Investigational New Drugs (2019) 37:452–460
https://doi.org/10.1007/s10637-018-0637-2

PRECLINICAL STUDIES

A natural compound derivative P-13 inhibits STAT3 signaling by covalently inhibiting Janus kinase 2

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Received: 28 April 2018 / Accepted: 4 July 2018 / Published online: 3 August 2018

Summary

We investigated the function and molecular mechanisms of 2-desoxy-4β-propylcarbamate-pulchellin (P-13), a sesquiterpene lactone derivative of 2-desoxy-4-epi-pulchellin from the traditional Chinese medicinal herb Carpesium abrotanoides L, in regulating STAT3 signaling and cancer cell growth. We found that P-13 inhibited the IL-6-induced, as well as the constitutive, STAT3 activation in a dose and time-dependent manner. In vitro kinase activity analyses demonstrated that P-13 directly inhibited JAK2 kinase activity. The inhibitory effects of P-13 on JAK2/STAT3 signaling could be blocked by reducing agents dithiothreitol (DTT) or glutathione (GSH), indicating an involvement of the thiol-reactive α-β unsaturated carbonyl group in P-13. Further analyses with mass spectrograph, as well as molecular docking, revealed that P-13 covalently bound with the C452 in the SH2 domain of JAK2. Furthermore, P-13 inhibited growth and induced death of many cancer cell lines, particularly those expressing constitutively activated STAT3. It also inhibited *in vivo* growth of human cancer cell xenografts. Taken together, these findings revealed P-13 as a novel covalent inhibitor of JAK2, uncovered a new mechanism to inhibit JAK2, and provided a promising anti-cancer drug candidate.

Keywords Natural compound · JAK2/STAT3 · Cancer · Covalent interaction · Signal transduction

Introduction

The Janus Kinase (JAK) / signal transducer and activator of transcription (STAT) signaling pathway plays key roles in immune responses and cancer development by regulating transcription of cell growth and differentiation-related genes [1, 2]. Aberrant activation of the JAK-STAT signaling pathway has been known to be involved in a variety of human diseases including cancer, autoimmune diseases, and chronic inflammatory diseases [3, 4]. In mammals, four JAKs (JAK1, JAK2, JAK3, TYK2) and seven STATs (STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, STAT6) are activated by various cytokines, growth factors, and microbial infections, respectively [5, 6]. After cytokine stimulation, the receptor-associated JAKs phosphorylate and activate each other, and then phosphorylate and activate STATs, leading to nuclear translocation of STATs and transcription of STAT-regulated genes [7, 8]. Among the seven STATs, STAT3 is the most frequently activated STAT in both solid and hematopoietic malignances and has been demonstrated as an oncogene [9–13]. JAKs are also found to be associated with cancers [14, 15]. JAK2 (V617F) mutation is found in a majority of patients with polycythemia vera, essential thrombocythemia, and idiopathic myelofibrosis [16, 17].

Therefore, considerable attentions have been paid to develop drugs to target the JAKs and STATs [18–21]. However, many of the drugs developed are targeting the conserved ATP pockets of the kinases and have poor specificity for the JAK/STAT pathways. Therefore, it is of great importance and

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Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10637-018-0637-2) contains supplementary material, which is available to authorized users.

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urgency to develop novel therapeutic agents with new mechanism and better specificity against the JAK/STAT signaling pathways.

Carpesium abrotanoides L. is a traditional Chinese medicinal herb and has been used to treat various inflammatory diseases, such as pneumonia and malaria [22]. One of its components, 2-desoxy-4-epi-pulchellin, has been reported to have anti-inflammatory, antiviral, and anti-cancer activities [23]. However, its molecular targets and modes of action have not been reported. We studied the effects of 2-desoxy-4-epi-pulchellin and its derivatives on JAK/STAT signaling and found that one of the derivatives, 2-desoxy-4β-propylcarbamate-pulchellin (P-13), was ten times more potent than 2-desoxy-4-epi-pulchellin in inhibiting the STAT3 signaling.

In this study, we investigated the molecular mechanisms of P-13 in inhibiting STAT3 signaling and cancer cell growth. We demonstrated that P-13 was a potent inhibitor of Janus kinase 2. It covalently bound to a specific cysteine in a noncatalytic region of JAK2 and inactivated its activity. In doing so, P-13 inhibited growth and induced death of cancer cells that had constitutively activated JAK2/STAT3. It also inhibited *in vivo* tumor growth. Our study uncovered a new mechanism to specifically inactivate JAK2 and supported P-13 to be a novel therapeutic agent for the treatment of cancer.

Materials and methods

Materials and methods are documented in the Supplementary Material.

Results

P-13 inhibited the IL-6-induced and constitutive STAT3 activation

We identified 2-desoxy-4β-propylcarbamate-pulchellin (P-13), a sesquiterpene lactone derivative of a natural compound 2-desoxy-4-epi-pulchellin from the traditional Chinese

