Establishment and Characterization of gc-006-03, a Novel Human Signet Ring Cell Gastric Cancer Cell Line Derived from Metastatic Ascites

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Abstract

Signet ring cell gastric cancer (SRCGC) is a special type of gastric cancer with rapid progression and poor prognosis. However, few available SRCGC cell lines from Chinese patients can be used for research, the molecular mechanism of its growth and metastasis is still incompletely understood. In this study, we established and characterized a novel SRCGC cell line, gc-006-03. The cells showed a tendency to pile up without contact inhibition. G-band karyotypes of gc-006-03 were revealed hypotriploid with a modal chromosome number of 51. Immunohistochemistry analysis showed that the cells were positive for CEA, CK7, CDX2 and Ki-67 (45%), and negative for CK20, TTF1 and Lí-cadherin. Flow cytometry analysis showed that gc-006-3 had 25% of CD44+ cells. The cells possessed strong clonality and high plating efficiency, and the doubling time was 36h. The cells grew vigorously for more than 100 passages in serial culture. Meanwhile, the cells showed a high rate of tumor formation. Tumors were observed in all of the nude mice (5/5) given injections of the cells. The metastatic capability of the cell line was found in zebrafish injected the cells. The results of whole genome sequencing revealed the unique genomic characteristics of gc-006-03. In summary, this new stable cell line may be useful in basic and clinical research on gastric signet ring cell carcinoma.

Key words: Signet ring cell gastric cancer, cell line, whole genome sequencing, TP53, CD44.

Introduction

Gastric cancer is the world’s second most deadly cancer, with poor survival especially in the advanced patients [1-3]. Signet ring cell gastric cancer (SRCGC) is a special type of gastric cancer diagnosed by its microscopic features of signet ring cells [4]. It is reported that the incidence of SRCGC has grown to 99.8% in recent five years [5]. SRCGC has a specific epidemiology and oncogenesis and has two forms: early gastric cancer and advanced gastric cancer. The two forms have different prognosis to non-SRCGC. Patients with SRCGC had a better survival than patients with other gastric adenocarcinomas in 1520 early gastric cancer patients [6]. However, more than 3500 patients with advanced SRCGC showed a significantly worse 5-year survival rate than in non-SRCGC [7, 8]. Unfortunately, SRCGC is more frequent in stage4, T3/T4 and N2 gastric cancers than in early gastric cancer. Meanwhile, SRCGC is thought to be less chemosensitive than non-SRCGC, but recent reports suggest that it could have a specific sensitivity profile and be more sensitive to taxane-based chemotherapy or antiangiogenics [9]. However, basic research on SRCGC is still incomplete due to the lack of the suitable cell model. Therefore, it is absolutely necessary to establish a stable and available SRCGC cell line.
Cell lines established from human gastric cancers may provide useful tools to study the biology of the disease and to develop and test new therapeutic approaches. A large bank of well-characterized cell lines should reflect the diversity of tumor phenotypes and provide adequate models for the study of tumor heterogeneity. Generally, well-characterized gastric carcinoma cell lines have proven to be more difficult to establish in long-term culture than colorectal carcinoma cell lines. The mostly number of gastric carcinoma lines have been described from Korea [10, 11] and Japan [12-15]. A few lines have been established in other countries, including China [16-24]. We have established three cell lines of gastric carcinoma derived from primary tumors and metastatic sites in 32 Chinese patients since 2016. But only the gc-006-03 cell line, which an immortal human signet ring cell gastric cancer cell line derived from metastatic ascites, was cultured with high efficiency and well-characterized. This line would serve as a valuable tool for gastric carcinoma research.

Herein we describe the establishment and characterization of the gc-006-03 cell line. The cell line has been analyzed with respect to the growth property, cellular ultrastructure, neoplastic behavior in athymic BALB/c nude mice and zebrafish assay, karyotype, the expression of tumor-associated antigen, cell Line authentication by short tandem repeat (STR) profiling, excepted mycoplasma contaminated.

