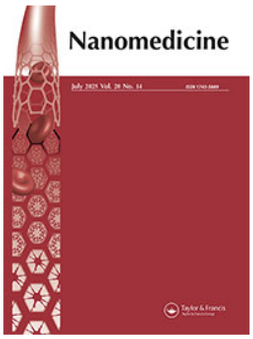

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RESEARCH ARTICLE

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Transcriptomics insight into occupational exposure to engineered nanoparticles

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ABSTRACT

Aim: To investigate the effect of acute (daily) inhalation of nanoparticles (NPs) on the transcriptomic profile of male nanocomposite research workers with a history of long-term exposure (years).

Materials & methods: Whole genome mRNA and miRNA expression changes were analyzed from blood samples collected before and after machining or welding. Exposure in the work environment was assessed using stationary and personal monitoring.

Results: Following PM0.1 exposure, a significant decrease in the expression of *DDIT4* and *FKBP5*, genes involved in the stress response, was detected in exposed workers. In the Machining group, the *DDIT4* expression correlated with the exposure dose. Increased levels of miR30-d-5p and miR-3613-5p (both involved in carcinogenesis) in welders were associated with the NP exposure dose, highlighting their potential suitability as inhalation exposure markers.

Conclusion: The results from this pilot transcriptomic analysis (mRNA and miRNA) indicate that exposure to NPs contributes to immune system deregulation and alters the pathways related to cancer. Therefore, the use of protective equipment, as well as obtaining more data by additional research, is highly recommended.

ARTICLE HISTORY

Received 24 April 2025
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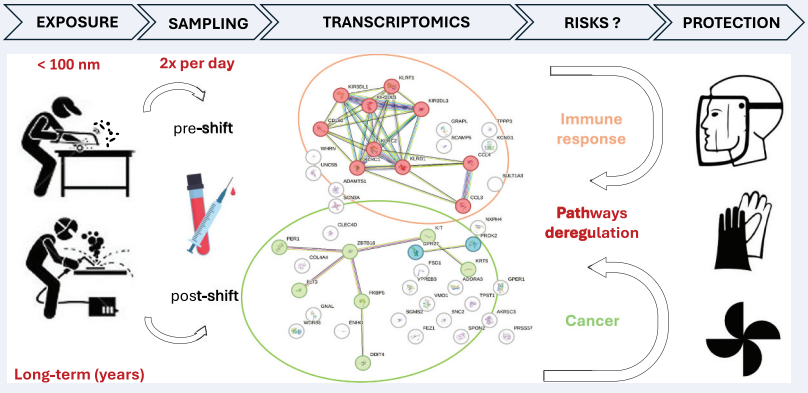
KEYWORDS

Occupational exposure; nanoparticles; transcriptome changes; welding; machining

PLAIN LANGUAGE SUMMARY

This is a follow-up study to our previous research that examined the acute effects of occupational inhalation exposure to nanoparticles (NPs) in females without a previous exposure history. This time, we reexamined the impacts of acute exposure in a group of 18 male workers, including welders and nanocomposite machinists with a long-term previous exposure history at the transcriptomic level. Whole genome transcriptomics studies the complete set of RNA molecules, or transcripts, produced in a cell or organism at a specific time. The analysis allows us to understand which genes are active/inactive, how they are regulated, and how they contribute to various biological processes or diseases. We looked at changes in mRNA and miRNA (types of RNA) from blood samples taken before and after workers were exposed to dust and fumes during machining and welding. We also monitored the exposure doses. The results suggest that inhaled NPs may present an occupational hazard to human health. The transcriptomic analysis shows that exposure to welding fumes and nanocomposite dust from machining affects the immune system and alters cancer-related pathways. Our research helps to understand NP exposure effects and may contribute to minimizing the negative health consequences of their inhalation.



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Article highlights

- Transcriptomic analysis (mRNA and miRNA) indicates that exposure to NPs contributes to immune system deregulation and alters cancer pathways.
- Differential gene expression (mRNAs and miRNAs) was studied in 18 volunteers, long-term employed male participants who worked with nanomaterials in their daily work routine.
- Acute exposure of chronically exposed volunteers to particulate matter, including NPs was monitored using personal and static air monitoring.
- During work shifts, the researchers were divided into two groups. The first workgroup (Machining) worked on grinding epoxy nanocomposite filled with SiO_2. The second group (Welding) focused on active metal gas welding of metal surfaces.
- In the subgroups, more mRNA deregulations were detected in the Welding group than in the Machining group.
- In exposed workers, a significant decrease in the expression of genes involved in the stress response *DDIT4* and *FKBP5* was detected in comparison before vs. after exposure.
- In the Machining group, the expression of *DDIT4* correlated with the exposure dose.
- In the Welders, the increased levels of miR30-d-5p and miR-3613-5p, involved in carcinogenesis, were associated with NP exposure dose, suggesting their suitability as an inhalation exposure marker.

1. Introduction

Nanotechnology has rapidly evolved since the 1980s [1]. Research consistently focuses on improving developed materials' electrical, mechanical, biomedical, and magnetic properties by enriching them with nanoparticles (NPs) [2]. Newly produced nanocomposites possess unique mechanical, electrical, thermal, optical, electrochemical, and catalytic properties. They are characterized by a multiphase structure, where at least one of the phases has one less than 100 nanometers in dimension. Nanocomposites can be divided into polymer matrix, polymer-layered silicate, ceramic-polymer, inorganic-organic-polymer, and inorganic-hybrid polymer composites [3]. These materials have been applied in many sectors of human production, such as automobile and transport, building, electronics, electrical, medical, and health products [1,4].

Researchers employed by nanotechnology industries could be exposed to uniquely designed materials with new biological, physical, and chemical properties [5]. The major route of NP exposure is inhalation, which may contribute to the processes of lung inflammation and fibrosis [6]. To date, the most valuable information about the possible toxicological impact of NPs is from *in vitro* experiments, while the data from *in vivo* studies are still limited. The animal inhalation model provides evidence of the NP's pulmonary toxicity [7,8], but overall, more research is needed to understand the impact of nanotechnology on human health, including determining appropriate exposure monitoring and control strategies. There are limited longitudinal epidemiological studies of nanomaterial workers exposed to low concentrations of engineered nanomaterials (ENM) with defined exposure [9]. The most significant exposure to NPs released from ENM is from the occupational environment, for which occupational exposure limits to NPs are missing. Current European chemical work environments are governed by national regulations that incorporate guidance documents and occupational exposure limits based on national and international classification and labeling systems, including the European framework for the Registration, Evaluation, Authorisation, and Restriction of Chemicals (REACH) [10]. In addition, the World Health Organization (WHO) has previously created general recommendations on effectively safeguarding workers from the potential risks associated with ENMs [11]. Despite this, occupational health risks associated with manufacturing and processing nanomaterials are still not clearly understood on a molecular basis. Epidemiological studies, however, have suggested potential negative health effects of NPs on nanocomposite workers [12–15].

In 2015–2020, extensive toxicological research was conducted on individuals developing nanocomposite materials. The results indicated that lung injury at the molecular level, induced by oxidative stress, was associated with NP exposure [14,16]. In a recently published study, personal NP exposure was found to be associated with elevated levels of glutathione, a key antioxidant molecule [17]. Other parts of the study indicated an increase in DNA single/double-strand breaks and micronuclei levels during welding processes [18,19], in addition to the initiation of adaptation processes following chronic NP exposure via DNA methylation alteration [20]. Although the various biomarkers discussed above were previously studied in a group of nanocomposite workers, human studies on the transcriptomic level are still scarce.

