An analysis of the effect of Oxaliplatin in inducing immunogenetic cell death and improving the efficacy of checkpoint inhibitor on SGC-7901

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Abstract

Previous studies demonstrated Oxaliplatin, a derivative of Cisplatin, to have anti-cancer properties in a Lewis Lung Carcinoma (LLC). It can induce immunogenic cell death (ICD) in tumor cells and co-administrate with checkpoint inhibitors to improve the therapeutic efficacy. This study aims to investigate Oxaliplatin's effect on a different gastric carcinoma, SGC-7901, and analyze the therapeutic efficacy of its co-administration with the checkpoint inhibitor. To evaluate the immunogenic cell death (ICD) induced by Oxaliplatin in SGC-7901, flow cytometry, HMGB1, ATP release, and immunoblotting were conducted. The effectiveness of Oxaliplatin was analyzed using a vaccination approach and subcutaneous tumor models to observe tumour regression. PD-L1 mRNA and protein levels in SGC-7901 were also examined. The therapeutic efficacy of Oxaliplatin in murine lung tumor models will be enhanced by co-administering with aPD-L1. Cisplatin will be employed as a positive control, while PBS will be a negative control. The result of the study will provide important insight into the experimental effectiveness of Oxaliplatin in SGC-7901, it also sets the basis for future experimental, preclinical, and clinical studies of the drug. Future studies should focus on practicing the actual experiment on the experimental effectiveness of Oxaliplatin in SGC-7901 and look for the applicability of the effectiveness of Oxaliplatin on other tumor cells.

Keywords: SGC-7901, Oxaliplatin, PD-L1, aPD-L1, checkpoint (inhibitor)

1. Introduction

Cancers have been the second lethality disease for decades, and the medical community has no definitive means to treat cancers. One of its main branches, known as gastric, is a devastating common cancer with high-mortality rate and high-morbidity rate in the recent 20 years. It is the fifth most frequent cancer and the third leading cause of cancer death in the world [1]. A 2000 estimation shows that there were about 10.4% of cancer deaths were due to gastric cancer [2]. Despite advances in diagnostic and therapeutic technologies, gastric cancer remains a significant public health challenge in the biological and medical community due to its high incidence, aggressive nature, and poor prognosis. Oxaliplatin is a new third-generation platinum complex that was used in the clinic to treat a variety of cancers, for instance: colon, esophagogastric, gastric ovarian, and pancreatic cancers. Oxaliplatin has been shown to have a great outcome when combine with 5-fluorouracil (5-FU). Unlike commonly known platinum such as Cisplatin, Oxaliplatin is a rare platinum-containing medicine with very few side effects. The safety profile of oxaliplatin contributes to its ideal candidate for combination therapy.

Immunogenic cell death (ICD) is a form of cell death that can combine with chemotherapy to stimulate a process called "immune response" by the obligatory activation of dendritic cells that provide antigens from dying tumor cells and activation of T lymphocyte cells. ICD can be marked by the release of damage-associated molecular patterns (DAMPs), a significant increase in the immunogenicity of dying cancer cells. Several important DAMPs, including box 1 (HMGB1) and ATP, are crucial in analyzing ICD [3-5]. Oxaliplatin has been shown to induce ICD in cancer cells. The immune systems are essential in controlling and eradicating cancer. Checkpoint, however, is a mechanism of immune suppression that can stop the immune system from successful antitumor immunity [6]. It is really important to use a checkpoint inhibitor to enhance the effect of chemotherapy. To be specific, scientists now developed anti-PD-1, a checkpoint inhibitor, to target the overexpression of a surface protein used to prevent the attack of other cells, known as PD-1, a surface protein used to prevent the attack of other cells [3]. In the previous study, had reported that Oxaliplatin can induce ICD in Lewis lung cancer and activate DCs as well as T cells, leading to an immune response and tumor regression in murine lung carcinoma model; Furthermore, the combination of Oxaliplatin and checkpoint inhibitor, aPD-L1, could greatly enhance the therapeutic efficacy of LLC in the same model [3]. In this study, the same therapeutic effect of Oxaliplatin in a very similar murine gastric carcinoma cell line (SGC-7901) was analyzed and the effect of the combination of Oxaliplatin and aPD-L1

(the checkpoint inhibitor for PD-L1) were predicted. It is predicted that the similar effect will be observed in SGC-7901 and the use of Oxaliplatin will enhance the therapeutic effect of the checkpoint inhibitor.

