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Full length article

Transcriptome alterations in long-term mining region residents: Insights into immune response and molecular pathways

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ABSTRACT

Pollution with metals and metalloids is a global problem that adversely affects human health and environment. Although several studies have reported gene expression changes in response to human exposures to metals, there are a limited number of studies exploring the effect of long-term residence in mining areas. The evidence of increased levels of several essential and non-essential metals in soil, water, and plants in Kapan mining area (Armenia) has been previously demonstrated in several environmental studies. Our study investigated the impact of long-term residence in this mining area on the transcriptome state of human peripheral blood mononuclear cells and the possible association of transcriptome changes with the blood metallome.

In total, 58 participants including 27 mining region residents (MRR) and 31 non-mining region residents (NMR) were selected for our study. Transcriptomic analysis of peripheral blood mononuclear cells was performed by mRNA sequencing. Differential expression analyses were conducted using generalized linear modeling, optimized for participant demographics, cell types, and sequencing technical factors, followed by pathway analysis.

The study revealed that long-term residence in a mining area is correlated with alterations in the blood transcriptome, with responses varying by sex. The identified transcriptome changes were enriched for pathways related to immune response and RNA translation. These changes correlated with higher blood levels of a mixture of non-essential metals, including arsenic, antimony, nickel, thallium, and beryllium. Additionally, the study identified differences in the transcriptome response between male and female MRR. While females exhibited a stronger immune response, males show dysregulation in ion transport and epigenetic modifications. Our findings contribute to understanding the effects of long-term residence in mining regions and can aid in developing more effective risk assessment and mitigation approaches in target populations.

1. Introduction

Metal and metalloid pollution is a global issue that negatively impacts human health and the environment. Growing evidence suggests that chronic human exposure to various metals and their mixtures leads to intertwined changes in gene expression and epigenetic regulation including DNA methylation, histone and RNA modifications, and non-coding RNA (ncRNA) expression (Bozack et al., 2021; Demanelis et al., 2019; Joneidi et al., 2019; Korashy et al., 2017; Ramírez et al., 2023; Renu et al., 2021).

While the majority of studies focus on *in vitro*, *in vivo*, and population approaches to examine the effect of metal and metal mixtures on molecular physiology of cells, relatively few have explored the association of long-term residence in mining regions with changes on transcriptome and epigenome levels (Korashy et al., 2017; Cai et al., 2022; Stepanyan et al., 2023). Factors such as the genetic structure of local populations, natural geochemical features of the local environment, exposure levels, types, and pathways that mediate these changes are diverse. Thus, there is a need for more inclusive and diverse studies to improve our knowledge on the molecular mechanisms of possible adverse effects related to long-term residence in mining regions.

In our previous study, we showed that methylation changes at CpG sites of the *NFKB1, CDKN2A, IGF2,* and *ESR1* genes are associated with multiple and specific blood metal levels among residents of the Kapan mining area (Armenia) (Stepanyan et al., 2023). The evidence of increased levels of several essential and non-essential metals in

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agricultural and urban soils (Ni, Mo, Cu, Cr, As, Cd, Pb, Zn, V, U), mine and river waters (Cd, As, Hg, Cu, Zn, V, Fe, Co), and agricultural crops, vegetables and fruits sold in markets (Cr, Ni, Pb, Hg, Cu, Mo, Cr, As, Cd) in this area has been also demonstrated in several environmental studies (Monitoring Kapan, 2007; Pipoyan et al., 2018; Belyaeva et al., 2019; Pipoyan et al., 2019; Meteomonitoring Report, 2022). The elevated levels for the mentioned elements were attributed to natural and technogenic factors as this region of Armenia is known for its diverse geological potential with a wide range of mining resources (Belyaeva et al., 2019; ITA, 2023).

Our study investigated the association between long-term residence in this mining area and the gene expression changes of human peripheral blood mononuclear cells (PBMCs), as well as the possible correlation between transcriptome changes and the blood metallome.

