Brusatol suppresses STAT3-driven metastasis by downregulating epithelial-mesenchymal transition in hepatocellular carcinoma

Jong Hyun Lee a,1, Chakrabhavi Dhananjaya Mohan b,1, Amudha Deivasigamani c, Young Yun Jung a, Shobith Rangappa d, Salundi Basappa e, Arunachalam Chinnathambi f, Tahani Awad Alahmadi g, Sulaiman Ali Alharbi f, Manoj Garg h, Zhi-Xiu Lin i, Kanchugarakoppal S. Rangappa j, Gautam Sethi k,*, Kam Man Hui c,l,m,n,o,*, Kwang Seok Ahn a,*

a Department of Science in Korean Medicine, College of Korean Medicine, Kyung Hee University, 24 Kyungheedae-ro, Dongdaemun-gu, Seoul 02447, Republic of Korea
b Department of Studies in Molecular Biology, University of Mysore, Manasagangotri, Mysore 570006, India
c Division of Cellular and Molecular Research, Humphrey Oei Institute of Cancer Research, National Cancer Centre, Singapore
d Adichunchanagiri Institute for Molecular Medicine, BG Nagara 571448, Nagamangala Taluk, India
e Department of Studies in Organic Chemistry, University of Mysore, Manasagangotri, Mysore 570006, India
f Department of Botany and Microbiology, College of Science, King Saud University, Riyadh 11451, Saudi Arabia
g Amity Institute of Molecular Medicine and Stem cell Research (AIMMSR), Amity University, Noida, Uttar Pradesh 201313, India
h Faculty of Medicine, The Chinese University of Hong Kong, rm 101, 1/F Li Wai Chun Building, CUHK, Shatin, N.T., Hong Kong
i Institute of Excellence, Vijnana Bhavan, University of Mysore, Manasagangotri, Mysore 570006, India
j Department of Pharmacology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore 117600, Singapore
k Institute of Molecular and Cell Biology, A*STAR, Biopolis, Singapore
l Program in Cancer & Stem Cell Biology, Duke-NUS Medical School, Singapore
m National University of Singapore, Dept of Biochemistry, Yong Loo Lin School of Medicine, Singapore

HIGHLIGHTS

- Brusatol affects migration and invasion ability of HCC cells.
- Brusatol affects EMT process through modulation of STAT3 activation pathway.
- Brusatol mitigates tumorigenesis and metastasis in HCC preclinical model.

GRAPHICAL ABSTRACT

---

Peer review under responsibility of Cairo University.

* Corresponding authors at: Department of Pharmacology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore 117600, Singapore (Gautam Sethi), Division of Cellular and Molecular Research, Humphrey Oei Institute of Cancer Research, National Cancer Centre, Singapore (Kam Man Hui), and Department of Science in Korean Medicine, College of Korean Medicine, Kyung Hee University, 24 Kyungheedae-ro, Dongdaemun-gu, Seoul 02447, Republic of Korea (Kwang Seok Ahn).

E-mail addresses: phgcs@nus.edu.sg (G. Sethi), cmrhkm@nccs.com.sg (K.M. Hui), ksahn@khu.ac.kr (K.S. Ahn).

1 JHL and CDM contributed equally to this work.

https://doi.org/10.1016/j.jare.2020.07.004
2090-1232/© 2020 The Authors. Published by Elsevier B.V. on behalf of Cairo University.
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
Introduction

Metastasis is a process of dislodging of cancer cells from the primary tumor and their dissemination to the different organs through lymphatic system or circulation [1–5]. Metastasis contributes to about 90% of cancer-related deaths [6,7]. The five-year survival rate of various early-stage cancers is above 50% and it falls to below 20% when cancer cells are metastasized to distant tissues [8,9]. During metastasis, the immobile epithelial cancer cell undergoes trans differentiation to attain a mesenchymal phenotype that can permeate via extracellular matrix (ECM) through a process of epithelial-mesenchymal transition (EMT) [10,11]. The mesenchymal phenotypes exhibit stem cell properties, enhanced production of ECM components along with increased cellular motility, and apoptotic resistance [12–15].

EMT can be modulated by diverse transcription factors including zinc-finger E-box-binding (ZEB), Twist, Snail, and Slug [16–18]. STAT3 is frequently overactivated in different tumors including hepatocellular carcinoma (HCC) and can positively correlate with tumorigenicity, EMT, antiapoptosis, and metastasis [19–22]. IL-6 activates STAT3 to promote EMT through the induction of Snail expression in cancers [8,23]. Activated STAT3 can also induce the transcription of the Twist gene to promote oncogenic functions [24]. Therefore, it may be concluded that targeting STAT3 may be an appropriate strategy to counteract EMT and metastasis in advanced cancers.

