MUS81 Participates in the Progression of Ovarian Cancer Associated with Dysfunctional DNA Repair Systems

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Introduction: Ovarian cancer (OC) is characterized by genome instability and heterogeneity. Novel insights into OC are required to reduce mortality rates and drug resistance. MUS81 is a structure-special endonuclease that plays important roles in DNA damage repair systems and the genome instability of cancer cells. Here, we found that MUS81 was involved in DNA damage repair pathway of ovarian cancer cells and associated with the susceptibility to HR inhibitors.

Methods: qRT-PCR was used to investigated MUS81 expression in OC and matched adjacent tissues (n=43). To evaluate the status of genome instability and DNA damage response in OC, RAPD analysis and comet assays were performed along with immunofluorescence analysis of proteins involved in DNA damage repair. Transcriptional profile analysis and protein interaction screening Chip was used to explore the potential pathways MUS81 involved. Experiments in vitro and vivo were performed to assess the sensitivity to camptothecin and HR inhibitors.

Result 1. Abnormal MUS81 expression correlates with malignant features in human OC and involves genomic instability

Result 2. Identifying interactions between MUS81 and cell cycle-related proteins

Result 3. Silencing MUS81 enhances dysfunction of the DNA repair system

Result 4. The combination of MUS81 and RAD51 suppression impacts the sensitivity of OC cells to PARP inhibitors

Fig 1. Abnormal MUS81 expression correlates with malignant features of human OC and involves genomic instability. A, MUS81 expression levels in matched non-tumor tissues were detected by qRT-PCR. The pie chart shows the proportion of OC samples showing up-regulation (red), no change (green) and down-regulation (blue). B, The level of MUS81 protein expression in transduced SKOV3 and HO8910 cells was determined by western blot. C, Genomic instability in OC was assessed by RAPD analysis. Band pattern changes in OC cells: 1: band missing, 2: band intensity change, 3: band shift; C, Control group, K.MUS81 knockdown group. (a-f) representative images of different primers. D, The expression profile of MUS81 in MUS81-Con (CON) and MUS81-KD1 (MUS-KD1) cells. E, Nucleotide excision repair, mismatch repair and homologous recombination (HR) etc pathways are involved in Signal Path-Net analysis.

Fig 2. Interactions between MUS81 and proteins involved in cell cycle progression. A, The cell cycle model of the Cell Cycle AntibodyArray™. B, The Cell Cycle AntibodyArray™ results. Proteins captured on the array were detected by immunoblotting, and the amount of target proteins was calculated by gray scanning. C, The association of MUS81 and BM28 was confirmed by western blot (I) and qRT-PCR (II), data are presented as the mean ± SD of three independent experiments (*P<0.05). D, The potential interaction of MUS81 and BM28 was analyzed by Discover Studio.

Fig 3. Silencing MUS81 involved in the dysfunction of the DNA repair system. A, MUS81 is involved in DNA repair system dysfunction. MUS81 was upregulation after UV exposure by immunofluorescence (I) and western blot (II) assay. B, Silencing MUS81 restrains the DNA repair system according to the comet assay. C, MUS81 knockdown affected cell cycle distribution after UV exposure. Cell cycle distribution was assessed by flow cytometry. Data are presented as the mean ± SD of three independent experiments (*P<0.05, **P<0.01).

Fig 4. The interaction between MUS81 and RAD51 influenced OC cell sensitivity to DNA-damaging agents. A, The association of MUS81 and RAD51 expression was evaluated. (I) Silencing MUS81 resulted in decreased RAD51 expression, (II) RAD51 knockdown was accompanied by a downregulation of MUS81. B, The co-localization of MUS81 and RAD51. MUS81 was co-localized with RAD51 in the cell nuclei. C, The potential interaction between MUS81 and RAD51 was analyzed by Discover Studio. D, MUS81 suppression was involved in OC cells sensitivity to DNA damage. (I) Silencing MUS81 reduced HR efficiency; data are presented as the mean±SD of three independent experiments. (II) MUS81 knockdown enhanced enhanced OC cells sensitivity to the DNA-damaging agent Olaparib; (III) simultaneous suppression of MUS81 and RAD51 in the transduced OC cells was associated with around 70% of cells undergoing apoptosis, whereas 30-40% of MUS-KD cells and only 5% of controls (CON) had evidence of apoptosis (*P<0.05, **P<0.01).