



Preclinical study of the antitumor effect of sphingosine-1-phosphate receptor 1 antibody (S1PR₁-antibody) against human breast cancer cells

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Summary

Sphingosine-1-phosphate (S1P) is a bioactive sphingolipid metabolite, which regulates a broad range of physiological and pathophysiological processes. The signaling of S1P via its cell surface receptor S1PR₁ has been identified to play an important role in carcinogenesis, cancer growth and survival, and tumor metastasis. In this study, we evaluated whether a monoclonal antibody against S1PR₁ (S1PR₁-antibody) could impose any effect on cell growth of human breast cancer SK-BR-3 and MDA-MB-231 cells. The S1PR₁-antibody exhibited cytostatic effect against both cell lines at the concentration of 4000 ng/mL. Co-administration of 4000 ng/mL of the S1PR₁-antibody not only potentiated the cytotoxicity of carboplatin towards the MDA-MB-231 cells but also increased the anti-proliferative effect of S1P towards the SK-BR-3 cells. Furthermore, we showed that co-administration of S1P did not sensitize the SK-BR-3 and MDA-MB-231 cells towards carboplatin.

Keywords S1PR₁-antibody · Sphingosine-1-phosphate · Carboplatin · HER2 subtype breast cancer · Triple-negative subtype breast cancer

Introduction

Sphingosine-1-phosphate (S1P) is a common product of sphingolipid catabolism and an important bioactive sphingolipid metabolite. It exerts its biological functions both intracellularly and extracellularly to regulate various physiological and pathophysiological processes [1–3]. The intracellular function of S1P is not clearly understood. There is evidence showing that S1P acts as an intracellular messenger to regulate cell growth, invasion and apoptosis [4–6]. However, the extracellular function of S1P has been well studied, and S1P employs an “inside-out” signaling mode for its biological functions [7–10]. Briefly, S1P is first synthesized inside cells, and then transported out of the cells to interact with a

family of five G protein-coupled sphingosine-1-phosphate receptors (S1PR_{1–5}) [9, 10]. Receptors S1PR_{1–3} are relatively ubiquitously expressed with S1PR₁ possessing the highest expression level; whereas S1PR₄ is mainly expressed in lymphoid tissues and blood cells and S1PR₅ is expressed in brain, skin and natural killer cells [11–13]. S1P promotes cell proliferation and survival, inhibits cell apoptosis, and enhances angiogenesis via binding S1PR₁ and S1PR₃ [9, 10, 14, 15]. However, upon binding S1PR₂, S1P inhibits cell proliferation and survival and induces cell apoptosis [9, 10, 16, 17].

Extensive studies have concluded that the S1P-S1PR signaling axis plays an important role in cancer development and progression [9, 10]. Sphingosine kinase 1 (SK1), which is a cytosolic enzyme catalyzing the synthesis of S1P, is upregulated in various types of cancer [18–20]. It has also been shown that overexpression of SK1 promotes cancer metastasis and is responsible for the poor prognosis of breast cancer [21]. SK1 is required for epidermal growth factor (EGF)-directed motility and acts as a key regulator of breast cancer progression [22]. These observations implicate that SK1 is a valid anticancer therapeutic target [23, 24]. Furthermore, antagonization of S1PR₁ by FTY720, a potent immunomodulator, caused internalization and desensitization of S1PR₁ and inhibited tumor-associated angiogenesis [25]. Thus, downregulation of the S1P-S1PR₁ signaling pathway would

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likely be an effective option to impede cancer development and progression.

In order to evaluate whether the expression status of S1PR_{1–3} affects human breast cancer patient's survival, we extracted the Kaplan-Meier plots of survival probability versus mRNA expression level for S1PR_{1–3} from patients' data deposited at The Human Protein Atlas (HPA) database (<https://www.proteinatlas.org/>). As shown in Fig. 1, the mRNA expression status of S1PR₁ does not have any significant effect on breast cancer patients' survival; however, both S1PR₂ and S1PR₃ are favorable prognostic factors for breast cancer patients' survival. Therefore, in the current study, we decided to undertake a preclinical evaluation on whether blocking receptor S1PR₁ using a monoclonal antibody (S1PR₁-antibody), while leaving receptors S1PR₂ and S1PR₃ unblocked, would exhibit any antitumor effect against two highly invasive human breast cancer cell lines, HER2 subtype SK-BR-3 cell line and triple-negative subtype MDA-MB-231 cell line.

Materials and methods

Materials

All chemicals including sphingosine-1-phosphate (S1P), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and carboplatin were purchased from Sigma-Aldrich Canada (Oakville, ON, Canada). The MTT stock solution was prepared by dissolving MTT powder in phosphate buffered saline (1X PBS, pH 7.4) with the final concentration of 5 mg/mL. Monoclonal antibody against human S1PR₁ (S1PR₁-antibody) was purchased from Abcam Inc. (Toronto, ON, Canada). Human breast cancer cell lines SK-BR-3 and MDA-MB-231 were purchased from the American Type Culture Collection (ATCC) (Manassas, VA, USA). The cell culture media for cell lines SK-BR-3 and MDA-MB-231 were purchased from Cedarlane Canada (Burlington, ON, Canada). CellTox™ Green Cytotoxicity Assay was purchased from Promega North America (Madison, WI, USA).

Cell culture

Human breast cancer cell lines SK-BR-3 and MDA-MB-231 were cultured in T-75 cell culture flasks under a humidified atmosphere at 37 °C. The SK-BR-3 cells were cultured in McCoy's 5A Modified Medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin under 5% CO₂; and the MDA-MB-231 cells were cultured in Leibovitz's L-15 Medium supplemented with 10% FBS and 1% penicillin under 0% CO₂. Cell culture media were changed every 2–3 days.

