



HAL
open science

Biomarkers involved in cell transformation induced by nano-silica materials in the murine Bhas 42 model

Anaïs Kirsch, Hélène Dubois-Pot-Schneider, Hami Musaoglu, Aurélie Remy, Caroline Fontana, Hervé Schohn, Laurent Gaté, Yves Guichard

► To cite this version:

Anaïs Kirsch, Hélène Dubois-Pot-Schneider, Hami Musaoglu, Aurélie Remy, Caroline Fontana, et al.. Biomarkers involved in cell transformation induced by nano-silica materials in the murine Bhas 42 model. 12th International Particle Toxicology Conference, IPTC 2019, Sep 2019, Salzburg, Austria. hal-02414017

HAL Id: hal-02414017

<https://hal.univ-lorraine.fr/hal-02414017>

Submitted on 16 Dec 2019

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Biomarkers involved in cell transformation induced by nano-silica materials in the murine Bhas 42 model

Anais Kirsch^{*,1,2}, Hélène Dubois-Pot-Schneider², Hami Musaoglu¹, Aurélie Remy¹, Caroline Fontana¹, Hervé Schohn², Laurent Gaté¹ & Yves Guichard¹

1. Institut National de Recherche et de Sécurité (INRS), CS 60027 Vandœuvre-lès-Nancy - Cedex – 2. Université de Lorraine, CNRS, CRAN, F-54000 Nancy, France

*anais.kirsch@inrs.fr

INTRODUCTION & OBJECTIVES

Synthetic amorphous silica nanomaterials (SAS) are used in many industrial sectors. However, we have previously found that SAS (namely, **NM-200**, **NM-202** and **NM-203**) led to cell transformation in the cell transforming assay developed by Sasaki et al (2015). Among them, **NM-203 was the most active** in triggering Bhas 42 cell transformation (Fontana et al., 2015) (Seidel et al., 2017).

In order to gain insight into the molecular mechanisms involved in the early stages of the SAS-induced cell transformation, we carried out a global and unsupervised transcriptomic analysis after a 48h-treatment with **NM-203**, **Min-U-Sil 5®** (a carcinogenic crystalline silica microparticle), **DE** (Diatomaceous Earth, an amorphous silica microparticle) and **TPA** (12-*O*-Tetradecanoylphorbol-13-acetate, a tumor promoter) (**Table 1**).

Genes regulated by NM-203, Min-U-Sil 5®, and TPA-induced cell transformation were validated by RT-qPCR. Finally, we analyzed whether similar changes were also found after cell exposure to **NM-200 and NM-202**.

Sasaki K et al, J Environ Sci Health C Environ Carcinog Ecotoxicol Rev, 2015.

Fontana C et al, Mutat Res, 2017.

Seidel C et al, Nanotoxicology, 2017.

