Format for ANSWERING REVIEWERS

July 6th, 2015

World Journal of Biological Chemistry

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 19999-Revised manuscript.docx).

Title: Targeting Amino Acid Metabolism in Cancer growth and Anti-Tumor Immune Response

Author: Elitsa Ananieva

Name of Journal: World Journal of Biological Chemistry

ESPS Manuscript NO: 19999

The format of the manuscript has been updated. Likewise, the manuscript has been revised and improved according to the suggestions of the reviewers as follows:

(1) Reviewer 02254242

Reviewer's comments:

The review manuscript, "Targeting Amino Acid Metabolism in Cancer growth and Anti-Tumor Immune Response" (Manuscript #20150527103026), authored by Dr. Ananieva describes targeting of amino acid metabolism and the potential use of that targeting as a cancer treatment by developing more effective immunotherapies. The author describes links that exist between the immune system and amino acids that either have been or have the potential to be exploited for therapeutic purposes. The brief review provides insight into the role of the immune system and targeting of tumors using inhibitors of metabolic enzymes. This is a promising area in which to develop new therapeutic agents and the role of immune system cells is discussed.

Answer: The author highly appreciates the positive comments of *Reviewer 02254242* and his/her suggestion for publication.

1. Overall, the manuscript is well-written. Minor issues that the author should consider addressing: The author should consider adding a figure or figures showing some of the metabolic pathways that are described in the text.

Answer: Figure 1. "Schematic representation of amino acid metabolic pathways

targeted in cancer therapy "was added to the manuscript.

2. Page 4. "…by being source of arginine…", is better as "…by being a source of arginine…".

Answer: This sentence was modified due to another reviewer's suggestion and no longer contains "by being source of".

3. Page 7. The review paper by Mider cited does not "show", because it is a review paper. Manuscripts concerning glutamine metabolism and cancer actually appeared prior to the review manuscript.

Answer: "Showed" was replaced by "described".

4. Page 7. Although glutamine is not essential, it can become a conditionally essential amino acid, which is later discussed. That conditionality may be important to mention in the same sentence.

Answer: The following sentence "As such, glutamine is a conditionally essential amino acid for the proliferating cells as well as critically ill humans [76]" was added to account for the conditionality of glutamine as an essential amino acid.

(2) Reviewer 00289737

Reviewer's comments:

This is brief review article that nicely describes the potential role of amino acid metabolism in cancer growth and immune response. Recent studies indicate that amino acid metabolism plays a significant role in the pathophysiology of cancer cachexia. Authors have done careful job in describing how some of the essential amino acids involved in the cancer growth.

Answer: The author highly appreciates the positive comments of *Reviewer 00289737* and his/her suggestion for publication.

Few minor comments are given below to improve the manuscript. Some of the sentences

are too long and not clear. For example; Page 4; the sentence that starts with " It is hypothesized that tumor associated myeloid cells (TAMCs), that consist of macrophages, monocytes, myeloid suppressor cells, and neutrophils and reside in the tumor microenvironment, may form metabolic relationship with tumor cells by being source of arginine, thus by-passing the effect of arginine deprivation"

Answer: The sentence was modified as follows: "It is hypothesized that tumor associated myeloid cells (TAMCs), that consist of macrophages, monocytes, myeloid suppressor cells, and neutrophils [24], form metabolic relationship with the tumor cells in the tumor microenvironment. They provide arginine to help the tumor cells by-pass the effect of arginine deprivation [25]".

Page 5; the sentence that starts with "As a result…..died".

Answer: The sentence was modified and more information was added to this paragraph to discuss the role of IDO in autoimmunity and inflammation as suggested by *Reviewer 00289387*. The following sentences replaced/ or were added to the text of the manuscript: "By degrading tryptophan, IDO inhibits T cell proliferation and plays a role in autoimmunity and anti-inflammatory responses [41-43]. For example, Apoe-/- mice treated with the IDO inhibitor, 1-methyl-Trp (1-MT), showed a significant increase in atherosclerotic lesions in the aortic arch and root of their hearts along with enhanced vascular inflammation [41]. Additionally, IDO-expressing dendritic cells suppressed the allograft rejection and increased the survival time of small bowel transplanted mice [44]".

