

RESEARCH

Open Access



# Cryptolepine inhibits hepatocellular carcinoma growth through inhibiting interleukin-6/STAT3 signalling

Seth A. Domfeh<sup>1,2</sup>, Patrick W. Narkwa<sup>3</sup>, Osbourne Quaye<sup>1,2</sup>, Kwadwo A. Kusi<sup>1,2,4</sup>, Gordon A. Awandare<sup>1,2</sup>, Charles Ansah<sup>5</sup>, Alimatu Salam<sup>6</sup> and Mohamed Mutocheluh<sup>3\*</sup>

## Abstract

**Background:** Diverse signalling pathways are involved in carcinogenesis and one of such pathways implicated in many cancers is the interleukin 6/signal transducer and activator of transcription 3 (IL-6/STAT3) signalling pathway. Therefore, inhibition of this pathway is targeted as an anti-cancer intervention. This study aimed to establish the effect of cryptolepine, which is the main bioactive alkaloid in the medicinal plant *Cryptolepis sanguinolenta*, on the IL-6/STAT3 signalling pathway.

**Methods:** First, the effect of cryptolepine on the IL-6/STAT3 pathway in human hepatoma cells (HepG2 cells) was screened using the Signal Finder Multi-Pathway Reporter Array. Next, to confirm the effect of cryptolepine on the IL-6/STAT3 signalling pathway, the pathway was activated using 200 ng/mL IL-6 in the presence of 0.5–2  $\mu$ M cryptolepine. The levels of total STAT3, p-STAT3 and IL-23 were assessed by ELISA.

**Results:** Cryptolepine downregulated 12 signalling pathways including the IL-6/STAT3 signalling pathway and upregulated 17 signalling pathways. Cryptolepine, in the presence of IL-6, decreased the levels of p-STAT3 and IL-23 in a dose-dependent fashion.

**Conclusion:** Our results demonstrated that cryptolepine inhibits the IL-6/STAT3 signalling pathway, and therefore cryptolepine-based remedies such as *Cryptolepis sanguinolenta* could potentially be used as an effective immunotherapeutic agent for hepatocellular carcinoma and other cancers.

**Keywords:** Cryptolepine, IL-6/STAT3, Carcinogenesis, Hepatoma, Anti-cancer

## Introduction

Interleukin 6 (IL-6) is produced by several cells such as tumour cells, adipocytes, fibroblasts, macrophages, hepatocytes and endothelial cells [1, 2]. This pleiotropic cytokine interacts with its receptor (IL-6R) to form the IL-6/IL-6R complex. The complex dimerises with glycoprotein 130 (GP130), a transmembrane protein, leading to the activation of Janus kinases (JAK),

which in turn phosphorylates the signal transducer and activator of transcription 3 (STAT3). The phosphorylated STAT3 (p-STAT3) homodimerises and moves into the nucleus where the homodimer binds to the promoter region of effector genes including, but not limited to, IL-23, c-Myc, Bcl-2 and HIF-1 $\alpha$  (reviewed in [3]). In normal liver cells, IL-6 plays a vital role in the defence against infection, liver regeneration and hepatocyte homeostasis [4, 5]. However, aberrant induction of IL-6 is injurious to the liver and could lead to the development of hepatocellular carcinoma (HCC) [6, 7]. Persistent hyperactivation of

\* Correspondence: [mmutocheluh.chs@knust.edu.gh](mailto:mmutocheluh.chs@knust.edu.gh); [mutocheluh@gmail.com](mailto:mutocheluh@gmail.com)

<sup>3</sup>Department of Clinical Microbiology, School of Medicine and Dentistry, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana  
Full list of author information is available at the end of the article



© The Author(s). 2021 **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated in a credit line to the data.

the IL-6/STAT3 signalling pathway also leads to angiogenesis [8], epithelial-mesenchymal transition [9] and stemness [10] in the tumour microenvironment.

Targeting the STAT3 signalling could be a novel approach to prevent and/or treat cancers since p-STAT3 contributes to malignant transformation and progression. Some studies have shown that serum IL-6 levels are related to the tumour stage and size, metastasis and survival of colon cancer patients [11], chemotherapy efficacy for advanced pancreatic cancer [12], neoadjuvant chemoradiotherapy for oesophageal carcinoma [13] and metastasis-related morbidity of breast cancer [14]. Currently, strategies are being developed to inhibit the STAT3 signalling pathway by targeting the activation of cytokine and growth factor receptors with monoclonal antibodies or receptor antagonists. For example, the use of IL-6R super-antagonists such as Sant7 inhibited IL-6-dependent human myeloma cell growth (IC<sub>50</sub> = 0.16 nM), as well as induced cell death as a pro-apoptotic factor through the STAT3 signalling [15].

Globally, HCC is among the most life-threatening cancers [16] because the condition causes a decline in the quality of life and normally leads to death after few months to a year from the time of diagnosis [17]. According to the GLOBOCAN 2018 Report, HCC is the sixth most common cancer and the fourth cause of cancer-related deaths worldwide [18]. Chronic viral hepatitis, mainly caused by hepatitis B and C virus (HBV and HCV), account for about 80% of HCC globally [19], and in low- and middle-income countries (LMICs), about two-thirds of HCCs are associated with HBV and/or HCV infection [20, 21]. The current interventions used in the management of HCC include targeted systemic chemotherapy, liver transplantation, surgical resection, percutaneous radiofrequency ablation, transarterial chemoembolization and radioembolization [22]. Besides the cost that is associated with these therapeutic interventions, they are unavailable in LMICs, and hence the need for other sources of therapy that are cheaper, readily accessible and culturally acceptable. Most persons living in LMICs depend on herbal medicine for their primary care needs and hence, we believe that repurposing locally acceptable herbal products will be a complementary approach in the management of HCC and other cancers. Moreover, these medicinal plants are in abundance and easily accessible.

Cryptolepine is the main bioactive alkaloid in *Cryptolepis sanguinolenta* (Lindl.) Schlechter (Apocynaceae), a medicinal plant that is indigenous to West Africa [23], and has been reported to exhibit numerous pharmacological properties such as anti-malarial, anti-bacterial, anti-fungal, anti-hyperglycaemic [reviewed in 23], and anti-inflammatory effects in different animal models [24, 25]. Due to the potent effects of cryptolepine, *C. sanguinolenta*

is used in Ghana and other West African countries for the treatment of malaria [26], and other ailments in West Africa including, but not limited to, pyrexia, jaundice, septicaemia, hypertension, tuberculosis, hepatitis and diabetes [27–29]. Cryptolepine and its derivatives have been reported to be toxic to mammalian cancer cells [30–32]; an anti-cancer effect that is exhibited by directly binding to DNA and thereby inhibiting DNA replication [30, 33]. Cryptolepine has also been reported to inhibit cancer cell growth by significantly reducing enhancers of mitochondrial biogenesis and by up-regulating p53 [30, 31].

