

Comparison of the effect of betanin on STAT3, STAT5, and KAP1 proteins in HepG2 and THLE-2 cells

Hanna Szaefer

Department of Pharmaceutical Biochemistry,
Poznan University of Medical Sciences, Poland

 <https://orcid.org/0000-0001-5875-3425>

Corresponding author: hszaefer@ump.edu.pl

Katarzyna Hadryś

Department of Pharmaceutical Biochemistry,
Poznan University of Medical Sciences, Poland

 —

Hanna Gajewska

Department of Pharmaceutical Biochemistry,
Poznan University of Medical Sciences, Poland

 —

Kinga Migdałek

Department of Pharmaceutical Biochemistry,
Poznan University of Medical Sciences, Poland

 —

Violetta Krajka-Kuźniak

Department of Pharmaceutical Biochemistry,
Poznan University of Medical Sciences, Poland

 <https://orcid.org/0000-0001-7275-0298>

 DOI: <https://doi.org/10.20883/medical.e805>

Keywords: betanin, STAT3, STAT5, KAP1, HepG2 cells, THLE-2 cells

Published: 2023-06-29

How to Cite: Szaefer H, Hadryś K, Gajewska H, Migdałek K, Krajka-Kuźniak V. Comparison of the effect of betanin on STAT3, STAT5, and KAP1 proteins in HepG2 and THLE-2 cells. *Journal of Medical Science*. 2023;92(2):e805. doi:10.20883/medical.e805



© 2023 by the author(s). This is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY-NC) licence. Published by Poznan University of Medical Sciences

ABSTRACT

Background. Several studies suggest that the pleiotropic properties of betanin may interfere with different signaling pathways. Our previous studies on human hepatocytes showed that betanin activated the nuclear factor erythroid-2-related factor 2 (Nrf2) signaling pathway. To further understand the exact mechanism of action of betanin, we evaluated its effect on the levels of signal transducers and activators of transcription (STATs) and KRAB domain-associated protein 1 (KAP1) in hepatoma cells (HepG2) and normal human hepatocytes (THLE-2).

Material and methods. HepG2 and THLE-2 cells were treated with 2 or 10 μ M betanin for 72 h. The levels of STAT3, STAT5a, STAT5b, and KAP1 proteins in cytosolic and nuclear fractions were assessed by Western blot.

Results. At a concentration of 10 μ M, betanin significantly decreased the levels of STAT3, STAT5a, and STAT5b proteins in the nuclear fraction of HepG2 cells. On the other hand, no significant changes in the levels of STAT proteins were observed in THLE-2 cells. In HepG2 cells, betanin at both tested doses increased the level of KAP1. In contrast, in THLE-2 cells, betanin at a dose of 10 μ M decreased the nuclear level of KAP1.

Conclusions. Betanin modulated the levels of STAT3, STAT5, and KAP1 proteins, especially in hepatoma cells. Thus, it may be considered a potential therapeutic agent for the treatment of hepatoma.

Introduction

Hepatocellular carcinoma (HCC) is the major form of primary liver cancer. It is one of the most serious human cancers, the pathogenesis of which

involves continuous hepatocyte death, inflammatory cell infiltration, and compensatory liver regeneration. Understanding the molecular signaling pathways that induce or mediate these

processes during liver carcinogenesis is essential for identifying novel therapeutic targets for this disease [1].

According to epidemiological studies, diet and physical activity influence the incidence of certain types of cancers, including liver cancer, and preventive measures should be taken in the early stages of these diseases [2]. Some prophylactic measures include a diet enriched with natural substances that can inhibit or reverse tumor development. For example, beetroot, a vegetable commonly included in the human diet, has many health-promoting properties. Besides antioxidant activity, it exhibits anti-inflammatory and detoxifying effects [3]. Many of the properties of beetroot are attributed to betalains, especially betanin. Betanin is widely used in the food industry as a coloring agent in fruit yogurt, ice cream, and cosmetic care products [4] and well-known for its anti-inflammatory and hepatoprotective functions in human cells. It has been reported that betanin inhibited cell proliferation in hepatoma cancer cells HepG2 [5] and ovarian cancer cell line PA-1 [6] and showed pro-apoptotic action in human lung cancer cell lines [7], U87MG human glioma cells [8], and oral squamous cancer cells SCC131 and SCC4 [9]. Another mechanism of the anticancer action of betanin is the modulation of signaling pathways. Betanin activated nuclear factor erythroid-2-related factor 2 (Nrf2) signaling pathway in hepatoma cancer cells HepG2 [10]. Additionally, the activation of NF- κ B/PI3K/Akt signaling pathway was observed in oral squamous cancer cells SCC131 and SCC4 [9]. Since epidemiological studies indicate that liver cancer still has a poor prognosis, intensive research is needed to develop new therapeutic solutions. One promising therapeutic strategy for the treatment of liver cancer is interfering with other signaling pathways.

Literature data highlight increased expression of signal transducers and activators of transcription (STATs) in liver tumors [11]. The upregulation of STAT signaling pathways promotes tumor growth and survival, due to the inhibition of apoptosis as well as increased cell proliferation, migration, and invasion [12].

The STATs family comprises seven members – STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, and STAT6. These cytoplasmic transcription factors mediate signal transduction from vari-

ous growth factors and cytokines to the nucleus [11]. Recent studies also indicate that STATs may interfere with the functions of KRAB domain-associated protein 1 (KAP1) [13–15], which promotes cell proliferation and metastatic progression in different cancers, including HCC [16].

