KRAS (G12D)-selective inhibitor MRTX1133 suppresses proliferation and differentially modulates chemosensitivity in ovarian mucinous carcinoma

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Abstract. Ovarian mucinous carcinoma (OMC) is a rare subtype of ovarian cancer characterized by frequent KRAS mutations and a poor response to platinum- and taxane-based chemotherapy, underscoring the need for novel therapeutic strategies. MRTX1133, a recently developed non-covalent and selective KRAS (G12D) inhibitor, has demonstrated potent antitumor activity in pancreatic and colorectal cancers; however, its efficacy in OMC remains unexplored. In the present study, the antitumor effects of MRTX1133 in the KRAS (G12D)-mutant OMC cell line MCAS and its interactions with conventional chemotherapeutic agents were evaluated. Cell viability was assessed using WST-1 assays, ERK phosphorylation was evaluated by western blotting and gene expression levels were analyzed by reverse transcription-quantitative PCR. Results indicated that MRTX1133 suppressed MCAS cell proliferation in a concentration-dependent manner, whereas proliferation of KRAS wild-type and KRAS (G12S)-mutant cells was not significantly inhibited. Treatment markedly inhibited ERK phosphorylation, suggesting suppression of the MAPK pathway. MRTX1133 reduced the mRNA expression of Ki-67 and numerous cyclins (D1, A2 and B1), suggesting attenuation of proliferative signaling. When combined with cytotoxic agents, including paclitaxel, SN38, gemcitabine and cisplatin, MRTX1133 reduced the sensitivity to cell cycle-dependent chemotherapeutic agents, namely paclitaxel, SN38 and gemcitabine, while not affecting the activity of the non-cell cycle-dependent agent cisplatin. The present study therefore provided preclinical evidence for the potential utility of KRAS (G12D)-targeted therapy in OMC and highlights the importance of sequential rather than concurrent scheduling of MRTX1133 with cell cycle-dependent chemotherapy to optimize therapeutic efficacy.

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Abbreviations: OMC, ovarian mucinous carcinoma; CDDP, cisplatin; GEM, gemcitabine; PTX, paclitaxel; RT-qPCR, reverse transcription-quantitative PCR

Key words: ovarian mucinous carcinoma, KRAS (G12D), MRTX1133, cell cycle suppression, chemotherapy

Introduction

Ovarian cancer is the sixth leading cause of cancer-related mortality among women in the United States, with ~21,000 new cases and 13,000 mortalities recorded annually (1). Early-stage ovarian cancer is often asymptomatic and numerous patients are diagnosed at advanced stages with peritoneal dissemination or ascites. Standard treatment for advanced ovarian cancer consists of cytoreductive surgery followed by combination chemotherapy with platinum-based and taxane-based agents. Although ovarian cancer is relatively chemosensitive and a number of patients achieve remission with this multimodal approach, the effects are often transient and >50% of patients ultimately relapse (2,3).

Ovarian mucinous carcinoma (OMC) is one of the histological subtypes of ovarian cancer, accounting for 10-14% of all cases (4,5). Compared with serous carcinoma, which constitutes the majority of ovarian cancers, OMC exhibits markedly low sensitivity to chemotherapy and advanced cases are associated with poor prognosis (6-9). Despite recent advances in ovarian cancer treatment, including poly (ADP-ribose) polymerase (PARP) inhibitors and immunotherapies (10), treatment options for OMC remain limited, highlighting the need for novel therapeutic strategies.

Activating mutations in KRAS are detected in ~25% of all cancer types, with high prevalence in pancreatic and colorectal cancers (11). In OMC, KRAS mutations are observed in ~50% of cases, with the G12D variant being the most frequent (11,12). MRTX1133 is a non-covalent, KRAS (G12D)-specific inhibitor that has demonstrated potent proliferation suppression in KRAS (G12D)-mutant pancreatic and

colorectal cancer models both *in vitro* and *in vivo* (13,14). Based on promising preclinical results demonstrating potent inhibition of cell proliferation, suppression of ERK phosphorylation and tumor growth reduction in KRAS (G12D)-mutant models (13), MRTX1133 was evaluated in a phase I/II clinical trial (clinicaltrials.gov ID NCT05737706) for patients with advanced KRAS (G12D)-mutant solid tumors, including pancreatic, colorectal and non-small cell lung cancers (15). Unlike pancreatic or colorectal cancers whereby MRTX1133 has demonstrated preclinical efficacy, to the best of our knowledge, the efficacy of MRTX1133 in OMC remains unexplored. OMC exhibits a distinct tumor microenvironment and chemoresistance profile, characterized by abundant mucin production and a poor clinical response to standard platinum- and taxane-based chemotherapy compared with other major ovarian cancer subtypes (16-18). These features make it important to evaluate whether KRAS (G12D) inhibition has comparable efficacy and how it can be optimally integrated with current treatment standards. Given the high prevalence of KRAS (G12D) mutations and the poor response of OMC to conventional chemotherapy, targeting KRAS (G12D) is a rational and required therapeutic approach. Thus, in the present study, the aim was to elucidate the antitumor effects and underlying mechanisms of the KRAS (G12D)-selective inhibitor MRTX1133 in OMC cells and further evaluate its interactions with conventional chemotherapeutic agents to establish a basis for combination strategies.

Materials and methods

Cell lines and culture. MCAS, the human OMC cell line and the human ovarian serous adenocarcinoma cell line OVKATE were purchased from the Japanese Collection of Research Bioresources Cell Bank. The human ovarian serous adenocarcinoma cell lines SHIN-3 (19) and TU-OS-4 (20) were obtained from the original investigators. These cells were cultured in DMEM/F12 (Thermo Fisher Scientific, Inc.) supplemented with 10% heat-inactivated FBS (Sigma-Aldrich; Merck KGaA) and 1% penicillin/streptomycin (Thermo Fisher Scientific, Inc.) at 37˚C under 5% CO_2.

KRAS gene mutations. OVKATE, SHIN-3 and TU-OS-4 cells were analyzed for KRAS gene mutations using a previously reported sequencing method (21). Genomic DNA was extracted from cells using a QIAamp DNA Mini Kit (Qiagen, Inc.). The hotspot region of the KRAS gene (exon 2) was amplified through PCR with EX Taq DNA polymerase (Takara Bio, Inc.) and primers as follows: Forward, 5'-GTGTGACATGTTCTA ATATAGTCA-3'; reverse, 5'-GAATGGTCCTGCACCAGT AA-3'. Thermocycling conditions were 94˚C for 30 sec, 64˚C for 30 sec and 72˚C for 30 sec for 45 cycles. PCR products were run on a 10% agarose gel and visualized under LED light to confirm a single band before direct sequencing. Sequencing was performed using the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems; Thermo Fisher Scientific, Inc.) on an ABI PRISM 310 genetic analyzer (Applied Biosystems; Thermo Fisher Scientific, Inc.). The sequencing chromatograms were visually inspected at the nucleotide positions corresponding to KRAS codons 12 and 13 to identify nucleotide substitutions.