Although some of the mechanisms of cryptolepine-associated anti-cancer properties have been reported, further studies are required to explore the anti-HCC effect of cryptolepine. Here we demonstrate the effect of cryptolepine on the IL-6/STAT3 signalling pathway.

## Materials and methods

### Chemical and reagents

The cryptolepine used in this study was donated by Professor Kwesi Mensah Boadu of the Faculty of Pharmacy and Pharmaceutical Sciences at the Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. The cryptolepine was prepared to a stock concentration of 861 µM in phosphate-buffered saline, filter sterilized, aliquoted into Eppendorf tubes, wrapped with aluminium foil and stored at –20 °C until used. Recombinant human IL-6 protein (cat # ab9627), niclosamide (cat # ab120868), STAT3 (Tyr705) In-Cell ELISA Kit (cat # ab126427) and IL-23 Human ELISA Kit (cat # ab64708) were purchased from Abcam (Cambridge, USA). Signal Finder Reporter Array Plate (cat # CCA-901 L) and Attractene Transfection Reagent (cat # 301005) were purchased from Qiagen (Germantown, USA). Dual Luciferase Reporter Assay System (cat# E1960) was purchased from Promega (Madison, USA) and Thiazolyl blue tetrazolium bromide (MTT) powder (cat # M5655-1G) was purchased from Sigma Aldrich (St. Louis, USA). All reagents and chemicals were prepared according to the manufacturers' instructions.

### Cell culture

The human hepatoma cell line (HepG2, cat # ATCC HB-8065) was purchased from American Type Culture Collection (ATCC, Manassas, USA). The cells were maintained in Eagle's Minimum Essential Medium (EMEM, Manassas, ATCC) that has been supplemented with 10% heat-inactivated foetal bovine serum (FBS, ATCC 30–2020), 1% MEM non-essential amino acids (ScienCell, Carlsbad, USA, cat # 0823), 100 IU/mL penicillin (Gibco Life Technologies Ltd., Paisley, UK, cat # 15140122) and 100 µg/mL streptomycin (Gibco Life Technologies Ltd., Paisley, UK, cat # 15140122), to make a complete growth medium, and were grown in 5%

carbon dioxide (CO<sub>2</sub>) under a humidified condition at 37 °C.

#### Cytotoxicity assay

The HepG2 cells were cultured at  $4 \times 10^4$  cells per well in 96-well plates in 100 µL complete growth medium. At 24 h post-incubation, the cells were treated with 0.5–8 µM cryptolepine or 4–32 µM niclosamide. Cell viability was assessed at 24, 48 and 72 h post-treatment using an MTT assay. A volume of 20 µL MTT solution (5 mg/mL MTT working solution in phosphate-buffered saline) was added to the wells and further incubated in 5% CO<sub>2</sub> under a humidified condition at 37 °C. After 3 h, the culture supernatants were carefully removed and 130 µL absolute isopropanol was added to the wells. The plate was incubated as above for 30 min and the optical density of the colour formed was read at 595 nm using an iMark Microplate Reader (Bio-Rad, USA).

#### Determination of cryptolepine effects on signal transduction pathways in HepG2 cells

The effects of cryptolepine on 45 signal transduction pathways in HepG2 cells were elucidated using the Cignal Finder Multi-Pathway Reporter Array. The reporter array has 45 inducible transcription factor-responsive constructs, encoding the firefly luciferase reporter gene, which have been dried down in duplicate wells of a 96-well plate. Also, the *Renilla* luciferase gene is included in the wells to normalise transfection efficiency. To determine the effects of cryptolepine on the signalling pathways, the constructs were reverse transfected into HepG2 cells at  $8 \times 10^4$  cells per well using the Attractene Transfection Reagent. At 24 h post-transfection, the cells were treated with 5 µM cryptolepine, and 24 h later, luciferase assay was performed using the Dual Luciferase Reporter Assay System following the manufacturer's instructions. The Berthold Orion Luminometer (Berthold Detection Systems, Germany) was used in the measurement of luminescence. Cells that were not treated with cryptolepine served as negative control and used to normalise the pathway activity in the cells that were treated with cryptolepine. The control experiment was done in parallel with the main experiment.

#### Determination of the effects of cryptolepine on the levels of p-STAT3 and IL-23 in the presence of IL-6

The next experiments were designed to validate the down-regulatory effect of cryptolepine on the IL-6/STAT3 signalling pathway since this pathway has been implicated in the pathogenesis of cancers including HCC [7, 34]. First, the concentration of IL-6 with optimal pathway activity was determined. The HepG2 cells were cultured at  $4 \times 10^4$  cells per well in 96-well plates in 100 µL complete growth medium. At 24 h post-

incubation, the cells were rinsed with PBS and serum-starved for another 24 h. The cells were stimulated with 50–200 ng/mL IL-6 in serum-free medium for 24 h, and the levels of phosphorylated and total STAT3 were assessed using the STAT3 (Tyr705) In-Cell ELISA Kit following the manufacturer's instructions. The phosphorylated STAT3 (p-STAT3) levels were normalised to the total STAT3 levels as previously done [35]. Levels of IL-23 (a responsive cytokine of the STAT3 pathway (reviewed in [36]) in culture supernatants were assessed using the IL-23 Human ELISA Kit following the manufacturer's instructions. Next, the suppression of p-STAT3 and IL-23 was determined using niclosamide, a known inhibitor of STAT3 signalling [37]. Hence, HepG2 cells were treated with 5 and 10 µM niclosamide in the presence of 200 ng/mL IL-6 in a serum-free medium. The levels of phosphorylated and total STAT3, as well as IL-23, were assessed after 24 h. Further, to determine the effect of cryptolepine on the IL-6/STAT3 signalling pathway, HepG2 cells were treated with 0.5–2 µM cryptolepine in the presence of 200 ng/mL IL-6 in serum-free medium. The additive effect of 0.5 µM cryptolepine and 5 µM niclosamide on the IL-6/STAT3 signalling pathway was also assessed by co-treating with the alkaloid and inhibitor in the presence of IL-6 and measuring the levels of total STAT3, p-STAT3 and IL-23 after 24 h.