Brusatol (BT) is a natural quassinoid that has been demonstrated as an inhibitor of nuclear factor erythroid 2-related factor-2 (Nrf-2) by several research groups [25]. BT can interfere with Nrf-2 signaling in cancer cells to enhance the chemotherapeutic potential of SDHB [26–28]. It was dissolved in dimethyl sulfoxide (DMSO) to prepare a stock (10 mM), stored at −80°C. Further, stock solution was diluted with culture medium as per experimental requirement.

Materials and methods

Reagents

Brusatol (BT) (CAS: 14907–98-3, purity ≥ 98% by HPLC analysis) was isolated from Bruecea.

Fructus in our laboratory and its structural identity was confirmed by comparing its NMR and HRMS data with those published previously [27]. It was dissolved in dimethyl sulfoxide (DMSO) to prepare a stock (10 mM), stored at −80°C. Further, stock solution was diluted with culture medium as per experimental requirement.

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Sodium dodeyl sulfate (SDS), DMSO, and ribonuclease A were obtained from Sigma–Aldrich (St. Louis, MO, USA). Anti-Fibronectin, anti-Vimentin, anti-E-cadherin, anti-N-cadherin, anti-Occludin, and anti-Twist antibodies (diluent, 1: 5000) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Anti-Snail, anti-p-STAT3 (Tyr 705), and anti-STAT3 anti-
bodies were purchased from Cell Signaling Technology (Beverly, MA, USA).

Cell culture

HCCLM3 cell line was obtained from Prof Zhao-You Tang’s laboratory at The Liver Cancer Institute and Zhongshan Hospital, Fudan University, Shanghai China. This cell line has been completely characterized and published previously by us and others [39,40]. They were maintained in DMEM containing 10% FBS and 1X penicillin/streptomycin.

Cell growth analysis

The growth behavior on BT treatment of cells was observed by xCELLigence RTCA DP instrument as done previously [41–44]. HCCLM3 cells (5 x 10^4 cells/well) were seeded on E-plate. Then cells were treated by BT (0, 5, 10 nM) for 72 h, and analyzed every 15 min time intervals.

Western blot analysis

Western blotting was executed as elaborated before [45–48].

Real-time polymerase chain reaction

Total RNA was extracted using Trizol and PCR was done as elaborated upon previously [49].

Immunocytochemistry (ICC) for Vimentin and Occludin localization

Immunocytochemistry was carried out as per the prior reported protocol [50].

Invasion assay

Roche xCELLigence Real-Time Cell Analyzer instrument was used to calculate invasion as reported formerly [51–53].

Boyden chamber assay

In vitro invasion assay was executed using micro chemotaxis Boyden chamber as described earlier [54]. Matrigel-coated 8-μm polycarbonate membrane was prepared on trans-well chamber. HCCLM3 cells (2 x 10^4 cells/well) were seeded on top chamber with BT (10 nM) in medium then incubated at 37 °C in 5% CO2 conditions.

siRNA transfections

siRNA transfection was carried out as described earlier [55]. To determine whether BT interferes with EMT through modulating STAT3 signaling, HCCLM3 cells were transfected with STAT3 siRNA (Santa Cruz Biotechnology [sc-29493]) and scrambled control with transfection reagent (Intron Biotechnology, Seoul, Korea).

Acute toxicity studies

The study was conducted as per the protocol approved by the SingHealth Institutional Animal Use and Care Committee (protocol number: 2013/SHS/870). Thereafter the experiments were performed using eight-week-old NCr nude female mice following treatment with intraperitoneal injections of 5 and 15 mg/kg of BT, and vehicle (0.1% DMSO) as described previously [56].

Preclinical experiments

In vivo experiments were performed as per the protocol approved by the SingHealth Institutional Animal Use and Care Committee (protocol number: 2013/SHS/870). NCr nude mice were injected subcutaneously with 100 μl of HCCLM3-Luc cells (5 x 10^6) in the right flank region. After tumor reaching the size of 1 cm^3, it was removed and cut into small pieces of 2 mm^3 and placed into the liver of NCr nude mice orthotopically. Tumor development was measured weekly twice by quantifying the bioluminescence signals after intraperitoneal injection of BT (1 mg/kg) twice a week, for four weeks.