Cell viability assay

Cell viability was determined using the MTT assay. Both SK-BR-3 and MDA-MB-231 cells were plated in 96-well plates at 8000 cells per well with the final cell culture volume of 100 µL and allowed to grow to 70–80% confluence before being subjected to different treatments. The treatment time was 48 h and 72 h, respectively. At the end of each treatment, the cell culture media were discarded and replaced with 100 µL MTT solution (prepared by adding 1 mL MTT stock solution to 9 mL cell culture media). The cell culture plates were then incubated at 37 °C for 3 h. After incubation, the MTT solution was discarded and 100 µL of dimethyl sulfoxide (DMSO) was added to each well to dissolve the MTT formazan. Subsequently, the cell culture plates were wrapped up in aluminum foil and shook on an orbital shaker at room temperature for 10 min. The absorbance was recorded at 570 nm on a BioTek® microplate reader (BioTek Canada, Winooski, VT, USA). The cell viability was calculated using Eq. 1.

Cell viability (%)

$$= \frac{\text{OD (sample)} - \text{OD (background)}}{\text{OD (vehicle control)} - \text{OD (background)}} \times 100\% \quad (1)$$

For the effect of the S1PR₁-antibody on cell viability of SK-BR-3 and MDA-MB-231 cells, the concentration of the S1PR₁-antibody was ranging from 16 ng/mL to 4000 ng/mL. 1X PBS buffer (pH 7.4), in which the S1PR₁-antibody was dissolved, was used as the vehicle control. For the effect of the combination of the S1PR₁-antibody and carboplatin on cell viability of SK-BR-3 and MDA-MB-231 cells, the S1PR₁-antibody concentration was 4000 ng/mL and the carboplatin concentration was 4 µM towards the SK-BR-3 cells and 67.5 µM towards the MDA-MB-231 cells. PBS buffer (1X, pH 7.4) was used as the vehicle control. For the effect of the combination of the S1PR₁-antibody and S1P on cell viability of SK-BR-3 and MDA-MB-231 cells, the concentration of the S1PR₁-antibody was 4000 ng/mL and the concentration of S1P was 10 µM/mL. Methanol with 120 mg/mL PEG 3350 (polyethylene glycol 3350), in which S1P was dissolved, was used as the vehicle control. For the effect of the combination of S1P and carboplatin on cell viability of SK-BR-3 and MDA-MB-231 cells, the S1P concentration was at 1 µM and 10 µM, respectively. The carboplatin concentration was ranging from 4 µM to 540 µM for the SK-BR-3 cells and from 67.5 µM to 1080 µM for the MDA-MB-231 cells, respectively. Methanol with 120 mg/mL PEG 3350 was used as the vehicle control.

Cytotoxicity assay

The SK-BR-3 and MDA-MB-231 cells were plated in 96-well plates at 8000 cells per well with the final culture volume of 100 µL and allowed to grow to 70–80% confluence before

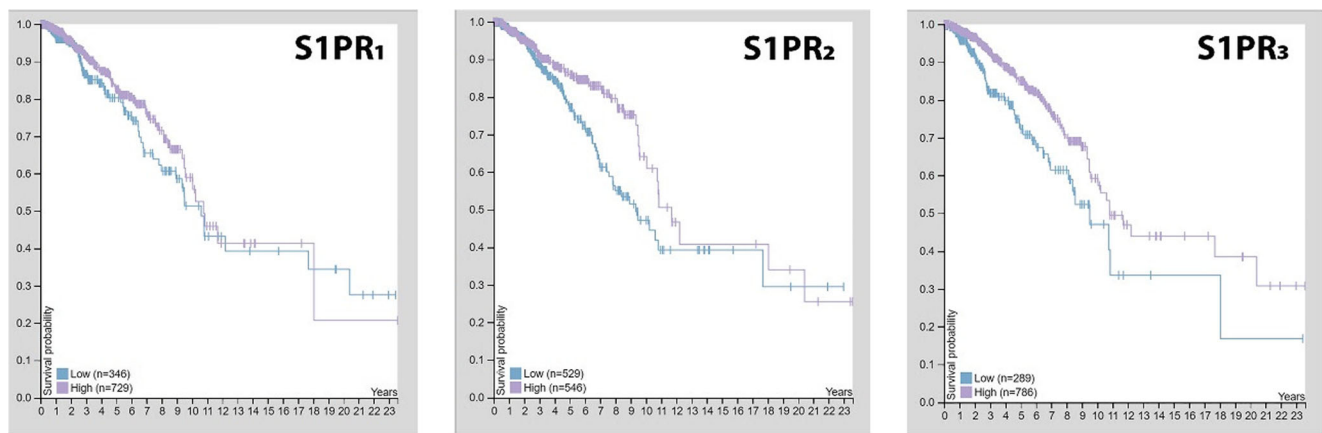


Fig. 1 The Kaplan-Meier plot of patient survival probability versus mRNA expression level for S1P receptors 1–3 (S1PR_{1–3}) based on breast cancer patients' data deposited at the Human Protein Atlas database (<https://www.proteinatlas.org/>)

being treated with S1PR₁-antibody for 48 h and 72 h, respectively. The concentration of S1PR₁-antibody was ranging from 16 ng/mL to 4000 ng/mL, with 1X PBS buffer (pH 7.4), in which S1PR₁-antibody was dissolved, as the negative control. The cytotoxicity was measured using the CellTox™ Green Cytotoxicity Assay from Promega and calculated using Eq. 2.