This study is a follow-up of our previous research [21] where we investigated the effects of acute NP exposure on transcriptome changes in a group of females without a previous NP inhalation history. We now focus on investigating the acute effects within the context of ongoing occupational inhalation exposure to NPs in a group of chronically exposed workers (years) on the overall transcriptomic level. The effects of exposure to NPs were studied in male volunteers exposed during metal welding and machining of epoxide resins enriched by nanoSiO_2. We aimed to search for differently expressed genes (mRNAs/miRNAs) by using whole genome next-generation sequencing. Post and pre-exposure samples were compared in the exposed group with/without consideration of the type of work (welding/machining). Moreover, the mRNA-altered biological pathways were described with the protein-protein interaction (PPI) database to construct the PPI networks of differentially expressed genes, which provides further comprehensive biological insight, helping to reveal the potentially toxic effects of occupational exposure to NPs.

2. Materials & methods

2.1. Study characterization and sample collection

In September 2020, nanocomposite researchers were sampled during their work routine [17]. A total of 18 male subjects aged between 24 and 67 years (mean 40.9 ± 10.9), with BMI between 19.7 and 37.6 (mean 27.5 ± 5.4), were selected for transcriptome assessment. Participant age and BMI are summarized in Supplementary Table S1.

All volunteers signed an informed consent form according to the Helsinki Declaration and filled out a detailed questionnaire with information about their type of exposure history, length of exposure, usage of medications, personal medical care, diet, and habits such as smoking and alcohol consumption. This study was approved by the Ethics Committees of the General University

Hospital (registration mark: 2/17 Grant GA CR – VFN) and Institute of Experimental Medicine (registration mark: 2021/04).

Whole venous blood samples were collected before (pre-shift) and after (post-shift) exposure to PAXgene Blood RNA Tubes (PreAnalytiX, Qiagen, Switzerland). The PAXgene tubes were stored at −20 °C until the RNA isolation procedure.

2.2. Exposure measurement

The study participants were exposed and monitored in three-hour blocks during two consecutive days/shifts. They were divided into two work groups based on the type of work (machining and welding). Only the active Welders worked with basic protective helmets and gloves; no protective equipment was used in the Machining group to reduce inhalation exposure. During work shifts, acute exposure to PM (particulate matter), including NPs was monitored. The first workgroup (machining) involved the grinding of epoxy nanocomposites filled with SiO2. The second group focused on metal active gas welding of metal surfaces. A detailed description of the nanocomposite material and the chemical content of the gas was published in previous studies [15–17]. Static air monitoring was conducted by online aerosol spectrometers, a Scanning Mobility Particle Sizer (SMPS, TSI, Inc., MN, USA), and an Aerodynamic Particle Sizer (APS, TSI, Inc., MN, USA) to determine aerosol particle number size distribution in the size range between 10 nm and 10 µm. Alternatively, mass size distributions were determined by Berner Low Pressure Impactor, separating particles into 10 size classes from 25 nm to approximately 13 µm (BLPI, Hauke, Austria). The personal nanoparticle sampler (PENS, Pluto Technology Co, Ltd, Taiwan) connected to the AirCheck XR5000 (SKC, Inc., USA) sampling pump measured researchers' exposure by collecting the respirable (PM4) and NP (PM0.1) fractions during work activity. An aluminum foil with a double-sprayed layer (Dekati DS-515, Kangasala, Finland) was used as the impaction substrate. A Teflon Filter cassette (SKC 225–1709, SKC, Inc., PA) with a 37 mm diameter was used for NP collection.

2.3. RNA extraction and quality analysis

RNA from whole blood samples was isolated using a PAXgene Blood miRNA Kit (PreAnalytiX, Qiagen, Switzerland). RNA concentration was determined with a Qubit 4.0 Fluorometer using a High-Sensitivity RNA kit by The Qubit® RNA HS (High Sensitivity) Assay Kit (both ThermoFisher Scientific, DE, USA). The quality of RNA was controlled with a Fragment Analyzer using an SS RNA kit (both Agilent Technologies, Santa Clara, CA, USA).

2.4. RNA library preparation and sequencing

The manufacturer's instructions were followed to prepare both types of libraries. One hundred ng of total RNA was the starting amount for miRNA library construction using QIAseq miRNA Library and QIAseq miRNA NGS 96 indexes (Qiagen, Hilden, Germany). Two hundred ng of total RNA was used for the mRNA libraries. mRNA was separated with NEBNext Poly(A) mRNA Magnetic isolation module, then mRNA libraries were processed with NEBNext Ultra II Directional RNA library prep with magnetic beads and NEBNext Multiplex oligos (New England Biolabs, Ipswich, MA, USA). The RNA library concentration was measured with the 1× dsDNA HS kit (Thermo Fisher Scientific, Wilmington, DE, USA) on the Qubit 4 fluorometer, and the quality was checked by the Fragment Analyzer with the HS NGS Fragment Kit (Agilent Technologies, Santa Clara, CA, USA). Pooled mRNA and pooled miRNA libraries were sequenced separately (pair-end for 2 × 60cycles, and single-end for 85 cycles, respectively) using the NovaSeq 6000 instrument and v1.5 chemistry (all Illumina, San Diego, CA, USA). The libraries were sequenced in isolated lanes. An nf-core/rnaseq pipeline [22] was utilized to process the RNA sequencing data. The pipeline takes a sample sheet and FASTQ files as input. It performs quality control (FastQC), trimming (TrimGalore!), and alignment (STAR, Salmon). The pipeline produces a transcript expression matrix and delivers an extensive QC report (MultiQC). The gene-level expression counts were obtained with a tximport package [23]. DESeq2 [24] with default parameter settings was applied to normalize the read counts and identify differences in gene expression between sample groups. The sister nf-core/smrnaseq pipeline was used to process miRNA sequencing data. Most steps were analogous to those described above, except for the read alignment to a reference genome, carried out using Bowtie; the mapped reads were annotated and quantified using the miRDeep2 tool.

2.5. Data analysis

Sequencing run, BMI, and age were used as covariates for mRNA deregulation. The significance was set to $p < 0.05$ with $\log_2$FoldChange >0.58 (upregulation) or < −0.58 (downregulation). Significant deregulation with the criterion of log2fold change > 0.3 or < −0.3 was set up for miRNA analysis.

The sample groups and workgroups were defined by sampling time (before and after exposure). Experiments where groups differed in sampling time only were designed as paired to maximize statistical power. The pairs were created with pre-shift and post-shift samples taken from the same volunteer, therefore, each participant served as a control for themself. Finally, the response to the PM0.1 exposure level was evaluated. In exposure graphs, the exposed samples were split into below-median exposure and above-median exposure bins to simplify the relationship. At the same time, the exposure influence was quantified with the Pearson correlation coefficient.

2.6. Analysis of the related affected mRNA-affected pathways and protein-protein interactions (PPIs)

Significantly affected pathways, functional analysis, and PPI networks were analyzed using online software; the Search Tool for Retrieval of Interacting Genes STRING (https://string-db.org/). This protein network determination provides valuable information for protein interactions. In addition, this online database generates acknowledged and expected PPI interactions together with other input data from the literature,

genome sequencing, laboratory experimental database sources, annotated pathways, and predicted co-expression interaction-based datasets [25]. The interaction score was set up as medium confidence 0.4, describing the possible link between two enzymes in the same metabolic outline. The PPI enrichment cluster was arranged at < 1.0–16 p-value.

