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REVIEW

Metformin and prostate cancer stem cells: a novel therapeutic target

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Prostate cancer is the second most frequently diagnosed cancer in the world. Localized disease can be effectively treated with radiation therapy or radical prostatectomy. However, advanced prostate cancer is more difficult to treat and if metastatic, is incurable. There is a need for more effective therapy for advanced prostate cancer. One potential target is the cancer stem cell (CSC). CSCs have been described in several solid tumors, including prostate cancer, and contribute to therapeutic resistance and tumor recurrence. Metformin, a common oral biguanide used to treat type 2 diabetes, has been demonstrated to have anti-neoplastic effects. Specifically, metformin targets CSCs in breast cancer, pancreatic cancer, glioblastoma and colon cancer. Metformin acts directly on the mitochondria to inhibit oxidative phosphorylation and reduce mitochondrial ATP production. This forces tumor cells to compensate by increasing the rate of glycolysis. CSCs rely heavily on mitochondrial oxidative phosphorylation for energy production. The glycolytic switch results in an energy crisis in these cells. Metformin could be used to exploit this metabolic weakness in CSCs. This would increase CSC sensitivity to conventional cancer therapies, circumventing treatment resistance and enhancing treatment efficacy. This review will explore the characteristics of prostate CSCs, their role in tumor propagation and therapeutic resistance and the role of metformin as a potential prostate CSC sensitizer to current anticancer therapies.

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INTRODUCTION

Prostate cancer (PCa) has a long natural history. When prostate cancer is localized and is classified as a low Gleason grade tumor, it can be monitored with active surveillance. Higher-grade cancers are effectively treated by surgical resection of the prostate (radical prostatectomy) or radiation therapy.[1,2] However, if the cancer invades through the capsule into surrounding tissue, or recurs after local therapy, it is much more difficult to treat. If the disease metastasizes, it is generally incurable.[3,4] Advanced-stage PCa is usually treated with androgen-deprivation therapy (ADT). Most patients with metastatic disease managed with ADT eventually relapse with castration-resistant prostate cancer (CRPC) and die of the disease.[3,4] CRPC can be treated with docetaxel, abiraterone plus prednisone, enzalutamide, and cabazitaxel, which provide significant survival benefits, but are not curative.[5] Thus, there is still a need to improve the therapeutic options available for advanced-stage prostate cancer patients.

Prostate cancer is a multifocal disease. Prostates often contain multiple independent and genetically distinct foci.[6] One model that explains this heterogeneity is the 'cancer stem cell' model. This model postulates that only a small subset of cancer cells within a tumor have the ability to sustain tumorigenesis, resulting in a hierarchical organization of primary tumors.[7,8] This subset of tumor-propagating cells are defined as cancer stem cells (CSCs), as they share a number of characteristics with normal stem cells, one such example being self-renewal.[7–12] CSCs were first identified and characterized in hematological malignancies by Bonnet and Dick.[13] Since then, CSCs have been identified in several solid tumors including breast, pancreas, colon, lung, brain and prostate.[14–19] The CSC model is intriguing because it provides an explanation for tumor recurrence and resistance to conventional cancer therapies.[8] The discovery of new agents that can sensitize CSCs to conventional cancer treatments holds therapeutic promise. Metformin, a common well-tolerated oral biguanide prescribed for type 2 diabetes, has been shown to selectively target cancer stem cells in several types of solid tumors and improve the efficacy of radiation and chemotherapy in breast cancer and colon cancer.[20–23]

Targeting therapy-resistant CSCs in prostate cancer provides a unique opportunity for novel therapeutic interventions. Metformin could be used to sensitize prostate CSCs to current conventional anticancer therapies and improve efficacy of treatment. This review evaluates the current evidence regarding the role of CSCs in prostate cancer and focuses on the following topics: (1) identification and characterization of prostate CSCs; (2) role that CSCs play in tumor propagation and therapeutic resistance; (3) role of metformin as a prostate CSC sensitizer for conventional therapy.