1.1 Research question

Since Oxaliplatin and checkpoint inhibitor have been found to contribute to the inhibitory effect of ICDs on grafted LLC in nude mice (and are more effective when used together than when used alone). Can the combination of Oxaliplatin and checkpoint inhibitor induce the same ICD inhibitory effect in subcutaneously (and orthotopically) SGC-7901 gastric cancer in nude mice?

1.2 Hypothesis

Since Oxaliplatin can induce an immune response to the xenografted Lewis Lung Carcinoma (LLC) by inducing ICDs, and also enhances anti-checkpoint activity. I predict that increasing concentrations and treatment durations with both oxaliplatin and anti-pdL1 will shrink tumor in SGC7901 xenograft similarly to LLC xenograft and both decrease ICD markers. Cisplatin is positive control and PBS is negative control. Measure ICD markers and tumor size with the two types of tumor xenografts, also remove treated tumors and do FACS for annexin V/PI.

2. Materials and methods

2.1 Drug treatments

Cisplatin (range from 3 mg/kg to 10 mg/k) can be obtained commercially from the pharmacy as the positive control drug treatment. Oxaliplatin can be obtained commercially from the pharmacy. PBS(0.9%)can be obtained commercially as the negative control drug treatment. The drug concentration will be ranged from 3 mg/kg to 10 mg/kg for different mice (3,4 5 6 7 8 9 10 mg/kg for 10 mice in a group. The therapeutic effect will be detected at different drug duration time, from 1 day to 30 days.

2.2 Cancer cell lines

Human gastric carcinoma cell lines, SGC-7901, will be used to analyze the effect of Oxaliplatin. The cancer cell should be cultured in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum, 100 μg/ml streptomycin, and 100 unit/ml penicillin in a humidified cell incubator at 37°C in an atmosphere of 5% CO2 [7,8].

2.3 Nude mice

C57BL/6 female mice will be obtained commercially.

2.4 Immunoblotting analyses

The immunoblotting experiments can be performed using specific antibodies against the PD-L1 primary antibody (R&D systems, AO1019, 1:2000 dilution, the internal control b-actin antibody [3].

2.5 Immunocytochemistry

The tumor sample will be harvested and fixed in phosphatebuffered 4% paraformaldehyde, then embedded by paraffin. The 6-lm thickness SGC-7901 tumor sections will be mounted on glass slides and performed IHC following the standardized protocols. The PD-L1 primary antibody will be ordered from R&D systems. The sections can be studied under the digital microscope [3].

2.6 Flow cytometry analysis

The procedure for preparing the SGC-7901 tumor tissues involves cutting them into small pieces, which are then subjected to digestion with collagenase type I (0.5 mg/mL, Sigma, Burlington, VT, USA) for 1 hour at 37°C. After digestion, the tissues are filtered using a 60-μm cell strainer to obtain single cells. The subsequent flow-cytometry analysis for the SGC-7901 tumor cells follows the same procedures as mentioned earlier. Specific antibody sources are utilized for the flow-cytometry analysis of the SGC-7901 tumors: CD80-APC (Thermo Fisher Scientific, 17–0801-82, 1:100), CD86- PE-Cyanine7 (Thermo Fisher Scientific, 25–0862-82, 1:100), CD8-PE (Novus Biologicals, NBP2-34588PE, 1:100), CD4-FITC (Thermo Fisher Scientific, 11–0042-82, 1:100), CD86-Alexa Fluor647 (Novus Biologicals, FAB141R, 1:200), CD80- Alexa Fluor488 (Novus Biologicals, NBP2-25255AF488, 1:200), CD11b-PE (BioLegend, #553311, 1:100), CD11c-V450 (BioLegend, #560521, 1:100), CD45-V450 (BioLegend, #560501, 1:100).

2.7 High mobility group box 1 (HMGB1) release assay

The Western blot method was utilized to quantify HMGB1 proteins that were released from cells and present in the supernatant. To ensure accuracy, BSA was utilized as an internal control. Specific antibodies directed against the S-HMGB1 primary antibody (Abcam, ab77302, dilution of 1:2000) and the internal control BSA (Thermo Fisher Scientific, A11133, dilution of 1:2000) were used in the immunoblotting experiments [3].