Cytotoxicity (%)

$$= \frac{\text{OD}(\text{experiment}) - \text{OD}(\text{negative control})}{\text{OD}(\text{maximum cell death}) - \text{OD}(\text{negative control})} \times 100\% \quad (2)$$

Statistical analysis

The statistical analysis of the data was carried out by two-way ANOVA with Tukey's post hoc analysis using GraphPad Prism 6 (GraphPad Software, La Jolla, CA, USA). The significance was set at $p \leq 0.05$ (*, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$; and ****, $p \leq 0.0001$).

Results and discussion

S1PR₁-antibody exhibited cytostatic effect

The promoting effect of S1P on cell proliferation, migration and metastasis of different types of cancer is mainly through S1PR₁ [9, 10, 26–28]. S1PR₁ antagonists were shown to be able to inhibit tumor angiogenesis in vivo and enhance the therapeutic efficacy of doxorubicin [29, 30]. Taking in consideration that S1PR₂ and S1PR₃ are favorable prognostic factors for human breast cancer (Fig. 1), we examined whether blocking S1PR₁ with a monoclonal S1PR₁-antibody and allowing endogenous S1P to interact selectively with S1PR₂

and S1PR₃ would inhibit the growth of the highly invasive human breast cancer SK-BR-3 (HER2 subtype) and MDA-MB-231 (triple-negative subtype) cells. We first assessed the effect of the S1PR₁-antibody on cell viability of SK-BR-3 and MDA-MB-231 cells using the MTT assay. As shown in Fig. 2a, SK-BR-3 cells exhibited a dose-dependent and time-dependent response towards S1PR₁-antibody; however, the response was relatively mild. The maximum effect of about 10% reduction in cell viability was achieved at the treatment of 4000 ng/mL S1PR₁-antibody for 72 h. For the MDA-MB-231 cells, the S1PR₁-antibody promoted cell growth at low concentrations (16–500 ng/mL) and inhibited cell growth at high concentrations (1000–4000 ng/mL) (Fig. 2b). The maximum promoting effect of cell growth (~10% increase in cell viability) was at treatment of 125 ng/mL S1PR₁-antibody for 72 h, whereas the maximum inhibiting effect on cell growth (~27% decrease in cell viability) was at treatment of 4000 ng/mL S1PR₁-antibody for 72 h. To further understand whether the inhibition of cell growth by the S1PR₁-antibody was due to cytostatic or cytotoxic effect, we measured the cytotoxicity of the S1PR₁-antibody towards the SK-BR-3 and MDA-MB-231 cells using the CytoTox™ Green Cytotoxicity Assay under the same experimental condition. The S1PR₁-antibody did not cause any cytotoxic effect towards either SK-BR-3 cells or MDA-MB-231 cells, except a small 10% cytotoxicity towards the MDA-MB-231 cells at treatment of 4000 ng/mL S1PR₁-antibody for 72 h (Fig. 2c, d). We speculated that this small cytotoxicity might be caused by the differences of S1PR₁ expression and internalization between the two cell lines. However, we could not completely rule out the possibility of that a different antigen possesses a fragment of sequence highly homologous to the epitope used in producing the S1PR₁-antibody and binds S1PR₁-antibody, even with much weaker binding affinity, in the MDA-MB-231 cells. In summary, we conclude that the inhibitory function of the S1PR₁-antibody on cell growth of the SK-BR-3 and MDA-MB-231 cells is likely through cytostatic effect. Further studies are warranted

