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World Journal of Clinical Oncology
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Subject: *Revised version of an invited editorial, ID: 00213126; Manuscript number: 1735*

Dear Prof Calderwood.

We greatly appreciate to thank you, editorial managers and the reviewers 1 and 2 for the manifestation of interest and pertinent suggestions and comments about our manuscript entitled: **"Development of animal models underlining mechanistic connections between prostate inflammation and cancer"** authored by **Murielle Mimeault and Surinder K. Batra**" for your consideration for publication in your journal, ***World Journal of Clinical Oncology***.

We have revised the manuscript according to the comments and suggestions indicated in the manuscript (detailed in your e-mail dated, January , 2012) and made by the editor and reviewer 2. The comments (italics) and the changes made in response to comments and suggestions from reviewers are listed below and indicated in font color (blue characters) in the revised version of manuscript.

Please find appended a "point by point" reply to the comments from editor and reviewer 2 in the cover letter. We are also returning online the revised version of our manuscript in which the changes made in responses to comments and suggestions from editor and reviewer 2 are indicated in blue characters. Moreover, we also send to you, a decomposable version of the figures 1 and 2 in power point format.

Editor's comments

Editor's comment no 1: *Title should be no more than 10~12 words/60 bytes. Please revise it.*

Our response: The title "…**Development of animal models underlining mechanistic connections between prostate inflammation and cancer: Persistent challenge and perspectives…"** has been by "…**Development of animal models underlining mechanistic**

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connections between prostate inflammation and cancer…" in order to consider the editor's comment.

Editor's comment no 2: *Only one is needed. Please revise it.*

Our response: Please conserve two corresponding authors as indicated on the first version of manuscript. This is very important for us. Thanks for your understanding.

Editor's comment no 3: Core tip: *Please write a summary of less than 100 words to outline the most innovative and important arguments and core contents in your paper to attract readers.*

Our response: A core tip has been included in the revised version of manuscript as suggested by editor. The changes made in the text of the manuscript and which are also indicated in blue characters in the revised version of manuscript are as following:

Page 3: "…Accumulating lines of evidence have revealed that the persistence of prostate inflammatory lesions may constitute one of the etiopathological causes that may promote prostate cancer initiation and progression. Some predisposing factors, including genetic and epigenetic alterations, infectious agents, high fat diet and dietary carcinogens, and the occurrence of hormonal imbalance, and more particularly with advancing age, have been associated with prostate inflammation, disorders and cancer. Consequently, the establishment of the mechanistic interconnections between intensive inflammatory response and prostate cancer development is of major importance to identify novel biomarkers and therapeutic targets and develop new chemopreventive and therapeutic treatments against prostate cancer…"

Editor's comment no 4: *The coordinate graphs supplied should be* ***decomposable*** *(each part of your figure could be moved so as to easily edited). You can send it as excel, word or powerpoint format so that I can edit them easily.*

Our response: A decomposable version of the figures 1 and 2 in power point format have been sent in attached files with the revised version of manuscript as suggested by editor.

Reviewer's Comments

Here are our responses to comments and suggestions from reviewers 1 and 2.

Reviewer 1

Reviewer 1: Comments to the Authors: *This review article deals a relevant topic. This paper is well-written and the topic is timely appropriate. I think that this paper is quite a competent one for being published in World Journal of Clinical Oncology.*

Our response: We greatly appreciate to thank this reviewer 1 for its positive comments and manifestation of interest on its very important topic.

Reviewer 2

Reviewer 2 Comments to the Authors: *Many review articles on prostate cancer have published, but reviews on prostatic diseases is rear. The manuscript could be strengthened by*

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including more literatures on the "mechanistic connections between prostate inflammation and cancer", as indicated in the title, not just describe a few models.

Our response: We greatly appreciate to thank this reviewer 2 for its positive comments and manifestation of interest on its very important topic. We have included as possible more literatures on the animal models underlining the mechanistic connections between prostate inflammation and cancer as suggested by this reviewer 2. The changes made in the text of the manuscript and which are also indicated in blue characters in the revised version of manuscript are as following:

Pages 6-7: "…In this regard, a treatment of immortalized, non-transformed and androgen-responsive rat NRP-152 prostatic epithelial cell line with 17β-estradiol at concentrations 1-3 μM for a period of 2-6 weeks has also been observed to induce their capacity of forming colonies in soft agar and tumors in immunodeficient nude mice[29]. The oncogenic effect of 17β-estradiol on NRP-152 cells was accompanied by an increase of expression levels of estrogen receptor-α (ER-α) and PC stem cell-like markers (integrins $\alpha_2\beta_1$, CD44, CD133, ABCG2 and CXCR4) but a decrease of ER-β and androgen receptor (AR) expression levels[29]…"