2.7. Identification of miRNA biological targets

The miRDB online database was used for potential biological targets of identified miRNAs [26]. The evaluation of miRNA regulatory roles and the identification of controlled miRNA pathways was completed using online software mirPath v.3 (https://dianalab.e-ce.uth.gr/html/mirpathv3/index.php?r=mirpath). mirPath applied the predicted miRNA targets in coding sequences (CDS) or 3′ untranslated regions (3′-UTR), depending on the DIANA-microT-CDS algorithm or the experimentally validated miRNA interactions from the DIANA-TarBase.

2.8. miRNA-mRNA interaction predictions

Correlation-based analyses between the deregulated mRNAs and miRNAs (identified in our dataset) were performed. The analyses were conducted on all available exposed samples, using the normalized expression values obtained via the median-of-ratios method implemented in DESeq2. Two complementary approaches were used: 1) Simple correlation without adjustment for treatment variables – pairwise Pearson correlations between miRNA and mRNA expression levels); 2) Linear modeling with treatment adjustment – linear models incorporating treatment status and exposure duration as covariates.

3. Results

3.1. Elemental composition and size distribution of PM

The results (Table 1) show that welding produced mainly fine particles (<1 µm), while the number concentrations of coarse particles (>1 µm) were negligible. The number concentrations of the fractions in the size range 10–100 nm and 100 nm – 1 µm were comparable (~10^4 #/cm^3). These particles most likely resulted from the agglomeration of primary particles of high concentrations. Mechanical machining produced higher amounts of coarse particles (>1 µm) with number concentrations corresponding in the range of 10^1 #/cm^3. However, the process was also a source of nanoparticles in the size range of 10–100 nm (~10^3 #/cm^3) that were likely produced by evaporation, caused by higher temperatures reached while using mechanical tools and following the condensation of released vapors. The accumulation mode (100 nm – 1 µm) in this case was most likely mainly formed by background sources. The mass size distributions measured during welding were almost monomodal with the maxima between 300–500 nm and a second, much lower mode around 5 µm. Alternatively, samples collected during machining were dominated by a coarse mode with maxima at about 5 µm and a not fully developed fine mode of around 300 nm (Figure 1).

3.2. Individual exposure

Personal exposure to PM0.1 fraction was established by the correlation of the concentration ratio calculated from the SMPS/APS system. The individual exposure dose of PM0.1 varied among the study participants in a range from 0.5 µg to 6.7 µg with a median of 1.72 µg (Figure 1). The type of work (machining or welding) and occupational classification/position (main operator) reflect the dose of NPs.

3.3. Transcriptomics – mRNA analysis

Blood samples from eight volunteers performing machining and ten performing welding, collected *before and after* three-hour shifts, were analyzed to discover the gene expression changes resulting from exposure to NP. The Exposed group contained both of these subgroups; the type of work was used as a covariate in the analysis. A total of 50 deregulated mRNAs were found in the Exposed group when samples collected before and after work shifts were compared (Table 2). In all these comparisons, mRNAs were downregulated rather than upregulated. In the subgroups, a higher number of deregulations was detected in the Welding, than in the Machining group. Twelve common deregulated mRNAs were identified among all three comparisons. A higher proportion of unique deregulated mRNAs was observed in the Welding group than in the Machining group (Figure 2).

A list of the three most significantly deregulated mRNAs for each comparison is summarized in Table 3 (a complete list of the deregulated genes is reported in Supplementary Table S2). In all comparisons, all of the top three genes were downregulated, and two of them (*DDIT4* and *FKBP5*) were common for all groups. On the contrary, the third one was unique for each comparison.

Table 1. The proportion of particle numbers in each size fraction was measured by online spectrometers (SMPS and APS) during the shift concerning the work process.

	Percentages of particles in the given size fraction (%)			Total number concentration in particular fractions (#/cm^3)		
Process	10 nm – 100 nm	100 nm – 1 µm	1 µm – 10 µm	10 nm – 100 nm	100 nm – 1 µm	1 µm – 10 µm
Welding A (workshop 1)	54.66	45.33	0.01	3.62×10^4	3.00×10^4	3.80×10^0
Welding B (workshop 1)	37.90	62.09	0.01	1.27×10^4	2.07×10^4	3.62×10^0
Welding background	72.74	27.26	0.01	2.44×10^3	9.14×10^2	2.21×10^1
Machining A (workshop 2)	66.89	32.83	0.28	3.89×10^3	1.91×10^3	1.60×10^1
Machining B (workshop 2)	76.34	23.51	0.15	6.93×10^3	2.14×10^3	1.40×10^1
Machining background	44.12	55.87	0.01	1.23×10^3	1.55×10^3	2.37×10^1

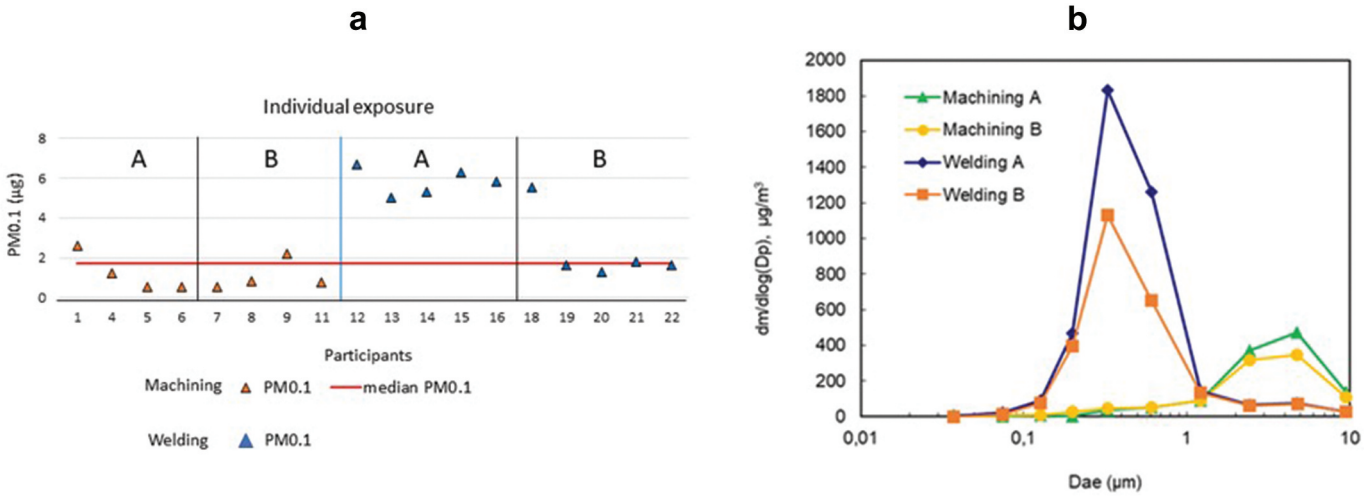

Figure 1. (a) Individual exposure dose of PM0.1 for both working groups/shifts (A- morning shift, B- afternoon shift). The horizontal red line represents the median exposure dose. The x-axis indicates the ID of the individual participants. (b) Particle mass size distribution for both operations, Welding and Machining, in morning and afternoon shifts, as determined by Berner Low Pressure Impactor.

3.4. The correlation between the exposure dose of PM0.1 and the gene expression level

We searched for a correlation between the PM0.1 NP exposure dose and the gene expression alteration. The correlation was checked for all three referred comparisons, including the three most significantly affected genes (Table 3). The only marginally significant correlation was detected for the *DDIT4* gene expression level in the Machining group. No significant correlation between exposure dose and expression level changes was observed in the other groups.

These nine examples of deregulation were chosen to manifest the individual variability of participants' gene expression response to PM0.1 exposure. Large interindividual variability in gene expression profiles within the studied participants was detected (Figure 3).

