

Inhibitory Effects of Circulating Natural Autoantibodies to CD47-derived Peptides on Oral Squamous Cell Carcinoma Cells

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Research

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Abstract

Natural autoantibodies serve as an important anti-tumorigenic system due to its immune surveillance function. The present study aims to investigate whether circulating natural IgG autoantibodies against cluster of differentiation 47 (CD47) could suppress the proliferation of oral squamous cell carcinoma (OSCC) cells. Three OSCC cell lines were used for this study. The expression of fourteen tumor related genes including CD47 were tested. OSCC cells were grown, respectively, with 20% human plasma positive and negative for anti-CD47 IgG. Cell proliferation, apoptosis and invasion/metastasis were examined. The results showed that CD47 presented highest expression among all 14 genes detected in OSCC cells. Plasma anti-CD47 IgG significantly inhibited the viability of all three OSCC cell lines. The proportions of apoptotic cells were remarkably higher in OSCC cells treated with anti-CD47 IgG-positive plasma than those treated with IgG-negative plasma. Furthermore, the cell invasion/metastasis was attenuated evidently with the attribution of plasma anti-CD47 IgG. In conclusion, natural autoantibodies against CD47 may be a potential target for OSCC immunotherapy.

1. Introduction

Oral squamous cell carcinoma (OSCC) is one of the most common cancers counting for more than 90% of all histological subtypes of oral cancer [1, 2]. About 300,000 new cases are estimated to be diagnosed annually worldwide, and locoregional recurrences may occur in 30-35% of patients, 20% of whom die of OSCC eventually [3, 4]. The high recurrence and metastasis rate of OSCC lead to the 5-year survival rate of less than 50% [5, 6]. In recent years, treatment options, particularly for advanced (III and IV) malignancies, have been applied including surgery, radiation and/or chemotherapy as well as targeted therapy. [1, 7]. Despite huge advances in diagnosis and treatment, the toxic side effects and drug resistance remain the main causes of high mortality and poor mortality in OSCC [1, 3]. Therefore, there is an urgent need for new actionable molecular targets or new therapeutic agents with better efficacy to prevent the recurrence and metastasis of OSCC.

Cluster of differentiation 47 (CD47), also called integrin-related signal transducer or protein (IAST or IAP), is composed of an extracellular N-terminal V-set immunoglobulin superfamily (IgSF) domain attached to five-time transmembrane domains and an intracellular C-terminus [8]. Being the principal ligand, CD47 expresses in several tumors and binds to the cytoplasmic tail of signal regulatory protein alpha (SIRP α) that is a surface glycoprotein and an immunoreceptor expressed on all myeloid cells. CD47-SIRP α interaction triggers a cascade of events and elicits a "do not eat me" signal to suppress macrophage phagocytosis, allowing cancer cells to escape the immune surveillance. Several lines of evidence have recently shown that overexpression of CD47 is associated with the pathogenesis of leukemia, non-Hodgkin's lymphoma (NHL), glioblastoma, bladder and breast cancer [9, 10]. Therefore, blocking CD47-SIRP α checkpoint has been applied for immunotherapy. Inhibition of CD47 binding to SIRP α activates both innate and adaptive immunity by promoting presentation of tumor antigens to CD8 T lymphocytes, resulting in cancer cell destruction by macrophage and anti-tumor cytotoxicity due to antigen-presentation by dendritic cells, respectively [11, 12]. There are several CD47 antagonists have shown

strong efficacy against solid and hematological tumors undergoing clinical trials, such as Hu5F9 and TTI-621 [9, 12]. Hu5F9 is a humanized monoclonal antibody binding to CD47 with high affinity and has been proposed to activate a pro-phagocytic signal pathway to eliminate the tumor cells [13]. TTI-621 is a recombinant fusion protein composed of human SIRPα N-terminal domain fused to the Fc receptor of IgG, reinforcing phagocytosis and antitumor activity by engagement of macrophage Fcγ receptors with IgG1 Fc receptor [13, 14]. However, the safety of these immune pharmaceuticals remains a key issue.

Natural autoantibodies are defined as immunoglobulins produced by B1 lymphocyte cells without the stimulation of exogenous antigens [15]. They are present in normal individuals and physiologically maintain the tissue homeostasis such as elimination of invading pathogenic agents, clearance of apoptotic cells and destruction of cancer cells [16, 17]. Recent studies suggested that the levels of natural anti-forkhead box P3 (FOXP3) IgG autoantibodies are significantly higher in patients with esophageal cancer [18]; in addition, anti-CD25 and anti-baculoviral IAP repeat-containing protein 5 (BIRC5) IgG levels are elevated obviously in patients with non-small cell lung cancer (NSCLC), who retained a better prognosis and longer overall survival [15, 19]. Our previous study showed that anti-vascular endothelial growth factor receptor 1 (VEGFR1) IgG positive plasma could significantly inhibit the proliferation of hepatocellular carcinoma (HCC) cells by induction of apoptosis and the progression-free survival of HCC patients received infusion with anti-VEGFR1 IgG positive plasma was prolonged [20]. However, studies of natural autoantibodies in OSCC cells are rarely documented. The present study was thus undertaken to analyze the inhibitory effects of anti-CD47 IgG-positive plasma on OSCC cell lines.

2. Materials And Methods

2.1. Cell culture. The human OSCC cell lines, CAL27, SCC9 and SCC25 (American Type Culture Collection, ATCC, Manassas, USA), were maintained at 37 °C in a humidified atmosphere of 5% CO₂ in Dulbecco's modified Eagle's medium (DMEM) (Hyclone, USA) for the first two cell lines and Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM/F-12) (Gibco, USA) for the last cell line and supplemented both with 1% (v/v) penicillin/streptomycin and 10% (v/v) fetal bovine serum (FBS, Gibco, USA) for growth.

2.2. Gene expression assay. To identify a target gene of interest for subsequent experiments, we used qRT-PCR technology to screen the expression of 14 target genes in OSCC cells. These tumor-targeted genes were obtained by analysis of gene expression profiling database through Oncomine™ Platform (<http://www.oncomine.org/resource/login.html>), including ABCA1, ABCA5, ABCB6, ABCC3, ABCC4, ABCC5, ABCC10, CD20, CD22, CD47, ERBB2, PIGR, GPNMB and NECTIN4 (Table 1).

RNA was isolated from OSCC cells lysates using the Trizol method (Invitrogen) after cells were incubated in a humidified atmosphere with 5% CO₂ at 37°C, and total RNA samples were quantified with the Nanodrop machine (Agilent Technologies, Santa Clara, CA). Next, the cDNA was generated with SuperRT cDNA Synthesis Kit (CWbiotech, Beijing, China), and UltraSYBR One Step qRT-PCR Kit (Low ROX, CWbiotech, Beijing, China) was then used to quantify the expression of a target gene. Glyceraldehyde-3-

phosphate dehydrogenase (GAPDH) was used as a housekeeping gene for normalization as previously described [21], and gene expression was determined by the $2^{-\Delta\Delta CT}$ method. qRT-PCR primer sequences are listed in Table 1.

2.3. Detection of plasma IgG levels. According to the results of qRT-PCR analysis, a linear peptide antigen of human CD47 was designed using a computational epitope prediction software (<http://www.iedb.org>) based on the features of the target proteins such as hydrophilicity, flexibility, surface accessibility and antigenicity [19]; plasma IgG against the extracellular domain of the CD47 protein was detected with in-house ELISA assay [22].

Human plasma samples were collected from healthy blood donors by the Blood Center of Dongguan, Guangdong Province, China. Pooled plasma from more than 100 randomly selected individuals was used as a quality control (QC) for relative quantification of plasma anti-CD47 IgG levels. This work was approved by the local Ethics Committee based in Dongguan and conformed to the requirements of the Declaration of Helsinki. Briefly, PBS containing 0.5% bovine serum albumin (BSA) was prepared as an analysis buffer and added to each negative control (NC) well, and a positive control (PC) sample was added to each PC well. The detection was performed according to the instructions of the ELISA kit, which was provided by Hailanshen Biotechnology Ltd, Qingdao, China. Optical density (OD) measurement was done on a microplate reader at 450 nm within 10 minutes at a reference wavelength of 620 nm and the specific binding ratio (SBR) was used to represent plasma anti-CD47 IgG levels. Plasma with the highest SBR value from two healthy donors was mixed as anti-CD47 IgG-positive plasma; anti-CD47 IgG negative plasma was taken from two healthy donors with the lowest SBR value and mixed properly as described in our previous study [15, 18, 21, 23, 24]. SBR calculation is as follows: $SBR = (OD_{\text{sample}} - OD_{\text{NC}}) / (OD_{\text{PC}} - OD_{\text{NC}})$.