#### Data analysis

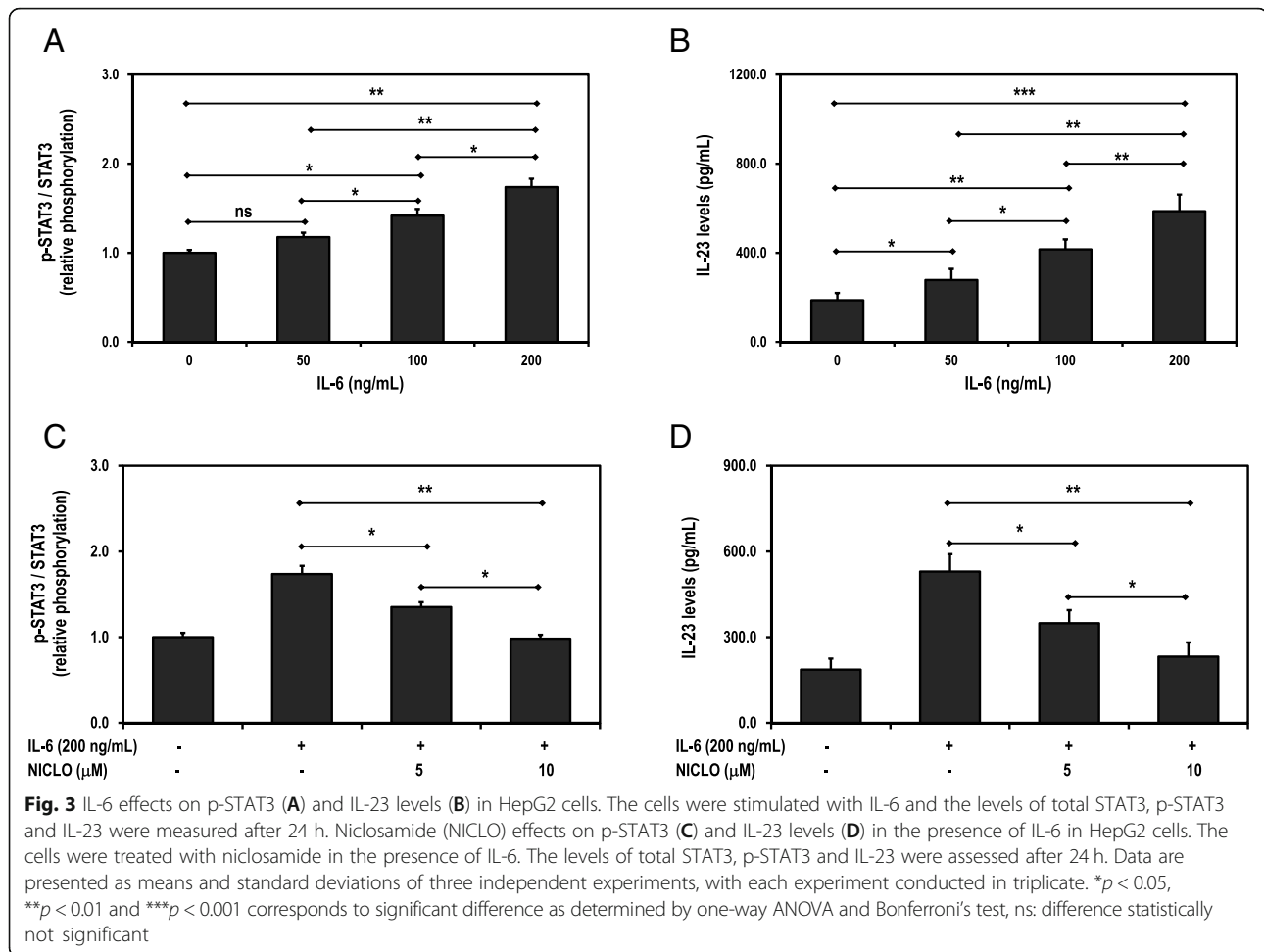
All the experiments were conducted on three different occasions and each assay was done in duplicate or triplicate. The data were analysed using Microsoft Excel (version 2019). Cell viability was determined by the ratio of cells cultured in the presence of cryptolepine to cells cultured in the absence of cryptolepine. Reporter array data were converted into fold change based on normalised firefly luciferase activity in the cells cultured in the presence of cryptolepine to the cells cultured in the absence of cryptolepine and presented on a log<sub>2</sub> scale. Pathways with fold change values that were greater than 1.5 and less than 0.7 were considered as upregulated and downregulated pathways, respectively [38]. The levels of p-STAT3/STAT3 were expressed relative to the untreated control cells. The actual protein levels of IL-23 (pg/mL) were presented. Multiple comparisons were assessed using the one-way ANOVA and Bonferroni's test for the post-hoc analysis. In all comparisons,  $p < 0.05$  was considered as statistically significant.

## Results

### Cryptolepine and niclosamide inhibit the growth of HepG2 cells

Cryptolepine inhibited the growth of HepG2 cells in a dose- and time-dependent fashion (Fig. 1A). IC<sub>50</sub> values