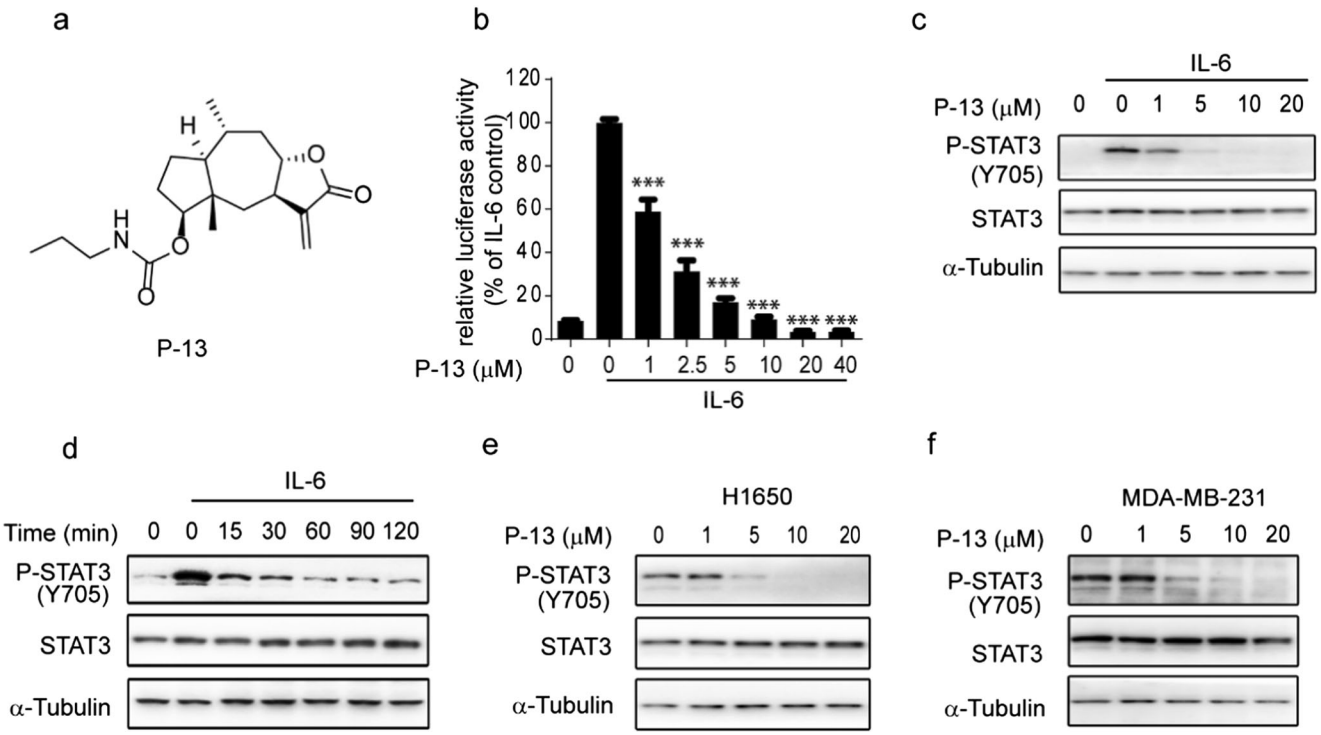

Fig. 1 P-13 inhibited the IL-6-induced and constitutive STAT3 activation. a Chemical structure of compound P-13. **b** HepG2/STAT3 cells were pretreated with P-13 at indicated concentrations for 2 h, and luciferase activity was measured following stimulation with IL-6 (10 ng/mL) for 4 h. Student's t-test was performed, ***$P < 0.001$. **c** HT29 cells were pretreated with P-13 at indicated concentrations for 2 h before stimulation with IL-6 (10 ng/mL) for 15 min. Whole cell lysates were processed for western blot analysis and probed with the indicated antibodies. Anti-α-Tubulin served as a loading control. **d** HT29 cells were pretreated with 10 μM P-13 for various lengths of time (0–120 min) before stimulation by IL-6 (10 ng/mL) for 10 min. Whole cell lysates were subjected to western blot analysis. **e** NCI-H1650 cells were treated with P-13 at indicated concentrations for 2 h. Whole cell lysates were processed for western blot analysis and probed with indicated antibodies. **f** MDA-MB-231 cells were treated with P-13 at indicated concentrations for 2 h. Whole cell lysates were processed for western blot analysis and probed with indicated antibodies

medicinal herb Carpesium abrotanoides L, as a potent inhibitor of STAT3 signaling with an IC_{50} (half-maximal inhibitory concentration) of 1.5 μM, using a STAT3-responsive luciferase gene reporter assay (Fig. 1a and b).

To understand the mechanisms of P-13 in inhibiting the STAT3 signaling, we analyzed the effects of P-13 on the tyrosine phosphorylation of STAT3. P-13 inhibited the IL-6-induced STAT3 Y705 (tyrosine 705) phosphorylation in a dose and time-dependent manner (Fig. 1c and d). It also inhibited the constitutive phosphorylation of STAT3 in the non-small cell lung cancer NCI-H1650 cells (Fig. 1e) and the breast cancer MDA-MB-231 cells (Fig. 1f), both of which have been reported to rely on the activated STAT3 for proliferation [24, 25].

P-13 inhibited Janus Kinase 2 activity

Because JAK2 is the major kinase of STAT3 [26], we analyzed the effects of P-13 on JAK2 phosphorylation/activation. P-13 effectively inhibited the phosphorylation of JAK2 in a dose-dependent manner (Fig. 2a). We next analyzed the effects of P-13 on the JAK2 kinase activity in an *in vitro* kinase assay. P-13 also inhibited the *in vitro* kinase activity of JAK2 (Fig. 2b), demonstrating that P-13 directly inhibited JAK2 kinase activity.

Reducing agents DTT and GSH blocked the inhibition of STAT3 phosphorylation by P-13

P-13 contains an α, β-unsaturated carbonyl group that has the potential to react with thiols of cysteines of a protein [27]. Reducing agents such as DTT and GSH can react with the α, β-unsaturated carbonyl group. To investigate whether P-13 functions through its α, β-unsaturated carbonyl group, we pre-incubated P-13 with DTT or GSH before adding it to the cells. DTT or GSH completely blocked the effect of P-13 on the Y705 phosphorylation of STAT3 (Fig. 3a). We further examined the products of the P-13 and GSH interaction using LC-MS. A major product with the mass signal at m/z 643 [P-13+GSH+H] was detected (Fig. 3b), indicating a reaction occurred between P-13 and the thiols of GSH. The essentiality of the double bond of the α, β-unsaturated carbonyl group of P-13 for the interaction was further confirmed by a hydrogenation modification of the double bond (Fig. 3c). The modified P13-H could no longer inhibit the IL-6-induced STAT3 phosphorylation (Fig. 3d). These data suggested that P-13 might interacted with JAK2 covalently through a Michael addition reaction using its α, β-unsaturated carbonyl group.

