Abstract

Since their discovery by Christian de Duve in the 1950s, the role of lysosomes in cellular function has been explored extensively, which led to the change of the view of lysosomes from a static digestive system to the dynamic regulator of cellular metabolism. As indicated in various studies, lysosomal dysfunctions are found to be linked with the group of metabolic disorders known as lysosomal storage diseases. Therefore, understanding lysosomal biology in both normal and pathogenic conditions is crucial to figuring out the mechanistic insights of lysosomal activity, to facilitate diagnostic methods or establish a new therapeutic strategy.

The rapid and efficient isolation of lysosomes is a prerequisite to identify lysosomal protein composition, using proteomic analysis to reveal their involvement in cellular functions or disease progression. So far, several strategies have been developed to isolate lysosomes, including density-gradient centrifugation, immunoaffinity purification, and magnetic nanoparticle-based fractionation. Among these approaches, a nanoparticle-based method that delivers magnetic nanoparticles to the lumen of lysosomes, through an endocytic pathway, followed by a separation process, using a magnetic column, has been proven to be able to isolate lysosomes with the highest yield and purity, while efficiently preserving their integrity. The traditional magnetic probes, such as superparamagnetic iron oxide nanoparticles (SPIONs), require surface modification by fluorescent dyes to enable the investigation of their intracellular trafficking, which has some disadvantages, including the possible alternation of their bio-interaction, and the instability of fluorescence properties in the lysosomal environment.

In this thesis, we have focused on developing a multifunctional magnetic probe with intrinsic imaging capabilities for tracking the intracellular transport of nanoparticles to lysosomes through endocytic pathways before performing magnetic separation. Notably, the kinetic transport is an important parameter that strongly affect the yield and purity of isolated fraction. In addition, we established the protocol to isolation of lysosomes as intact as possible.

Firstly, the magnetic-plasmonic AgFeCoAg nanoparticles (MPNPs) were prepared using polyol method. The obtained MPNPs was then undergone the encapsulation in phospholipid micelles, followed by the conjugation of amino dextran (aDxt) for targeting lysosomes. The hydrodynamic size of particles after encapsulation and conjugation process are 33.9 ± 2.6 nm and 52.4 ± 7.8 nm, respectively. The zeta potential was positive charge after conjugating aDxt. We observed that the dispersion of aDxt-MPNPs in culture medium, DMEM (+10% FBS), would suppress the cytotoxicity of nanoparticles, cell viability was above 70% even after 24 h incubation with nanoparticle concentration, CNPS = 100 μg/mL. Furthermore, the aDxt-MPNPs was also highly stable in culture medium which was very important to maintain the particle uptake. The number of aDxt-MPNPs internalized was almost double when extending the loading time from 1 h to 8 h.

Next, the intracellular trafficking of aDxt-MPNPs to a cell model (COS-1 cells) was investigated using the pulse-chase experiment and colocalization analysis. The colocalization between nanoparticle and organelles was determined by Manders' coefficient (R_t). As the result, the time-lapse colocalization of aDxt-MPNPs and early endosomes (EE), late endosomes (LE) and lysosomes (L) were constructed, which indicated that the aDxt-MPNPs arrived at lysosomes after a chasing period of 7 h. Furthermore, a simple mathematic model based on stretch exponential functions has been established to derive time constant that represented the speed with which nanoparticles were transported to EE, LE and L. Finally, TEM and EDS analysis of aDxt-MPNPs-treated COS-1 cells after 1 h loading and 7 h chase was performed to confirm the result of colocalization analysis.

Finally, after understanding the transport kinetics of aDxt-MPNPs to lysosomes in the cell model. Cells were completely homogenized using syringe with 23G needle after 15 passages, which was confirmed by the bright-field microscope. Subsequently, lysosomes were isolated using a magnetic column. The integrity was qualitatively screened by confocal laser scanning microscopy (CLSM), while the Western blot results confirmed the high purity of the isolated fraction. Additionally, it is concluded that to isolate lysosomes as intact as possible, the lysosomes should be isolated within 30 min after homogenization at 4^{o}C. Furthermore, our established protocol was demonstrated to be superior to the density gradient centrifugation method (DGC) in term of number of starting materials, isolation yield, purity, and time. Lysosomes were also isolated from HEK293 cells to confirm the versatility of the established methods.

Keywords: *magnetic isolation, lysosomes, intracellular trafficking, bioimaging, nanoparticles*