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Combating metastasis of breast cancer cells with a carboplatin analogue containing an all-trans retinoic acid ligand†

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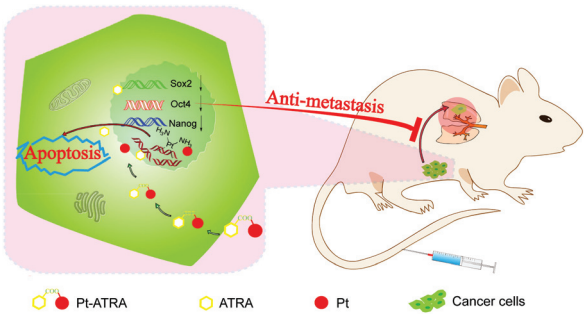
Pt-ATRA, a carboplatin analogue containing an all-trans retinoic acid (ATRA) derivative ligand, was synthesized *via* a click reaction. Upon cellular internalization, Pt-ATRA exhibits a dual function, releasing an active Pt(II) moiety to induce cell apoptosis and ATRA to inhibit tumor metastasis.

Breast cancer is the most common malignancy in women and has a high mortality rate.[1–3] Notably, among various causes, metastasis is responsible for 90% of deaths of breast cancer.[4] Therefore, combating cancer metastasis is an urgent need for the treatment of breast cancer in addition to the inhibition of the proliferation of cancer cells. Although many advanced methods, such as immunotherapy and targeted therapy, have been developed for cancer treatments, the most widely used agents for metastatic breast cancer are chemotherapeutics. For instance, paclitaxel and platinum based anticancer drugs are approved by the FDA for the treatment of metastatic breast cancer.[5–8] The application of platinum drugs and paclitaxel demonstrates an effective antitumor efficacy; however, it has been reported that the chemotherapy could even increase the motility and invasion of cancer cells.[9,10] While reducing the number cancer cells, the increased proportion of cancer stem cells (CSCs) during chemotherapy is associated with cancer recurrence and metastasis.[11,12] Therefore, improving the anti-metastasis activity of platinum drugs is highly desired.

All-trans retinoic acid (ATRA) is a clinically used anti-cancer agent with limited toxicity.[13] ATRA has been found to induce differentiation and arrest the proliferation of cancer cells;[14] this activity could also render cancer cells more susceptible to other cytotoxic therapies.[15–17] For instance, ATRA strongly enhances the apoptosis induced by arsenic trioxide or cisplatin in various human cancer cells.[18,19] Indeed, ATRA was found to modulate the plasticity and inhibit the motility of breast cancer cells,[20] implying an anti-metastasis application.[21,22] Based on these finding, we hypothesized that the combination of ATRA could enhance the anti-metastasis activity of platinum drugs. Therefore, we designed a carboplatin analogue (Pt-ATRA), in which the ligand cyclobutane-1,1-dicarboxylic acid (CDBCA) of carboplatin was replaced by an ATRA-bis(carboxylato) derivative. Upon internalization, Pt-ATRA is hydrolyzed in cells to release the activated platinum moiety and ATRA (Scheme 1). The inhibition of the proliferation and metastasis of breast cancer cells has been analyzed, and the anti-metastasis mechanism has been investigated.

Pt-ATRA is prepared *via* a click reaction between the propynyl ester of retinoic acid and a carboplatin analogue containing azide. The preparation of Pt-ATRA was designed using mild experimental conditions since ATRA is sensitive to light and heat. Therefore, the Cu-catalytic azide–alkyne click (CuAAC) reaction was employed. In brief, diethyl 2-(6-bromohexyl)malonate was synthesized by the reaction of diethyl malonate with



Scheme 1 A schematic illustration of the anti-tumor and anti-metastasis actions of Pt-ATRA.

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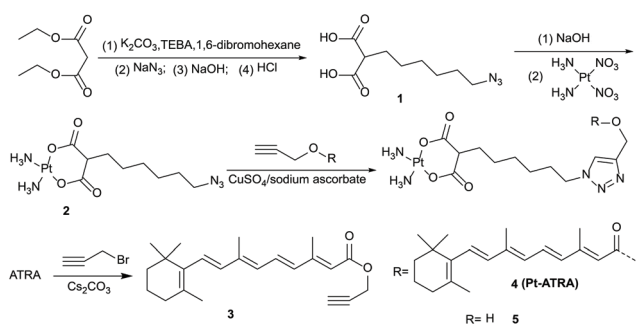
1,6-dibromohexane under the conditions of potassium carbonate and benzyl triethyl-ammonium bromide (TEBA),[23] and then reacted with sodium azide to replace the bromine atoms to obtain diethyl 2-(6-azidohexyl)malonate (Scheme 2).