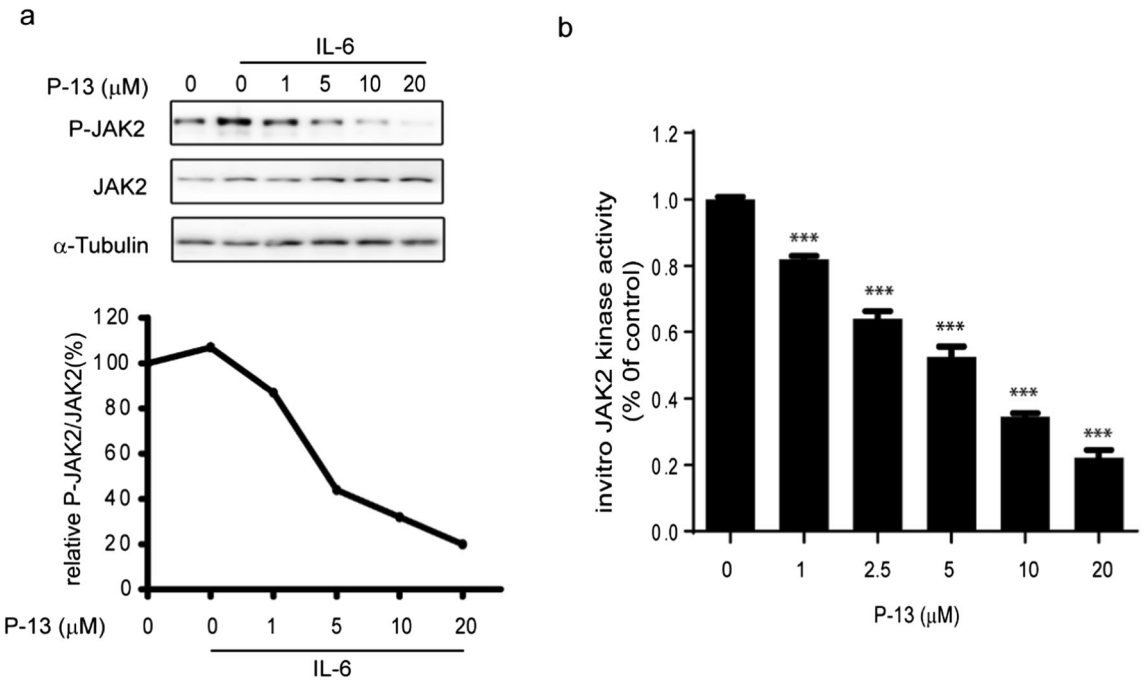

Fig. 2 P-13 inhibited Janus Kinase 2 activity. **a** HT29 cells were pretreated with P-13 at indicated concentrations for 2 h before stimulation with IL-6 (10 ng/mL) for 15 min. Whole cell lysates were processed for western blot analysis and probed with the indicated antibodies. Protein levels were quantitated densitometrically and graphed below the corresponding blots. Anti-α-Tubulin served as a loading control. **b** JAK2 protein was overexpressed in HEK293 cells for 24 h, then immunoprecipitated and subjected to an *in vitro* kinase assay in the presence of indicated concentrations of P-13. Student's t-test was performed, $^{***}P < 0.001$