2.4. Cell proliferation assay. 96-well plates were used to seed three OSCC cell lines in 100 μl /well with a density of 3×10^4 cells/ml, 5×10^4 cells/ml and 5×10^4 cells/ml, respectively, and then cultured in complete medium for 24 h under the same conditions as mentioned above; the medium containing 20% human plasma either negative or positive for anti-CD47 IgG antibodies was then used to culture OSCC cells for 48 h under the same conditions as indicated above [21]. Cell counting kit-8 (CCK-8) (Vazyme, Nanjing, China) was used to detect cell viability. Briefly, 10 μl CCK-8 solution was mixed with complete medium at a ratio of 1:10 and then added to each well. After incubation at 37°C for 2 h, the optical density (OD) was measured at a wavelength of 450 nm. Cell viability measurements were used to present data and calculated as follows: $\text{Cell viability} = (OD_{\text{positive}} - OD_{\text{blank}}) / (OD_{\text{negative}} - OD_{\text{blank}})$.

2.5. Analysis of apoptosis. 6-well dishes were used to seed three OSCC cell lines seeded in 2 ml/dish with a density of 2×10^5 cells/ml, 2.5×10^5 cells/ml and 3×10^5 cells/ml, respectively, for initial culture for 24 h as mentioned above. Cells were then treated with the medium containing 20% human plasma either positive or negative for anti-CD47 IgG and harvested at 48 h; the apoptosis rate was evaluated using the Annexin V-FITC/PI Apoptosis Detection kit (BD Biosciences, Franklin Lakes, NJ, USA) according to the instructions from the manufacturer. In brief, 5 μl Annexin V-FITC and 5 μl PI were added to the buffer and

incubated at room temperature for 15 min in the dark. Cells were analyzed by flow cytometry (BD FACSCanto) within 1 h. Annexin V-FITC +/PI- staining and Annexin V-FITC +/PI+ staining were used to define early apoptosis and late apoptosis.

2.6. Transwell assay. Cell invasion assays were performed in Transwell chambers (24-well format; 8 mm pore size; Corning, NY, USA) coated with Matrigel (1 mg/ml; Corning Incorporated, Corning, NY, USA) in triplicate. After treatment with the medium containing 20% human plasma either positive or negative for anti-CD47 IgG for 48 h, OSCC cells (5×10^5 cell/well) were planted into the upper chamber and 1000 μ l complete culture was added to the lower chamber, and then incubated in a humidified atmosphere with 5% CO₂ at 37°C for additional 48 h. After that, polyoxymethylene and Giemsa (Salarbio, Beijing, China) were used for the fixation and staining of invading cells on the underside of the membrane. Five visual fields were randomly selected and the average number of invading cells was calculated with an inverted microscope (Olympus Corporation, Tokyo, Japan, $\times 200$).

2.7. Statistical analysis. All experimental data were expressed as mean \pm standard deviation (SD). Student's t-test (two-tailed) and one-way analysis of variation were applied to examine the differences in cell viability, percentage of apoptotic cells and cell invasion/metastasis between OSCC cells treated with anti-CD47 IgG positive and negative plasma as well as in gene expression. $P < 0.05$ (*) and $P < 0.01$ (**) were considered to be statistically significant. All experiments were repeated at least three times.

3. Results

3.1. Expression of the target genes in OSCC cell lines. All 14 genes studied were expressed in three OSCC cell lines, in which compared with the expression of the ABCA1 gene in each cell line, the top three levels of gene expression detected in CAL27 cells were CD47 ($P=2.62 \times 10^{-8}$), ABCA5 ($P=3.13 \times 10^{-6}$) and ABCC4 ($P=1.47 \times 10^{-6}$) (Figure 1(a)); the top three levels of gene expression detected in SCC9 cells were CD47 ($P=3.35 \times 10^{-6}$), ABCA5 ($P=1.24 \times 10^{-5}$) and ABCC10 ($P=1.24 \times 10^{-7}$) (Figure 1(b)); the top three levels of gene expression detected in SCC25 cells were CD47 ($P=3.92 \times 10^{-8}$), ABCC3 ($P=3.41 \times 10^{-5}$) and ABCC5 ($P=0.00028$) (Figure 1(c)). Nevertheless, CD47 gene expression showed the highest level concertedly in all three OSCC cell lines. It is notable that CD47 expression was 1800-fold higher than ABCA1 in CAL27 cells. Based on the high expression of CD47 in three OSCC cells, we subsequently detected natural IgG autoantibodies against CD47 in human plasma and then analyzed the inhibitory effects of anti-CD47 IgG-positive plasma on OSCC cells.

3.2. Inhibitory effects of anti-CD47 IgG plasma on proliferation of OSCC cells. In comparison with anti-CD47 IgG-negative plasma, anti-CD47 IgG-positive plasma could significantly inhibit the proliferation of CAL27 ($P=0.0067$) (Figure 2(a)), SCC9 ($P=3.42 \times 10^{-13}$) (Figure 2(b)) and SCC25 ($P=1.87 \times 10^{-6}$) cells (Figure 2(c)). The cell viabilities of CAL27 and SCC25 cells were decreased by more than 25%. Notably, SCC9 cell proliferation was reduced almost by 50%.

3.3. Cell apoptosis induced by anti-CD47 IgG plasma. Because of the inhibitory effects of anti-CD47 IgG-positive plasma on the proliferation of OSCC cells, we investigated their apoptosis induced by anti-CD47 IgG-positive plasma in OSCC cells. The proportion of apoptotic cells was significantly higher in CAL27, SCC9 and SCC25 cells treated with anti-CD47 IgG-positive plasma than those treated with anti-CD47 IgG-negative plasma ($P=0.00027$ for CAL27 cells, $P=0.028$ for SCC9 cells, and $P=0.0017$ for SCC25 cells) (Figure 3). In comparison with SCC9, the apoptosis of CAL27 and SCC25 cells was induced more remarkably by anti-CD47 IgG-positive plasma.

3.4. Suppressive effects of anti-CD47 IgG plasma on cell invasion of OSCC cells. Transwell assay showed that the invasive number of CAL27, SCC9 and SCC25 cells was reduced significantly in OSCC cells treated with anti-CD47 IgG-positive plasma compared with those treated with anti-CD47 IgG-negative plasma ($P=5.42\times 10^{-11}$ for CAL27 cells, $P=1.52\times 10^{-17}$ for SCC9 cells, and $P=5.02\times 10^{-6}$ for SCC25 cells). Additionally, the inhibition rate of invasion was about 65% in CAL27 cells, about 80% in SCC9 cells and about 60% in SCC25 cells (Figure 4).

3.5. Figure summary. This study described the screening processes of plasma enriched with natural anti-CD47 IgG autoantibodies and analyzed the effects on cell proliferation, apoptosis and invasion/metastasis of three OSCC cell lines in three stages. In order to depict the content of this study and highlight the strategy more clearly, a diagram was constructed and shown in Figure 5.

4. Discussion

Natural autoantibodies are considered to be important antitumor systems *in vivo* showing a decrease in early-stage cancer patients while a gradual increase during the tumor progresses [18, 25]. A number of studies have been concerned about the role of natural autoantibodies in NSCLC, esophageal cancer, breast cancer and liver cancer, including those for p16, FOXP3, CD25, ABCC3, ANXA1, BIRC5, MYC and VEGFR1 [18, 25-29]. It has been supposed that natural autoantibodies may be useful anticancer agents for postoperative therapies to prevent the recurrence of human cancer.

The present study has demonstrated that some healthy individuals have remarkably high levels of natural IgG autoantibodies against CD47, which could significantly inhibit the proliferation of OSCC cell lines, such as SCC9 cells. Our previous study also showed that natural anti-ABCC3 IgG-positive plasma had similar suppressive effects on the OSCC cells previous [21]. The observation was interpreted with study of apoptosis and gene expression in cells. CD47 showed the highest expression levels in all three OSCC cell lines studied, consistent with other reports that CD47 was expressed more significantly in oral cancer tissue than normal mucosal tissues [30]. Plentiful CD47 was expressed on the surface of three OSCC cells and targeted by anti-CD47 IgG autoantibodies. Notably, SCC9 cell viability was suppressed most obviously whereas the apoptosis was not significantly altered, suggesting that distinct mechanisms are likely to be involved in inhibitory effects of anti-CD47 IgG autoantibodies on the proliferation of different OSCC cells. Cell migration and invasion are the key processes of tumor evolution contributing to poor prognosis of OSCC [31-33]. The natural anti-CD47 IgG-positive plasma could efficiently inhibit the

invasion and migration of three OSCC cell lines *in vitro*, especially SCC9 cells. Therefore, we hypothesize natural IgG autoantibodies for CD47 may have potential to treat OSCC. To our knowledge, this is the first report on inhibitory effects of natural anti-CD47 autoantibodies on the proliferation and invasion of OSCC cells *in vitro*.