Page 8"the sentence that starts with "The cytoplasmic branched chain aminotransferase (BCATc), that catalyzes leucine transamination, was induced in activated T cells, where it regulated leucine supply to complex 1 of the mTOR pathway, providing negative feedback regulation of T cell activation"

Answer: The sentence was shorten for more clarity and now reads as follows: "The cytoplasmic branched chain aminotransferase (BCATc), that catalyzes leucine transamination, was induced in activated T cells, where it regulated leucine supply to complex 1 of the mTOR pathway".

A schematic diagram showing the links between amino acid metabolism, inflammation and cancer will be great help for the readers.

Answer **Figure 1. "Schematic representation of amino acid metabolic pathways targeted in cancer therapy "was added to the manuscript.**

(3) Reviewer 00289387

Reviewer's comments:

This manuscript provides up-to-date information of amino acid metabolism in cancer development. Dr. Ananieva discussed four main types of amino acid metabolism including arginine and tryptophan, serine and glycine, glutamine, and branched chain amino acids; also presented several lines of drug evidence that currently targets these metabolic pathways in clinical trials. The paper focuses primarily on amino acid metabolism by which both cancer cells and immune cells grow and differentiate, indicating the potential competition for sharing same nutrients. In general, the paper has a clear objective and is a well-written review in which the organization of the manuscript makes the material easy to follow.

Answer: The author highly appreciates the positive comments of *Reviewer 00289387* and his/her suggestion for publication.

A few minor concerns may be addressed. 1) These amino acids are essential for almost all of different types of body cells in biological, physiological and pathological development. Abnormal regulations of these metabolic processes must occur in a variety of human diseases, not limited to cancer; therefore, the other diseases associated with dysfunction of these metabolic pathways should be briefly discussed. Indeed, some diseases are largely connected with carcinogenesis such as inflammation and cancer.

Answer: The author agrees that many other diseases are associated with dysfunctions of amino acid metabolic pathways and there are excellent reviews in the literature. However, the objective of this minireview is to discuss the importance of amino acid metabolism in the tumor microenvironment in relationship to the immune system and to highlight clinical relevance. Other disorders of amino acid metabolism are out of the scope of this minireview. The author took in consideration the reviewer's question about inflammation and added a small paragraph on the role of IDO in inflammation and it reads as follows: "By degrading tryptophan, IDO inhibits T cell proliferation

and plays a role in autoimmunity and anti-inflammatory responses [41-43]. For example, Apoe-/- mice treated with the IDO inhibitor, 1-methyl-Trp (1-MT), showed a significant increase in atherosclerotic lesions in the aortic arch and root of their hearts along with enhanced vascular inflammation [41]. Additionally, IDO-expressing dendritic cells suppressed the allograft rejection and increased the survival time of small bowel transplanted mice [44]".

2) The paper discussed some clinical results that unveil differential expression levels of amino acid metabolic enzymes in cancer such as PHGDH, IDO, TDO, etc. Are there studies or data that evaluate abnormal levels (either up or down regulation) of corresponding amino acids in tumor or blood? These kinds of evidence may hold great value for establishing cancer biomarkers.

Answer: The differential expression levels of PHGDH, IDO, and TDO are found in tumor samples/tumor microenvironment.

3) It is nice to have a summery table listing a number of drugs used in clinical trials, which specifically target individual amino acid metabolism. Are these drugs limited to cancer patient treatment? As stated earlier, other cells like immune cells also share the same material for cell metabolism. As such, it is curious to know if some of these drugs are engaged in clinical practice for immune disorders or others.

Answer: Please refer to Table 1.