Statistical analysis

The significance of differences between groups was evaluated by Student’s t-test and one-way analysis of variance (ANOVA) test. p < 0.05 was considered as statistically significant. * p < 0.05; ** p < 0.01 and *** p < 0.001. All results are presented as the mean ± S.D. of three independent experiments.

Results

BT moderately affects proliferation of HCC cells

Firstly, the action of BT (structure shown in Fig. 1A) on viability/proliferation of HCC cells was elucidated. BT modestly decreased the cell viability of HCCLM3 cells (Fig. 1B), and the differences in the proliferation were observed at 5 and 10 nM doses (Fig. 1C).

BT alters the transcription and protein expression of EMT-related proteins

We then evaluated the effects of BT on EMT markers. It reduced the protein expression of Fibronectin, Vimentin, N-cadherin, Twist, and Snail (Fig. 1D) and increased expression of Occludin, and E-cadherin (Fig. 1E). In addition, we also noted that mRNA levels of Fibronectin, Vimentin, N-cadherin were attenuated (Fig. 1F left) whereas Occludin and E-cadherin mRNAs were elevated upon BT exposure (Fig. 1F right). We analyzed the expression of Vimentin and Occludin in control and BT-treated cells using immunofluorescence. BT impeded the level of Vimentin but triggered that of Occludin (Fig. 1G) and thus can influence the EMT process.

BT suppresses migration as well as invasion in HCC cells

Next, whether BT regulates HCCLM3 cell migration was explored using xCELLigence RTCA DP and Boyden chamber assay. Interestingly, it was also noted that BT substantially counteracted the invasiveness of HCCLM3 cells (Fig. 2A). In addition, HCCLM3 cells appeared to be able to migrate efficiently as noted in Boyden chamber assay but BT inhibited the cell migration (Fig. 2B). These data suggested that BT can reduce cancer cell motility in vitro.

BT inhibits constitutively active STAT3 in HCC cells

Our previous report suggests that BT can modulate STAT3 signaling in HNSCC cells and since this transcription factor can regulate the EMT process. Therefore, the action of BT on phosphorylation of STAT3 Y705 in HCCLM3 cells was deciphered. It was noted that BT concentration-dependently inhibited constitutive STAT3 activation (Fig. 2C), thus suggesting that BT may affect EMT through targeting STAT3 pathway.

BT regulates EMT through affecting STAT3 signaling

To decipher the possible role of STAT3 in modulating EMT, we carried out the transient transfection using STAT3 siRNA. Fig. 2D indicates that STAT3-siRNA transfection successfully depleted STAT3 from the cells. In parallel, knockdown of STAT3 using siRNA can substantively reverse the alteration of EMT markers expression by BT (Fig. 2E and 2F).
Fig. 1. BT changes the levels of EMT markers. (A) The structure of BT. (B) HCCLM3 cells were exposed to BT (0, 1, 3, 5, 10, 25, 50, 100 nM) for 24 h and viability was calculated by MTT method. (C) HCCLM3 cells were exposed to BT and proliferation assay was performed using RTCA for 72 h. (D-E) HCCLM3 cells were exposed to BT for 24 h and Western blotting was executed. (F) Total RNA was measured via real-time PCR for levels of different genes. * p < 0.05; ** p < 0.01 and *** p < 0.001 as measured by (G) HCCLM3 cells were exposed to 10 nM of BT for 24 h, and then distribution of Vimentin and Occludin was studied by immunocytochemistry.
**BT did not exhibit toxicity in preclinical studies**

Initially, we conducted acute toxicity studies to identify if any adverse effects can be noted in mice treated with BT. We found that no mortality in mice was observed and BT-treated animals did not show major alterations in feed and water consumption, and body weight. We also noticed that there were no variations in the biochemical parameters of serum in BT-treated groups such as blood urea nitrogen (BUN), alanine aminotransferase (ALT), and aspartate aminotransferase (AST). Overall, these results suggest that BT treatment did not impart notable toxicity in tested mice (Fig. 3).

**BT attenuates tumorigenesis and metastasis in vivo**

We next established an orthotopic HCC mouse model as described in methods and examined the antitumor activity of BT. The intraperitoneal injection of BT (1 mg/kg, twice a week for four weeks) dramatically reduced tumor burden (Fig. 4A-C). The tumor burden was quantified by measuring photon counts before the first administration of BT and at the last dose as described in our previous studies [57]. We also observed a slight increase in body weight of mice in the brusatol treated group compared to vehicle control mice, however no significant difference observed. The mice in both the groups were found to be healthy (Fig. 4D). Interestingly, a significant decrease in metastasis to lungs upon BT exposure as compared to the vehicle-treated group (0.1% DMSO) was also noted (Fig. 4E).