In function, CD47 plays a critical role in the self-recognition process of tumors [34]. The expression of CD47 has been exploited in order to escape immunological eradication in various solid and hematologic cancers; its overexpression is clinically related to poor prognosis [34]. Although CD47 is involved in the regulation of cancer cell growth, its most important function related to tumor development is to prevent phagocytosis via ligating SIRP α with surrounding phagocytes [10]. Ligation of CD47 to SIRP α on the surface of macrophages promotes phosphorylation of the intracellular ITIM, subsequently recruit and activate inhibitory phosphatases SHP-1 and SHP-2 protein to produce a series of cascade reactions that inhibit phagocytosis of macrophages [9, 35]. Based on the characteristics of CD47 and the mechanism behind natural autoantibodies against tumors, natural anti-CD47 IgG autoantibodies might play an anti-tumor role by mediating antibody-dependent cell-mediated cytotoxicity (ADCC) and complement dependent cytotoxicity (CDC). ADCC is a set of mechanisms that target cells coated with IgG antibodies of the proper subclasses to be the prey of cell-to-cell cytolysis executed by immune cells expressing FcRIIIA (CD16A) [36]. CDC is initiated when C1q, the initiating component of the classical complement pathway, fixed to the Fc portion of target-bound antibodies [37], which triggers a cascade, leading to the assembly of the membrane attack complex (MAC) and cell lysis occurs as the MAC disrupts the plasma membrane of the target cell [38, 39]. In addition, combination of autoantibodies to CD47 triggering the ADCC or CDC could compete the CD47-SIRP α interaction. Although our primary research has shown the effects of natural anti-CD47 IgG autoantibodies on OSCC cells, further research needs to be done to reveal the exact mechanism by which natural anti-CD47 IgG autoantibodies destroy OSCC cells, laying a solid foundation for future animal experiments and clinical trials.

5. Conclusions

Natural anti-CD47 IgG-positive plasma could inhibit cell proliferation, induce apoptosis of OSCC cells, and inhibit their invasion. However, restriction of human plasma utility clinically may compromise the anti-tumor effects of natural anticancer antibodies. Therefore, the intravenous immune globulin (IVIG) produced from plasma rich in natural anti-tumor antibodies may become a promising agent for immunotherapy of oral cancer and other types of cancer in the future.

Declarations

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Disclosure

A preprint version of this study has been submitted to <https://www.researchsquare.com/article/rs-59440/private/draft>. This manuscript is the result interpretation of our study.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Table

Table 1. Amplification primers for quantitative real-time PCR

Gene abbr.	Gene name	NCBI accession	Forward (5'-3')	Reverse (5'-3')
ABCA1	ATP binding cassette subfamily A member 1	NC_000009.12	ACGACCACCATGTCAATCCT	AGCATGTCAAACAGCACGTT
ABCA5	ATP binding cassette subfamily A member 5	NC_000017.11	GCTGGCTGTTCAAATCATGTG	GACTCCAGCTCCTTCCAAAG
ABCB6	ATP binding cassette subfamily B member 6	NC_000002.12	GATCAAGTTCAGGCACAGCC	AACCTGCTGGCCCAAGTC
ABCC3	ATP binding cassette subfamily C member 3	NC_000017.11	TTCTGGGACTCCAACCTGTC	TAGAGCAAGTAGCAGGGCAG
ABCC4	ATP binding cassette subfamily C member 4	NC_000013.11	TGGCGAATTGTTAGCTGTGG	CAGGGCTGCTGAGACACATA
ABCC5	ATP binding cassette subfamily C member 5	NC_000003.12	GGGAGAGAACCAGCACTTCT	TGCATGGAGGCATCAAGAGA
ABCC10	ATP binding cassette subfamily C member 10	NC_000006.12	CCAACAAGACAGTGCTGACC	TACCACTCTCCCCGCTTGTA
CD20(MS4A1)	Membrane spanning 4-domains A1	NC_000011.10	AGCTGGCATCGTTGAGAATG	TGTTTCAGTTAGCCCAACCAC
CD22	CD22 molecule	NC_000019.10	CATACCACGAAGTGGAGATGC	TGACGTTCTCATAGTCGCC
CD47	CD47 molecule	NC_000003.12	CATGGCCCTCTTCTGATTTC	GGAGGTTGTATAGTCTTCTGATTGG
ERBB2	Erb-b2 receptor tyrosine kinase 2	NC_000017.11	CTGGCCTGCCTCCACTT	ATTGGGCATGGACTCAAACG
PIGR	Polymeric immunoglobulin receptor	NC_000001.11	CAGATCAACGGGAGAGAAGG	ACTCTTCGTGGAGATGGCT
GPNMB	Glycoprotein nmb	NC_000007.14	TCACGAGCACCTGATTCT	ACACCAAGAGGGAGATCACA
NECTIN4	Nectin cell adhesion molecule 4	NC_000001.11	ATGGGGACACTTTGGGCTTT	GTCTTCTGGGGTCAAGAA
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	NC_000012.12	AGGTCGGTGTGAACGGATTG	TGTAGACCATGTAGTTGAGGTCA

Figures

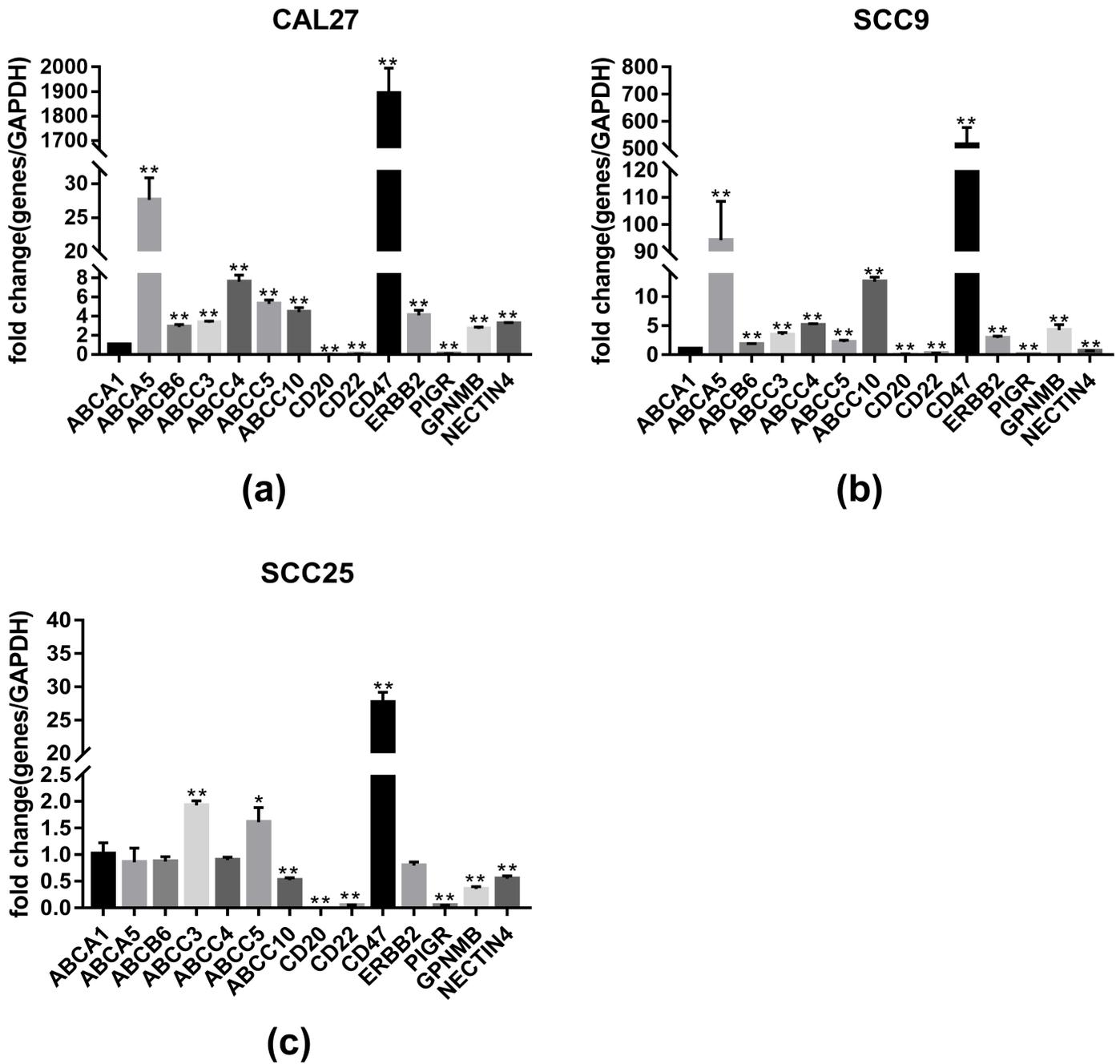


Figure 1

Expression of the 14 genes in oral squamous cell carcinoma cells. (a) CAL27 cells, (b) SCC9 cells and (c) SCC25 cells. Data of gene expression were expressed as mean \pm SD in FC. * $P \leq 0.05$, ** $P \leq 0.01$.