Western blotting analysis. Effects of MRTX1133 on the phosphorylation of ERK, which acts downstream of KRAS in the MAPK pathway, were examined. MCAS cells were seeded onto six-well plates at $2x10^5$ cells/well, incubated at 37˚C for 24 h, then cultured in medium containing 10% FBS with 0-100 nM MRTX1133 (TargetMol Chemicals, Inc.) at 37˚C for 3 h. Cells were lysed using lysis buffer (1% NP-40, 150 mM NaCl and 50 mM Tris-HCl; pH 8.0). Extracted proteins (10 μg per lane) were mixed with 1% SDS sample buffer (10 mM Tris-HCl; pH 7.5; 150 mM NaCl; 1% SDS) supplemented with an EDTA-free protease inhibitor cocktail (Roche Diagnostics), separated by electrophoresis using 10% polyacrylamide gels and transferred onto PVDF membranes (Merck KGaA). Protein concentrations were determined prior to loading using a Bradford assay. Membranes were incubated at room temperature for 1 h in PVDF Blocking Reagent for Can Get Signal® (Toyobo Co., Ltd.), washed thrice using Tris-buffered saline-Tween-20 (TBS-T) and incubated overnight with the following antibodies diluted 1:1,000 at room temperature in Can Get Signal® Immunoreaction Enhancer Solution 1 (Toyobo Co., Ltd.): Anti-phospho-ERK (D13.14.4E; Cell Signaling Technology, Inc., cat. no. 4370), anti-ERK (137F5; Cell Signaling Technology, Inc., cat. no. 4695) and anti-α-actin antibody (Sigma-Aldrich; Merck KgaA, cat. no. A2066). After the reaction, membranes were washed three times with TBS-T and incubated with HRP-labeled anti-rabbit antibodies (Rabbit IgG HRP Linked F(ab')2; cat. no. NA9340V; Cytiva) diluted 1:1,000 in Can Get Signal® Immunoreaction Enhancer Solution 2 (Toyobo Co., Ltd.) at room temperature for 1 h. Membranes were then washed three times with TBS-T, incubated with ECL Prime Western Blotting Detection reagent (Cytiva) and imaged using a cooled charge-coupled device system (ImageQuant™; LAS-4000mini; Cytiva).

Cell viability. First, the effect of MRTX1133 alone on numerous ovarian cancer cells was evaluated. MCAS cells (500 cells/well) seeded into 96-well plates were exposed to MRTX1133 at concentrations of 10-400 nM for 72 h at 37˚C. Cell viability was assessed by colorimetric assay using the Premix WST-1 Cell Proliferation Assay System (Takara Bio, Inc.) with data presented as a percentage of the untreated (MRTX1133-free) control. The combined effects of MRTX1133 and numerous anticancer drugs on MCAS cells were then investigated. Cells (500 cells/well) seeded into 96-well plates were exposed to cisplatin (CDDP; Nichi-Iko Pharmaceutical Co. Ltd.) at concentrations of 4-128 μM, paclitaxel (PTX; Sawai Pharmaceutical Co., Ltd.) at concentrations of 4-128 nM, SN38, the active metabolite of irinotecan (Tokyo Chemical Industry Co., Ltd.) at concentrations of 0.05-1.6 μM or gemcitabine (GEM; Nichi-Iko Pharmaceutical Co. Ltd.) at concentrations of 4-128 μM, with or without MRTX1133 at concentrations of 100 nM for 72 h at 37˚C. Cell viability was assessed as aforementioned and expressed as a percentage of the corresponding drug-untreated control.

Programmed cell death detection. To investigate whether cell death pathways contribute to the effect of MRTX1133, programmed cell death following MRTX1133 administration was investigated. MCAS cells (1,000 cells/well) seeded into a 96-well plate were exposed to the pan-caspase

Table I. List of primer sequences used in the present study.

Gene	Direction	Primer sequence (5'-3')
Ki-67	Forward	GAAAGAGTGGCAACCTGCCTTC
Ki-67	Reverse	GCACCAAGTTTTACTACATCTGCC
Cyclin D1	Forward	TCTACACCGACAACTCCATCCG
Cyclin D1	Reverse	TCTGGCATTTTGGAGAGGAAGTG
Cyclin A2	Forward	CTCTACACAGTCACGGGACAAAG
Cyclin A2	Reverse	CTGTGGTGCTTTGAGGTAGGTC
Cyclin B1	Forward	GACCTGTGTCAGGCTTTCTCTG
Cyclin B1	Reverse	GGTATTTTGGTCTGACTGCTTGC
GAPDH	Forward	ACCACAGTCCATGCCATCAC
GAPDH	Reverse	CATCACGCCACAGTTTCCCG

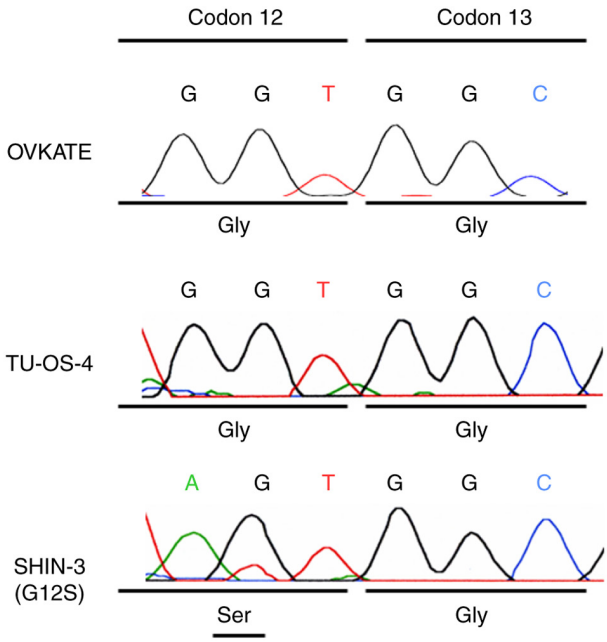

Figure 1. KRAS gene mutation status in ovarian cancer cell lines. KRAS mutations were analyzed in OVKATE, TU-OS-4 and SHIN-3 cell lines. Genomic DNA was extracted and the KRAS codon 12 in exon 2 was sequenced. No mutations were detected in OVKATE or TU-OS-4, whereas SHIN-3 harbors a G12S mutation. Ser, serine; Gly, glycine.