2. Material and methods

2.1. Study participants and analysis of chemical elements in plasma

Study groups, inclusion and exclusion criteria used, and the details about the analysis of chemical elements in plasma are described in detail previously (Stepanyan et al., 2023). The study cohorts were composed of healthy volunteers to minimize the influence of pre-existing conditions that could alter the transcriptome as well as the confounding effects of diseases. In total, 58 participants including 27 mining region residents (MRR), and 31 non-mining region residents (NMR) were recruited (**Additional file 1: Table S1-S2)**. The concentrations of 16 chemical elements (Ca, Mg, Co, Fe, I, Mn, Cu, Mo, Se, Zn, V, Sb, Tl, Be, Ni, As) in plasma samples were analyzed using ICP-MS, and the data were filtered based on the limit of quantification (Stepanyan et al., 2023). At the time of sample collection, all subjects provided informed consent about the use of the blood for the planned analysis. This study was approved by the Ethics Committee of the Institute of Molecular Biology NAS RA (IRB/IEC:IRB00004079, Approval#4/2021).

2.2. Library preparation and sequencing

PBMCs were isolated from venous whole blood on a Histopaque gradient, washed, and stored in RNAlater (Sigma-Aldrich, Darmstadt, Germany) at −40 °C. PBMC collection for RNA extraction was done at the same time as blood plasma samples collection for analysis of metal levels. RNA was extracted using the RNeasy Plus Micro Kit (QIAGEN, Germantown, Maryland, USA) according to the manufacturer's instructions. Poly-A selected strand-specific mRNA library was prepared with Illumina Stranded mRNA Prep kit (Illumina, San Diego, California, USA). The quality and concentration of both RNA samples and libraries were checked before downstream processing using an Agilent 2100 Bioanalyzer with appropriate kits. The sequencing was performed on the Illumina NovaSeq-6000 platform with 38.5 million single-end 100 bp reads on average generated per sample. The runs and sequencing quality were within normal ranges, i.e., Q30 % greater than 90 %.

2.3. Sequencing data processing

Quality checking of the sequencing data was performed using Illumina Basespace reports and the program FastQC (V0.10.1). Reads were aligned against the human genome hg38 using STAR (version 2.7.1a) with the parameters "–outSAMtype BAM SortedByCoordinate'' and "–quantMode GeneCounts TranscriptomeSAM" specified but otherwise default parameters (Dobin et al., 2013). Adapter sequences were not trimmed. The median unique alignment rate was 89.53 % (+/− 2.84 %). For estimating cell type composition, TPM values were calculated using RSEM with the GFF file GCF_000001405.40_GRCh38.p14_genomic.gff and the parameter "–trusted-sources BestRefSeq" (Li and Dewey, 2011).

2.4. Estimation of cell type composition

The cell type composition in PBMC samples was estimated using MuSiC (1.0.0) (Wang et al., 2019). The reference set of single-cell expression data for PBMCs was obtained from the Gene Expression Omnibus (GEO) at NCBI (https://www.ncbi.nlm.nih.gov/geo) under accession number GSE190992 (Vasaikar et al., 2023). Briefly, using the single-cell data we performed 1-to-1 differential expression comparisons between all cell type pairs using Seurat (5.0.0) FindMarkers function (Satija et al., 2015). For each cell type, we then assessed the marker genes found in all comparisons involving the cell type and retained marker genes that were always expressed at least 2x fold lower in other cell types. If a cell type did not yield enough marker genes (5 or more), then that cell type was not further considered in the composition estimation process. Typically the cell types that were dropped were subtypes of cell types rather than distinct types. Then MuSIC was used to estimate the cell type proportions with the selected marker genes. Cell types of interest for use as factors in the differential expression analyses were then selected. In general, we retained cell types that comprised at least 1 % of the PBMC samples. In addition, we included erythrocytes and platelets as technical factors since, although steps were taken during sample and RNA preparation to remove these cell types, in practice, RNA from these cell types remains and varies from sample to sample at levels not related to their original biological levels. Cell-type proportions used for differential composition analysis were re-normalized after excluding erythrocyte and platelet fractions. Cell-type proportions estimated by MuSiC were used as covariates in the differential expression analysis.