After hydrolysis of diethyl 2-(6-azidohexyl)malonate with NaOH, the resulting compound **1** reacted with [Pt$(NH_3)_2(NO_3)_2$] to form intermediate compound **2**. Another branch of synthesis is esterification of ATRA with propargyl bromide under the conditions of Ce_2CO_3 to form an intermediate compound **3**.[24] After the preparation of compounds **2** and **3**, Pt-ATRA was generated *via* a click reaction catalyzed by sodium ascorbate and copper sulfate. In addition, we synthesized compound **5** as a control using the same synthetic method as that for Pt-ATRA. All the reactions were conducted under ambient conditions in order to reduce the destruction of ATRA at high temperatures. The products were verified by NMR and ESI-MS (Fig. S1–S6, ESI†). Pt-ATRA is a light yellow solid and characterized as follow: ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.00 (s, 6H), 1.23 (brs, 6H), 1.42 (m, 2H), 1.56 (m, 2H), 1.67 (s, 2H), 1.79 (brs, 4H), 1.98 (m, 6H), 2.30 (s, 3H), 3.50 (t, J = 6.9 Hz, 1H), 4.14 (m, 6H), 4.33 (t, J = 7.0 Hz, 2H), 5.14 (s, 2H), 5.84 (s, 1H), 6.19 (m, 3H), 6.41 (d, J = 15.3 Hz, 1H), 7.08 (dd, J = 15.1, 11.4 Hz, 1H), 8.16 (s, 1H). ESI-MS: m/z = 794.8 (calculated 794.3).

The *in vitro* cytotoxicity of Pt-ATRA was evaluated on 4T1 breast cancer cells. MTT assay showed that the IC_{50} value of Pt-ATRA (68.07 ± 3.92 μM) was comparable to those of carboplatin (50.62 ± 4.99 μM) and compound **5** (60.24 ± 6.35 μM) (Fig. 1A). This result suggests that these Pt(II) chelation complexes have a similar potency in the inhibition of cell proliferation. The cellular drug internalization analysis showed that the treatment of Pt-ATRA resulted in more platinum accumulation in cells in comparison to that with carboplatin, while a slightly lower DNA platination level was detected by the treatment of Pt-ATRA (Fig. S7†). The DNA platination levels are consistent with the results of the cytotoxicity assay.

The *in vivo* antitumor effect of these compounds was assessed on the 4T1 breast tumor bearing mice model. These compounds were administered through intravenous injection every other day 6 times. The average tumor size and body weight were monitored to evaluate the drug efficacy and toxicity effects. The results showed that Pt-ATRA has a slightly



Scheme 2 Synthetic route to Pt-ATRA and compound **5**.

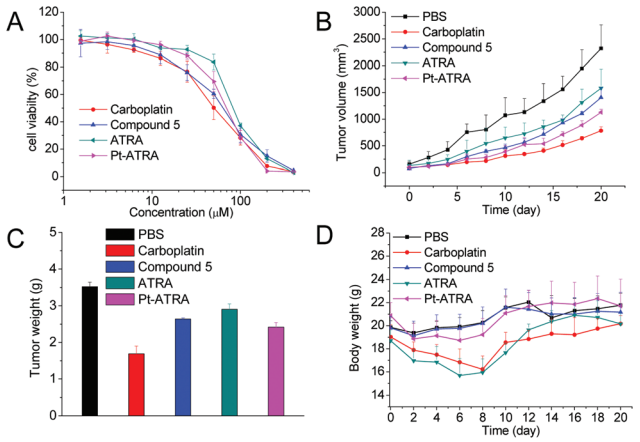


Fig. 1 *In vitro* and *in vivo* anti-tumor growth efficacy. (A) 4T1 cell viability under different concentrations of the compounds from MTT assay. (B) Tumor growth curves show the effects of the different treatments on tumor volume. (C) The tumor weight in each group at the end of the experiments. (D) The body weights of mice during the treatments. Balb/c mice bearing 4T1 tumors were injected through the tail vein with PBS, carboplatin, compound 5, ATRA and Pt-ATRA. The Pt(II) dose per injection was equivalent to 1.5 mg kg^{-1} body weight and the ATRA dose per injection was equivalent to 5.4 mg kg^{-1} body weight (n = 5). PBS was used as a control. Mice were treated 6 times, every other day. Error bars denote standard deviations.