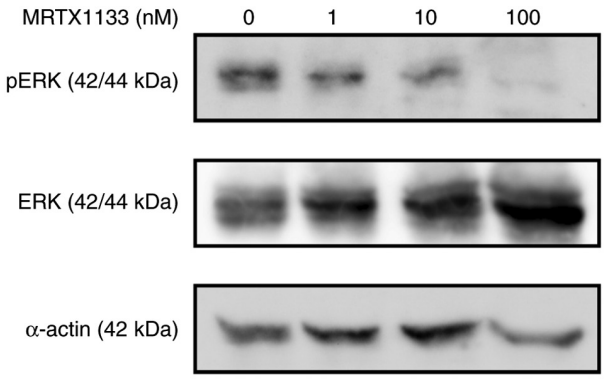

Figure 2. MRTX1133 inhibits ERK phosphorylation in KRAS (G12D)-mutant MCAS cells. MCAS cells were incubated with MRTX1133 at 0-100 nM for 3 h in DMEM/F12 containing 10% FBS. Protein lysates were prepared using NP-40 lysis buffer and subjected to SDS-PAGE. ERK phosphorylation was detected by western blotting using anti-phospho-ERK and anti-ERK antibodies, followed by HRP-conjugated secondary antibodies. The levels of phosphorylated ERK were normalized to total ERK. α-actin was used as a loading control. MRTX1133 reduced ERK phosphorylation in a concentration-dependent manner.

inhibitor Z-VAD-FMK (Abcam), caspase-1 specific inhibitor Z-YVAD-FMK (Selleck Chemicals), ferroptosis inhibitor ferrostatin-1 (Cayman Chemical Company) or necroptosis inhibitor necrostatin-1 (Abcam) at concentrations of 100 μM at 37˚C. After 1 h, the medium was removed and cancer cells were exposed to MRTX1133 (100 nM) for 48 h at 37˚C. Viable cell count was measured as aforementioned and expressed as a percentage of the control untreated with MRTX1133 and the programmed cell death inhibitors.

Reverse transcription-quantitative PCR (RT-qPCR). Next, the expression levels of numerous cell cycle-related genes were analyzed. MCAS cells ($5x10^5$ cells/well) seeded into a six-well plate were exposed to MRTX1133 at concentrations of 100 nM for 24 h at 37˚C. Cellular mRNA was extracted using the RNeasy Mini Kit (Qiagen, Inc.) according to the manufacturer's instructions. Reverse transcription and subsequent qPCR were performed using the One Step TB Green PrimeScript RT-PCR Kit II (Perfect Real Time; cat. no. RR066A; Takara Bio, Inc.), which includes TB Green as the fluorophore, according to the manufacturer's protocol. RT-qPCR was performed using the Thermal Cycler Dice Real Time System II (Takara Bio, Inc.) following the manufacturer's instructions. PCR was performed using 40 cycles of heating at 95˚C for 15 sec, 58˚C for 15 sec and 72˚C for 20 sec. mRNA levels were determined relative to the fluorescence signal level of GAPDH using the comparative $2^{-\Delta\Delta Cq}$ method (22). Primer sequences are shown in Table I.

Statistical analysis. Statistical analysis was performed using EZR software, version 4.5.2 (Saitama Medical Center; Jichi Medical University). Data for cell viability and mRNA expression assays are presented as the mean ± SD of three independent experiments. Unpaired Student's t-tests were used for comparisons between two groups. For comparisons involving multiple groups Bonferrnoi correction was applied.

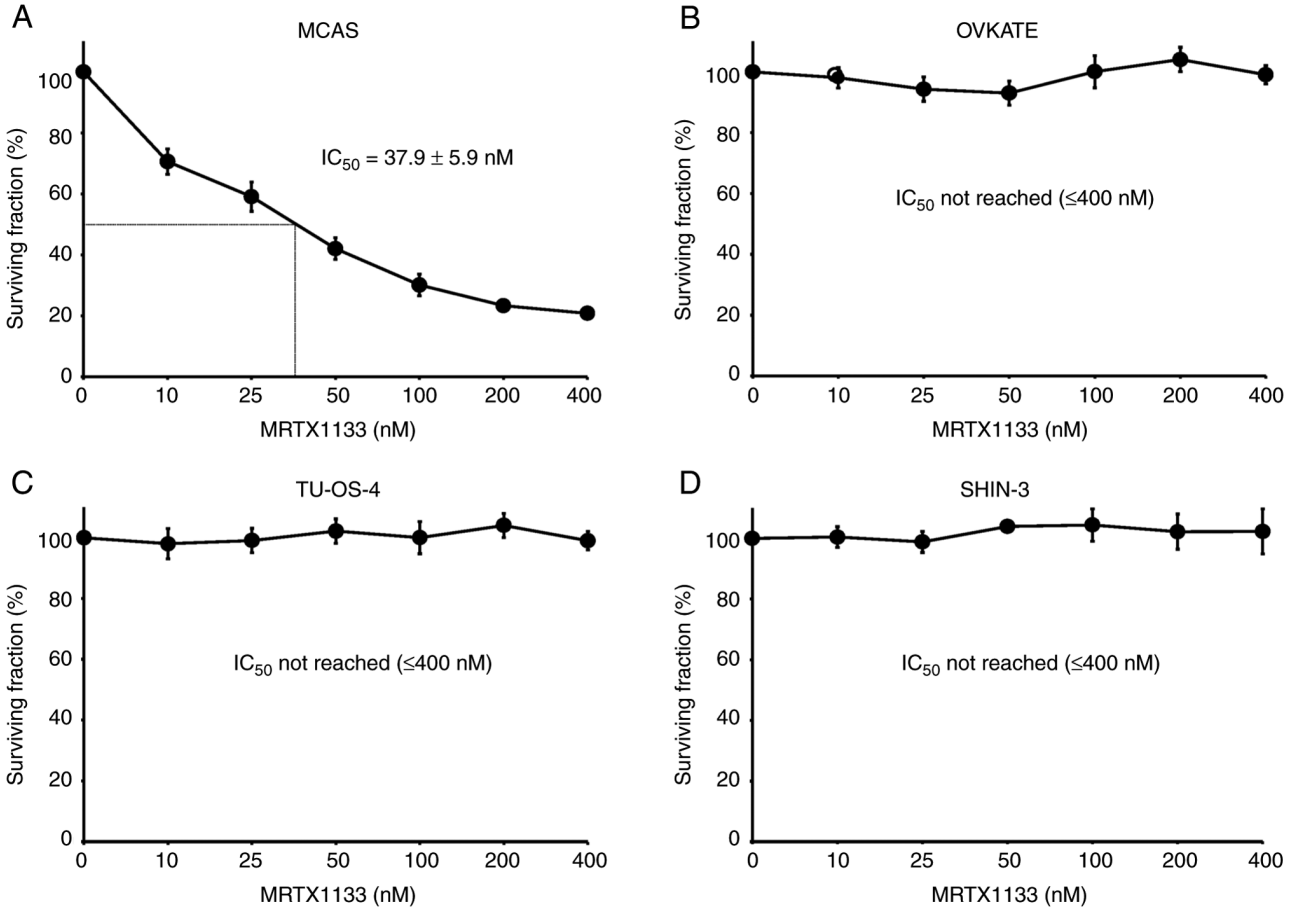

Figure 3. Selective proliferation inhibition of KRAS (G12D)-mutant MCAS cells by MRTX1133. (A) MCAS, (B) OVKATE, (C) TU-OS-4 and (D) SHIN-3 cells were seeded at 500 cells/well in 96-well plates and treated with 10-400 nM MRTX1133 for 72 h. Cell viability was measured using a water soluble tetrazolium salt-1 colorimetric assay and normalized to untreated controls. The IC_{50} was determined as the drug concentration required to reduce cell viability by 50%. MRTX1133 reduced the viability of MCAS cells, with an IC_{50} of 37.9±5.9 nM, whereas no IC_{50} was reached for OVKATE, TU-OS-4 or SHIN-3 cells within the tested concentration range. Data are presented as the mean ± SD (n=3).