Therefore, to further explore the mechanism of action of betanin, we assessed the effect of betanin on other signaling pathways such as STAT3, STAT5a, and STAT5b, as well as their dependence on KAP1 protein, in hepatoma cells (HepG2) and normal human hepatocytes (THLE-2).

Materials and methods

Chemicals

Betanin, dimethyl sulfoxide (DMSO), Tris, antibiotic solution (10⁴ U penicillin, 10 mg streptomycin, 25 μ g amphotericin B), Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), and trypsin were supplied by Sigma-Aldrich (USA). Bronchial Epithelial Cell Growth Basal Medium (BEGM) and Bullet Kit were purchased from Lonza/Clonetics Corporation (USA). Primary antibodies against STAT3 (sc-483), STAT5a (sc-1081), STAT5b (sc-1656), β -Actin (sc-7210), lamin (sc-20680), and secondary alkaline phosphatase labeled antibodies were obtained from Santa Cruz Biotechnology (Dallas, TX, USA). Primary antibody against KAP1 (ab10484) was obtained from Abcam (Cambridge, UK). The protein molecular weight marker was supplied from EURx (Gdańsk, Poland).

Cell Culture

The hepatocellular carcinoma cells – HepG2 (ATCC HB 8065, USA) were cultured in DMEM with 10% FBS and 1% antibiotic solution and grown under standard conditions (37°C, 5% CO₂). Normal human hepatocytes THLE-2 (ATCC CRL-2706, USA) were maintained in BEGM supplemented with Bullet Kit and 5 ng/mL EGF, 70 ng/mL phosphoethanolamine, and 10% FBS (37°C, 5% CO₂). After the 24 hours of initial incubation, the cells (1 \times 10⁶ cells per 100 mm culture dish) were treated with 2 or 10 μ M of betanin, incubated for a further 72h, and harvested. Control cells were treated with 0.1% DMSO. The dimethyl sulfoxide 100 mM stock solutions of betanin were used and stored at –20°C.

The doses of betanin were selected based on the MTT viability assay, which was carried out in our previous research [10].

Nuclear and Cytosolic Fractions Preparation

The subcellular extracts from HepG2 and THLE-2 cells were prepared using the Nu-clear/Cytosol Fractionation Kit (BioVision Research, Mountain-View, CA, USA) according to the manufacturer's protocol. Protein concentration was assessed using the Lowry method, and the samples were stored at -80°C for further analysis.

Western Blot Analysis

Nuclear fractions for STAT3, STAT5a, STAT5b, KAP1, lamin, or cytosolic fractions for STAT3, STAT5a, STAT5b, KAP1, β -Actin protein detection, were separated on 10% SDS-PAGE slab gels. β -Actin and lamin were used as a loading control. The amount of cytosolic and nuclear fractions was 100 μg of protein per well. Proteins were transferred to the nitrocellulose Immobilon P membrane (Millipore, Bedford, MA, USA). After blocking for 2 hours with 10% skimmed milk, proteins were probed with rabbit anti-STAT3, rabbit anti-STAT5a, mouse anti-STAT5b, rabbit anti- β -Actin, rabbit anti-lamin (Santa Cruz Biotechnology, Dallas, TX, USA), rabbit anti-KAP1 (Abcam, Cambridge, UK) antibodies (dilution 1:1000). The alkaline phosphatase-labeled anti-mouse IgG and anti-rabbit IgG (dilution 1:5000) were used as the secondary antibodies. Bands were visualized using the AP Conjugate Substrate Kit NBT/BCIP (BioRad Laboratories, Hercules, CA, USA). The amount of immunoreactive products in each lane was determined using the ChemiDoc Imaging System (BioRad Laboratories, Hercules, CA, USA). Values were calculated as relative absorbance units (RQ) per mg of protein and expressed as a percentage of the control.

Statistical Analysis

GraphPad InStat version 3.10 (GraphPad Software, San Diego, CA, USA) was used to perform statistical analysis. The data are shown as the means \pm SEM. To assess the significance of the differences in the evaluated parameters, one-way ANOVA with Dunnett's post hoc test was performed with the significance level of $p < 0.05$.

Results

Effect of betanin on STAT3 protein level in HepG2 and THLE-2 cells

The level of STAT3 protein in cytosolic and nuclear fractions of HepG2 and THLE-2 cells was investigated by Western blot using a specific antibody against this protein. As shown in **Figure 1**, HepG2 cells incubated with either of the tested doses of betanin showed no significant changes in the cytosolic level of STAT3 protein. However, an $\sim 19\%$ reduction in the nuclear STAT3 protein level was observed in these cells after treatment with betanin at the dose of 10 μM .

In THLE-2 cells, both cytosolic and nuclear levels of STAT3 protein remained unchanged following treatment with betanin at both tested doses.

Effect of betanin on STAT5a and STAT5b protein levels in HepG2 and THLE-2 cells

Western blot analysis revealed a significant increase in the cytosolic level of STAT5a protein in HepG2 tumor cells under the influence of 10 μM betanin (see **Figure 2**).