**BT alters the levels of various proteins in tumor tissues**

We determined the levels of EMT and proliferation markers in the tumor tissues. The intensity of Ki-67, Vimentin, and Twist was markedly reduced by BT treatment, whereas E-cadherin was markedly elevated in the tissues (Fig. 5A and 5B). Besides, the level of mesenchymal markers was downregulated and epithelial markers were upregulated in BT-treated group. These observations are consistent with our in vitro findings (Fig. 5C and 5D).

**Discussion**

EMT can control embryonic development, wound healing, tissue remodeling, repair, and malignant transformation. Improper activation of EMT in cancer cells can contribute to their metastasis [58]. We report here that BT can significantly alter EMT through affecting STAT3 activation. An initial evaluation revealed that BT can suppress cell proliferation only at lower doses. Western blotting, Real-Time PCR, and ICC analysis suggested that BT attenuated the levels of mesenchymal markers with a subsequent increase in epithelial markers (Fig. 6). An elevated N-cadherin expression can be positively linked with metastasis in HCC and colon cancer tissues with poor survival rates [59,60]. Fibronectin and integrin levels are often augmented in tumors and can increase regulate abnormal proliferation [61]. In addition, Fibronectin may also promote EMT in breast cancer cells [62].

Vimentin is ubiquitously expressed in non-diseased mesenchymal cells and overexpressed in a broad range of epithelial cancers, which can be positively correlated with elevated tumor proliferation, metastasis, and reduced survival [63,64]. A decrease in E-cadherin can lead to the promotion of invasiveness, and resistance to standard chemotherapeutics in colorectal cancer cells [65], and knockdown of Occludin can contribute to the progression of breast cancer [66]. Next, we were interested to investigate the cause behind the altered expression of EMT-related proteins. Therefore, we deciphered the levels of major transcription factors that can affect EMT such as Snail and Twist. Interestingly, expression of both these proteins was downmodulated thereby indicating that EMT-related proteins may be suppressed by BT at the transcription level. For instance, Snail can repress the levels of E-cadherin and Occludin, and induce that of fibronectin, and MMP-9 [67]. The expression of N-cadherin is dependent on the integrin-mediated nuclear translocation of Twist1 [68]. Besides, Twist can regulate the levels of E-cadherin and may contribute to altered levels of various mesenchymal proteins [69].

STAT3 is a major transcription factor that promotes malignant progression, antiapoptosis, angiogenesis, and metastasis [70-76]. Activation of STAT3 can be achieved by forming a positive feedback loop and crosstalk with other oncogenic mediators in the tumor microenvironment [77-82]. Moreover, HCC patients with increased phosphorylated STAT3 in tumor tissues showed poor prognosis after transarterial chemoembolization and post-liver resection [83]. In addition, hyperactivated STAT3 signaling contributed to EMT in the same study [84]. IL-6, that can stimulate STAT3 activation [85,86], can promote metastasis by promoting EMT through JAK-STAT3 axis in HNSCC [8]. In addition, TGFβ-induced EMT is also dependent on JAK-STAT3 cascade in lung cancer [87]. In our previous investigation, we identified that STAT3 signaling can be downmodulated by BT in HNSCC cells [38]. Here we found that BT suppressed the phosphorylation of STAT3 Tyr705 in HCC cells that can contribute to its effect on various hallmarks of cancer, specifically EMT. The knockdown of STAT3 using STAT3 targeted siRNA caused a substantial decline of epithelial and increase in mesenchymal markers thus indicating that the STAT3 can modulate EMT. In parallel, we also observed a reduction in the levels of Snail and Twist. This effect could be due to the regulation of Snail and Twist expression by STAT3 [8,23,24].

It has also been previously documented that STAT3 can affect EMT by modulating Snail gene expression in pancreatic cancer [88]. Similarly, bergamottin, a furanocoumarin present in grapefruits, attenuated STAT3 signaling and mitigated metastasis through inhibition of EMT [49]. In addition, we have previously demonstrated that several STAT3 signaling inhibitors can suppress metastatic ability of cancer cells [89-93]. Furthermore, we evaluated the effect of BT on the invasive and migratory potential and the results demonstrated a significant decrease in cellular motility. The alterations of EMT-related proteins by BT may mediate its repressive actions on the invasive ability of HCC cells.