3.5. Analysis of the related mRNA-affected pathways and protein-protein interactions (PPIs)

The results of the online tool STRING for functional enrichment analysis are the three clusters shown in Figure 2. A summary of basic information about the created PPI network is presented in Table 4. The individual involvement of deregulated genes in connected pathways is summarized in Supplementary Table S3. Cluster one includes 10 nodes and is associated with immune response processes (e.g., MHC class I-like antigen recognition-like, MHC class Ib receptor activity, Antigen processing and presentation, Natural killer cell mediated cytotoxicity, Immune System, and Prostaglandin signaling). Cluster two contains 7 nodes, related mainly to cancer processes (e.g., Acute myeloid leukemia, Mast-cell leukemia, and FLT3 signaling through SRC family kinases, or Pathways in cancer). No significant enrichment was determined in Cluster 3.

Table 2. The total number of deregulated mRNAs.

	Down	Up	Total
Machining	18	8	26
Welding	23	12	35
Exposed	35	15	50

3.6. miRNA analysis

Compared to mRNA, most of the discovered miRNAs were upregulated. No deregulated miRNA was found in the Machining group. Eight altered miRNAs were identified in the Welding group. After exposure, miRNA hsa-miR-3613-5p was detected as the most upregulated, and in contrast, hsa-miR-4646-3p was found to be the most downregulated. In the Welding group, hsa-miR-3613-5p was the most upregulated, and only one miRNA (hsa-miR-4448) was downregulated. The highest number (20) of deregulated miRNAs was identified in the Exposed group. We detected six overlapping miRNAs commonly deregulated in both groups (Figure 4).

3.7. miRNA target prediction

Gene target detection is an important process in miRNA functional studies. For the selected three most up- and down-regulated miRNAs, target mRNA prediction from the MiRDB database with the algorithm MirTaget2 was performed. The results are presented in Supplementary Table S4 with common targets predicted by an algorithm, prepared to provide insight into the mRNA target. Most gene targets (18) were identified for hsa-miR-30e-5p. According to the STRING database, the target genes are involved in the O-linked glycosylation of mucins, the Oligosaccharide metabolic process, and the Glycosphingolipid biosynthesis pathways.

3.8. Interindividual miRNA expression variability

A significant correlation between the miRNA expression changes and the exposure dose of PM0.1 was studied with the top five significantly deregulated miRNAs (Table 5). Two

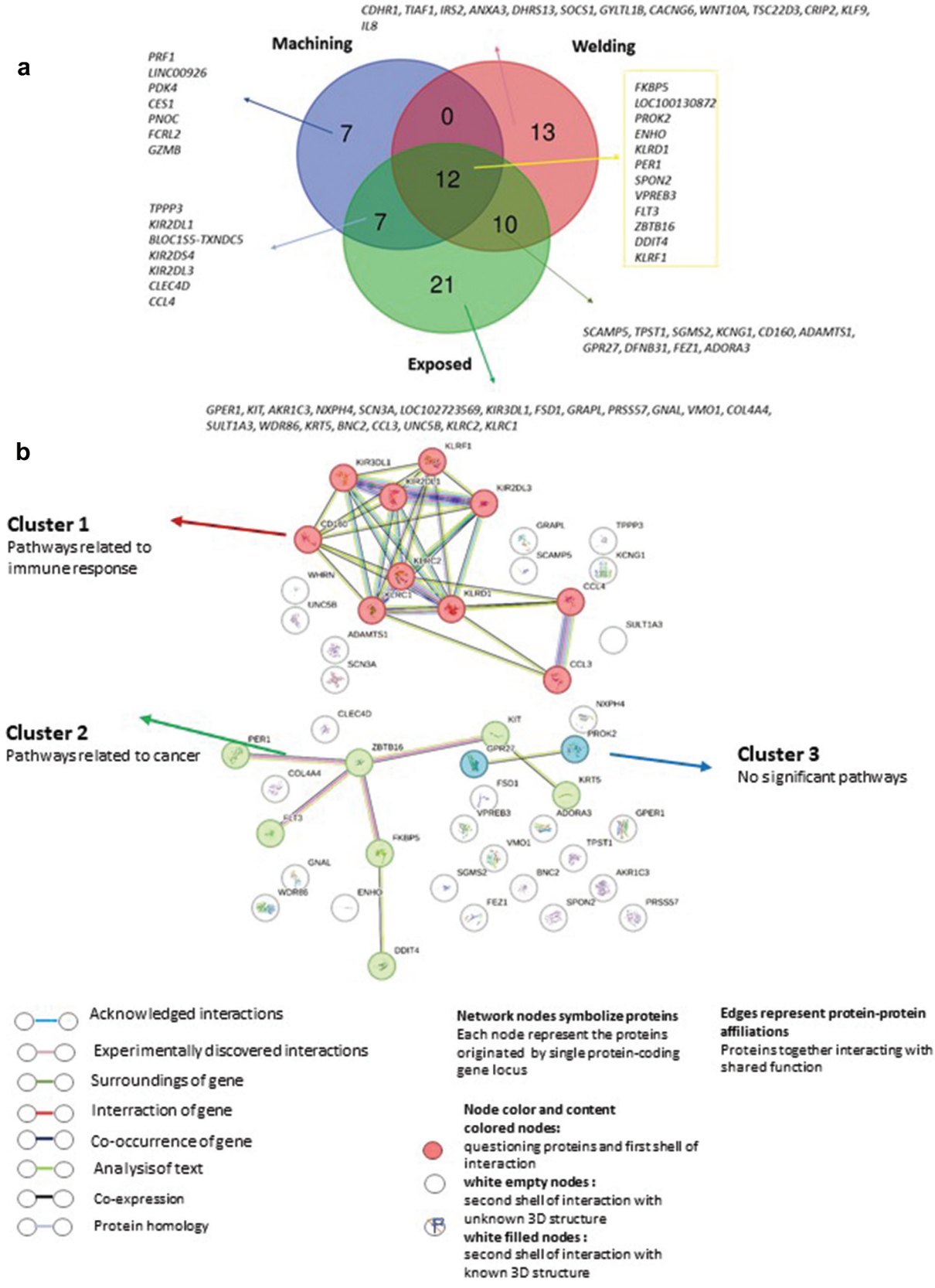

Figure 2. (a) The Venn diagram demonstrates the distribution of common and unique mRNA deregulation. (b) The three biologically relevant clusters derived from the Exposed group PPI network analysis.

Table 3. The top three most significantly modulated mRNAs for each group.

				deregulation		correlation with the dose of PM0.1	
	Name	Full name	Function	Log2FC	padj	correlation	p-value
Machining	*FKBP5*	FKBP Prolyl Isomerase 5	immunoregulation	-1.09	2.59E-15	0.25	0.55
	DDIT4	DNA Damage Inducible Transcript 4	response to virus; negative regulation of TOR signaling; response to hypoxia	-1.01	7.24E-10	-0.70	0.05
	KLRD1	Killer Cell Lectin Like Receptor D1	regulation of NK cell function	-0.76	4.96E-05	-0.19	0.66
Welding	*DDIT4*	DNA Damage Inducible Transcript 4	response to virus; negative regulation of TOR signaling; response to hypoxia	-1.23	2.77E-35	0.01	0.99
	IRS2	Insulin Receptor Substrate 2	control of various cellular processes by insulin	-0.68	1.31E-12	0.29	0.43
	FKBP5	FKBP Prolyl Isomerase 5	immunoregulation	-1.14	1.50E-12	0.10	0.79
Exposed	*DDIT4*	DNA Damage Inducible Transcript 4	response to virus; negative regulation of TOR signaling; response to hypoxia	-1.13	4.56E-36	0.02	0.94
	FKBP5	FKBP Prolyl Isomerase 5	immunoregulation	-1.12	8.27E-36	0.25	0.31
	FLT3	Fms Related Receptor Tyrosine Kinase 3	regulation of hematopoiesis	-1.07	9.56E-21	0.21	0.40

The green color indicates downregulation; the red color highlights a significant correlation with the exposure dose.

miRNAs (hsa-miR-3613-5p and hsa-miR-30d-5p) correlated significantly with the exposure dose. In addition, we detected interindividual variability in the miRNA expression profile changes among the studied participants. To demonstrate the different responses to PM0.1 exposure on the individual molecular level, the top three miRNA expression-exposure dose correlations are presented in Figure 5.