In contrast, an opposite trend of changes was observed in the nuclear levels of STAT5a and STAT5b proteins. At a dose of 10 μM , betanin significantly decreased (by 20–22%) the nuclear levels of both tested STAT5 isoforms in HepG2 cells (see **Figures 2 and 3**).

Similar to STAT3, no significant changes in both cytosolic and nuclear levels of STAT5a and STAT5b proteins were observed in THLE-2 cells after incubation with betanin at any of the tested doses (see **Figures 2 and 3**).

Effect of betanin on KAP1 protein level in HepG2 and THLE-2 cells

As shown in **Figure 4**, a significant increase in the nuclear level of KAP1 protein was noted in HepG2 cells after incubation with 2 or 10 μM betanin (by 38% and 52%, respectively), compared to the cells treated with DMSO.

However, in THLE-2 cells, a significant decrease in the nuclear KAP1 protein level was observed after treatment with betanin at a dose of 10 μM .

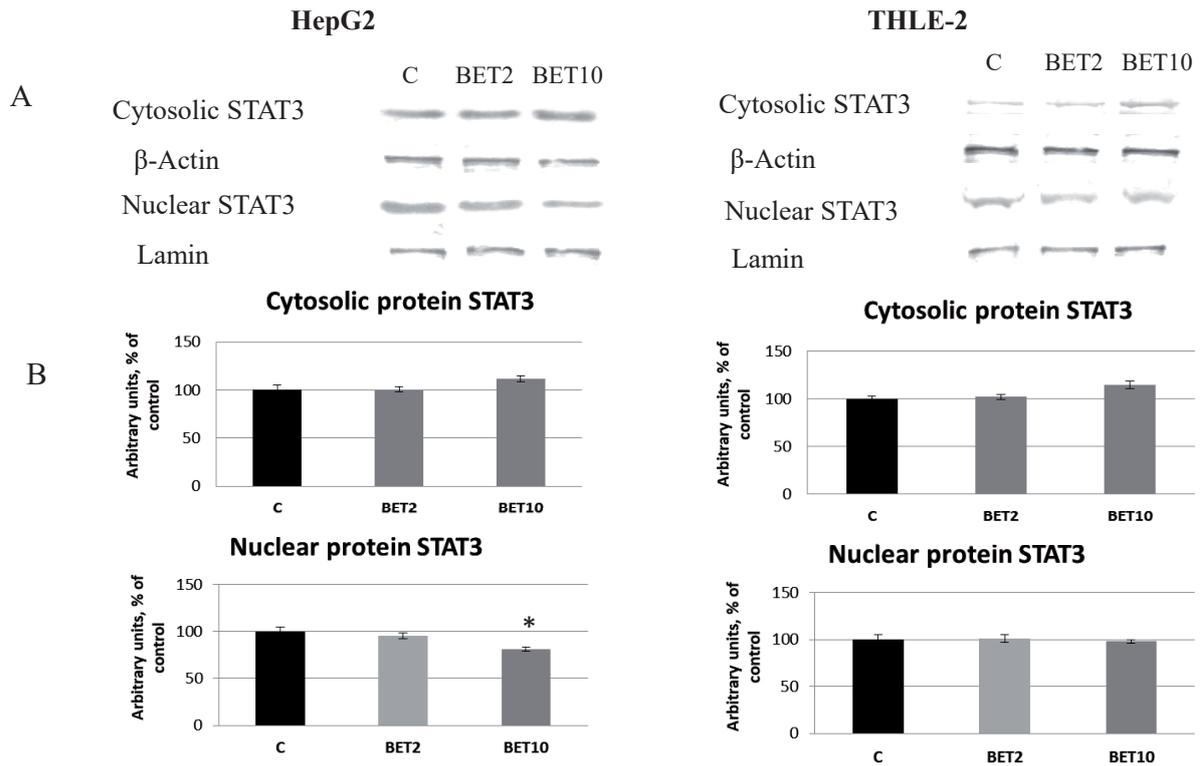


Figure 1. The effect of betanin on the level of the STAT3 protein in HepG2 and THLE-2 cells. A. Representative immunoblots showing the cytosolic and nuclear levels of STAT3 protein. B. Data (mean \pm SEM) from three separate experiments in comparison to the control cells set to 100%. Asterisk (*) above the bar indicates statistically significant differences from the control group, $p < 0.05$.