Massively sequencing technologies promise the capacity to paint a genome-wide portrait of mutation in gastric cancer. In order to comprehensively characterize the genome of this cell line and to serve as a model of SRCGC genome sequencing, we have generated 30× genomic sequence coverage using a PE150 sequence strategy. Here we counted results for point mutations, small insertions/deletions, copy number variation and structure variation in this cell. According to some well-known driver genes in gastric cancer (GC), status of these exon alterations in this cell line were presented for details, and organized mutated genes from the perspective of related biological pathways.

Materials and Methods

Establishment and cell culture conditions

The cell line was derived from metastatic ascites of a 55-year-old Chinese female patient with SRCGC, taken after 5-fluorouracil(5-FU) combined with oxaliplatin chemotherapy, and informed consent was signed by the patient before the study. First we extracted the patient’s ascites, then separated the cells by partial solution of 1.077g density. Cancer cells were cultured in 1640 medium (Gibco) with 10% fetal bovine serum (FBS, Gibco), 10mM HEPES (Gibco), 100u/ml penicillin G (Gibco), 100µg/ml streptomycin sulfate (Gibco) and 0.25µg/ml amphotericin B (Gibco). After that, the cells were incubated in an atmosphere containing 5%CO2 at 37°C. By repeating subculture of the cells in vitro, we established a signet ring cell gastric cancer cell line named gc-006. Furthermore, picked distinct monoclonal cells from gc-006 cell line were cultured into coated poly-L-sine in a 24-well plate containing 1ml of 1640 complete medium. And so gc-006-03 cell line (the subline of gc-006) was established.

Ultrastructural studies

Cell pellets were fixed in 2.5% glutaraldehyde, postfixed in 1% osmium tetroxide, stained with 1% uranyl acetate, and thin sections were examined by a Siemens 1A electron microscope.

Cell Line Authentication by Short Tandem Repeat (STR) profiling

The cell line, together with positive and negative control were amplified using GenePrint 10 System (Promega). Then, amplified products were processed using the ABI3730xl Genetic Analyzer. Data were analyzed using GeneMapper4.0 software and then compared with ATCC, DSMZ or JCRB databases for reference matching. STR assay at China Center for Type Culture Collection (Wuhan, China).

Chromosomal analysis

The logarithmic phase cells of 5, 10, 25 passages were taken for karyotype analysis,respectively. The cells were treated with colchicine for 2h,then with 0.075mol/L KCL solution, fixed with acetic acid glacial, dropped with ice wet glass, disposed with pancreatic enzyme after aging at room temperature, finally, utilized with Giemsa staining for banding analysis.

Immunohistochemical analysis

Morphology of the cells was confirmed using antibodies directed against target protein at the 30th passage. Cells were plated in 100-mm plates and grew to approximately 90% confluence. For immunohistochemical staining, tumor cells were collected by centrifuging as cell pellet. Slowly added neutral formalin into the tube and fixed the cell pellet overnight. Then processed the cell pellet to paraffin block for embedding which could be used for making histological sections. The sections were dewaxed with xylene and rehydrated with graded alcohol, then briefly immersed in water. Endogenous peroxidase activity was blocked by incubating the sections with 3% hydrogen peroxide for 10 min. For all biomarkers,
heat-mediated antigen retrieval was performed by
heating the sections (immersed in a 0.01 mol/L citrate
buffer, pH 6.0) in a microwave oven (450 W) for 10
min. The slides were then washed with phosphate-buffered saline (PBS) before incubation
with respective primary antibody overnight at 4 °C.
After being washed with PBS, the slides were
incubated for 1 h with the secondary antibody, and
then further washed for 3 x 5 min with PBS.
Peroxidase reaction was developed in PBS using
hydrogen peroxide as a substrate and DAB as a
chromogen. Sections were counterstained with
hematoxylin, dehydrated, and evaluated under a light
microscope. Negative control sections were processed
immunohistochemically after the primary antibody
was replaced with PBS. Anti-vimentin (MA5-11883,
Invitrogen), anti-CK7 (9021, abcom), anti-CK20 (854,
abcom), anti-TTF1 (72876, abcom), anti-Li-cadherin
(204960, abcom), anti-CDX2 (157524, abcom),
anti-CEA (4451, abcom) and anti-Ki-67 (8191, abcom)
were used in this study.