Page 8: "…In the same way, the induction of bacterial prostatitis in C3H/HeOuJ mice by intraurethral inoculation of *Escherichia coli* has also been associated with a marked decrease of the expression level of Nkx3.1 tumor suppressor protein in infected prostate lobes and development of chronic inflammatory response within 14 days postinoculation[33]. The down-regulation of Nkx3.1 also correlated with an increased expression of a proliferation marker, reduction of AR level and a marked increase in the basal cell marker p63[33]. Hence, the decrease expression of key tumor suppressor products, including $p27^{Kip1}$, PTEN and Nkx3.1 in these animal models of bacterial prostatitis that are frequently down-regulated during PC development provide a potential link between the persistence of prostate inflammation and carcinogenesis…"

Pages 15-21: "…Moreover, it has also been observed that the $Vav3^{+/+}$ transgenic mice generated by overexpressing a constitutive active form of guanine nucleotide exchange factors for Rho family GTPases, Vav3 under the control of ARR_2-*PB* promoter in the prostatic epithelium exhibited a marked activation of AR, NF-κB and phosphatidylinositol 3-kinase (PI3K)-Akt signaling elements[78]. These molecular events led to the development of nonbacterial chronic prostatitis in the prostate gland which was associated with the infiltration of monocytes, lymphocytes, and plasma cells as well as the formation of PIN lesions and invasive PCs at the age as early as 3 months[78]. In addition, it has also been reported that fibroblast growth factor-8b (FGF-8b)$^{+/+}$ transgenic mice overexpressing FGF-8b in the prostate epithelium exhibited activated stroma containing increased proportion of fibroblastic cells, collagen deposition, and aggregates of inflammatory cells, including T cells, B cells and macrophages and intensive neoangiogenesis[79]. The intensive stromal changes and inflammation in $FGF\text{-}8b^{+/+}$ transgenic mice preceded the development of PIN lesions that culminated to the tumor formation with phenotypical features of adenocarcinoma and sarcoma[79]. These data suggest that the overexpression and secretion of FGF-8b by prostate epithelial cells can promote prostate carcinogenesis in part *via* the stromal activation and induction of an inflammatory response. On the other hand, several investigations have also revealed the major contribution of the activation of AR in the stromal cells and recruitment of bone marrow (BM)-derived cells in the induction of inflammatory response and PC progression.

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Functions of AR in the modulation of the development of prostatic inflammatory lesions and PC

The sustained activation of AR expressed by epithelial and adjacent stromal fibromuscular cells in the prostate gland, which plays critical functions in the modulation of stromal-epithelial interactions for the maintaining of normal prostate homeostasis, also can promote the development of prostatic inflammatory lesions, including inflammation-associated benign prostatic hyperplasia (BPH) and PCs[80-84]. For instance, it has been observed using an *in vitro* cell co-culture system that immortalized and non-tumorigenic BPH-1 human prostate epithelial cells significantly increased the migration of THP-1 macrophages which, in turn, induced the EMT markers such as N-cadherin, snail and TGF-β2 and proliferation of BPH-1 sphere cells[80]. Moreover, the exogenous expression of AR in BPH-1-AR also promoted the THP-1 cell migration and enhanced EMT marker expression and sphere-forming ability of BPH-1-AR cells relative to BPH-1-vector cells used as control[80]. Conversely, the BPH-1/THP-1 co-culture in the presence of an anti-TGF-β2 antibody or silencing of AR function in BHP-1-AR cells using AR degradation enhancer, ASC-J9, has also been observed to decrease the THP-1 macrophage migration and suppress the induction of EMT marker expression in BPH-1 cells[80]. Moreover, the results from *in vivo* tissue recombination studies have indicated that the combination of BPH-1 cells with human PC-associated fibroblasts from PC surgical specimens generated large tumors while no tumor was formed by BPH-1 cells in the presence of normal prostatic fibroblasts[84]. The data from investigations performed with tissue recombinants composed of mouse or rat urogenital sinus mesenchyme expressing ARs and estrogen receptors (ERs) and BPH-1 cells showing undetectable levels of ARs and ER levels grown under the kidney capsule of male athymic nude mice have also revealed that a treatment with 17β-estradiol plus testosterone induced only invasive PC development in the presence of functional mesenchymal AR[80-83]. It has also been noted that rat UGM plus BPH-1 tumors metastasized to lymph nodes, liver and lungs(81). Additionally, the selective AR knockout in fibroblasts and smooth muscle cells in the *dARKO/PTEN*$^{+/-}$ mouse model of PC has also been observed to inhibit the prostate epithelial cell proliferation concomitant with a decrease of the development of low- and high-grade PIN lesions and low-grade PIN progression as compared to wild-type *AR/Pten*$^{+/-}$ mice[85]. The AR deletion in fibromuscular cells of *dARKO/PTEN*$^{+/-}$ mice was also accompanied by a reduction of the extracellular matrix (ECM) remodelling, collagen deposition and number of infiltrating immune cells, including T cells, B cells and macrophages and neovasculature formation in the stromal compartment of prostate gland[85]. Moreover, it has also been shown that the AR activation by 4-DHT in prostate stromal cells (PrSCs) isolated from *Pten*$^{+/-}$ mouse prostates up-regulated the expression of pro-inflammatory cytokines and chemokines such as macrophage inflammatory protein-1α (MIP-1α), MIP-1β, MIP-2 and IL-10[85]. These pro-inflammatory factors, in turn, induced an immune cell recruitment and inflammatory response that promoted the PIN development in *Pten*$^{+/-}$ mouse prostate[85]. Of therapeutic interest, it has also been noted that the down-regulation of AR in stromal fibromuscular cells and prostate epithelial cells with the AR degradation enhancer, ASC-J9, was effective at reducing the stromal remodeling and PIN development and progression in *Pten*$^{+/-}$ mice[85]. Hence, together these data supports the benefit to target AR in stromal and epithelial cells of the prostate to suppress the inflammatory response and prevent PC development.