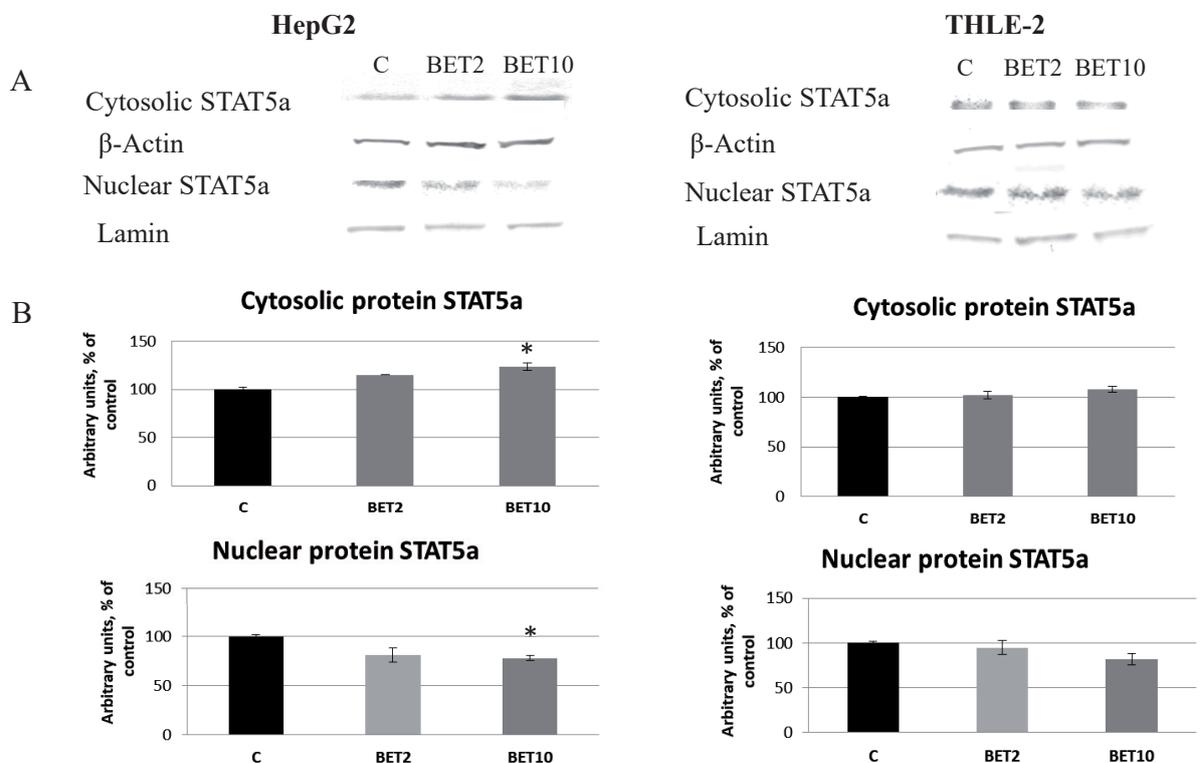


Figure 2. The effect of betanin on the level of the STAT5a protein in HepG2 and THLE-2 cells. A. Representative immunoblots showing the cytosolic and nuclear levels of STAT5a protein. B. Data (mean \pm SEM) from three separate experiments in comparison to the control cells set to 100%. Asterisk (*) above the bar indicates statistically significant differences from the control group, $p < 0.05$.

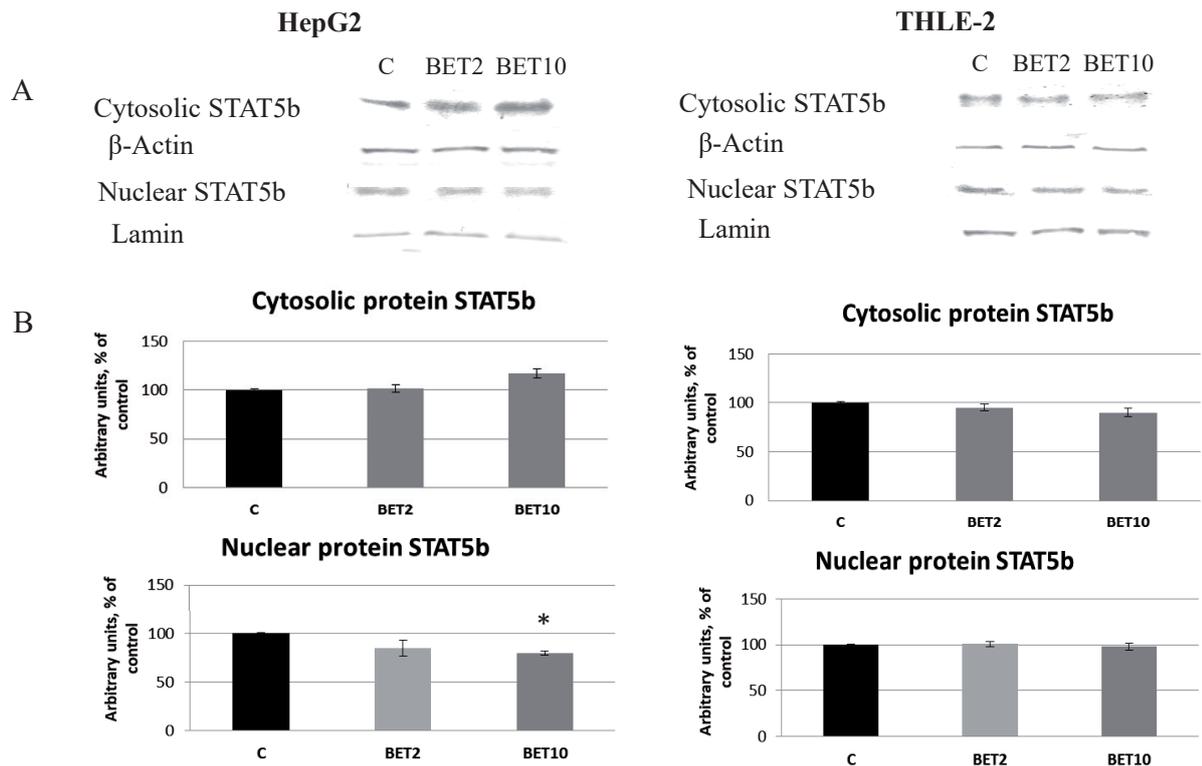


Figure 3. The effect of betanin on the level of the STAT5b protein in HepG2 and THLE-2 cells. A. Representative immunoblots showing the cytosolic and nuclear levels of STAT5b protein. B. Data (mean±SEM) from three separate experiments in comparison to the control cells set to 100%. Asterisk (*) above the bar indicates statistically significant differences from the control group, $p < 0.05$.

