Format for ANSWERING REVIEWERS

May 22, 2015

Dear Editor,

Please find enclosed the edited manuscript in Word format (File Name: Hassan et al -review.doc).

Title: Endoplasmic reticulum stress-mediated pathways to both apoptosis and autophagy: significance for melanoma treatment

Authors: Mohamed Hassan, Denis Selimovic, Matthias Hannig, Youssef Haikel, Robert T. Brodell, Mossaad Megahed

Name of Journal: *World Journal of Experimental Medicine*

ESPS Manuscript NO: 17858

The manuscript has been improved according to the suggestions of reviewers:
1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer

(1) Reviewer: 00504024

Comments: It is comprehensive and easily understandable. Thus, it is Acceptable for publication

Authors' response: Thank you very much for your valuable comment

(2) Reviewer: 00503927

Comment: The article subject is pertinent. New treatment approach for melanoma is needed. Authors used very recent bibliography. Still, I consider important to refer to old and previous researches regarding chemotherapy. There are a lot of typos. The manuscript must be revised carefully. I highlighted some of them on the text. Others observations on the text.

Authors' response: Thank you very much for your valuable comment. Accordingly, we revised the language of the manuscript and corrected all typing errors**.** In addition we added some information about the old chemotherapy.

See Page: 3; Lines: 1-19 from the bottom and Page: 4; Lines: 1-3 from the top. The following paragraph has been added to the manuscript**: [**Metastatic melanoma demonstrates particularly poor response rates to single chemotherapeutic agents [14, 15]. For instance, dacarbazine (DTIC) demonstrates no impact on survival, though it is considered to be one of the most effective agents that

is used as standard therapy for the treatment of metastatic melanoma [16, 17]. Other anticancer agents such as cisplatin, carmustine and the vinca alkaloids (e.g. vindesine and vinblastine) fail to show any therapeutic advantage over DTIC [18], though several combination chemotherapy regimens demonstrate a modest increased response rate [19].

Melanoma's resistance to therapy is the results of an upregulation in pro-survival factors, which potentiate tumor maintenance and progression [20]. One of these factors is the inducible transcription factor NF-κB that is responsible for the regulation of the expression of genes related to apoptosis [21]. It is also, central to the development of tumor resistance to alkylating agents such as DTIC [22-24]. Accordingly, the inhibition of NF-κB pathway may improve the cytotoxic efficacy of alkylating agent- based therapy. To that end, preclinical studies in vitro and in vivo using human melanoma tumor models revealed that the therapeutic efficiency of DTIC or temozolomide is enhanced with the addition of the proteasome inhibitor, bortezomib [25, 26].

Traditional mono- or multi-chemotherapy regimens are also associated with the development of significant adverse effects [27, 28]. The development of new tumor types in these patients is attributed to the molecular action of the anticancer agents leading to the induction and/or destruction of aberrant signaling pathways.

The molecular action of chemotherapy in tumor cells is commonly associated with phenotypic alterations including cell death and survival-dependent mechanisms including apoptosis and autophagy [12, 13].**]**

(3) Reviewer: 000504030

Major comments

Comment: 1. there are many grammar errors and inappropriate use of English throughout the text. The authors should correct them carefully.

Authors' response: Thank you very much for your comment. As required, we proofed the English language throughout the manuscript.

Comment: 2. what's the meaning of the sentences with red font? It makes the manuscript looks like a draft.

Authors' response: Thank you very much for your comment. We apologize for this occurrence. Accordingly, we corrected it.

Comment: 3. In [Introduction] part, the authors should introduce the role of apoptosis and autophagy in melanoma or melanoma treatment, and mention the significance and link of ER stress to these cellular processes.

Authors' response: Thank you very much for your comment. As required we added a paragraph about the role of apoptosis and autophagy regarding to the key role of ER stress in the modulation of these processes

See Page: 3, Lines: 1-12 from the bottom and Page: 4; Lines: 1-30 from the top. The following paragraph has been added. **[**Melanoma's resistance to therapy is the results of an upregulation in

