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Original Articles

Molecular characterization of 7 new established cell lines from high grade serous ovarian cancer

Caroline Kreuzinger [a], Magdalena Gamperl [a], Andrea Wolf [a], Georg Heinze [b], Angelika Geroldinger [b], Diether Lambrechts [c,d], Bram Boeckx [c,d], Dominiek Smeets [c,d], Reinhard Horvat [e], Stefanie Aust [a], Gerhard Hamilton [f], Robert Zeillinger [a,f], Dan Cacsire Castillo-Tong [a,*]

[a] Molecular Oncology Group, Department of Obstetrics and Gynecology, Comprehensive Cancer Center, Medical University of Vienna, Waehringer Guertel 18-20, 5Q, A-1090 Vienna, Austria
[b] Center for Medical Statistics, Informatics and Intelligent Systems, Medical University of Vienna, Vienna, Austria
[c] Vesalius Research Center, VIB, Leuven, Belgium
[d] Laboratory for Translational Genetics, Department of Oncology, KU Leuven, Leuven, Belgium
[e] Department of Clinical Pathology, Medical University of Vienna, Vienna, Austria
[f] Ludwig Boltzmann Cluster of Translational Oncology, Vienna, Austria

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ABSTRACT

Cancer cell lines are good *in vitro* models to study molecular mechanisms underlying chemoresistance and cancer recurrence. Recent works have demonstrated that most of the available ovarian cancer cell lines are most unlikely high grade serous (HGSOC), the major type of epithelial ovarian cancer. We aimed at establishing well characterized HGSOC cell lines, which can be used as optimal models for ovarian cancer research.

We successfully established seven cell lines from HGSOC and provided the major genomic alterations and the transcriptomic landscapes of them. They exhibited different gene expression patterns in the key pathways involved in cancer resistance. Each cell line harbored a unique *TP53* mutation as their corresponding tumors and expressed cytokeratins 8/18/19 and EpCAM. Two matched lines were established from the same patient, one at diagnosis and being sensitive to carboplatin and the other during chemotherapy and being resistant. Two cell lines presented respective *BRCA1* and *BRCA2* mutations.

To conclude, we have established seven cell lines and well characterized them at genomic and transcriptomic levels. They are optimal models to investigate the molecular mechanisms underlying the progression, chemo resistance and recurrence of HGSOC.

Introduction

Epithelial ovarian cancer (EOC) is the most lethal type of ovarian cancer and accounts for 4% of cancer deaths in women [1]. High grade serous ovarian cancer (HGSOC) is the most frequent histological type, accounting for about 70% of all EOC [2]. Standard therapies include surgery and platinum-based chemotherapy. Although most of the patients show complete clinical response after the first-line treatment, nearly all of them relapse and develop resistant disease which eventually causes death. The very high rate of resistance and early recurrence are the major reasons for the very low 5-year survival rate of around 30% [3].

Platinum-based drugs bind to DNA, produce inter- and intrastrand adducts and ultimately induce cell death. The mechanisms of platinum resistance and recurrence of HGSOC are not completely understood [4]. Various pathways have been proposed to be involved in platinum resistance [5] including DNA repair [6–10], cell cycle control and apoptosis [11,12]. Despite the fact that p53 plays a central role in most of these processes and that almost all HGSOC harbor mutations in the *TP53* gene [13], no direct link between *TP53* mutations and carboplatin resistance could be determined so far.

It is thus of utmost importance to identify key genes or pathways involved in platinum resistance to open the way to develop new drugs to be used alone or in combination with platinum to eliminate the tumor mass along with resistant cells [14].

Cancer cell lines are good *in vitro* models to study molecular mechanisms underlying chemoresistance and tumor recurrence, provided that they have been well characterized [15]. For decades, cell lines have been used to generate our knowledge on ovarian cancer.

* Corresponding author. Tel.: +43 1 4040078330; fax: +43 1 4040078320.
E-mail address: dan.cacsire-castillo@meduniwien.ac.at (D. Cacsire Castillo-Tong).

