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Graphene oxide-chloroquine induced p62/SQSTM1 mediated necroptotic cell death in A549 lung cancer cells

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Graphene oxide (GO) alters the autophagy response through toll like receptor signaling, lysosomal dysfunction, mitochondrial destabilization and NF-κB pathways. On the other hand, chloroquine (Chl) an FDA approved drug inhibits the autophagy and has also shown anticancer potential. In the present study, we endeavored to conjugate Chl with highly exfoliated GO nanosheets and test its antiproliferative activity on A549 lung cancer and BEAS-2B normal lung cell lines respectively. Morphological parameters have been analyzed through HRTEM, FESEM and AFM techniques. Also, TEM has been employed for analysis of cellular components on exposure of GO-Chl nanoconjugate. Further, structural, functional and optical properties of GO, Chl and GO-Chl have been investigated using Raman, FTIR and UV-Vis spectroscopy respectively. MTT assay has been performed for *in-vitro* cytotoxicity evaluation of GO, Chl and GO-Chl exposure on A549 and BEAS-2B cell lines and demonstrated that GO-Chl treatment exhibits significant cell death in A549 lung cancer cells, in contrast to an almost 90% cell survival in normal BEAS-2B cells. The flow cytometric and DCFDA assay reveals that GO-Chl has been internalized through clathrin mediated endocytosis mechanism and indicates enhanced level of ROS. Further, the autophagy response in A549 cells due to GO-Chl treatment is investigated through fluorescence microscopic analysis (MDC staining and GFP-LC3 plasmid), TEM observations and immunoblot analysis. Enhanced level of LC-3 I/II and Atg-5 markers signifies the autophagosomes formation and elevated expression of p62/SQSTM1 indicates the inhibition of autophagy at later stage. The co-immunoprecipitation assay reveals necroptotic cell death in A549 cell lines on exposure of GO-Chl.

Biography

Brahamdutt Arya is currently pursuing his Doctoral Degree under the supervision of Dr. Surinder P Singh at CSIR-National Physical Laboratory, New Delhi, India. He received his Bachelor's Degree in Chemistry Honors from MDU, Rohtak, India and Master's Degree with specialization in Organic Chemistry from University of Delhi, New Delhi, India. At present, he is working on the fabrication of gold and graphene oxide based on multifunctional nanomaterials and exploring their applications in bio imaging, drug delivery, and photothermal therapy for development of targeted and personalized nanomedicines. Also, he is developing the Indian National Standard for the gold nanoparticles.

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