p53 Promotes Ferroptosis in Macrophages Treated with Fe3O4 Nanoparticles

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Research Article

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Abstract

Background: Fe_3O_4 nanoparticles (NPs, also known as iron oxide NPs; IONPs) have high biocompatibility and low biotoxicity. They are widely used in the field of biotechnology for targeted delivery, image formation, and photothermal therapy. NP biodistribution is determined by macrophage capture in vivo, and recently, the induction of macrophage polarization into the M1 phenotype by IONPs has become a hot topic in research. Previous research has shown that IONPs can induce ferroptosis of ovarian cancer cells and ischemic cardiomyocytes. In this study, we exposed macrophages to synthesized Fe_3O_4 NPs (100 nm in diameter) and determined the effects of NPs in inducing cell death by RNA sequencing.

Results: We observed that after 48 h exposure to NPs, there was a change in the macrophage phenotype and a reduction in cell viability. Then, we demonstrated that NPs could induce macrophage cell damage by increasing intracellular reactive oxygen species and by repressing the mitochondrial membrane potential. Furthermore, we investigated the underlying mechanisms of ferroptosis of macrophages using RNA sequencing and change in ultrastructural morphology, and found that ferroptosis was caused by the upregulation of p53 expression and inhibition of SLC7A11 expression, as their protein levels after 48 h exposure to Fe_3O_4 NPs were consistent with erastin-induced ferroptosis.

Conclusions: These results provide an insight into the molecular mechanisms underlying ferroptosis induced by Fe_3O_4 NPs in macrophages and provide a basis for the biotoxicity study of Fe_3O_4 NPs in vivo.

Full Text

Due to technical limitations, full-text HTML conversion of this manuscript could not be completed. However, the manuscript can be downloaded and accessed as a PDF.