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However, previously established cell lines are insufficiently characterized, missing important information on tumors and genomic characteristics such as histopathological type, clinical outcome of the patients and *TP53* mutation status. A systematic genomic analysis on a panel of 47 ovarian cancer cell lines and the comparison with the TCGA dataset suggested that most of the commonly used "ovarian cancer" cell lines were most unlikely to originate from HGSOC and thus are not optimal models for studying the disease [16]. Furthermore, discrepancies and difficulties in identifying cell origin, histological type, mutation status or clinical data of the donor patients in different cell banks question the use of the available cell lines as proper models of HGSOC [15,17].

A considerable study on tumor heterogeneity and clonal evolution in ovarian cancer has been performed [18] using matched cell lines established at the end of the 1980s [19] and new cell line series derived from the same patient have been established and characterized [20,21]. These approaches provide new opportunities to study HGSOC. However, the unavailability of histopathological confirmation, the non-standard treatment and lack of information on patients' clinical outcome are still persisting obstacles. New cell lines with well-defined molecular and cellular characteristics, complete clinical documentation of the corresponding tumors and the patients are urgently needed. Particularly, matched cell lines established from tumor materials taken from different time points from the same patient will certainly provide advantages to study the clonal evolution of tumor cells.

In this work, we established cell lines from ascites or tumor tissue from patients with HGSOC, and characterized them regarding gene mutations, mRNA expression, protein expression and chemosensitivity.

Materials and methods

Patients and clinical materials

Informed consents were obtained from all patients with HGSOC included in this study in the Department of Obstetrics and Gynecology, Medical University of Vienna. The study protocol was approved by the ethics committees (EK Nr. 366/2003 and 260/2003). During cytoreductive surgery, tumor tissues were directly transferred to the Department of Pathology, Medical University of Vienna. After confirming the histological type, the materials were sent to the laboratory. Ascites was collected from the clinic and directly sent to the laboratory. The clinical response of the patients was evaluated following the standard guidelines [22].

Establishment and maintenance of cell lines

Ascites was centrifuged and the red blood cells were depleted with a centrifugation step with Histopaque 1077 (Sigma-Aldrich, St. Louis, USA).

Tumor tissues were cut into small pieces and digested with collagenase (1 mg/mL, 1453 CDU/mg, Sigma-Aldrich) at 37 °C for about 1 h.

Cells were cultivated in DMEM medium, with 10% fetal bovine serum (FBS), 100 units/mL penicillin and 100 µg/mL streptomycin (PS; all from Gibco by Life Technologies, CA, USA) at 37 °C and 5% CO_2.

VenorGeM Classic Mycoplasma Detection Kit for conventional PCR (Minerva Biolabs, Berlin, Germany) was used to control mycoplasma contaminations.

Authentication of cell lines

Short tandem repeat (STR) analyses of 7 markers (TPOX, vWA, CSF180, D16S539, D7S820, D13S317, D5S818, Applied Biosystems Life Technologies) were performed using ABI Prism 310 Genetic Analyzer (Applied Biosystems, Life Technologies).

Scratch assay

Cell culture at 100% confluency was scratched with a Pasteur pipette and pictures were taken at the time of scratching and 48 h afterwards. The web based Software WimScratch (ibidi, Munich, Germany) was used to determine the confluency of the cells on the scratched area. The scratched surface in each cell culture flask was defined as 100% and the proportion of the remaining cell free area after 48 h was calculated.

DNA and RNA isolation

Homogenized fresh frozen tumor tissue (Mikro-Dismembrator U; B.Braun Biotech International, Melsungen, Germany) lysate and cell pellet lysate were processed for DNA and RNA isolation using the AllPrep DNA/RNA Mini Kit (Qiagen, Hilden, Germany). The nucleic acid concentrations were measured by a BioPhotometer (Eppendorf, Hamburg, Germany).

Determination of gene mutation

TP53 mutation was determined by a modified p53 functional yeast assay [23,24], and Sanger sequencing. In addition, ddPCR systems for each unique *TP53* mutation were established to determine the percentage of the *TP53* mutant cells in cell culture (Table 1).

BRCA1 and BRCA2 mutations were determined by Sanger sequencing [25].