Since BT did not display any major toxic effects (up to 15 mg/kg), we next investigated its antitumor actions in HCC model. BT imparted significant antitumor potential in orthotopic model at a very low dose of 1 mg/kg. Lu and colleagues also reported the non-toxic nature of BT (2 mg/kg) in nude mice when intraperitoneally administered for 28 consecutive days [94]. Importantly, lung metastasis was also significantly inhibited with an alteration in the expression profile of Ki-67, Vimentin, Twist, and E-cadherin. The modulation in the expression of these proteins is consistent with our in vitro experimental findings.

**Conclusion**

EMT has been linked with metastasis of cancer cells and commonly observed in advanced tumors. Blocking of STAT3 activation by BT may interfere with mesenchymal phenotype and can downmodulate metastasis potential. Our results demonstrate that BT can attenuate STAT3-driven metastasis by altering the levels of EMT-related proteins in HCC preclinical settings.
Fig. 2. BT reduces invasion and blocks the STAT3 pathway. (A) HCCLM3 invasive activity in Matrigel-coated plate was determined. (B) HCCLM3 cells were exposed to 10 nM of BT for 8 h and invasion assay was done. (C) HCCLM3 cells were exposed to BT for 4 h and Western blot was executed. (D) HCCLM3 cells were transiently transfected with scrambled or STAT3 siRNA and then exposed to 10 nM of BT for 4 h and blotting was carried out. (E-F) Transfection was done with 50 nM STAT3 siRNA or scrambled siRNA for 24 h as narrated above in D. The cells were processed as narrated in C and blotting was conducted.
Fig. 3. The consequence of intraperitoneal administration of BT on body weight change and various biochemical parameters was measured. The nude mice n = 5 per group were exposed to one single dose of BT (5 or 15 mg/kg) and 0.1% DMSO control.
Fig. 4. Effect of BT on tumor development. (A) Bioluminescence images of tumors in mice. HCCLM3-Luc cells-induced tumors are orthotopically placed followed by treatment with 0.1% DMSO (n = 7) or BT (n = 7) (administered 1 mg/kg intraperitoneally, twice a week, for four weeks). Lung tissues were also analyzed for metastasis using bioluminescence imaging (B) The scattered plot indicates the tumor burden was quantified by measuring photon counts before the first administration of BT and at the last dose (**p < 0.01). (C) Tumor burden was recorded in vehicle-treated or BT-treated tumor-bearing mice throughout the study duration. (D) The graph represents the body weight of experimental animals throughout the study duration. (E) The quantitative estimation of lung tumor burden after BT treatment.
Fig. 5. The action of BT on EMT in tissues. (A) Analysis of EMT-related proteins by IHC. Magnification 200x. (B) Quantification of IHC. (C-D) The levels of various proteins was checked in tumor tissues.
Compliance with ethics requirements

All Institutional and National Guidelines for the care and use of animals (fisheries) were followed.

Declaration of Competing Interest

The authors have declared no conflict of interest.

Acknowledgements

K.S.R. thanks the University Grants Commission, New Delhi for providing the Basic Science Research faculty fellowship. K.S.R., and C.D.M., thanks DST-Promotion of University Research and Scientific (PURSE), Institution of Excellence, University of Mysore for arranging laboratory facility. H.K.M is supported by grant from the National Medical Research Council of Singapore (NRNMRRP18101). The authors also thank the International Scientific Partnership Program ISPP at King Saud University for funding this research work through ISPP# 0091.

Authors Contributions

Experiments performed by: J.H.L., C.D.M., A.D., Y.Y.J.
Study design, data interpretation: S.R., S.B., A.C., T.A.A., S.A.A., M. G., Z.X.L., K.S.R.
Writing of the manuscript: J.H.L., C.D.M., G.S., K.M.H., K.S.A.

Funding

This work was supported by a National Research Foundation of Korea (NRF) grant funded by the Korean government (MSIP) (NRF-2018R1D1A1B07042969).