Figures

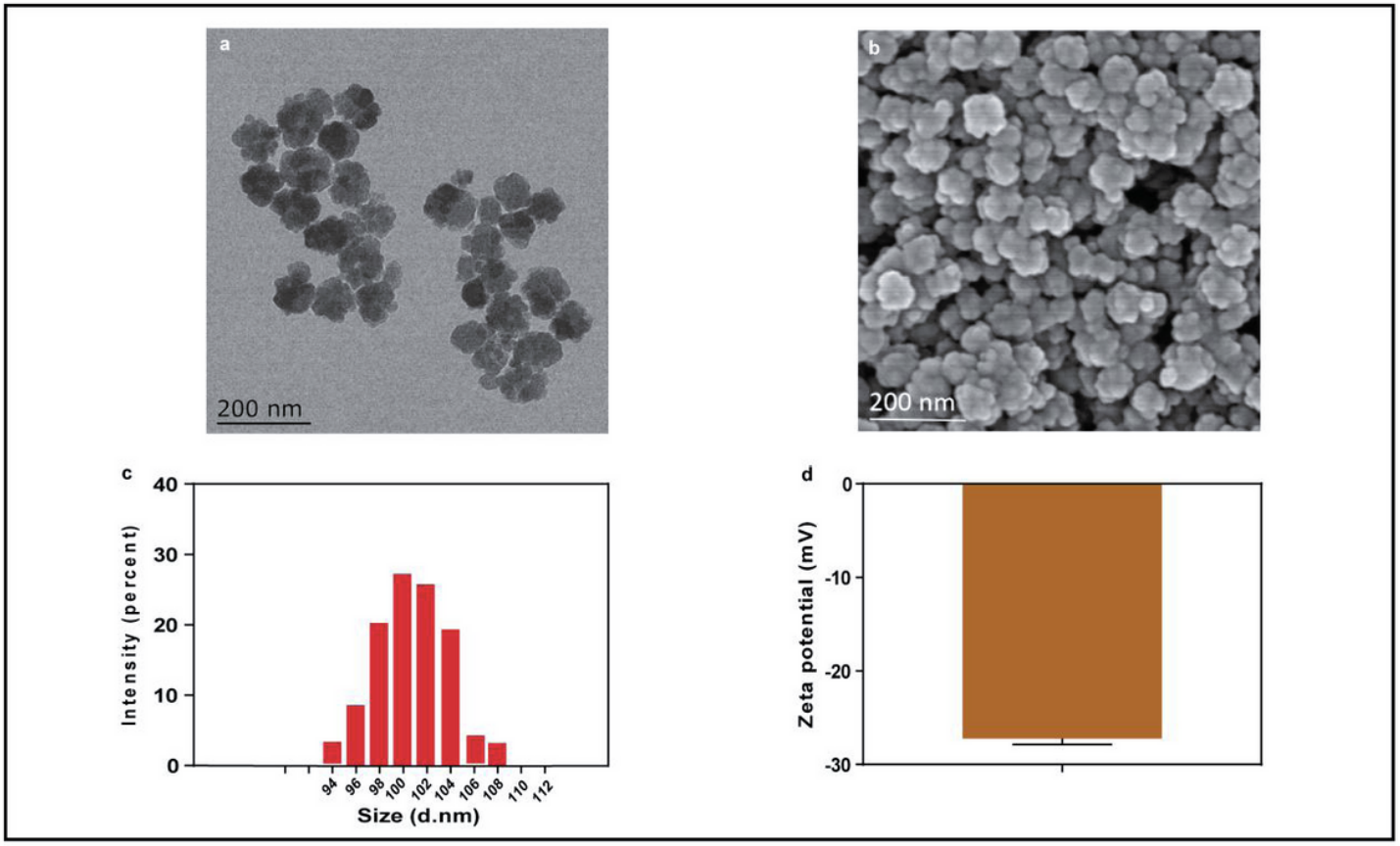

Figure 1

Characterization of Fe3O4 NPs. (a) Transmission electron micrograph, Ob: 50000×; (b) Scanning electron micrograph, Ob: 50000×; (c) ζ-potential of NPs in Milli-Q water; (d) determination of NPs size by DLS.

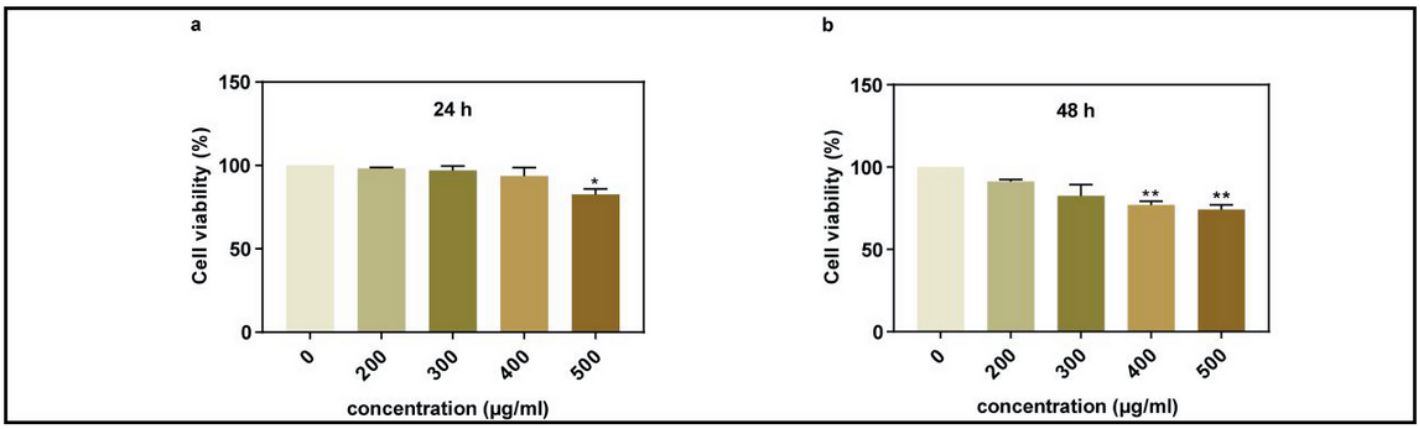

Figure 2

Effects of gradient concentrations of nanoparticles (NPs) on cell viability after (a) 24 h and (b) 48 h. Control cells cultured in nanoparticle-free medium were processed in parallel with the treatment groups. Results of the CCK-8 assay are expressed as percentage relative to the control. Data are expressed as means ± SEM, *p < 0.05; **p < 0.01 vs Control.

