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Prostate cancer: radioresistance molecular target-related markers and foreseeable modalities of radiosensitization

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Abstract. – OBJECTIVES: Though the external beam radiation therapy is a standard treatment option for both organ-confined and regionally advanced prostate cancer, unluckily, despite more and more effective advances in radiation delivery procedures, the prostate cancer radioresistance still occurs in a significant amount of patients undergone radiotherapy. This review aims to highlight the molecular aberrations of prostate cancer cell growth- and apoptosis signaling pathways that might induce, together with both prostate cancer cell/cancer stem cells gene- and surrounding microenvironment crucial implications, the tumor radioresistance.

METHODS: An up-dated review of several thorough studies on such matter.

RESULTS: The plenty of intriguing knowledge acquisitions about the prostate cancer radioresistance depending on cancer cell growth/apoptosis signaling pathway-related molecular aberrations together with prostate cancer cell/cancer stem cell abnormal gene profile, may be the premise leading – on the basis of preclinical research in animal models – to clinically overcome the tumor radioresistance.

CONCLUSIONS: Current developments of radiosensitizer agents focusly targeting prostate cancer cell radioresistance-associated specific molecular/gene aberrations are directed to improve, by implementing customized tumor radiosensitization modalities, the radiation therapy outcomes.

Key words:
Cell growth pathway, Apoptosis pathway, Cancer stem cells, Radiosensitizers.

Introduction

Though the general cancer incidence rates, during the five-year period, slightly declined in North American men, by 0.6% per year, with male cancer death rates lowering by 1.8% per year, and were, instead, stable in women with a female cancer death decrease by 1.4% per year, the *prostate carcinoma* (PCa) remains a considerable health burden in developed countries, it representing, after the skin tumors, the most common cancer in men, about 1 man in 7 running into PCa onset during lifetime. According to American Cancer Society, PCa is the second leading cause of cancer death in North-American men, after the lung cancer[1]. In European Union, the PCa incidence sets at fourth site among the most common malignancies including female breast-, colorectal-, lung tumors[2]. Organ-confined PCa affects around 70% of cases whose the majority shows low-to-intermediate disease clinico-pathologic features[3].

External beam radiation therapy (EBRT) is a standard treatment option for both organ-confined and regionally advanced PCa, while brachytherapy strategies, with either 125 I or 103 Pd low dose rate (LDR) permanent implant and 192 Ir high dose rate (HDR) temporary implant, may be applied to treat low-to-intermediate risk intraprostatic tumor.

Despite radiation delivery technological advances, the rate of biochemical/clinical relapse for a significant number of PCa patients undergone radiotherapy unfortunately remains high[3-6]. Given that gene expression profiles may predict, for PCa patients, an individual possible radioresistance, the identification of aberrant both cancer cell growth or death signaling pathway-related specific biomarkers can implement customized radiosensitization modalities by the resort to molecular targeted agents (7). Moreover, the more and more thorough characterization of prostate cancer stem cells (PCSCs) suggest that they may significantly be responsible for both radio- and chemoresistance, hence it following that PCSC radioresistant subpopulation-targeting strategies can improve PCa radiotherapy[7-9].

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Aberrant cell growth signaling pathway-related prostate cancer radioresistance

Radioresistant prostate cancer cells often exhibit high epidermal growth factor (EGF) receptor levels, hence resulting a considerable activation of phosphatidylinositol 3-kinase (PI3K)-Akt and mammalian target of rapamicin (mTOR) – PI3K-Akt/mTOR – pathway, besides mitogen-activated protein kinase(MAPK) and Janus tyrosine kinase (Jak)/signal transducer activator of transcription (STAT) – Jak-STAT – pathways[10-12]. Otherwise, even the exposure of cells to ionizing radiations may activate such signaling pathways – among which particularly the MAPK/ERK(extracellular-regulated kinase)-mediated one – with various downstreams that result to be dependent on different expression of cell plasma-membrane growth factor receptors and influence of autocrine ligands, such as transforming growth factor-alpha or beta, or even triggered by Ras mutations[10-14].