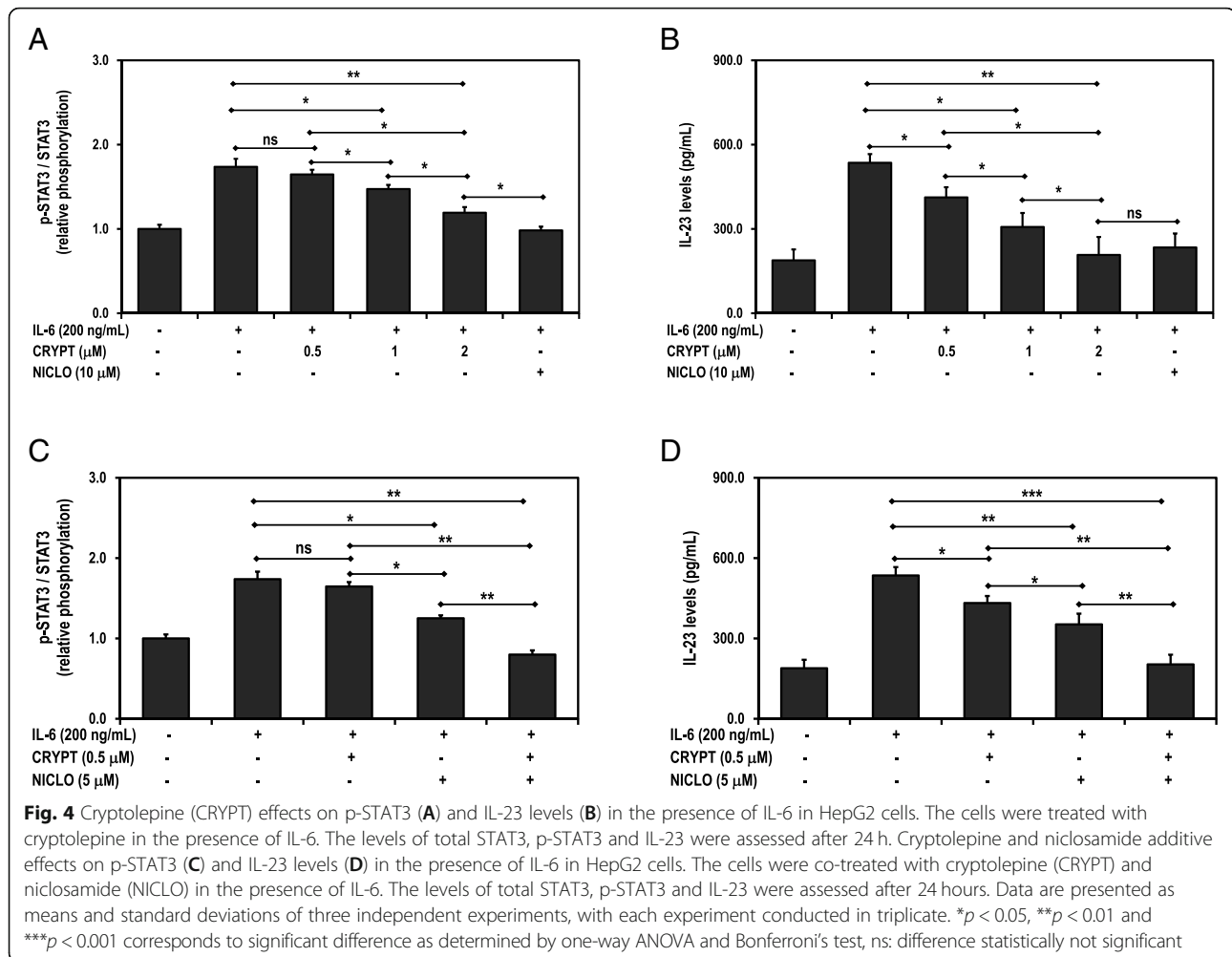




have made a key impact in pharmacotherapy, predominantly in infectious diseases as well as cancers (reviewed in [40]). Furthermore, herbal medicines are emerging as new and innovative compounds with amazing pharmacological effects that can be exploited to halt human ailments (reviewed in [41]), and one such candidate that needs to be explored is cryptolepine. Although there is limited data on the modulatory effects of cryptolepine on signal transduction pathways, few studies have delved into the cytotoxic effects of cryptolepine in vitro and its anti-cancer potential. A previous study [31] has established that cryptolepine inhibits melanoma cells in a time and dose-dependent manner, which is consistent with the findings of this current study. The mechanism of cryptolepine inhibition of melanoma cell growth has been shown to involve reducing the enhancers of mitochondrial biogenesis and the reduction of the c-Myc levels [31].

In this current study, cryptolepine up-regulated anti-cancer pathway reporters such as p53, SP1 and ISRE. Cryptolepine has been shown to impede cancer cell growth via the induction of the p53 signalling pathway

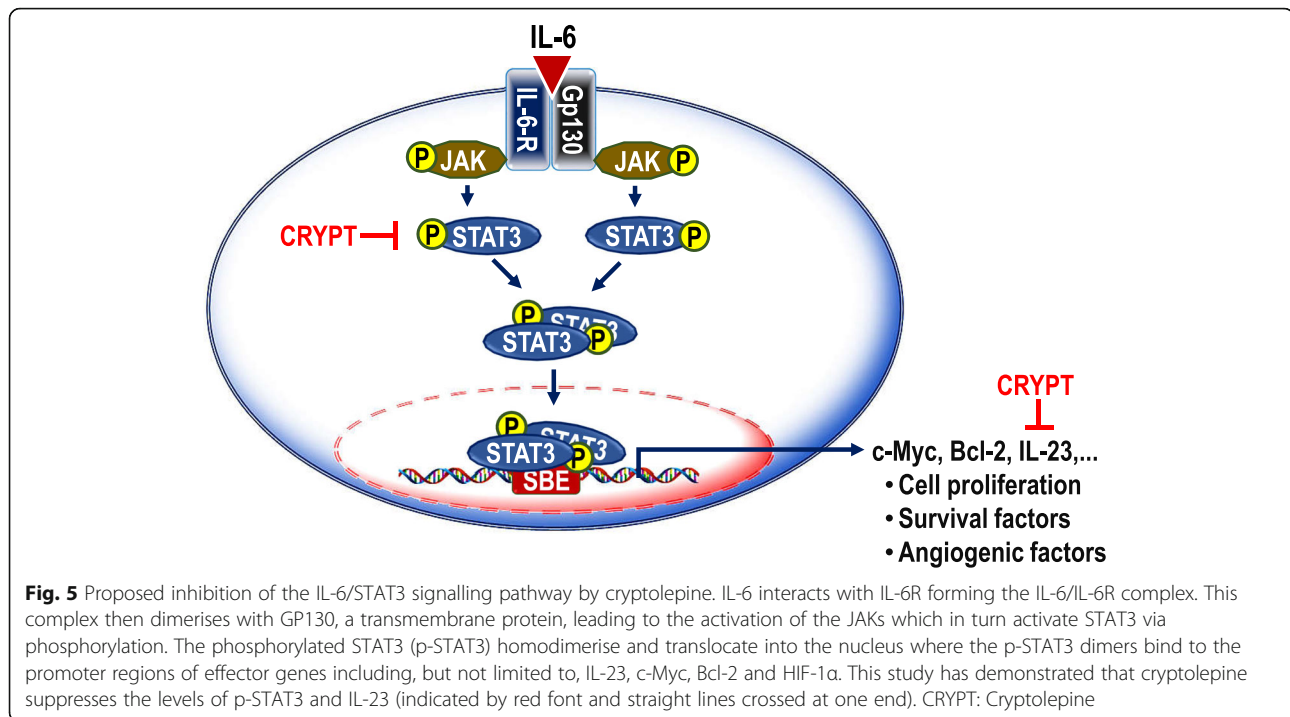
[30, 32] which is consistent with the findings of this study. The p53 signalling induces apoptosis and cell cycle arrest thereby serving as a tumour suppressing pathway [42], and this accounts for the *TP53* mutation in about 50% of cancers [43]. The SP1 signalling is another pathway of interest because SP1 dysregulation has been reported in many diseases and cancers [44]. This pathway plays a role in immune responses, apoptosis, and cell growth and differentiation [45]. Overexpression of SP1 has been reported to induce p53-dependent apoptosis in tumours [46] and hence, this could be another mechanism via which cryptolepine exhibits its anti-cancer effects which need to be explored. Induction of the type 1 interferon response signalling pathway leads to antiviral, anti-cancer, and anti-inflammatory mechanisms [47–49]. Thus, the up-regulation of this multi-functional innate immune pathway is essential in immunotherapeutic strategies [50] and interestingly, cryptolepine up-regulated the reporter (ISRE) of this pathway. Currently, experiments are ongoing in our laboratory to validate the up-regulatory effect of cryptolepine on this innate immune pathway.