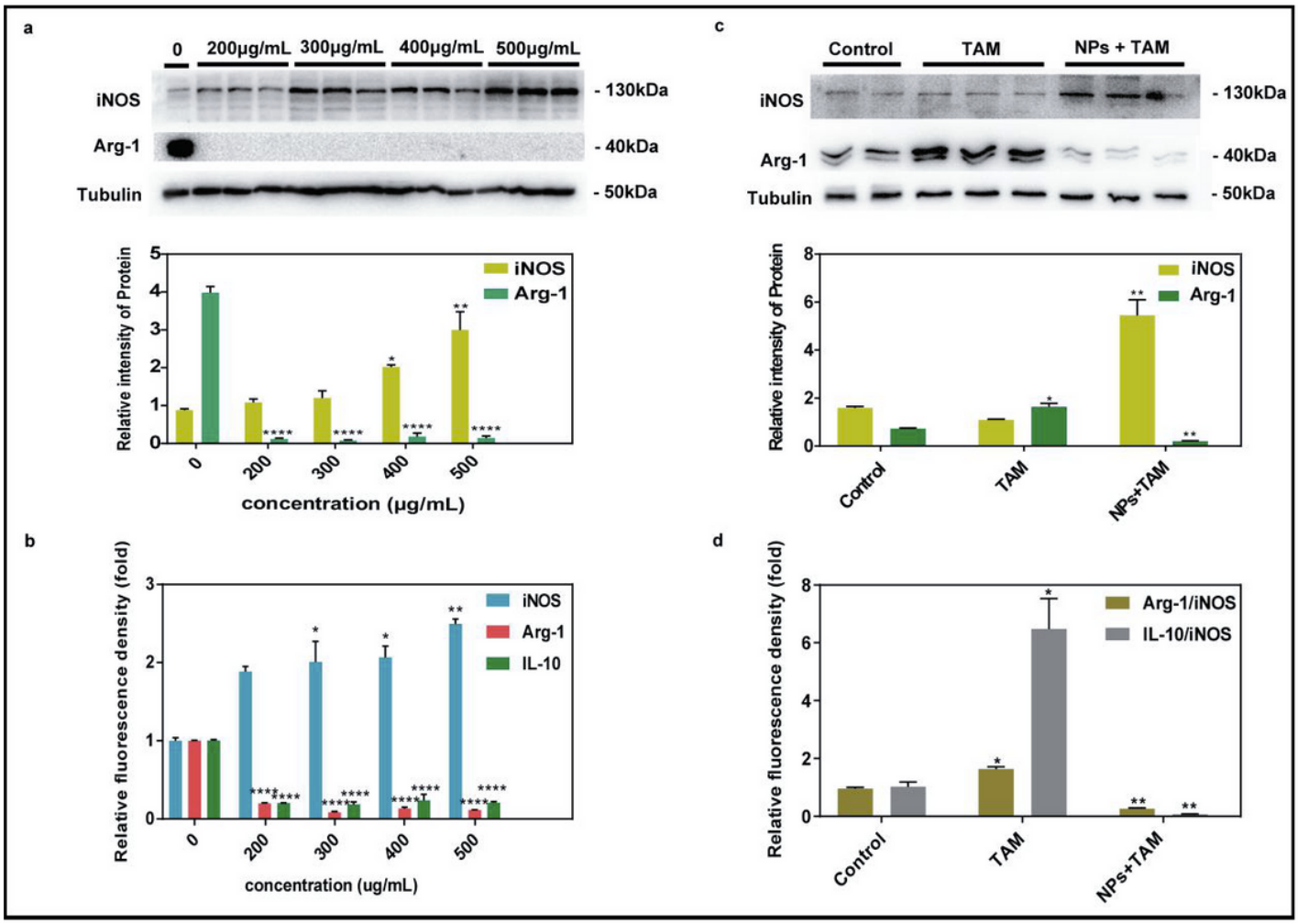

Figure 3

Effect of NPs on the change of macrophages phenotype. (a) Protein levels of iNOS and Arg-1 in Ana-1 cells incubated with 200, 300, 400, and 500 μg/mL NPs examined by western blotting; (b) mRNA levels of iNOS, Arg-1, and IL-10 in Ana-1 cells treated with various concentrations of NPs; (c) Expression levels of iNOS and Arg-1 in the tumor-associated macrophage (TAM) model incubated with or without NPs; (d) The ratios of Arg-1/iNOS and IL-10/iNOS assayed by reverse transcription–quantitative PCR. Protein levels are shown relative to untreated Ana–1 cells as control. The quantification of protein levels was achieved by calculating the signal intensity ratio of iNOS and Arg-1 to the internal control (Tubulin); The mRNA levels were normalized to those of GAPDH. Data from at least three independent experiments were used for quantification. Data are expressed as means ± SEM, and *$p < 0.05$; **$p < 0.01$; ****$p < 0.001$.