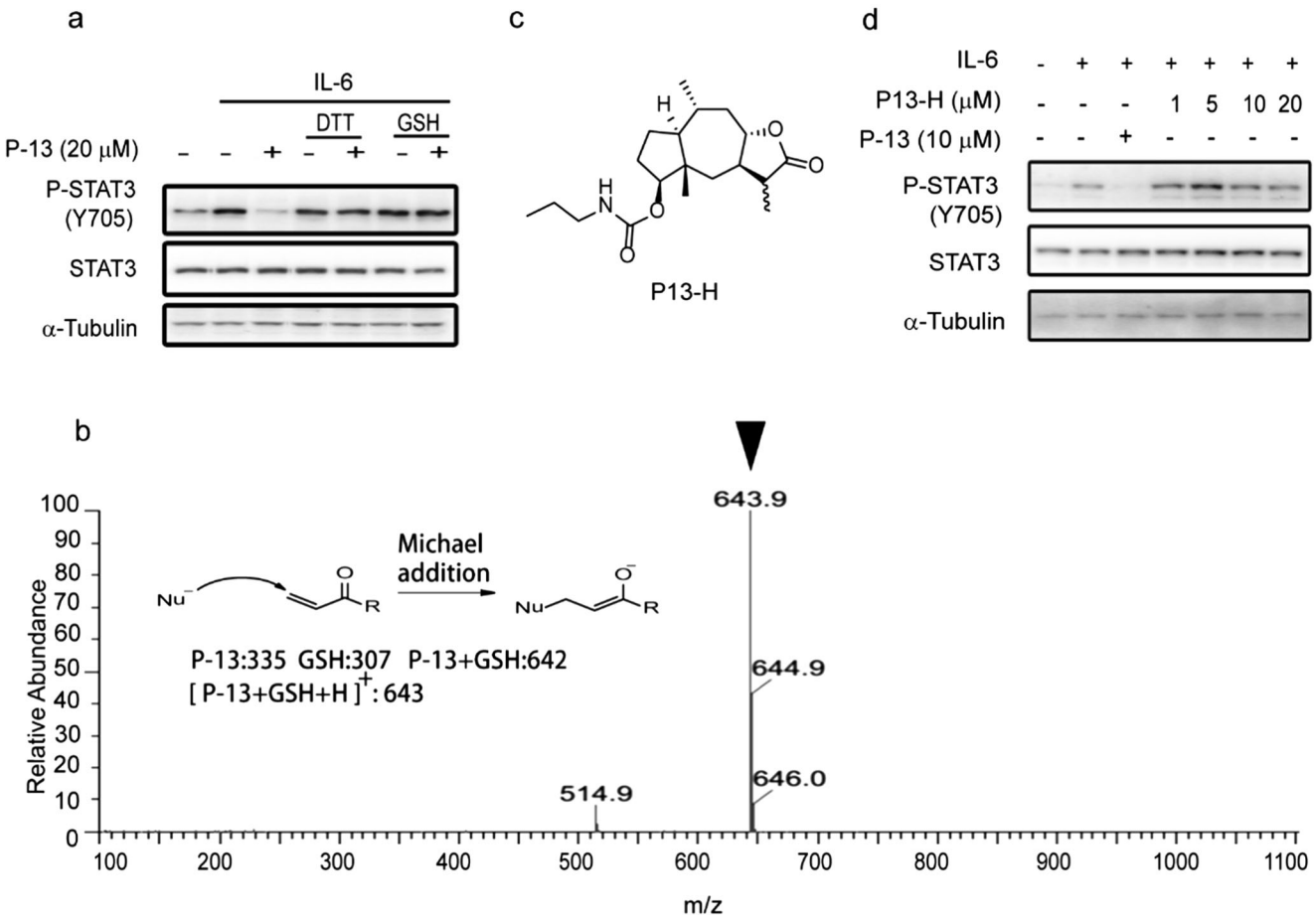

Fig. 3 DTT and GSH blocked the inhibition of STAT3 phosphorylation by P-13. **a** HT29 cells were pretreated with DTT (1 mM), GSH (1 mM), P-13 (20 μM) or their mixture (P-13 and DTT/GSH were pre-incubated at 37°C for 30 min) for 2 h and then stimulated with IL-6 (10 ng/mL) for 15 min. Whole cell lysates were processed for western blot analysis using antibodies as indicated. Anti-α-Tubulin served as a loading control. **b** P-13 (20 μM) was incubated with GSH (250 μM) at 37°C for 2 h, and the mixture was resolved by mass spectrometry. A possible Michael addition reaction between P-13 and GSH is illustrated, the arrow indicated the molecular weight of P-13+GSH+H. **c** Chemical structure of compound P13-H. **d** HT29 cells were pretreated with P13-H and P-13 at indicated concentrations for 2 h before stimulation with IL-6 (10 ng/mL) for 15 min. Whole cell lysates were processed for western blot analysis

P-13 covalently interacted with JAK2

To explore the possibility that P-13 covalently interacts with JAK2, we modified P-13 with an addition of an alkyne group (Fig. 4a, upper panel) to examine whether JAK2 could be pulled-down with the modified P13-alk. The modified P13-alk fully retained the biological activities of P-13 and blocked the IL-6-induced STAT3 activation at the same concentration as that of P-13 (Fig. 4b).

We then performed pull-down experiments using the P13-alk as a probe, which could react with an azide-biotin catalyzed by CuAAC [28], and the P13-alk-interacted proteins could then be pulled-down by streptavidin-coated agarose beads. We found that JAK2 interacted with the P13-alk in a dose-dependent manner (Fig. 4c), and the interaction could be competed away by the unlabeled P-13 (Fig. 4d). We also modified P13-alk to P13-alkH by hydrogenating the double bond of the α, β-unsaturated carbonyl group (Fig. 4a, lower panel), and found that the P13-alkH could not interact with the JAK2 protein (Fig. 4d). Furthermore, by using MS spectra, we found that P-13 covalently bound with C452 (cysteine 452) of JAK2 (Fig. 4e and supplementary Fig. S1).

In addition to the covalent interaction with C452, compound P-13 might also form non-covalent interactions with JAK2, including two hydrogen bonds with residue H451 and hydrophobic interactions with residue F439, L464, L454, L453, and F436, as predicted by the structure model of JAK2 (Fig. 4e).