2.8 ATP release assay

To detect the presence of released ATP in SGC-7901, an ATP Determination Kit was utilized following the manufacturer's instructions. Briefly, both control and drug-treated samples were added to a reaction solution that contained 0.5-mM D-luciferin, 1-μM dithiothreitol, 1.25 μg/mL firefly luciferase, 25-mM Tricine buffer (pH 7.8), 5-mM MgSO4, and 100-mM EDTA. The luminescence was measured according to the manufacturer's protocol,

and the concentration of released ATP was determined using a standard curve generated from ATP standard solutions [3].

2.9 Measurement of intracellular NAD+ levels and released ATP levels

To measure intracellular NAD+ levels, the EnzyChrom NAD+/NADH assay kit from BioAssay Systems was used. Cell pellets were homogenized with 100 µl of NAD extraction buffer and then heated at 60°C for 5 minutes. To neutralize the extracts, 20 µl of assay buffer and 100 µl of NADH extraction buffer were added. After centrifugation, 40 µl of supernatant from each sample was mixed with 80 µl of the working reagent, and the optical density was measured at 570 nm at time zero (OD0) and after a 15-minute incubation (OD15). The DOD for each standard and sample was calculated by subtracting OD0 from OD15, and the NAD+ concentration of the sample was determined by plotting the standard curves.

To quantify ATP levels, the ATP determination kit from Beyotime was used with the luciferin/luciferase method. After centrifuging cell lysates, 10 µl of supernatant was mixed with 100 µl of ATP detection solution. Relative light units (RLU) were measured using a GloMax-Multi Jr Luminometer from Promega [9,10].

2.10 Immunotherapy

To establish the timeline for mouse immunotherapy, the surface exposure of CRT was evaluated by flow cytometry after treating SGC-7901 cells with Cisplatin (100 µM) or Oxaliplatin (50 µM) for 24 hours. After this, 2*10^6 dying SGC-7901 cells were injected subcutaneously into the left flank of the mice, followed by the injection of 2*10^6 living SGC-7901 cells into the contralateral/right side seven days later. Tumor growth was measured every 3 days using a digital caliper. On day 27, treated mice were euthanized, and their tumor tissues were harvested for subsequent analysis [3].

3. Statistical Analysis

All reported results are representative of three independent experiments, and the data were expressed as the mean ± standard deviation (SD) [11]. Differences between groups were analyzed using one- or two-way analysis of variance (ANOVA). Statistical significance thresholds were set at $*p < 0.05$ [3].

Table 1. Possible combination of results

	Observations				
Possible Results		Oxaliplatin and aPDL1 reduce SGC7901 tumor size identically to LLC	Oxaliplatin and aPDL1 reduce SGC7901 HMGB1 by western identically to LLC	Oxaliplatin and aPDL1 reduce SGC7901 released ATP by ATP determination Kit identically to LLC	Support hypothesis
	1	+	+	+	YES
	2	+	+	-	P
	3	+	-	+	P
	4	+	-	-	P
	5	-	+	+	P
	6	-	+	-	P
	7	-	-	+	P
	8	-	-	-	NO

Note: A "+" indicated a positive result where Oxaliplatin treatment of SGC-7901 performs similarly to that of LLC and Cisplatin. A "-" indicates a negative result where Oxaliplatin treatment fails to perform similarly to Oxaliplatin treatment of SGC-7901 and instead performs similarly to PBS treatment. A "P" indicates partial support where Oxaliplatin and aPDL-1either fail to reduce SGC-7901tumor size, reduce SGC-7901 HMGB1, or reduce SGC-7901 released ATP.

3.1 Possible Results

PR1 Oxaliplatin and aPDL1 reduce SGC-7901 tumor size, HMGB1, and released ATP identically to that of LLC.

PR2 Oxaliplatin and aPDL1 reduce SGC-7901 tumor size and HMGB1 but fail to reduce its released ATP identically to that of LLC.

PR3 Oxaliplatin and aPDL1 reduce SGC-7901 tumor size and released ATP, but fail to reduce HMGB1 identically to that of LLC.

PR4 Oxaliplatin and aPDL1 reduce SGC-7901 tumor size but fail to reduce HMGB1 and released ATP identically to that of LLC.
PR5 Oxaliplatin and aPDL1 fail to reduce SGC-7901 tumor size but successfully reduce its HMGB1 and released ATP identically to that of LLC.
PR6 Oxaliplatin and aPDL1 fail to reduce SGC-7901 tumor size and its released ATP but successfully reduce its HMGB1.
PR7 Oxaliplatin and aPDL1 fail to reduce SGC-7901 tumor size and its HMGB1 but successfully reduce its released ATP
PR8 Oxaliplatin and aPDL1 fail to reduce SGC07901 tumor size, its HMGB1 and it released ATP.