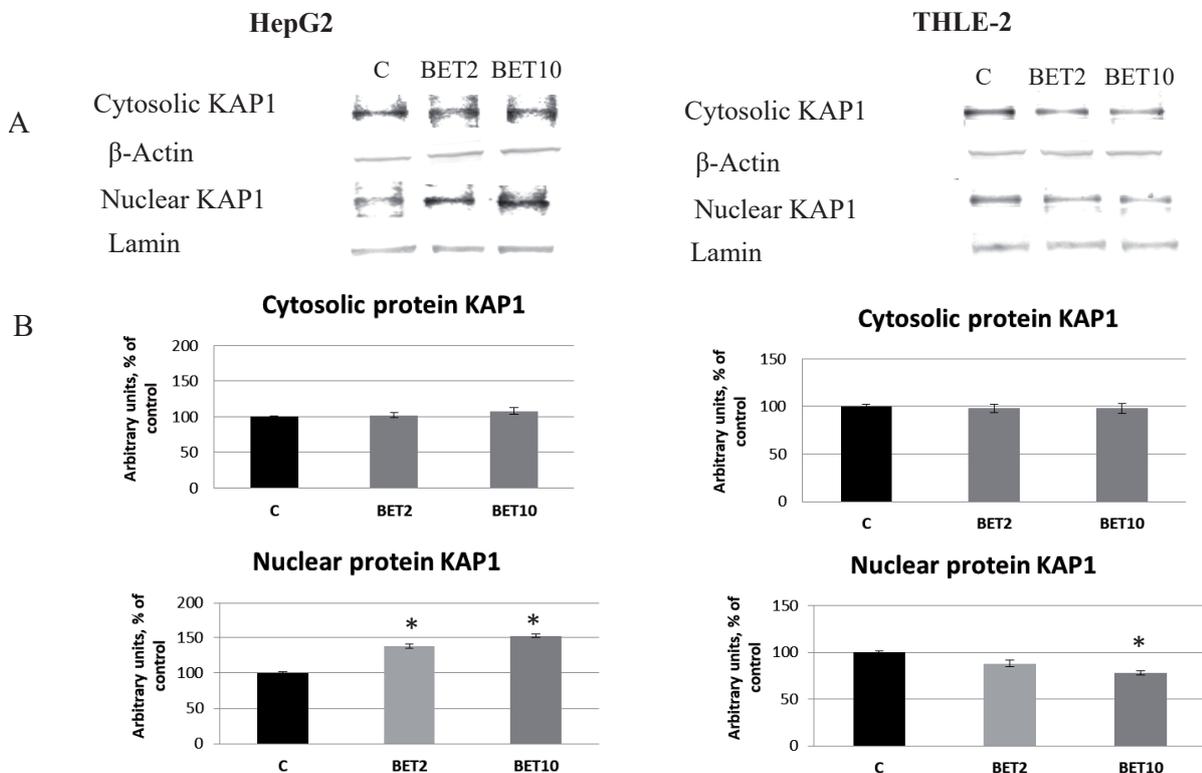


Figure 4. The effect of betanin on the level of the KAP1 protein in HepG2 and THLE-2 cells. A. Representative immunoblots showing the cytosolic and nuclear levels of KAP1 protein. B. Data (mean±SEM) from three separate experiments in comparison to the control cells set to 100%. Asterisk (*) above the bar indicates statistically significant differences from the control group, $p < 0.05$.

Discussion

Many studies have investigated the involvement of STAT3 in tumor development [17–19]. Recent evidence indicates that STAT5 plays a significant role in progression of several cancers, such as breast, colorectal, lung, prostate, and liver cancer [12].

In the case of liver cancer, STAT5 plays a dual role. First, STAT5 can act as an oncogene promoting tumor development and progression by enhancing cell proliferation of cancer stem cells (CSCs), chemoresistance, and epithelial–mesenchymal transition, a key mechanism that could lead to increased invasion and metastasis [20–22]. Fu et al. [20] demonstrated that in HCC increased STAT5 expression regulated by GRAM domain-containing 1A (GRAMD1A), a cholesterol transporter, could induce tumor growth, and chemoresistance and increased CSC side population and tumor cell survival by upregulating cyclin D1, Bcl-2, c-Myc, and c-Jun, as well as downregulating caspase 3 and poly (ADP-ribose) polymerase (PARP). Second, STAT5 can act as a tumor suppressor in the liver. Studies by Yu et al. [23] showed the upregulation of NADPH oxidase 4 (NOX4), an enzyme involved in the generation of reactive oxygen species, as well as the induction of pro-apoptotic proteins: p53-upregulated modulator of apoptosis (PUMA) and Bcl-2-interacting mediator of cell death (BIM).

These arguments convinced us to search for potential inhibitors of the abovementioned transcription factors. Betanin is one such compound, which is found in beetroot and exhibits pleiotropic effects [3, 24], including on transcription factors.