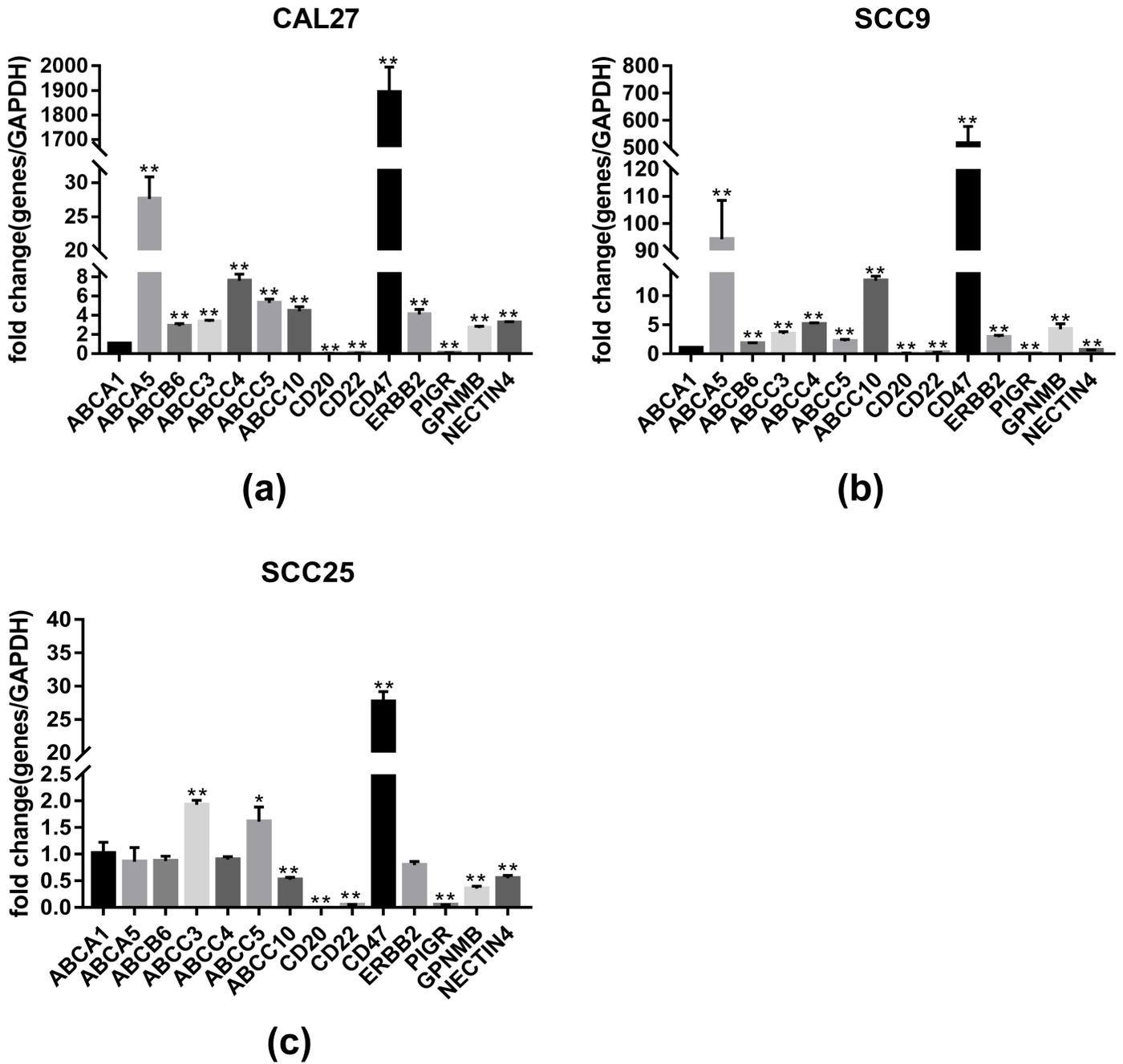
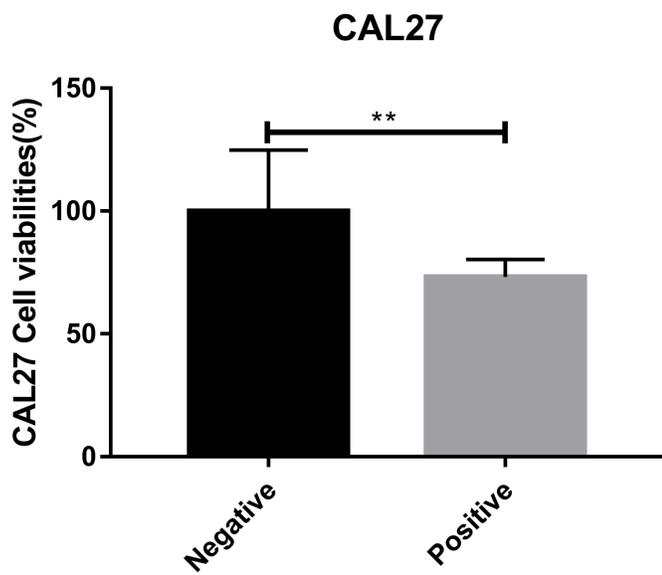
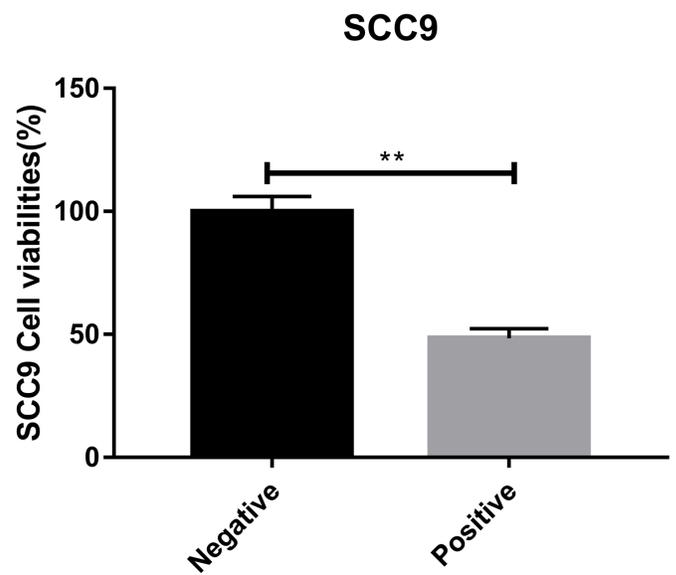


Figure 1

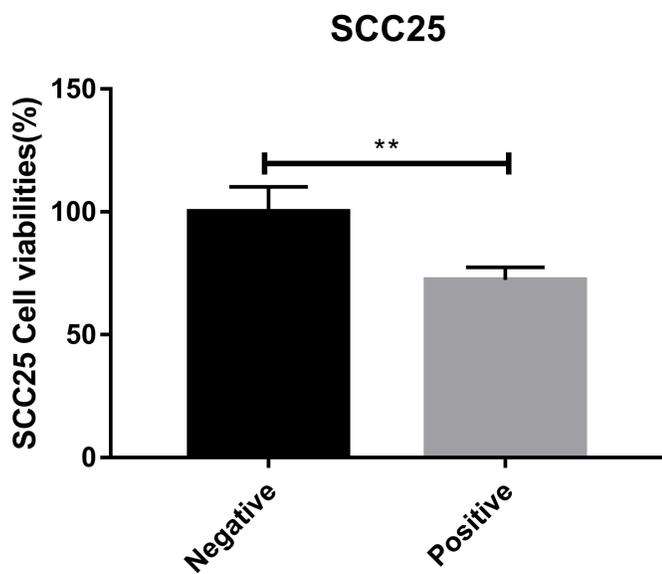
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(a)



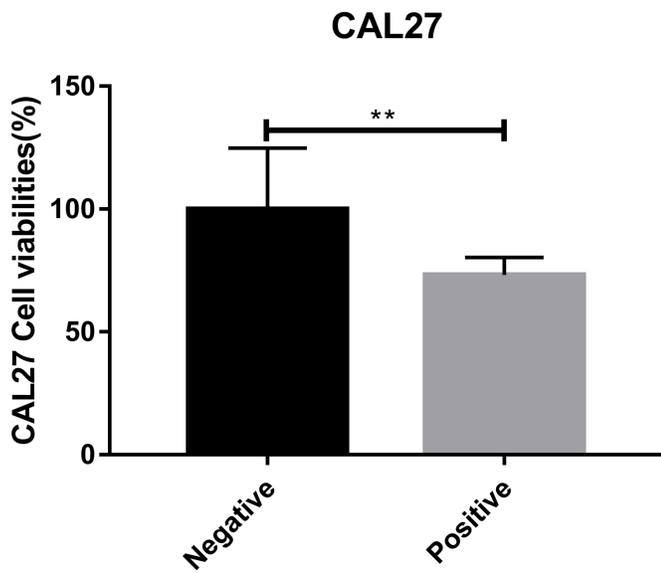
(b)