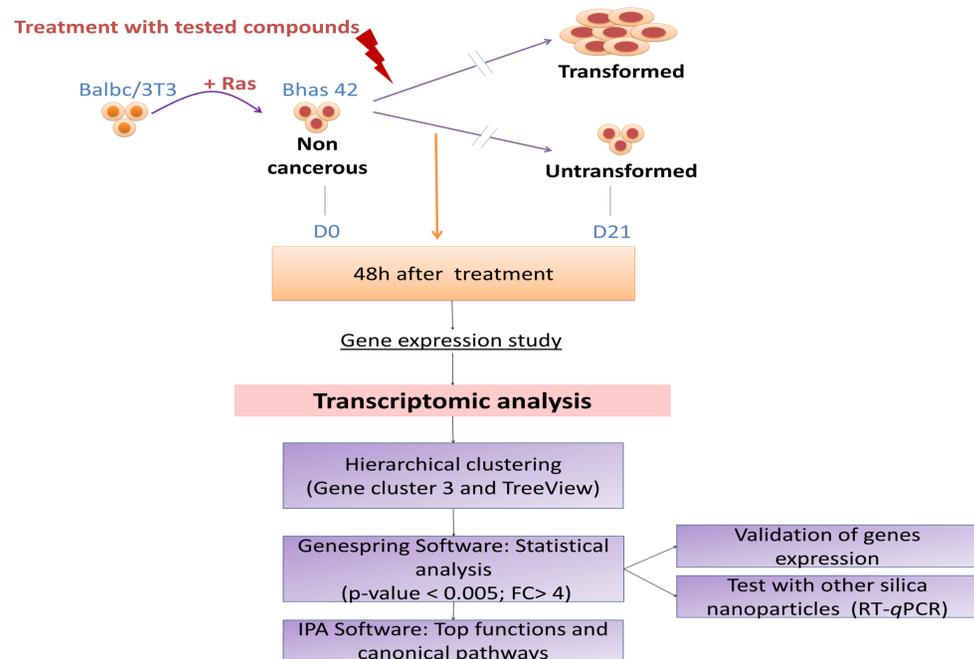


Figure 1: Bhas 42 cell transformation assay and experimental strategy: The Bhas 42 cell line is a fibroblastic murine cell line derived from Balbc / 3T3 cells transfected with the viral oncogene v-Ha-ras (Sasaki et al., 2015). Bhas 42 cells are then considered to be “initiated” cells. This cell line was used to study the effect of diverse SAS.

MATERIALS & METHODS

Table 1 : Physical characteristics of silica particles used in this study and cell transforming effect of compounds tested

Silica/ Promoter agent	Bhas 42 assay	Main phase	Primary particle size	Impurities (wt%)	BET SSA (m ² /g)	DLS	
						Z-average in Water (nm)	Polydispersity index
NM-200	+	Amorphous	18.3 ± 4.5 nm	Al (0.87); Na (0.88); S (0.46)	189	ND	ND
NM-202	+	Amorphous	17.7 ± 2.5 nm	Al (0.45); Ca (0.18)	204	232.8 ± 7.3	0.441 ± 0.012
NM-203	+	Amorphous	24.7 ± 17.7 nm	Al (0.43); S (0.04)	204	318.7 ± 4.9	0.423 ± 0.026
Min-U-Sil 5®	+	Crystalline	1.33 ± 0.85 µm	Al (0.16); Fe (0.05); Ca (0.04); Mg (0.01)	5	1385.7 ± 377.0	0.318 ± 0.130
DE	-	Amorphous	1.35 ± 1.48 µm	Al (1.4); Fe (1.3); Ca (2.2); Ti (0.3); Mg (0.2)	20	ND	ND
TPA	+						

The Bhas 42 cell transformation assay was carried out as followed: Cells were seeded at high density and treated for 48 h with NM-203 at 1 and 2 µg/cm² (non cell transforming doses) and at 5 µg/cm² (a cell transforming dose), DE at 5 µg/cm², Min-U-Sil 5® at 20 µg/cm² and TPA at 0.05 µg/ml (**Figure 1**). Characteristics of tested chemicals are presented in Table 1.

RESULTS

Hierarchical clustering showed that non cell transforming treatments (NM-203 at 1 or 2 µg/cm², DE and controls) are closed and interestingly cell transforming treatments (NM-203 at 5 µg/cm², Min-U-Sil 5® and TPA) induced similar changes in gene expression (**Figure 2**). Several genes were **differentially expressed** after treatment with **NM-203, Min-U-Sil 5® and TPA**, while we did **not find any differentially expressed genes with DE**. Furthermore, IPA analysis showed that the main pathways common to NM-203, Min-U-Sil 5® and TPA treatments were involved in **cell proliferation**. Finally, twenty genes are common to all three conditions and among them, **twelve** relevant genes were selected (**Figure 3**). The expression of these genes was validated by RT-qPCR. In addition, deregulation of selected genes were also found with **NM-200 and NM-202** as shown for two selected genes POSTN (Periostin) and AUNIP (Aurora kinase A and ninein interacting protein) (**Figure 4**).