PROSTATE STRUCTURE AND DEVELOPMENT

The prostate is a glandular organ comprised of three distinct epithelial cell types. Basal cells are found along the basement membrane of each prostatic duct and express CK5, CK14, CD44,

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CD133, p63, Bcl-2 and low or undetectable levels of androgen receptor (AR).[1,6,7,24] Luminal cells form a layer above the basal cells and are the predominant cell type of the prostate.[1,24] Luminal cells express high levels of AR, CK8, CK18, CK19, CD57 and produce proteins such as PSA and prostatic acid phosphatase (PAP) that are secreted into the luminal space.[1,6,7,24] Neuroendocrine cells are a rare population, which is dispersed among the basal layer, are androgen-independent, express chromogranin A and secrete neuroendocrine peptides.[1,6,24]

The primary survival of basal cells following androgen deprivation led to the hypothesis that prostate stem cells reside within the basal layer.[7,24] Collins *et al*.[25] identified a small population of basal prostate epithelial stem cells, which expressed high levels of $\alpha_2\beta_1$. This was further corroborated by the identification of a small (1%) population of human prostate basal stem cells, which expressed CD133 and were restricted to the $\alpha_2\beta_1$ population.[26] Leong *et al*.[27] demonstrated that a single stem cell with the Lin-/Sca-1+/CD133+/CD44+/CD117+ phenotype was able to regenerate a prostate after transplantation *in vivo*. Lineage tracing has shown that multipotent basal stem cells give rise to basal, luminal and neuroendocrine cells, as well as unipotent luminal progenitors, which cause the epithelial expansion observed during postnatal prostate development.[28] Wang *et al*.[29] also demonstrated that basal cells and luminal cells undergo different types of division. Basal cells can divide symmetrically to produce two daughter basal cells or asymmetrically to produce one basal cell and one luminal cell.[29] However, luminal cells can only undergo symmetric divisions to produce two luminal cells and cannot produce any basal cells.[29] These results lend support to the theory that there is a hierarchy of epithelial lineages containing both multipotent and unipotent stem cells in the developing prostate.[29]

ROLE OF CSCs IN PROSTATE CANCER

CSCs are defined as a subset of tumor cells that have the capacity to self-renew and generate the heterogeneous cell lineages that comprise a tumor.[10,30] The term 'cancer stem cell' was established for this unique subpopulation of tumor cells because they possess properties similar to those of normal tissue stem cells, such as self-renewal by symmetric or asymmetric division, high proliferative potential and the capacity to differentiate into multiple cell lineages.[10,12,30,31] The CSC model postulates that CSCs give rise to a hierarchically organized, heterogeneous tumor, with the CSCs at the top of the hierarchy.[30] The CSCs then proliferate and generate PCa progenitor cells, which despite only having limited self-renewal abilities, can differentiate into many different mature cells types, such as CD57+AR+PSA+ luminal secretory cells.[12] The CSCs themselves are typically quiescent, whereas the majority of the proliferating population of tumor progenitor cells are generated by the CSCs.[12] CSCs are also thought to have an important role in cancer progression. Colombel *et al*.[32] demonstrated that primary prostate carcinomas contain a subset of CSCs, which have a role in local invasion, specifically seminal vesicle invasion and bone metastasis. The percentage of CSCs had a prognostic impact, particularly on the risk of progression of bone metastases.[32] The percentage of cells expressing a stem cell phenotype in bone marrow metastases of prostate cancer patients has been shown to be predictive of bone metastases progression.[33] There is also a small population of docetaxel-resistant cells, likely CSCs, that have been shown to be higher in metastasic compared to primary patient samples, and, in primary untreated samples, the percentage of these cells was associated with prognostic factors and time to biochemical relapse.[34]