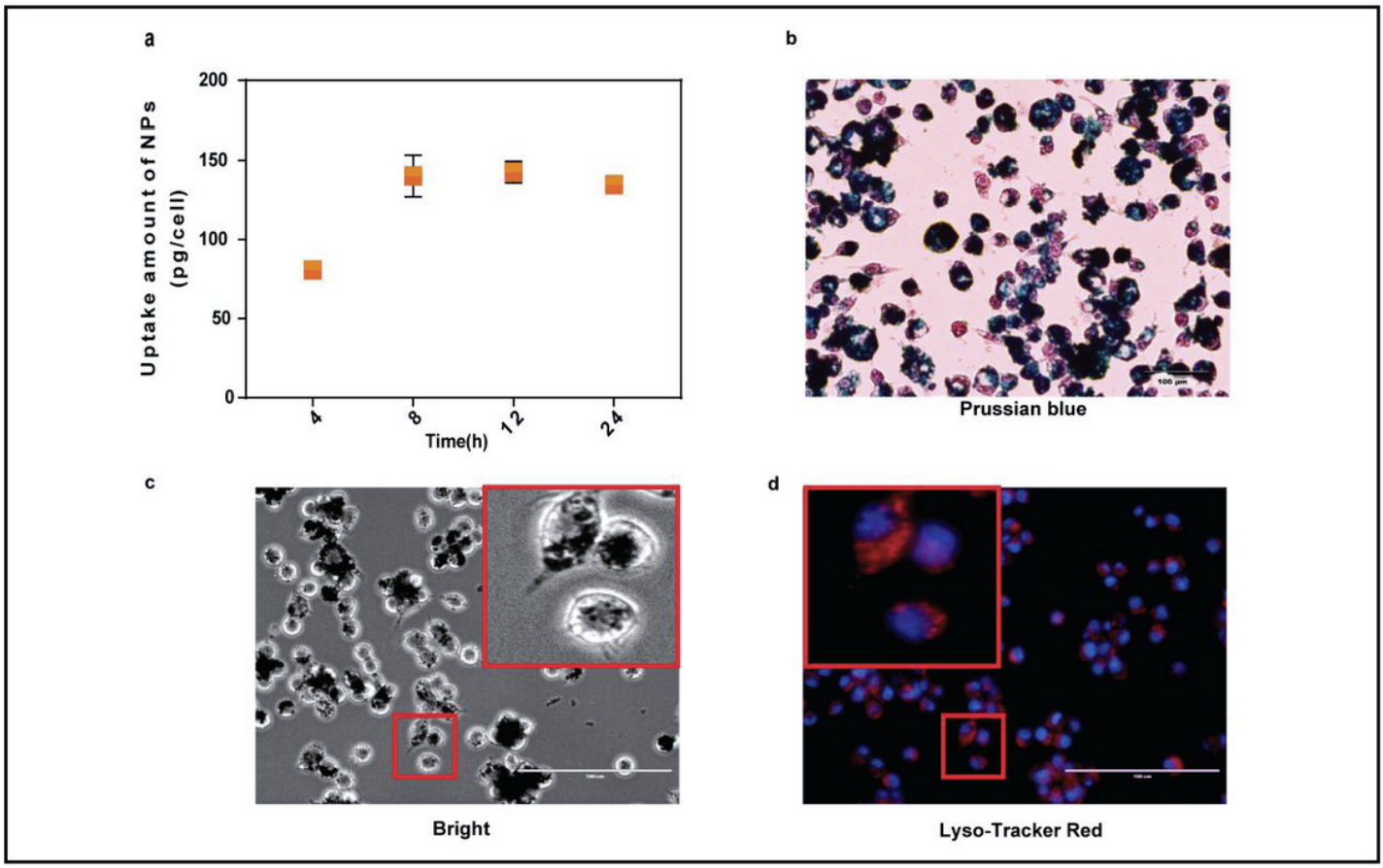

Figure 4

Quantification of intracellular localization of NPs. (a) Cellular iron contents measured by ICP-ACE; (b) Presence of iron in cells are visualized by Prussian blue staining; (c, d) Colocalization of intracellular NPs with lysosomes revealed by staining with Lyso-Tracker Red fluorescence probes; Ob: 200×.