3.9. miRNA related pathways

Functional enrichment analysis using significantly deregulated miRNAs in the Exposed and Welding groups was performed (Figure 4, Supplementary Table S5). Among these groups, 24 enriched KEGG pathways were shared. The exposure may have altered a total of 39 pathways in the Exposed group. In the Welding group, 32 pathways were identified. The Exposed group showed the most alteration of pathways associated with the TGF-beta signaling pathway, ECM-receptor interaction, glioma, Proteoglycans in cancer, and the Hippo signaling pathway. The Welding group showed possible changes in pathways associated with cancer-related signaling pathways. The most common pathways were related to immune system signaling in cancer and different types of carcinogenesis (glioma, prostate cancer, small cell lung cancer, etc.).

3.10. miRNA-mRNA interaction predictions

The prediction was implemented in the Exposed group, where 50 mRNA and 20 miRNAs were found deregulated. Based on the "Simple correlation without adjustment for treatment variables," a network including 20 significant negative correlations are presented in Supplementary Figure S1 with details in Supplementary Table S6. While this approach benefits from clear statistical significance, it may be confounded by treatment effects. Although no correlations reached statistical significance after correction in "Linear modeling with treatment adjustment," we report the 25 strongest negative interactions in Supplementary Figure S2 with details in Supplementary Table S7. This later approach provides more reliable evidence of miRNA – mRNA regulation independent of experimental design biases. Together, these analyses offer a more integrated view of potential miRNA – mRNA interactions in our dataset. However, given that the treatment-adjusted models did not yield statistically significant correlations despite their methodological rigor, the unadjusted correlations are likely confounded by treatment effects.

4. Discussion

Previous human biomonitoring studies have indicated that occupational exposure to TiO_2 and FeO NPs may induce oxidative damage to DNA, lipids, and proteins in the exhaled breath condensate of workers during NP processing [15–18,20]. Furthermore, these exposures may initiate adaptation processes associated with long-term chronic exposure [17,27]. The current study serves as follow-up research on this well-documented group of nanocomposite researchers from the Czech Republic. We compared the effects of two work activities (Machining and Welding) using transcriptomic data to investigate the impact of acute exposure to NPs on long-term employees and daily occupationally exposed researchers. Our focus was on PM0.1 released during metal active gas welding and machining of epoxy resins enriched with SiO_2. To date, no transcriptomics analysis of mRNA and miRNA expression regarding occupational exposure to NPs has been conducted.

Toxicological data have generally demonstrated the potential harmful effects of NP inhalation on human health, ranging from mild tissue inflammation to chronic systemic disorders [28–30]. A higher incidence of respiratory diseases and lung cancer among welders has been noted [31–33]. The health

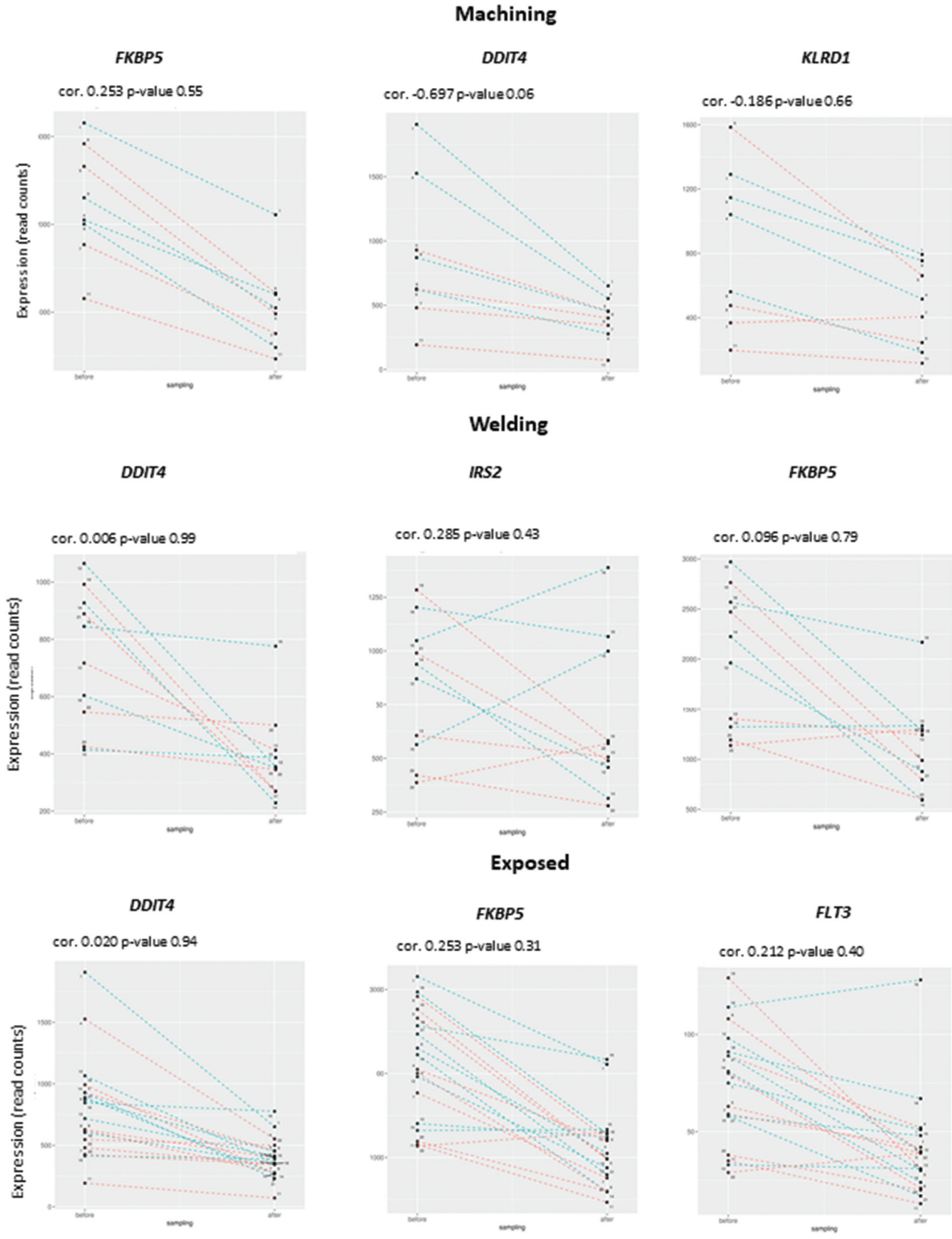

Figure 3. Interindividual variability in expression profile changes of the top three most significantly deregulated genes affected by PM0.1 exposure in Machining, Welding, and Exposed groups. "Before" and "after" indicate the expression values before and after exposure. Each participant is represented as a number and a black dot. The color of the line indicates the exposure dose – blue for high (above median) and orange for low dose (below median).

Table 4. A summary of the basic information of the PPI network.

