Mitochondrial protein 18 (MTP18) plays a pro-apoptotic role in chemotherapy-induced gastric cancer cell apoptosis

SUPPLEMENTARY MATERIALS

Supplementary Figure 1: Doxorubicin induces apoptosis in gastric cancer cells. (A) and (B) Doxorubicin (DOX) exposure induces caspase-3 and PARP1 cleavage. (A) AGS cells were stimulated with the indicated doses of DOX, and harvested after 12h for immunoblotting. (B) AGS cells were stimulated with 1μmol/L DOX and then harvested at the indicated time for immunoblotting. (A) and (B) (up panels) Show caspase-3 and PARP1 cleavage. β-actin served as a loading control. (A) and (B) (low panels) Show densitometry analysis. The densitometry data were expressed as the mean±SEM of three independent experiments. (C) AGS cells were stimulated with 1μmol/L DOX and then harvested at the indicated time for detection of apoptosis related DNA fragmentation using the cell death detection ELISA. (C) shows percentage of apoptosis related DNA fragmentation. Data were expressed as the mean±SEM of three independent experiments. *P<0.05, **P<0.01, ***P<0.001, and ****P<0.0001 compared to non-treated control.
Supplementary Figure 2: DRP1 (DNM1L) was predicted to be the MTP18 (MTFP1)'s target protein. Analysis of protein interaction using String v10 (http://string.embl.de/). (A) shows schematic representation of target protein interaction network of MTP18 (also known as MTFP1). (B) Shows the confidence interaction scores of potential functionally associated proteins. DRP1 (alternative name DNM1L) shows highest estimated confidence score among all of the potential functionally associated proteins. DRP1 or DNM1L= dynamic-related protein 1 or dynamin 1-like protein; MTP18 or MTFP1= mitochondrial protein 18 or mitochondrial fission protein 1.
Supplementary Figure 3: MTP18 promotes doxorubicin-induced DRP1 accumulation in mitochondria. (A) and (B) Densitometry analysis of DRP1 expression in mitochondria and cytosolic fraction. (A) Shows an increase in DRP1 accumulation in mitochondria upon enforced expression of MTP18 (correspond to immunoblot data of Figure 6C). (B) Shows a reduction in DRP1 accumulation when MTP18 was knocked down (correspond to immunoblot data of Figure 6D). Cytochrome c oxidase (COXIV) served as a loading control for mitochondria-enriched heavy membranes (HM) and β-actin served as a loading control for cytosolic fraction. Data were expressed as the mean±SEM of three independent experiments. *P<0.05, ***P<0.001, and ****P<0.0001
Supplementary Figure 4: Fluorouracil treatment downregulates MTP18 expression. (A-C) Densitometry analysis of MTP18 expression. (A) and (B) Show kinetics of MTP18 expression in AGS cell line (correspond to immunoblot data of Figure 8E) and (C) shows kinetics of MTP18 expression in NCI-N87 cell line (correspond to immunoblot data of Figure 8F) in response to 5-FU exposure. Cells were stimulated with indicated dose of fluorouracil (5-FU) and then harvested at the indicated time for immunoblotting. β-actin or tubulin served as a loading control. Data were expressed as the mean±SEM of three independent experiments. ***P<0.001, and ****P<0.0001 compared to non-treated.