higher antitumor activity than that of compound **5** and ATRA, and was almost equivalent to that of carboplatin (Fig. 1B and C). More importantly, the body weights of the mice clearly decreased during the treatment with carboplatin or ATRA, while little affected by Pt-ATRA, implying the low toxicity of Pt-ATRA at the dosage used in this measurement (Fig. 1D). These observations suggest that Pt-ATRA possesses the same anticancer activity but with a lower toxicity relative to carboplatin at the same dosage.

Pt-ATRA is formed by the coordination of the Pt$(NH_3)_2$ moiety with a chelation leaving group. This leaving group of the ATRA derivative contains a bis(carboxylato) ligand, and therefore the coordination structure of Pt-ATRA is similar to that of carboplatin. It has been well-investigated that the equation of carboplatin results in the release of the CDBCA ligand and generates the active Pt(II) moiety for DNA binding.[25,26] On the other hand, the leaving group of Pt-ATRA is formed through an ester bond between ATRA and the hydroxyl group of a malonic acid derivative. Ester bonds are typically designed in prodrugs since they can be broken by an esterase catalyzed hydrolysis in the cellular environment.[27–29] Therefore, Pt-ATRA can produce both an active Pt(II) moiety and ATRA in cells. Since ATRA is able to inhibit the motility of breast cancer cells, the anti-metastasis effect of Pt-ATRA was evaluated with a scratch assay on monolayer cells. The control experiment showed that the scraped cells completely recovered after 24 hours. The treatment of ATRA reduced the wound healing rate to 13.53%, showing the inhibition of cell migration by ATRA. The complex Pt-ATRA also reduced the ability for migration and inhibited the wound healing rate to

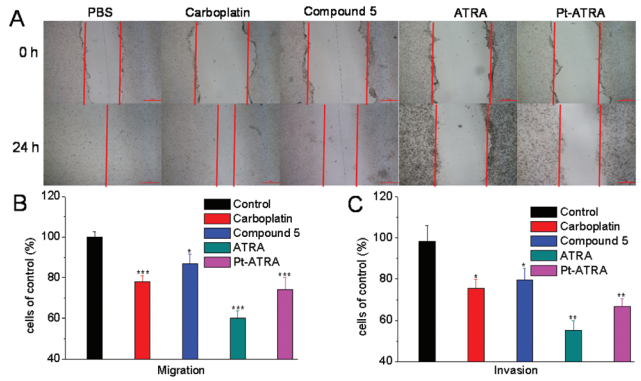


Fig. 2 The effects of compounds on the migration and invasion of cancer cells measured using a scratch test and transwell assay. 4T1 cells were pre-incubated for 24 h with drugs at a dose of 50 μM. Analyses of the lateral migratory cells (A) were obtained by measuring the wound closure rate. Quantitative analyses of migratory cells (B) and invasive cells (C) were obtained by measuring the UV absorbance at 550 nm of crystal violet dye that was extracted from the migration or invasion cells on the lower surface of the membrane. Error bars denote the standard deviations of three independent experiments and asterisks indicate *p*-values relative to the control group (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$, *T* test).

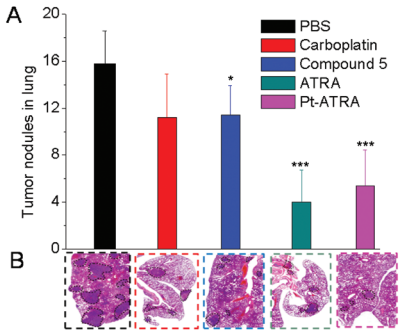


Fig. 3 *In vivo* anti-tumor metastasis effects. (A) The number of metastatic tumor nodules on the lungs of mice at the end of treatment. Error bars denote standard deviations. Asterisks indicate *p*-values relative to the control group (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$). (B) Representative histopathologic examinations of the lungs. The metastatic tumors in the lungs are marked with black dotted circles.

