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Summary and Future prospects

NLRs are cytoplasmic regulators of inflammation expressed in many cell types, including dendritic cells, macrophages, endothelial cells, and fibroblasts[Guo *et al.*, 2015]. NLRs have been shown to play an important role in various inflammatory as well biological processes such as embryo development, cell death, regulation of adaptive immune response and antigen presentation[Motta *et al.*, 2015]. Aberrant NLR signaling can lead to adverse outcomes in various diseases such as silicosis, colon cancer, and Alzheimer's disease. The aim of this study was to elucidate the importance of NLRs and associated proteins in two different scenarios: 1) Cellular and molecular pathways of innate immunity in response to amorphous nanosilica exposure in different cell types 2) cellular and molecular pathways of innate immunity in the context of glioma pathophysiology.

5.1 Summary and future prospects

Silica nanoparticles have been widely used in various industries such as cement, cosmetics, and biopesticides. They are also being used for a wide variety of therapeutic and diagnostic purposes. Micron sized crystalline silica causing cell cytotoxicity and NLR based inflammatory response has been well studied yet studies on the effects of amorphous nanosilica remains limited. Amorphous nanosilica have a higher surface reactivity, and larger surface to volume ratio, their potential cellular response, when detected as DAMP, can differ significantly. Our study revealed a dose-dependent increase in nanosilica induced endothelial cell death. We also highlight that the silica nanoparticles intake is partially dependent on phagocytosis. The finding also identifies an increase in expression of the adaptor protein, ASC following amorphous nanosilica exposure. Different sized silica nanoparticles contributed to a distinct mode of cell death, i.e., the 12nm amorphous silica nanoparticles cause necrotic cell death, while 22nm amorphous silica nanoparticles apoptotic cell death. The results summarize and characterize the potential inflammatory response against amorphous silica nanoparticles in a size-dependent and dose-dependent manner. We should be concerned about the health hazardous of amorphous silica nanoparticles considering the potential applications of silica nanoparticles in different industries .Future research can be focused on detailed characterization of the cell death pathways following amorphous nanosilica exposure on different cell types. Since phagocytosis does not play a primary role in the internalization of 12nm and 22nm amorphous silica nanoparticles further studies on how these particles are internalized inside the cells is necessary. A detailed analysis of distinct inflammatory response based on the size of amorphous silica nanoparticles is also required. Role of other NLRs in the amorphous nanosilica detection and subsequent inflammatory response can also be studied.

Dysregulated and chronic inflammation can lead to the initiation and progression of cancer. Activation of NLRs can have both pro-tumorigenic and anti-tumorigenic roles in cancer [Allen *et al.*, 2010; Janowski *et al.*, 2013]. The role of inflammation in glioma progression remains less studied. To closely analyze the role and expression of NLRs and related proteins in glioma, our lab first performed a TCGA data analysis [Sharma *et al.*, 2019]. The network analysis showed differential expression and methylation patterns of

NLRs and associated genes in different grade of gliomas. The study also identifies NLRP12 as a potential prognostic marker for glioma progression. Since TCGA data analysis only an overall tumor based NLRs expression, we utilized the human glioma tissue-based approach to identify a cell-specific expression of NLRs in glioma pathology cell. We identified that NLRP3, NLRC4, AIM2, ASC, and NLRP12 protein expression increased in grade IV glioma tissues sections. Co-localization of these proteins was also seen with microglia and astrocytes in a cell-specific manner. Multicytokine based microarray identified an increase in proliferation and invasion proteins such as VEGF, RANTES, GM-CSF, G-CSF, and MCP-1. These proteins support tumor progression and invasion by supporting tumor microenvironment and recruiting microglia and macrophages. The levels of inflammatory proteins such as IL-6, IL-1β, IL-17, TNF-α, IFN-γ, and IL-12 also increased in human glioma tissue lysates. The pro-inflammatory microenvironment helps in priming an effective adaptive immune response and further supporting tumor progression. Future studies should focus on the understanding of cellular and molecular mechanisms regulating NLRs and NLR-associated pathways during glioma pathogenesis. Considering the contributions of NLR regulation in glioma, possible therapeutic strategies can be devised targeting NLRs and associated proteins.