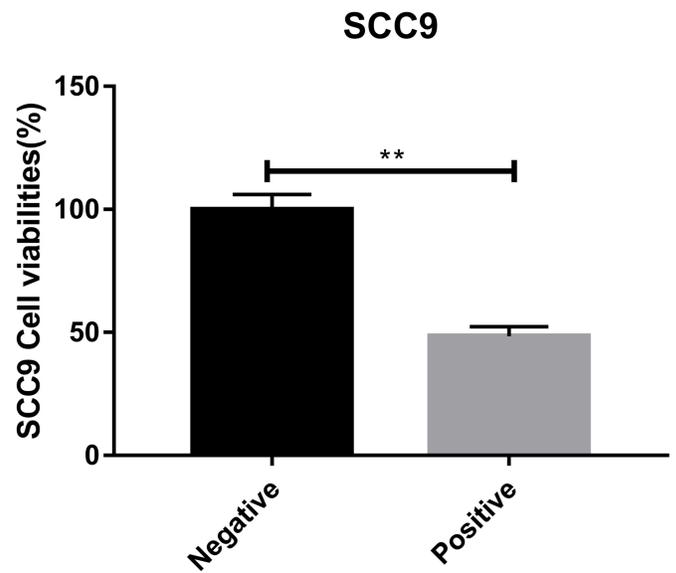
(c)

Figure 2

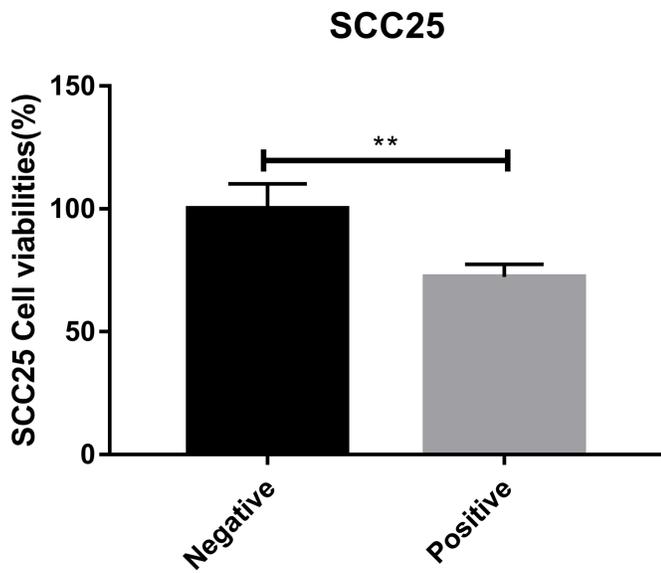
Inhibitory effects of anti-CD47 IgG-positive plasma on the proliferation of three OSCC cell lines. (a) CAL27 cells, (b) SCC9 cells and (c) SCC25 cells. These 3 cell lines were treated with either anti-CD47 IgG-positive or negative plasma for 48 h. The data of cell proliferation were expressed as mean \pm standard deviation in cell viability; * $P < 0.05$, ** $P < 0.01$.



(a)



(b)



(c)

Figure 2

Inhibitory effects of anti-CD47 IgG-positive plasma on the proliferation of three OSCC cell lines. (a) CAL27 cells, (b) SCC9 cells and (c) SCC25 cells. These 3 cell lines were treated with either anti-CD47 IgG-positive or negative plasma for 48 h. The data of cell proliferation were expressed as mean \pm standard deviation in cell viability; * $P < 0.05$, ** $P < 0.01$.

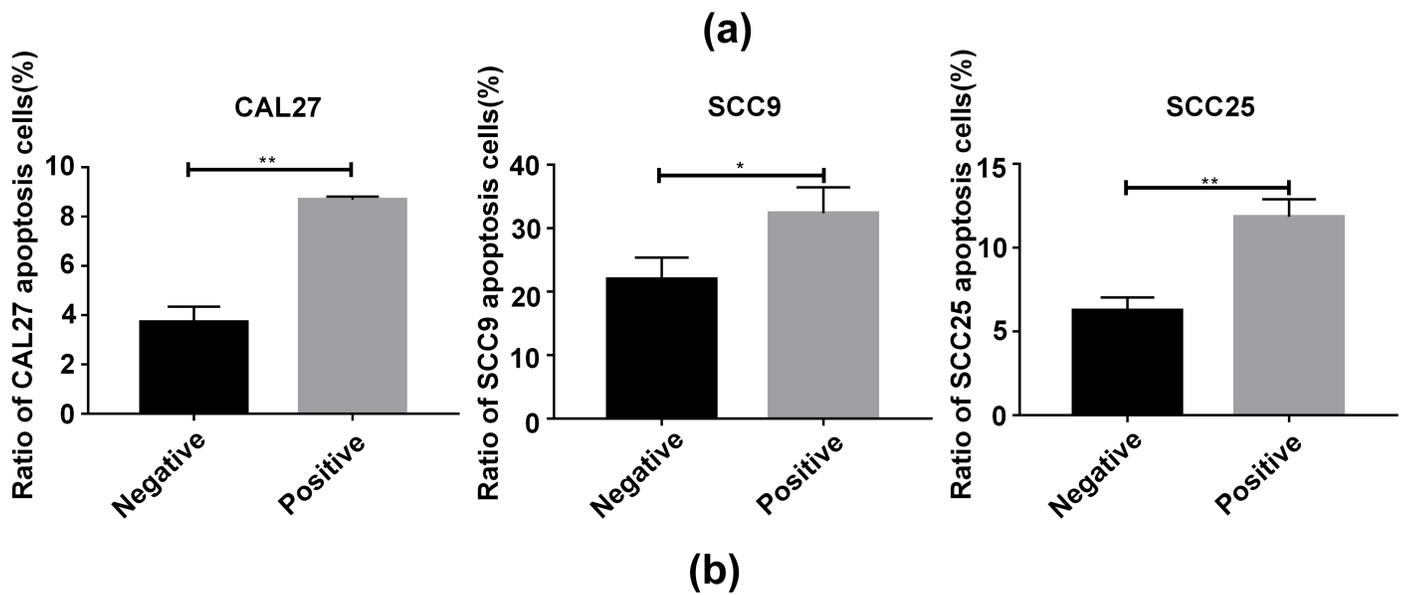
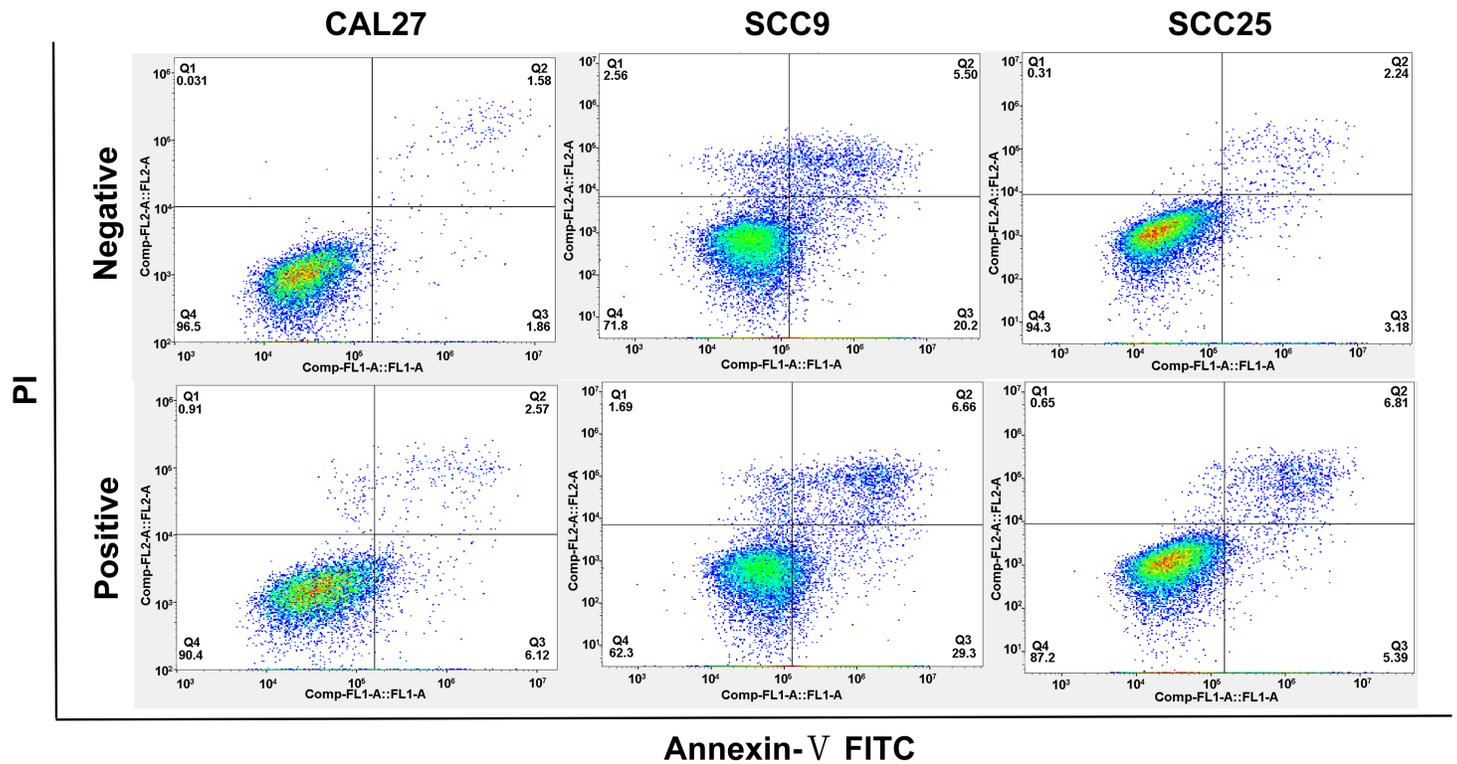


Figure 3

Apoptosis of OSCC cells induced by anti-CD47 IgG-positive plasma. (a) Apoptotic cells ratio in CAL27 cells, SCC9 cells and SCC25 cells were treated with either anti-CD47 IgG-positive or negative plasma for 48 h, respectively. (b) The data of cell apoptosis were expressed as mean \pm standard deviation; * $P < 0.05$, ** $P < 0.01$.