P<0.01 was considered to indicate a statistically significant difference.

Results

KRAS mutation status in ovarian cancer cell lines. In each ovarian cancer cell line the KRAS gene status was examined. While no mutations at codon 12 in exon 2 were detected in OVKATE or TU-OS-4 cells, a single point G12S mutation, GGT (glycine) to AGT (serine) was identified in SHIN-3 cells at codon 12 in exon 2 of the KRAS gene (Fig. 1). In our previous study, the KRAS (G12D) gene mutation, in which codon 12 is substituted from GGT (glycine) to GAT (aspartic acid), was detected in the MCAS cell line (21).

Suppression of ERK phosphorylation by MRTX1133 in KRAS (G12D)-mutant MCAS cells. Treatment with MRTX1133 led to a concentration-dependent suppression of ERK phosphorylation in MCAS cells (Fig. 2), indicating effective inhibition of the MAPK pathway by MRTX1133 in KRAS (G12D)-mutant cells.

Selective proliferation inhibition of KRAS (G12D)-mutant cells by MRTX1133. Sensitivity results of each ovarian cancer cell line to MRTX1133 are presented in Fig. 3. MRTX1133 exhibited a concentration-dependent proliferation inhibitory effect exclusively in MCAS cells, which harbor the KRAS (G12D) mutation, with an IC_{50} value of 37.9±5.9 nM. By contrast, the proliferation of OVKATE, TU-OS-4 and SHIN-3 cells was unaffected and IC_{50} values were not reached within the tested concentration range (up to 400 nM), indicating that MRTX1133 selectively suppressed the proliferation of KRAS (G12D)-mutant MCAS cells.

Attenuation of cell cycle-dependent chemotherapeutic efficacy by MRTX1133. Chemosensitivity results of MCAS cells to anticancer agents, with or without MRTX1133, are shown in Fig. 4. The IC_{50} values for PTX, SN38 (the active metabolite of irinotecan) and GEM significantly increased in the presence of MRTX1133: PTX IC_{50} increased from 10.8±3.1 to 30.9±1.1 nM (2.9-fold), SN38 IC_{50} increased from 0.2±0.1 to 1.4±0.2 μM (7.0-fold) and GEM IC_{50} increased from 0.8±0.0 to 3.3±0.6 μM (4.1-fold). By contrast, no significant change was observed for CDDP (9.4±1.3 μM without MRTX1133 vs. 7.6±1.5 μM with MRTX1133). Collectively, these results indicated that MRTX1133 significantly increased the IC_{50} values of cell cycle-dependent chemotherapeutic agents (PTX, SN38 and GEM), while the efficacy of the non-cell cycle-dependent agent CDDP remained unaffected.

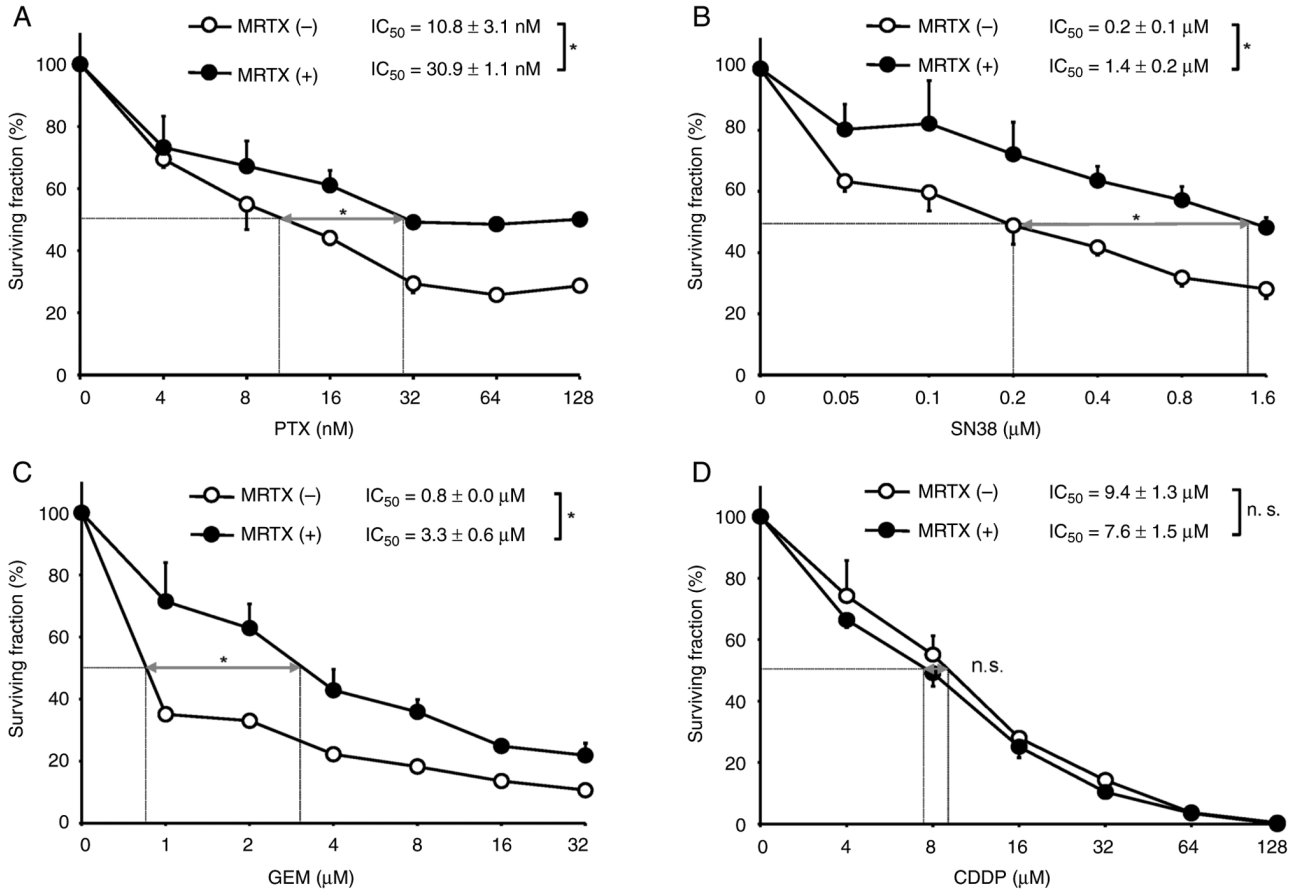

Figure 4. MRTX1133 differentially modulates chemosensitivity in MCAS cells. MCAS cells were incubated with (A) PTX (4-128 nM), (B) SN38 (0.05-1.6 μM), (C) GEM (1-32 μM) or (D) CDDP (4-128 μM) for 72 h, with or without 100 nM MRTX1133. Viability was measured using a water soluble tetrazolium salt-1 assay and IC_{50} was calculated. MRTX1133 significantly increased the IC_{50} of cell cycle-dependent drugs (PXT, SN38 and GEM) but not that of CDDP. Data are presented as the mean ± SD (n=3). *P<0.01. n.s., not significant; PTX, pixatel; GEM, gemcitabine; CDDP, cisplatin.