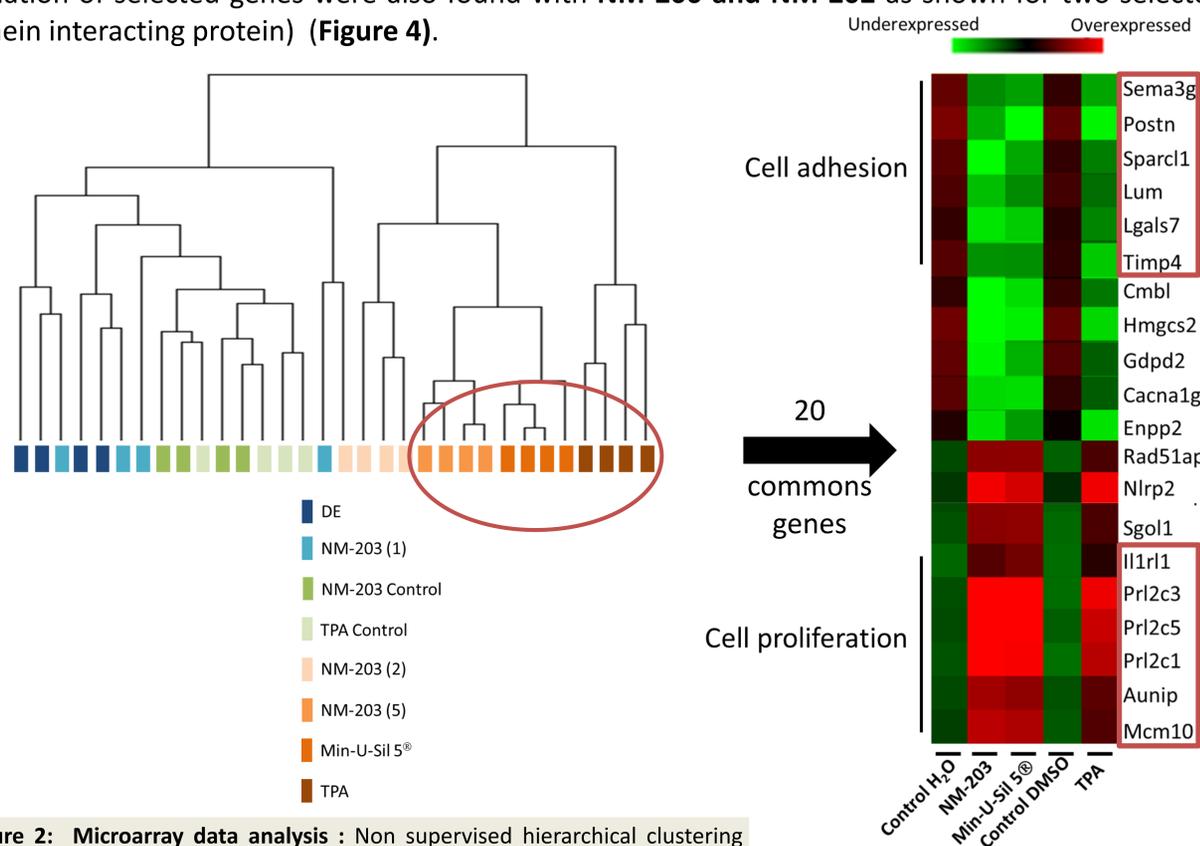


Figure 2: Microarray data analysis : Non supervised hierarchical clustering was obtained after filtration of microarray data (clustering was performed using a Gene Cluster 3 software and visualized with Treeview). Red circle show similar signature of three compounds NM-203, Min-U-Sil 5® and TPA.