Implications of BM-derived adult stem/progenitor cells in the development of prostatic inflammatory lesions and PCs

Several investigations have revealed that circulating BM-derived adult stem/progenitor cells, including hematopoietic stem/progenitor cells (HSCs and HPCs), mesenchymal stem cells

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(MSCs), endothelial progenitor cells (EPCs) and/or myeloid cells may be recruited at prostatic inflammatory lesions and contribute to the PC development and metastases[86-93]. In fact, although BM-derived adult stem/progenitor cells appear to play only minimal roles in prostate epithelial regeneration after severe prostate inflammation and glandular disruption *via* cell fusion with prostate epithelial cells or transdifferentiation into prostate epithelial-like cells, they can exhibit immunosuppressive and angiogenic effects that promote prostate carcinogenesis[86-93]. More specifically, the release of soluble pro-inflammatory chemokines and cytokines acting as chemoattractant factors, such as SDF-1 (CXCL12), chemokine (C-C motif) ligand 5 (CCL5, RANTES) and monocyte chemotactic protein-1 (CCL2/MCP-1) by prostate epithelial cells, activated stromal cells and/or immune cells may recruit BM-derived adult stem/progenitor cells expressing their cognate receptors at injured prostate site and PCs[88-90]. More specifically, it has been observed that PC-derived stromal cells, which express fibroblast activation protein-α (FAP)-, CD90-, CD73- and CD105 but undetectable levels of CD14-, CD20-, CD34-, CD45- and human leukocyte antigen (HLA-DR) exhibited morphological and phenotypic features comparable to BM-derived MSCs[94]. It has also been noted that PC-derived stromal cells represented about 0.01-1.1% of the total cells present in core biopsies from primary human PC specimens[94]. Moreover, the stimulation of murine RM-1 PC cells by inflammatory cytokines, such as interferon-γ and tumor necrosis factor-α has been shown to be accompanied by the production of platelet-derived growth factor-BB (PDGF-BB) that in turn promoted the proliferation of MSCs *in vivo* and *in vitro*[88]. The exogenous and endogenous MSCs recruited into the tumor microenvironment was also able to promote the tumor growth of RM-1 cells subcutaneously implanted in mice[88]. These data suggest the potential implication of the recruitment of MSCs in prostate inflammatory microenvironment induced *via* the proinflammatory mediators in the induction of immunosuppressive effects that can allow PC cells to escape the immune surveillance and favor PC development.

In addition, it has been observed that CXCR-4$^+$/sca-1$^+$, VEGFR-2$^+$/CD34$^+$ and VEGFR-2$^+$/CD117$^+$ BM-derived cell subpopulations were increased in the peripheral blood of SCID mice bearing PC cell xenografts and contributed to the tumor growth by promoting neoangiogenesis[91-93]. The treatment of tumor-bearing mice for 5 days with doxorubicin or daunorubicin was however effective at reducing the tumor vascularization at least in part by inhibiting the recruitment of BM-derived cells at tumor *via* the inhibition of HIF-1α[92]. Moreover, the data from BM transplantation/reconstitution and genetic lineage-tracing experiments have also revealed that BM-derived myelomonocytic cells can transform into lymphatic endothelial cells and integrated into PC-associated lymphatic vessels in the TRAMP-C1 cell transplantation model and thereby contribute to lymphangiogenesis[93].

Of therapeutic interest, it has also been shown that parental or genetically-engineered MSCs and EPCs, which show an innate tropism for damaged epithelial tissues, including PCs may be exploited as vehicles for targeted-delivery of anti-inflammatory, cytotoxic and/or anti-angiogenic agents at injured prostatic sites. For instance, it has been observed that MSCs engineered for expressing secreted frizzled related protein-2 (SFRP-2) suppressed the tumor growth and increased apoptosis and necrosis within tumors formed by C4-2B human castration-resistant-PC cells orthotopically implanted into the prostates of castrated host SCID mice[89]…"

We believe that we have considered all the comments from editor and reviewer 2. These comments were very important for us and have improved the manuscript. ***We sincerely hope that this manuscript will really aid the interested researchers on the development of new effective therapies for treating diverse disorders associated with persistent prostate inflammation, including PCs and thereby prevent disease progression to metastatic and lethal PCs.***

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We thank you for your manifestation of interest on very important topic, and would highly appreciate your kind consideration for publication of this editorial in your journal, ***World Journal of Clinical Oncology***.

If you have any question/concern, please contact us.

Very Sincerely and Cordially,

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