2.5. Statistical analysis

We performed a principal components analysis (PCA) of the gene expression data and when possible determined the correlation between the principal components (PCs) and sample annotations. Differential expression analysis (DEA) was performed using the R package edgeR (3.40.2) and limma (3.54.2) (McCarthy et al., 2012; Ritchie et al., 2015). Genes with a total count across all samples less than 1 CPM were excluded. Technical factors associated with the library preparation and sequencing process consisted of the RNA concentration and RNA integrity number (RIN) as well as the library average length, concentration, molarity, adapter dimer fraction, and non-dimer molarity measured using an Agilent BioAnalyzer. As expected, the library concentration and molarity values are highly correlated, so only molarity was considered in the model. The adapter dimer fraction was negatively correlated with molarity, so was also not used. Moreover, the sequence depth in millions of reads was considered. Technical, participant demographic (age, sex, smoking, BMI), and cell-type factors were included as described in the results. Throughout this article, we collectively refer to them as DTC (Demographic, Technical, Cell type composition) factors or covariates. The decision to include or exclude factors in the final analysis model was based on the Nguyen Backward Selection Algorithm (NBSA) designed for the optimal selection of factors (Nguyen et al., 2015). The algorithm works by performing a series of generalized linear modeling (GLM) analyses of expression data using the main factor(s) of interest (MFI) as well as a set of user-supplied covariates that may influence the apparent effects of the MFIs (Nguyen et al., 2015). The first round of NBSA analysis includes all potential covariates. Then, the least important factor (the one associated with the least amount of potentially significant differentially expressed genes) is removed, and a new GLM analysis is performed with the remaining factors. This process is repeated until the MFI is the least important factor. At this point the algorithm has identified any remaining covariates as obligatory as well as putting the less essential factors in order of importance. The second backtracking phase of the algorithm is to look back through the series of excluded factors to identify the point where the number of potentially differentially expressed genes (DEGs) for the MFI reaches a maximum. The optimal set is the obligatory covariates and the series of helpful

backtrack factors.

DEA were either done using the participant's sex as a factor or in a stratified manner where separate analyses were done for males and females. Similarly, differential PBMC cell-type compositions were either done with all participants together or for males and females separately.

Metal mixture cumulative Z-Score was calculated as the mean of Z-Scores of the five metals (As, Sb, Ni, Tl, Be) (Krauskopf et al., 2020).

The Benjamini-Hochberg procedure was used to compute the false discovery rate (FDR), with FDR < 0.05 considered statistically significant.

Pathway analysis was performed using the R package fgsea (1.24.0) and the Msigdb database file sigdb.v2023.2.Hs.symbols.gmt downloaded from https://www.gsea-msigdb.org/gsea/msigdb/collections.jsp (Subramanian et al., 2005; Sergushichev, 2021). We used the log fold change or regression beta coefficient as the gene rank. We conducted Gene Set Enrichment Analysis (GSEA) using the KEGG, HALLMARK, and REACTOME pathway databases and Gene Ontology terms (note that the GSEA algorithm considers the ranks of all genes when assessing enrichment). Pathway analysis was performed using the optimal set of factors of DTC. Data visualization was done using R (4.2.2) and the SRplot web server (Tang et al., 2023).