The role of prostate CSCs in recurrence and development of CRPC is likely due to their intrinsic therapeutic resistance. Conventional anticancer therapies such as radiation, androgen deprivation and chemotherapy target the rapidly proliferating bulk of tumor cells, but they may not affect the small quiescent, androgen-independent CSC population. This reservoir of CSCs could then in the future, after therapy has been halted, begin proliferating again and give rise to another tumor, which would have survived selective pressure from therapy. The selection theory of androgen resistance postulates that there is a set of pre-existing ADT-resistant cancer cells in tumors.[35] Androgen depletion, therefore, provides selective pressure, killing the androgen-dependent cancer cells while androgen-independent tumor cells survive and can then give rise to castration-resistant tumors.[35] Prostate CSCs are candidates for these pre-existing ADT-resistant cells that could give rise to CRPC since they have self-renewal and tumor-propagating capabilities, as well as a lack of or very low AR expression.[35] Recurrent PCa tissue samples are enriched in CSCs, which are likely responsible for chemoresistance.[36]

An important distinction should be made between CSCs and the cancer cell of origin. The cell of origin refers to the initial cell that is the target of genetic alteration(s) that result in malignant transformation.[7,30] CSCs, in contrast, are tumor-propagating cells that sustain malignant growth.[37] CSCs may arise from a number of different types of cells including normal stem cells, restricted progenitors or differentiated cells.[30,38,39]

IDENTIFICATION AND CHARACTERIZATION OF PROSTATE CSCs

CSCs can only be identified and characterized experimentally.[40] The gold standard for defining CSCs functionally is serial transplantation in immunodeficient mice. This approach confirms the properties of self-renewal and tumor propagation.[4,10] Many CSC studies have used experimental methods that were established for enriching normal stem cells including transplantation of flow cytometry-sorted CSCs, side-population and ALDEFLUOR assays, and lineage-tracing studies.[7]

A number of different populations of prostate CSCs have been identified.[41] A population of CD44+/$\alpha_2\beta_1^{high}$/CD133+ cancer stem cells from human prostate tumor samples has been identified in human prostate tumor tissue.[19,42] The CD44+/$\alpha_2\beta_1^{high}$ population was also shown to be enriched in CSCs in LAPC-9 cells.[43] Populations of CD44+ prostate cancer stem cells have been shown to be more proliferative, tumorigenic and metastatic than CD44 – prostate cancer cells, which are all typical characteristics of CSCs.[44,45] Aldehyde dehydrogenase (ALDH) has also been shown to be a marker for prostate CSCs. ALDH+ prostate cancer cells have been shown to exhibit several CSC characteristics and is considered a marker for prostate CSCs.[46,47] ALDH+ prostate cancer cells have been shown to possess enhanced clonogenicity, migration, tumorigenicity and readily form metastases *in vivo*.[47] Qin *et al*.[48] also identified highly tumorigenic castration-resistant $PSA^{-/lo}$ CSCs, which can be further enriched to include a population of ALDH+/CD44+/$\alpha_2\beta_1$+ cells. ATP-binding cassette (ABC) transporter ABCG2+ prostate CSCs have also been isolated using the side-population method, which allows for the isolation of cells that can efflux Hoechst 33342 dye more efficiently because of elevated ABC transporter expression.[49,50] Stemness markers have also been used to characterize CSC populations in the prostate. Overexpression of Nanog in LNCaP and DU145 human prostate cancer cell lines promoted CSC properties and enhanced the expression of CD133, CD44, ALDH1A1 and ABCG2.[51] Prostate CSCs have also been shown to express Oct4 and Sox2 along with Nanog.[52,53] Guzel *et al*.[36] also showed an increased expression of Sox2, Oct4, Nanog, and ABCG2 in recurrent prostate cancer tissue samples, indicating an enrichment in prostate CSCs. A number of other cell surface markers identify prostate CSCs. These are included in Table 1.