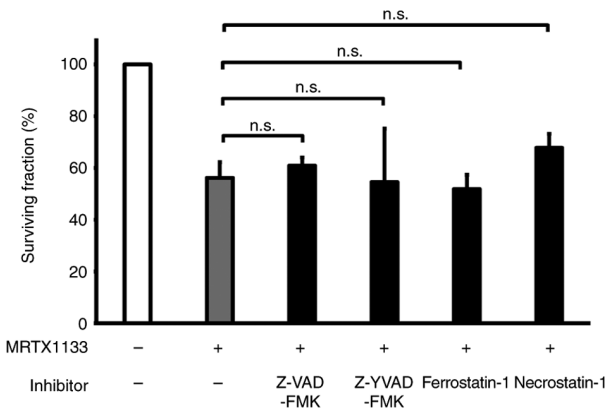

Figure 5. MRTX1133-mediated proliferation inhibition is not reversed by inhibition of programmed cell death pathways. MCAS cells were pretreated with inhibitors of apoptosis (Z-VAD-FMK, pan-caspase inhibitor), pyroptosis (Z-YVAD-FMK, caspase-1 inhibitor), ferroptosis (ferrostatin-1, lipid peroxidation inhibitor) or necroptosis (necrostatin-1, RIPK1 inhibitor) at 100 μM for 1 h, followed by exposure to 100 nM MRTX1133 for 48 h. Cell viability was measured using a water soluble tetrazolium salt-1 assay. None of the inhibitors restored cell viability. Data are presented as the mean ± SD (n=3). n.s., not significant.

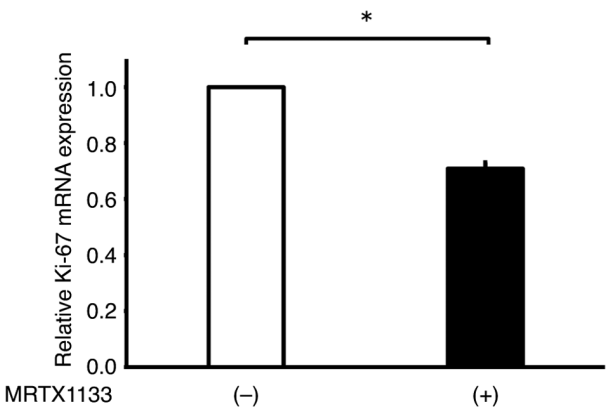

Figure 6. MRTX1133 suppresses Ki-67 mRNA expression in MCAS cells. MCAS cells were incubated with 100 nM MRTX1133 for 24 h and Ki-67 mRNA expression was quantified using reverse transcription-quantitative PCR. Total RNA was extracted using the RNeasy Mini Kit, reverse transcribed and amplified using specific primers. Expression levels were normalized to GAPDH. MRTX1133 significantly reduced Ki-67 mRNA levels. Data are presented as the mean ± SD (n=3). *P<0.01.

Limited evidence for programmed cell death following MRTX1133 treatment. To determine whether programmed cell death contributed to MRTX1133-mediated proliferation inhibition, MCAS cells were incubated with specific inhibitors of apoptosis, pyroptosis, ferroptosis and necroptosis. Results indicated that none of these inhibitors significantly restored cell viability following MRTX1133 treatment (Fig. 5), suggesting

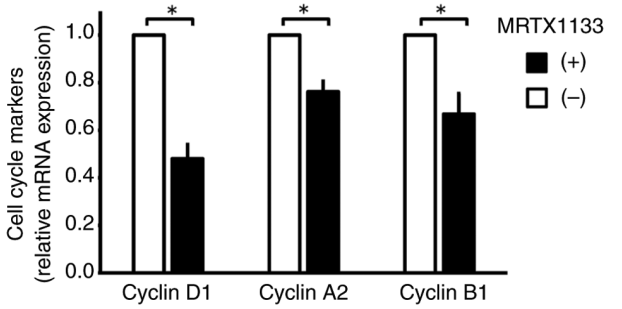

Figure 7. MRTX1133 downregulates phase-specific cyclin mRNA expression in MCAS cells. MCAS cells were incubated with 100 nM MRTX1133 for 24 h. Cyclin D1 (G_1 phase), cyclin A2 (S/G_2 phase) and cyclin B1 (G_2/M phase) mRNA levels were measured using reverse transcription-quantitative PCR with GAPDH normalization. MRTX1133 reduced the mRNA expression of all three cyclins. Data are presented as the mean ± SD (n=3). *P<0.01.

that robust activation of these programmed cell death pathways was not observed under the experimental conditions.

Suppression of proliferation- and cell cycle-related mRNA expression by MRTX1133. Next, the expression of proliferation- and cell cycle-associated markers was evaluated. Ki-67 is a well-established proliferation marker (23,24) expressed in all active phases of the cell cycle (G_1, S, G_2 and M), but not in quiescent cells (G_0) (25). In the present study, MRTX1133 reduced Ki-67 mRNA expression in MCAS cells (Fig. 6). The expression of phase-specific cyclins was next analyzed with results indicating that cyclins D1, A2 and B1 were upregulated in G_1, S/G_2, and G_2/M phases, respectively (26). MRTX1133 treatment decreased the mRNA expression of all three cyclins (Fig. 7).

Discussion

OMC, which frequently harbors KRAS mutations and shows poor response to platinum- and taxane-based chemotherapy (6-9,27), remains a malignancy with limited therapeutic options, highlighting the need for novel strategies. In the present study, it was shown that MRTX1133 selectively inhibited the proliferation of KRAS (G12D)-mutant OMC cells. Mechanistically, MRTX1133 suppressed ERK phosphorylation in a concentration-dependent manner, suggesting that the anti-proliferative effect was at least partly mediated through MAPK pathway inhibition. Given that KRAS (G12D) mutations have been studied in diverse solid tumors, including pancreatic, colorectal, bile duct and endometrial cancers (11), the present findings in OMC further extend the potential applicability of KRAS (G12D)-targeted therapy to this rare ovarian cancer subtype. The mutation-selective effects observed in the present study are consistent with previous reports demonstrating the specificity of MRTX1133 for KRAS (G12D), supporting the interpretation that the observed proliferation inhibition is primarily attributable to on-target KRAS (G12D) blockade (13,28). Given that KRAS activates a number of downstream pathways, including PI3K/Akt/mTOR signaling, ERK phosphorylation was used as a representative and widely accepted pharmacodynamic readout of MAPK signaling inhibition (13,29,30). Although total ERK protein levels increased following MRTX1133 treatment, perhaps reflecting compensatory feedback regulation within the MAPK pathway (31), ERK phosphorylation was suppressed, supporting inhibition of ERK phosphorylation and downstream MAPK signaling activity.