3. Results

3.1. Differential cell type composition analysis

The estimation of cell type composition in PBMC samples revealed cell proportions of 15 types of cells: erythrocytes; type 2 conventional dendritic cells (cDC2); MAITs; regulatory T cells (Treg); CD16+ monocytes; natural killer cells (NK); CD8+ memory T cells; CD4+ memory T cells; CD14 + monocytes, platelets, B memory cells, B intermediate, B naive, CD8 naive and CD4 naive (Fig. 1, **Additional file 1:** Fig. S1). According to the differential cell type composition analysis results, there were significantly decreased numbers of cDC2, MAIT and CD4+ memory cells ($p_{nominal} = 0.02$, $p_{nominal} = 0.001$ and $p_{nominal} = 0.003$, respectively) and an increased number of NK cells ($p_{nominal} = 0.049$) in MRR group. After multiple-testing adjustment FDR values remain significant only for MAIT and CD4+ memory cells (FDR = 0.012 and FDR = 0.026, respectively). In the sex-stratified analysis, no significant differences in cell types were observed in MRR vs. NMR after adjustment (**Additional file 1: Table S3**).

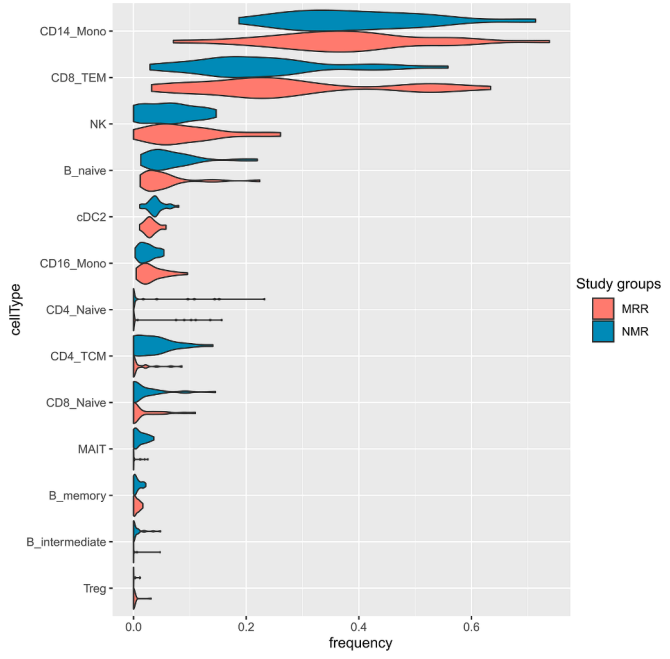

Fig. 1. The distribution of cell types across study groups. The cell types are ordered by mean proportion and color-coded, with each study group represented by a unique color. The key is shown on the right: mining region residents (MRR) (red) and non-mining region residents (NMR) (green). Cell types are displayed on the y-axis: CD14 + monocyte (CD14_Mono); CD8 + memory T cell (CD8_TEM); natural killer cell (NK); type 2 conventional dendritic cell (cDC2); CD16 + monocyte (CD16_Mono); CD4 + memory T cell (CD4_TCM); mucosa-associated invariant T cell (MAIT); regulatory T cell (Treg). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.2. Principal component analysis

PCA of the entire set of expression data showed that the first 5 to 10 PCs explain 40 to 50 % of the variation and revealed at least 9 PCs with significant variance (**Additional file 1:** Fig. S2**)**. Residence status (MRR or NMR) was correlated (Spearman, two-sided, using R cor.test function) with PC5 (r = 0.678 and $p_{nominal} = 4.92e-09$) and participant sex was associated with PC4 (r = 0.866, $p_{nominal} = 1.649e-18$). In the PC4 vs. PC5 scatter plot, the MRR samples were cleanly separated from the NMR samples for both sexes (Fig. 2). Next, we looked to identify other DTC factors that may explain PC1, PC2, and PC3 as well as the remaining PCs.