DOC2/DAB2 interactive protein (DAB2IP), that normally has a control over the PI3K/Akt pathway, is often down-regulated in aggressive PCa, from it resulting the onset of PCa cell radioresistance due to easier DNA double-strand break repair – so it maintaining the telomere stability – together with apoptosis evasion. Therefore, adjuvant treatment with *NU7441,* as a novel agent leading to ineffective DNA repair besides blocking overactivated PI3K-Akt/mTOR pathway, can overcome DAB2IP loss-induced PCa cell radioresistance[15,16]. Moreover, radiosensitizing effects may be achieved by targeting PI3K-Akt/mTOR pathway by imidazol-quinoline derivative *NVP-BEZ 235*, which, in addition, has the potential to inhibit the expression of HIF-1alpha (hypoxia-inducible factor-1alpha), another intriguing target for tumor radiosensitization[11,13,17,18].

Regarding the Jak-STAT persistent activation-promoted PCa cell survival against ionizing radiation, the radiosensitization could be reached by block such pathway by resorting to *AG 490*, a STAT 3 inhibitor[8].

As for cell cycle modulation and DNA repair, P53 gene, through its own codified p53 transcriptional factor protein, plays a central role by regulating the expression of different genes concerning either cell growth or apoptosis, that's why P53 deletion/mutation-induced loss of p53 functions may promote dramatic effects, among which the tumorigenesis with development of cancer cell radioresistance. What's particularly due to increased interactions between p53 and MDM2 (mouse double minute 2) protein, that, when aberranttly overexpressed as it sometimes occurs under the radiation stress, may induce the p53 inactivation, through its ubiquitination and intraproteasome degradation, with subsequent lack of p53-dependent normal functions. It follows that the use of drugs preventing the p53-MDM2 interactions – such as the anticancer *Nutlins,* cis-imidazoline analogs – can lead, by allowing the p53 accumulation/activation, to the p53-mediated efficient block of cancer cells growth meanwhile with activation of their apoptosis mechanisms[19-22]. Otherwise, the P53 gene mutations can themselves make radioresistant the cancer cells, that's why the restoration of wild-P53 can induce their radiosensitization as it has been shown in human PCa xenograft animal models[5-12]. Moreover, given that Reprimo (RPRM) tumor suppressor gene is normally involved in p53-mediated radiation-due G2/M phase cell cycle arrest, an aberrant epigenetic methylation of DNA-RPRM promoter region, can facilitate both tumor progression and apoptosis evasion together with development of cancer cell radioresistance. In this regard, the nucleotide analog *5-azacytidine,* as methyl-transferase inhibitor, can avoid the aberrant DNA-RPRM silensing, thus it radiosensitizing the cancer cells[23,24].

Aberrant cell death signaling pathway-related prostate cancer radioresistance

For the androgen-sensitive prostate carcinoma, the androgen deprivation therapy (ADT) leads to apoptosis of hormone-dependent cancer cells meanwhile unfortunately selecting out, after an average of 18 to 22 months, hormone-refractory cell clones, thus the cancer cell apoptotic evasion contributing, together with abnormal cancer cell proliferation, to tumor progression. Ionizing radiation can not only act on the nucleus-leaving signalling pathways, by inducing the DNA damage, but also by activating, even at the plasma membrane level, the apoptotic process-driving one[25,26].

