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Formation of nucleoplasmic protein aggregates impairs nuclear function in response to SiO_2 nanoparticles

Min Chen, Anna von Mikecz*

Institut für umweltmedizinische Forschung (IUF) at Heinrich-Heine-University Düsseldorf, Auf'm Hennekamp 50, D-40225 Düsseldorf, Germany

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Abstract

Despite of their exponentially growing use, little is known about cell biological effects of nanoparticles. Here, we report uptake of silica (SiO_2) nanoparticles to the cell nucleus where they induce aberrant clusters of topoisomerase I (topo I) in the nucleoplasm that additionally contain signature proteins of nuclear domains, and protein aggregation such as ubiquitin, proteasomes, cellular glutamine repeat (polyQ) proteins, and huntingtin. Formation of intranuclear protein aggregates (1) inhibits replication, transcription, and cell proliferation; (2) does not significantly alter proteasomal activity or cell viability; and (3) is reversible by Congo red and trehalose. Since SiO_2 nanoparticles trigger a subnuclear pathology resembling the one occurring in expanded polyglutamine neurodegenerative disorders, we suggest that integrity of the functional architecture of the cell nucleus should be used as a read out for cytotoxicity and considered in the development of safe nanotechnology.

Keywords: Cell nucleus; Cellular senescence; Nanoparticles; Nanotechnology; Nuclear domains; Protein aggregation; Proteasomes; Transcription

Introduction

Nanotechnology is expected to represent one of the leading technologies of the 21st century [1]; however, we should be aware that nanoparticles (NPs) are already broadly distributed in our environment [2]. Diesel exhaust NPs occur in fuel combustion, whereas silica (SiO_2) NPs are produced on industrial scale serving as additives to cosmetics, drugs, printer toners, varnishes, and even food. While investigation on adverse health effects and toxicokinetics of occupational and environmental particles was focused on fine and coarse particles of 0.5 to 10 μm for decades [3], the growing abundance and industrial application of nanotechnology urged a recent shift of focus towards NPs sized between 1 and 100 nm [4–6]. In addition to their ambient occurrence, NPs, including SiO_2 NPs, are being developed for a host of biomedical and biotechnological applications such as cancer therapy, DNA transfection, drug delivery, and enzyme immobilization [7–10].

In contrast to the growing literature on application of NPs and nanotechnology, there is little information about biological effects of NPs. Recent reports indicate endosomal uptake of nanoparticles into cells. Triple labeling confocal microscopy in live cells revealed localization of micellar nanocontainers, sized between 20 and 45 nm, in cytoplasmic organelles such as endoplasmatic reticulum (ER), golgi, lysosomes, and mitochondria, but not within the cell nucleus [11]. Fluorescently tagged polyethylenimine(PEI) NPs were shown to attach to cell surfaces and migrate into clumps that are endocytosed. Endocytosed PEI NPs move in vesicles and undergo nuclear localization in ordered structures which may explain their excellent properties as gene-delivery vectors [12]. In order to define the size of macromolecules that can be transported by the nuclear pore complex (NPC), Panté and Kann found using electron microscopy that gold particles with a diameter close to 39 nm could be translocated to the cell nucleus [13]. Consistent with the idea of nuclear localization of NPs, covalent conjugation of PEI chains to

* Corresponding author. Fax: +49 211 3190910.
E-mail address: mikecz@uni-duesseldorf.de (A. von Mikecz).

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gold NPs enhances PEI-mediated transfer of DNA into mammalian cells [14].

Increasing evidence for translocation of NPs into the cell nucleus raises the question whether nanoparticles influence nuclear structure and function. The mammalian cell nucleus is a membrane-bound organelle that contains the machinery essential for gene expression, ribosome biogenesis, and maintenance of the genetic material. A hallmark of the nucleus is the presence of visually and functionally defined structural compartments that are dynamic and form in response to gene expression [15]. Subnuclear compartments include nuclear speckles involved in mRNA biogenesis [16], and a variety of subnuclear foci or nuclear bodies each containing specific sets of proteins and/or RNA, such as the Cajal body [17], and the promyelocytic leukemia nuclear body (PML NB; [18]). Components of the ribosome biogenesis pathway are predominantly confined to the nucleolus [19]. Besides subnuclear compartments, the cell nucleus also contains the components of the ubiquitin-proteasome system such as ubiquitin [20], proteasomes [21], the regulator subunit PA28 [22], and the 19S regulatory complex [23]. 20S proteasomes are localized throughout the nucleoplasm in nuclear speckles, but not within nucleoli, nor the nuclear envelope [24–26]. Proteasomes are ATP-driven, multisubunit proteolytic machines that degrade endogenous proteins into peptides [27,28]. In most cultured mammalian cells, 80–90% of the protein breakdown occurs by the proteasome pathway [29]. It is generally accepted that proteasomal degradation is present in the cytoplasm, whereas the role of nuclear proteasomes and components of the ubiquitin–proteasome system is less clear. Consistent with the idea of proteasomal proteolysis in the cell nucleus, it could be shown that (1) proteasome-dependent degradation of nuclear proteins is correlated with their subnuclear localization [30], and (2) the xenobiotic mercury chloride induces recruitment of nucleolar protein fibrillarin to nucleoplasmic proteasomes and its degradation by the ubiquitin–proteasome pathway [26]. Proteasomes serve as a quality control system that rapidly eliminates misfolded or damaged proteins whose accumulation would interfere with normal cell function and viablity [31]. This quality control is of particular importance in protection of cells against harsh conditions such as heat shock and/or oxidative stress and in a variety of diseases, for example, the major degenerative diseases that are characterized by intranuclear accumulations of insoluble aggregated polyglutamine (polyQ)-containing proteins along with other proteins [32].