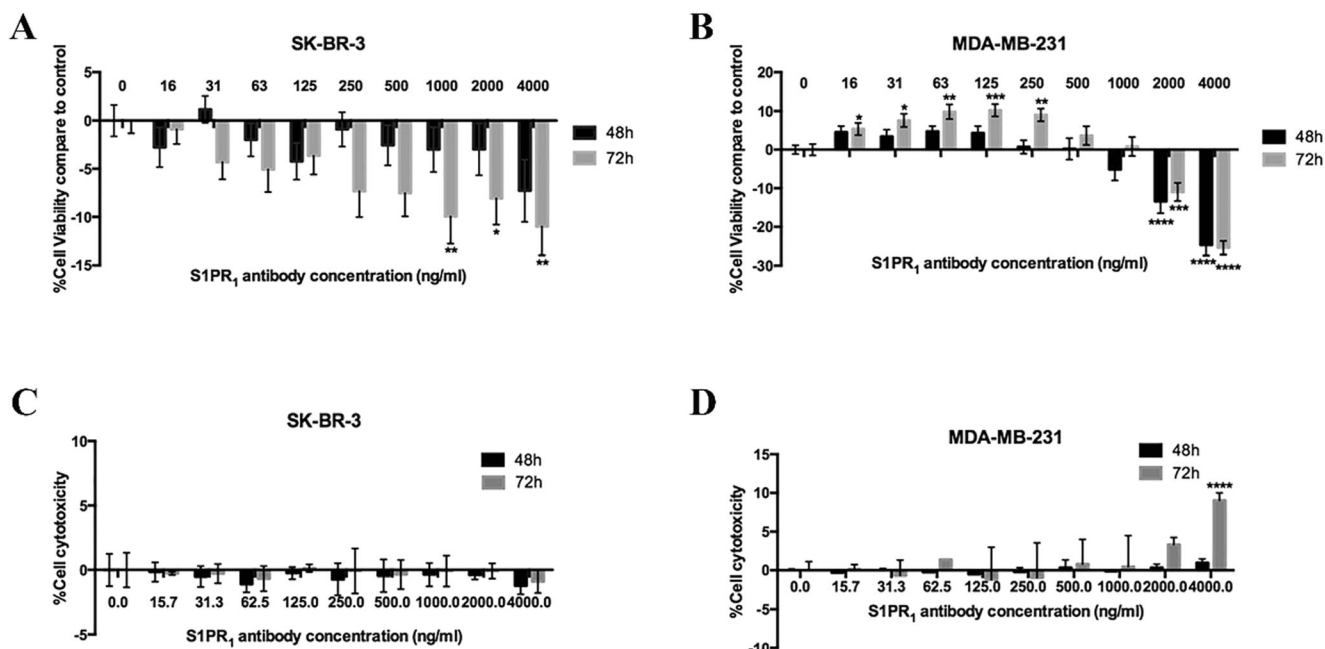


Fig. 2 The effect of the S1PR₁-antibody on cell viability towards breast cancer cell lines SK-BR-3 (a) and MDA-MB-231 (b), and the effect of the S1PR₁-antibody on cytotoxicity towards breast cancer cell lines SK-BR-3 (c) and MDA-MB-231 (d) at 48 h and 72 h of treatment. The effect on

cell viability was measured using the MTT assay and the effect on cytotoxicity was measured using the CytoTox™ Green Cytotoxicity Assay, respectively. PBS buffer (1X, pH 7.4) was used as the vehicle control

to identify whether blocking S1PR₁ by the S1PR₁-antibody would change endogenous S1P synthesis and/or affect S1P-S1PR₂ and S1P-S1PR₃ signaling in human breast cancer cells.

S1PR₁-antibody potentiated the cytotoxicity of carboplatin towards MDA-MB-231 cells

Platinum-based chemotherapy drugs are a family of alkylating agents widely used to treat different types of cancer, such as small cell lung cancer, head and neck cancer and ovarian cancer. However, breast cancer does not respond well towards platinum-based drugs; and carboplatin, usually in combination with other chemotherapy drugs, is the only platinum-based drug approved to treat advanced-stage breast cancer

[31, 32]. Since antagonization of S1PR₁ has been observed to enhance the efficacy of doxorubicin [25, 30], we decided to evaluate whether the S1PR₁-antibody could potentiate the cytotoxic effect of carboplatin towards SK-BR-3 and MDA-MB-231 cells. The IC₅₀ of carboplatin was determined to be 80.0 μM (48 h) and 30.6 μM (72 h) against the SK-BR-3 cells and 834 μM (48 h) and 294 μM (72 h) against the MDA-MB-231 cells, respectively (supplementary Fig. S1). It is extremely high compared to the reported IC₅₀ of carboplatin towards several cell lines derived from ovarian cancer patients, which is around 1 μM for pre-chemotherapy cell lines and 5 μM for post-chemotherapy cell lines [33]. This implicates that carboplatin might not be a first choice for treating HER2 or triple-negative subtype breast cancer, although it is approved for advanced-stage breast cancer.

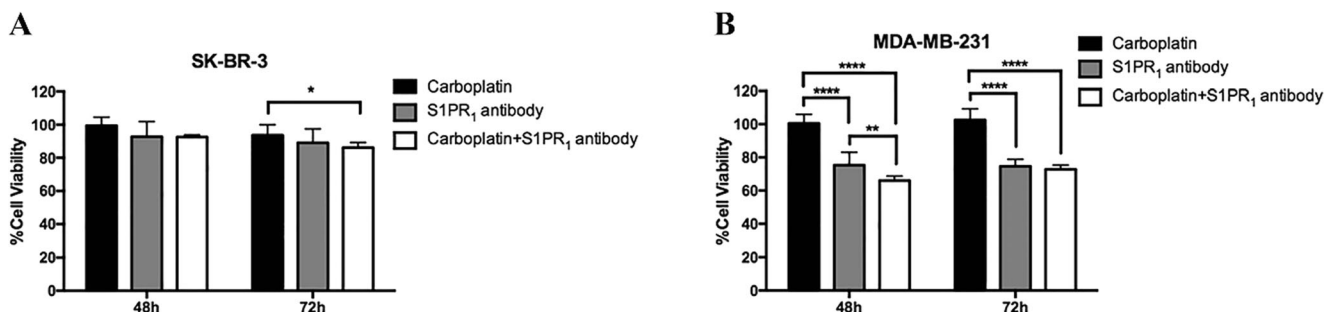


Fig. 3 The effect of carboplatin, S1PR₁-antibody and their combination on cell viability of the SK-BR-3 (a) and MDA-MB-231 (b) cells at 48 h and 72 h of treatment. For the SK-BR-3 cells, the carboplatin concentration was 4 μM and the S1PR₁-antibody concentration was

4000 ng/mL; whereas for the MDA-MB-231 cells, the carboplatin concentration was 68 μM and the S1PR₁-antibody concentration was also 4000 ng/mL. The effect on cell viability was measured using the MTT assay with PBS buffer (1X, pH 7.4) as the vehicle control

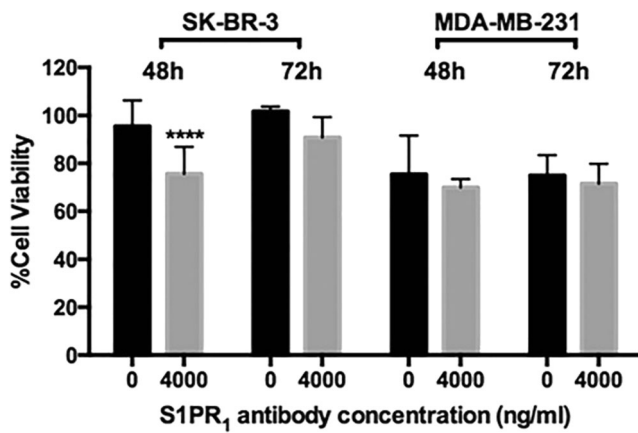


Fig. 4 The effect of S1P (concentration: 10 μ M), with or without S1PR₁-antibody (concentration: 4000 ng/mL) on cell viability of the SK-BR-3 and MDA-MB-231 cells at 48 h and 72 h of treatment. The effect on cell viability was measured using the MTT assay with methanol containing 120 mg/mL PEG 3350 as the vehicle control