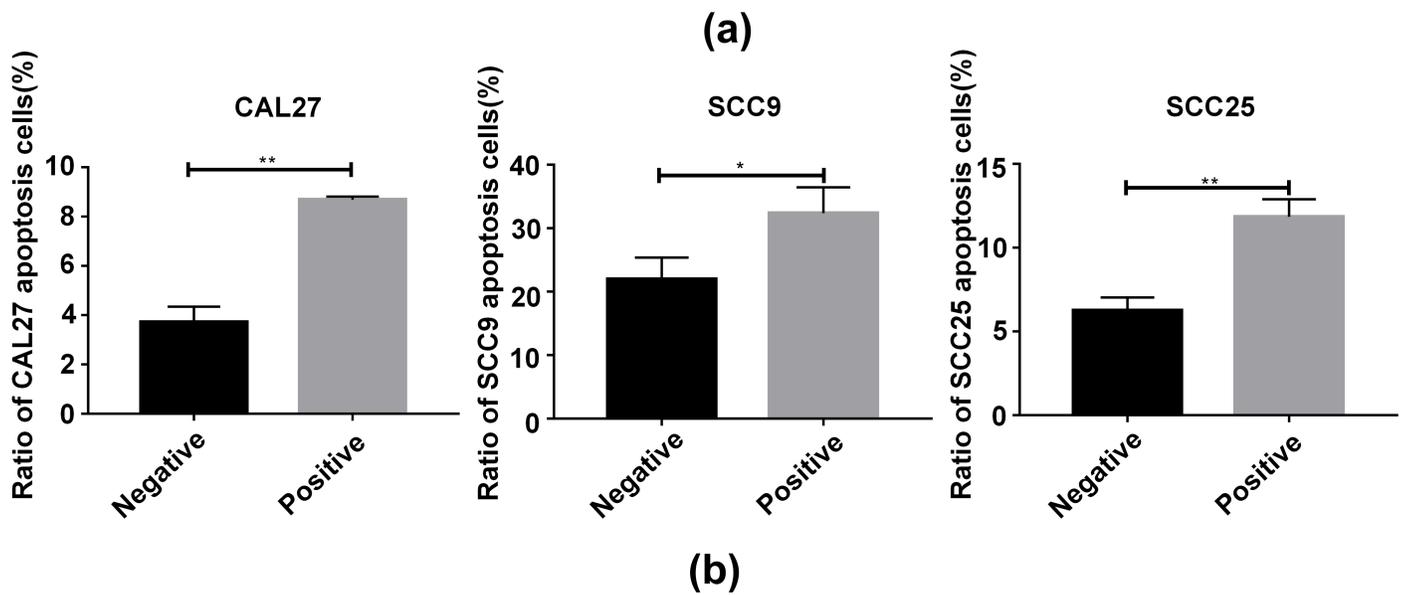
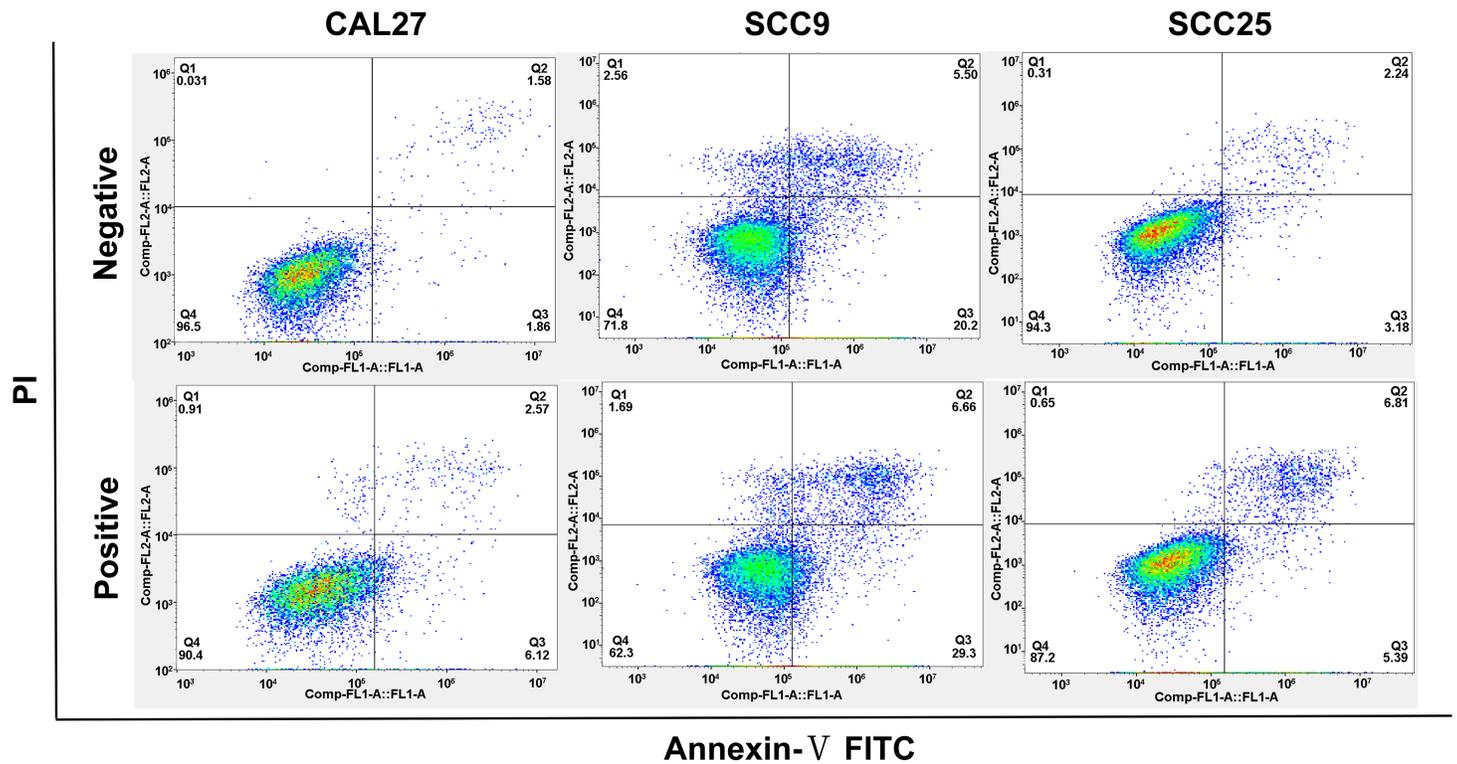
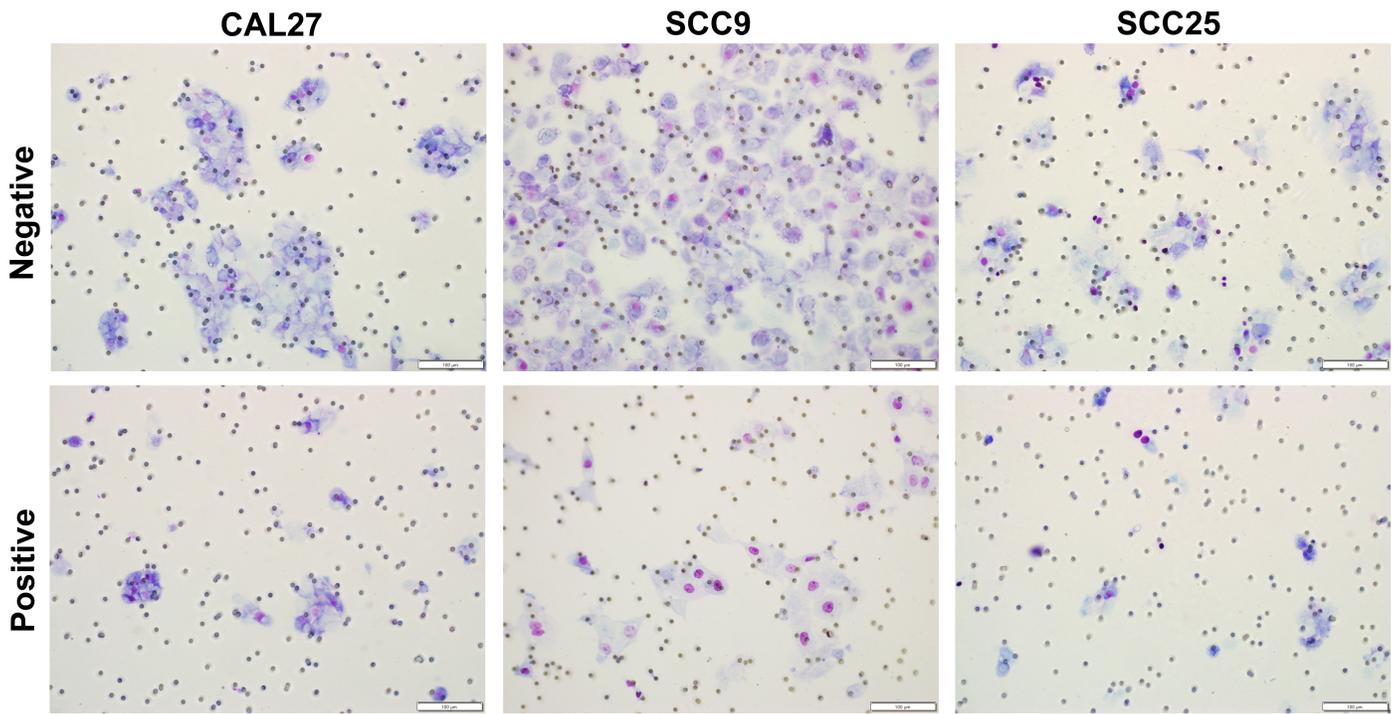
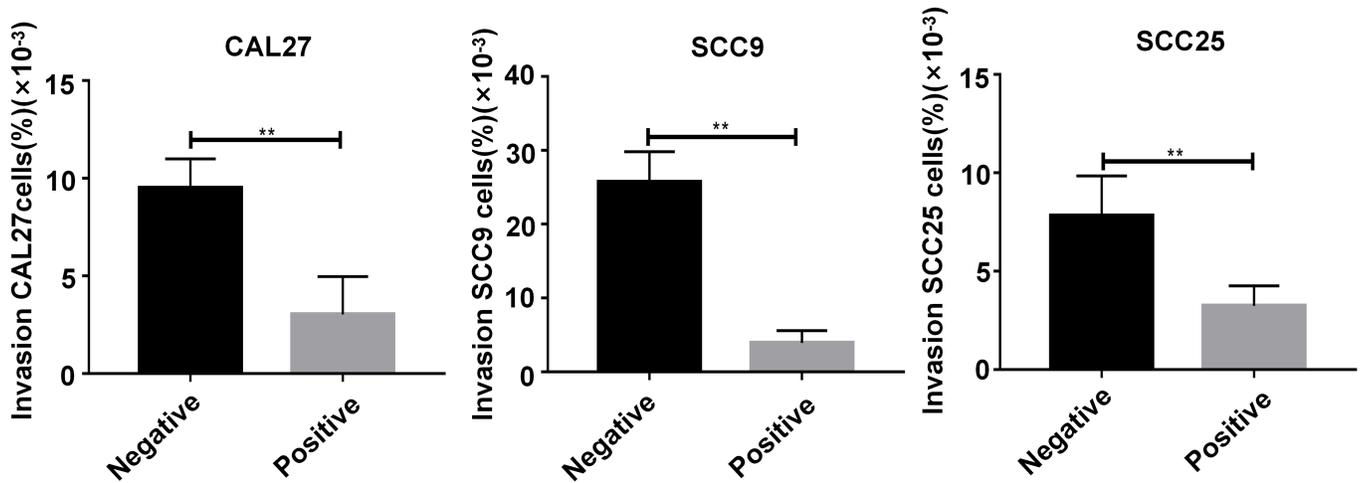