On the contrary, cryptolepine down-regulated pro-cancer pathway reporters such as STAT3, NF- $\kappa$ B and c-Myc. Chronic activation of NF- $\kappa$ B signalling results in the pathogenesis of numerous inflammatory and autoimmune diseases [51]. Hence, the suppression of this signalling is indispensable in the management of cancers and autoimmune diseases. The down-regulation of the NF- $\kappa$ B signalling in this current study is in consonance with a previous study [52]. Also in animal models, *C. sanguinolenta* and cryptolepine have been shown to suppress inflammation [24]. In over 50% of cancers, the oncogenic c-Myc is deregulated and this is often linked to unfavourable prognosis and poor patient survival [53]. The c-Myc plays a key function in oncogenesis and hence, c-Myc inhibition has been proposed as a powerful approach in cancer management [54]. The down-regulation of c-Myc as reported in this study agrees with a previous study which reported that cryptolepine reduces c-Myc levels in melanoma cells both in vitro and in vivo [31].

STAT3 functions in cell differentiation, proliferation and survival. Hence within the normal hepatocytes,

STAT3 signalling promotes liver regeneration, glucose homeostasis and hepatic lipid metabolism [55]. However, aberrant IL-6 mediated STAT3 signalling has been reported to suppress anti-cancer immunity in HCC [56], making the STAT3 signalling a target in cancer treatment [57, 58]. Also, studies have reported that the IL-6/STAT3 pathway activated genes arrest apoptosis, enhance angiogenesis and promote cell survival [reviewed in 3]. For example, a responsive cytokine of the IL-6/STAT3 signalling pathway, IL-23 has been reported to promote the malignant properties of hepatoma cells [34]. Also, increased levels of IL-23 have been reported to promote HCC development after chronic hepatitis B virus infection [59]. Hence, the down-regulation of the IL-6/STAT3 signalling is critical in cancer management [60, 61]. Natural products exhibit potent anticancer effects through diverse mechanisms. However, data on the therapeutic potency of phytochemicals as inhibitors of the IL-6/STAT3 signalling is limited [reviewed in 41]. According to our results, cryptolepine inhibited the IL-6/STAT3 signalling pathway by suppressing the levels of



p-STAT3 and IL-23. Our result is similar to a previous study which reported that  $\beta$ -escin (a compound present in the medicinal plant *Aesculus hippocastanum*) induced cytotoxicity in human hepatocellular carcinoma via the suppression of STAT3 activation [62]. Similarly, diosgenin (a compound present in the medicinal plant *Trigonella foenum-graecum*) has been shown to inhibit the growth of human hepatocellular carcinoma via inhibiting both inducible and constitutive activation of STAT3 [63]. STAT3 signalling has a close association with inflammation which is subsequently linked with tumour initiation and tumorigenesis (reviewed in [64]). Hence, the suppression of the IL-6/STAT3 signalling pathway by cryptolepine (Fig. 5), as well as inflammation [24], suggest that cryptolepine could be exploited as a cheaper and novel anti-cancer agent in the management of HCC and other cancers. Also, cryptolepine and niclosamide additively inhibited the IL-6/STAT3 signalling pathway which is an interesting finding and could be further exploited as a combination therapy. Niclosamide is an FDA-approved anthelmintic drug that has been reported to inhibit the IL-6/STAT3 signalling pathway [65].

Based on the present data and previous reports, we propose that inhibition of the IL-6/STAT3 signalling pathway may promote the introduction of anti-tumour immunity into the tumour microenvironment. Moreover, enhancement of anti-cancer pathways and inhibition of pro-cancer pathways by cryptolepine may be a promising basis for the development of more effective immunotherapeutic strategies including repurposing

cryptolepine-based remedies such as *Cryptolepis sanguinolenta* for the management of hepatocellular carcinoma and other cancer patients.

#### Abbreviations

Bcl: B-cell lymphoma; FDA: Food and drugs authority; HCC: Hepatocellular carcinoma; HIF: Hypoxia-inducible factors; IL: Interleukin; ISRE: Interferon stimulated response elements; JAK: Janus kinase; NF- $\kappa$ B: Nuclear factor-kappa B; SBE: STAT3 binding element; SP: Specificity protein; STAT: Signal transducer and activator of transcription

#### Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12906-021-03326-x>.

**Additional file 1: S1.** Signal transduction pathways up-regulated by cryptolepine. **S2.** Signal transduction pathways down-regulated by cryptolepine. **S3.** Signal transduction pathways unaffected by cryptolepine.

#### Acknowledgements

Not applicable.

#### Authors' contributions

MM conceived and supervised the work. SAD and PWN planned and carried out the experiments and analysed the data. SAD and MM wrote the manuscript. MM, PWN, OQ, KAK, CA, AS and GAA reviewed and edited the manuscript. GAA and MM provided the funding for the work. All author(s) read and approved the final manuscript.

#### Authors' information

Not applicable.

#### Funding

This study was supported by a Wellcome/African Academy of Sciences Developing Excellence in Leadership Training and Science (DELTA) grant (DEL-15-007 and 107755/Z/15/Z: Awandare). Additional support came from

the Kwame Nkrumah University of Science and Technology Research Fund (KReF to Mohamed Mutocheluh).

#### Availability of data and materials

The datasets used during the current study are available from the corresponding author on reasonable request.

#### Declarations

#### Ethics approval and consent to participate

Not applicable.

#### Consent for publication

Not applicable.

#### Competing interests

The authors declare that they have no competing interests.