The apoptotic mechanism encompasses crucial molecular events particularly involving two distinct routes, where various caspases (interleukin converting enzyme, ICE-like cysteine proteases) have an important role:the one – intrinsic/cell surface receptor-indipendent, mitochondrial activity involving, pathway – including translocation of Bax (Bcl 2-associated x-protein) from cytosol into mitochondria, hence activation of mitochondrial protein Bak (Bcl 2 antagonist killer)with subsequent release of several mito-

chondrial pro-apoptotic proteins such as cytochrome C and SMAC (second mitochondria-derived activator of caspases), that lead to activate the procaspase 9 – caspase 9 – caspase 3 sequence, while the other – extrinsic/cell surface receptor-dipendent pathway – resulting from stress/cytotoxic agent (among which ionizing radiation)-induced activation of transmembrane "death receptors", including Fas/APO 1 (apoptosis inducing protein 1), also known TNFRSF6 (tumor necrosis factor-receptor super-family 6) as crosslinked by tumor necrosis factor-alpha (TNF alpha). The apoptotic signal down-stream emerging from such plasma membrane receptor activation, sequentially includes procaspase 8 – caspase 8, down to caspase 3, that, as a crucial key protease mediator of both intrinsic and extrinsic pathways, promotes the proteolytic cleavage of poly(ADP-ribose)polymerase-1(PARP-1), thus allowing the apoptotic process closing endonuclease-mediated DNA fragmentation[27-29].

The anti-apoptotic gene Bcl 2(B-cell lymphoma) overexpression may promote an aggressive behaviour of PCa cells with their heavy radio/chemo-resistance, that's why a recently identified Bcl 2 inhibitor *HA14-1* can improve the cancer cells apoptosis meanwhile enhancing their radiosensitivity(30). Otherwise, the clusterin, a glycoprotein overexpressed in various malignancies, protects, by interfering with Bax proapoptotic activity, the cancer cells from TGF beta(transforming growth factor-beta)-promoted apoptotic mechanisms, it following that, by down-regulating its expression through specific antisense nucleotide *OGX-011,* the apoptosis might be restored together with cancer cell radio- and chemo-sensitivity[15,31-33]. Furthermore, also the survivin can facilitate the cancer cell survival – though upon cell death stimuli such as ionizing radiation – by interfering with the caspase activity, hence it resulting that the survivin inhibitor *YM155* can sensitize PCa cells to radiation[9]. Otherwise, the over-expression of cell surface membrane integrin"alpha v beta 3" significantly prevents the radiation-induced downregulation of survivin, that's why c*RGDfV*, as an antagonists of such integrin and survivin-mediated anti-apoptopic signaling, may allow the achievement of radiation-promoted PCa cell apoptosis[34].

Given that the above-mentioned PARP-1 may play an antiapoptotic role, as preventing caspase/endonuclease-induced DNA fragmentation, its block by specific inhibitors – such as *veliparib, rucaparib, niraparib* and particularly *olaparib* – can maximize the DNA damage-related cancer cell death, so it reaching an effective PCa cell radiosensitation in both EBRT and alpha-emitter Ra 223 radiopharmaceutical treatment[35,36].

Among various molecular factors involved in the apoptotic process, the *ceramide* plays a crucial role just regarding its changeable conditions under radiation treatment. Indeed, the radiation stress promotes the membrane-associated sphingomyelin hydrolysis with generation of ceramide that acts as a potent proapoptotic mediator by inhibiting Bcl 2 protein-induced mitochondrial depolarization, therefore the ceramide production during radiotherapy predicting a treatment favourable outcome[37,38]. Unfortunately, the surrounding tumor microenvironment ceramide accumulation, induces, by feed-back, the acid ceramidase (N-acylsphingosine amidohydrolase) gene up-regulation, that, in turn, leads to production of ceramide catabolite sphingosine and its mitogenic phosphorilated derivative sphingosine-1-phosphate which, besides its ineffectiveness to maintain the ceramide's role in the apoptosis, may activate the Akt pathway, hence it enhancing cancer cells proliferation together with supporting their radioresistance. It follows that acid ceramidase proteolytic degradation promoters, such as lysosomotropic agents *LCL 521* and *LCL 385,* could maintain the ceramide-associated apoptotic process meanwhile radiosensitizing PCa cells[39,40]. In addition, even the *toremifene,* a structurally tamoxifen-like antiestrogen, has been recognized to be an efficacious inhibitor of the acid ceramidase activity, thus its use allowing to restore both the cancer cells death and PCa radiosensitation [41].