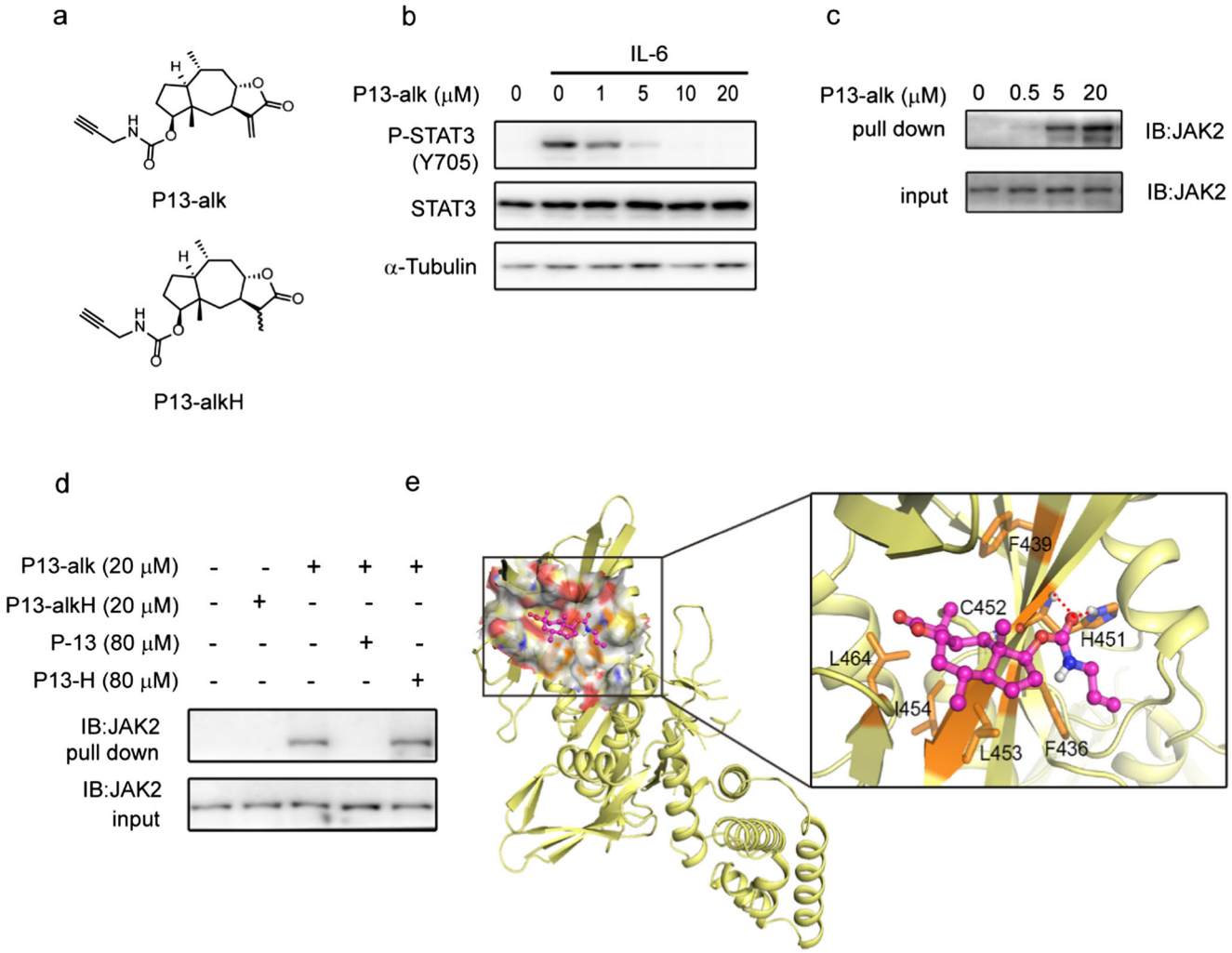

Fig. 4 P-13 covalently interacted with Janus kinase 2. **a** Chemical structure of probe P13-alk (upper panel) and P13-alkH (lower panel). **b** HT29 cells were pretreated with P13-alk at indicated concentrations for 2 h before stimulation with IL-6 (10 ng/mL) for 15 min. Whole cell lysates were processed for western blot analysis using antibodies as indicated. Anti-α-Tubulin served as a loading control. **c** HT29 cells were incubated with P13-alk at indicated concentration or DMSO for 2 h, the lysates were then precipitated with streptavidin-agarose, and the precipitates were analyzed by western blotting using an anti-JAK2 antibody as indicated. **d** HT29 cells were incubated with P13-alkH or P13-alk in the absence or presence of P-13 or P13-H for 2 h at 37°C, the lysates were precipitated with streptavidin-agarose, and the precipitates were analyzed by western blotting using the antibody as indicated. **e** Putative binding mode of P-13 to JAK2 (PDB code: 4Z32), (Left panel) surface presentation of the binding pocket, (Right panel) interaction of P-13 with JAK2. P-13 was shown in pink sticks. Residues of JAK2 were shown in orange sticks

P-13 preferentially inhibited growth and induced apoptosis of human cancer cells with activated STAT3

Because STAT3 is frequently activated in cancer cells and its activation is important for the survival and growth of cancer cells, we analyzed the effects of P-13 on the growth and survival of a panel of human cancer cell lines. P-13 effectively inhibited the growth of the human cancer cell lines with IC_{50} values ranging from 3 to 21 μM (Fig. 5a). Further analyses demonstrated that P-13 induced cell apoptosis, as indicated by the cleavage of PARP (Fig. 5b).

We next examined whether the growth inhibitory effects of P-13 on cancer cells were related to the activation status of STAT3. As shown in Fig. 5c and d, the breast cancer cell line MDA-MB-231 and the colon cancer cell line HT-29, which had constitutively activated STAT3, were more sensitive than the breast cancer cell line MDA-MB-453 and the liver cancer cell line BEL7404, which lacked the constitutively activated STAT3, to the growth inhibition of P-13.

P-13 inhibited *in vivo* tumor growth

Finally, we evaluated the effects of P-13 on tumor growth *in vivo*. The nude mice bearing the HT29 human colon adenocarcinoma xenografts were treated with vehicle or P-13 (25 mg/kg). As shown in Fig. 6, tumor growths were inhibited in