References

[1] Chaffer CL, Weinberg RA. A perspective on cancer cell metastasis. Science 2011;331(6024):1559–64.
[2] Mittal V. Epithelial mesenchymal transition in tumor metastasis. Annual Rev Pathol 2018;13:395–412.
[3] Chua AW, Hay HS, Rajendran P, Shanmugam MK, Li F, Bist P, et al. Butein downregulates chemokine receptor CXCR4 expression and function through suppression of NF-kB activation in breast and pancreatic tumor cells. Biochem Pharmacol 2010;80(10):1553–62.
[4] Syn N, Wang L, Sethi G, Thiery JP, Goh BC. Exosome-mediated metastasis: from epithelial-mesenchymal transition to escape from immunosurveillance. Trends Pharmacol Sci 2016;37(7):606–17.
[5] Shanmugam MK, Manu KA, Ong TH, Ramachandran L, Surana R, Bist P, et al. Inhibition of CXCR4/CXCL12 signaling axis by ursolic acid leads to suppression of metastasis in transgenic adenocarcinoma of mouse prostate model. Int J Cancer 2011;129(7):1552–63.
[6] Seyfried TN, Huygens LJ. On the origin of cancer metastasis. Crit Rev Oncog 2013;18(1–2):43–73.
[7] Gupta GP, Massagué J. Cancer metastasis: building a framework. Cell 2006;127(4):679–95.
[8] Yadav A, Kumar B, Datta J, Teknos TN, Kumar P. IL-6 promotes head and neck tumor metastasis by inducing epithelial-mesenchymal transition via the JAK-STAT3-SNAIL signaling pathway. Molecule Cancer Res MCR 2011;9(12):1658–67.
[9] Moore W, Talati R, Bhattacharji P, Bilfinger T. Five-year survival after cryoablation of stage i non-small cell lung cancer in medically inoperable patients. J Vasc Interv Radiol 2015;26(3):312–9.
[10] Diepenbruck M, Christofori G. Epithelial-mesenchymal transition (EMT) and metastasis: yes, no, maybe?. Curr Opin Cell Biol 2016;43:7–13.
Liu Y, Weinberg RA. Epithelial-to-mesenchymal transition in cancer: complexity and opportunities. Front Medicine 2018;12(2):361–73.

Kalluri R, Weinberg RA. The basics of epithelial-mesenchymal transition. J Clin Investig 2009;119(4):729–38.

Cheng J-T, Wang L, Wang H, Tang F-R, Cai W-Q, Sethi G, et al. Insights into biological role of LncRNAs in epithelial-mesenchymal transition. Cells 2019;8(10):1788.

Baek SH, Ko JH, Lee JH, Kim C, Lee H, Nam D, et al. Ginkgolic acid inhibits invasion and migration and TGF-β-induced EMT of lung cancer cells through PI3K/Akt/mTOR inactivation. J Cell Physiol 2017;232(2):346–54.

Dai X, Aho KS, Rangappa S, Kim C, Devasagaiam A, Arfuso F, et al. Ascorchlorin enhances the sensitivity of doxorubicin leading to the reversal of epithelial-to-mesenchymal transition in hepato cellular carcinoma. Mol Cancer Ther 2016;15(12):2966–76.

Itoh Y, Saitoh M, Kawamura K. Smad3-STAT3 crosstalk in pathological contexts. Acta Biochim Biophys Sin 2018;50(1):82–90.

Loh C-Y, Chai JY, Tang TF, Wong WF, Sethi G, Shanmugam MK, et al. The E-cadherin and N-cadherin switch in epithelial-to-mesenchymal transition: signaling, therapeutic implications, and Challenges. Cells 2019;8(10):1118.

Lee JH, Chinnathambi A, Alharbi SA, Shair OHM, Sethi G, Aho KS. Farnesol abrogates epithelial to mesenchymal transition process through regulating Akt/mTOR pathway. Pharmacol Res 2019;150:105404.

Lee JH, Kim F, Lee J, Lim YJ, Sethi G, Aho KS, Actin is a pharmacological inhibitor of STAT3 phosphorylation at tyrosine 705 residue and potentiates bortezomib-induced apoptotic and anti angiogenic effects in human multiple myeloma cells. Phytomed Int J Phytotherapy Phytomedical 2019;25:282–92.

Lee M, Hirpara JL, Eu JQ, Sethi G, Wang L, Goh BC, et al. Targeting STAT3 and oxidative phosphorylation in oncogene-addicted tumors. Redox Biol 2019;25:101073.

Baburajeev CP, Mohan CD, Patel GS, Ran gappa S, Pandey V, Sebastian A, et al. Nano-cuprous oxide catalyzed one-pot synthesis of a carbazole-based STAT3 inhibitor: a facile approach via intramolecular C-N bond formation reactions. RSC Adv 2016;6(3):36775–85.

