

Supplementary data

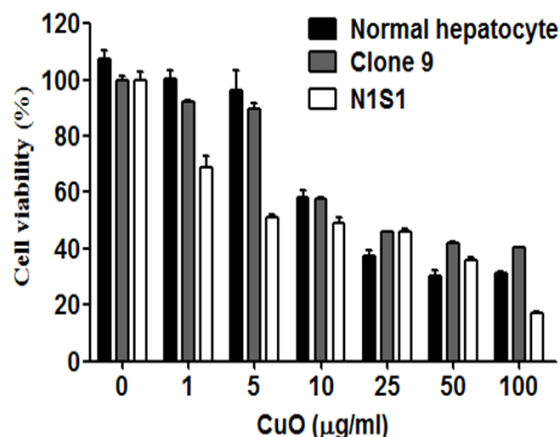


Fig S1. Comparison of survival impact of CuO NPs on the primary rat hepatocyte, the normal liver cell line (Clone 9), and malignant hepatoma cell line (N1S1). After 24h treatment of CuO NPs, normal and Clone 9 cells show a greater tolerance toward CuO-induced toxicity than malignant N1S1 cells.

Supplementary material and methods

Animals and cell culture

Animal care and experimental procedures were approved by the Institutional Animal Care and Use Committee of National Sun Yat-Sen University. We ensured that all animals received humane care and that study protocols complied with the institution's guidelines. Primary hepatocytes were isolated from male Sprague–Dawley (SD; 220–260 g) rats by a two-step in situ collagenase perfusion method¹. Primary hepatocytes were grown in DMEM medium with low glucose (Gibco, Bethesda, MD) containing 10% fetal bovine serum (Hyclone). N1-S1 cells (rat HCC) were incubated in RPMI-1640 medium (Gibco, Bethesda, MD) containing 10% calf serum (Hyclone). Clone-9 (rat hepatocyte) cells were maintained in F12K medium (Gibco, Bethesda, MD) containing 10% fetal bovine serum (Hyclone). All the media for cell culture were supplemented with 2 mM l-glutamine (Hyclone), 100 mg/mL streptomycin (Hyclone), and 100 U/mL penicillin (Hyclone). All cells were maintained under humidified conditions in 95% air and 5% CO₂ at 37°C. For the MTT cytotoxicity assay, cells were plated at a density of 2×10^4 cells/well onto 96-well, while primary hepatocytes were plate onto 96-well which pre-coated with rat tail type I collagen. After incubated with various concentrations of CuO NPs (0, 1, 5, 10, 25, 50, 75 and 100 μg/ml) for 24 h. Cells were further subjected to MTT assay.

1. Y. H. Kao, C. L. Chen, B. Jawan, Y. H. Chung, C. K. Sun, S. M. Kuo, T. H. Hu, Y. C. Lin, H. H. Chan, K. H. Cheng, D. C. Wu, S. Goto, Y. F. Cheng, D. Chao and M. H. Tai, *J. Hepatol.*, 2010, 52, 96-105.