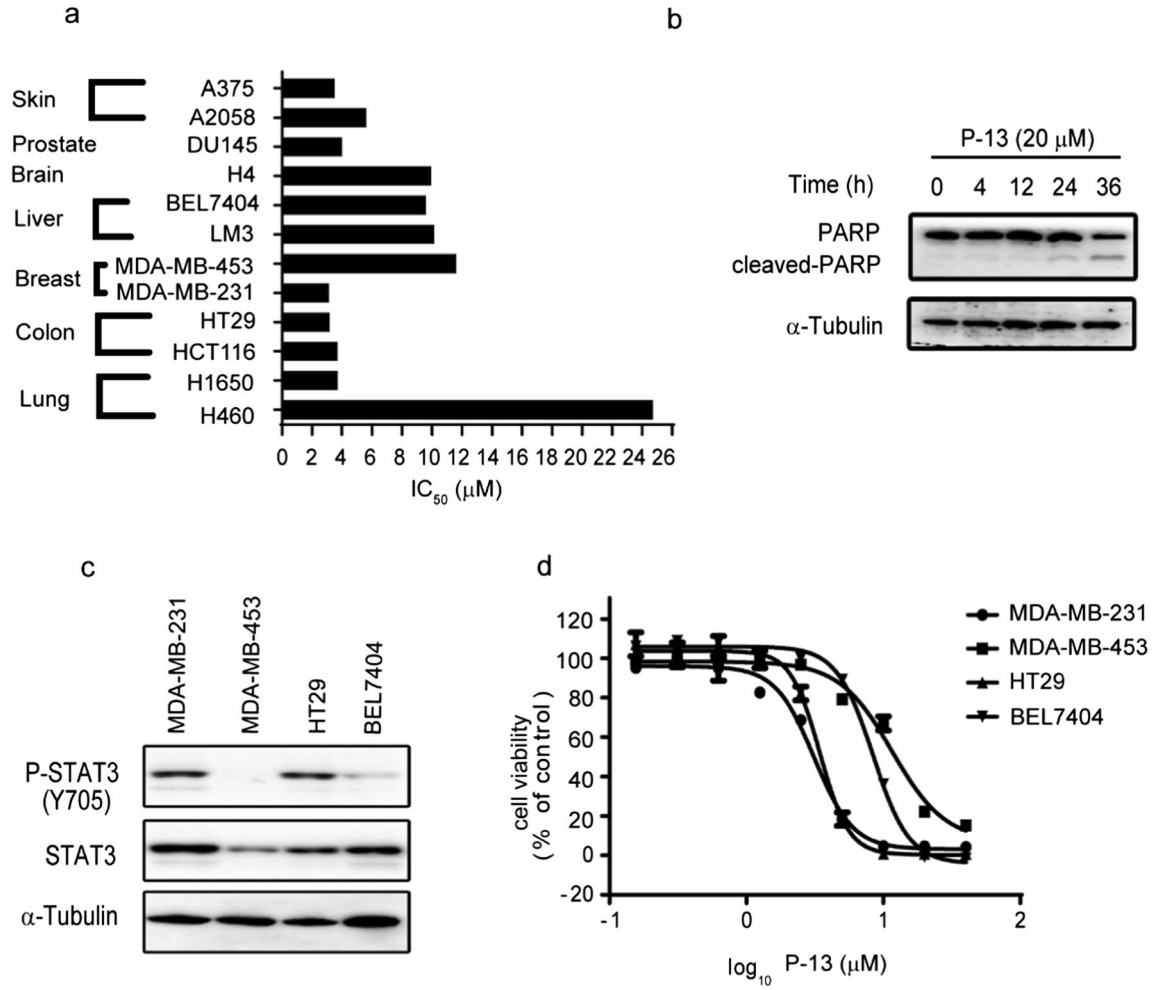

Fig. 5 P-13 inhibited growth of human cancer cells. **a** IC_{50} of P-13 on viability of different human tumor cell lines for 72 h. **b** HT29 cells were treated with 20 μM of P-13 for various time. Whole cell lysates were processed for western blot analysis and probed with an anti-PARP antibody. Anti-α-tubulin served as a loading control. **c** Lysates from human breast, colon and liver cancer cell lines MDA-MB-231, MDA-MB-453, HT29 and BEL7404 were processed for western blot analysis using specific antibodies as indicated. **d** MDA-MB-231, MDA-MB-453, HT29 and BEL7404 cells were treated with P-13 at various concentrations for 72 h and processed for MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay

all of the mice treated with P-13, as compared with the vehicle-treated mice. The body weights were not affected by the treatment of P-13 (Fig. 6c) and no organ injuries were observed, indicating that P-13 was relatively safe.

Discussion

Identification and development of STAT3 signaling inhibitors to inhibit cancer cell growth have attracted much attention because aberrant activations of STAT3 are associated with cancer formation and progression [29–32]. We have identified a natural compound derivative, 2-desoxy-4β-propylcarbamate-pulchellin (P-13), as a novel STAT3 signaling inhibitor. The goal of this study was to understand the molecular mechanisms of P-13 in inhibiting STAT3 signaling and to evaluate its potential to be an anti-cancer drug candidate. We presented sufficient evidences to demonstrate that the direct target of P-13 was the major STAT3 kinase JAK2. P-13 covalently interacted with the cysteine 452 in the SH2 domain of JAK2 and inactivated its kinase activity, leading to blockage of STAT3 signaling and inhibition of cancer cell growth *in vitro* as well as *in vivo*.

The unique feature of P-13, comparing with other JAK2 inhibitors, is its mode of action and its binding site on JAK2. Most of the JAK2 inhibitors developed so far are noncovalent ATP competitive inhibitors targeting the ATP binding region in the catalytic center of the enzyme [33, 34]. These inhibitors however often cross-react with other kinases because the structures of the ATP binding pockets of kinases are highly conserved [35]. P-13, on the other hand, covalently interacted with a specific cysteine, cysteine 452, in the non-ATP pocket region of JAK2. The C452 and its surrounding sequences are only present in two of the four JAK family members, JAK2

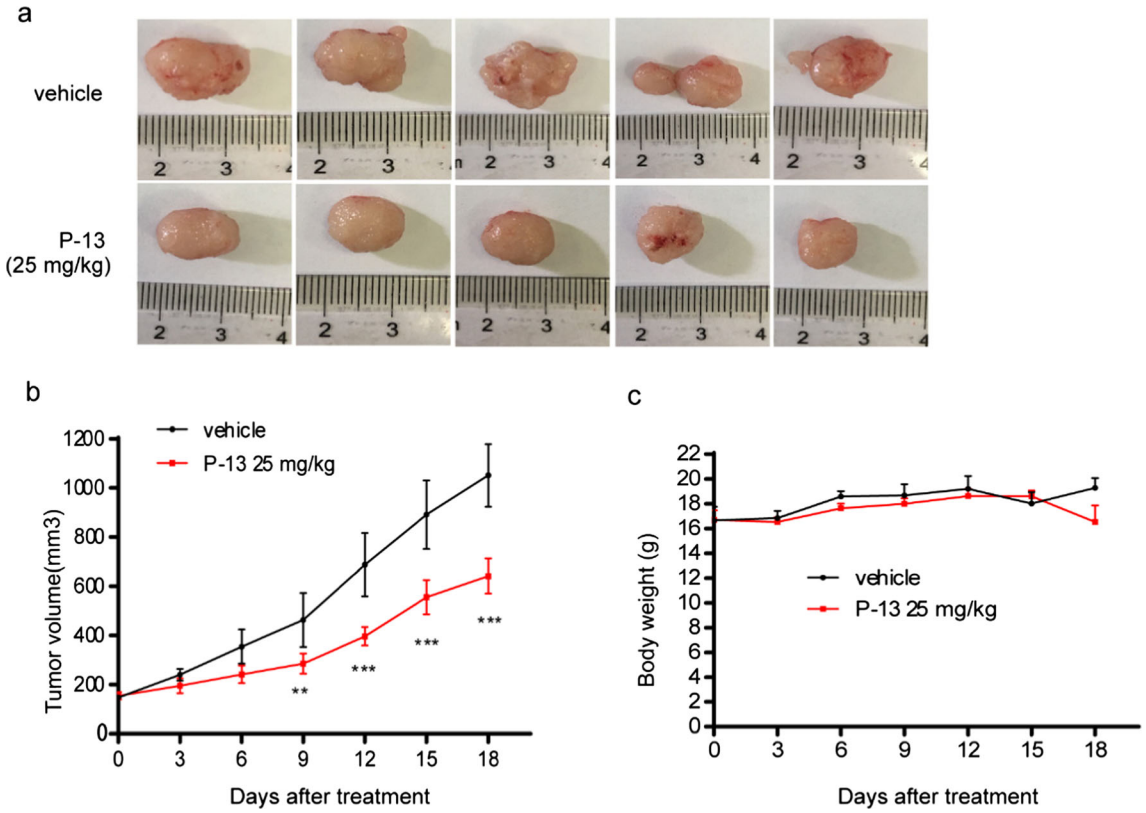

Fig. 6 P-13 inhibited *in vivo* tumor growth. HT29 xenograft nude mice were treated with vehicle, P-13 (25 mg/kg) for 18 days (IV; once per two days). Tumors were excised and photographed (**a**), tumor volumes and body weights of the mice during drug treatment were shown in (**b**) and (**c**), respectively. Two-way ANOVA with Tukey *post hoc* test was used. *($P < 0.05$); **($P < 0.01$); ***($P < 0.001$)

and JAK3. The other kinases, including the other two JAK family members JAK1 and TYK2, do not have such a structure motif. Therefore, P-13 may specifically interact with JAK2 and JAK3 without affecting other kinases. Indeed, our analyses of the effects of P-13 on the receptor tyrosine kinase EGFR and the ser/thr kinase IKK, two representatives of the tyrosine kinase and the ser/thr kinase families respectively [36, 37], supported this possibility (supplementary Fig. S2).

