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

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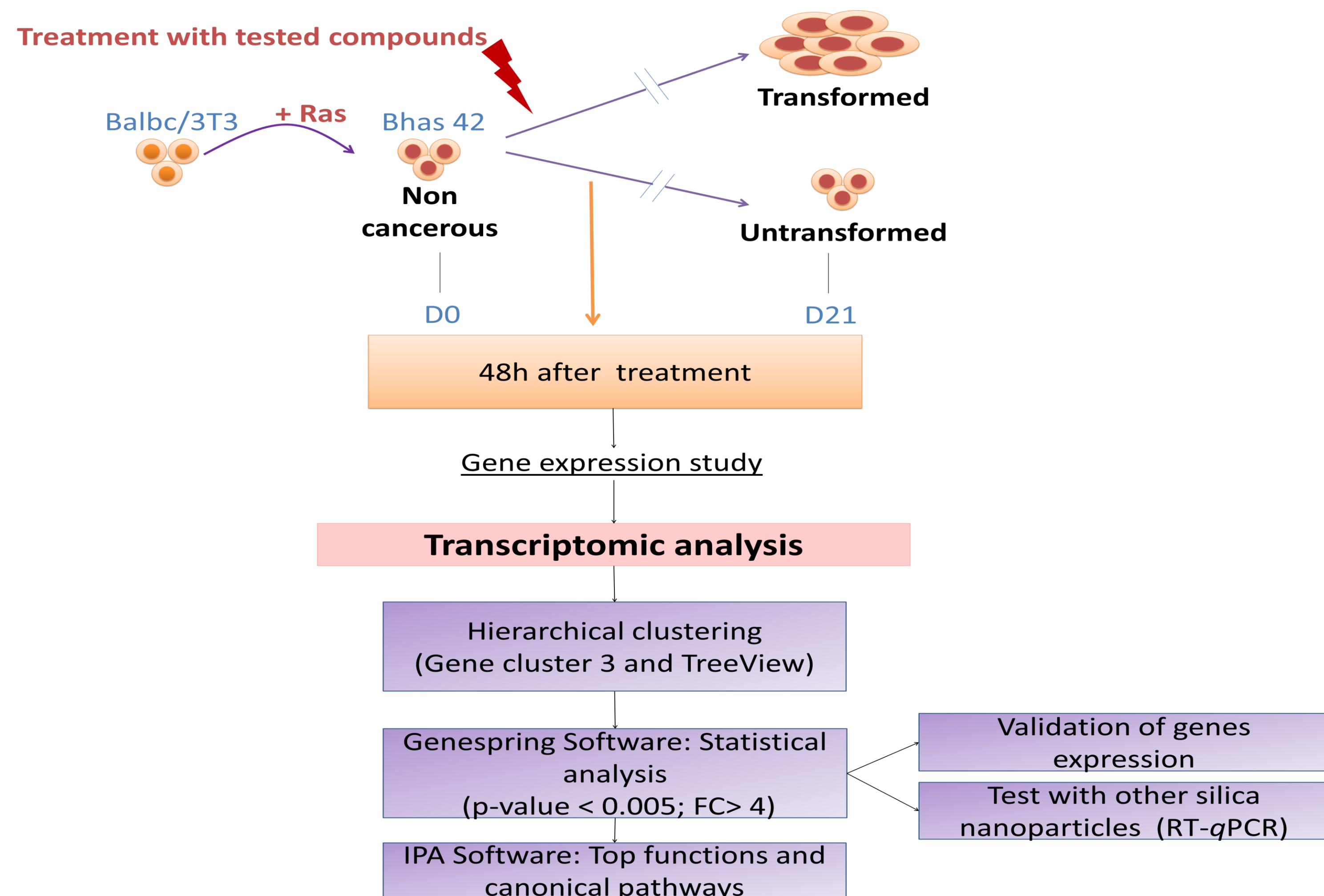
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INTRODUCTION & OBJECTIVES

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**).