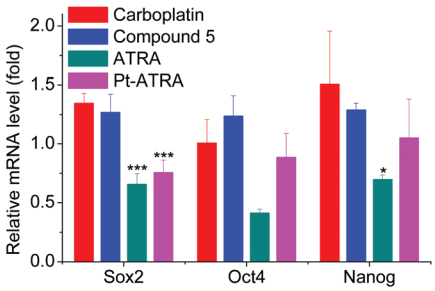


Fig. 4 The expression of stemness-associated genes measured *via* qRT-PCR on an mRNA level. Three genes (Sox2, Oct4 and Nanog) were analyzed on 4T1 cells that were treated with equivalent doses of Pt(II) (0.5 mg mL^{-1}) and/or ATRA (1.9 mg mL^{-1}) in different formulations for 24 h. Asterisks indicate *p*-values relative to the carboplatin group (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$).

15.62%, which is comparable to that of ATRA (Fig. 2A and Fig. S8†). By comparison, carboplatin and compound **5** demonstrated a much lower inhibition effect on the cell migration, confirming the function of ATRA derivative as a ligand on the platinum complex. Cell migration and invasion were also measured using a transwell experiment to further validate the anti-metastasis effect of Pt-ATRA. In agreement with the scratch assay results, ATRA and Pt-ATRA effectively inhibited the cell migration and invasion, while carboplatin and compound **5** showed much less activity (Fig. 2B, C and Fig. S9†).

The anti-metastasis effect of Pt-ATRA was also assessed *in vivo* on the 4T1 breast tumor bearing mice model. The tumor nodules were clearly observed on the surface of the lungs in the control group, showing a strong pulmonary metastasis of 4T1 cells. Consistent with the *in vitro* assay, ATRA inhibited the anti-metastasis and reduced the number of tumor nodules from 16 to 4 (Fig. 3A and Fig. S10†). Carboplatin and compound **5** only reduced the number of tumor nodules to 13, indicating the marginal anti-metastasis activity of these Pt(II) complexes. The coordination of the ligand of the ATRA derivative significantly enhanced the anti-metastasis activity of the Pt(II) complex, Pt-ATRA reduced the tumor nodules to 5, which is similar to the treatment of free ATRA. Furthermore, the tumor burden inside the lung tissue was analyzed using H&E staining. The results clearly showed that the treatment of ATRA and Pt-ATRA prevented tumor growth inside the lung tissue more efficiently than compound **5** and carboplatin. These results confirmed that the ligand of the ATRA derivative designed in this work results in the remarkable anti-metastasis effect of ATRA.

As cancer stem cells (CSCs) are believed to be involved in tumor metastasis,[30–34] the expression of CSC-associated genes was measured in order to understand the cause of the anti-metastasis effect of Pt-ATRA. Three CSC-associated genes, including Sox2, Oct4 and Nanog, were analyzed since they are highly expressed in breast CSCs.[35–40] The RT-qPCR measurement showed that the treatments of carboplatin and compound **5** can slightly increase the gene expression level, whereas the treatments of ATRA and Pt-ATRA suppressed the expression of the correlated genes (Fig. 4). This result suggested that ATRA and Pt-ATRA can reduce the stemness of cancer cells, which is probably associated with the anti-metastasis activity of Pt-ATRA.

Conclusions

In summary, a carboplatin analogue (Pt-ATRA) was synthesized in this work. Pt-ATRA is constructed by the coordination of Pt(II) to a chelation ligand containing an all-trans retinoic acid

(ATRA) derivative. This design endows Pt-ATRA with both anti-proliferation and anti-metastasis functions toward tumor cells. Upon cellular internalization, Pt-ATRA is hydrolyzed to release the activated platinum moiety and ATRA, exerting simultaneously anti-proliferation and anti-metastasis. *In vitro* cytotoxicity assays showed that Pt-ATRA has a similar potency to that of carboplatin toward tumor cells. *In vivo* assays demonstrated that Pt-ATRA inhibited tumor growth similarly to the effect of carboplatin, while Pt-ATRA exhibited a lower toxicity than carboplatin. Wound-healing and transwell assays confirmed that Pt-ATRA can inhibit the migration and invasion of tumor cells *in vitro*. The *in vivo* assay showed that Pt-ATRA can reduce the lung metastasis of tumors in a mouse model. Further mechanistic investigations revealed that Pt-ATRA can suppress the expression of cancer stem cell related genes, as these genes are associated with tumor metastasis.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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Notes and references