The C452 is in the SH2 domain of JAK2, which is outside of the catalytic domain and interacts with phosphorylated tyrosine residues [38]. It is not clear how binding of P-13 to the SH2 domain may inhibit the catalytic activity of JAK2. It is however clear that P-13 acted as an allosteric inhibitor. Binding of P-13 to the C452 may alter the tertiary structure of JAK2 and interfere with its interactions with its substrates, including its autophosphorylation sites. Therefore, the covalent nature of P-13 to interact with JAK2 may ensure its binding affinity and potential efficacy as a therapeutic agent, while the specific interaction with the C452 of JAK2 may ensure its specificity and potential therapeutic safety, which was also supported by our *in vivo* experiments (Fig. 6).

In conclusion, we have discovered a novel STAT3 signaling inhibitor P-13, which covalently inactivated JAK2 and induced apoptosis of human cancer cells, especially those with constitutively activated STAT3. Moreover, P-13 inhibited cancer cell growth both *in vitro* and *in vivo*, supporting its potential to be an anti-cancer drug candidate.

Acknowledgments We thank Prof. Xinyuan Fu (National University of Singapore) for providing the HepG2/STAT3 cell line. We also thank Mingwei Sun (Shanghai Institute of Materia Medica) for the help of LC-MS analysis and Yuqi Yu (Shanghai Institute of Materia Medica) for the help of the molecular docking.

Funding This work was supported by the National Natural Science Foundation of China (grant 81673465 to Q. Yu) and the China Ministry of Science and Technology Key New Drug Creation and Manufacturing Program (No. 2014ZX9102001002 to JY. Yuan).

Compliance with ethical standards

Conflict of interest The authors have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

References

1. Villarino AV, Kanno Y, O'Shea JJ (2017) Mechanisms and consequences of Jak-STAT signaling in the immune system. Nat Immunol 18(4):374–384. https://doi.org/10.1038/ni.3691