In lung cancer, KRAS (G12C) mutations are found in 7% of cases (11). The KRAS (G12C)-specific inhibitor sotorasib has demonstrated efficacy in patients previously treated with chemotherapy or immune checkpoint inhibitors (32) and is currently being used in clinical settings (33,34). These clinical successes of mutation-specific KRAS inhibition collectively support the overall feasibility of targeting oncogenic KRAS and provide a conceptual and preliminary rationale for the development of KRAS (G12D)-directed therapies for OMC. Notably, KRAS (G12D)-negative ovarian cancer cell lines were unaffected by MRTX1133 in the present study, suggesting potential selectivity for the G12D mutation. This observation aligns with the mutation-specific profiles reported for KRAS (G12C) inhibitors (34-36), such as sotorasib, which are known for their relatively manageable safety profiles in clinical settings (13,32). While the biochemical properties of KRAS (G12D) and KRAS (G12C) differ and the clinical safety of KRAS (G12D) inhibitors remains to be fully established, the present *in vitro* data suggest a limited impact on KRAS wild-type cells. This selectivity could potentially translate into a wider therapeutic window and support the rationale for combination strategies with conventional cytotoxic agents, including platinum-based and taxane-based agents. Such combinations may be particularly beneficial for regimens limited by overlapping hematological toxicities; however, *in vivo* validation would be required to assess systemic safety.

The combination of MRTX1133 with chemotherapeutic agents commonly used in ovarian cancer was evaluated. While MRTX1133 did not affect the activity of CDDP, a non-cell cycle-dependent agent, it reduced sensitivity to agents whose efficacy depends on active cell cycle progression such as PTX, SN38 and GEM. To explore the underlying mechanisms, numerous forms of programmed cell death, including apoptosis, pyroptosis, ferroptosis and necroptosis, were evaluated using specific inhibitors in the present study (37,38). Inhibition of these pathways did not restore cell viability following MRTX1133 treatment, suggesting that overt activation of canonical programmed cell death pathways was unlikely to be the predominant mechanism of proliferation suppression under the experimental conditions tested, which may instead occur through alternative mechanisms such as cell cycle suppression.

Next, markers associated with cell cycle regulation were examined. In the present study, it was found that MRTX1133 reduced Ki-67 expression in KRAS (G12D)-mutant OMC cells, indicating diminished proliferative activity. In addition, the mRNA expression of phase-associated cyclins was assessed; cyclins D1, A2 and B1 are predominantly associated with the G_1, S/G_2 and G_2/M phases, respectively (26). Consistent with the reduced Ki-67 expression, MRTX1133 treatment resulted in coordinated downregulation of these cyclins at the mRNA level, suggesting broad attenuation of cell cycle-related transcriptional programs rather than discrete phase-specific blockade.

Chemotherapeutic agents are classified as cell cycle-dependent and non-cell cycle-dependent. PTX, SN38

and GEM are cell cycle-dependent, whereas CDDP is non-cell cycle-dependent (39). MRTX1133 reduced the sensitivity of KRAS (G12D)-mutant OMC cells to PTX, SN38 and GEM, but not to CDDP. These results indicated that reduced proliferative activity induced by MRTX1133 may attenuate the effects of cell cycle-dependent chemotherapeutic agents while minimizing the effects on non-cell cycle-dependent agents. As a DNA-crosslinking agent, CDDP likely maintains its efficacy regardless of cell cycle status. Therefore, concurrent administration of MRTX1133 with cell cycle-dependent agents may be counterproductive and sequential administration (cytotoxic chemotherapy followed by KRAS inhibition) could potentially maximize efficacy while minimizing antagonism. From a scheduling perspective, sequential administration may be preferable, in which cell cycle-dependent cytotoxic agents are given during active proliferation, followed by MRTX1133 to suppress residual tumor growth through sustained KRAS pathway inhibition. Such strategies warrant systematic evaluation in future *in vivo* studies to determine optimal timing and therapeutic benefit.

Acquired and intrinsic resistance to KRAS-targeted therapies also represents an important challenge in clinical translation. Clinical experience with KRAS (G12C) inhibitors, such as sotorasib, has revealed certain mechanisms including secondary KRAS mutations, MAPK pathway reactivation and bypass signaling through receptor tyrosine kinases or alternative oncogenic drivers. Reported alterations include MET amplification, activating mutations in NRAS, BRAF, MAP2K1 and RET, oncogenic fusions involving ALK, RET, BRAF, RAF1 and FGFR3, and loss-of-function mutations in NF1 and PTEN (40). Although resistance to KRAS (G12D) inhibitors remains poorly characterized, similar adaptive responses may emerge, underscoring the need for combination approaches and longitudinal molecular monitoring in future studies.

The present study exhibits certain limitations. First, all experiments were conducted *in vitro* and primarily relied on a single KRAS (G12D)-mutant ovarian cancer cell line MCAS. To the best of our knowledge, MCAS is currently the only available OMC cell line harboring this specific mutation. Although this model provides a valuable platform for investigating KRAS (G12D)-targeted therapy, it does not fully recapitulate the tumor microenvironment, systemic drug exposure or intertumoral heterogeneity observed *in vivo*. Therefore, validation in additional models, including patient-derived samples, organoids or xenograft systems, are necessary to further determine therapeutic efficacy and safety.

Second, formal genetic rescue experiments were not performed to further validate target specificity. While overexpression of wild-type KRAS or a drug-resistant KRAS mutant would provide additional mechanistic demonstration, such experiments require stable genetic manipulation and extensive optimization. Given that the primary aim of the present study was to evaluate the biological consequences of pharmacological KRAS (G12D) inhibition rather than to re-establish the biochemical specificity of MRTX1133 (already extensively validated in previous studies) (13,28), the present study instead relied on mutation-selective proliferation responses across numerous ovarian cancer cell lines. Despite this, the absence of genetic rescue experiments represents a limitation and should be addressed in future mechanistic studies.

Third, evidence for cell cycle suppression was primarily based on transcriptional changes in proliferation- and cell cycle-associated genes. Functional analysis of cell cycle distribution by flow cytometry was not feasible due to the biological characteristics of the MCAS cell line. MCAS cells exhibit marked cell-cell adhesion and abundant extracellular matrix production (18,41,42), with repeated attempts to generate single-cell suspensions requiring extensive enzymatic and mechanical dissociation. These procedures have consistently resulted in notable debris formation and persistent cell aggregates, which severely compromise DNA histogram resolution and prevent reliable flow cytometric analysis, including PI staining-based cell cycle analysis. In addition, our repeated preliminary western blotting analyses (18) of a number of key cell cycle regulators, including cyclin D1 and cyclin A2, yielded inconsistent or non-specific signals despite optimization efforts, precluding robust protein-level validation. Consequently, the present findings supported transcriptional suppression of proliferative signaling but did not establish definitive protein-mediated or phase-specific cell cycle arrest.