1 H. Sancho-Garnier and M. Colonna, *Presse Med.*, 2019, **48**, 1076–1084.
2 N. Li, Y. Deng, L. Zhou, T. Tian, S. Yang, Y. Wu, Y. Zheng, Z. Zhai, Q. Hao, D. Song, D. Zhang, H. Kang and Z. Dai, *J. Hematol. Oncol.*, 2019, **12**, 140.
3 Z. Cai and Q. Liu, *Sci. China: Life Sci.*, 2019, 1–4.
4 B. L. Eckhardt, P. A. Francis, B. S. Parker and R. L. Anderson, *Nat. Rev. Drug Discovery*, 2012, **11**, 479–497.
5 K. D. Miller, L. Nogueira, A. B. Mariotto, J. H. Rowland, K. R. Yabroff, C. M. Alfano, A. Jemal, J. L. Kramer and R. L. Siegel, *Ca-Cancer J. Clin.*, 2019, **69**, 363–385.
6 F. Cardoso, S. Kyriakides, S. Ohno, F. Penault-Llorca, P. Poortmans, I. T. Rubio, S. Zackrisson, E. Senkus and E. G. Committee, *Ann. Oncol.*, 2019, **30**, 1194–1220.
7 S. Adams, M. E. Gatti-Mays, K. Kalinsky, L. A. Korde, E. Sharon, L. Amiri-Kordestani, H. Bear, H. L. McArthur, E. Frank, J. Perlmutter, D. B. Page, B. Vincent, J. F. Hayes, J. L. Gulley, J. K. Litton, G. N. Hortobagyi, S. Chia, I. Krop, J. White, J. Sparano, M. L. Disis and E. A. Mittendorf, *JAMA Oncol.*, 2019, **5**, 1205–1214.
8 M. Abotaleb, P. Kubatka, M. Caprnda, E. Varghese, B. Zolakova, P. Zubor, R. Opatrilova, P. Kruzliak, P. Stefanicka and D. Büsselberg, *Biomed. Pharmacother.*, 2018, **101**, 458–477.
9 L. Volk-Draper, K. Hall, C. Griggs, S. Rajput, P. Kohio, D. DeNardo and S. Ran, *Cancer Res.*, 2014, **74**, 5421–5434.
10 H. Kajiyama, K. Shibata, M. Terauchi, M. Yamashita, K. Ino, A. Nawa and F. Kikkawa, *Int. J. Oncol.*, 2007, **31**, 277–283.
11 K. Sampieri and R. Fodde, *Semin. Cancer Biol.*, 2012, **22**, 187–193.
12 P. B. Gupta, T. T. Onder, G. Jiang, K. Tao, C. Kuperwasser, R. A. Weinberg and E. S. Lander, *Cell*, 2009, **138**, 645–659.
13 L. Degos, H. Dombret, C. Chomienne, M. T. Daniel, J. M. Miclea, C. Chastang, S. Castaigne and P. Fenaux, *Blood*, 1995, **85**, 2643–2653.
14 X. H. Tang and L. J. Gudas, *Annu. Rev. Pathol.: Mech. Dis.*, 2011, **6**, 345–364.
15 R. Wang and C. Liu, *Oncol. Lett.*, 2019, **18**, 3646–3654.
16 S. R. Modarai, A. Gupta, L. M. Opdenaker, R. Kowash, G. Masters, V. Viswanathan, D. Zhang, J. Z. Fields and B. M. Boman, *Oncotarget*, 2018, **9**, 34658–34669.
17 X. Chen, Z. Zhang, S. Yang, H. Chen, D. Wang and J. Li, *Onco Targets Ther.*, 2018, **11**, 6177–6187.
18 S. Kozono, Y. M. Lin, H. S. Seo, B. Pinch, X. Lian, C. Qiu, M. K. Herbert, C. H. Chen, L. Tan, Z. J. Gao, W. Massefski, Z. M. Doctor, B. P. Jackson, Y. Chen, S. Dhe-Paganon, K. P. Lu and X. Z. Zhou, *Nat. Commun.*, 2018, **9**, 3069.
19 S. Aebi, R. Kroning, B. Cenni, A. Sharma, D. Fink, G. Los, R. Weisman, S. B. Howell and R. D. Christen, *Clin. Cancer Res.*, 1997, **3**, 2033–2038.
20 A. Zanetti, R. Affatato, F. Centritto, M. Fratelli, M. Kurosaki, M. M. Barzago, M. Bolis, M. Terao, E. Garattini and G. Paroni, *J. Biol. Chem.*, 2015, **290**, 17690–17709.
21 M. Ryuto, S. Jimi, M. Ono, S. Naito, Y. Nakayama, Y. Yamada, S. Komiyama and M. Kuwano, *Jpn. J. Cancer Res.*, 1997, **88**, 982–991.
22 T. Palomares, I. Garcia-Alonso, R. San Isidro, J. Mendez and A. Alonso-Varona, *J. Surg. Res.*, 2014, **188**, 143–151.
23 Y. Wang, D. Liu, Q. Zheng, Q. Zhao, H. Zhang, Y. Ma, J. K. Fallon, Q. Fu, M. T. Haynes, G. Lin, R. Zhang, D. Wang, X. Yang, L. Zhao, Z. He and F. Liu, *Nano Lett.*, 2014, **14**, 5577–5583.
24 A. M. Lone, N. J. Dar, A. Hamid, W. A. Shah, M. Ahmad and B. A. Bhat, *ACS Chem. Neurosci.*, 2016, **7**, 82–89.
25 J. Kuduk-Jaworska, J. J. Janski and S. Roszak, *J. Inorg. Biochem.*, 2017, **170**, 148–159.
26 A. Ciancetta, C. Coletti, A. Marrone and N. Re, *Dalton Trans.*, 2012, **41**, 12960–12969.
27 M. Takahashi, T. Uehara, M. Nonaka, Y. Minagawa, R. Yamazaki, M. Haba and M. Hosokawa, *Eur. J. Pharm. Sci.*, 2019, **132**, 125–131.
28 M. A. M. Subbaiah, S. Mandlekar, S. Desikan, T. Ramar, L. Subramani, M. Annadurai, S. D. Desai, S. Sinha, S. M. Jenkins, M. R. Krystal, M. Subramanian, S. Sridhar, S. Padmanabhan, P. Bhutani, R. Arla, S. Singh, J. Sinha,