3.2 Possible results for increasing treatment concentration and duration

PR1-1 Increasing either treatment concentration or treatment duration can shrink the SGC-7901 tumor size, reduce HMGB1 and released ATP.
PR1-2 Increasing either treatment concentration or treatment duration cannot shrink the SGC-7901 tumor size, reduce HMGB1 and released ATP.
PR1-3 Increasing either treatment concentration or treatment duration can shrink the SGC-7901 tumor size, reduce HMGB1 but fail to reduce released ATP.
PR1-4 Increasing either treatment concentration or treatment duration can shrinl the SGC-7901 tumor size and released ATP but fail to reduce HMGB1.
PR1-5 Increasing either treatment concentration or treatment duration can reduce the tumor size but fail to reduce HMGB1 and released ATP.
PR1-6 Increasing either treatment concentration or treatment duration cannot reduce the tumor size but can reduce HMGB1 and released ATP.
PR1-7 Increasing either treatment concentration or treatment duration cannot reduce the tumor size and released ATP but can reduce HMGB1.
PR1-8 Increasing either treatment concentration or treatment duration cannot reduce tumor size and HMGB1 but can reduce released ATP.
PR2-1 Increasing either treatment concentration or treatment duration can reduce tumor size and HMGB1.
PR2-2 Increasing either treatment concentration or treatment duration can reduce tumor size but fail to reduce HMGB1.
PR2-3 Increasing either treatment concentration or treatment duration cannot reduce tumor size but can to reduce HMGB1.
PR2-4 Increasing either treatment concentration or treatment duration cannot reduce tumor size and HMGB1
PR3-1 Increasing either treatment concentration or treatment duration can reduce tumor size and released ATP.
PR3-2 Increasing either treatment concentration or treatment duration can reduce tumor size but fail to reduce released ATP.
PR3-3 Increasing either treatment concentration or treatment duration cannot reduce tumor size but can to reduce released ATP.
PR3-4 Increasing either treatment concentration or treatment duration cannot reduce tumor size and released ATP.
PR4-1 Increasing either treatment concentration or treatment duration can reduce tumor size
PR4-2 Increasing either treatment concentration or treatment duration cannot reduce tumor size
PR5-1 Increasing either treatment concentration or treatment duration can reduce HMGB1 and released ATP.
PR5-2 Increasing either treatment concentration or treatment duration can reduce HMGB1 but fail to reduce released ATP.
PR5-3 Increasing either treatment concentration or treatment duration cannot reduce HMGB1 but can to reduce released ATP.
PR5-4 Increasing either treatment concentration or treatment duration cannot reduce HMGB1 and released ATP.
PR6-1 Increasing either treatment concentration or treatment duration can reduce HMGB1
PR6-2 Increasing either treatment concentration or treatment duration cannot reduce HMGB1
PR7-1 Increasing either treatment concentration or treatment duration can reduce released ATP
PR7-2 Increasing either treatment concentration or treatment duration cannot reduce released ATP

4. Discussion

PR1 and PR1-1-PR1-8. PR1 fully supports the hypothesis. Oxaliplatin and aPDL1 reduce SGC-7901 tumor size, HMGB1, and released ATP identically to that of LLC, meaning that Oxaliplatin and aPDL1induces cell death in SGC-7901 and improves anti-SGC-7901 immunity in tumor model and lower the cell activity. This shows that Oxaliplatin and aPDL1 can successfully induce tumor cell death and inhibit cancer cell growth, induce ICD, and lower the cancer cell respiration rate. Further measurements of intracellular NAD^+ levels, released ATP level and tumor cell size are required. In addition to this, increasing either treatment concentration or treatment duration may or may not play a role in therapeutic efficacy, given that Oxaliplatin and aPDL1 can reduce SGC-7901 tumor size, HMGB1, and released ATP

identically to that of LLC. Further measurements of intracellular NAD^+ levels, released ATP level and tumor cell size of different treatment concentration and treatment duration are required.