Mohan CD, Bharathkumar H, Bulusu KC, Pandey V, Rangappa S, Fuchs JE, et al. Development of a novel azaspirane that targets the Janus kinase-signal transducer and activator of transcription (STAT) pathway in hepatocellular carcinoma in vitro and in vivo. J Biol Chem 2018;293(49):3296-307.

Lamouille S, Xu J, Derynck R. Molecular mechanisms of epithelial-mesenchymal transition. Nat Rev Mol Cell Biol 2014;15(3):178–96.

Cheng GZ, Zhang WZ, Sun M, Wang Q, Coppola D, Mansour M, et al. Twist is transcriptionally upregulated by activation of STAT3 and mediates STAT3 oncogenic function. J Biol Chem 2008;283(21):14665–73.

Olayanju A, Coppie IM, Bryan HK, Edge GT, Sison RL, Wong MW, et al. Brusatol provokes a rapid and transient inhibition of Nrf2 signaling and sensitizes mammary cells to chemical toxicity-implications for therapeutic targeting of Nrf2. Free Radic Biol Med 2015;78:174–86.

Chen HM, Lai QZ, Liao HJ, Xie JH, Xian YF, Chen YL, et al. Synergistic anti tumor effect of brusatol combined with cisplatin on colorectal cancer cells. Int J Mol Sci 2019;20(1):100.

Ren D, Villeenueve NF, Jiang T, Wu T, Lai A, Toppin HA, et al. Brusatol enhances the efficacy of chemotherapy by inhibiting the Nrf2-mediated defense mechanism. PNAS 2011;108(4):1423–7.

Xiaoyan Ye, Wu Y, Hou D, Chen H, Deng T, et al. Brusatol enhances the chemotherapeutic efficacy of gemcitabine in pancreatic cancer via the Nrf2 signalling pathway. Oxidative medicine and cellular longevity. 2018; 2018.

Sun X, Wang Q, Yang Y, Du L, Xu C, Liu Q, Brusatol enhances the chemosensitivity of colorectal cells by promoting ROS and enhancing DNA damage. Int J Mol Cell Sci 2016;17(7).

Ye R, Dai N, He Q, Guo P, Xiang Y, Zhang Q, et al. Comprehensive anti-tumor effect of Brusatol through inhibition of cell viability and promotion of apoptosis caused by autophagy via the PI3K/Akt/mTOR pathway in hepatocellular carcinoma. Biomed Pharmacother 2018;105:962–73.

Vartanian S, Ma TP, Lee J, Haverty PM, Kirkpatrick DS. Application of Mass Spectrometry Profiling to Establish Brusatol as an Inhibitor of Global Protein Synthesis, characterization and cytotoxicity studies of 1,2,3-triazoles and 1,2,3-triazoles. 2016;15(4):1220–31.

Ma TK, Vartanian S, Devasagaiam A, Khatrikeyan C, Trivedi P, et al. Discovery of a small-molecule inhibitor of specific serine residue BAD phosphorylation. PNAS 2018;115(44):E10505–14.

Babarajeev CP, Dhananjaya Mohan C, Ananda H, Rangappa S, Fuchs JE, Jagdish S, et al. Development of Novel Triazolo-Thiazolodiheteroarenes from Heterogeneous “Green” Catalysis as Protein Tyrosine Phosphatase 1B Inhibitors. Sci Rep 2015;5:14195.

Mohan CD, Srivavana R, Rangappa S, Moven L, Mohan S, Paricharak S, et al. Trisubstituted-imidazoles induce apoptosis in human breast cancer cells by targeting the oncogenic PI3K/Akt/mTOR signaling pathway. PLoS ONE 2016;11(4):e0151355.

Lee JH, Kim C, Sethi G, Aho KS. Brassinin inhibits STAT3 signaling pathway through modulation of PI3K and SOSC-3 expression and sensitizes human lung cancer xenograft in mice to paclitaxel. Oncotarget. 2015; 6:8366–405.

Heo JY, Kim HJ, Kim SM, Park KR, Park SY, Kim SW, et al. Embelin suppresses STAT3 signaling, proliferation, and survival of multiple myeloma via the protein tyrosine phosphatase PTPN11. Cancer Lett 2011;308:71–80.

Mohan CD, Anil Kumar NC, Rangappa S, Shanmugam MK, Mishra S, Chinnathambi A, et al. Novel 1,3,4-oxadiazole induces anticancer activity by targeting NF-κB in hepatocellular carcinoma cells. Front Oncol 2015;8:42.