(4) Reviewer 03283891

Reviewer's comments:

Overall this is a well written review with targeted therapies in cancer being a very timely topic. ?There are several times that preclinical studies are mentioned and listed the references. The table provides are a great summary of these, but as a review article it would be good to take a more consistent and comprehensive approach. For example, under arginine you list preclinical studies in hepatocellular carcinoma and pancreatic cancer- without having to look up the references it would be nice to know what model was used (in vivo/in vitro). Then under tryptophan you list the actual cell line P815B- what tumor site it this? oConsider revising the table to include the disease site and the

model for each study. ?It is mentioned under tryptophan and glutamine that there are safety cancers/ and general toxicity. Again, as a review article this should be a comprehensive source where the reader can easily identify the issues. As a clinical provider involved in both cytotoxic chemotherapy trials and targeted trials I am continually amazed what patients are willing to endure for the chance at more time alive. Are the toxicities in these clinical studies grade 3 or 4? What about in the preclinical studies, is it animal weight that is monitored, or are they resulting in animal fatality?

Answer: The author highy values *Reviewer 03283891*'s suggestion to make Table 1 more comprehensive source of information for the readers. The author understands the need cancer patients have for information and hopes that this minireview, and in particular Table 1, will be helpful. Table 1 was revised to include two new columns (Drug toxicity/adverse events and Response rate). Additionally, more information was included about animal models, disease models, cancer cell lines etc., In terms of animal models, tumor size and tumor growth are the parameters commonly monitored as opposed to the tumor site. Attempt was made to include information about the tumor site as well.

Thank you again for publishing this manuscript in the World Journal of Biochemistry.

Sincerely yours,

Elitsa Ananieva, PhD
Biochemistry Department
Des Moines University
3200 Grand Avenue
Des Moines, IA
Fax: 515.271.4215
E-mail: Elitsa.ananieva-stoyanova@dmu.edu

September 2nd, 2015

World Journal of Biological Chemistry

Dear Editor in-chief,

Please find enclosed the edited manuscript in Word format (file name: 19999-Review-09-02-2015.docx):

Title: Targeting Amino Acid Metabolism in Cancer growth and Anti-Tumor Immune Response

Author: Elitsa Ananieva

Name of Journal: World Journal of Biological Chemistry

ESPS Manuscript NO: 19999

The format of the manuscript has been revised and updated according to your recommendations. The author is very grateful for the opportunity to revise the manuscript and answer additional questions and suggestions. So far, the manuscript received: 1 Grade A (Excellent), 2 Grades B (very good) and 1 Grade C (Good) under classification. Under language evaluation, the manuscript received 3-Grades A (Excellent) and 1 Grade B (Very good). One of the reviewers suggested high priority for publication while the rest of the reviewers suggested minor revisions, which the author has completely addressed in the final revised version of the manuscript as well as below:

Editor in-chief comments:

1). The author only partly answered the "Reviewer 00289387" comments 2 and 3. Especially, for question 2: "Are there studies or data that evaluate abnormal levels (either up or down regulation) of corresponding amino acids in tumor or blood? These kinds of evidence may hold great value for establishing cancer biomarkers."

Answer: The studies included in this mini review described abnormal levels of amino acid metabolic enzymes at the tumor site and surrounding tumor environment. Because of the abnormal expression of these enzymes, the tumor microenvironment can

become depleted of amino acids (especially arginine and tryptophan). Targeted cancer immunotherapy using inhibitors of these enzymes aims at helping the immune cells eliminate cancer cells from the tumor microenvironment.

In the majority of cancer trials, the blood amino acid levels should not be impacted following targeted cancer therapy unless there are systemic changes (either inhibition or overexpression) of the corresponding amino acid enzymes. One of the goals of targeted cancer therapy is to actually target the cancer cells without disturbing the surrounding normal cells including peripheral tissues and organs. While this is ideal, it is not always possible. Therefore, the author researched several more studies and found an increased number of immune cells overexpressing IDO as well as increased arginase activity in lymph nodes and peripheral blood of cancer patients; however no data on blood amino acid levels were reported in these studies [1,2]. This can be explained with the notion that current cancer trials do not select patients based on assessment of systemic activity of amino acid metabolic enzymes or by analysis of their metabolites in patients' serum[3].