In order to get a glimpse on whether the S1PR₁-antibody could improve the response of SK-BR-3 and MDA-MB-231 cells towards carboplatin, we evaluated the effect of carboplatin in combination of 4000 ng/mL of S1PR₁-antibody on the cell viability of SK-BR-3 and MDA-MB-231 cells. For both cell lines, we chose a concentration of carboplatin significantly lower than the IC₅₀ (4 μ M for SK-Br-3 and 68 μ M for MDA-MB-231), which does not impose any cytotoxicity to the cells. The reason for this type of selection is to ensure that

this research would lead to further in vivo and even clinically meaningful studies if the S1PR₁-antibody indeed sensitized the breast cancer cells to carboplatin, as the IC₅₀ of carboplatin was only around 1 μ M towards patient-derived ovarian cancer cell lines [33]. As shown in Fig. 3, co-administration of S1PR₁-antibody did not sensitize the SK-BR-3 cells towards carboplatin treatment. For MDA-MB-231 cells, the co-administration of S1PR₁-antibody and carboplatin decreased the cell viability by more than 35% compared to carboplatin alone ($p \leq 0.0001$) and almost 10% compared to S1PR₁-antibody alone ($p \leq 0.01$) at 48 h of treatment (Fig. 3). This implicated that S1PR₁-antibody enhanced the cytotoxic effect of carboplatin. However, at 72 h of treatment, we did not observe any significant difference in cell viability between S1PR₁-antibody alone and its combination with carboplatin. We speculated that this was due to the cytostatic effect of the S1PR₁-antibody, as carboplatin targets fast-growing cells and prolonged treatment with the S1PR₁-antibody made the MDA-MB-231 cells insensitive towards carboplatin. Further studies are warranted to identify the optimal conditions, such as S1PR₁-antibody and carboplatin concentrations and the sequential order of the administration, to achieve the best efficacy (i.e. synergistic/additive effect) of the cytostatic effect of S1PR₁-antibody and cytotoxic effect of carboplatin. In addition, we have already imitated a study to evaluate whether the S1PR₁-antibody could exhibit any synergistic/additive effect with other chemotherapy drugs, such as docetaxel,

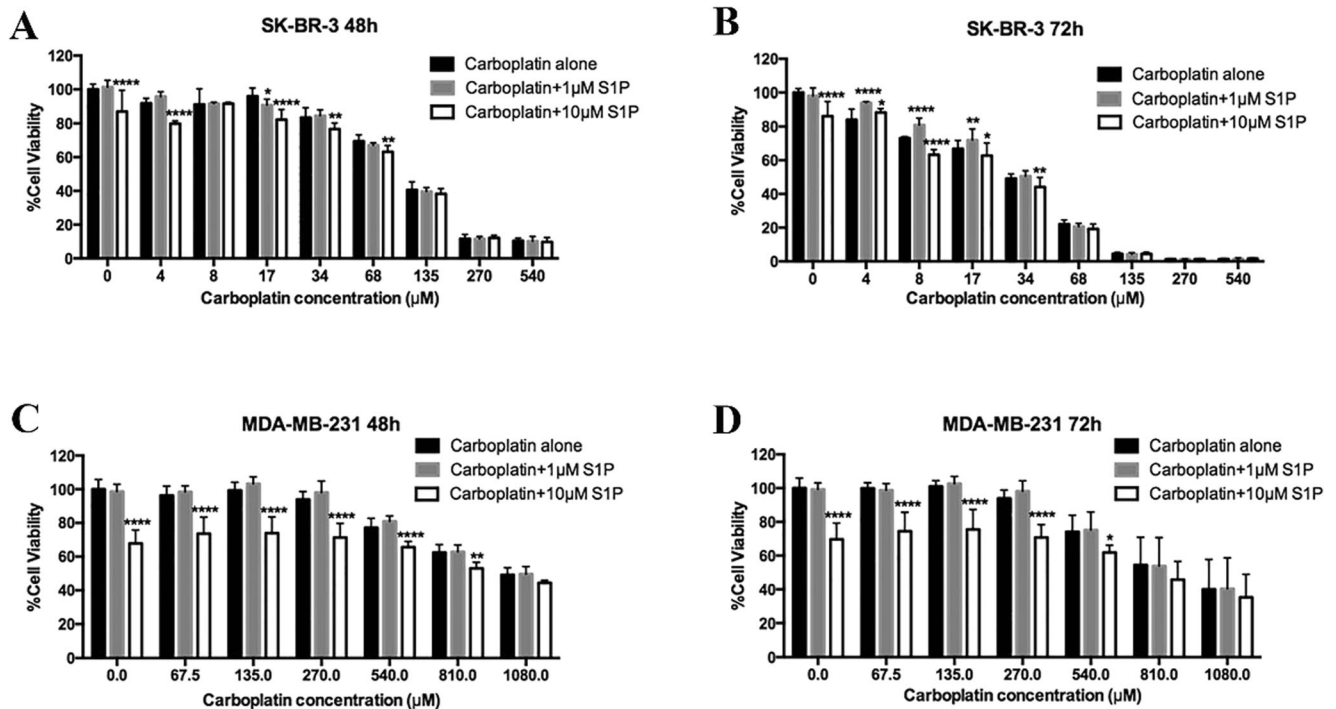


Fig. 5 The effect of carboplatin and its combinations with 1 μ M S1P and 10 μ M S1P on cell viability of the SK-BR-3 and MDA-MB-231 cells at 48 h and 72 h of treatment. The concentration of carboplatin was ranging from 4 μ M to 540 μ M for the SK-BR-3 cells and from 67.5 μ M to

