Author’s response to reviews

Title: The adipokine vaspin reduces apoptosis in human hepatocellular carcinoma (Hep-3B) cells, associated with lower levels of NO and superoxide anion

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Author’s response to reviews:

Dear Editor

BMC Pharmacology and Toxicology.

I would like to thank you for the opportunity to revise our manuscript, titled "The adipokine vaspin reduces apoptosis in human hepatocellular carcinoma (Hep-3B) cells, associated with lower levels of NO and superoxide anion." (PHAT-D-18-00156R1). After carefully revision according to your recommendation we have made appropriate changes. We believe we have improved the manuscript what may allow to be published in BMC Pharmacology and Toxicology.

Sincerely,

Magdalena Skonieczna.

The affiliation was changed, because of changes in the structure of Institute.
Page 1

Line 10: “Biosytems Group” replaced with “Systems Engineering Group”;

Editor Comments:

1- In abstract, the background is very long. Authors should be focused on the apoptotic effect of vaspin.
Response: We have shortened an abstract with focusing on the apoptotic effect of vaspin.

Page 2

Line 26: “for apoptotic pathway. Among of them,” was added;
Line 26-27: omentin, chemerin, visfatin, and adiponectin were removed;
Line 27: “regulate” was replaced with “regulates”;
Line 29: “and related metabolic disorders, diabetes, liver fibrosis” were removed;
Line 31-32: “, and displays up- or down-regulation in different tissues.” were removed;
Line 33: and apoptosis, was added;
Line 34: “some hormones, such as vaspin, reduce apoptosis,” were added;
Line 36-37: “reactive oxygen species” was added;

Editor Comments:

2- In the Introduction the relationship between of vaspin and cancer not clear, the author should be focused on this link.
Response: We have focused on the relationship between vaspin and cancer by removing some statements from Background:

Page 3

Line 53-58: “In a well-developed countries, one of the actual health problems is population obesity, with accompanying health disorders such as increased risk of colon and breast cancers (Flegal at al., 2016; Vucenik et al., 2012). The United State’s population suffer from chronic
obesity, and one third of cancers are classified as obesity-connected metabolic dysfunctions or chronic inflammation (Hursting at al., 2012).” were removed;

Line 63-66: “Adipose tissue, which is also a residuary of an adipose-derived stem cells (ASCs) could be a promising candidate for autologous cell-based regeneration therapies (Pérez at al., 2013). The existence of a feedback loop involving nuclear factor (NF-κB), Lin28/Let7 and tumor necrosis factor α (TNF-α) has been described in ASC transformation (Iliopoulos at al., 2009).” Were removed;

Editor Comments:

3- I think this statement is not necessary in background and it is not clear the authors mentioned this statement: "Addition of plant-derived curcumin during anticancer therapy of Hep-3B cells inhibited cell death via the apoptotic pathway".

Response: We have removed that statement.

Page 4

Line 85-86: Addition of plant-derived curcumin during anticancer therapy of Hep-3B cells inhibited cell death via the apoptotic pathway (Liou at al., 2017). Was removed;

Editor Comments:

4- In some part of background is far away the main of the study "the effect of vaspin in survival of liver cancer cell.”

Response: previously we would like to show a wide spectrum of activities of vaspin, however now we focused on main aspect of manuscript.

Page 4

Line 73-78: Hepatocellular carcinoma (HCC), also called hepatoma (Hiron et al., 1992), is a high mortality liver cancer (Sun at al., 2017), with associations to hepatitis B (HBV) and C (HCV) viral infections and chronic alcohol intake (Yang et al., 2010). On the metabolic background of HCC, there are correlations to obesity, metabolic syndrome with insulin resistance and nonalcoholic fatty liver disease (NAFLD) (Mittal et al., 2013, Derra et al. 2018). were removed;

Line 88-91: “Osteogenic differentiation of MC3T3-E1 cells was examined in the molecular pathway PI3K-Akt under miR-34c control. This molecular loop was down-regulated by vaspin, which could inhibit bone reconstruction and regeneration in vitro (Liu et al., 2016). Were removed;
Page 6
Line 142: “was” replaced with “is”

Page 7
Line 105-173: Materials section was moved from the end of the manuscript and placed before Results section according to the Journal’s preparation notes.

Results
Page 8
Line 176: “(Fig. 1A and 1B)” replaced with “(Fig. 1 A, B and C)”
Line 179-180: “(Fig. 1B)” replaced with “(Fig. 1 C)”
Page 9
Line 195: “(Fig. 3A)” replaced with “(Fig. 3 A and B)”
Line 196: “(Fig. 3A)” replaced with “(Fig. 3 B)”
Line 199: “(Fig. 3B)” replaced with “(Fig. 3 C and D)”
Line 202: “(Fig. 3B)” replaced with “(Fig. 3 D)”

Editor Comments:
5- The histogram of flow cytometry for apoptosis assay should be presented in the figure section.
Response: Appropriate plots for apoptosis were added to the Figure section. Figure 1 was improved and flow cytometry dot plots were added.