#### Author details

<sup>1</sup>West African Centre for Cell Biology of Infectious Pathogens, University of Ghana, Legon, Ghana. <sup>2</sup>Department of Biochemistry, Cell and Molecular Biology, School of Biological Sciences, University of Ghana, Legon, Ghana. <sup>3</sup>Department of Clinical Microbiology, School of Medicine and Dentistry, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. <sup>4</sup>Department of Immunology, Noguchi Memorial Institute for Medical Research, College of Health Sciences, University of Ghana, Legon, Ghana. <sup>5</sup>Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, College of Health Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. <sup>6</sup>Manhyia Government Hospital, Kumasi, Ghana.

Received: 14 March 2021 Accepted: 11 May 2021

Published online: 02 June 2021

#### References

- Yin Y, Yao S, Hu Y, Feng Y, Li M, Bian Z, et al. The immune-microenvironment confers chemoresistance of colorectal cancer through macrophage-derived IL-6. *Clin Cancer Res*. 2017;23(23):7375–87. <https://doi.org/10.1158/1078-0432.CCR-17-1283>.
- Feng B, Zhu Y, Yan L, Yan H, Huang X, Jiang D, et al. Ursolic acid induces the production of IL-6 and chemokines in both adipocytes and adipose tissue. *Adipocyte*. 2020;9(1):523–34. <https://doi.org/10.1080/21623945.2020.1814545>.
- Carpenter RL, Lo H-W. STAT3 target genes relevant to human cancers. *Cancers*. 2014;6(2):897–925. <https://doi.org/10.3390/cancers6020897>.
- Fazel Modares N, Polz R, Haghghi F, Lamertz L, Behnke K, Zhuang Y, et al. IL-6 trans-signalling controls liver regeneration after partial hepatectomy. *Hepatology*. 2019;70(6):2075–91. <https://doi.org/10.1002/hep.30774>.
- Peppler WT, et al. Acute administration of IL-6 improves indices of hepatic glucose and insulin homeostasis in lean and obese mice. *Am J Physiology-Gastrointestinal Liver Physiol*. 2019;316(1):166–78.
- Zhou M, et al. Non-cell-autonomous activation of IL-6/STAT3 signalling mediates FGF19-driven hepatocarcinogenesis. *Nat Commun*. 2017;8(1):1–16.
- Gai X, Zhou P, Xu M, Liu Z, Zheng X, Liu Q. Hyperactivation of IL-6/STAT3 pathway led to the poor prognosis of post-TACE HCCs by HIF-1 $\alpha$ /SNAI1 axis-induced epithelial to mesenchymal transition. *J Cancer*. 2020;11(3):570–82. <https://doi.org/10.7150/jca.35631>.
- Gao P, Niu N, Wei T, Tozawa H, Chen X, Zhang C, et al. The roles of signal transducer and activator of transcription factor 3 in tumour angiogenesis. *Oncotarget*. 2017;8(40):69139–61. <https://doi.org/10.18632/oncotarget.19932>.
- Liu H, Ren G, Wang T, Chen Y, Gong C, Bai Y, et al. Aberrantly expressed Fra-1 by IL-6/STAT3 transactivation promotes colorectal cancer aggressiveness through epithelial-mesenchymal transition. *Carcinogenesis*. 2015;36(4):459–68. <https://doi.org/10.1093/carcin/bgv017>.
- Yao C, Su L, Shan J, Zhu C, Liu L, Liu C, et al. IGF/STAT3/NANOG/slug signalling axis simultaneously controls epithelial-mesenchymal transition and stemness maintenance in colorectal cancer. *Stem Cells*. 2016;34(4):820–31. <https://doi.org/10.1002/stem.2320>.
- Knüpfner H, Preiss R. Serum interleukin-6 levels in colorectal cancer patients—a summary of published results. *Int J Color Dis*. 2010;25(2):135–40. <https://doi.org/10.1007/s00384-009-0818-8>.
- Mitsuhashi S, Ikeda M, Shimizu S, Ohno I, Furuse J, Inagaki M, et al. Serum levels of IL-6 and IL-1 $\beta$  can predict the efficacy of gemcitabine in patients with advanced pancreatic cancer. *Br J Cancer*. 2013;108(10):2063–9. <https://doi.org/10.1038/bjc.2013.174>.
- Makuuchi Y, Honda K, Osaka Y, Kato K, Kojima T, Daiko H, et al. Soluble interleukin-6 receptor is a serum biomarker for the response of esophageal carcinoma to neoadjuvant chemoradiotherapy. *Cancer Sci*. 2013;104(8):1045–51. <https://doi.org/10.1111/cas.12187>.
- Gupta N, Goswami B, Mittal P. Effect of standard anthracycline based neoadjuvant chemotherapy on circulating levels of serum IL-6 in patients of locally advanced carcinoma breast—a prospective study. *Int J Surg*. 2012;10(10):638–40. <https://doi.org/10.1016/j.ijsu.2012.11.007>.
- Sporeno E, Savino R, Ciapponi L, Paonessa G, Cabibbo A, Lahm A, et al. Human interleukin-6 receptor super-antagonists with high potency and wide spectrum on multiple myeloma cells. *Blood*. 1996;87(11):4510–9. <https://doi.org/10.1182/blood.V87.11.4510.bloodjournal87114510>.
- Fathy AH, Bashandy MA, Bashandy SAE, Mansour AM, Elsadek B. Sequential analysis and staging of a diethylnitrosamine-induced hepatocellular carcinoma in male Wistar albino rat model. *Can J Physiol Pharmacol*. 2017;95(12):1462–72. <https://doi.org/10.1139/cjpp-2017-0413>.
- Bosch FX, Ribes J, Cléries R, Diaz M. Epidemiology of hepatocellular carcinoma. *Clinics in Liver Disease*. 2005;9(2):191–211. <https://doi.org/10.1016/j.cld.2004.12.009>.
- Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *Cancer J Clin*. 2018;68(6):394–424. <https://doi.org/10.3322/caac.21492>.
- Luo GG, Ou J-h J. Oncogenic viruses and cancer. *Virology*. 2015;30(2):83–4. <https://doi.org/10.1007/s12250-015-3599-y>.
- Plummer M, et al. Global burden of cancers attributable to infections in 2012: a synthetic analysis. *Lancet Global Health*. 2016;4(9):609–16.
- Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. *Cancer J Clin*. 2015;65(2):87–108. <https://doi.org/10.3322/caac.21262>.
- Daher S, et al. Current and future treatment of hepatocellular carcinoma: an updated comprehensive review. *J Clinical & Transl Hepatol*. 2018;6(1):69–78.
- Osafo N, Mensah KB, Yeboah OK. Phytochemical and Pharmacological Review of *Cryptolepis sanguinolenta* (Lindl.) Schlechter. *Adv Pharmacol Sci*. 2017;2017:3026370–83.
- Olajide OA, Ajayi AM, Wright CW. Anti-inflammatory properties of cryptolepine. *Phytother Res*. 2009;23(10):1421–5. <https://doi.org/10.1002/ptr.2794>.
- Olajide OA, Bhatia HS, de Oliveira ACP, Wright CW, Fiebich BL. Anti-neuroinflammatory properties of synthetic cryptolepine in human neuroblastoma cells: possible involvement of NF- $\kappa$ B and p38 MAPK inhibition. *Eur J Med Chem*. 2013;63:333–9. <https://doi.org/10.1016/j.ejmech.2013.02.004>.
- Bugyei K, Boye G, Addy M. Clinical efficacy of a tea-bag formulation of *Cryptolepis sanguinolenta* root in the treatment of acute uncomplicated falciparum malaria. *Ghana Med J*. 2010;44(1):3–9. <https://doi.org/10.4314/gmj.v44i1.68849>.
- Paulo A, Pimentel M, Viegas S, Pires I, Duarte A, Cabrita J, et al. *Cryptolepis sanguinolenta* activity against diarrhoeal bacteria. *J Ethnopharmacol*. 1994;44(2):73–7. [https://doi.org/10.1016/0378-8741\(94\)90071-X](https://doi.org/10.1016/0378-8741(94)90071-X).
- Silva O, Duarte A, Cabrita J, Pimentel M, Diniz A, Gomes E. Antimicrobial activity of Guinea-Bissau traditional remedies. *J Ethnopharmacol*. 1996;50(1):55–9. [https://doi.org/10.1016/0378-8741\(95\)01323-7](https://doi.org/10.1016/0378-8741(95)01323-7).
- Claude K, et al. Efficacy of *Cryptolepis sanguinolenta* root extract on slow-growing rifampicin-resistant mycobacterium tuberculosis. *J Med Plants Res*. 2012;6(7):1140–6.
- Pal HC, Katiyar SK. Cryptolepine, a plant alkaloid, inhibits the growth of non-melanoma skin cancer cells through inhibition of topoisomerase and induction of DNA damage. *Molecules*. 2016;21(12):1758. <https://doi.org/10.3390/molecules21121758>.
- Pal HC, Prasad R, Katiyar SK. Cryptolepine inhibits melanoma cell growth through coordinated changes in mitochondrial biogenesis, dynamics and metabolic tumour suppressor AMPK $\alpha$ 1/2-LKB1. *Sci Rep*. 2017;7(1):1498–502. <https://doi.org/10.1038/s41598-017-01659-7>.
- Zhu H, Gooderham NJ. Mechanisms of induction of cell cycle arrest and cell death by cryptolepine in human lung adenocarcinoma A549 cells. *Toxicol Sci*. 2006;91(1):132–9. <https://doi.org/10.1093/toxsci/kj1146>.