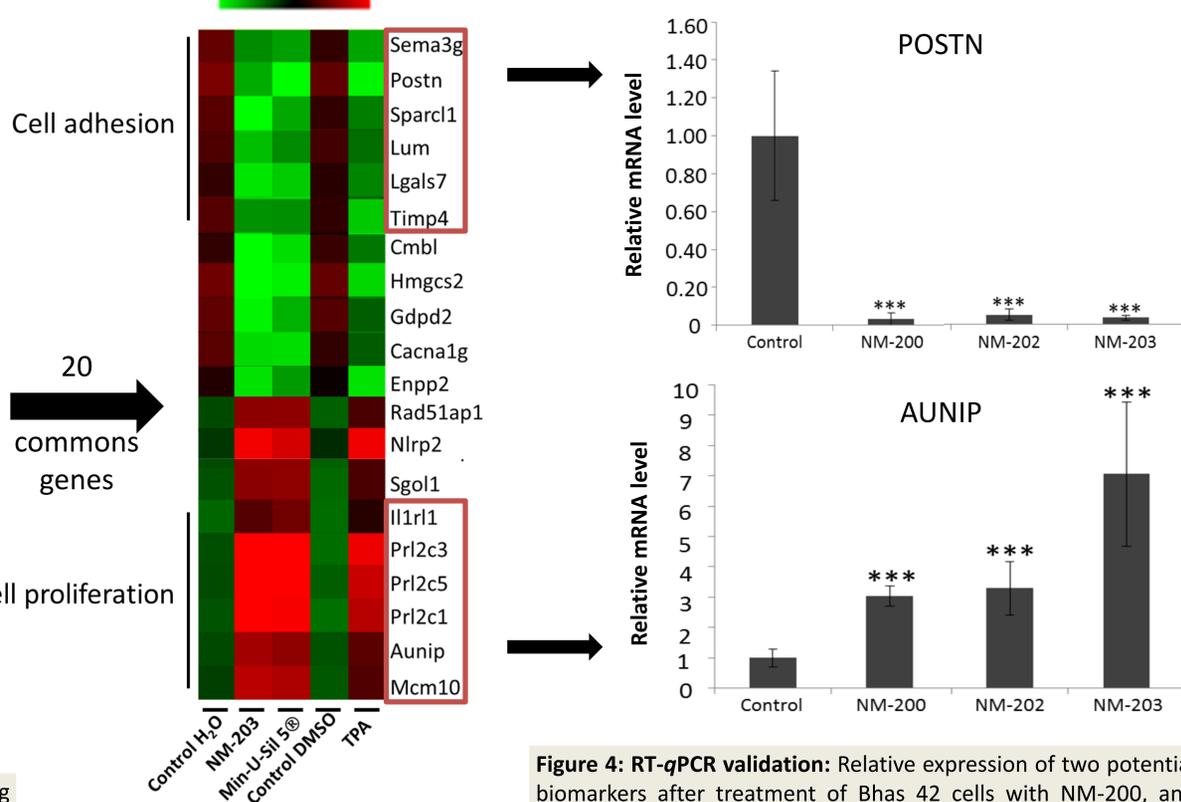


Figure 3: Heatmap of 20 common genes regulated by NM-203, Min-U-Sil 5® and TPA. p < 0.005; FC > 4

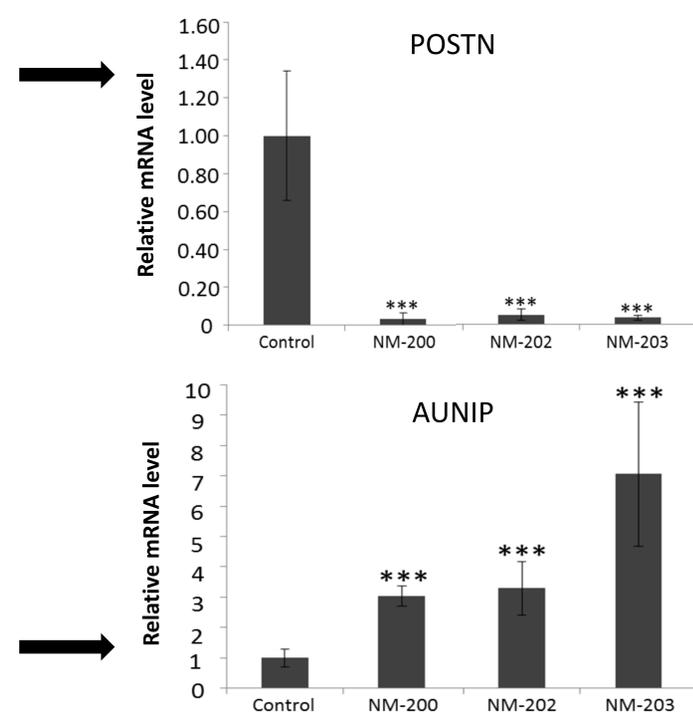


Figure 4: RT-qPCR validation: Relative expression of two potential biomarkers after treatment of Bhas 42 cells with NM-200, and NM-202. *** indicates p < 0.005 versus untreated cells after a mixed linear regression model followed by a Bonferroni multiple comparison test.

CONCLUSIONS

- ✓ For the first time, 20 common genes potentially involved in cell transformation were identified by a transcriptomic analysis after a 48h-treatment of Bhas 42 cells with **NM-203, Min-U-Sil 5® and TPA**.
- ✓ Deregulation of common genes has been found for two other SAS (**NM-200 and NM-202**).
- ✓ A set of 12 genes could be used as potential biomarkers of the early stages of Bhas 42 cell transformation for other SAS or other transforming nanomaterials.