Karlsruhe Institute of Technology

Institute of Applied Biosciences
Department of Food Chemistry and Toxicology

Elucidating the impact of nanomaterials on genomic stability using high-throughput RT-qPCR and Alkaline Unwinding

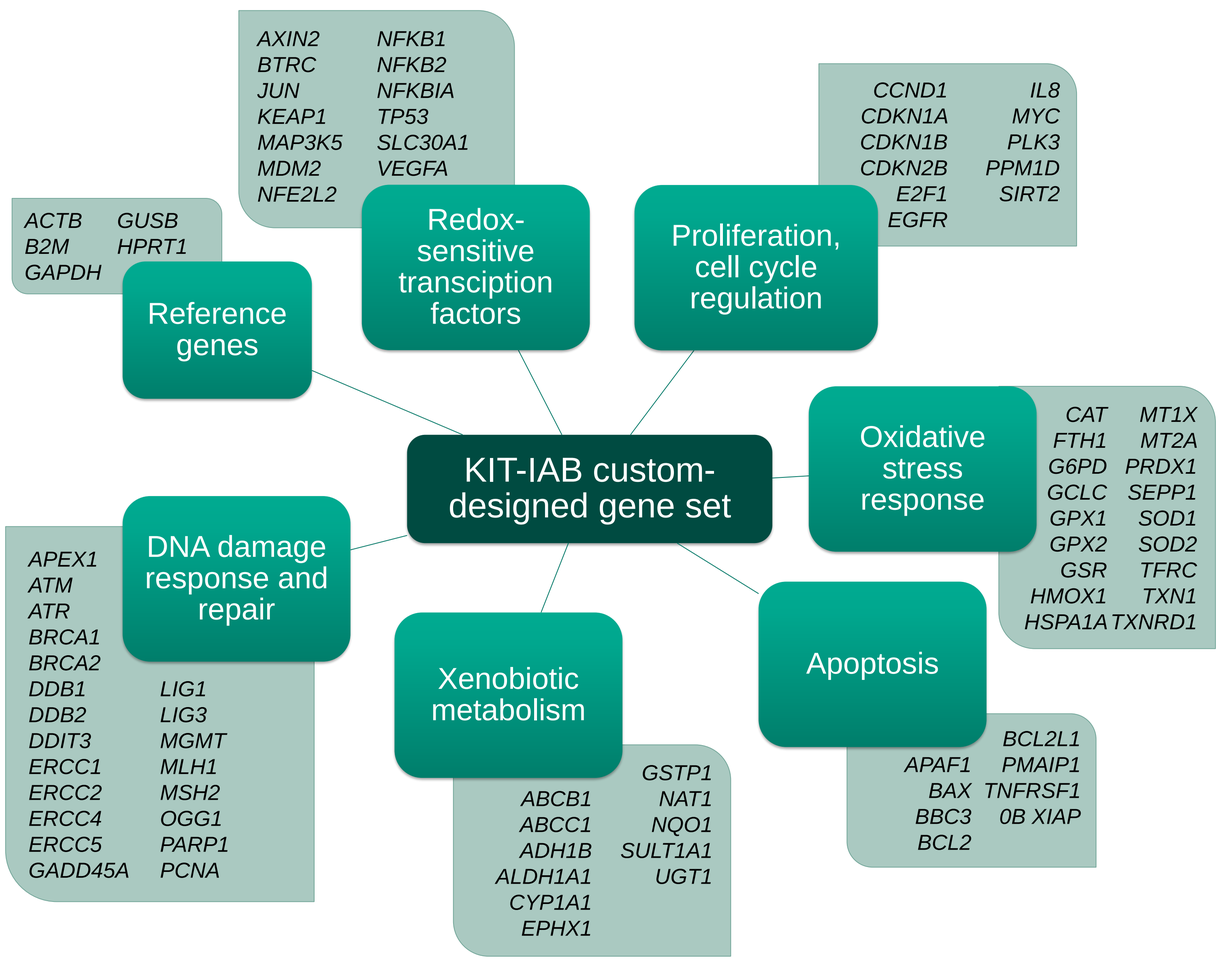
Matthias Hufnagel, Prof. Dr. Andrea Hartwig