	Cluster 1	Cluster 2	Cluster 3
Number of nodes	10	7	2
Average node degree	6.8	1.71	1
PPI enrichment p-value	<1.0E−16	0.00006	0.00162
Top 5 hub nodes	*KLRC1*	*ZBTB16*	*GPR27*
	KLRD1	*FKBP5*	*PROK2*
	KLRC2	*KIT*	
	CD160	*DDIT4*	
	KIR2DL1	*FLT3*	

risks associated with NP exposure are corroborated by toxicological research detailing the toxicity of specific particles. A basic chemical analysis of aerosols produced by welding shows that they mainly contain iron (Fe), manganese (Mn), and nickel (Ni), whereas the machining process generates a markedly different pattern of chemical compounds [19]. A strong correlation between respirable dust and manganese, which exhibits neurotoxic effects, was observed during steel welding [34]. Other epidemiological studies identified even more types of artificially released NPs during daily work activities in the manufacturing or welding industry [35–37].

As a result of both work activities in this study (Machining and Welding), a significant number of NPs were produced, and the exposure dose of PM0.1 varied among individual participants. The specific properties of the materials used and the work activities contribute to the quantity of released NPs [27]. We assume that the type of work (Machining or Welding) and occupational position (main operator) reflect the dose of respirable NPs. Welding is associated with a higher exposure dose of PM0.1 compared to Machining. The small size of ultrafine particles (smaller than 0.1 µm) allows them to pass through the alveoli, the most distal regions of the lungs, evade the primary airway defense mechanisms of the respiratory system [30], enter the bloodstream, and come into direct contact with the vascular endothelium [29]. The toxicity of NPs is mediated by altering the immune response, leading to pathological conditions (allergies, autoimmunity, tumors) [28]. Stainless steel particles from welding fumes have been found to generate reactive oxygen species (ROS) and cause DNA damage, lung macrophage cytotoxicity, and *in vivo* lung cell apoptosis [31,32]. The immunotoxicity of SiO_2, released during the machining of the nanocomposite, may contribute to the proinflammatory responses and the generation of ROS [33]. Acute exposure to silica particles can result in inflammatory responses in macrophages, leading to conditions such as silicosis, and increase the risk of autoimmune diseases or other chronic lung conditions [34]. Inhalation exposure of workers to high levels of silica dust over extended periods is associated with an increased risk of silicosis, lung cancer, and other respiratory diseases [35,37].

In the current study, we identified a significant alteration in the expression levels of mRNAs among the participants following exposure to PM0.1 from machining and welding. Fifty deregulated genes (predominantly downregulated) were discovered when both groups were analyzed together. We assume that these changes at the mRNA level reflect the individual response variability concerning inhalation. In the Welding group, we observed a greater number of deregulated mRNAs than in the Machining group, corresponding to the higher individual exposure dose.

A significant decrease in the expression of *DDIT4* (DNA damage-inducible transcript 4) after exposure to PM0.1 was noted in all comparisons (Machining, Welding, and Exposed – a combination of both subgroups). Interestingly, similar effects were observed in our previous human transcriptomic study, where female volunteers without a previous record of exposure history were acutely exposed to nanoparticles while grinding dental nanocomposites [21]. *DDIT4* is involved in the negative regulation of the TOR signaling pathway and is highly expressed in response to stress conditions like hypoxia and DNA damage [38]. In clinical studies related to cancer research, the same direction of gene deregulation was reported. The *DDIT4* downregulation contributed to different types of cancers, such as lung cancer, acute myeloid leukemia, bladder cancer, gastric, ovarian, and triple-negative breast cancer [39–42]. A borderline significant negative correlation was identified between exposure to PM0.1 and mRNA expression levels in the Machining group.

In addition, decreased expression of mRNA encoding FK506 binding protein 5 (*FKBP5*) after exposure to PM0.1 was observed. Such alteration was also detected in our previous study [21]. FKBP5 is a protein, encoded by the highly conserved *FKBP* gene in eukaryotes, that plays an important role in the control of cellular processes, especially in the stress response and immunoregulation. It is a member of the large immunophilin family known for its ability to influence steroid response pathways. The essential role of FKBP proteins is in regulating glucocorticoid signaling, canonical and non-canonical NF-κB signaling, mTOR/AKT signaling, and TGF-β signaling [43]. The role of FKBP5 in occupational exposure confirms its general importance in biological processes, especially concerning stress response, inflammatory signaling pathways, glucose homeostasis, and immune response [44]. Changes in the *FKBP* expression levels have been connected to different types of cancers, psychiatric disorders, cardiovascular diseases, asthma, obesity, and diabetes [45–47]. However, the functional validation at the protein level must be experimentally performed to support our preliminary data.

As aforementioned, we also searched for a correlation between the exposure dose and the gene expression changes. The global knowledge of molecular processes and the exact mechanism of inhaled NP toxicity is not completely understood. The individual response to inhalation exposure is a process influenced by many factors such as genetic polymorphisms, the setting of the immune system, the nature of the chemical substances, dosage, route of exposure, gender, age and health condition, and lifestyle [48]. These factors, as well as the sample size, could be a reason why we detected only a borderline significant correlation between exposure to PM0.1 and mRNA expression changes (*DDIT4*). Due to the relatively small sample size in this study, only BMI and age were used as covariates, so other, non-included factors may play a role in the correlation of exposure to PM0.1 and expression levels.

Based on our transcriptomic data and STRING analysis, the pathways associated with immune response and cancer-related processes were found to be possibly altered. This