Flow cytometry analysis

Ninety percent confluent culture cells in a
100-mm cell plate were dissociated from plates using
trypsin-EDTA, centrifuged, resuspended up to 10^7
nucleated cells per 100 µL of buffer, and then stained
with 10 µL of human CD44-FITC (130-098-210,
Miltenyi Biotec), isotype control antibody Mouse
IgG1-FITC (130-098-847, Miltenyi Biotec) or human
CD326 (EpCAM)-PE (130-098-115, Miltenyi Biotec),
isotype control antibody Mouse IgG1-PE (130-098-845,
Miltenyi Biotec) at 4°C for 10 min, respectively.
Analyses were performed using a BD FACSCanto II
(BD Biosciences).

Cell proliferation and colony formation assay

Suspensions of 1x10^3 logarithmic phase cells
were placed on 24 well plates to ensure 1000ul per
well, with 5 repeating wells each, respectively. Then,
the cells were placed in JuLi Stage real-time cell count
meter, counted every hour and recorded for total
eight days. The population doubling time was
calculated from the growth curve, which was drawn
by cells confluence and integration time, according to
the formula that (PDT)=(t-t0)lg2/(lgNt-lgN0). For
studies of plating efficiency, 100 single suspension
cells were placed onto three-60mm dishes each and
cultured for 9 days. Plating efficiency was calculated
by the ratio of the number of clones (more than 50
cells after crystal violet stain) to the total number of
inoculated cells.

Nude mouse tumorigenicity assay

Tumorigenicity of gc-006-03 cell line was tested
by inoculating 5x10^6 cells s.c. into one side shoulder
and the contralateral hip of each of 5 male athymic
nude mice, BALB/c background (Comparative
Medicine Centre of Yangzhou University, Jiangsu,
China) and observing one weekly for progressive
growth. All animal studies were performed according
to protocols approved by Animal Ethical and Welfare
Committee of Nanjing Medical University (NJMU).

Zebrafish assay

Zebrafish zygotes were harvested and incubated
for 24h in an incubator at 28.5°C. 1-Phenyl-3-
(2-thiazolyl) -2-thiourea (PTU)(sigma) was added to
water of the incubator to make its final concentration
0.2mM, and the zygotes were incubated for another
24h. Then, removed zebrafish embryonic membrane
and injected tumor cells of gc-006-03 which had
treated with Dil solutions (C7000, Invitrogen) before
into zebrafish yolk sac. Four days after injection,
Fluorescence microscopy (D-eclipse Ci, Nikon) was
used and the fluorescence intensity was analyzed
using the image pro plus (6.0) software. All animal
studies were performed according to protocols
approved by Animal Ethical and Welfare Committee
of NJMU.

DNA extraction

DNA was extracted with Qiagen extraction kits
Qiagen GmbH, Hilden, Germany) from the cell line.

Whole genome sequencing

Library preparation and sequencing were
performed at Bionova Company.In brief, genomic
DNAs were sheared using Covaris instrument to
average size of 500 bp, fragment purified, ends
repaired, A-tailed, ligated with appropriate adapters
and amplified using Illumina sequencing primers.
The libraries were applied to pair-end sequencing
with read lengths of 150 nt on Illumina HiSeqXten
platform.

Results

Cytomorphology

Phase contrast microscope revealed that the
gc-006-03 cell line was strictly anchorage-
independent, and formed multi-layered sheets with
clusters upon confluence, was without obvious
contact inhibition phenomenon (Figure 1A). Stained
with hematoxylin and eosin, the cells presented
morphology of malignant epithelial cells, and showed
typical signet ring cell morphology (Figure 1B).
Organelles were visible under the transmission
electron microscopy, such as ribosome, rough
endoparasitic reticulum and golgi complexes (Figure
1C).
STR matching analysis

By comparing the results of STR matching analysis with the information of cells in ATCC, DSMZ and JCRB, there had not found any gc-006-03 homologous cells. This proved the uniqueness of the cell line.