The author modified the last three sentences on page 8 as follows: "Thus far, IDO inhibitors, specifically designed for cancer immunotherapy, have been broadly used in preclinical and clinical trials alone or in combination with T cell checkpoint inhibitors [52-54], while systemic inhibition of TDO, although promising, may raise safety concerns [54] (Table 1). Another limitation of cancer trials targeting tryptophan metabolism is that they do not select cancer patients based on assessment of systemic IDO/TDO activities or by analysis of their metabolites in patients' serum [54]. Nevertheless, combinatorial approaches targeting tryptophan metabolism will continue to deliver novel therapeutic avenues in cancer therapy.

Comment 3: "Are these drugs limited to cancer patient treatment? As stated earlier, other cells like immune cells also share the same material for cell metabolism. As such, it is curious to know if some of these drugs are engaged in clinical practice for immune disorders or others.

Answer: The cancer therapy drugs discussed in this review are with selective anti-cancer activity and have been successfully used in preclinical and clinical cancer studies alone or in combination with other chemotherapy/ immunotherapy drugs (arginine depleters, IDO, and TDO inhibitors). Because most of the drugs discussed in this review

are either recently explored (TDO inhibitors), specifically designed for cancer immunotherapy (IDO inhibitors), or no longer in use (L-DON, azaserine, and acivicin), little information can be found in terms of their potential future usage associated with other diseases.

The only drug the author found to be associated with treatment of other diseases beyond cancer is ADI-PEG20. ADI-PEG20 has two versions: one is with anti-cancer and the other is with anti-viral activity against hepatitis C virus (http://polarispharma.com/~polari14/pipeline/adipeg20av.php).

The author added the information about ADI-PEG20 and its anti-viral activity on page 7 and it reads as follows"ADI-PEG20 is not limited to cancer therapy as there is ADI-PEG20 with anti-viral activity designed for treatment of hepatitis C by Polaris group. "

Also, the author not clearly replied the reviewer 4 questions. The authors reviewed 4 kinds of amino acid metabolism. I agree with Reviewer 4, could the author list the actual tumor site for each tumor cell?

Answer: The tumor sites for P815B (peritoneal cavity), B16F10 (flank), U-87MG (intracerebral transplantation), Panc-1 (right flank) are listed in Table 1.

Otherwise, the author should draw a scheme of the metabolon for each amino acid.

Answer: Defined details for each individual amino acid metabolic pathway would be more appropriate for a review on recent advances in amino acid metabolism but not in the context of cancer therapy since not all of the steps are explored/and or relevant to cancer. The author's aim was to emphasize major enzymes and steps in the pathway of each amino acid explored in cancer, make interconnections with other major metabolic pathways, and show the readers potential target points for cancer interventions.

Please refer to Figure 1 that illustrates steps in each amino acid metabolic pathway relevant to cancer. It includes major cellular intermediates and precursors of biosynthetic pathways important for tumor growth and proliferation. The amino acid

metabolic enzymes targeted for cancer therapy are also shown in Figure 1 and are discussed in detail in the text of this review.

References

1. Porembska Z, Luboinski G, Chrzanowska A, Mielczarek M, Magnuska J, Baranczyk-Kuzma A. Arginase in patients with breast cancer. *Clin Chim Acta* 2003; **328**: 105-111 [PMID: 12559605 DOI:S0009898102003911 [pii]]

2. Yu J, Du W, Yan F, Wang Y, Li H, Cao S, Yu W, Shen C, Liu J, Ren X. Myeloid-derived suppressor cells suppress antitumor immune responses through IDO expression and correlate with lymph node metastasis in patients with breast cancer. *J Immunol* 2013; **190**: 3783-3797 [PMID: 23440412 DOI:10.4049/jimmunol.1201449 [doi]]

3. Platten M, von Knebel Doeberitz N, Oezen I, Wick W, Ochs K. Cancer Immunotherapy by Targeting IDO1/TDO and Their Downstream Effectors. *Front Immunol* 2015; **5**: 673 [PMID: 25628622 DOI:10.3389/fimmu.2014.00673 [doi]]