M phase) and apoptosis.

Survival benefit achieved by docetaxel-based chemotherapy in patients with CRPC have denied
the burden of CRPC as a chemoresistant cancer. However, tumors characterized by P-glycoprotein (Pgp) overexpression show usually a docetaxel-refractoriness. Cabazitaxel, a novel taxane drug, unlike other taxane-based agents, exhibits low propensity for Pgp-mediated plasmalemmal drug efflux pump, thus avoiding the development of taxane-resistance, therefore showing an antitumor activity even in docetaxel-resistant CRPC. Cabazitaxel combined with prednisone can induce a median survival of 15.1 months, compared with that of 12.7 months achieved through mitoxantrone, as it results from phase III-TROPIC trial. 

Intriguingly, in the treatment of CRPC, microtubule-targeted taxane chemotherapy seems also to interfere with androgen-mediated signaling because taxanes may prevent the androgen-dependent nuclear translocation of the androgen-receptor (AR) by targeting AR connection with tubulin. In fact, AR, in the absence of ligand DHT, is primarily located, bound with Hsp90 (heat shock protein 90) chaperone, onto the cytoskeleton, while the link with the ligand results in AR homodimerisation and translocation in the nucleus, where binds to specific androgen-dependent genes with following proliferative and trophic effects. Incidentally, Hsp90 chaperone plays a critical role in prostate cancer cell survival response to chemotherapy and radiation, whereas Hsp90 inhibitors, such as I7-AAG (17-allylamino-17-demethoxygeldanamycin), can sensitize cancer cells to cytotoxic agents, including taxanes and radiation therapy.

RNA (RNA interfering)-mediated STMN1 microtubule-gene silencing can have synergistic effects with paclitaxel against the prostate cancer cells. Also histone deacetylase inhibitors, such as suberoylanilide hydroxamic acid, potentiate the taxane (docetaxel) anticancer activity in CRPC, by both acetylating tubulin and inhibiting Bel-2 antiapoptotic protein.

Unfortunately, cremophor, a nonionic polyethoxylated castor oil used as solubilizer/emulsifier of taxanes, has been associated with severe hypersensitivity reactions, sometimes including anaphylaxis and cardiac collapse. Recently, to avoid such drawbacks, it has been shown that paclitaxel delivery in cancer cells may result significantly enhanced by its binding with albumin nanoparticles (Abraxane). Nevertheless, endotoxin-like properties of taxol in itself, such as macrophage activation with production of inflammatory cytokines and nitric oxide, can induce hypersensitivity conditions.

Epothilones are a family of novel microtubule-targeting agents originally identified as metabolites of myxobacterium Sorangium cellulosum. Like taxanes, epothilones stabilize the microtubules and inhibit their dynamic function at the mitotic spindle, thus preventing cancer cells from mitosis. Particularly, once epothilones bind to β-tubulin subunit, the rate of its dissociation decreases with following tubulin polymerization, hence microtubule stabilization and suppression of microtubule detachment from centrosomes, with cell cycle arrest at the G2/M transition phase.

Both semisynthetic and total-synthetic epothilone analogs (ixabepilone, sagopilone, patupilone), currently undergoing various clinical development phases to treat different cancers, exhibit a cytotoxic activity even towards taxane-refractory cancer cell lines, without showing taxane-like endotoxin-properties.

Differences between epothilones and taxanes in drug-resistance mechanisms have been elucidated by recent research findings on the which basis epothilone cytotoxicity appears to be unaffected by alanine-to-threonine substitution at residue 364 – microtubule β-tubulin which, instead, is responsible for chemoresistance to taxanes. It follows that epothilones, unlike taxanes, maintain their cytotoxic performance even in cancers overexpressing Pgp.

Given their good water solubility, epothilones do no need cremophor-based solvents sometimes causing hypersensitivity reactions.

It has been demonstrated an epothilone-induced radiosensitivity enhancement in radioreistant human prostate cancer cells, because such agent is able to delay DNA-strand break repair together with prolonging cell cycle arrest and enhancing cell death.

Noscapine, a microtubule-modulating alkaloid, displays synergistic effects with docetaxel in anticancer chemotherapy, moreover exhibiting cytotoxic activity even in paclitaxel-resistant tumors.