Chromosomal analysis

Chromosomes of 50 metaphase plates were analyzed for passage 5, 10, 25 respectively, indicating gc-006-03 relative stability. The karyotypes of over 58.5% of the cells fell within the modal chromosome number of 51±2, and showed a unimodal distribution. Representative karyotype of the clone with 54 chromosomes in gc-006-03 has been observed as 54, XX, +3, +7, +7, +8, +13, +18, +19, 20 (Figure 2A and 2B). And also they were founded double centromere, ectopic and derivative phenomenon.

Figure 1. Cytomorphology of cells from gc-006-03. (A) Cells were photographed under microscope. Scale bar, 50μm. (B) H&E staining of the gc-006-03 cells showed typical signet ring cell morphology (indicated by the arrows). Scale bar, 10μm. (C) Cell structure under the transmission electron microscopy. Scale bar, 1μm.

Figure 2. (A, B) Representative karyotype of the gc-006-03 cells. Approximately 58.5% of the gc-006-03 cells possessed the modal chromosomes number of 51±2.
Figure 3. The cell line showed the characteristic of the epithelial tumor cells: keratin (+) (A), vimentin (-) (B). Immunohistochemical staining with anti-ck7 (C), anti-ck20 (D), anti-ttf1 (E), anti-li-cad (F), anti-adx2 (G), anti-CEA (H) and anti-Ki-67 (I). Scale bar, 10 μm.

**Immunohistochemistry**

The gc-006-03 cell line was confirmed to the characteristic of epithelial tumor cells: cytokeratin (+), vimentin (-) (Figure 3A and 3B). Because of the cell line was source from the ascites of advanced patient with signet ring cell gastric cancer, so CK7, CK20 and TTF1 testing was needed to identify the source of the cells. Immunohistochemical analysis showed CK7 (2+), CK20 (-) and TTF1 (-) in gc-006-03 (Figure 3C, 3D and 3E), conformed to the characteristics of gastric carcinoma. Furthermore, the cell line presented Li-cadherin (+), CDX2 (2+), CEA (3+) and 45% positive of Ki-67 (Figure 3F, 3G, 3H and 3I), which showed more biological characteristics of gastric carcinoma.

**CD44 and CD326 expression assay**

We performed the Flow cytometry analysis to confirm the source of the cell line and clarity the proportion of cancer stem cells (CSCs) in gc-006-03. The results showed that nearly 100% of CD326+ in gc-006-03 cells were compared to the control group (Figure 4), meaning that gc-006-03 derived from epithelial cells. Meanwhile, about 25.5% of CD44+ in gc-006-03 cells were compared to the control group (Figure 4). Therefore, this cell line could be used in the study of cancer stem cells theory.

**Cell proliferation and clone formation ability**

The average of doubling time for the gc-006-03 cells was 36h (Figure 5A). And the cells had a strong ability of clone formation, with approximately 70% cloning efficiency in soft agar (data was not shown here), while 88% in a 60mm cell plate (Figure 5B).

**Tumorigenicity and metastatic capability in vivo**

To confirm its tumorigenicity in vivo, we injected viable cells (5×10⁶/site) into nude mice. Strikingly, xenograft tumors initiated with 100% incidence (5/5) after 2 weeks (Figure 6A). The hematoxylin- and eosin-stained tissue sections of the mice tumor xenografts showed histologic features of human signet ring cell gastric carcinoma, a few signet ring cells (Figure 6B). We used zebrafish to study the metastatic capability of gc-006-03 cells. After 7 days post injection (dpi), cancer cells showed a tendency to metastasise in zebrafish (Figure 6C).