PR2 and PR2-1-PR2-4. PR2 partially supports the hypothesis. Oxaliplatin and aPDL1 reduce SGC-7901 tumor size and HMGB1 but fail to reduce its released ATP identically to that of LLC, meaning that Oxaliplatin and aPDL1 induces cell death in SGC-7901 and improve anti-SGC-7901 immunity in tumor model but fail to reduce the activity of the cancer cell. This shows that Oxaliplatin and aPDL1 can successfully induce tumor cell death and inhibit cancer cell growth, induce ICD, but fail to lower the cancer cell respiration rate. Further measurements of intracellular NAD^+ levels, released ATP level and tumor cell size are required. In addition to this, increasing either treatment concentration or treatment duration may or may not play a role in therapeutic efficacy, given that Oxaliplatin and aPDL1 reduce SGC-7901 tumor size and HMGB1 but fail to reduce its released ATP identically to that of LLC. Further measurements of intracellular NAD^+ levels, released ATP level and tumor cell size of different treatment concentration and treatment duration are required.

PR3 and PR3-1-PR3-4 partially supports the hypothesis. Oxaliplatin and aPDL1 reduce SGC-7901 tumor size and released ATP, but fail to reduce HMGB1 identically to that of LLC, meaning that Oxaliplatin and aPDL1 induce cell death in SGC-7901 and lower the activity of the cancer cell but fail to improve anti-SGC-7901 immunity. This shows that Oxaliplatin and aPDL1 can successfully induce tumor cell death and inhibit cancer cell growth, and lower the cancer cell respiration rate, but fail to induce ICD. Further measurements of intracellular NAD^+ levels, released ATP level and tumor cell size are required. In addition to this, increasing either treatment concentration or treatment duration may or may not play a role in therapeutic efficacy, given that Oxaliplatin and aPDL1 reduce SGC-7901 tumor size and released ATP, but fail to reduce HMGB1 identically to that of LLC. Further measurements of intracellular NAD^+ levels, released ATP level and tumor cell size of different treatment concentration and treatment duration are required.

PR4 and PR4-1-PR-4-4 partially supports the hypothesis. Oxaliplatin and aPDL1 reduce SGC-7901 tumor size but fail to reduce HMGB1 and released ATP identically to that of LLC, meaning that Oxaliplatin and aPDL1 can induce ICD but fail to improve anti-SGC-7901 immunity and lower its activity. This shows that Oxaliplatin and aPDL1 can successfully induce tumor cell death and inhibit cancer cell growth, but fail to lower the cancer cell respiration rate and fail to induce ICD. Further measurements of intracellular NAD^+ levels, released ATP level and tumor cell size are required. In addition to this, increasing either treatment concentration or treatment duration may or may not play a role in therapeutic efficacy, given that Oxaliplatin and aPDL1 reduce SGC-7901 tumor size but fail to reduce HMGB1 and released ATP identically to that of LLC. Further measurements of intracellular NAD^+ levels, released ATP level and tumor cell size of different treatment concentration and treatment duration are required.

PR5 and PR5-1-PR5-4 partially supports the hypothesis. Oxaliplatin and aPDL1 fail to reduce SGC-7901 tumor size but successfully reduce its HMGB1 and released ATP identically to that of LLC, showing that Oxaliplatin and aPDL1 fail to induce ICD but have improvement in SGC-7901 immunity and lower the cancer cell activity. This shows that Oxaliplatin and aPDL1 cannot induce tumor cell death and inhibit cancer cell growth, but can lower the cancer cell respiration rate and induce ICD. Further measurements of intracellular NAD+ levels, released ATP level and tumor cell size are required. In addition to this, increasing either treatment concentration or treatment duration may or may not play a role in therapeutic efficacy, given that Oxaliplatin and aPDL1 fail to reduce SGC-7901 tumor size but successfully reduce its HMGB1 and released ATP identically to that of LLC. Further measurements of intracellular NAD^+ levels, released ATP level and tumor cell size of different treatment concentration and treatment duration are required.

PR6 and PR6-1-PR6-2 partially supports the hypothesis. Oxaliplatin and aPDL1 fail to reduce SGC-7901 tumor size and its released ATP but successfully reduce its HMGB1, meaning that Oxaliplatin and aPDL1 cannot induce ICD or lower the cell but can improve the SGC-7901 immunity. This shows that Oxaliplatin and aPDL1 cannot induce tumor cell death and inhibit cancer cell growth, or lower the cancer cell respiration rate but can induce ICD. Further measurements of intracellular NAD^+ levels, released ATP level and tumor cell size are required. In addition to this, increasing either treatment concentration or treatment duration may or may not play a role in therapeutic efficacy, given that Oxaliplatin and aPDL1 fail to reduce SGC-7901 tumor size and its released ATP but successfully reduce its HMGB1. Further measurements of intracellular NAD^+ levels, released ATP level and tumor cell size of different treatment concentration and treatment duration are required.