Dai X, Wang L, Devasagaiam A, Looy CK, Kirthikeyan C, Trivedi P, et al. A novel benzimidazole derivative, MBIC inhibits tumor growth and promotes apoptosis via activation of ROS-dependent JNK signaling pathway in hepatocellular carcinoma. Oncotarget 2017;8(8):12831–42.

Dai X, An KS, Kim C, Siven K, Ongh TH, Shanmugam MK, et al. Ascorchlorin, an isoprenoid antibiotic inhibits growth and invasion of hepatocellular carcinoma by targeting STAT3 signaling cascade through the induction of PI3AS. Mol Cancer 2015;2015.10:817.

Wendt MK, Balinas N, Carlin CR, Schiempmann WP, STAT3 and epithelial-mesenchymal transitions in carcinomas. JAKSTAT 2014;3(1):e28975.

Zhuo H, Jiang K, Dong L, Zhi Y, Liu L, et al. Overexpression of N-cadherin is correlated with metastasis and worse survival in colorectal cancer patients. J Cancer Sci Bull 2013;58(28):3290–294.

Sebastian A, Pandey V, Mohan CD, Chia YT, Rangappa S, Mathai J, et al. Novel adamantryl-benzo[b]thiazolyl pyrazoles targeting EGR in triple-negative breast cancer. ACS Omega 2015;10:83–90.

Wang JP, Hsieh A, Miki E, Hohorm B: how its aberrant expression in tumors may improve therapeutic targeting. J Cancer 2017;8(4):674–82.

Li CL, Yang D, Cao X, Wang F, Hong DY, Wang J, et al. Fibrosectin induces epithelial-to-mesenchymal transition in human breast cancer MCF-7 cells via activation of calpain. Oncology Lett 2017;13(5):3889–95.

Wei X, Jiu W, Mub M, Zhang Y, Li Q, Liu P, et al. Overexpression of vimentin contributes to prostate cancer invasion and metastasis via snc regulation. Anticancer Res 2008;28(1a):327–34.
Shanmugam MK, Li F, Rajendran P, Kim C, Sikka S, Siveen KS, et al. Abrogation of STAT3 signaling regulates adipocyte induced epithelial-mesenchymal transition in breast cancer cells. Sci Rep 2018;8(1):3859.

Lee JH, Kim C, Kim SH, Sethi G, Ahn KS. Farnesol inhibits tumor growth and enhances the anticancer effects of bortezomib in multiple myeloma xenograft mouse model through the modulation of STAT3 signaling pathway. Cancer Lett 2015;360(2):280–93.

Zheng X, Xu M, Yao B, Wang C, Jia Y, Liu Q. IL-6/STAT3 axis initiated CAFs via up-regulating TIMP-1 which was attenuated by acetylation of STAT3 induced by PCAF in HCC microenvironment. Cell Signal 2016;28(9):1314–24.

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-1α/SNAI1 axis-induced epithelial to mesenchymal transition. J Cancer 2020;11(3):570–82.

Kim C, Cho SK, Kapoor S, Kumar A, Vali S, Abbasi T, et al. β-Caryophyllene oxide inhibits constitutive and inducible STAT3 signaling pathway through induction of the SHP-1 protein tyrosine phosphatase. Mol Carcinog 2014;53(10):793–806.

Lee JH, Chang SY, Nam D, Chung WS, Lee J, Na YS, et al. Capilarinis inhibits constitutive and inducible STAT3 activation through induction of SHP-1 and SHP-2 tyrosine phosphatases. Cancer Lett 2014;345(1):140–8.

Lee JH, Kim J, Cho SK, Kapoor S, Lee JH, Ahn KS. Farnesol inhibits tumor growth and enhances the anticancer effects of bortezomib in multiple myeloma xenograft mouse model through the modulation of STAT3 signaling pathway. Cancer Lett 2015;360(2):280–93.

Chai EZ, Shanmugam MK, Arfuso F, Dharmarajan A, Wang C, Kumar AP, et al. Targeting transcription factor STAT3 for cancer prevention and therapy. J Pharmacol Experimen Therapeutics 2010;334(1):285–93.

Lu Z, Lai Z-Q, Leung AWN, Leung PS, Li Z-S, Lin Z-X. Exploring brusatol as a new anti-pancreatic cancer adjuvant: biological evaluation and mechanistic studies. Oncotarget 2017;8(49):84974–85.