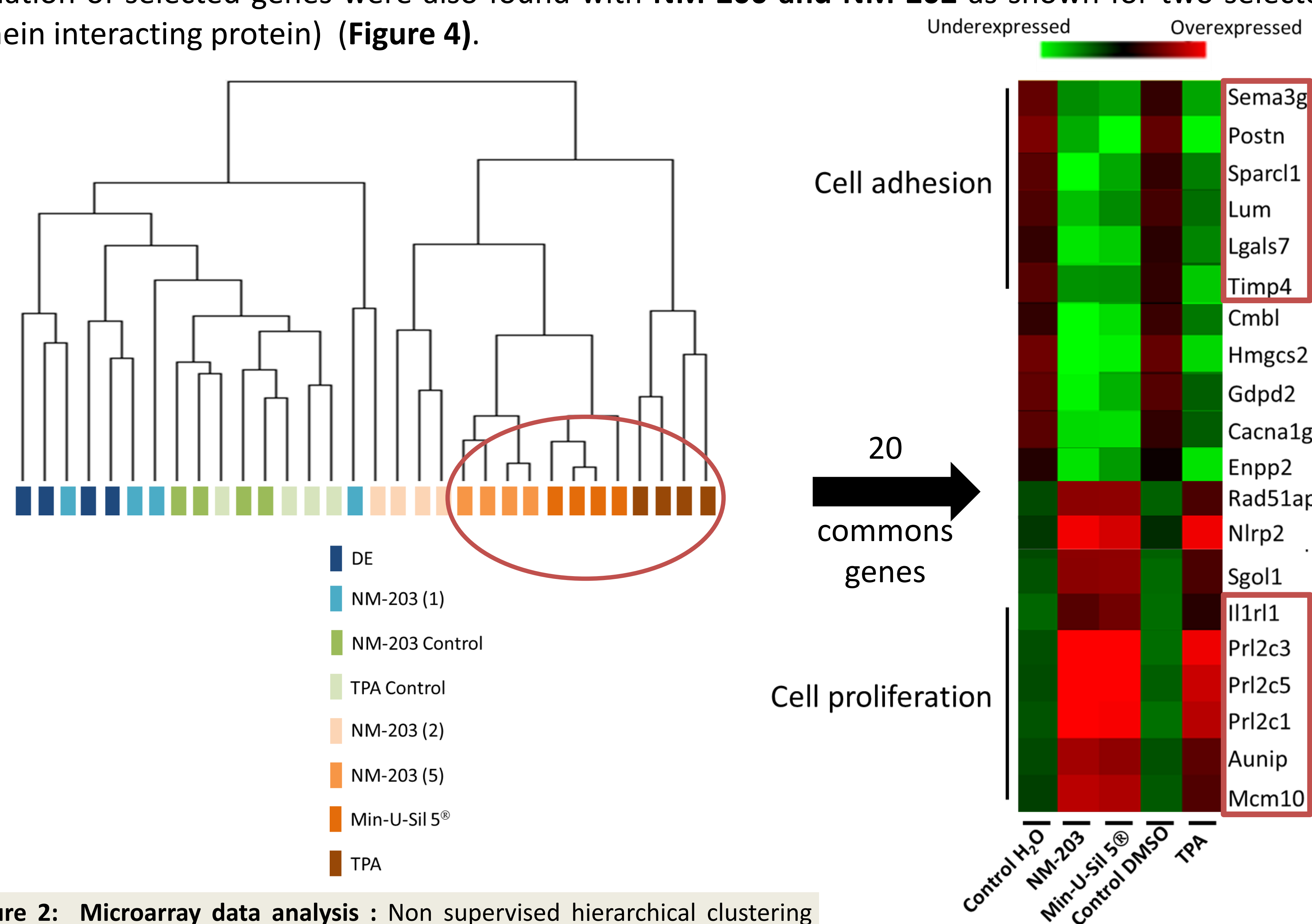


Figure 2: Microarray data analysis : Non supervised hierarchical clustering was obtained after filtration of microarray data (clustering was performed using a Gene Cluster 3 software and visualized with Treeview). Red circle show similar signature of three compounds NM-203, Min-U-Sil 5® and TPA.

