Cationic polystyrene nanosphere induces autophagy through inhibition of the Akt/mTOR and activation of the AMPK signaling pathways in macrophage and epithelial cells

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ABSTRACT

Nanoparticles have been used to produce a wide range of products. Those include applications in imaging and drug delivery in medicine. However, the possible adverse biological effects in human being remain unclear. Autophagy is an important catabolic process responsible for degrading and recycling long-lived proteins, cellular aggregates and damaged organelles. In addition to the well-documented role of autophagy in cell survival, a function for autophagy in cell death has long been proposed. Polystyrene could be used as biosensor and drug delivery carrier. It has been reported that cationic polystyrene (NH_2-PS) could induce cell death in RAW 264.7 and BEAS-2B cells through apoptotic and necrotic cell death. Our current study further demonstrated that autophagic cell death could also be induced by NH_2-PS. We applied bafilomycin A1, an inhibitor of autophagosome-lysosome fusion, and 3-MA, an initiator of autophagy, to determine whether inhibition of autophagy alters NH_2-PS treatment-induced cytotoxicity. The results indicated a decreased autophagy flux by bafilomycin A1and an increased cell viability by 3-MA which confirm the autophagic cell death treated with NH_2-PS. In addition, ER stress and signaling pathways related to the process of autophagy induced by NH_2-PS in RAW 264.7 and BEAS-2B cells were examined. We found that NH_2-PS significantly increased the staining of ER-specific dye and IRE1α protein expression. Meanwhile, the phosphorylation of Akt/mTOR decreased and the phosphorylation of AMPK increased. Taken together, our results indicate that NH_2-PS-induced autophagic cell death was mainly occurred through inhibition of the Akt/mTOR and activation of the AMPK signaling pathways. Specifically, NH_2-PS induced ER stress in RAW and BEAS-2B cells. Thus, autophagy can be considered as an additional mechanism providing intracellular selectivity for introduced NH_2-PS nanospheres.

Keywords: cationic polystyrene nanosphere; autophagy; endoplasmic reticulum stress

1 INTRODUCTION

Nanoparticles (NPs) have been used to produce a wide range of products. Their industrial and commercial applications include catalysis, sensors, environmental remediation, personal care products, cosmetics, and they are showing great prospect in the field of medicine, including imaging and drug delivery [1]. It is not a surprise, therefore, that academia, government, industry, and members of the public have expressed great concern about the possibility that engineered nanomaterials (NMs) could lead to adverse biological effects in humans and the environment. Development of nanotechnology calls for a comprehensive understanding of the impact of NMs on biological systems.

It is now known that different modalities of cell death (apoptosis, necrosis, autophagy) contribute to the pathophysiology of different human disorders [2]. Autophagy is an important catabolic process responsible for degrading and recycling long-lived proteins, cellular aggregates and damaged organelles. In addition to the well-documented role of autophagy in cell survival, a function for autophagy in cell death has long been proposed [3]. In recent years, the role of autophagy as an alternative cell death mechanism has been a topic of debate. Activation of the PI3 kinase/Akt pathway, a well-known method of inhibiting apoptosis, also inhibits autophagy [4]. Akt phosphorylates mammalian target of rapamycin (mTOR), which has been reported to inhibit the induction of autophagy [5]. In mammalian cells, AMPK is also required for autophagy [6]. During energy stress, AMP accumulation causes activation of the LKB1-AMPK pathway, which inhibits mTOR by activating TSC1/TSC2 [7]. AMPK is a key regulator for balancing intracellular energy when cells are under metabolic stresses, such as hypoxia, glucose deprivation, heat shock, or mitochondrial dysfunction, which result in depleting cellular ATP and elevating AMP levels [8]. Recently, NPs from various sources can induce autophagy in treated cells [9]. In addition, previous studies have demonstrated that the endoplasmic reticulum (ER) stress response, in combination with autophagy, represents an adaptive mechanism for supporting cell survival in

response to a great variety of detrimental conditions [10]. However, if the cells are exposed to prolonged or robust ER stress, the cells die by apoptosis [11]. Increasing evidence has indicated that ER stress is also a potent trigger of autophagy [12]. A better understanding of the interaction of NPs with these pathways of cell injury can help in developing the counter strategies for different human disorders and will help in the formulation of safe and consumer friendly nanotechnology. In this study, RAW 264.7 (mouse macrophage cell line) and BEAS-2B (human bronchial epithelial cell line) cells were used to investigate the autophagic effects and ER stress of NH_2-labeled polystyrene (NH_2-PS) nanospheres. We examined whether NH_2-PS-induced autophagy serves as a cell death mechanism. Furthermore, we also examined the signaling pathways related to the process of autophagy induced by NH_2-PS in RAW 264.7 and BEAS-2B cells.