Table 1. Markers for isolation of prostate CSCs

Marker	*Expression level*	*Characteristics*	*Reference*
ALDH	High	Enzyme oxidizes aldehydes	Li *et al.*,[46] Qin *et al.*,[48] Finones *et al.*[92]
ABCG2	High	ATPase transporter	Foster *et al.*,[49] Gangavaparu *et al.*[50]
CD44	+	Cell adhesion and signaling	Patrawala *et al.*,[44] Patrawala *et al.*,[43] Collins *et al.*,[19] Hurt *et al.*[45]
CD133 (Prominin-1)	+	Marker normal stem cells and CSCs	Richardson *et al.*,[26] Collins *et al.*[19]
c-Kit (CD117)	+	Receptor tyrosine kinase	Finones *et al.*[92]
Integrin $\alpha_2\beta_1$ (CD49b)	High	Collagen receptor	Collins *et al.*,[19] Patrawala *et al.*,[44] Guzman-Ramirez *et al.*[42]
CD49f	High	Laminin binding	Guzman-Ramirez *et al.*[42]
CD166	+	Cell adhesion	Jiao *et al.*[93]
PSA	−/lo	Glycoprotein	Qin *et al.*[48]
CK5/14	+	Cytokeratin	Tokar *et al.*[94]
CK8/18	+	Cytokeratin	Tokar *et al.*[94]
Nestin	+	Intermediate filament protein	Guzman-Ramirez *et al.*[42]
SCA-1	+	Cell surface marker	Lawson *et al.*,[95] Xin *et al.*[96]
SMO (Smoothened)	+	G-protein-coupled receptor	Patrawala *et al.*[44]
Sox2	+	Transcription factor (self-renewal)	Rybak and Tang[53]
Oct4	+	Transcription factor (self-renewal)	Patrawala *et al.*[44]
Nanog	+	Transcription factor (self-renewal)	Jeter *et al.*[51]

Abbreviation: ALDH, aldehyde dehydrogenase; CSC, cancer stem cell.

PROSTATE CSCs AND THERAPEUTIC RESISTANCE

Prostate CSCs are resistant to most conventional cancer therapies. Although there is some evidence that CSCs are radioresistant in breast cancer and glioblastoma cell lines, this has not yet been demonstrated in prostate cancer.[54–56]

Prostate CSCs are thought to contribute to the development of CRPC. Seiler *et al.*[57] demonstrated that ADT resulted in castration resistance and overexpression of stemness markers such as Sox2 and Oct4, indicating a role for prostate CSCs in ADT resistance. *In vivo* studies have demonstrated enhanced expression of stem cell markers in resistant tumors in castrated mice.[58] The peak expression of these markers also occurred soon after ADT, suggesting that prostate CSCs may have a role as an adaptive survival mechanism.[58]

CSCs may confer resistance to chemotherapy.[59] One contributing factor to this chemoresistance is the expression of ABC transporters. ABC transporters are membrane bound and can pump various small molecules, such as cytotoxic drugs and dyes, out of cells at the expense of ATP hydrolysis.[60] CSCs express high levels of ABC transporters. These pump chemotherapeutic drugs out of the cytoplasm, resulting in reduced intracellular drug concentrations.[60,61] Zhang *et al.*[62] demonstrated that tumorspheres from PCa cell lines expressing high levels of CD44 and ABCG2 were chemoresistant, likely due to the elevated expression of ABCG2. A subset of cells with high tumor-initiating capacity, likely CSCs, have been identified in both DU145 and 22RV1 human prostate cancer cells, as well as in human PCa samples. These cells contribute to docetaxel resistance and tumor re-initiation.[34]

METFORMIN AND PROSTATE CANCER

Metformin, a commonly prescribed and well-tolerated oral biguanide used to treat type 2 diabetes, has been shown to exert anti-neoplastic effects in several types of cancer. Metformin reduces hepatic gluconeogenesis, increases glucose uptake in peripheral tissue such as skeletal muscle and increases insulin sensitivity.[63,64] Evans *et al.*[65] showed a reduced cancer burden in diabetic patients treated with metformin compared with those treated with other diabetic therapies.[63] Since that initial paper, metformin has been shown to have anti-neoplastic properties in breast cancer,[66,67] ovarian cancer,[68] pancreatic cancer[69] and prostate cancer.[70] In prostate cancer, metformin inhibits the proliferation of LNCaP, DU145 and PC3 human prostate cancer cell lines and also reduced tumor growth in LNCaP xenografts.[70] In prostate cancer patients, metformin use is associated with a decreased risk of PCa diagnosis while other oral diabetes medications are not.[71] Increased cumulative metformin exposure after PCa diagnosis has been associated with decreased all-cause and PCa-specific mortality in diabetic patients.[72] A meta-analysis by Yu *et al.*[73] showed that metformin was associated with a significant reduction in cancer risk and biochemical recurrence.