Hot spot mutations in *KRAS* (c.35G>C, c.34G>C, c.35G>A, c.34G>T, c.34G>A; 35G>T, c.34G>C; 35G>T, c.34G>A, c.35G>T, c.38G>A, c.37G>T) and *BRAF* (V600E, c.1799T>A) were examined with a reverse oligonucleotide hybridization assay (KRAS-BRAF StripAssay (ViennaLab Diagnostics GmbH, Vienna, Austria)).

Immunohistochemical staining (IHC)

Formalin fixed paraffin embedded (FFPE) tissues were sectioned at 3 µm. The IHC was performed with the Dako LSAB+ System-HRP kit (Code K0690; Dako, CA, USA) and all steps were performed according to the manufacturer's instructions.

Cytospin preparations were fixed in 4% formaldehyde and incubated with 0.5% X Triton X-100 for 10 min before further processed.

Primary antibodies were diluted with Dako REAL Antibody diluent (Agilent Technologies, St. Clara, California) and incubated overnight at 4 °C. FLEX Negative Control Mouse Cocktail (Agilent Technologies) and Negative Control Rabbit IgG (Biocare Medical, Concord, USA) were used as isotype controls.

Nuclei were stained with hematoxylin solution modified according to Gill III (Merck Millipore Darmstadt, Germany) before mounting the slide with Kaisers Glyceringelatine (Merck Millipore).

Antibodies: anti-cytokeratin 8/18/19 (IgG1, mouse, clone A45-B/B3; AS Diagnostik, Hueckeswagen, Germany) at 1:100; anti-vimentin, ready to use (CONFIRM Anti-vimentin (V9) Primary Antib, Ventana, Roche Diagnostics, Basel, Switzerland); CD44 (IgG1, mouse, clone 8E2F3; ProMab, Richmond, USA) at 1:4000; EpCAM (IgG, rabbit, clone E144; abcam, Cambridge, UK) at 1:300; CA125 (IgG, rabbit, clone OV185:1; Leica Biosystems, Nussloch, Germany) at 1:200.

The staining was scored by a semi quantitative method as described previously [26].

In vitro chemosensitivity assay

A total of 1×10^4 cells/well were seeded in 96-well plates. Carboplatin (Enzo Life Sciences, NY, USA) at concentrations of 20, 10, 5, 2.5, 1.25, 0.6, 0.3, 0.16, 0.08, and 0.04 µg/mL was added in quadruplicates. Cells were incubated at 37 °C and 5% CO_2

Table 1
ddPCR systems for individual *TP53* mutation.

Mutation	Forward primer	Reverse primer	Probe 1 FAM	Probe 2 VIC
Cd_del170	5′-cgccatggccatctacaag-3′	5′-gctcaccatcgctatctgagc-3′	5′-FAM-agcacatggaggttg-3′-MGB	5′-VIC-gcacatgacggaggt-3′-MGB
Cd_187_Intron Splice site, ggt>gat	5′-gcagtcacagcacatgacgg-3′	5′-cagtgaggaatcagaggcctg-3′	5′-FAM-agatagcgatgatgagc-3′-MGB	5′-VIC-agatagcgatggtgagc-3′-MGB
Cd_193, cat>cct	5′-ccaggcctctgattcctcac-3′	5′-catagggcaccaccacactatg-3′	5′-FAM-tcctcagcctcttat-3′-MGB	5′-VIC-tcctcagcatctta-3′-MGB
Cd_273, cgt>cat	5′-gtggtaatctactgggacgg-3′	5′-cggagattctcttcctctgt-3′	5′-FAM-tgaggtgcatgtttg-3′-MGB	5′-VIC-tgaggtgcgtgtttg-3′-MGB
Cd_333-del c	5′-gtcagctgtataggtacttgaagtgcag-3′	5′-gctctcggaacatctcgaagc-3′	5′-FAM-ctgcagatcgtgggc-3′-MGB	5′-VIC-gcagatccgtgggc-3′-MGB
Cd_340_343, gag-del10-ag-ctg	5′-ctcctctgttgctgcagatcc-3′	5′-ctggagtgagccctgctcc-3′	5′-FAM-cttcgagagctgaatg-3′-MGB	5′-VIC-cttcgagatgttccgagag-3′-MGB