**Whole genome sequencing**

The DNA sample of the cell line was processed for the whole genome sequencing, the results showed 143,111.94Mb of the original bases. After the removal of low-quality reads, the sample got 922,451,234 clean reads (138,367.69Mb). The clean reads of the sample had high value of Q20 and Q30, indicating that sequencing data had good quality. The average content of GC was 40.91%. The statistics for the distribution of SNP, InDel, CNV and SV were shown in Table 1. Also, we showed the circo plot of whole genome sequencing (Figure 7). Meanwhile, we selected 23 important gastric cancer driven genes, and showed their corresponding SNP, InDel, CNV or SV variants in exon regions (Table 2). (We named this cell line gc-006-03 and the company labeled this cell line as gc_006_03).
Figure 4. Flow cytometry of CD44 and CD326 expression. a and d Histograms showing absence of CD44, CD326 marker respectively in gc-006-03 cells, when no antibody were used (technical control). b, e Histograms of mouse IgG1 stained with FITC-conjugated, with PE-conjugated mouse monoclonal antibody respectively, against Human CD44, CD326 respectively (isotype control). c and f Histograms of gc-006-03 cells stained with FITC-conjugated, PE-conjugated mouse monoclonal antibody respectively, against Human CD44 and CD326 showing.

Figure 5. Proliferation and clone formation ability of gc-006-03 cells. (A) Growth curves of gc-006-03 cells. Doubling time was 36h. (B) 100 cells was used to clone formation assay in a 60mm cell plate.

Discussion

Despite the cell lines may only present a part of the characteristics of the tumor [25], it is still necessary to establish a stable model of advanced SRCGC to study the mechanism of its genesis and development, and then so as to guide the clinical treatment. Which cells of SRCGC established from primary tumors are relatively difficult to culture in vitro as permanent cell lines because of overgrowth of fibroblasts. The use of body fluids as a starting material for cell culture provides many technical advantages and has already been used for the establishment of signet ring cell carcinoma of the stomach [22, 26]. In this literature, for the first time, we isolated and established a new cancer cell line from metastatic ascites of the Chinese female patient with SRCGC, diagnosed by its microscopic characteristics as described by the World Health Organization, with a worse prognosis than other forms of gastric cancer in Asia and the United States [27, 28], named gc-006-03. And we confirmed that the cell line was not contaminated with mycoplasma, bacteria and fungi.
Figure 6. Tumorigenicity and metastatic capability in vivo. (A) The gc-006-03 cells formed tumors in 100% of mice (5 to 5 mice). (B) H&E staining of the tumor specimens in mice showed obvious signet ring cell characteristics (indicated by the arrows). (C) The gc-006-03 cells in zebrafish metastasized to its brain (indicated by the arrows).

Figure 7. Circos plot of whole genome sequencing. Chromosome ideograms are shown around the outer ring and are oriented pter–qter in a clockwise direction. Other tracks, which were divided into five parts, contain somatic alterations from outside to inside. SNP was indicated by the black spots according to the position. InDel results were represented in dark-blue and yellow layers, where dark-blue indicated deletion, yellow indicated insertion. Both light-blue and light-red layers represent these results of CNV, with blue rectangles representing genomic deletion of CNV, and red rectangles representing genomic duplication of CNV. Both light-blue and green layer and the lines of innermost represented the relative position of SV, blue rectangles indicated chromosomal deletion, dark-green rectangles indicated chromosomal inversion, and the line represented chromosomal translocations.
We used a series of immune histochemical markers to confirm that the cell line has an epithelial nature of the stomach, instead of derived from the lungs or the thyroid gland. Furthermore, gc-006-03 expressed CDX2, Li-cadherin, and strongly expressed CEA. All of these three tumor markers are important to diagnosis, therapy control and monitoring of gastric cancer. However, CDX2 suggests a good prognosis of gastric cancer while Li-cadherin and CEA not. In general, different markers suggested different prognosis of gastric cancer. Therefore, in addition to gastric cancer, we still need to find more useful markers for signet ring cell gastric cancer. Moreover, gc-006-03 slightly expressed Ki-67. Ki-67 protein is widely used as a cellular marker for proliferation [29], and it is often studied in breast cancer, which is used as a marker of cellular proliferation and response to chemotherapy, and also is associated with patient prognosis [30-35]. However, the role of Ki-67 in gastric cancer is still not clear except that it can be used as a biomarker for predicting the prognosis of early gastric cancer [36]. Therefore, this cell line is helpful for the study of the mechanism of Ki-67 in cellular proliferation in gastric cancer.

