Supplemental Information

LINC01234/MicroRNA-31-5p/MAGEA3 Axis

Mediates the Proliferation and Chemoresistance

of Hepatocellular Carcinoma Cells

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Supplementary Figure 1. Depleted MAGEA3 represses the proliferation and invasion but potentiates the apoptosis of Huh7 cells. A, transfection efficiency of siRNAs targeting MAGEA3 (si-MAGEA3-1, si-MAGEA3-2, si-MAGEA3-3) in Huh7 cells determined using RT-qPCR; B-D, viability (B), apoptosis (C) and invasion (× 200; D) of Huh7 cells in response to MAGEA3 depletion, as measured using MTT assay, flow cytometry and Transwell assay, respectively; E, protein expression of E-cadherin in Huh7 cells in response to MAGEA3 depletion measured using Western blot analysis. Measurement data were expressed as mean ± standard deviation. Data in panel B were analyzed using repeated-measures ANOVA along with Tuckey’s post hoc test and data among groups in other panels were compared using one-way NA0VA together with Tuckey’s
post hoc test. The experiment was repeated three times. $p < 0.05$ vs. mock- or si-NC-transfected Huh7 cells.
Supplementary Figure 2. MAGEA3 silencing reduces chemoresistance of Huh7 cells to cisplatin. A, CCK-8 assay showing viability and the IC\textsubscript{50} of Huh7 cells transfected with mock, si-NC or si-MAGEA3 upon treatment with cisplatin at different concentrations; B, the mRNA levels of MRP2, MRP3 and MDR1 in Huh7 cells following silencing of MAGEA3 determined by RT-qPCR; C, the protein levels of MRP2, MRP3, and MDR1 in Huh7 cells transfected with si-MAGEA3 measured by Western blot analysis. D, ALB content in the supernatant of Huh7 cells in response to si-MAGEA3 transfection detected by ELISA. Measurement data were expressed as mean ± standard deviation. The comparisons among multiple groups were assessed with one-way ANOVA, followed by Tukey’s post hoc test. Each experiment was repeated three times. * p < 0.05 vs. the mock- or si-NC-transfected Huh7 cells.
Supplementary Figure 3. miR-31-5p dampens Huh7 cell proliferation and reduces chemoresistance to cisplatin. A-C, viability (A), invasion (× 200; B) and cisplatin-induced apoptosis (C) of Huh7 cells in response to miR-31-5p mimic transfection using MTT assay, Transwell assay and flow cytometry, respectively; D, IC_{50} value of Huh7 cells in response to miR-31-5p mimic transfection; E, protein expression of MRP2, MRP3, MDR-1, and E-cadherin in Huh7 cells in response to miR-31-5p mimic transfection measured by Western blot analysis; F, content of ALB in the supernatant of Huh7 cells in response to miR-31-5p mimic transfection detected using ELISA. Measurement data were expressed as mean ± standard deviation. The comparison between two groups was analyzed by independent sample t-test and the comparisons among multiple groups by one-way ANOVA, followed by Tukey’s post hoc test. Data in panel C were analyzed using repeated-measures ANOVA with Tuckey’s post hoc test. Each experiment was repeated three times. * p < 0.05 vs. NC-transfected Huh7 cells.
Supplementary Figure 4. Reduction of LINC01234, results in suppression of HCC progression via miR-31-5p-mediated inhibition of MAGEA3. A, the subcellular localization of LINC01234 in Huh7 cells detected using FISH (× 400); B, the luciferase activity of LINC01234 in Huh7 cells...
upon miR-31-5p mimic transfection detected by dual-luciferase reporter gene assay; C, the binding between LINC01234 and miR-31-5p in Huh7 cells detected by RNA pull-down assay; D, the binding between LINC01234 and Ago2 or DICER in Huh7 cells detected by RIP assay; E, MAGEA3 expression in Huh7 cells measured using RT-qPCR; F, binding of miR-31-5p to MAGEA3 in Huh7 cells detected using RNA pull-down; G-I, the viability (G), invasion (× 200; H) and cisplatin-induced apoptosis (I) of Huh7 cells upon silence of LINC01234 and/or miR-31-5p evaluated by MTT assay, Transwell assay (× 200) and flow cytometry, respectively; J, IC_{50} value of Huh7 cells upon silence of LINC01234 and/or miR-31-5p; K, protein expression of MRP2, MRP3, MDR-1, and E-cadherin in Huh7 cells determined using Western blot analysis; L, content of ALB in the supernatant of Huh7 cells detected by ELISA. Measurement data were expressed as mean ± standard deviation. Differences among groups at different points of time were assessed with repeated-measures ANOVA. The comparison between two groups was analyzed by independent sample t-test and the comparisons among multiple groups by one-way ANOVA, followed by Tukey’s post hoc test. Each experiment was repeated three times. *p < 0.05 vs. the IgG immunoprecipitates or the NC- or si-ctrl-transfected cells; #p < 0.05 vs. the si-LINC01234-transfected cells.