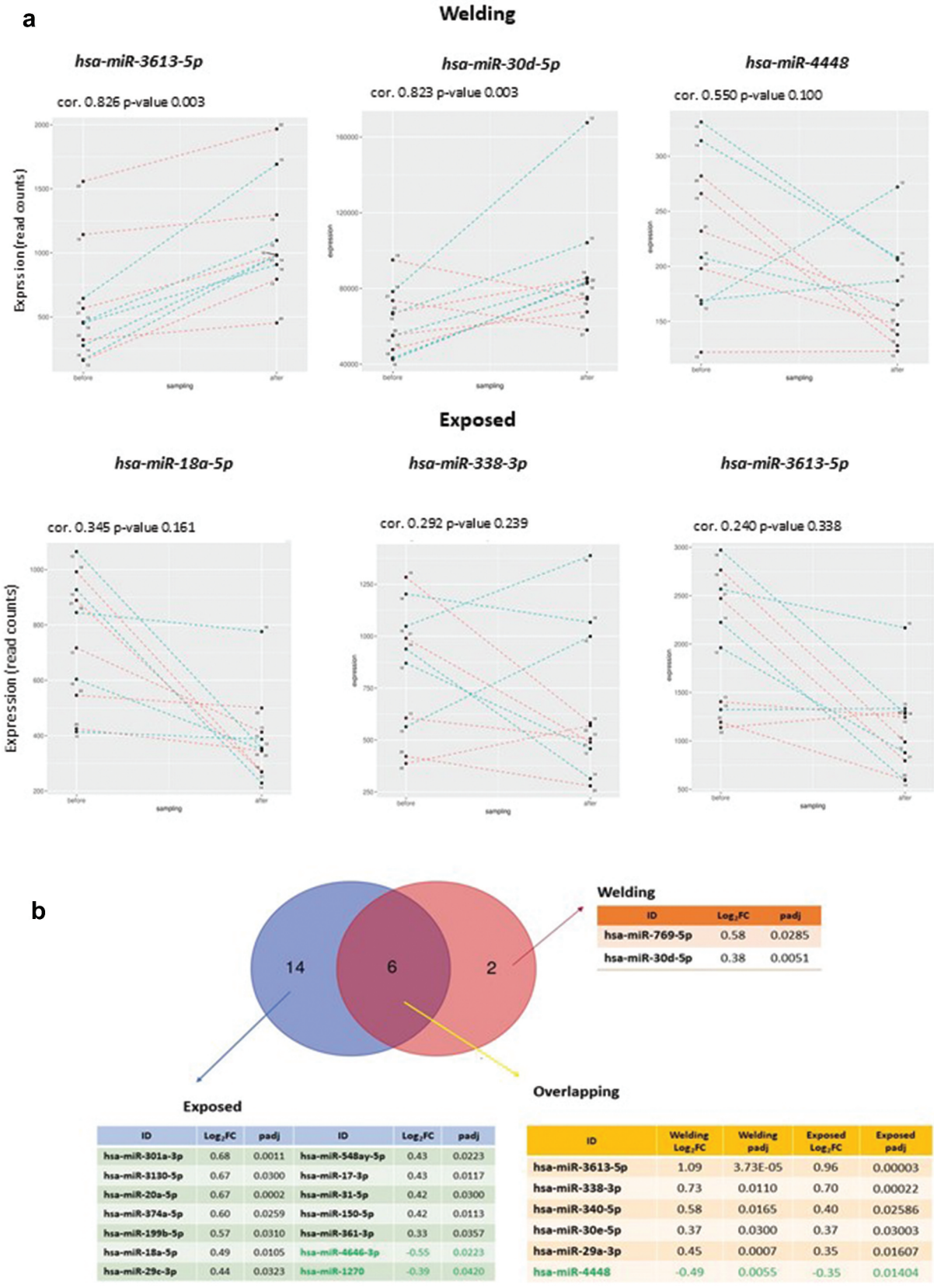

Figure 4. (a) Interindividual variability in the expression profile changes of the top three most significantly deregulated genes affected by PM0.1 exposure in Machining, Welding, andExposed groups. "Before" and "after" indicate the expression values before and after exposure. Each participant is represented as a number and a black dot. The color of the line indicates the exposure dose – blue for high (above median) and orange for low dose (below median). (b) Venn diagram showing the number of detected common and unique deregulated miRNAs with their Log2FC. Overlapped miRNAs are also summarized. The green numbers indicate downregulated miRNAs.

Table 5. The top five most significantly deregulated miRNAs in correlation with the PM0.1 exposure dose.

	miRNA	correlation	p-value
Welding	hsa-miR-3613-5p	0.826	0.003
	hsa-miR-30d-5p	0.823	0.003
	hsa-miR-4448	0.550	0.100
	hsa-miR-30e-5p	0.545	0.104
	hsa-miR-29a-3p	0.512	0.131
Exposed	hsa-miR-18a-5p	0.345	0.161
	hsa-miR-338-3p	0.292	0.239
	hsa-miR-3613-5p	0.240	0.338
	hsa-miR-20a-5p	0.237	0.343
	hsa-miR-301a-3p	-0.012	0.961

The red highlighted numbers show significant correlations with the exposure dose.

observation is under the fact that NPs can be eliminated by macrophages and the intestinal-lymphatic system, or potentially distributed to other organs [49,50], thus employing an immune response. The alterations of the carcinogenesis-related pathways are in line with the carcinogenicity of welding fumes, as classified by the International Agency for Research[* [51] on Cancer (IARC, 2018). However, the association between welding and cancer development is influenced by multiple factors. Studies have shown a modestly increased risk of chronic myeloid leukemia, primarily due to exposure to benzene, PAHs, and heavy metals, known risk factors for hematologic malignancies [52]. Changes in the FLT3 signaling pathway, through SRC family kinases (SFKs), play a crucial role in cellular processes such as proliferation, survival, and differentiation, especially in hematopoietic cells. Alternation of this pathway has been implicated in hematologic malignancies [53,54].

Engineered nanoparticles (NPs) were identified in various human biological fluids, including exhaled breath condensate, blood serum, and urine, in the studied groups following occupational exposure [55]. Previous studies have indicated that particulate matter (PM) may promote epigenetic changes in blood cells, including DNA methylation, histone and chromatin structure alterations, and miRNA modifications [56]. Dysregulation of miRNAs is associated with several diseases, such as respiratory diseases, cardiovascular disorders, and cancers. Similar to mRNAs, we observed interindividual differences in miRNA expression changes among study participants. While no miRNA expression changes were noted in the Machining group, the miRNA (hsa-miR-3613-5p) exhibiting the highest levels of upregulation was found in the Welding group. Machining has been linked to lower PM0.1 exposure compared to the welding process, possibly explaining the absence of effects on miRNA expression in this group. In this study, expression of hsa-miR-3613-5p positively correlates with PM0.1 exposure. miRNAs have been recognized for their dual roles in cancer, functioning either as tumor-suppressors or as oncogenes, depending on how they influence the expression of their targeted genes. Nevertheless, recent studies have described miR-3613-5p as both an oncogene and a tumor suppressor in several carcinomas. Dysregulation has been observed in various cancers, including colorectal cancer, glioblastoma, and lung cancer. In pancreatic cancer, this molecule has been associated with tumor suppressor activity, as it inhibits the invasion and migration of pancreatic cancer cells by targeting the CDK6 gene [57]. Upregulated hsa-miR-3613-5p was identified in lung adenocarcinoma, promoting cell proliferation through a positive feedback loop involving RELA/JUN/miR-3613-5p/NR5A2/AKT1/MAPK3/1, resulting in continual NF-κB activation [58]. Its upregulation has been correlated with poor prognosis for patients with renal clear cell carcinoma [59]. Thus, alterations in hsa-miR-3613-5p expression may contribute to the development of renal disease. In other types of cancers, its downregulation was observed [57,60]. In our study, we identified significant downregulation of hsa-miR-4448 only in the Welding group. This miRNA has been previously shown to inhibit Girdin-mediated epithelial-mesenchymal transition and suppress tumorigenic Akt signaling. Its downregulation has been implicated in small-cell lung carcinoma, suggesting a potential tumor-suppressive role [61]. Welding fume exposure is associated with oxidative stress and epigenetic dysregulation [62], so we propose that silencing of this miRNA after exposure may contribute to oncogenic signaling pathways.

As exposure to air pollution and changes in miRNA expression have been linked to lung cancer progression and respiratory diseases [63], we speculate that miR-3613-5p and miR-30d-5p could serve as potential markers for occupational exposure to PM0.1 due to their correlation with the PM0.1 exposure dose. However, further more complex investigation of the miRNA-mRNA and miRNA-miRNA interactions is needed to confirm these suggestions, because miRNAs are negative regulators of mRNA expression affecting various biological processes. miRNAs could be downregulated or upregulated in different types of cells, and their target predictions should be experimentally validated. The level of mRNA expression could be decreased by miRNAs interaction with the 3´-untranslated region of target mRNA, but also a single miRNA can affect more mRNAs and the opposite way [64].

Several scientific studies have noted the sex-specific differences in mRNA and miRNA expression patterns in response to particulate matter (PM) exposure. These studies highlight the influence of molecular responses to environmental pollutants, which vary by gender, and potentially affect disease susceptibility and progression. These differences are likely shaped by hormonal regulation, sex chromosome complement, and epigenetic landscapes. Notably, in the male cohort, some miRNAs that affect mRNA expression may show opposite deregulations compared to females [65–69].

Finally, comparing the current results with our recent report [21], in both studies, we identified the impact of NPs released during the processing of nanomaterials on transcriptomic profiles. Consistently for both studies, expression changes of *DDIT4* and *FKBP5* indicate that NP exposure can affect inflammatory signaling pathways and immune responses regardless of gender. The observed effects depend on the type of material, work style, interindividual biological variability of the study subjects, and potential sex-biased mRNA and miRNA expression.