Figure 3

Apoptosis of OSCC cells induced by anti-CD47 IgG-positive plasma. (a) Apoptotic cells ratio in CAL27 cells, SCC9 cells and SCC25 cells were treated with either anti-CD47 IgG-positive or negative plasma for 48 h, respectively. (b) The data of cell apoptosis were expressed as mean \pm standard deviation; * $P < 0.05$, ** $P < 0.01$.



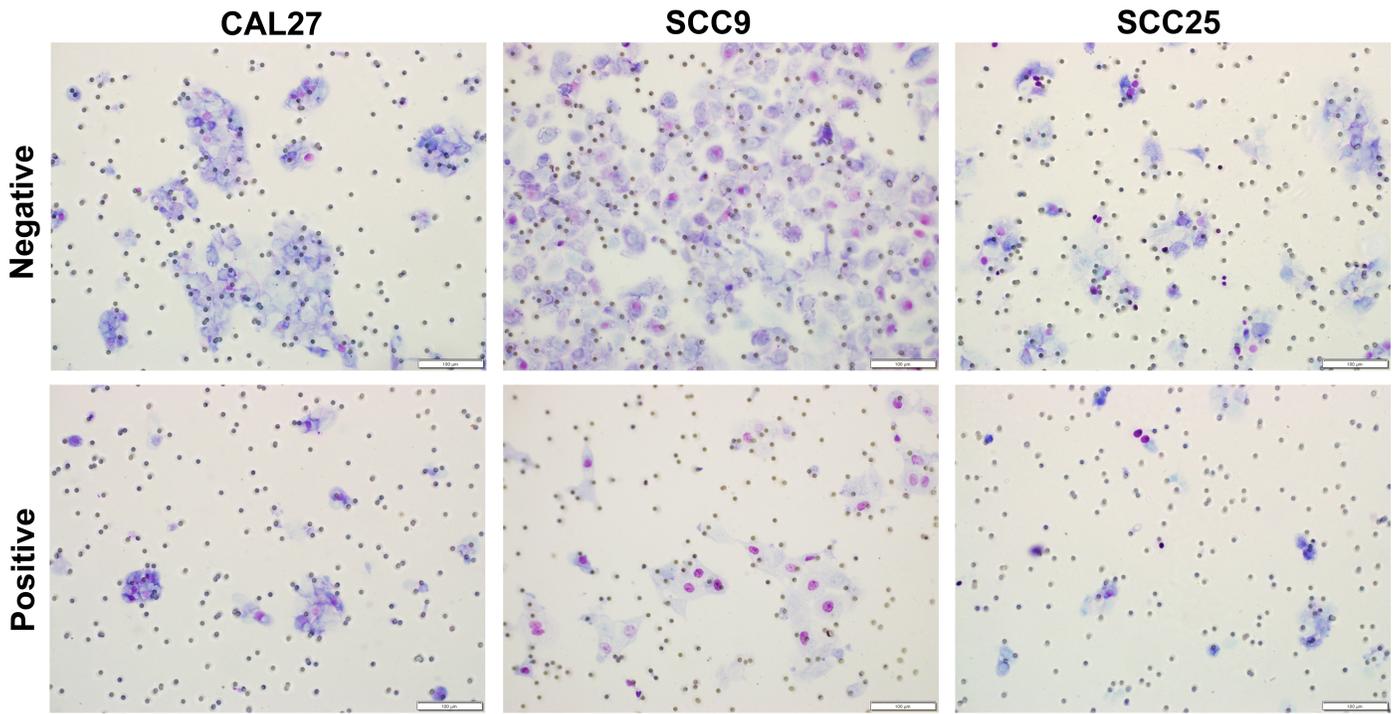
(a)



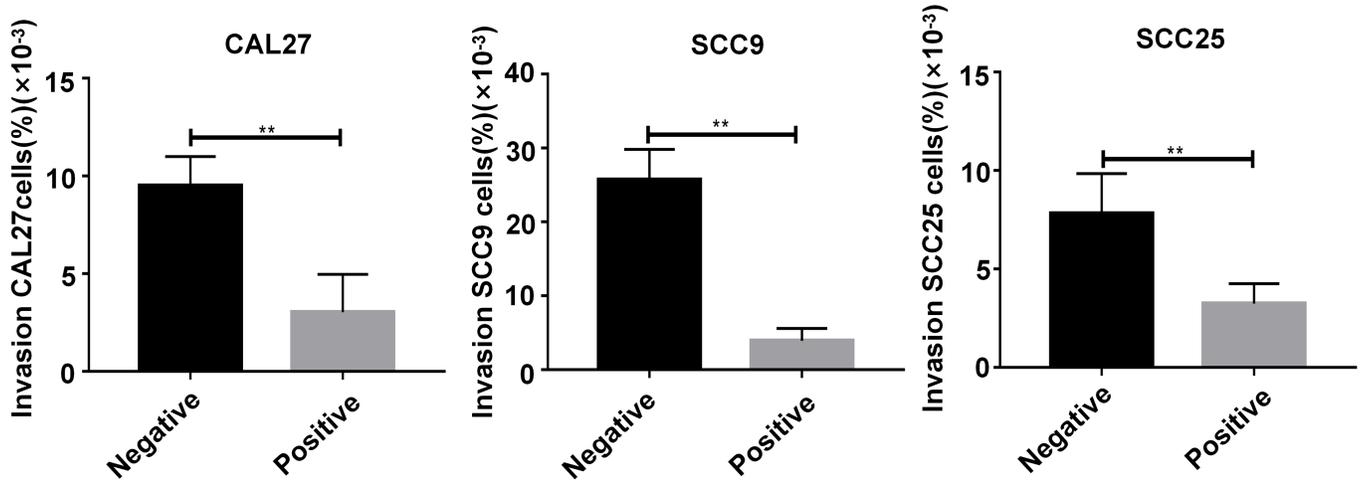
(b)

Figure 4

Transwell assay in OSCC cells treated with anti-CD47 IgG-positive plasma or IgG-negative plasma. The data of invasion were expressed as mean \pm standard deviation; * $P \leq 0.05$, ** $P \leq 0.01$.



