SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Plasmids

Myc-DDK-tagged-human DDX1 expression plasmid was purchased from Origene (RC208769). Flag-tagged human Drosha expression plasmid was kindly provided by N. Kim at Seoul National University and pri-miR-16 and pri-miR-145 containing pmirGLO plasmids were kindly provided by S. Kawai at Osaka University. To generate pmirGLO-pri-miR-200a and -pri-miR-200b, pri-miR-200a and pri-miR-200b sequences were inserted into the 3′ untranslated region of firefly luciferase of pmirGLO vector (#E1330, Promega).

Antibodies

Anti-DDX1 (A300-521A), Anti-Drosha (A301-886A), Anti-DGCR8 (A302-468A) and anti-ATM (A300-299A) antibodies were purchased from Bethyl Laboratories. Anti-Rad50 (ab89) and anti-NBS1 (ab23996) antibodies were purchased from Abcam. Anti-E-Cadherin (24E10), anti-Vimentin (D21H3) and anti-ZEB1 (D80D3) and anti-Ki67 (D3B5) antibodies were purchased form Cell Signaling. Anti-Lamin B (sc-6216), anti-p53 (sc-126), anti-GAPDH (sc-48166), HRP-anti-goat IgG (#2020), HRP-anti-rabbit IgG (#2054) and HRP-anti-mouse IgG (#2055) antibodies were purchased from Santa Cruz.

Generation of stable DDX1 knocked-down cell lines

Lentiviral pGIPZ vector expressing control or DDX1 shRNAs were obtained from the MD Anderson shRNA and ORFome Core Facility (originally from Open Biosystem). Cells were infected with lentiviruses in the presence of polybrene (8 μg/ml). To establish stable knockdown cell line, lentiviral shRNA-transduced cells were selected with puromycin (2 μg/ml) 48h post-infection and individual colonies were propagated and validated for DDX1 expression by Western blotting (protein) and qRT-PCR (mRNA).

RNA isolation, qRT–PCR, and miRNA PCR array

Total RNA was isolated using Trizol reagent according to the manufacturer's instructions (Invitrogen) and was then reverse transcribed with a Universal cDNA Synthesis Kit II (Exiqon). cDNA was used for qPCR using SYBR Green master mix (Exiqon) on ABI4900 real-time PCR cycler. miRNA LNA PCR primer sets (Exiqon) and gene specific primers were used for

detecting miRNA and mRNA levels and data was normalized to internal control, U6 (miRNA) or GAPDH (mRNA). Genome-wide miRNA expression profiling analyses were performed using miRNA Ready-to-Use PCR arrays (Human panel I+II, V2.M, Exiqon) according to the manufacturers' instructions. The real-time PCR reactions were performed on ABI 7900HT and data pre-processing, normalization and statistical analysis were performed using Exiqon GenEx qPCR software. RT-PCR primers are shown in Supplemental Table S2.

RNA-binding assays

In vitro transcribed pri-miRNAs were incubated with GST or GST-DDX1 bound beads in a 20-µl reaction mixture that contained 10 mM Tris-HCl (pH 8.0), 25 mM KCl, 10 mM NaCl, 1 mM $MgCl_2$, 0.5 mM dithiothreitol and 10 % glycerol at 25 °C for 30 min. After washing beads three times, RNA was extracted with Trizol and quantified by RT-qPCR.

RIP (RNA immunoprecipitation) assay

RIP assay was performed as described previously (Zhang et al., 2011). Briefly, cells were crosslinked for 20 min with 1% formaldehyde, and cell pellets were resuspended in buffer B (1% SDS, 10 mM EDTA, 50 mM Tris-HCl (pH 8.1), protease inhibitor, 50 U/ml RNase inhibitor). After 10-min incubation in ice, the pellets were disrupted by sonication, and the lysates were subjected to immunoprecipitation with control IgG or anti-DDX1 or anti-Drosha antibody, followed by stringent washing, elution, and reversal of crosslinking. The RNA was resuspended in 20 µl of TE buffer (10 mM Tris-HCl, pH 7.5, 1 mM EDTA, 50 U/ml RNase inhibitor) and incubated with DNase I for 30 min at 37°C to remove any remaining DNA. After extraction with phenol:chloroform:isoamyl alcohol (25:24:1), RNA was precipitated with ethanol and dissolved in 20 µl of DEPC-treated water. RNA (5 µl) was used for the cDNA synthesis reaction. Quantitative PCRs were then performed on real-time PCR machine.

Northern blotting analysis of miRNAs

Total RNA (5 µg) from HCT116 cells was loaded onto 15% TBE-Urea gel (Invitrogen), electrophoresed with 1 X TBE buffer, and semi-dry transferred onto nylon membrane. After RNA was cross-linked to the membrane using EDC reagents (2 h at 60°C), the membrane was incubated with 0.5 nM DIG-labelled LNA-DNA probe (Exiqon) in hybridization buffer (DIG

Easy Granules, Roche) at 50°C for overnight. The membrane was washed twice with low stringent buffer (0.1X SSC, 0.1% SDS) and high stringent (2X SSC, 0.1 % SDS) buffer at 37°C for 10 min, respectively. It was then incubated with Blocking buffer (Roche) for 3 h at room temperature and incubated with DIG antibody solution by mixing DIG antibody (Roche) and blocking solution (1:10,000) for 30 min. Finally, the membrane was washed with wash buffer (Roche), developed with CSPD (Roche), and exposed to X-ray film.