Unlike cancer cell apoptosis, the autophagy – as lysosomal machinery driving to ubiquitin-mediated degradation of cell'own components and cytoplasm sequestration into autophagosomes with vacuolated appearance – maintains cells alive in response to different stress stimuli, including the ionizing radiation, what contributing to cancer cell radioresistance. Therefore, autophagy blockers, such as a lysosomotropic antimalarian chloroquine-like *bafilomycin,* may act as cancer cell radiosensitizers[8,9].

Cancer stem cells and radioresistance

The prostate cancer growth, as well as the any tumor one, is driven by the subpopulation of prostate cancer stem cells (PCSCs) – also called tumor-initiating cells – that, in addition, provide a cellular reservoir to promote tumor recurrence after therapy[18,42].

Today's more and more reliable characterization of PCSCs – by taking advantage from stem cell sphere-forming assay – particularly with identification of their specific markers expression, suggests that they are highly responsible for both radio- and chemoresistance, by enhancing the cancer cell proliferation and supporting the apoptosis evasion[8,9,43]. PCSCs, indeed, by their ATR (Ataxia-telangiectasia mutated/Rad-related kinase protein)-mediated DNA repair mechanisms, make sure their own survival, in niche-guarted quiescent state (late "S" cell cycle phase), against ionizing radiation, compared, instead, with the high radio- and chemosensitivity of proliferating cells in G2/M phase[8,42,44]. Otherwise, the specific gene mutation-dependent over-activation of stem cell specific pathways – such as Wnt/beta catenin-, Hedgehog- and Notch pathways – play an important role in facilitating both PCSCs self-renewal and radioresistance. As for the beta-catenin, its abnormal accumulation, resulting from over-activated Wnt signaling, can increase the PCa progression, as well as of other tumors, by enhancing the specific cell growth-dependent transcriptional activity meanwhile supporting the production of survivin that, in turn, can interfere, as it has been above mentioned, with the apoptosis-associated caspase protein family; that's why the survivin inhibitor *YM155* may induce anticancer-radiosensitizer effects[9]. Furthermore, the *perifosine,* besides blocking the Akt pathway, can also inhibit the Wnt signaling with following restoration of tumor radiation sensitivity[8]. On the other hand, the aberrant activation of Hedgehog pathway seems to lead to development of various malignancies, including PCa, through the transformation of adult stem cells into PCSCs, which, because of own inside decrease in E-cadherin (E-calcium-dependent adhesion glycoprotein) and, instead, increase in pro-cell growth cyclins, give rise to a tumor onset with radioresistance. Regarding the Notch pathway – as a family of cell transmembrane protein-receptors – its disregulation, apart from maintaining the cancer cell self-renewal stemness, can facilitate the tumor radioresistance onset together with metastasis[45,46].

Recent intriguing studies have allowed to identify some PCSC radioresistance-related genes, such as PCSC-1 and PSCS-3 RAN (Ras-associated nuclear protein)-signaling genes, that are involved in the DNA synthesis and in cell cycle promotion. It follows that the PCSC-1 and PCSC-3 RAN signaling genes might be an important target to hopefully reach the PCa radiosensitization[9]. In addition, CXCR4 (chemokine CXC motif receptor 4) has been recently recognized as a biomarker for both drug- and radioresistant cancer stem cells, the interaction of such receptor with its ligand (CXCL12) playing a crucial role in protecting them from anticancer agents and radiation. Therefore, a feasible therapeutic block of the CXCR4/CXCL12 signaling pathway should represent a promising opportunity to refine the PCa radiation therapy[47].

