SUPPLEMENTAL MATELIALS AND METHODS

Affinity purification of HERC2 complex and Mass Spectrometry

Cell lysates form 3×10^7 HeLa cells and HCT116 cells treated or not with MMC (0.5 μg/ml) for 16 h or MG132 (5 μmol/L) for 12 h were prepared with 3 ml of the 0.5% NP-40 buffer. After clarify with centrifugation the lysates were immunoprecipitated with antibody to HERC2 or control IgG covalently coupled with Dynabeads (Thermo Fisher), washed, eluted with an elution buffer (0.5 mol/L NH_4OH, 0.5 mmol/L EDTA) according to the manufacturer's instructions. Samples were concentrated with an evaporator and subjected to 4–12% NuPAGE Bis-Tris gels (Life Technologies) with a short run (1 cm). The gels were stained with Bio-Safe Coomassie Stain (Bio-Rad). After the gels were extensively washed with Milli-Q water (Millipore), the gel regions of entire lane were excised, cut into 1 mm^3 pieces, destained for 1 h with 1 ml 50 mmol/L ammonium bicarbonate (AMBC)/30% acetonitrile (ACN) with agitation and then further washed for 1 h with 1 ml 50 mmol/L AMBC/50% ACN. Finally, a 100% ACN wash was performed to ensure complete gel dehydration. Trypsin solution (Promega, 20 ng/μl in 50 mM AMBC/5% ACN) was subsequently added to the gel pieces at approximately equivalent volumes and incubated on ice for 30 min. Another small volume of trypsin solution was added to the gel samples and incubated at 37°C overnight. Digests were extracted by addition of 100 μl 50% ACN/0.1% trifluoroacetic acid (TFA) for 1 h with shaking. The peptides were recovered into fresh Eppendorf tubes, and an additional extraction step was performed with 70% ACN/0.1% TFA for 30 min. The extracted peptides were concentrated using a speed-vac, and subjected to shotgun MS analysis on a Q Exactive mass spectrometer coupled with an EASY-nLC 1000 liquid chromatograph and nanoelectrospray ion source (Thermo Scientific). The mobile phases were 0.1% formic acid (FA) in water (Solvent A) and 0.1% FA in 100% ACN (Solvent B). Peptides were directly loaded onto a C18 analytical column (ReproSil-Pur 3 μm, 75 μm inner diameter and 12 cm length, Nikkyo Technos) and separated using a 80 min two-step gradient (0–35% Solvent B for 70 min and 35–100% for 10 min) at a constant flow rate of 300 nl/min. The Q Exactive was operated in the data-dependent MS/MS mode, using Xcalibur software, with survey scans acquired at a resolution of 70,000 at m/z 200. The top 10

most abundant isotope patterns with charge 2–5 were selected from the survey scans with an isolation window of 2.0 m/z, and fragmented by HCD with normalised collision energies of 28. The maximum ion injection times were 60 ms for both survey and MS/MS scans, and the AGC values were set to 3×10^6 and 5×10^5 for the survey and MS/MS scans, respectively. Ions selected for MS/MS were dynamically excluded for 10 sec. Proteome Discoverer software (ver. 1.3, Thermo Scientific) was used to generate peak lists. The MS/MS spectra were searched against a Swiss-Prot database (version 2012_10 of UniProtKB/Swiss-Prot protein database) using the MASCOT search program (Matrix Science). The precursor and fragment mass tolerances were set to 10 ppm and 20 mmu, respectively. Maximum missed cleavage site of trypsin was set to two. Acetylation of N-terminal protein, oxidation of methionine, ubiquitylation/acetylation of lysine, phosphorylation of serine/threonine, pyroglutamylation of N-terminal glutamine were set as variable modifications for database searching. Peptide identification was filtered at a 1% false discovery rate.

Sister chromatid exchange (SCE) assays

Exponentially growing HeLa-shHERC2 cells induced or not with doxycyclin for 48 hours, or WT or HERC2$^{\Delta E3/\Delta E3}$ HCT116 cells were grown for additional 48 hours in the presence of 20 μmol/L or 90 μmol/L BrdU, respectively. Cells were incubated with 0.2 μg/ml colcemid for the last one hour before harvest. Cells were collected with trypsin, incubated for 20 min in 75 mmol/L KCl and gently fixed for 30 min in methanol:acetic acid (3:1). Cells were dropped onto ethanol-treated glass slides, air dried, and aged for 3 days. Nuclei were sensitized with 10 μg/ml Hoechst 33258 in 0.5 x saline sodium citrate (SSC) for 30 min at room temperature. The slides were then bleached with a 352 nm black light for 2h, heat-treated at 70°C for 60 min, stained with 3% Giemsa for 15 min, and mounted with glass coverslips in Malinol. Slides were analyzed with Olympus BX53F microscope equipped with a 100x objective.