| Classification | Gene symbol | Status of Genetic alterations (SNP, InDel, CNV and SV) |
|----------------|-------------|---------------------------------------------------|
| Cell cycle regulation | TP53 | p.Pro72Arg/c.215C>G |
| Cell cycle regulation | PTEN | Wild-type |
| Cell cycle regulation | TTK | Wild-type |
| Cell cycle regulation | CDKN2A | p.Glu338c.97delG |
| Cell cycle regulation | BRCA2 | p.Asn289His/c.865A>C, p.Asn991Asp/c.2971A>G, p.Val2466Ala/c.7977G>C, |
| Cell cycle regulation | ARID1A | ARID1A-Def |
| Cell cycle regulation | Cell adhesion | CDH1 | Wild-type |
| Cell cycle regulation | Cell adhesion | FAT4 | p.Arg172Cys/c.514C>T, p.Gln453Leu/c.1538A>T, p.Ala807Val/c.2420C>T, p.Gly833Glu/c.2498G>A, p.Gln257Glu/c.776C>G, p.Gly3526Asp/c.10577C>A, p.Ile3604Leu/c.10810A>C, p.Cys3928Tyr/c.11793C>G, |
| Cell cycle regulation | Cell adhesion | CTNNB1 | Wild-type |
| Cell cycle regulation | Cell adhesion | CD44 | p.Lys417Arg/c.1250A>G, p.Ile479Thr/c.1436T>C, |
| Cell cycle regulation | Cytoskeleton and cell motility | RHO A | Wild-type |
| ALK pathway | CTNNB1 | Wild-type |
| ALK pathway | APC | p.Val1822Asp, p.Ile169fs/c.505_508delATAG, p.Glu1554_Lys1556fs/c.4661_4662insA |
| ALK pathway | RNF43 | p.Leu418Met/c.1252C>A, p.Arg117His/c.350G>A, |
| ALK pathway | MAD4 | Wild-type |
| PIK3 pathway | PIK3CA | p.Glu543Gly/c.1634A>G, p.Ala1045Thr/c.3135G>A, p.Lys1046fs/c.3141delA, |
| PIK3 pathway | ERBB2 | p.Ala1045Thr/c.3135G>A, |
| PIK3 pathway | KRAF | Wild-type |
| PIK3 pathway | NRAS | Wild-type |
| DNA replication | MSH6 | Wild-type |
| DNA replication | MSH3 | p.Ile79Val/c.225A>G, p.Gln94Arg/c.2846A>G, p.Ala1045Thr/c.3135G>A, p.Lys1046fs/c.3141delA, |
| DNA replication | MUTYH | p.Glu324His/c.972G>C, |
| Fas signaling | MET | Wild-type |

Annotate: ITX: intrachromosomal translocation; fs: frameshift; ins: insert; del: deletion.

* copy number losses of ARID1A at the chromosome 1 27022501-27022500
* structural variation of CD44 and SLC1A2 occurred intrachromosomal translocation

CD44 is a multi-structural and multifunctional cell surface molecule and the major cell surface receptor for hyaluronic acid [37, 38]. Many results show that CD44 plays an important role in Cancer stem cells (CSCs), including mediation of adhesion and indirect enhancement of the expression of antiapoptotic proteins [39-41]. Meanwhile, CD44 may be a new target for the treatment of gastric cancer [42]. We found that the positive rate of CD44 in gc-006-03
was 25%. So, this cell line may be a useful model for the study of CSCs and the targeted therapy of gastric cancer. Apart from this, structural variation in CD44-SLC1A2 gene was found in the data of structure variation (SV) and CD44-SLC1A2 may represent a class of gene fusions in gastric cancer that establish a pro-oncogenic metabolic milieu favoring tumor growth and survival [43].