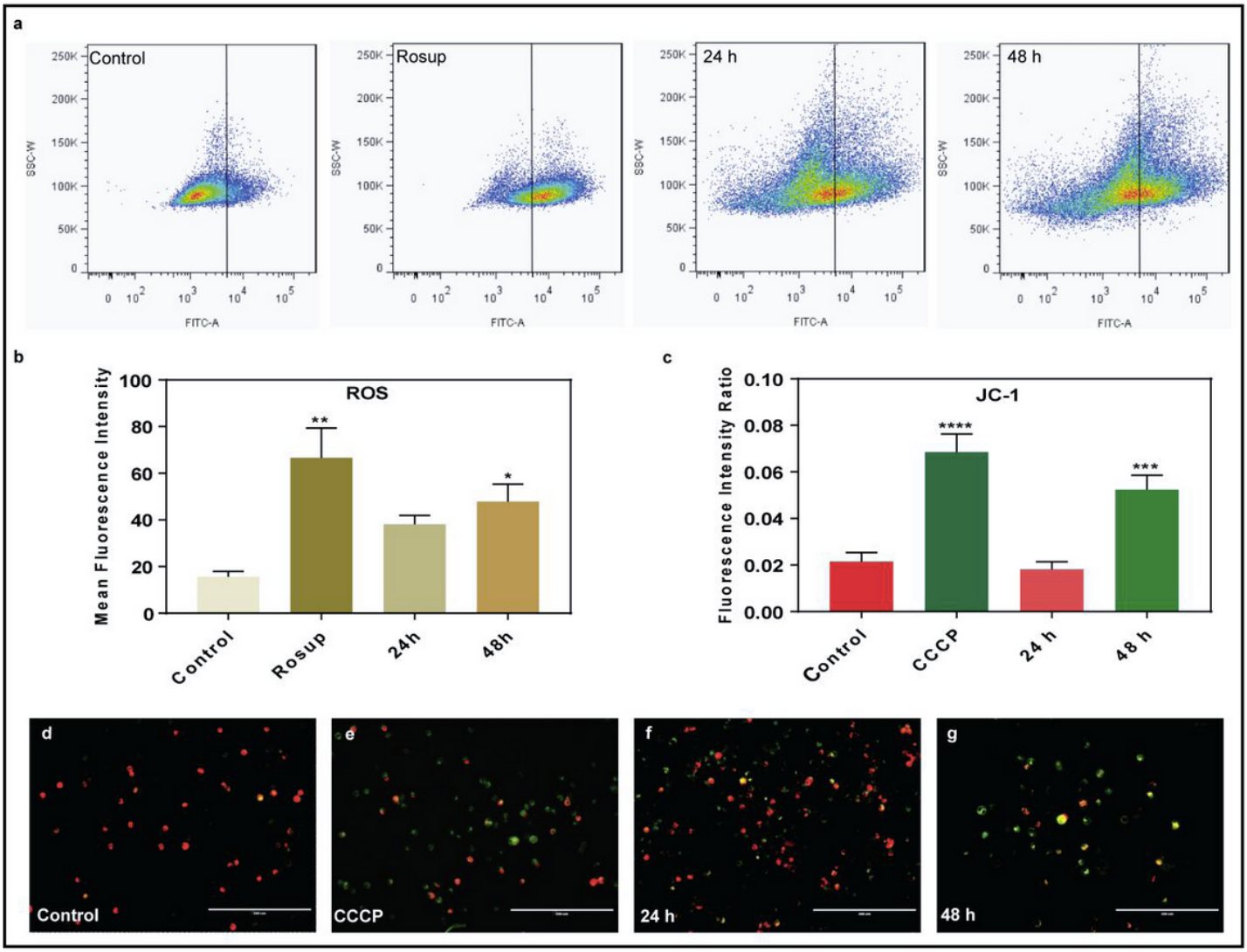

Figure 5

Determination of intracellular ROS and MMP levels. (a, b) Trends in ROS generation detected by FCM. When the MMP is high, the JC-1 probe is dispersed in a multimeric form in the mitochondrial matrix (red fluorescence; Ex = 585 nm, Em = 590 nm). JC-1 is dispersed in the mitochondrial matrix in a free state when the MMP is low (green fluorescence; Ex = 514 nm, Em = 529 nm); (c) Graphical representation of the green/red fluorescence intensity ratios; (d) Untreated cells used as negative control; (e) CCCP treatment for 20 min used as positive control; JC-1 stained micrographs at 24 h (f) and 48 h (g); Ob: 200×; *p < 0.05; **p < 0.01; ***p < 0.005; ****p < 0.001 vs Control.