METFORMIN TARGETS CSCs

In breast cancer, metformin effectively targets breast CSCs and enhances the effectiveness of some therapies. Song *et al.*[20] demonstrated that metformin was cytotoxic to radioresistant breast CSCs and increased the efficacy of radiation in suppressing tumor growth *in vivo*, likely by reducing the population of CSCs. Hirsch *et al.*[21] also showed that metformin targeted breast CSCs in four cell lines, enhanced the tumor-suppressing effect of doxorubicin and prolonged remission *in vivo*. Furthermore, metformin combined with a fourfold lower dose of doxorubicin was shown to be as effective as the standard dose of doxorubicin treatment alone *in vivo*.[74] Metformin has also been shown to enhance the effect of paclitaxel and carboplatin by targeting CSCs in breast cancer xenografts at doses comparable to the dose per kg used in type 2 diabetes patients.[74] In pancreatic cancer, metformin has been shown to significantly decrease cell survival, clonogenicity, wound-healing and sphere-forming capacity in pancreatic CSCs and also decreased the expression of CSC markers such as CD44, CD133, ALDH1, Nanog and Oct4.[22,75–78] In glioblastoma cell lines, metformin effectively reduced the proliferation of CD133+ CSCs in an Akt-dependent manner.[79] The CD133+ population of colon cancer CSCs have been shown to be significantly reduced by treatment with metformin and it enhanced the antiproliferative effect of 5-fluorouracil on colon CSCs.[22] In addition, metformin acts synergistically with 5-fluorouracil and oxaliplatin to inhibit cell proliferation and tumor growth of chemoresistant colorectal cancer cells by reducing the viability of colorectal CSCs.[23]

MECHANISM OF ACTION OF METFORMIN IN CSCs

Biguanides, such as metformin, exert their effects by decreasing oxidative phosphorylation which induces energetic stress.[80] This leads to a number of secondary cell lineage-specific effects.[81] Metformin action is mediated through direct effects on the

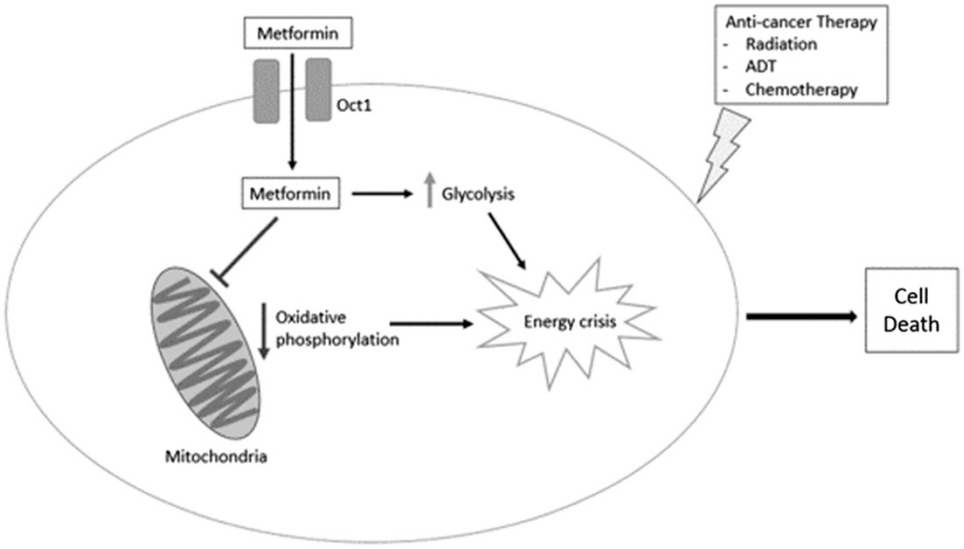

Figure 1. Mechanism of action of metformin in prostate cancer stem cells. ADT, androgen-deprivation therapy. Oct, organic cation transporter.