1080 μ M for the MDA-MB-231 cells. The effect on cell viability was measured using the MTT assay with methanol containing 120 mg/mL PEG 3350 as the vehicle control

doxorubicin and lapatinib, against both HER2 subtype and triple-negative subtype human breast cancer cell lines.

S1PR₁-antibody enhanced the effect of S1P towards SK-BR-3 cells

In our previous studies, we showed that S1P selectively induced cell apoptosis in breast cancer MCF7 cells and exhibited synergistic effect with chemotherapy drugs towards breast cancer MCF7 and MDA-MB-361 cells at a concentration higher than 1 μ M, especially at 10 μ M [34, 35]. Therefore, we decided to evaluate whether there could be any synergistic/additive effect between S1PR₁-antibody (concentration: 4000 ng/mL) and S1P (concentration: 10 μ M). For the SK-BR-3 cells, S1P did not exhibit any significant effect on cell viability compared to the vehicle control at either 48 h or 72 h treatment; however, co-administration of S1PR₁-antibody decreased the cell viability by almost 20% ($p \leq 0.0001$) at 48 h of treatment (Fig. 4). At 72 h of treatment, the co-administration of S1PR₁-antibody reduced the cell viability by approximately 10%; however, this decrease is not statistically significant (Fig. 4). For the MDA-MB-231 cells, S1P induced about 20–25% reduction in cell viability compared to the vehicle control at 48 h or 72 h of treatment; however, co-administration of S1PR₁-antibody did not further reduce the cell viability at either treatment time (Fig. 4). This study implicated that S1PR₁-antibody was able to enhance the effect of S1P against the SK-BR-3 cells. A study has been initiated to understand how the S1PR₁-antibody affects the intracellular function of S1P, as our studies suggested that the selective cytotoxic effect of S1P towards human breast cancer cells at concentrations higher than 1 μ M is highly likely through its intracellular function (data not published).

S1P did not elevate the cytotoxicity of carboplatin

S1P has been shown to potentiate the cytotoxic effects of docetaxel, doxorubicin and cyclophosphamide towards human breast cancer cells at high concentrations ($\geq 1 \mu$ M) [34, 35]. In the current study, we also evaluated whether S1P (concentration: 1 μ M and 10 μ M) could improve the sensitivity of the SK-BR-3 and MDA-MB-231 cells to carboplatin, as neither type of cells is responding well to carboplatin treatment (supplementary Fig. S1) in spite of that carboplatin has been approved for advanced-stage breast cancer. Neither 1 μ M nor 10 μ M concentration of S1P was able to improve the response of the SK-BR-3 and MDA-MB-231 cells to carboplatin treatment (Fig. 5). However, S1P (concentration: 10 μ M) decreased the cell viability of the SK-BR-3 and MDA-MB-231 cells by approximately 15 and 30%, respectively at both 48 h and 72 h treatments. For both cell lines, when carboplatin concentration was less than the IC₅₀ values, the effect of the co-administration of S1P (concentration: 10 μ M) and carboplatin

on cell viability was dominated by S1P. Whereas the carboplatin concentration was increased higher than the IC₅₀ values, the effect of the co-administration of S1P (concentration: 10 μ M) and carboplatin on cell viability was controlled by carboplatin. Therefore, in contrary to our previous studies that S1P can increase the cytotoxic activities of docetaxel, doxorubicin and cyclophosphamide, co-administration of high concentrations of S1P does not improve the cytotoxic activity of carboplatin.

Conclusion

In this study, we showed that the S1PR₁-antibody exhibited cytostatic effect towards both HER2 subtype SK-BR-3 cell line and triple-negative subtype MDA-MB-231 cell line. Co-administration of 4000 ng/mL of the S1PR₁-antibody not only potentiated the cytotoxicity of carboplatin towards cell line MDA-MB-231 but also increased the anti-proliferative effect of S1P towards cell line SK-BR-3. Furthermore, co-administration of S1P did not improve the response of the SK-BR-3 and MDA-MB-231 cells towards carboplatin treatment.

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Author contributions This research work was designed by Jian Yang and carried out by Shujun Xiao.

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Compliance with ethical standards

Conflict of interest Shujun Xiao declares that she has no conflict of interest. Jian Yang declares that he has no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

References

1. Pyne NJ, El Buri A, Adams DR, Pyne S (2017) Sphingosine 1-phosphate and cancer. *Adv Biol Regul* 68:97–106. <https://doi.org/10.1016/j.jbior.2017.09.006>
2. Maceyka M, Spiegel S (2014) Sphingolipid metabolites in inflammatory disease. *Nature* 510:58–67. <https://doi.org/10.1038/nature13475>
3. Weske S, Vaidya M, Reese A, von Wnuck Lipinski K, Keul P, Bayer JK, Fischer JW, Flögel U, Nelsen J, Epple M, Scatena M, Schwedhelm E, Dörr M, Völzke H, Moritz E, Hannemann A, Rauch BH, Gräler MH, Heusch G, Levkau B (2018) Targeting sphingosine-1-phosphate lyase as an anabolic therapy for bone loss. *Nat Med* 24:667–678. <https://doi.org/10.1038/s41591-018-0005-y>