pro-survival factors, which potentiate tumor maintenance and progression [20]. One of these factors is the inducible transcription factor NF-κB that is responsible for the regulation of the expression of genes related to apoptosis [21]. It is also, central to the development of tumor resistance to alkylating agents such as DTIC [22-24]. Accordingly, the inhibition of NF-κB pathway may improve the cytotoxic efficacy of alkylating agent- based therapy. To that end, preclinical studies in vitro and in vivo using human melanoma tumor models revealed that the therapeutic efficiency of DTIC or temozolomide is enhanced with the addition of the proteasome inhibitor, bortezomib [25, 26]. Traditional mono- or multi-chemotherapy regimens are also associated with the development of significant adverse effects [27, 28]. The development of new tumor types in these patients is attributed to the molecular action of the anticancer agents leading to the induction and/or destruction of aberrant signaling pathways. The molecular action of chemotherapy in tumor cells is commonly associated with phenotypic alterations including cell death and survival-dependent mechanisms including apoptosis and autophagy [12, 13]. Apoptosis and autophagy occur in normal. These are essential physiological mechanisms required for the maintenance of organismal and cellular homeostasis [29]. Current information about autophagy in melanoma focuses on autophagosome formation and/or autolysosome degradation in response to a variety of therapeutic agents using melanoma derived cell lines [13, 30, 31]. Chemotherapy induction of autophagy serves to protect melanoma cells from intendent chemotherapy- induced apoptosis. In fact the induction of autophagy following the treatment of melanoma cells with bortezomib reduces bortezomib-induced apoptosis [13]. Similarly, the induction of autophagy by esomeprazole, a proton pump inhibitor, blocks melanoma cell death [32]. Based on this preclinical evidence, the modulation of autophagy-associated pathways offers a promising treatment strategy to increase treatment efficiency by overcoming melanoma resistance to chemotherapy.

The involvement of ER stress in the modulation of apoptotic mechanisms leading to melanoma cell death has been reported in several studies [12, 13, 33]. This may result from the induction of BH3 proteins such as Noxa and Puma leading to the inhibition of Bcl-2 localization at the ER membrane, alterations in the distribution of the calcium flux which produce ER stress [13, 34].

Although ER stress and autophagy are capable of modulating each other in tumor tissues, their specific function is thought to be tumor type and stage-dependent [34-36]. The clinical potential of ER stress and /or autophagy-associated pathways as therapeutic target for melanoma treatment has been reported in several studies [37-39]. For example, BRAF wild type (wt) melanoma is more sensitive to ER stress-based therapies than melanoma with hyperactivating BRAF mutations [40]. The frequency of BRAF mutation seems to be associated with elevated levels of autophagy in melanoma. Accordingly, ER stress-induced apoptosis of melanoma cells harboring oncogenic BRAF is lower than those observed in BRAF wt melanoma cells [40-42]. Accordingly, the inhibition of autophagy is a good strategy to sensitize BRAF wt melanoma cells to ER stress- mediated apoptosis. In addition, the development of anti-cancer agents based on the enhancement or suppression of these processes may be relevant therapeutic strategies. [38, 43, 44].**]**

Comment: 4. As mentioned by the authors 'Figure 1 outlines the ER stress-associated pathways in normal and tumor cells', is there any difference between normal and tumor cells when ER stress occurs? If yes, it will be better to present it in separate schematic diagrams.

Authors' response: Thank you very much for your comment. Based the current publication there is no difference between normal and tumor cells regarding the outcome of ER stress

Comment: 5. the authors should put their emphasis on introducing ER stress and its role in tumor instead of ER classification.

Authors' response: Thank you very much for your comment. As required, we highlighted the mechanistic role of ER stress in tumor cells.

See Page: 4; Lines: 2-23 from the bottom. The following sentence has been added [The involvement of ER stress in the modulation of apoptotic mechanisms leading to melanoma cell death has been reported in several studies [12, 13, 33]. This may result from the induction of BH3 proteins such as Noxa and Puma leading to the inhibition of Bcl-2 localization at the ER membrane, alterations in the distribution of the calcium flux which produce ER stress [13, 34].

Although ER stress and autophagy are capable of modulating each other in tumor tissues, their specific function is thought to be tumor type and stage-dependent [34-36]. The clinical potential of ER stress and /or autophagy-associated pathways as therapeutic target for melanoma treatment has been reported in several studies [37-39]. For example, BRAF wild type (wt) melanoma is more sensitive to ER stress-based therapies than melanoma with hyperactivating BRAF mutations [40]. The frequency of BRAF mutation seems to be associated with elevated levels of autophagy in melanoma. Accordingly, ER stress-induced apoptosis of melanoma cells harboring oncogenic BRAF is lower than those observed in BRAF wt melanoma cells [40-42]. Accordingly, the inhibition of autophagy is a good strategy to sensitize BRAF wt melanoma cells to ER stress- mediated apoptosis. In addition, the development of anti-cancer agents based on the enhancement or suppression of these processes may be relevant therapeutic strategies. [38, 43, 44].

Tumor resistance or response to available therapeutic modalities depends on the balance between apoptosis and autophagy-associated mechanisms [45, 46]. Although the development of the most available therapeutic approaches focuses on the excessive activation of mitochondrial dysregulation-dependent pathways leading to apoptosis, there is increasing evidence that ER stress-associated pathways represent an important therapeutic target for melanoma treatment [13, 47].]