mitochondria, either by inhibiting mitochondrial complex 1 or the redox shuttle enzyme mitochondrial glycerophosphate dehydrogenase.[63,81–84] This causes inhibition of oxidative phosphorylation and reduced ATP production and oxygen consumption.[63] The decline in mitochondrial ATP production triggers activation of the cellular energy regulator AMP-activated protein kinase (AMPK) thereby directing cells towards an antiproliferative 'energy-saving' phenotype characterized by the downregulation of ATP-consuming processes such as protein and fatty acid synthesis.[63,80] The antiproliferative effect of this metabolic reprogramming by AMPK was thought to contribute to the anti-neoplastic effect of metformin. However, the activation of AMPK can enhance cell survival under the conditions of energetic stress and could in fact have a pro-survival effect.[85] The effect of metformin in PCa cells has been shown to be independent of the AMPK pathway.[86] Tumors with loss of LKB1, an upstream kinase required for AMPK activation, are hypersensitive to biguanides, which indicates that cancer cells deficient in AMPK will be less likely to reduce energy consumption due to biguanide-induced reduction in ATP production and would increase the likelihood of these cells experiencing an energy crisis.[85] More research is still needed to elucidate the antiproliferative and pro-survival effect of AMPK in cancer cells and the specific downstream effects of metformin-mediated inhibition of mitochondrial oxidative phosphorylation.

This mechanism of action of metformin has been evaluated in cancer. In human breast cancer cells, metformin directly inhibits mitochondrial complex 1 and citric acid cycle function, which reduces mitochondrial respiration.[81] Reduced mitochondrial oxidative phosphorylation results in impaired mitochondrial ATP production and forces cancer cells to compensate by increasing aerobic glycolysis.[81] Therefore, cells which rely more heavily on mitochondrial oxidative phosphorylation are more sensitive to metformin treatment and CSCs are included in this category.[81] Pancreatic cancer stem cells have been reported to have a highly mitochondrial-dependent metabolic profile.[78] This is quite different from the majority of cancer cells that proliferate uncontrollably and limit their metabolism primarily to glycolysis even in the presence of oxygen (i.e., aerobic glycolysis), known as the Warburg effect.[87] CSCs do not rapidly divide and therefore do not undergo this metabolic shift. If CSCs continue to rely on mitochondrial ATP production, they would be more sensitive to metformin treatment and would not be able to compensate effectively by switching to aerobic glycolysis. There are two possible scenarios related to the role of AMPK as the expression of AMPK in CSCs has not been fully elucidated. If CSCs do have functional AMPK, the activation of AMPK would likely prevent CSCs from shifting to aerobic glycolysis, which would cause an energy crisis and render CSCs incapable of compensation.[88] If the CSCs are deficient in AMPK, they would have an innate inability to compensate for an energy crisis and AMPK-deficient CSCs would, therefore, be more sensitive to metformin treatment.[88] Both scenarios would ultimately result in making CSCs more sensitive to additional stressors after metformin treatment, suggesting that metformin could be used as a CSC sensitizer for various types of cancer therapy (Figure 1).