Fourth, direct biochemical assays of apoptosis were not performed. In the present study, programmed cell death was primarily evaluated using pharmacological inhibitors targeting numerous pathways. However, inhibitor-based approaches alone cannot definitively exclude apoptosis or other forms of cell death. Under the experimental conditions tested, MRTX1133 induced sustained proliferation inhibition consistent with a predominantly cytostatic phenotype, without clear evidence of cytotoxicity. Previous studies have shown that MRTX1133 markedly suppresses KRAS-MAPK signaling and tumor cell proliferation in KRAS (G12D)-mutant models, a finding that supports a predominantly cytostatic response *in vitro* (13,28). Accordingly, biochemical assays of apoptosis were not prioritized in the present study. Nevertheless, the absence of direct biochemical assays, such as caspase-3/7 activity or detection of cleaved caspase-3 and PARP, represents a limitation, therefore low-level or delayed apoptotic signaling cannot be completely excluded.

Finally, although the reduced sensitivity to cell cycle-dependent chemotherapeutic agents observed after MRTX1133 treatment may partly reflect suppression of proliferative signaling, alternative mechanisms of drug interaction were not directly investigated. In particular, KRAS signaling has been reported to modulate the expression and activity of drug efflux transporters, including ATP-binding cassette (ABC) transporters, such as ABCB1 and ABCG2 (43). These transporters can reduce intracellular drug accumulation by actively exporting cytotoxic agents from cancer cells, thereby decreasing effective intracellular drug concentrations and attenuating cytotoxicity (43). In addition, clinical and preclinical work on KRAS inhibitors has highlighted a range of potential adaptive and bypass signaling mechanisms, including MAPK reactivation and signaling through alternative oncogenic drivers, which have been implicated in resistance to KRAS-targeted therapies (44). While these mechanisms primarily relate

to resistance to KRAS inhibition itself, such adaptive signaling changes may conceivably interact with cellular responses to cytotoxic agents when used in combination. Comprehensive evaluation of these mechanisms would require additional approaches, such as transporter expression profiling, intracellular drug accumulation assays and metabolomic analyses, which were beyond the scope of the present study.

Despite these limitations, the present findings provided initial mechanistic insights into the biological effects of KRAS (G12D) inhibition in OMC and support further investigation of KRAS (G12D)-targeted therapeutic strategies in this rare malignancy.

In summary, MRTX1133 selectively reduced proliferation of KRAS (G12D)-mutant OMC cells accompanied by inhibition of ERK phosphorylation and downstream MAPK signaling activity. These findings provide preclinical evidence for the potential utility of KRAS (G12D)-targeted therapy in OMC and suggest that treatment scheduling may influence the efficacy of combination strategies with cell cycle-dependent chemotherapy. Given the clinical investigation of KRAS (G12D) inhibitors, including the phase I/II trial of MRTX1133 (NCT05737706), the present results support further exploration of this therapeutic approach in OMC. Further *in vivo* and clinical studies are required to fully realize the therapeutic potential of this approach and to guide the development of precision therapeutic strategies for KRAS (G12D)-driven OMC.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

YS and RW performed the experiments, analyzed the data and wrote the manuscript. YS and HM conceived and designed the present study, contributed to the concept, analyzed the results and revised the manuscript. All authors read and approved the final version of the manuscript. YS and RW confirm the authenticity of all the raw data.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Siegel RL, Kratzer TB, Giaquinto AN, Sung H and Jemal A: Cancer statistics, 2025. CA Cancer J Clin 75: 10-45, 2025.
2. Cannistra SA: Cancer of the ovary. N Engl J Med 351: 2519-2529, 2004.
3. Banerjee S and Gore M: Recent advances in systemic treatments for ovarian cancer. Cancer Imaging 12: 305-309, 2012.
4. McGuire V, Jesser CA and Whittemore AS: Survival among U.S. women with invasive epithelial ovarian cancer. Gynecol Oncol 84: 399-403, 2002.
5. Chan JK, Teoh D, Hu JM, Shin JY, Osann K and Kapp DS: Do clear cell ovarian carcinomas have poorer prognosis compared to other epithelial cell types? A study of 1411 clear cell ovarian cancers. Gynecol Oncol 109: 370-376, 2008.
6. Hess V, A'Hern R, Nasiri N, King DM, Blake PR, Barton DP, Shepherd JH, Ind T, Bridges J, Harrington K, *et al*: Mucinous epithelial ovarian cancer: A separate entity requiring specific treatment. J Clin Oncol 22: 1040-1044, 2004.
7. Pectasides D, Fountzilas G, Aravantinos G, Kalofonos HP, Efstathiou E, Salamalekis E, Farmakis D, Skarlos D, Briasoulis E, Economopoulos T and Dimopoulos MA: Advanced stage mucinous epithelial ovarian cancer: The hellenic cooperative oncology group experience. Gynecol Oncol 97: 436-441, 2005.
8. Shimada M, Kigawa J, Ohishi Y, Yasuda M, Suzuki M, Hiura M, Nishimura R, Tabata T, Sugiyama T and Kaku T: Clinicopathological characteristics of mucinous adenocarcinoma of the ovary. Gynecol Oncol 113: 331-334, 2009.
9. Bamias A, Psaltopoulou T, Sotiropoulou M, Haidopoulos D, Lianos E, Bournakis E, Papadimitriou C, Rodolakis A, Vlahos G and Dimopoulos MA: Mucinous but not clear cell histology is associated with inferior survival in patients with advanced stage ovarian carcinoma treated with platinum-paclitaxel chemotherapy. Cancer 116: 1462-1468, 2010.
10. Konstantinopoulos PA and Matulonis UA: Clinical and translational advances in ovarian cancer therapy. Nat Cancer 4: 1239-1257, 2023.
11. Li S, Balmain A and Counter CM: A model for RAS mutation patterns in cancers: Finding the sweet spot. Nat Rev Cancer 18: 767-777, 2018.
12. Suzuki M, Saito S, Saga Y, Ohwada M and Sato I: Mutation of K-RAS protooncogene and loss of heterozygosity on 6q27 in serous and mucinous ovarian carcinomas. Cancer Genet Cytogenet 118: 132-135, 2000.
13. Hallin J, Bowcut V, Calinisan A, Briere DM, Hargis L, Engstrom LD, Laguer J, Medwid J, Vanderpool D, Lifset E, *et al*: Antitumor efficacy of a potent and selective non-covalent KRASG12D inhibitor. Nat Med 28: 2171-2182, 2022.
14. Kataoka M, Kitazawa M, Nakamura S, Koyama M, Yamamoto Y, Miyazaki S, Hondo N, Tanaka H and Soejima Y: Cetuximab enhances the efficacy of MRTX1133, a novel KRAS(G12D) inhibitor, in colorectal cancer treatment. Anticancer Res 43: 4341-4348, 2023.
15. Wei D, Wang L, Zuo X, Maitra A and Bresalier RS: A small molecule with big impact: MRTX1133 targets the KRASG12D mutation in pancreatic cancer. Clin Cancer Res 30: 655-662, 2024.
16. Wang Y, Liu L and Yu Y: Mucins and mucinous ovarian carcinoma: Development, differential diagnosis, and treatment. Heliyon 9: e19221, 2023.
17. Meagher NS, Hamilton P, Milne K, Thornton S, Harris B, Weir A, Alsop J, Bisinoto C, Brenton JD, Brooks-Wilson A, *et al*: Profiling the immune landscape in mucinous ovarian carcinoma. Gynecol Oncol 168: 23-31, 2023.
18. Saga Y, Takei Y, Fujiwara H and Suzuki M: Treatment of clear cell adenocarcinoma and mucinous adenocarcinoma. Nihon Rinsho 70 (Suppl 4): S634-S637, 2012 (In Japanese).
19. Imai S, Kiyozuka Y, Maeda H, Noda T and Hosick HL: Establishment and characterization of a human ovarian serous cystadenocarcinoma cell line that produces the tumor markers CA-125 and tissue polypeptide antigen. Oncology 47: 177-184, 1990.