Comment: 6. P5-6, in the [Induction of ER stress-associated pathways by anticancer agents] part, the authors should list the inducer or stimulator and briefly introduce the effects. It will make the contents more relational to the heading.

Authors' response: Thank you very much for your comment. As required, we ……

See Page: 5, Lines: 2-11 from top. The following paragraph has been added. [Dysregulation of ER homeostasis is a primary pathophysiological mechanism responsible for the initiation of an ER stress response that leads to the development of a number of human diseases including cancer[59]. The induction of ER stress by anti-cancer agents and other stimuli has been reported in several studies. The anti-cancer agent's bortezomib, vinblastine and taxol trigger ER stress in melanoma cells [13, 60,

61]. Similarly, caffeic acid phenethyl ester, the BH3 mimetic Obatoclax and the Abbott Compound ABT-737 have been reported to induce ER stress in melanoma [33, 62]. Interestingly, the induction of ER stress in melanoma cells by these agents is correlated with the deregulation of ER stress associated pathways including elF2α eukaryotic translation initiation factor 2α (elF2α) and Protein kinase RNA-like endoplasmic reticulum kinase (PERK).]

Minor comments:

Comment: 1. P3, paragraph 1, line 7, the sentence 'Thus, ……, the prognosis is poor.' can be deleted.

Authors' response: Thank you very much for your comment. We re-edit the mentioned sentences and modified the whole paragraph.

See Page: 3: Lines: 6-14 from the top. The following paragraph has been re-edited [Early detection and surgical excision of early stage disease offers the best hope of cure in patients with primary melanoma [4]. Even with new targeted therapies, the prognosis for advanced metastatic malignant melanoma. [5]. the available options for these patients provide limited therapeutic benefit with successful treatments often being measured in months of increased survival rather than years [6-8]. The potential to develop resistance mechanisms that counteract drug-induced apoptosis and evade host immunological responses is particularly devastating [9]. Accordingly, the replacement of single agent chemotherapy with targeted therapies is revolutionizing systemic therapy [10].**]**

Comment: 2. P3, paragraph 2, what is the purpose to state the last sentence "In recent past, …… the best treatment for systemic therapy."

Authors' response: Thank you very much for your comment. We deleted it and re-edit accordingly the sentence.

See Page: 2 Lines: 10-11 from the top. The following sentence has been re-edited. [However, the early detection and surgical management of early stage disease increase cure prospects of patients with primary melanoma [7]. **]**

Comment: 3. P3, paragraph 3, be careful with the use of 'however' and 'thus' as well as 'although'. Besides, mitochondrial deregulation-dependent pathways are often regarded as significant participants of apoptosis.

Authors' response: Thank you very much for your comment. As required we have re-edit this paragraph.

See Page: 4: Lines: 2-13 from the bottom. The following paragraph has been re-edited. [Accordingly, ER stress-induced apoptosis of melanoma cells harboring oncogenic BRAF is lower than those observed in BRAF wt melanoma cells [40-42]. Accordingly, the inhibition of autophagy is a good strategy to sensitize BRAF wt melanoma cells to ER stress- mediated apoptosis. In addition, the development of anti-cancer agents based on the enhancement or suppression of these processes may be relevant therapeutic strategies. [38, 43, 44].

Tumor resistance or response to available therapeutic modalities depends on the balance between

apoptosis and autophagy-associated mechanisms [45, 46]. Although the development of the most available therapeutic approaches focuses on the excessive activation of mitochondrial dysregulation-dependent pathways leading to apoptosis, there is increasing evidence that ER stress-associated pathways represent an important therapeutic target for melanoma treatment [13, 47].**]**

Comment: 4. P3, paragraph 4, lines 3 and 4, is there any difference between 'induces cell proliferation' and 'promotes proliferation' here? In addition, the authors should provide the reference(s) for the last sentence.

Authors' response: Thank you very much for your comment. We apologize for the repeated phrase. We deleted "promotes proliferation" and re-edited the sentence

See Page: 5: Lines: 1-4 from the top. The following paragraph has been corrected and re-edited is mediated in response to the enhancement of protein synthesis through the activation of mitogen-activated protein kinase kinase/extracellular signal-regulated kinase (MEK/ERK) pathway that, in turn, induces cell proliferation, a mechanism that can block ER stress-induced apoptosis [48].]

Comment: 5. P6, heading 'Endoplasmic stress-mediated pathways to apoptosis in melanoma' - does Endoplasmic stress means ER stress?

Authors' response: Thank you very much for your comment. We apologize for this. We corrected this mistake.

See Page: 6; Line: 1 from the top. [ER stress-mediated pathways to apoptosis in melanoma].

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Experimental Medicine*

Sincerely yours,

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