The pharmacokinetics of metformin also play an important role in its mechanism of action as different tissues have varying levels of exposure to biguanides, such as metformin.[80] This is due to the fact that metformin requires membrane transport proteins such as organic cation transporter 1 (OCT1) to enter cells.[80] The varying expression level of transport proteins between cell types can affect the bioavailability of metformin. For example, hepatocytes express high levels of OCT1, which allows for enhanced cellular uptake of metformin and the accumulation of relatively high metformin concentrations in the liver.[85] Once metformin has entered a cell, the mitochondrial membrane potential causes an increased uptake of metformin into the organelle and results in the mitochondria being the site of the highest concentration of the drug within a cell.[85] The accumulation of metformin in the mitochondria may contribute to its ability to effectively inhibit oxidative phosphorylation and cause an energy crisis in CSCs. It is also possible that if CSCs have a higher OCT1 expression level, the effect of metformin on these cells would be enhanced. However, the OCT1 expression levels of CSCs have not been elucidated.

ROLE OF METFORMIN IN PROSTATE CANCER THERAPY

A few studies suggest that metformin may enhance the effectiveness of prostate cancer therapies. Metformin has been shown to enhance the effectiveness of ADT. Research conducted in our laboratory has shown that metformin combined with bicalutamide significantly reduces prostate cancer cell growth more effectively than either metformin or bicalutamide alone.[89] In addition, the combined treatment affected AR-positive LNCaP cells by altering cell proliferation, whereas combined treatment in

AR-negative PC3 cells promoted apoptosis.[89] Nguyen *et al*.[90] also showed that combined treatment of enzalutamide and metformin significantly reduced tumor size in enzalutamide-resistant LNCaP C4-2B xenografts, and combined treatment was more effective than enzalutamide or metformin alone. Iliopoulos *et al*.[74] demonstrated that metformin combined with doxorubicin suppressed tumor growth in PC3 xenografts.

Radiosensitization by targeting prostate CSCs is another potential benefit. Reducing oxygen consumption may have an impact on prostate CSCs as reducing oxygen supply would impair mitochondrial oxidative phosphorylation. This would result in CSCs compensating by using glycolysis, which would cause a cellular energy crisis that would make CSCs more sensitive to additional stressors such as radiation. In one study, prostate cancer patients taking metformin during radiotherapy had a significant reduction in early biochemical relapse.[91]

When considering future clinical trials, an important consideration is the potential benefit of conducting trials assessing the effect of metformin on prostate CSCs. Currently two trials are registered on clinicaltrials.gov assessing the effect of metformin combined with anticancer therapies for prostate cancer. TAXOMET is a Phase II trial recruiting patients to assess the effect of metformin combined with Taxotere (generic name of docetaxel) on PSA response rate, biochemical and clinical progression-free survival, overall survival and tolerance. The MetAb-Pro trial is a Phase II pilot study assessing the impact of combining metformin with abiraterone treatment on survival in patients with metastatic CRPC. This is indicative of the definite interest in the use of metformin as a sensitizing agent for anticancer therapies. However, there is no focus on prostate CSCs in either of the trials mentioned above. Currently, very few trials are registered that assess the effect of metformin on CSCs. A randomized clinical trial on colon cancer patients currently in the data analysis phase assessed the effect of metformin administered before surgery on the proportion of CSCs identified in tumors. There is also a Phase II trial currently recruiting ovarian, primary peritoneal and fallopian tube cancer patients to assess whether metformin combined with chemotherapy will prevent relapse by targeting CSCs. However, again there are no trials exploring the effect of metformin on prostate CSCs. If metformin does, in fact, have a CSC-specific effect when combined with other anticancer therapies, this would provide evidence for a novel therapeutic approach for prostate cancer that would warrant assessment in clinical trials.

CONCLUSION

Great strides have been made in establishing expression profiles for prostate CSC isolation and characterization. Therapeutic resistance continues to be a problem in the treatment of prostate cancer, and prostate CSCs are likely an important factor in treatment resistance, cancer recurrence and progression. The ability to selectively eliminate prostate CSCs holds therapeutic promise. Metformin has been shown to target CSCs in a number of different solid tumors although there is limited data in prostate cancer. Establishing the effect of metformin as a potential sensitizer for prostate CSCs and combining metformin with other anticancer therapies to enhance their effectiveness provides an opportunity to develop improved therapeutic options for prostate cancer patients.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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