In this study, we examined the translocation of STAT3 and STAT5 from the cytosol to the nucleus. Our results showed that betanin decreased the nuclear levels of STAT3 and STAT5 in HepG2 hepatoma cells. The most significant inhibitory effect of betanin was observed at the higher dose (10 μ M), at which the nuclear level of STAT3 was decreased in HepG2 cells. It should be emphasized that the inhibitory effect of betanin on STATs has not been explored in hepatoma cells. In normal hepatocytes, THLE-2, we did not observe any significant changes in the levels of STATs. A similar effect has been reported for other components of sugar beet, namely betavulgarin in breast CSCs [25]. In addition, studies have demonstrated STAT inhibition in HepG2 cells using other phy-

tochemicals or active biological compounds. For instance, a study by Aggarwal and team [26] showed a reduction in the nuclear level of STAT3 in HepG2 cells after treatment with *Aegle marmelos* leaf extract, rutin, and quercetin. Soni et al. [27] observed a similar inhibitory effect of curcumin on the STAT3 protein level in the liver cancer line HepG2 and T-cell lymphoma line HuT78.

Some studies verified the possible involvement of Ser727 phosphorylation of STAT3 in cell survival and activities. For example, a study showed the reduction of phosphorylation of STAT3 at position Ser727 in the HepG2 cell line under the influence of resveratrol at a concentration of 25 μ M [28]. Additionally, Liu et al. [29] examined the effect of sulforaphane, which is abundant in cruciferous vegetables, such as cauliflower and broccoli, on the levels of total and phosphorylated STAT3 in the HepG2 cell line. The authors observed a reduction in the level of both proteins after treatment with sulforaphane at a concentration range of 5–20 μ M.

Understanding the role of STAT3 could be crucial to determine the potential application of STAT3 as a marker in liver cancer.

Studies conducted so far on the effect of phytochemicals on the JAK/STAT signaling pathway in an *in vitro* model mainly focused on the STAT3 protein, while very few concerned the STAT5 protein. Therefore, the effect of betanin on STAT5 may be of interest.

The present study confirmed the same direction of change for STAT5 also. At a dose of 10 μ M, betanin significantly decreased the nuclear level of STAT5a and STAT5b proteins in HepG2 cells. Jung et al. [30] also reported the inhibition of STAT5 protein, but with other compounds and in different cell lines. The authors showed that oxymatrine, a major alkaloid found in radix *Sophorae flavescens* extract, inhibited the activation of STAT5 and its binding to DNA in the lung cancer cell line A549. Sulforaphane has also been shown to inhibit STAT5 in the human leukemia cell line K562 [31].

Recent studies suggest that the KAP1 protein may interfere with STATs. KAP1 is a universal corepressor protein for the transcriptional repressors belonging to the KRAB zinc finger protein superfamily [13,15]. Tsuruma et al. [13] observed that endogenous KAP1 was associated with endogenous STAT3 in an *in vivo* model. KAP1 is susceptible to several posttranslational

modifications, including phosphorylation, which directly alters its biological functions. This protein is responsible for differentiation, transcriptional regulation, gene silencing, and response to DNA damage. It also plays a role in the control of oxidative stress and carcinogenesis [32]. Hence, there has been increasing interest in using the KAP1 protein for diagnostic purposes or as a marker for cancer treatment.

In this study, we assessed the effect of betanin on the level of KAP1. Our densitometric analysis showed that betanin increased the nuclear level of this protein in hepatoma cells while it decreased its level in normal cells. This discrepancy may be due to the different mechanisms of action of betanin in cancer and normal cells or its interaction with other transcription factors. It should be emphasized that our study is the first to determine the effect of betanin on KAP1 levels in hepatoma cells.

Few studies have been conducted by others to determine the level of KAP1 protein. Wang et al. [16] reported the overexpression of KAP1 in a human hepatoma cell line. In addition, comparisons between cancer and non-cancerous tissues proved that the expression level of KAP1 was significantly higher in tumor tissues obtained from HCC patients. These data confirm that KAP1 plays an important role in the development of HCC and could be a valuable biomarker for tumor diagnosis and prognosis prediction, as well as a potential therapeutic target for the treatment of this disease.

Similarly, in other research models, the expression of KAP1 has been shown to be higher in cancer cells of various tissues (e.g. lung cancer cells) compared to noncancerous cells [33].

In summary, natural compounds can be effective in the prevention and treatment of liver cancer. Our study highlighted the inhibitory effect of betanin on STAT3 and STAT5 in HepG2 cells, thus confirming its potential therapeutic effect. However, further *in vitro* and *in vivo* studies must be carried out to explain the mechanisms of chemopreventive and antitumor activity of phytochemicals, including betanin.

Acknowledgements

Conflict of interest statement

The authors declare no conflict of interest.

Funding sources

The present study was supported by research funding from Poznan University of Medical Sciences, Poznan, Poland.

Author Contributions

Conceptualization: V. Krajka-Kuźniak and H. Szaefer; methodology: V. Krajka-Kuźniak, K. Hadryś, H. Gajewska, K. Migdałek and H. Szaefer; validation: V. Krajka-Kuźniak and H. Szaefer; formal analysis: V. Krajka-Kuźniak and H. Szaefer; investigation: V. Krajka-Kuźniak and H. Szaefer; writing original draft preparation: V. Krajka-Kuźniak and H. Szaefer.