Introduction

The increasing use of nanomaterials in various industries requires appropriate test systems to elucidate the toxicological potential of these innovative materials. Especially for airborne nanomaterials the standard *in vitro* testing under submerged conditions represent a rather artificial test system. To simulate a more realistic exposure scenario, cell cultivation and exposure at an air-liquid interface appears more appropriate. Along with the improvement of cell cultivation and exposure, there is a need to establish applicable toxicological endpoints to evaluate the toxicological potential of nanomaterials. Therefore, we established the use of a high-throughput RT-qPCR method as well as the quantitative detection of DNA strand breaks (Alkaline Unwinding) for ALI exposed cells in our working group in order to investigate the impact on genomic stability.

High-throughput RT-qPCR

The use of a specially designed high-throughput RT-qPCR method to investigate the impact of nanoparticles on genomic stability has already been published for submerged cultivation conditions[1,2]. The system is applicable to examine the impact of 96 samples on 95 genes, consisting of seven well selected gene clusters, in parallel.

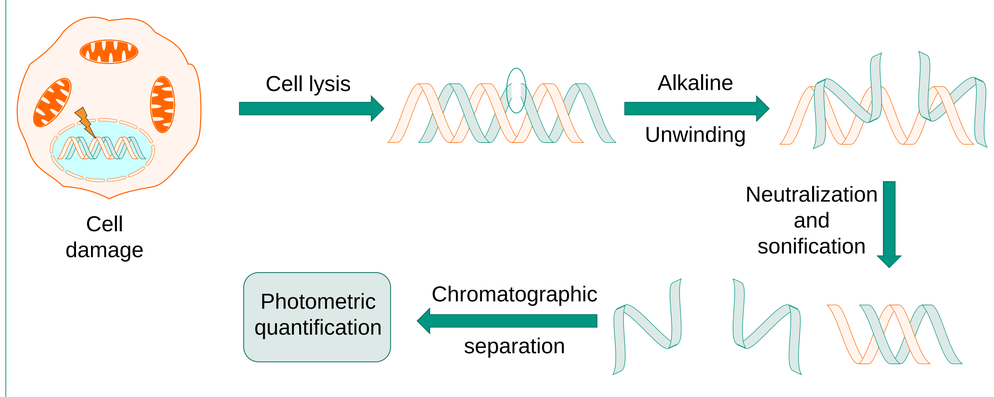


The system was validated by exposing the cells to TiO_2 nanoparticles (NP) as negative and CuO NP as positive control via the Vitrocell CLOUD.

No impact by TiO_2 NP, but pronounced dose-dependent alterations on gene expression profiles by CuO NP.

Alkaline Unwinding

Alkaline Unwinding is a fast and sensitive procedure to quantify DNA strand breaks. This method has also already been used for studies on nanomaterials under submerged conditions[3].



The quantification of DNA strand breaks per 10^6 base pairs is calculated by the ratio of double-stranded and single-stranded DNA after a chromatographic separation[4].

Analogous to the validation of the high-throughput RT-qPCR, the system was also validated using CuO and TiO_2 NP as controls. The particles were again applied via an ALI exposure using the Vitrocell CLOUD.

Dose-dependent increase of DNA strand breaks after exposure to CuO NP, no induction by TiO_2 NP.

Reference

[1] Fischer, B.M., Neumann, D., Piberger, A.L. et al. Arch. Toxicol. (2015)
[2] Strauch et al. Part. Fibre Toxicol. (2017)
[3] Semisch et al. Part. Fibre Toxicol (2014)
[4] Hartwig et al. Carcinogenesis (1993)

Aknowledgement

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