2. Villarino AV, Kanno Y, Ferdinand JR, O'Shea JJ (2015) Mechanisms of Jak/STAT signaling in immunity and disease. J Immunol 194(1):21–27. https://doi.org/10.4049/jimmunol.1401867
3. Lachance C, Leclerc P (2011) Mediators of the JAK/STAT signaling pathway in human spermatozoa. Biol Reprod 85(6):1222–1231. https://doi.org/10.1095/biolreprod.111.092379
4. Slattery ML, Lundgreen A, Kadlubar SA, Bondurant KL, Wolff RK (2013) JAK/STAT/SOCS-signaling pathway and colon and rectal cancer. Mol Carcinog 52(2):155–166. https://doi.org/10.1002/mc.21841
5. Aaronson DS, Horvath CM (2002) A road map for those who don't know JAK-STAT. Science 296(5573):1653–1655. https://doi.org/10.1126/science.1071545
6. Rawlings JS, Rosler KM, Harrison DA (2004) The JAK/STAT signaling pathway. J Cell Sci 117(8):1281–1283. https://doi.org/10.1242/jcs.00963
7. Derenzini E, Lemoine M, Buglio D, Katayama H, Ji Y, Davis RE, Sen S, Younes A (2011) The JAK inhibitor AZD1480 regulates proliferation and immunity in Hodgkin lymphoma. Blood Cancer J 1(12):e46. https://doi.org/10.1038/bcj.2011.46
8. Quan YD, Xu HT, Han YS, Mesplede T, Wainberg MA (2017) JAK-STAT signaling pathways and inhibitors affect reversion of envelope-mutated HIV-1. J Virol 91(9). https://doi.org/10.1128/JVI.00075-17
9. Jia ZH, Jia Y, Guo FJ, Chen J, Zhang XW, Cui MH (2017) Phosphorylation of STAT3 at Tyr705 regulates MMP-9 production in epithelial ovarian cancer. PLoS One 12(8):e0183622. https://doi.org/10.1371/journal.pone.0183622
10. Tong MT, Wang J, Jiang NY, Pan HM, Li D (2017) Correlation between p-STAT3 overexpression and prognosis in lung cancer: a systematic review and meta-analysis. PLoS One 12(8):e0182282. https://doi.org/10.1371/journal.pone.0182282
11. Valiulyte I, Steponaitis G, Skiriute D, Tamasauskas A, Vaitkiene P (2017) Signal transducer and activator of transcription 3 (STAT3) promoter methylation and expression in pituitary adenoma. BMC Med Genet 18:72. https://doi.org/10.1186/s12881-017-0434-3
12. Zulkifli AA, Tan FH, Putoczki TL, Stylli SS, Luwor RB (2017) STAT3 signaling mediates tumour resistance to EGFR targeted therapeutics. Mol Cell Endocrinol 451(C):15–23. https://doi.org/10.1016/j.mce.2017.01.010
13. Bromberg JF, Wrzeszczynska MH, Devgan G, Zhao YX, Pestell RG, Albanese C, Darnell JE (1999) Stat3 as an oncogene. Cell 98(3):295–303. https://doi.org/10.1016/S0092-8674(00)81959-5
14. Klisch TJ, Vainshtein A, Patel AJ, Zoghbi HY (2017) Jak2-mediated phosphorylation of Atoh1 is critical for medulloblastoma growth. Elife 6:e31181. https://doi.org/10.7554/eLife.31181
15. Wang SY, Liang K, Hu QS, Li P, Song J, Yang YD, Yao J, Mangala LS, Li CL, Yang WH, Park PK, Hawke DH, Zhou JW, Zhou Y, Xia WY, Hung MC, Marks JR, Gallick GE, Lopez-Berestein G, Flores ER, Sood AK, Huang SY, Yu DH, Yang LQ, Lin CR (2017) JAK2-binding long noncoding RNA promotes breast cancer brain metastasis. J Clin Invest 127(12):4498–4515. https://doi.org/10.1172/JCI91553
16. Bose P, Verstovsek S (2017) JAK2 inhibitors for myeloproliferative neoplasms: what is next? Blood 130(2):115–125. https://doi.org/10.1182/blood-2017-04-742288
17. Yao H, Ma Y, Hong Z, Zhao L, Monaghan SA, Hu MC, Huang LJ (2017) Activating JAK2 mutants reveal cytokine receptor coupling differences that impact outcomes in myeloproliferative neoplasm. Leukemia 31(10):2122–2131. https://doi.org/10.1038/leu.2017.1
18. Rosenthal A, Mesa RA (2014) Janus kinase inhibitors for the treatment of myeloproliferative neoplasms. Expert Opin Pharmacother 15(9):1265–1276. https://doi.org/10.1517/14656566.2014.913024
19. Banerjee S, Biehl A, Gadina M, Hasni S, Schwartz DM (2017) JAK-STAT signaling as a target for inflammatory and autoimmune diseases: current and future prospects. Drugs 77(5):521–546. https://doi.org/10.1007/s40265-017-0701-9
20. Boluda JCH, Gomez M, Perez A (2016) JAK2 inhibitors. Med Clin 147(2):70–75. https://doi.org/10.1016/j.medcli.2016.02.014
21. Liu Z, Wang H, Guan LN, Chen SY, Lai MD (2018) A novel small molecular STAT3 inhibitor, 5Br-6b, induces apoptosis and inhibits migration in colorectal cancer cells. Anti-Cancer Drugs 29(5):402–410. https://doi.org/10.1097/Cad.0000000000000605
22. Lee EK, Jeong DW, Lim SJ, Gu GJ, Ahn SI, Kim JS, Paek JH, Kim S, Hong JS, Lim SS, Youn HS (2014) Carpesium abrotanoides extract inhibits inducible nitric oxide synthase expression induced by toll-like receptor agonists. Food Sci Biotechnol 23(5):1637–1641. https://doi.org/10.1007/s10068-014-0223-0
23. Wang JF, He WJ, Zhang XX, Zhao BQ, Liu YH, Zhou XJ (2015) Dicarabrol, a new dimeric sesquiterpene from Carpesium abrotanoides L. Bioorg Med Chem Lett 25(19):4082–4084. https://doi.org/10.1016/j.bmcl.2015.08.034
24. Kunigal S, Lakka SS, Sodadasu PK, Estes N, Rao JS (2009) Stat3-siRNA induces Fas-mediated apoptosis in vitro and in vivo in breast cancer. Int J Oncol 34(5):1209–1220. https://doi.org/10.3892/ijo_00000249
25. Lieblein JC, Ball S, Hutzen B, Sasser AK, Lin HJ, Huang THM, Hall BM, Lin JY (2008) STAT3 can be activated through paracrine signaling in breast epithelial cells. BMC Cancer 8:302. https://doi.org/10.1186/1471-2407-8-302
26. O'Shea JJ, Schwartz DM, Villarino AV, Gadina M, McInnes IB, Laurence A (2015) The JAK-STAT pathway: impact on human disease and therapeutic intervention. Annu Rev Med 66:311–328. https://doi.org/10.1146/annurev-med-051113-024537
27. Wei X, Yin H (2015) Covalent modification of DNA by alpha, beta-unsaturated aldehydes derived from lipid peroxidation: recent progress and challenges. Free Radic Res 49(7):905–917. https://doi.org/10.3109/10715762.2015.1040009
28. Bateman LA, Zaro BW, Miller SM, Pratt MR (2013) An alkyne-aspirin chemical reporter for the detection of aspirin-dependent protein modification in living cells. J Am Chem Soc 135(39):14568–14573. https://doi.org/10.1021/ja408322b
29. Banerjee K, Resat H (2016) Constitutive activation of STAT3 in breast cancer cells: a review. Int J Cancer 138(11):2570–2578. https://doi.org/10.1002/ijc.29923
30. Geiger JL, Grandis JR, Bauman JE (2016) The STAT3 pathway as a therapeutic target in head and neck cancer: barriers and innovations. Oral Oncol 56:84–92. https://doi.org/10.1016/j.oraloncology.2015.11.022
31. Kim KJ, Kwon SH, Yun JH, Jeong HS, Kim HR, Lee E, Ye SK, Cho CH (2017) STAT3 activation in endothelial cells is important for tumor metastasis via increased cell adhesion molecule expression. Oncogene 36(39):5445–5459. https://doi.org/10.1038/onc.2017.148
32. Ouedraogo ZG, Biau J, Kemeny JL, Morel L, Verrelle P, Chautard E (2017) Role of STAT3 in genesis and progression of human malignant gliomas. Mol Neurobiol 54(8):5780–5797. https://doi.org/10.1007/s12035-016-0103-0
33. Clark JD, Flanagan ME, Telliez JB (2014) Discovery and development of Janus kinase (JAK) inhibitors for inflammatory diseases. J Med Chem 57(12):5023–5038. https://doi.org/10.1021/jm401490p

34. Ghoreschi K, Laurence A, O'Shea JJ (2009) Janus kinases in immune cell signaling. Immunol Rev 228:273–287. https://doi.org/10.1111/j.1600-065X.2008.00754.x
35. Garuti L, Roberti M, Bottegoni G (2010) Non-ATP competitive protein kinase inhibitors. Curr Med Chem 17(25):2804–2821. https://doi.org/10.2174/092986710791859333
36. Wieduwilt MJ, Moasser MM (2008) The epidermal growth factor receptor family: biology driving targeted therapeutics. Cell Mol Life Sci 65(10):1566–1584. https://doi.org/10.1007/s00018-008-7440-8
37. Karin M, Delhase M (2000) The I kappa B kinase (IKK) and NF-kappa B: key elements of proinflammatory signalling. Semin Immunol 12(1):85–98. https://doi.org/10.1006/smim.2000.0210
38. Yamaoka K, Saharinen P, Pesu M, Holt VET, Silvennoinen O, O'Shea JJ (2004) The Janus kinases (Jaks). Genome Biol 5(12):253. https://doi.org/10.1186/Gb-2004-5-12-253