M. Thakur, J. F. Kadow and N. A. Meanwell, *J. Med. Chem.*, 2019, **62**, 3553–3574.
29 P. Huang, D. Wang, Y. Su, W. Huang, Y. Zhou, D. Cui, X. Zhu and D. Yan, *J. Am. Chem. Soc.*, 2014, **136**, 11748–11756.
30 G. J. Yoshida and H. Saya, *Cancer Sci.*, 2016, **107**, 5–11.
31 P. S. Steeg, *Nat. Rev. Cancer*, 2016, **16**, 201–218.
32 N. Reymond, B. B. d'Agua and A. J. Ridley, *Nat. Rev. Cancer*, 2013, **13**, 858–870.
33 J. Lathia, H. P. Liu and D. Matei, *Oncologist*, 2019, **24**, 1–9.
34 Y. C. Chen, S. Sahoo, R. Brien, S. Jung, B. Humphries, W. Lee, Y. H. Cheng, Z. Zhang, K. E. Luker, M. S. Wicha, G. D. Luker and E. Yoon, *Analyst*, 2019, **144**, 7296–7309.
35 F. Yang, J. Zhang and H. Yang, *OncoTargets Ther.*, 2018, **11**, 7873–7881.
36 M. U. Oliphant, A. Pandey, K. Johnson, R. Powers, M. Galbraith, J. Costello and H. L. Ford, *Cancer Res.*, 2018, **78**, 5001.
37 C. G. Liu, Y. Lu, B. B. Wang, Y. J. Zhang, R. S. Zhang, Y. Lu, B. Chen, H. Xu, F. Jin and P. Lu, *Ann. Surg.*, 2011, **253**, 1165–1171.
38 G. Q. Ling, D. B. Chen, B. Q. Wang and L. S. Zhang, *Oncol. Lett.*, 2012, **4**, 1264–1268.
39 O. Leis, A. Eguiara, E. Lopez-Arribillaga, M. J. Alberdi, S. Hernandez-Garcia, K. Elorriaga, A. Pandiella, R. Rezola and A. G. Martin, *Oncogene*, 2012, **31**, 1354–1365.
40 N. A. Abd El Moneim, S. E. El Feky, F. A. E. M. R. Ibrahim, M. A. E. R. Ahmed, K. K. Kadhem and S. A. Sheweita, *Breast*, 2017, **32**, S52.