4. Igarashi N, Okada T, Hayashi S, Fujita T, Jahangeer S, Nakamura S (2003) Sphingosine kinase 2 is a nuclear protein and inhibits DNA synthesis. *J Biol Chem* 278:46832–46839. <https://doi.org/10.1074/jbc.M306577200>
5. Ohotski J, Rosen H, Bittman R, Pyne S, Pyne NJ (2014) Sphingosine kinase 2 prevents the nuclear translocation of sphingosine 1-phosphate receptor-2 and tyrosine 416 phosphorylated c-Src and increases estrogen receptor negative MDA-MB-231 breast cancer cell growth: The role of sphingosine 1-phosphate receptor. *Cell Signal* 26:1040–1047. <https://doi.org/10.1016/j.cellsig.2014.01.023>
6. Pébay A, Toutant M, Prémont J, Calvo CF, Venance L, Cordier J, Glowinski J, Tencé M (2001) Sphingosine-1-phosphate induces proliferation of astrocytes: regulation by intracellular signalling cascades. *Eur J Neurosci* 13:2067–2076. <https://doi.org/10.1046/j.0953-816x.2001.01585.x>
7. Takabe K, Paugh SW, Milstien S, Spiegel S (2008) “Inside-out” signaling of Sphingosine-1-phosphate: therapeutic targets. *Pharmacol Rev* 60:181–195. <https://doi.org/10.1124/pr.107.07113>
8. Pyne NJ, McNaughton M, Boomkamp S, MacRitchie N, Evangelisti C, Martelli AM, Jiang H-R, Ubhi S, Pyne S (2016) Role of sphingosine 1-phosphate receptors, sphingosine kinases and sphingosine in cancer and inflammation. *Adv Biol Regul* 60: 151–159. <https://doi.org/10.1016/j.jbior.2015.09.001>
9. Strub GM, Maceyka M, Hait NC, Milstien S, Spiegel S (2010) Extracellular and intracellular actions of sphingosine-1-phosphate. *Adv Exp Med Biol* 688:141–155
10. Pyne NJ, Pyne S (2010) Sphingosine 1-phosphate and cancer. *Nat Rev Cancer* 10:489–503. <https://doi.org/10.1038/nrc2875>
11. Olesch C, Ringel C, Brüne B, Weigert A (2017) Beyond immune cell migration: the emerging role of the Sphingosine-1-phosphate receptor S1PR4 as a modulator of innate immune cell activation. *Mediat Inflamm* 2017:6059203. <https://doi.org/10.1155/2017/6059203>
12. Rivera J, Proia RL, Olivera A (2008) The alliance of sphingosine-1-phosphate and its receptors in immunity. *Nat Rev Immunol* 8:753–763. <https://doi.org/10.1038/nri2400>
13. Di Pardo A, Castaldo S, Amico E, Pepe G, Marracino F, Capocci L, Giovannelli A, Madonna M, van Bergeijk J, Buttari F, van der Kam E, Maglione V (2018) Stimulation of S1PR5 with A-971432, a selective agonist, preserves blood-brain barrier integrity and exerts therapeutic effect in an animal model of Huntington’s disease. *Hum Mol Genet*. <https://doi.org/10.1093/hmg/ddy153>
14. Sassoli C, Pierucci F, Tani A, Frati A, Chellini F, Matteini F, Vestri A, Anderloni G, Nosi D, Zecchi-Orlandini S, Meacci E (2018) Sphingosine 1-phosphate receptor 1 is required for MMP-2 function in bone marrow mesenchymal stromal cells: implications for cytoskeleton assembly and proliferation. *Stem Cells Int* 2018: 5034679. <https://doi.org/10.1155/2018/5034679>
15. Hirata N, Yamada S, Shoda T, Kurihara M, Sekino Y, Kanda Y (2014) Sphingosine-1-phosphate promotes expansion of cancer stem cells via S1PR3 by a ligand-independent Notch activation. *Nat Commun* 5:4806. <https://doi.org/10.1038/ncomms5806>
16. Takuwa N, Du W, Kaneko E, Okamoto Y, Yoshioka K, Takuwa Y (2011) Tumor-suppressive sphingosine-1-phosphate receptor-2 counteracting tumor-promoting sphingosine-1-phosphate receptor-1 and sphingosine kinase 1 - Jekyll Hidden behind Hyde. *Am J Cancer Res* 1:460–481
17. Arikawa K, Takuwa N, Yamaguchi H, Sugimoto N, Kitayama J, Nagawa H, Takehara K, Takuwa Y (2003) Ligand-dependent inhibition of B16 melanoma cell migration and invasion via endogenous S1P 2 G protein-coupled receptor. *J Biol Chem* 278:32841–32851. <https://doi.org/10.1074/jbc.M305024200>
18. Madhunapantula SV, Hengst J, Gowda R, Fox TE, Yun JK, Robertson GP (2012) Targeting sphingosine kinase-1 to inhibit melanoma. *Pigment Cell Melanoma Res* 25:259–274. <https://doi.org/10.1111/j.1755-148X.2012.00970.x>
19. Marfe G, Di Stefano C, Gambacurta A, Ottone T, Martini V, Abruzzese E, Mologni L, Sinibaldi-Salimei P, de Fabritis P, Gambacorti-Passerini C, Amadori S, Birge RB (2011) Sphingosine kinase 1 overexpression is regulated by signaling through PI3K, AKT2, and mTOR in imatinib-resistant chronic myeloid leukemia cells. *Exp Hematol* 39:653–665.e6. <https://doi.org/10.1016/j.exphem.2011.02.013>