PR7 and PR7-1-PR7-2 partially supports the hypothesis.

Oxaliplatin and aPDL1 fail to reduce SGC-7901 tumor size and its HMGB1 but successfully reduce its released ATP, meaning that Oxaliplatin and aPDL1 cannot induce ICD or improve the SGC-7901 immunity but can lower the cell activity. This shows that Oxaliplatin and aPDL1 fail to induce tumor cell death and inhibit cancer cell growth, induce ICD, but can lower the cancer cell respiration rate. Further measurements of intracellular NAD+ levels, released ATP level and tumor cell size are required. In addition to this, increasing either treatment concentration or treatment duration may or may not play a role in therapeutic efficacy, given that Oxaliplatin and aPDL1 fail to reduce SGC-7901 tumor size and its HMGB1 but successfully reduce its released ATP.

PR8 fully rejects the hypothesis. Oxaliplatin and aPDL1 fail to reduce SGC-7901 tumor size and fail to lower released HMGB1 and ATP, meaning that they fail to induce ICD, improve the SGC-7901 immunity and activity. This shows that Oxaliplatin and aPDL1 cannot induce tumor cell death and inhibit cancer cell growth, induce ICD, and lower the cancer cell respiration rate. Further measurements of intracellular NAD+ levels, released ATP level and tumor cell size are required.

5. Conclusion

In conclusion, this paper analyzes the potential of Oxaliplatin's treatment on SGC-7901 gastric cancer. The results of the study would indicate whether Oxaliplatin can potentially be used as a treatment for SGC-7901 gastric cancer to reduce the metastatic behavior by inducing ICD and co-work with the checkpoint inhibitor. Notably, Oxaliplatin-induced ICD provides an immunogenic microenvironment, which enhances the therapeutic efficacy of the checkpoint inhibiton. The combination of Oxaliplatin and aPD-L1 provides a potential therapeutic strategy of gastric cancer treatment.

References

[1] Siegel, Rebecca L., Kimberly D. Miller, and Ahmedin Jemal. "Cancer statistics, 2019." CA: a cancer journal for clinicians 69.1 (2019): 7-34.

[2] Parkin, D. Maxwell. "Global cancer statistics in the year 2000." The lancet oncology 2.9 (2001): 533-543.

[3] Sun, Fengfei, et al. "Oxaliplatin induces immunogenic cells death and enhances therapeutic efficacy of checkpoint inhibitor in a model of murine lung carcinoma." journal of Receptors and Signal transduction 39.3 (2019): 208-214.

[4] Kroemer, Guido, et al. "Immunogenic cell death in cancer therapy." Annual review of immunology 31 (2013): 51-72.

[5] Garg, Abhishek D., et al. "Immunogenic cell death." International Journal of Developmental Biology 59.1-2-3 (2015): 131-140.

[6] Postow, Michael A., Margaret K. Callahan, and Jedd D. Wolchok. "Immune checkpoint blockade in cancer therapy." Journal of clinical oncology 33.17 (2015): 1974.

[7] Misset, J. L. "Oxaliplatin in practice." British journal of cancer 77.4 (1998): 4-7.

[8] Wang, Junqing, et al. "Suppressive effects on cell proliferation and motility in gastric cancer SGC-7901 cells by introducing ulinastatin in vitro." Anti-Cancer Drugs 27.7 (2016): 651-659.

[9] Wu, Kun, Lin-Hong Yuan, and Wei Xia. "Inhibitory effects of apigenin on the growth of gastric carcinoma SGC-7901 cells." World Journal of Gastroenterology: WJG 11.29 (2005): 4461.

[10] Wu, Ping, et al. "Oxaliplatin triggers necrosis as well as apoptosis in gastric cancer SGC-7901 cells." Biochemical and biophysical research communications 460.2 (2015): 183-190.

[11] Li, Qi, et al. "P38 signal transduction pathway has more cofactors on apoptosis of SGC-7901 gastric cancer cells induced by combination of rutin and oxaliplatin." BioMed Research International 2019 (2019).