33. Lisgarten JN, Coll M, Portugal J, Wright CW, Aymami J. The antimalarial and cytotoxic drug cryptolepine intercalates into DNA at cytosine-cytosine sites. *Nat Struct Mol Biol.* 2002;9(1):57–68. <https://doi.org/10.1038/nsb729>.
34. Jiang Q, Sun Y, Guo Z, Chen R, Ma S, Fu M, et al. IL-23 enhances the malignant properties of hepatoma cells by attenuation of HNF4 $\alpha$ . *Oncotarget.* 2018;9(47):28309–21. <https://doi.org/10.18632/oncotarget.24875>.
35. Yamamoto Y, et al. Lipopolysaccharide shock reveals the immune function of indoleamine 2, 3-dioxygenase 2 through the regulation of IL-6/stat3 signalling. *Sci Rep.* 2018;8(1):1–10.
36. Tang C, Chen S, Qian H, Huang W. Interleukin-23: as a drug target for autoimmune inflammatory diseases. *Immunology.* 2012;135(2):112–24. <https://doi.org/10.1111/j.1365-2567.2011.03522.x>.
37. Ren X, Duan L, He Q, Zhang Z, Zhou Y, Wu D, et al. Identification of niclosamide as a new small-molecule inhibitor of the STAT3 signalling pathway. *ACS Med Chem Lett.* 2010;1(9):454–9. <https://doi.org/10.1021/ml100146z>.
38. Protasio AV, Dunne DW, Berriman M. Comparative study of transcriptome profiles of mechanical-and skin-transformed *Schistosoma mansoni* schistosomula. *PLoS Negl Trop Dis.* 2013;7(3):2091–102.
39. Mbele M, Hull R, Dlamini Z. African medicinal plants and their derivatives: current efforts towards potential anti-cancer drugs. *Exp Mol Pathol.* 2017; 103(2):121–34. <https://doi.org/10.1016/j.yexmp.2017.08.002>.
40. Atanasov AG, et al. Natural products in drug discovery: Advances and opportunities. *Nat Rev Drug Discov.* 2021;20:200–2016.
41. Bose S, Banerjee S, Mondal A, Chakraborty U, Pumarol J, Croley CR, et al. Targeting the JAK/STAT signalling pathway using phytocompounds for cancer prevention and therapy. *Cells.* 2020;9(6):1451–91. <https://doi.org/10.3390/cells9061451>.
42. Oei AL, van Leeuwen CM, ten Cate R, Rodermond HM, Buist MR, Stalpers LJA, et al. Hyperthermia selectively targets human papillomavirus in cervical tumours via p53-dependent apoptosis. *Cancer Res.* 2015;75(23):5120–9. <https://doi.org/10.1158/0008-5472.CAN-15-0816>.
43. Conlin A, Smith G, Carey FA, Wolf CR, Steele RJ. The prognostic significance of K-ras, p53, and APC mutations in colorectal carcinoma. *Gut.* 2005;54(9): 1283–6. <https://doi.org/10.1136/gut.2005.066514>.
44. Vizcaíno C, Mansilla S, Portugal J. Sp1 transcription factor: a long-standing target in cancer chemotherapy. *Pharmacol Ther.* 2015;152:111–24. <https://doi.org/10.1016/j.pharmthera.2015.05.008>.
45. Meinders M, Kulu DI, van de Werken HJG, Hoogenboezem M, Janssen H, Brouwer RWW, et al. Sp1/Sp3 transcription factors regulate hallmarks of megakaryocyte maturation and platelet formation and function. *Blood.* 2015;125(12):1957–67. <https://doi.org/10.1182/blood-2014-08-593343>.
46. Chuang JY, Wu CH, Lai MD, Chang WC, Hung JJ. Overexpression of Sp1 leads to p53-dependent apoptosis in cancer cells. *Int J Cancer.* 2009;125(9): 2066–76. <https://doi.org/10.1002/ijc.24563>.
47. Enomoto H, Tao L, Eguchi R, Sato A, Honda M, Kaneko S, et al. The in vivo antitumor effects of type I-interferon against hepatocellular carcinoma: the suppression of tumour cell growth and angiogenesis. *Sci Rep.* 2017;7(1): 12189. <https://doi.org/10.1038/s41598-017-12414-3>.
48. Guarda G, Braun M, Staehli F, Tardivel A, Mattmann C, Förster I, et al. Type I interferon inhibits interleukin-1 production and inflammasome activation. *Immunity.* 2011;34(2):213–23. <https://doi.org/10.1016/j.immuni.2011.02.006>.
49. Xia Y, Cheng X, Blossley CK, Wisskirchen K, Esser K, Protzer U. Secreted interferon-inducible factors restrict hepatitis B and C virus entry in vitro. *J Immunol Res.* 2017;2017:1–12. <https://doi.org/10.1155/2017/4828936>.
50. Ye Z, Dong H, Li Y, Ma T, Huang H, Leong HS, et al. Prevalent homozygous deletions of type I interferon and defensin genes in human cancers associated with immunotherapy resistance. *Clin Cancer Res.* 2018;24(14): 3299–308. <https://doi.org/10.1158/1078-0432.CCR-17-3008>.