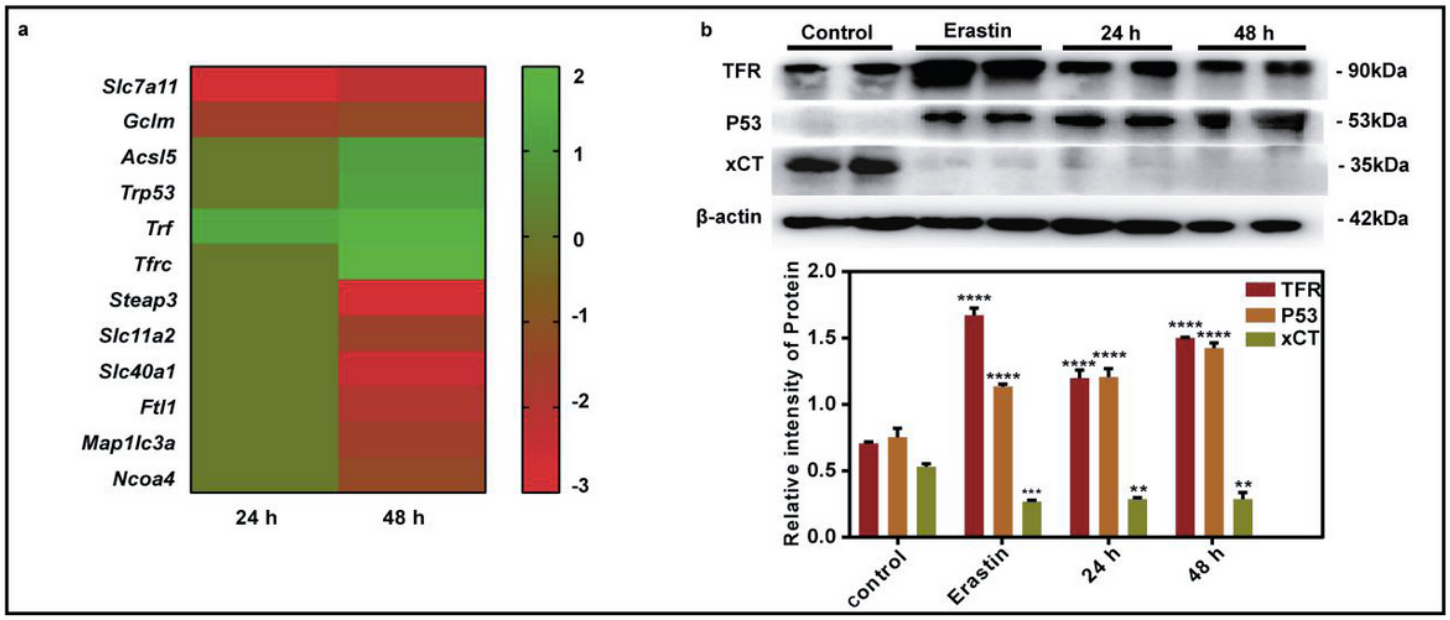

Figure 6

RNA-seq, ultrastructural change of mitochondria, and verification of protein expression associated with ferroptosis. (a) Heat map visualization of the expression of various key genes of the ferroptosis pathway; (b) Protein levels of TFR, p53, and xCT (SLC7A11) in the 24 h, 48 h, and erastin groups. Untreated Ana-1 cells used as control; quantification of protein levels was achieved by calculating the signal intensity ratio of target protein bands to the internal control bands (β-actin). Data are expressed as means ± SEM, and **p < 0.01; ***p < 0.005, ****p < 0.001 vs Control.

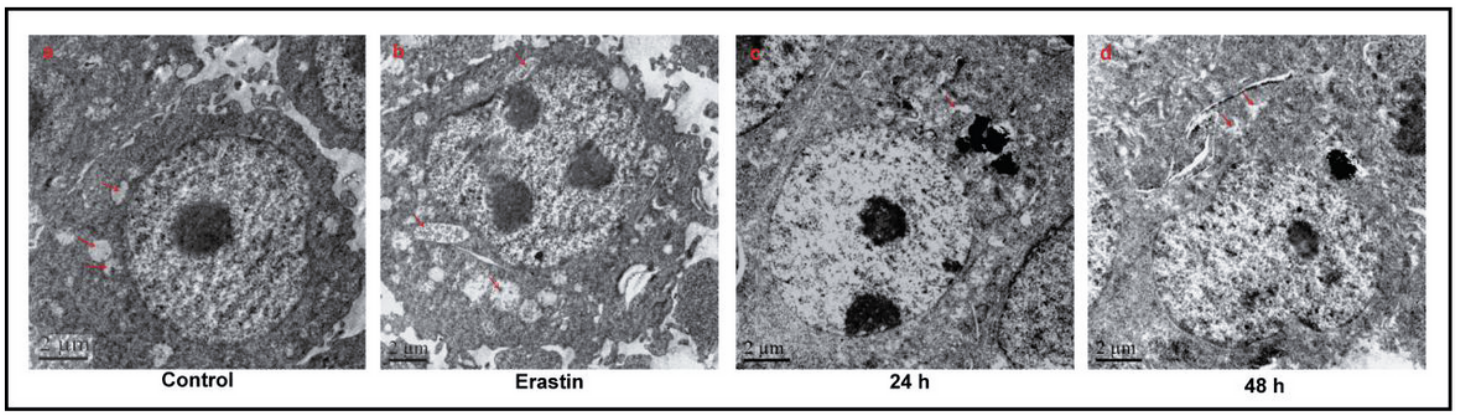

Figure 7

Morphology of mitochondria (red arrow) in nanoparticle (NP)-treated macrophages. (a–d): Untreated Ana-1 as control group (a), Ana-1 treated with erastin (25 μM) for 24 h (b); Transmission electron micrographs of NP treated cells for 24 h (c), 48 h (d). Ob: 2500×.

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