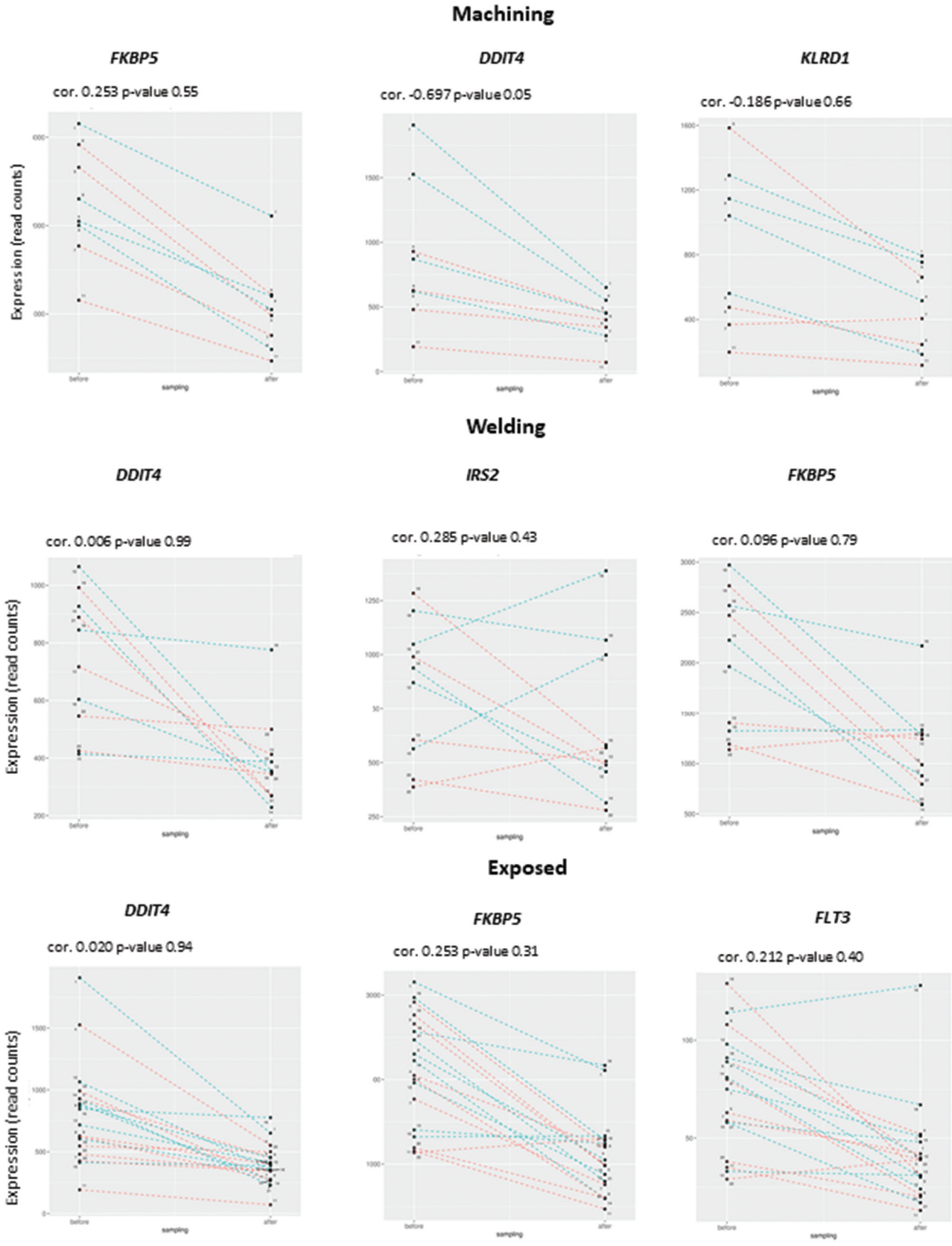

Figure 5. Interindividual variability in expression profile changes of the top three miRNA expression-exposure dose correlations. "Before" and "after" indicate the expression values before and after exposure. Each participant is represented as a number and a black dot. The line color indicates the exposure dose – blue for high (above median) and orange for low dose (below median).

Due to challenges in participant recruitment (missing female nanocomposite workers), this study included a relatively small sample size of a single gender. This constraint introduces potential sampling bias and may limit the generalizability of the results of differential gene expression analysis and the correlation analysis. While the findings offer preliminary insights, they should be interpreted with caution. Future research with larger and more representative cohorts is necessary to validate and expand upon these results.

5. Conclusion

In summary, we investigated the impact of acute exposure to NPs released during machining and welding on human transcriptome changes within the context of ongoing chronic exposure. Differential gene expression (mRNAs and miRNAs) was studied in 18 volunteers; long-term employed male participants working with nanomaterials in their daily work routine. While the majority of deregulated miRNAs were upregulated, the opposite occurred in the case of mRNAs. Interestingly, similar results at the mRNA levels (*DDIT4* and *FKBP5* downregulation) have been described in previous transcriptomic studies dealing with short-term occupational exposure to NPs [21]. Based on literature, the roles of altered mRNAs are mainly related to the pathways associated with immune response and cancer. The analysis of the deregulated miRNA pathways in Welding workers showed a probable alteration in cancer-related signaling pathways. A significant correlation between two miRNA expression changes and an exposure dose of PM0.1 was identified.

The result of the transcriptomic analysis (mRNAs and miRNAs) seems to support the evidence that exposure to welding fumes and nanocomposite dust can contribute to immune dysregulation and alteration in the pathways related to carcinogenesis. Although there is a long-term effort to reduce employees' occupational exposure, more efforts should be made to prevent inhalation exposure to NPs to minimize the health risks [30]. Personal protection equipment, measures reducing exposure, and sufficient ventilation systems should be used to avoid nanocomposite dust and welding fumes based on safety principles.

5.1. Limitations

There are some limitations of this study that need to be acknowledged. (1) Changes in mRNA expression levels may not directly reflect the phenotype, therefore, result validation using other techniques (qPCR, protein expression) should be performed. (2) The results represent the entire set of mRNAs/miRNAs at a defined/single time point. (3) Due to challenges in participant recruitment, this study included a relatively small sample size. This constraint introduces potential sampling bias and may limit the generalizability of the results. While the findings offer preliminary insights, they should be interpreted with caution. Future research with larger, more representative cohorts is necessary to validate and expand upon these results.

Author contributions

Conceptualization: A.R., M.S., Z.S., D.P., V.Z.; Formal Analysis: J.K., J.S., L.M.; Funding Acquisition: A.R.; Investigation (sampling): D.P., P.K., S.D., V.Z., J.S., A.R.; Investigation (mRNA, miRNA): Z.S., M.S.; Project Administration: A.R.; Software: J.K.; Supervision: A.R., D.P., V.Z.; Visualization: Z.S., M.S., J.K.; Writing – Original Draft Preparation: Z.S.; Writing – Review & Editing: Z. S., A.R., M.S., P.R., J.K., D.P., V.Z., L.M., J.S. The authors critically revised the manuscript and approved the final version.

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Reviewer disclosure

Peer reviewers on this manuscript have no relevant financial or other relationships to disclose.

Ethical disclosure

This study was approved by the Ethics Committees of the General University Hospital (registration mark: 2/17 Grant GA CR – VFN) and Institute of Experimental Medicine (registration mark: 2021/04). All volunteers signed an informed consent form according to the Helsinki Declaration.

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Disclosure statement

The authors have no competing interests or relevant affiliations with any organization or entity with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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Data availability statement

The research data will be available upon request.

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