51. Zhang H, Sun S-C. NF- $\kappa$ B in inflammation and renal diseases. *Cell Bioscience.* 2015;5(1):63. <https://doi.org/10.1186/s13578-015-0056-4>.
52. Olajide OA, Bhatia HS, de Oliveira ACP, Wright CW, Fiebich BL. Inhibition of neuroinflammation in LPS-activated microglia by cryptolepine. *Evid Based Complement Alternat Med.* 2013;2013:1–10. <https://doi.org/10.1155/2013/459723>.
53. Chen H, Liu H, Qing G. Targeting oncogenic Myc as a strategy for cancer treatment. *Signal Transduction targeted therapy.* 2018;3(1):1–7.
54. Koh CM, Sabò A, Guccione E. Targeting MYC in cancer therapy: RNA processing offers new opportunities. *Bioessays.* 2016;38(3):266–75. <https://doi.org/10.1002/bies.201500134>.
55. Hong F, Kim WH, Tian Z, Jaruga B, Ishac E, Shen X, et al. Elevated interleukin-6 during ethanol consumption acts as a potential endogenous protective cytokine against ethanol-induced apoptosis in the liver: involvement of induction of Bcl-2 and Bcl-xL proteins. *Oncogene.* 2002; 21(1):32–44. <https://doi.org/10.1038/sj.onc.1205016>.
56. Sun X, Zhang J, Hou Z, Han Q, Zhang C, Tian Z. miR-146a is directly regulated by STAT3 in human hepatocellular carcinoma cells and involved in anti-tumour immune suppression. *Cell Cycle.* 2015;14(2):243–52. <https://doi.org/10.4161/15384101.2014.977112>.
57. Laudisi F, Cherubini F, Monteleone G, Stolfi C. STAT3 interactors as potential therapeutic targets for cancer treatment. *Int J Mol Sci.* 2018;19(6):1787. <https://doi.org/10.3390/ijms19061787>.
58. Lin S, Yang L, Yao Y, Xu L, Xiang Y, Zhao H, et al. Flubendazole demonstrates valid antitumor effects by inhibiting STAT3 and activating autophagy. *J Exp Clin Cancer Res.* 2019;38(1):293. <https://doi.org/10.1186/s13046-019-1303-z>.
59. Zang M, et al. IL-23 production of liver inflammatory macrophages to damaged hepatocytes promotes hepatocellular carcinoma development after chronic hepatitis B virus infection. *Biochimica et Biophysica Acta (BBA)-Molecular Basis Dis.* 2018;1864(12):3759–70.
60. Zhang W, Yu W, Cai G, Zhu J, Zhang C, Li S, et al. A new synthetic derivative of cryptotanshinone KY23 as STAT3 inhibitor for triple-negative breast cancer therapy. *Cell Death Dis.* 2018;9(11):1098. <https://doi.org/10.1038/s41419-018-1139-z>.
61. Khan MW, Saadalla A, Ewida AH, al-Katranji K, al-Saoudi G, Giaccone ZT, et al. The STAT3 inhibitor pyrimethamine displays anti-cancer and immune stimulatory effects in murine models of breast cancer. *Cancer Immunol.* 2018;67(1):13–23. <https://doi.org/10.1007/s00262-017-2057-0>.
62. Tan SM-L, Li F, Rajendran P, Kumar AP, Hui KM, Sethi G. Identification of  $\beta$ -escin as a novel inhibitor of signal transducer and activator of transcription 3/Janus-activated kinase 2 signalling pathway that suppresses proliferation and induces apoptosis in human hepatocellular carcinoma cells. *J Pharmacol Exp Ther.* 2010;334(1):285–93. <https://doi.org/10.1124/jpet.110.165498>.
63. Li F, Fernandez PP, Rajendran P, Hui KM, Sethi G. Diosgenin, a steroidal saponin, inhibits STAT3 signalling pathway leading to suppression of proliferation and chemosensitization of human hepatocellular carcinoma cells. *Cancer Lett.* 2010;292(2):197–207. <https://doi.org/10.1016/j.canlet.2009.12.003>.
64. Loh C-Y, et al. Signal transducer and activator of transcription (STATs) proteins in cancer and inflammation: functions and therapeutic implication. *Front Oncol.* 2019;9(48):1–15.
65. Gyamfi J, et al. Niclosamide reverses adipocyte induced epithelial-mesenchymal transition in breast cancer cells via suppression of the interleukin-6/STAT3 signalling axis. *Sci Rep.* 2019;9(1):1–14.

## Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

**Ready to submit your research? Choose BMC and benefit from:**

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

**At BMC, research is always in progress.**

Learn more [biomedcentral.com/submissions](https://biomedcentral.com/submissions)