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

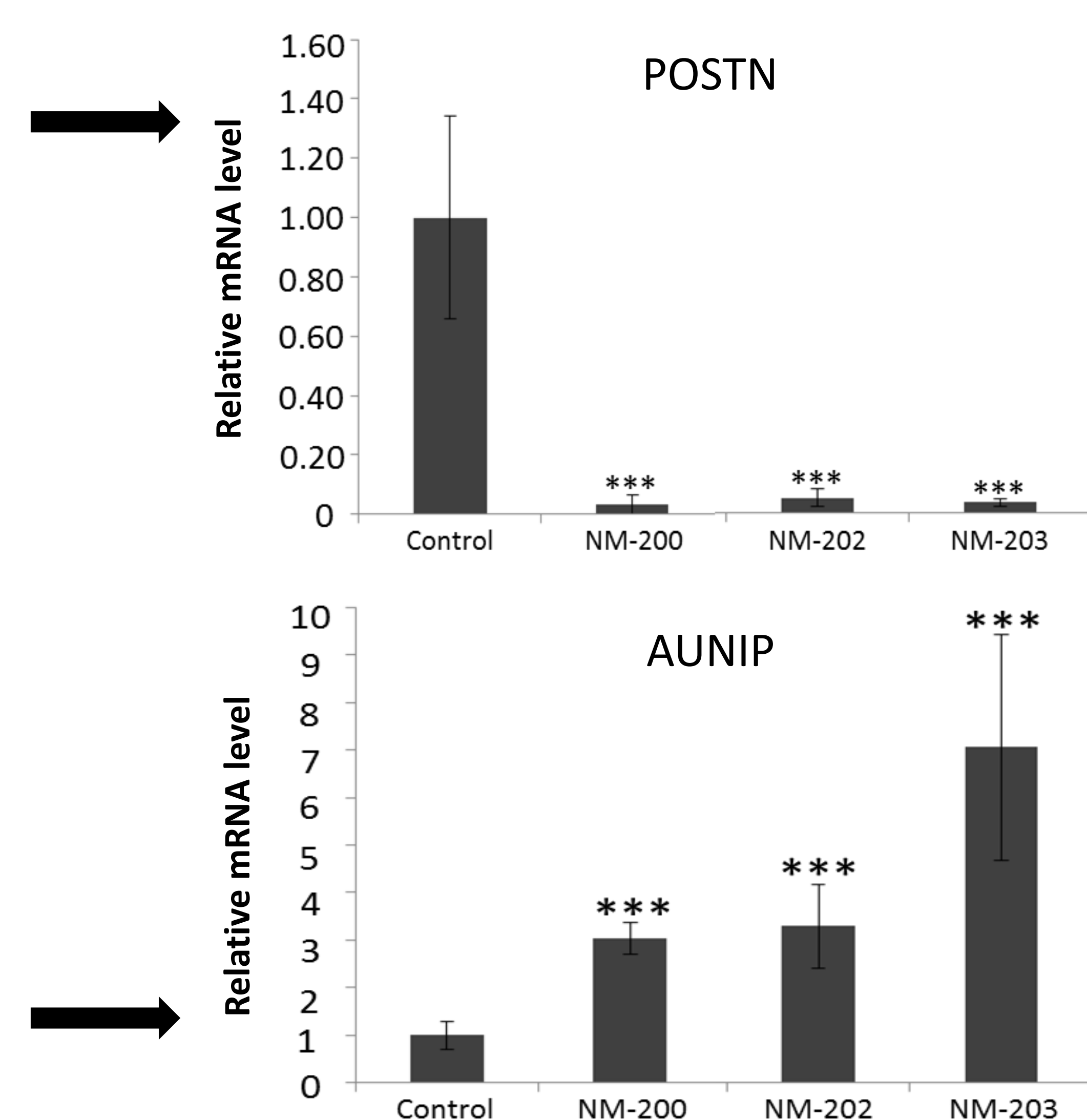


Figure 4: RT-qPCR validation: Relative expression of two potential biomarkers after treatment of Bhas 42 cells with NM-200, and NM-202. *** indicates p < 0.005 versus untreated cells after a mixed linear regression model followed by a Bonferroni multiple comparison test.

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.