2 MATERIALS AND METHODS

NH_2-PS solutions were fresh prepared from stock solutions (5 mg/mL) and sonicated for 30 s before addition to cell cultures. Cellular viability was determined by the MTS assay. ER stress was observed by fluorescent dyes (ER Tracker Blue White DPX dye). Immunofluorescence microscopy was used to determine autophagy. Western blotting was applied to determine the protein expression related to ER stress and autophagy. Furthermore, to further confirm the role of autophagy in NH_2-PS-induced cytotoxicity, we used bafilomycin A1 (BAF), an inhibitor of autophagosome-lysosome fusion, to inhibit the autophagy flux. In addition, we used 3-methyladenine (3-MA), an inhibitor of autophagy, to determine whether inhibition of autophagy alters NH_2-PS-induced cytotoxicity.

3 RESULTS

Our results showed that NH_2-PS reduced the viability of RAW 264.7 and BEAS-2B cells in the concentration-and time-dependent manner. Furthermore, we investigated whether or not NH_2-PS could induce autophagy. Microtubule‐ associated protein light chain 3 (LC3) is now widely used to monitor autophagy [13]. Thus, we applied fluorescence microscopy to determine the percentage of cells with punctate LC3 staining. After sixteen hours of NH_2-PS treatment (20 μg/mL), the percentage of LC3 punctate cells increased to ~50% in RAW 264.7 and BEAS-2B cells. To detect the expression of autophagic-related protein (LC3, Beclin 1 and p62), we performed western blotting with lysates from RAW 264.7 and BEAS-2B cells receiving different concentrations of NH_2-PS. The expression levels of the LC3-II, p62 and Beclin 1 proteins increased with NH_2-PS treatment. In addition, we used bafilomycin A1 to inhibite the autophagy flux and found a positive result showing that the LC3-II levels in cells treated with NH_2-PS were elevated by bafilomycin A1. Next, we used 3MA, an initiator of autophagy, to determine whether inhibition of autophagy alters NH_2-PS treatment-induced cytotoxicity. The results indicated that NH_2-PS treatment with 3-MA revealed a significant decrease of LC3-II expression compared to NH_2-PS treatment. Similar results could also be found in the viability assay with the same treatment. Previous reports have demonstrated that the induction of ER stress by treatment with thapsigargin, an ER $Ca2^+$-ATPase inhibitor, increases the fluorescence intensity of ER-Tracker Blue-White DPX, an ER-specific dye [14]. Our results showed that the treatment of cells with NH_2-PS significantly increased the staining intensity of this dye, suggesting a possible induction of ER stress. Furthermore, we detected the expression of ER stress-related proteins in RAW264.7 and BEAS-2B cells and found that IRE1α increased with NH_2-PS compared with control, thus confirming that the NH_2-PS induced ER stress in both cell lines. Previous studies have also demonstrated that the Akt/mTOR and AMPK pathway is involved in regulating autophagy [15]. Therefore, to investigate whether the Akt/mTOR and AMPK signaling pathway were involved in the NH_2-PS-induced autophagy, we performed western blotting to detect the protein phosphorylation status. The results indicated that phosphorylation of Akt, mTOR and p70S6K decreased and phosphorylation of AMPK increased in cells treated with NH_2-PS compared with control.

4 CONCLUSION

Taken together, our results indicate that NH_2-PS-induced autophagic cell death mainly occurred through inhibition of the Akt/mTOR and activation of the AMPK signaling pathways. Specifically, NH_2-PS induced autophagy could be related to er stress in raw and BEAS-2B cells. Thus, autophagy should be considered as an additional mechanism, providing intracellular selectivity for introduced NH_2-PS nanospheres.

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