Editor Comments:
6- The authors should use of appropriate positive control for ROS or RNS formation.
Response: On the Figure 3 an appropriate positive control was added, and examples of flow cytometry histograms are now presented. Positive control from cells treated for 5 minutes with H2O2 at concentration of 100 µM.

Discussion

Editor Comments:

7- The discussion should be focused on carcinogenesis of vaspin and resistance in cancer therapy by oxidative agents like drug induced ROS formation and induction of apoptosis by them.

Response: In section Vaspin enhances hepatoma cell viability and proliferation of Discussion section this aspect was developed and appropriate references were added.

Page 11

Line 262-275: “Only a few studies suggest a stimulatory effect of vaspin on proliferation of cancer cells (Booth at al., 2015; Erdogan et al., 2011; Fazeli et al., 2013). An elevated vaspin level was reported in colorectal cancer (Harwood et al., 2012) but a lower level in endometrial cancer (McCullough et al., 2008). Vaspin also increased the proliferation of rat insulinoma cells (Liu et al., 2017). Angiogenesis, new blood vessels formation, seems to be essential in cancer development and progression. Vaspin was positively related to intensity of angiogenesis in chronic hepatitis (Kukla et al., 2011) via association of adipokines with angiogenesis. The main risk factor of HCC in chronic liver diseases is advanced fibrosis/cirrhosis, which is strictly associated with angiogenesis intensity (Gabriel et al., 2009, Kukla et al. 2010). Vaspin serum levels and mRNA liver expression were found to be increased in patients with advanced fibrosis or cirrhosis in the course of chronic hepatitis (Kukla et a. 2012, Waluga et al. 2017). Pointing to these results, vaspin plays pivotal role in HCC development. Increased vaspin levels and expression in advanced liver disease may protect cancer cells against apoptosis and facilitate their proliferation.” This fragment was added.

Editor Comments:

8- Also necessary in discussion authors discuss which how vaspin reduce ROS and RNS radicals and apoptosis in cancer cells. This action could be useful for cancer treatment.

Response: In section Role of adipokines in regulating oxidative stress and next Vaspin down-regulates apoptotic pathways in Hep-3B cells of Discussion sections this aspect was pointed with appropriate references.

Page 13

Line 301: “which might be crucial” replaced with “this might be crucial”
Line 311-13: “Consequence for that could be also elevated pro-inflammatory cytokines production and vaspin inhibited that inflammatory process (Qi et al., 2017; Liu et al., 2014; Kiluk et al., 2017). This fragment was added.

Page 14

Line 337-345: Moreover, vaspin levels increased in definite nonalcoholic steatohepatitis when compared to simple steatosis and in patients with hepatocyte ballooning, which may reflect oxidative stress and mitochondrial dysfunction in hepatocytes (Kukla et al. 2010). The role of vaspin, either anti/pro-inflammatory, anti/pro-oxidative, or anti/pro-apoptotic is still unclear, but in Hep-3B cells it is rather protective (Fig. 6). Elevated serum concentrations of vaspin are associated with obesity and impaired insulin sensitivity in humans and it has therefore been postulated that increased vaspin expression and secretion could represent a compensatory mechanism associated with obesity, severe insulin resistance, and type 2 diabetes (Heiker et al., 2013). This fragment was modified.

Editor Comments:

9- Also the role of oxidative radicals in carcinogenesis must discuss in the discussion section.

Response: This aspect was pointed in first section of discussion Adipose tissue delivers regulatory factors with appropriate references.

Editor Comments:

10- In the figure for ROS formation there are no signs of statistical analyses. Also the name figure (Figure 1) is not above every figure.

Response: All Figures were statically analyzed, when there were no significant changes there were no asterisks added on charts (on the Figure’s legend it was appropriate described, also in text). Every Figure possesses appropriate titles and description. On the Figure 1 the titles were added, on the Figure 4 there were no significant changes in comparison to the untreated control.

Response: In References section all changes were made according to the journal style (Vancouver style).

Response: All necessary declarations were added previously.
Response: Funding section was changed; because my foundation sources were expired (I have mentioned that previously in my correspondence e-mails with Editorial Office), I have to involve additionally founding’s:

Page 15

Line 355-372 list of abbreviations was added.

Page 17

Line 362-369:

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