TCGA data analysis

Analyses were carried out in R statistical environment (version 3.0.1) (http://www.r-project.org/). All tests were two-sided and considered statistical significant at the 0.05 level. The heat maps were generated using the package gplots. For TCGA analysis, we downloaded and analysed data publicly available from the Cancer Genome Atlas (TCGA; http://tcga-data.nci.nih.gov/) for ovarian serous cystadenocarcinoma or kidney renal clear cell carcinoma. Level 3 Illumina HiSeq RNASeq "gene.quantification" files were used to extract mRNA expression for DDX1. Level 3 Illumina HiSeq miRNASeq data were used to obtain miRNA expression. We derived from the "isoform_quantification" files the "reads_ per_ million_ miRNA_ mapped" values for mature forms of individual microRNAs in the list. The Log-rank test was employed to determine the relationship between DDX1 expression and overall survival and the Kaplan-Meyer method was used to generate survival curves. We split randomly the entire population in training/validation cohorts (2/3, 1/3) and checked for a relation with the survival as follows. In both cohorts patients were divided into percentiles according to DDX1 expression. Using the training set, we considered any cut-off between 25th and 75th to split significantly the samples into two groups and checked for statistical significance in the validation set. We then chose the cut-off to optimally split the samples in both cohorts. Thus, each of the cohorts was dichotomized into "DDX1 low" and "DDX1 high" groups. For each cohort, the Shapiro-Wilk test was applied to verify if miRNA data followed a normal distribution in each group. The t-test was applied to normally distributed data; otherwise the nonparametric Mann-Whitney-Wilcoxon test was applied to assess the relationship of miRNA levels with the groups.

Immunohistochemistry, immunocytochemistry, and *in situ* hybridization

The tumors were fixed in 10% neutral buffered formalin and embedded in paraffin and 5-μm tissue sections were serially cut and mounted on slides. The sections were de-paraffinized in xylene, re-hydrated, and boiled for 10 min in antigen retrieval buffer (BD1000 S-250, Borg Decloaker, BioCare Medical). After retrieval, the sections were washed with distilled water and endogenous peroxidase activity was blocked using 3% H_2O_2 in TBS for 15 min and then blocked with blocking solution (1% bovine serum albumin, 10 % normal serum in 1X TBS). Samples were incubated with primary antibodies overnight at 4°C, washed three times with TBST buffer, and then incubated with biotinylated goat anti-rabbit or anti-mouse IgG (GR608H, BioCare Medical). A streptavidin–biotin peroxidase detection system with 3, 3′-diaminobenzidine as substrate was used according to the manufacturer's instructions (DAB Peroxidase Substrate Kit, Vector Laboratory). Sections were counterstained with hematoxylin. For immunocytochemistry, cells on chamber slide were fixed in 3.7% paraformaldehyde for 10 min, and permeablized by 0.2% Triton X-100 for 5 min and then blocked with blocking solution. After incubation with primary antibodies, cells were washed with PBS three times and incubated with Alexa fluor 488 or 594 conjugated antibodies (Life Technologies) and counter stained with Hoechst 33258. After mounting, signals were observed under microscope. Ovarian tumour tissue microarray (BC110118) was purchased from Biomax, including 72 cases of ovarian tumour samples. Signals of immunohistochemistry data in tumour cells were visually quantified using a scoring system from 0 to 9, multiplied intensity of signal, and percentage of positive cells (signal: 0 = no signal, 1 = weak signal, 2 = intermediate signal, and 3 = strong signal; percentage: 0 = 0%, 1 = <25%, 2 = 25%-50%, and 3 = >50%).

Immunoprecipitation

Cells were lysed on ice for 30 min in IP buffer (1% NP-40, 50 mM Tris-HCl, 500mM NaCl, 5mM EDTA) containing protease inhibitor cocktail. Cell lysates (700 μg) were incubated overnight with 3 μg of antibodies or normal IgG at 4°C with rotary agitation. Protein A-sepharose beads were added to the lysates and incubated for additional 4 h. Beads will be washed three times with IP buffer and boiled for 10 min in 3% SDS sample buffer. Total cell lysates and immunoprecipitates were separated by SDS–PAGE and analysed by Western blotting.

In vivo phosphorylation assay

In vivo phosphorylation assay was performed as described previously[53]. Briefly, cells expressing DDX1-FLAG were washed with phosphate-free DMEM (#11971-025, Invitrogen), and incubated with 0.5 mCi/ml of ^{32}P-orthophosphate in the phosphate-free DMEM containing 10% dialyzed FBS (#26400-036, Invitrogen) for 4 h at 37°C. Cells were harvested and cell lysates were immunoprecipitated by anti-FLAG beads. The DDX1 immunoprecipitates were run in SDS-PAGE and then dried for X-ray film exposure.

Homologous recombination (HR) repair assay

DR-U2OS cell line containing a single copy of the homologous recombination (HR) repair reporter substrate DR-GFP in a random locus was generated and previously described (Wan et al., 2013a; Wan et al., 2013b). DR-U2OS cells DR-U2OS cells were infected with lentiviruses expressing control or DDX1 shRNAs. Cells were re-seeded 24 h post-infection and were transfected with control or I-SceI-expressing plasmid. Flow cytometric analysis was performed to detect GFP positive cells on Guava EasyCyte Flow Cytometer (Millipore, USA) and relative HR frequencies were calculated.

Matrigel invasion assays

Matrigel Invasion assays in matrigel matrix chamber were performed according to the manufacture's instruction (#354480, BD Biocoat Matrigel).

SUPPLEMENTAL REFERENCES

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