Appendix 1 Materials and methods

Cell lines

Human ovarian cancer cell lines OVCAR8 & OVCAR4 were obtained from the NIGMS Human Genetic Cell Repository of the NIH, Bethesda, Maryland USA, and kindly provided by Rolf Müller, Marburg. The pancreatic cancer cell line PANC1 was purchased from the Leibniz Institute DSMZ, German Collection of Microorganisms and Cell Cultures GmbH, Braunschweig, Germany (Number ACC 783). OVCAR4 and 8 were cultured in RPMI and PANC-1 in DMEM with 10% fetal calf serum and 1% penicillin/streptomycin at 37 ℃ and 5% CO_2.

Therapeutic agents

Cells were treated with 100 nM LBH589 (SEL-S1030, Selleck Chemicals) or 300 nM & 600 nM APTO253 (S6963, Selleck Chemicals) for 24 hours. Both therapeutic agents were dissolved in dimethyl sulfoxide (DMSO).

RNA isolation and reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) analysis

Total RNA extraction was done using TRIzol (Thermo Fisher Scientific), in combination with NucleoSpin RNA Kit (Macherey–Nagel). First-strand cDNA synthesis was performed using the RevertAid RT Kit (Thermo Fisher Scientific) according to the supplier's instructions. For *Figure 1* and *Figure 4*, real-time quantitative PCR reactions were carried out using SYBR Green (Thermo Fisher Scientific) and a Thermo Cycler Mx3005P (Stratagene). The $2^{-\Delta\Delta Ct}$ method was used to measure the expression level of the target gene and normalized to the housekeeping gene *RPL27*. All experiments were performed in at least three independent biological replicates. For *Figure 3*, the PCR reactions were carried out on a Q – qPCR Instrument (QuantaBio). The following primers were used: *MICA* forward (fw.) 5'-CTGCAGGAACTACGGCGATATCT-3', reverse (rev.) 5'-CCCTCTGAGGCCTCGCTG-3'; *KLF4* fw. 5'-GAAATTCGCCCGCTCCGATGA-3', rev. 5'-CTGTGTGTTTGCGGTAGTGCC-3'; *c-MYC* fw. 5'-CCTCCACTCGGAAGGACTATC-3', rev. 5'-TGTTCGCCTCTTGACATTCTC-3'; *ULBP2* fw. 5'-TCATCATCCTCCCCTGCTTC-3', rev. 5'-AGACAGAAGGGCGAGTTTGA-3'; *ULBP5* fw. 5'-TGAGGACTTCTTGATGGGCA-3', rev. 5'-ATGAGGAGGCAGCAAAGGAT-3'; *ULBP6* fw. 5'-GGAGACTGCATAGGATGGCT-3', rev. 5'-ATGAGGAGGCAGCAAAGGAT-3'; *MICB* fw. 5'-TCTCTGTGTCCCTTGTTGCA-3', rev. 5'-GCTGTAGAGTCTAGGTGCCC-3'; *GAPDH* fw. 5'-TGCACCACCAACTGCTTAGC-3', rev. 5'-GGCATGGACTGTGGTCATGAG-3'; *RPL27* fw. 5'-AAAGCTGTCATCGTGAAGAAC-3', rev. 5'-GCTGTCACTTTGCGGGGGTAG-3', and *U6* fw. 5'-CTCGCTTCGGCAGCACATA-3', rev. 5'-GCTTCACGAATTTGCGTGTCA-3'. For *Figure 3*, the relative expression level was calculated by the $E^{-\Delta\Delta Ct}$ method in which *GAPDH*, *RPL27*, and *U6* were used for normalization.

Flow cytometry-based detection of MIC proteins

1.5×10^5 cells were seeded in 6-well plates. The next day, cells were treated with LBH589 or APTO253 for 24 hours. The cells were harvested and incubated with 1 µL MICA/B-Alexa Fluor® 647 (BioLegend, Clone#6D4, 320914) in MACS buffer for 30 min on ice in the dark. Cells were washed with 1 mL MACS buffer and then measured by flow cytometry (BD Canto II) and analyzed using FlowJo 10.8.1 software (Becton Dickinson & Company).

https://dx.doi.org/10.21037/atm-24-20