Surrounding microenvironment-dependent tumor radioresistance

Different both cell components of tumor surrounding microenvironment – such as fibroblasts, endothelial cells and vascular network, infiltrating immune cells – and bioactive factors, including growth factors, cytokines, hormones, variable level of oxygen/ROS (reactive oxygen species, generated by radiation-induced ionization of water molecules), can significantly influence the cancer growth, particularly with the involvement of CSC behaviour. In this regard, it has been highlighted that fibroblast- and endotelial cell-made niches of CSCs, protect them from radiation stress with following up-regulation of CSC-associated specific signaling pathways – such as Wnt/beta catenin-, Hedgehog- and Notch pathways – that accelerate the CSC self-renewal[8,9].

Given that oxygen is a potent ROS-mediated tumor radiosensitizer, a hypoxic microenvironment, with subsequent low ROS levels, prevents cancer cells from radiation-induced oxidative DNA damage, so driving them to radioresistance, moreover their survival advantage also resulting from HIF 1alpha expression-mediated inhibition of apoptotic process[48-50]. The significance of antitumor DNA oxidative damage-mediated effects – at least partly – of radiation and also of some anticancer agents, appears to be understandable considering that their activity results to be often made ineffective by ROS-scavengers – such as N-acetylcysteine or glutathione or even dihydrolipoic acid – what recently has been shown by the evaluation of Alternol anticancer activity in several PCa cell lines[51]. On the other hand, the microenvironmental ROS-scavenger glutathione depletion by *buthionine-sulfoximine* can reverse the radioresistant cancer cell condition[8].

To increase the radiation effects in hypoxic cancer cells, may be useful the resort to administration of the imidazol-quinoline derivative *NVP-BEZ 235,* which, a part from the block on PI3K/mTOR pathway, as it has been above mentioned, can also inhibit the HIF1alpha in hypoxic cancer cells, so improving the radiotherapy outcomes[17].

Some significant biomarkers of prostate cancer radioresistance

The identication of cancer cell radioresistance-related predictive biomarkers allows the potential of either select alternative treatment modalities or, at least, plan a radiation therapy in combination with specific radiosensitizer agents. Besides the above mentioned cell growth/cell death signaling pathway-related molecular aberrations, recent research results suggest that acquisition of EMT (epithelial-mesenchymal transition) from cancer cells is highly indicative of their radioresistance. Such cancer cell occurrence is characterized, indeed, by a down-expression of E-cadherin compared with the mesenchyme-peculiar expression of vimentin[18].

A significant predictor of PCa cell- and, particularly, of PCSC-radioresistance is represented by their CD44-v6 marker, that appears to be positively significant when microenvironmental basal or radiation-induced ROS levels are relatively low(8). Knock down of CD44-v6 in PCa cell lines can suppress, by following down-regulation of both Wnt/beta catenin- and PI3K-Akt/mTOR signaling pathways together with drop in EMT, tumor cell proliferation meanwhile enhancing PCa cell line radio- and chemosensitivity[52].

Besides the CD44-v6, other biomarkers, particularly the PCSC-associated ones – such as CD133, ABCG2 – may be a crucial factor for a radio-curative option[43].

The discovery of PCSC-peculiar radioresistance genes, among which the PCSC 1- and PCSC 2 RAN signaling ones, provides a further explanation of post-radiotherapy PCa recurrence (9,53). In addition, even the above mentioned CXCRA over-expression from PCSCs is highly indicative of PCa radioresistance, that's why the foreseeable inhibition of CXCRA/CXC ligand pathway might play a radiosensitizer role[47].

In the field of today's normal laboratory procedures, an elevated, immunoreactivity-tested, PCa cell Bcl2/Bax ratio, may be predictive of a worse response to radiation, it, for a long time now, suggesting the usefulness of such biomarker for prognostic evaluation of PCa radiotherapy[54].