20. Li W, Yu C-P, Xia J-t, Zhang L, Weng G-X, Zheng H-q, Kong Q-l, Hu L-j, Zeng M-S, Zeng Y-x, Li M, Li J, Song L-B (2009) Sphingosine kinase 1 is associated with gastric cancer progression and poor survival of patients. *Clin Cancer Res* 15:1393–1399. <https://doi.org/10.1158/1078-0432.CCR-08-1158>
21. Zhu Y-J, You H, Tan J-X, Li F, Qiu Z, Li H-Z, Huang H-Y, Zheng K, Ren G-S (2017) Overexpression of sphingosine kinase 1 is predictive of poor prognosis in human breast cancer. *Oncol Lett* 14:63–72. <https://doi.org/10.3892/ol.2017.6134>
22. Long JS, Edwards J, Watson C, Tovey S, Mair KM, Schiff R, Natarajan V, Pyne NJ, Pyne S (2010) Sphingosine kinase 1 induces tolerance to human epidermal growth factor receptor 2 and prevents formation of a migratory phenotype in response to sphingosine 1-phosphate in estrogen receptor-positive breast cancer cells. *Mol Cell Biol* 30:3827–3841. <https://doi.org/10.1128/MCB.01133-09>
23. Gao Y, Gao F, Chen K, Tian M, Zhao D (2015) Sphingosine kinase 1 as an anticancer therapeutic target. *Drug Des Devel Ther* 9:3239–3245. <https://doi.org/10.2147/DDDT.S83288>
24. Ogretmen B (2018) Sphingolipid metabolism in cancer signalling and therapy. *Nat Rev Cancer* 18:33–50. <https://doi.org/10.1038/nrc.2017.96>
25. LaMontagne K, Littiewood-Evans A, Schnell C, O’Reilly T, Wyder L, Sanchez T, Probst B, Butler J, Wood A, Liau G, Billy E, Theuer A, Hla T, Wood J (2006) Antagonism of sphingosine-1-phosphate receptors by FTY720 inhibits angiogenesis and tumor vascularization. *Cancer Res* 66:221–231. <https://doi.org/10.1158/0008-5472.CAN-05-2001>
26. Xu X, Huang C, Zhang Y, Chen L, Cheng H, Wang J (2016) S1PR1 mediates anti-apoptotic/pro-proliferative processes in human acute myeloid leukemia cells. *Mol Med Rep* 14:3369–3375. <https://doi.org/10.3892/mmr.2016.5629>
27. Cheng N, Wang G-H (2016) miR-133b, a microRNA targeting S1PR1, suppresses nasopharyngeal carcinoma cell proliferation. *Exp Ther Med* 11:1469–1474. <https://doi.org/10.3892/etm.2016.3043>
28. Go H, Kim P-J, Jeon YK, Cho YM, Kim K, Park B-H, Ku JY (2015) Sphingosine-1-phosphate receptor 1 (S1PR1) expression in non-muscle invasive urothelial carcinoma: association with poor clinical outcome and potential therapeutic target. *Eur J Cancer* 51: 1937–1945. <https://doi.org/10.1016/j.ejca.2015.07.021>
29. Fujii Y, Ueda Y, Ohtake H, Ono N, Takayama T, Nakazawa K, Igarashi Y, Goitsuka R (2012) Blocking S1P interaction with S1P1 receptor by a novel competitive S1P1-selective antagonist inhibits angiogenesis. *Biochem Biophys Res Commun* 419:754–760. <https://doi.org/10.1016/j.bbrc.2012.02.096>
30. Katsuta E, Yan L, Nagahashi M, Raza A, Sturgill JL, Lyon DE, Rashid OM, Hait NC, Takabe K (2017) Doxorubicin effect is enhanced by sphingosine-1-phosphate signaling antagonist in breast cancer. *J Surg Res* 219:202–213. <https://doi.org/10.1016/j.jss.2017.05.101>
31. Eckstein N (2011) Platinum resistance in breast and ovarian cancer cell lines. *J Exp Clin Cancer Res* 30:91. <https://doi.org/10.1186/1756-9966-30-91>
32. Lehmann BD, Bauer JA, Chen X, Sanders ME, Chakravarthy AB, Shyr Y, Pietenpol JA (2011) Identification of human triple-negative breast cancer subtypes and preclinical models for selection of

- targeted therapies. *J Clin Invest* 121:2750–2767. <https://doi.org/10.1172/JCI45014>
33. Létourneau IJ, Quinn MCJ, Wang L-L, Portelance L, Caceres KY, Cyr L, Delvoye N, Meunier L, de Ladurantaye M, Shen Z, Arcand SL, Tonin PN, Provencher DM, Mes-Masson A-M (2012) Derivation and characterization of matched cell lines from primary and recurrent serous ovarian cancer. *BMC Cancer* 12:379. <https://doi.org/10.1186/1471-2407-12-379>
 34. Ling B, Chen L, Alcorn J, Ma B, Yang J (2011) Sphingosine-1-phosphate: a potential therapeutic agent against human breast cancer. *Investig New Drugs* 29:396–399. <https://doi.org/10.1007/s10637-009-9375-9>
 35. Sultan A, Ling B, Zhang H, Ma B, Michel D, Alcorn J, Yang J (2013) Synergistic effect between sphingosine-1-phosphate and chemotherapy drugs against human brainmetastasized breast cancer MDA-MB-361 cells. *J Cancer* 4:315–319. <https://doi.org/10.7150/jca.5956>