References

1. He G, Karin M. NF- κ B and STAT3 – key players in liver inflammation and cancer. *Cell Res.* 2011 Jan;21(1):159-168. doi:10.1038/cr.2010.18.
2. Kotecha R, Takami A, Espinosa JL. Dietary phytochemicals and cancer chemoprevention: a review of the clinical evidence. *Oncotarget.* 2016 Aug 9;7(32):52517-52529. doi: 10.18632/oncotarget.9593.
3. Hadipour E, Taleghani A, Tayarani-Najaran N, Tayarani-Najaran Z. Biological effects of red beetroot and betalains: A review. *Phytother Res.* 2020 Aug;34(8):1847-1867. doi: 10.1002/ptr.6653.
4. Santamaria P. Nitrate in vegetables: Toxicity, content, intake and EC regulation. *J Sci Food Agric.* 2006; 86: 10-17. doi: 10.1002/jsfa.2351.
5. Lee EJ, An D, Nguyen CTT, Patil BS, Kim J, Yoo KS. Betalain and betaine composition of greenhouse- or field-produced beetroot (*Beta vulgaris* L.) and inhibition of HepG2 cell proliferation. *J Agric Food Chem.* 2014 Feb 12;62(6):1324-31. doi: 10.1021/jf404648u.
6. Rakshanaa R, Shafreen M, Kumar N. Inhibition of Proliferation in Ovarian Cancer Cell Line (PA-1) by the Action of Green Compound "Betanin". *Appl Biochem Biotechnol.* 2022 Jan;194(1):71-83. doi: 10.1007/s12010-021-03744-0.
7. Zhang Q, Pan J, Wang Y, Lubet R, You M. Beetroot red (betanin) inhibits vinyl carbamate- and benzo(a) pyrene-induced lung tumorigenesis through apoptosis. *Mol Carcinog.* 2013 Sep;52(9):686-91. doi: 10.1002/mc.21907.
8. Salimi A, Bahiraei T, Ahdeno S, Vatanpour S, Pourahmad J. Evaluation of Cytotoxic Activity of Betanin Against U87MG Human Glioma Cells and Normal Human Lymphocytes and Its Anticancer Potential Through Mitochondrial Pathway. *Nutr Cancer.* 2021;73(3):450-459. doi: 10.1080/01635581.2020.1764068.
9. Zou X, Yu K, Chu X, Yang L. Betanin alleviates inflammation and ameliorates apoptosis on human oral squamous cancer cells SCC131 and SCC4 through the NF- κ B/PI3K/Akt signaling pathway. *J Biochem Mol Toxicol.* 2022 Aug;36(8):e23094. doi: 10.1002/jbt.23094.
10. Krajka-Kuźniak V, Paluszczak J, Szaefer H, Baer-Dubowska W. Betanin, a beetroot component, induces nuclear factor erythroid-2-related factor 2-mediated expression of detoxifying/antioxidant enzymes in human liver cell lines. *Br*