20. Itamochi H, Kato M, Nishimura M, Oishi T, Shimada M, Sato S, Naniwa J, Sato S, Nonaka M, Kudoh A, *et al*: Establishment and characterization of a novel ovarian serous adenocarcinoma cell line, TU-OS-4, that overexpresses EGFR and HER2. Hum Cell 25: 111-115, 2012.
21. Sato N, Saga Y, Mizukami H, Wang D, Fujiwara H, Takei Y, Machida S, Ozawa K and Suzuki M: Cetuximab inhibits the growth of mucinous ovarian carcinoma tumor cells lacking KRAS gene mutations. Oncol Rep 27: 1336-1340, 2012.
22. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25: 402-408, 2001.
23. Gerdes J, Lemke H, Baisch H, Wacker HH, Schwab U and Stein H: Cell cycle analysis of a cell proliferation-associated human nuclear antigen defined by the monoclonal antibody Ki-67. J Immunol 133: 1710-1715, 1984.
24. Suzuki M, Tsukagoshi S, Saga Y, Ohwada M and Sato I: Assessment of proliferation index with MIB-1 as a prognostic factor in radiation therapy for cervical cancer. Gynecol Oncol 79: 300-304, 2000.
25. Gerdes J, Schwab U, Lemke H and Stein H: Production of a mouse monoclonal antibody reactive with a human nuclear antigen associated with cell proliferation. Int J Cancer 31: 13-20, 1983.
26. Hartwell LH and Kastan MB: Cell cycle control and cancer. Science 266: 1821-1828, 1994.
27. Gorringe KL, Cheasley D, Wakefield MJ, Ryland GL, Allan PE, Alsop K, Amarasinghe KC, Ananda S, Bowtell DDL, Christie M, *et al*: Therapeutic options for mucinous ovarian carcinoma. Gynecol Oncol 156: 552-560, 2020.
28. Wang X, Allen S, Blake JF, Bowcut V, Briere DM, Calinisan A, Dahlke JR, Fell JB, Fischer JP, Gunn RJ, *et al*: Identification of MRTX1133, a noncovalent, potent, and selective KRAS(G12D) inhibitor. J Med Chem 65: 3123-3133, 2022.
29. Ash LJ, Busia-Bourdain O, Okpattah D, Kamel A, Liberchuk A and Wolfe AL: KRAS: biology, inhibition, and mechanisms of inhibitor resistance. Curr Oncol 31: 2024-2046, 2024.
30. Li Q, Li Z, Luo T and Shi H: Targeting the PI3K/AKT/mTOR and RAF/MEK/ERK pathways for cancer therapy. Mol Biomed 3: 47, 2022.
31. Ryan MB, Fece de la Cruz F, Phat S, Myers DT, Wong E, Shahzade HA, Hong CB and Corcoran RB: Vertical pathway inhibition overcomes adaptive feedback resistance to KRAS(G12C) inhibition. Clin Cancer Res 26: 1633-1643, 2020.
32. Skoulidis F, Li BT, Dy GK, Price TJ, Falchook GS, Wolf J, Italiano A, Schuler M, Borghaei H, Barlesi F, *et al*: Sotorasib for lung cancers with KRAS p.G12C Mutation. N Engl J Med 384: 2371-2381, 2021.
33. Noordhof A, Asmara OD, de Jong K, Gijtenbeek RG, Huitema YM, van Vollenhoven FH, Venmans BJ, Hendriks L and van Geffen WH: KRAS G12C inhibitors versus chemotherapy in second line and beyond in adults with advanced or metastatic non-small cell lung cancer (NSCLC) harbouring the KRAS G12C mutation. Cochrane Database Syst Rev 2: CD016054, 2026.
34. de Langen AJ, Johnson ML, Mazieres J, Dingemans AC, Mountzios G, Pless M, Wolf J, Schuler M, Lena H, Skoulidis F, *et al*: Sotorasib versus docetaxel for previously treated non-small-cell lung cancer with KRAS(G12C) mutation: A randomised, open-label, phase 3 trial. Lancet 401: 733-746, 2023.
35. Liu J, Kang R and Tang D: The KRAS-G12C inhibitor: activity and resistance. Cancer Gene Ther 29: 875-878, 2022.
36. Tenekeci AK, Unal AA, Ceylan F and Nahit Sendur MA: An updated overview of K-RAS G12C inhibitors in advanced stage non-small cell lung cancer. Future Oncol 20: 3019-3038, 2024.
37. Tang D, Kang R, Berghe TV, Vandenabeele P and Kroemer G: The molecular machinery of regulated cell death. Cell Res 29: 347-364, 2019.
38. Lomphithak T and Fadeel B: Die hard: Cell death mechanisms and their implications in nanotoxicology. Toxicol Sci 192: 141-154, 2023.
39. Tulk A, Watson R and Erdrich J: The influence of statin use on chemotherapeutic efficacy in studies of mouse models: A systematic review. Anticancer Res 43: 4263-4275, 2023.
40. Awad MM, Liu S, Rybkin II, Arbour KC, Dilly J, Zhu VW, Johnson ML, Heist RS, Patil T, Riely GJ, *et al*: Acquired resistance to KRAS(G12C) inhibition in cancer. N Engl J Med 384: 2382-2393, 2021.
41. Klymenko Y, Kim O, Loughran E, Yang J, Lombard R, Alber M and Stack MS: Cadherin composition and multicellular aggregate invasion in organotypic models of epithelial ovarian cancer intraperitoneal metastasis. Oncogene 36: 5840-5851, 2017.
42. Valmiki S, Aid MA, Chaitou AR, Zahid M, Valmiki M, Fawzy P and Khan S: Extracellular matrix: A treasure trove in ovarian cancer dissemination and chemotherapeutic resistance. Cureus 13: e13864, 2021.
43. Robey RW, Pluchino KM, Hall MD, Fojo AT, Bates SE and Gottesman MM: Revisiting the role of ABC transporters in multidrug-resistant cancer. Nat Rev Cancer 18: 452-464, 2018.
44. Yang X and Wu H: RAS signaling in carcinogenesis, cancer therapy and resistance mechanisms. J Hematol Oncol 17: 108, 2024.