(a)



(b)

Figure 4

Transwell assay in OSCC cells treated with anti-CD47 IgG-positive plasma or IgG-negative plasma. The data of invasion were expressed as mean \pm standard deviation; * $P \leq 0.05$, ** $P \leq 0.01$.

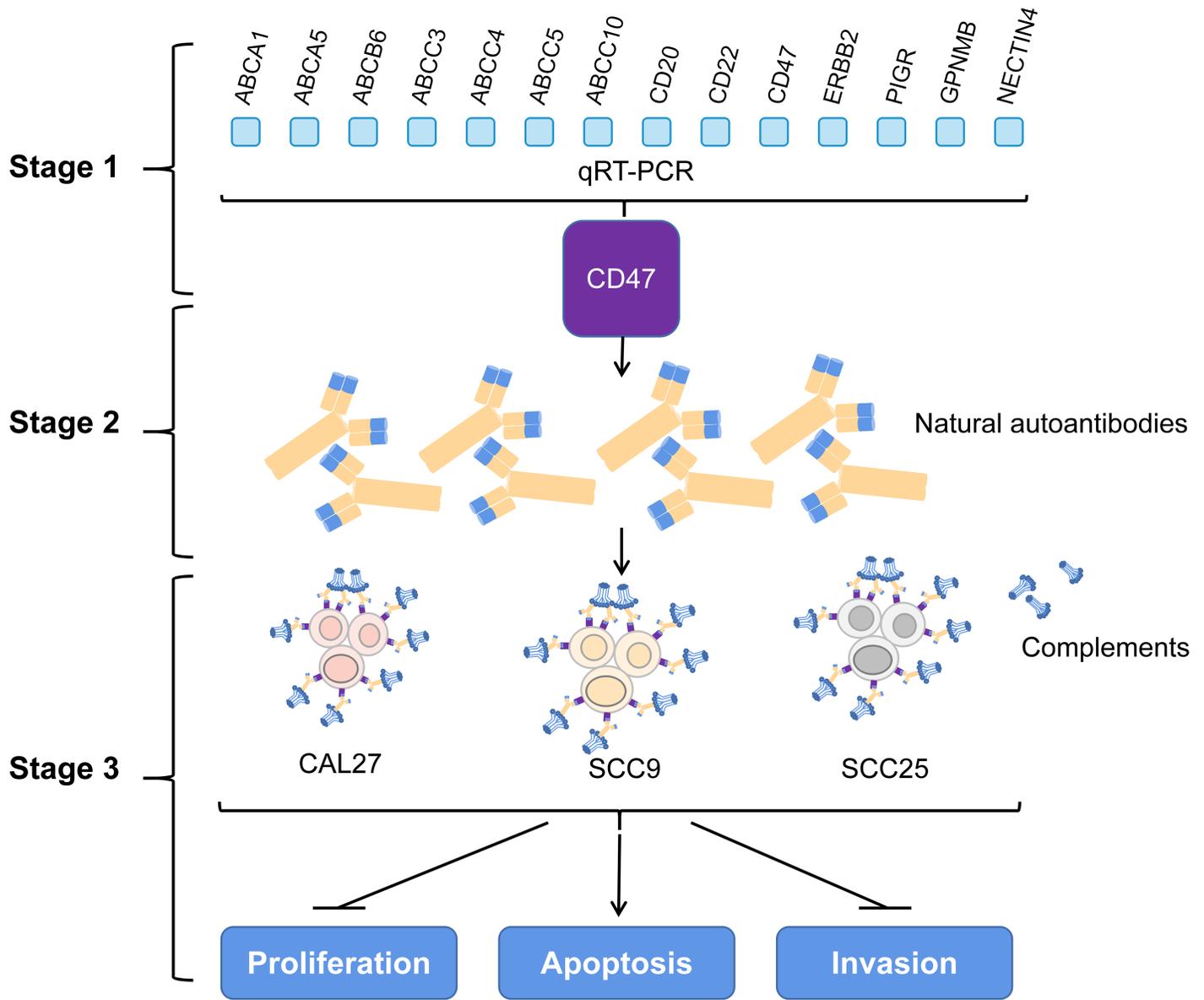


Figure 5

Schematic model for the findings of this study. Stage one is to screen tumor-associated genes and selected CD47 with the highest expression in three OSCC cell lines as an anticancer target. Stage two is to detect human plasma rich in natural anti-CD47 IgG autoantibodies. Third stage is to carry out analysis of cell proliferation, apoptosis and invasion in OSCC cells treated with human plasma.

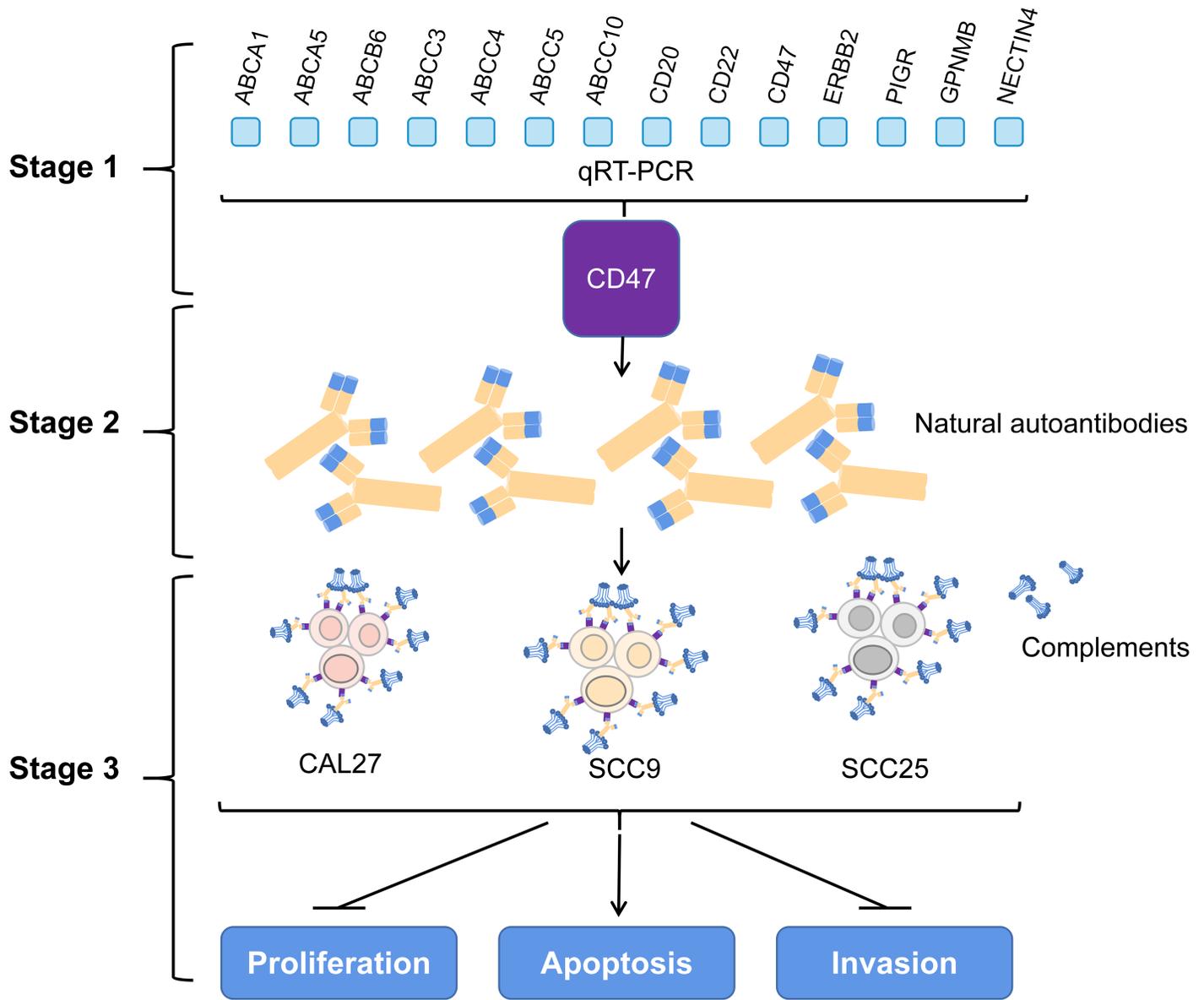


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