- J Nutr. 2013 Dec;110(12):2138-49. doi: 10.1017/S0007114513001645.
11. Svinka J, Mikulits W, Eferl R. STAT3 in hepatocellular carcinoma: new perspectives. *Hepat Oncol*. 2014 Jan;1(1):107-120. doi: 10.2217/hep.13.7.
 12. Halim CE, Deng S, Ong MS, Yap CT. Involvement of STAT5 in Oncogenesis. *Biomedicines*. 2020 Aug 28;8(9):316. doi: 10.3390/biomedicines8090316.
 13. Tsuruma R, Ohbayashi N, Kamitani S, Ikeda O, Sato N, Muromoto R, Sekine Y, Oritani K, Matsuda T. Physical and functional interactions between STAT3 and KAP1. *Oncogene*. 2008 May 8;27(21):3054-9. doi: 10.1038/sj.onc.1210952.
 14. Kamitani S, Ohbayashi N, Ikeda O, Togi S, Muromoto R, Sekine Y, Ohta K, Ishiyama H, Matsuda T. KAP1 regulates type I interferon/STAT1-mediated IRF-1 gene expression. *Biochem Biophys Res Commun*. 2008 May 30;370(2):366-70. doi: 10.1016/j.bbrc.2008.03.104.
 15. Kamitani S, Togi S, Ikeda O, Nakasuji M, Sakauchi A, Sekine Y, Muromoto R, Oritani K, Matsuda T. Krüppel-associated box-associated protein 1 negatively regulates TNF- α -induced NF- κ B transcriptional activity by influencing the interactions among STAT3, p300, and NF- κ B/p65. *J Immunol*. 2011 Sep 1;187(5):2476-83. doi: 10.4049/jimmunol.1003243.
 16. Wang Y, Jiang J, Li Q, Ma H, Xu Z, Gao Y. KAP1 is overexpressed in hepatocellular carcinoma and its clinical significance. *Int J Clin Oncol*. 2016 Oct;21(5):927-933. doi: 10.1007/s10147-016-0979-8.
 17. Bromberg J. Stat proteins and oncogenesis. *Clin Invest*. 2002 May;109(9):1139-42. doi: 10.1172/JCI15617.
 18. Chai E Z P , Shanmugam MK , Arfuso F, Dharmarajan A, Wang C, Kumar AP, Samy RP, Lim LHK, Wang L, Goh BC, Ahn KS, Hui KM , Sethi G. Targeting transcription factor STAT3 for cancer prevention and therapy. *Pharmacol Ther*. 2016 Jun;162:86-97. doi: 10.1016/j.pharmthera.2015.10.004.
 19. Thilakasiri PS, Dmello RS, Nero TL , Parker MW, Ernst M, Chand AL. Repurposing of drugs as STAT3 inhibitors for cancer therapy. *Semin Cancer Biol*. 2021 Jan;68:31-46. doi: 10.1016/j.semcancer.2019.09.022.
 20. Fu B , Meng W, Zhao H , Zhang B , Tang H , Zou Y, Yao J , Li H, Zhang T. GRAM domain-containing protein 1A (GRAMD1A) promotes the expansion of hepatocellular carcinoma stem cell and hepatocellular carcinoma growth through STAT5. *Sci Rep*. 2016 Sep 2;6:31963. doi: 10.1038/srep31963.
 21. Zhao C, Wang Q, Wang B, Sun Q, He Z, Hong J , Kuehn F, Liu E, Zhang Z. IGF-1 induces the epithelial-mesenchymal transition via Stat5 in hepatocellular carcinoma. *Oncotarget*. 2017 Dec 5;8(67):111922-111930. doi: 10.18632/oncotarget.22952.
 22. Lee TK, Man K, Poon R TP, Lo C M, A Yuen AP, Ng IO, Ng KT, Leonard W, Fan ST. Signal transducers and activators of transcription 5b activation enhances hepatocellular carcinoma aggressiveness through induction of epithelial-mesenchymal transition. *Cancer Res*. 2006 Oct 15;66(20):9948-56. doi:10.1158/0008-5472.CAN-06-1092.
 23. Yu JH, Zhu BM, Riedlinger G, Kang K, Hennighausen L. The liver-specific tumor suppressor STAT5 controls expression of the reactive oxygen species-generating enzyme NOX4 and the proapoptotic proteins PUMA and BIM in mice. *Hepatology*. 2012 Dec;56(6):2375-86. doi: 10.1002/hep.25900.
 24. Lechner JF, Stoner GD. Red Beetroot and Betalains as Cancer Chemopreventative Agents. *Molecules*. 2019 Apr 23;24(8):1602. doi: 10.3390/molecules24081602.
 25. Liu R, Choi HS, Zhen X, Kim S-L, Kim J-H, Ko Y-Ch, Yun B-S, Lee DS. Betavulgarin Isolated from Sugar Beet (*Beta vulgaris*) Suppresses Breast Cancer Stem Cells through Stat3 Signaling. *Molecules*. 2020 Jul; 25(13): 2999. doi: 10.3390/molecules25132999.
 26. Aggarwal H, Nair J, Sharma P, Sehgal R, Naeem U, Rajora P, Mathur R. Aegle marmelos differentially affects hepatic markers of glycolysis, insulin signalling pathway, hypoxia, and inflammation in HepG2 cells grown in fructose versus glucose-rich environment. *Mol Cell Biochem*. 2018 Jan;438(1-2):1-16. doi: 10.1007/s11010-017-3108-8.
 27. Soni VK, Shukla D, Kumar A, Vishvakarma NK. Curcumin circumvent lactate-induced chemoresistance in hepatic cancer cells through modulation of hydroxycarboxylic acid receptor-1. *Int J Biochem Cell Biol*. 2020 Jun;123:105752. doi: 10.1016/j.biocel.2020.105752.
 28. Li Y, Zhu W, Li J, Liu M, Wei M. Resveratrol suppresses the STAT3 signaling pathway and inhibits proliferation of high glucose-exposed HepG2 cells partly through SIRT1. *Oncol Rep*. 2013 Dec;30(6):2820-2828. doi: 10.3892/or.2013.2748.
 29. Liu P, Atkinson SJ, Akbareian SE, Zhou Z, Munsterberg A, Robinson SD, Bao Y. Sulforaphane exerts anti-angiogenesis effects against hepatocellular carcinoma through inhibition of STAT3/HIF-1 α /VEGF signalling. *Sci Rep*. 2017 Oct 4;7(1):12651. doi: 10.1038/s41598-017-12855-w.
 30. Jung YY, Shanmugam MK, Narula AS, Kim Ch, Lee JH, Namjoshi OA, Blough BE, Sethi G, Ahn KS. Oxymatrine attenuates tumor growth and deactivates STAT5 signaling in a lung cancer xenograft model. *Cancers (Basel)*. 2019 Jan 7;11(1):49. doi: 10.3390/cancers11010049.
 31. Pinz S, Unser S, Rasclé A. The natural chemopreventive agent sulforaphane inhibits STAT5 activity. *PLoS One*. 2014 Jun 9; 9(6): e99391: 1-14. doi: 10.1371/journal.pone.0099391.
 32. Cheng CT, Kuo CY, Ann DK. KAP1 in charge of multiple missions: Emerging roles of KAP1. *World J Biol Chem*. 2014 Aug 26;5(3):308-20. doi: 10.4331/wjbc.v5.i3.308.
 33. Wu GJ, Pen J, Huang Y, An S, Liu Y, Yang Y, Hao Q, Guo X-X, Xu T-R. KAP1 inhibits the Raf-MEK-ERK pathway to promote tumorigenesis in A549 lung cancer cells *Mol Carcinog*. 2018 Oct;57(10):1396-1407. doi: 10.1002/mc.22853.