In this study, effects of nanoparticles on structure, function, and proteasomal proteolysis in the cell nucleus were investigated by incubation of cell lines with unlabeled and fluorescently labeled SiO_2 particles of different sizes.

Materials and methods

Particles

Plain (unlabeled) and FITC-labeled silica particles sized 0.05 μm, 0.07 μm, 0.2 μm, 0.5 μm, 1 μm, and 5 μm were purchased from Kisker (Steinfurt, Germany) and Postnova (Landsberg/Lech, Germany). Unlabeled silica microspheres sized 0.05 μm were purchased from Polysciences (Warrington, PA). Silica particles defined as 0.07 or 0.05 μm contain subfractions of nanoparticles sized between 40 and 80 nm. Silica particles were added directly to the culture medium at a concentration of 25 μg/ml. With the exception of the fluorescence microscopy shown in Fig. 1A, all experiments were carried out with unlabeled particles.

Chemicals

Suc-Leu-Leu-Val-Tyr-7-amido-4-methylcoumarin (Suc-LLVY-AMC) and lactacystin were obtained from Affiniti (Exeter, UK) and Alexis Biochemicals (San Diego, CA), respectively. To determine reversibility of protein aggregation, cells were further cultivated in SiO_2-NP-free medium containing 100 μM Congo red (Sigma, St. Louis, MO) or 100 μM trehalose (Sigma) after an initial incubation with SiO_2 NPs for 4 h.

Cells

HEp-2 cells (human epithelial) and RPMI 2650 cells (epithelial, human nasal septum) were routinely cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS). A549 cells (human lung epithelial) were maintained in DMEM medium supplemented with 10% FBS, glutamine and penicillin and streptomycin (pen/strep). RLE-6TN (rat lung epithelial) cells were cultured in Ham's F12 medium supplemented with 5% FBS, glutamine and

Fig. 1. SiO_2 nanoparticles induce nucleoplasmic clusters of topoisomerase I (topo I) in airway epithelial and neuronal cells. (A) HEp-2 cells were left untreated or treated with fluorescently labeled SiO_2 particles of different sizes (0.07–5 μm, green) for 4 h and fluorescence was detected by confocal laser scanning microscopy. Nuclear structure was observed by double labeling with anti-topo I antibodies (red). Note that exclusive nuclear appearance of fluorescently labeled SiO_2 nanoparticles (0.07 μm) is due to confocal sectioning. (B) HEp-2 cells were left untreated or treated with SiO_2 nanoparticles and analyzed for occurrence of nucleoplasmic topo I clusters at different time points (0–24 h). (C) Indirect immunofluorescence of topo I in untreated and SiO_2-nanoparticle-treated human nasal (RPMI 2650), human lung (A549), rat lung (RLE-6TN) epithelial cells, and murine neuronal cells (N2a). (D) Indirect immunofluorescence of topo I in SiO_2-NP-treated HEp-2 cells indicating time-dependent enlargement of nucleoplasmic topo I clusters. Insets display blowups of representative topo I clusters: after 4 h SiO_2 NP exposure, small clusters with diameters <1.4 μm are observed in 100% of affected cells, whereas 60% of affected cells contain large topo I clusters with a diameter of 1.4–2 μm after 24 h. Experiments (B–D) were performed with unlabeled SiO_2 NPs. Micrographs and insets show representative cells or blowups thereof, respectively. 0.07 μm, 0.2 μm, 0.5 μm, 1.0 μm, and 5 μm, SiO_2 particle sizes. Scale bars, 5 μm.