Pertenoctoxins, besides targeting cytoskeletal actin, can induce apoptotic effects by increasing caspase-3 activity together with poly (ADP-ribose) polymerase (PARP) cleavage.

Implications for Current Approaches and Future Research Directions

Although among the different CRPC therapy modalities, the chemotherapy regimens might appear conceptually outclassed as exhibiting a scant tumor cell-selectivity if compared with new molecular mechanism-targeting agents – so-called smart
drugs – nevertheless “combo-strategies”, that combine the chemotherapeutic highly killing potential with specific mechanism-based products, seem to be effective antumor measures, moreover allowing a lower cytotoxic drug doses with following more limited drug-related toxic side effects. So, both microtubule (taxanes, epothilones, Vinca-derivatives)- and actin filament meshwork (pertenotoxins, cytochalasin D)-targeting agents may supply valuable outcomes in CRPC – as well as in other advanced malignancies – either alone or in combination with “smart drugs” such as tyrosine- or multi-kinase receptor blockers, mTOR (mammalian target of rapamycin) inhibitors, monoclonal antibodies against various growth factor signaling receptors⁴⁸,⁶⁵,⁷⁰.

Currently, other cytoskeleton targeting products are under investigation to use them in cancer chemotherapy, among which some antimycotics (benomyl, griseofulvin) and antimicrobials (sulfonamides), that, what’s more, seem to exhibit less toxic side effects than taxanes and Vinca alcaloids⁶⁷.

Recent studies show that microtubule-targeting drugs, besides the suppression of microtubule dynamics with following cell cycle arrest at G2/M phase, can promote cell apoptosis via intrinsic mitochondrial, cell death-receptor-independent, pathway by inducing the translocation of Bax (Bcl associated x-protein) from cytosol into mitochondria and, sequentially, Bak (Bcl-2 antagonist killer) mitochondrial protein activation with release into cytosol of some pro-apoptotic factors such as Mg²⁺-dependent endonuclease G and SMAC (second mitochondria-derived activator of caspase), that, in turn, promote the 9 → 3 caspase cascade⁶⁸,⁶⁹.

As far as taxanes are concerned, it’s now arousing a great interest that their microtubule-inhibiting effects may be enhanced by albumin nanoparticle carriers that, moreover, allow to avoid the use of solubilizing cremophors sometimes inducing severe hypersensitivity effects⁵⁴,⁷². Other nanoparticle – based carriers, such as nanoporous silicon particles, for chemotherapeutic shunting, have been recently proposed⁷².

Epothilones, as well as other microtubule-stabilizing agents (taxanes, nocapine), prolong activation of the spindle assembly checkpoint with following cancer cell death in mitosis, and, in addition, show a certain activity also in truly taxane-refractory patient cohorts, thus representing a potential therapeutic niche when a Pgp-induced taxane-resistance unfortunately develops⁶⁷. It has also been demonstrated an epothilone B-induced radiosensitivity enhancement in radiorefractory prostate cancer cells, however the radiation therapy resulting to be excluded from the treatment of metastatic CRPC while it might have therapeutic effects on locally-advanced CRPC⁶³.

The application of chemotherapy regimens in CRPC, as well as of other anticancer modalities, requires a preventive assessment of their possible effectiveness, individually considering tumor distant invasive propensity, about it today playing an important role the detection of both circulating prostate cancer cells through PSAmRNA/RT-PCR (reverse transcriptase-polymerase chain reaction) assay and, more thoroughly, circulating cancer stem cells (CSCs) responsible for resistance to chemotherapy and radiation. Indeed, cancer cells expressing pluripotent stem cell transcription factors, such as OCT3/4 and SOX2, exhibit quite uncontrollable aggressive aptitudes³⁵.

Conclusions

The development of techniques capable of molecularly identifying prostate CSCs not only may provide relevant predictive informations to make proper clinical-therapeutic decisions, together with allowing potential indications of efficacy, but also can entail the expansion of studies on CSC-targeted drugs³,⁷¹,⁷³. The constant strong challenges in the discovery of more and more efficacious agents to manage CRPC, moreover tailoring the treatment to the individual patient gene-molecular cancer cell features, reflect the frustrating condition due to drug-resistance development in addition to hormone-refractoriness⁷³,⁷⁵.

Conflict of Interest

None to declare.

References

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