Concluding remarks and new directions

In the last decades, more and more intersting and effective EBRT advances – from the 3D-conformal radiotherapy to the intensity-modulated one and even to the image-guided radiation delivery – together with adopting a suitable dose hypofractionation modality given that low alpha/beta PCa cell radiosensitivity ratio, have significantly improved, compared to the past, both biochemical and clinical outcomes for patients with organ-confined/locally advanced PCa undergone to radiation therapy[4,6,55,56]. Nevertheless, despite progress in the EBRT procedures, the PCa radioresistance – with the disease progression or local recurrence, both emerging by PSA's levels continuously rising above the radiation therapy-reached nadir and pointed up by prostatic biopsy – still occurs up to third of PCa patients undergone radiotherapy[3,11,37,57]. More thorougly, according to recent data[58,59], the biopsy-defined primary Gleason (pG) grade may represent an indipendent predictor for biochemical recurrence-free survival (BRfs), distant metastasis(DMfs)and PCa-specific mortality (PCa-SM), as 8-year BRfs rate for pG3 amounting 77.6% versus pG4 (61.3%), pG3-DMfs 96.8% versus pG4 (84.3%), pG3 PCa-SM 3.7% versus pG4 81.00%.

The role of different kinase hyperactivity – such as of PI3K, MAPK/ERK, Janus tyrosine kinase – in sometimes inducing PCa radioresistance has been highlighted in detail as far as the aberrations of cell growth signaling pathway[10-14,17], as well as the antiapoptotic-radioresistance promoting effects of Bcl-2, clusterin, survivin and, in addition, HIF1 alpha – compared with proapoptotic effects of Bax, Bak, caspase sequence – have been clearly taken into consideration in regard to aberrant cell death signaling pathway[25-38,49,50].

Current genetic profile/transcriptional pattern studies can provide reliable specific data predictive of radiotherapy long-term outcomes, thus it allowing to identify PCa patients who, as not expected to be responsive to radiation, might be undergone an alternative therapy such as surgery or hormone/anticancer chemotherapeutic agents[5]. In this regard, EBRT combination with either neoadjuvant- or adjuvant ADT/androgen receptor antagonists, such as the novel enzalutamide, can sometimes lead to more effective PCa local control and disease-free survival, with particularly favourable prognosis for high-risk PCa patients[4,22,60,61].

It's of the utmost importance to take into consideration that in elderly cancer patients, different comorbidities can influence both the acute and late

radio- or combined radio-chemotherapy dependent toxicity, it leading to carry out customized radiotherapy/chemotherapy treatments[62-64].

The implications of PCSCs in the tumor radioresistance development, with local recurrence and metastatic spread after radiotherapy, make more and more useful the resort to the identification of radioresistance-peculiar PCSC genes, such as the PCSC1- and PCSC2-RAN ones, highly predictive of the radiotherapy ineffectiveness[8].

In the field of gene therapy, radiosensitizer siRNA-based novel bio-approaches are today under study, particularly to suppress CSC-associated specific signaling pathways[8,9,45,46].

Foreseeably, further developments of *customized* radiosensitizers – focusly targeting the highlighted PCa cell radioresistance-linked specific molecular aberrations – will improve the PCa radiotherapy outcome[4,6,9]. In addition to various radiosensitizers acting at above-mentioned different signaling pathway levels, novel anticancer agents, such as cytoskeletal microtubule-stabilizing epothilone B-derived drugs, have been preclinically recognized to be provided with high potential of radiosensitizing various cancer cell types, among which the PCa cell ones, by inducing the G2-M cell cycle arrest and tumor growth delay[65-67].

On the basis of deepened radiobiological features – such as high linear energy transfer, radiobiological effectiveness, tumor dose delivery targeting, tumor/healthy tissue damage ratio – both hadron beam proton/neutron- and carbon ion radiation-therapy seem to successfully overcome both tumor intrinsic and extrinsic radioresistance classical conditions (CSC-related risk, crucial alpha/beta cancer cell sensitivity ratio, microenvironmental hypoxia, etc.) meanwhile better sparing pelvic organs in comparison with EBRT[68-72].

Conflict of Interest

The Author declares that he has no conflict of interests.

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