We have sequenced the whole genome of the cell line and obtained the total results of SNP, InDel, CNV and SV in our study. Meanwhile, we listed the common mutation sites in the exon of 23 driver genes of gastric cancer, such as TP53, APC, ERBB2 and RNF43. Among them, TP53 Pro72Arg polymorphism (rs1042522) was the most widely studied. By encoding a protein called p53, TP53 gene involved in cell cycle control, apoptosis, senescence, and maintenance of DNA integrity [44]. Many studies have been performed to investigate the association between this TP53 polymorphism and the risk of cancer, including cervical cancer, colorectal cancer, breast cancer, lung cancer, ovarian cancer, and endometrial cancer [45]. FAT4 plays a crucial role in carcinogenesis as a key component of the Hippo signaling pathway [46, 47]. FAT4 Ala807Val polymorphism (rs1039808) is associated with the risk of esophageal cancer in non-drinkers in an Eastern Chinese population [48].

The function of MSH3 is to eliminate replication errors and maintain genomic stability and its gene polymorphisms have been studied in many kinds of tumors [49, 50]. Hirata et al. found evidence for an increased risk of sporadic prostate cancer with single nucleotide polymorphisms (SNPs) in the MSH3 variants rs1805355 and rs26279 [50]. MSH3 Ala1045Thr polymorphism (rs26279) was associated with the sensitivity to platinum-based chemotherapy in advanced non-small cell lung cancer patients [51]. MUTYH, as a base excision gene, plays an important role in many cancers and loss of MUTYH function in human cells could lead to accumulation of oxidative damage and genetic instability [52]. Many studies have revealed the relationship between MUTYH Gln338His polymorphisms (rs3219489) and the risk of many cancers, such as breast cancer [53], esophageal adenocarcinoma [54] and colorectal cancer [55]. Thus, these mutations in gc-006-03 also reveal its potential for further biological research.

In addition, many new mutations were found in these driven genes, which may reveal some new mechanism in cancer. CDKN2A, as an important tumor suppressor, prevents carcinogenesis via induction of cell growth arrest and senescence [56, 57]. The BRCA2 gene encodes a tumor suppressor protein which helps repair damaged DNA and ensures stability of cellular genetic material, and its mutations may increase or reduce the risk of cancer at various sites [58]. RNF43, as a tumor suppressor gene, restricts Wnt/β-catenin signaling pathway by removing Wnt receptors from the cell surface [59]. The ERBB2 gene promotes cell proliferation and suppresses apoptosis by encoding HER2 protein in gastric cancer [60-62]. Because some of these mutations are novel, they require further functional characterization. In our study, we only analyzed a very small fraction of the whole genome sequence, so we were looking for a platform to save the full results for your future research to download.

In summary, the cell line gc-006-03 was derived from metastatic ascites of an advanced patient with SRCGC. Gc-006-03 is a well-established cell line with its specific biological characteristics. Studying protein profile of this cell line may provide some new tumor-associated markers. Also this cell line might be a good model for the study of cancer stem cells and targeted therapy of SRCGC. At the same time, the whole genome sequencing of cell line also provides the direction for the study of the gene of SRCGC. However, according to the results of genome sequencing, we still need more studies to further explore its genetic characteristics.

**Abbreviations**

SRCGC: signet ring cell gastric cancer; CEA: carcinoembryonic antigen; CK7: cytokeratin 7; CK20: cytokeratin 20; CDX2: caudal type homeobox transcription factor 2; TTF1: human transcription termination factor 1; STR: short tandem repeat; GC: gastric cancer; PDT: population doubling time; CSCs: cancer stem cells; SNP: single nucleotide polymorphisms; InDel: insertion-deletion; CNV: copy number variations; SV: structure variation.

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**Competing Interests**

The authors have declared that no competing interest exists.
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