As seen in Fig. S3, in the unstratified samples cell type composition was significantly correlated with PC1, PC2, and PC3 to varying degrees (**Additional file 1:** Fig. S3). Sequencing-related technical factors correlated with PC2, library length (r = −0.488, $p_{nominal} = 1.006e-04$), library molarity (r = −0.398, $p_{nominal} = 1.950e-03$), but CD14-Mono proportion (r = −0.553, $p_{nominal} = 6.768e-06$) was also correlated. As noted PC4 is correlated with sex, but also has correlations with smoking (r = −0.784, $p_{nominal} = 3.326e-13$) and weight (r = −0.371, $p_{nominal} = 4.174e-03$) as well as several metals, Fe (r = -0.533, $p_{nominal} = 1.633e-05$) and Co (r = 0.574, $p_{nominal} = 2.450e-06$), and finally a technical factor erythrocytes (r = 0.543, $p_{nominal} = 1.056e-05$). In our study, the only smokers were men and men are typically heavier which explains

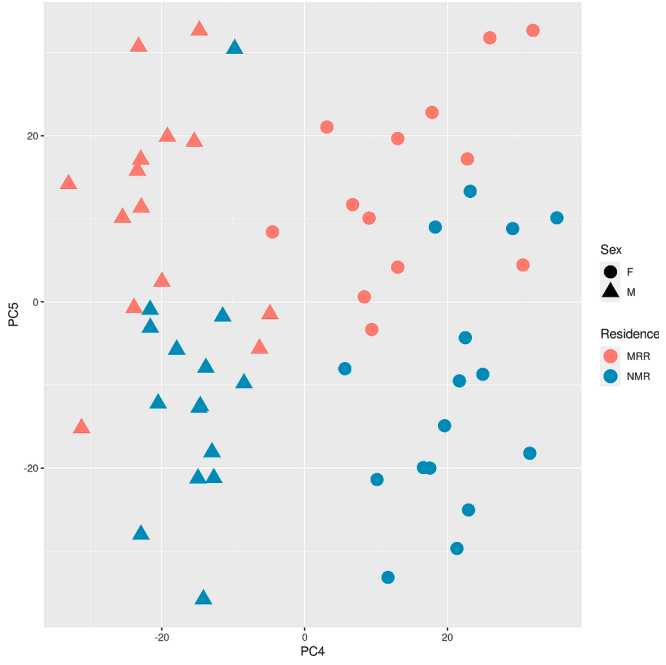

Fig. 2. The score plot for the principal component 4 (PC4) and PC5. The graph shows that the fourth principal component separates the data into two clusters: the cluster on the left represents males (triangles), while the right part shows females (circles). The PC5 separates data into mining region residents (MRR) (green) and non-mining region residents (NMR) (red) clusters. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

the observed correlations. Two metals, Fe and Co, have known sex differences, so their correlation with PC4 supports the observed differences in blood levels for these metals in males and females (Lee and Sex-specific, 2014; Chen et al., 2021). PC5 is correlated with residency status ($r = 0.678$, $p_{nominal} = 4.922e\text{-}09$) as well as cell type composition. In particular, mucosa-associated invariant T cell (MAIT) strongly correlated with PC5 ($r = -0.476$, $p_{nominal} = 1.617e\text{-}04$). PC7, PC8, PC10, and PC11 correlated with technical factors (estimated erythrocyte and platelet content, RNA RIN). Since these PCs represent minor variations compared to PC5, we conclude that the quality of sequencing data was high.

3.3. Factor optimization

The factor optimization analysis was performed for DEG discovery as described in the Methods. We applied the NBSA to the DTC factors and residence as the MFI (**Additional file 1:** Fig. S4**, A**). The algorithm selected Erythrocytes, Smoking, CD16+ monocytes, library length, CD4+ T memory cells, B memory, CD8+ T effector memory cells, and CD14+ monocytes as obligatory factors; the library molarity and NK cells were assigned as backtrack factors. The selected cell type proportion factors included the majority of the cell types that are most common in PBMCs, except for cDC2 and B naive cells.

We suspect that Smoking is a stand-in for sex since it is correlated with sex in our participants (all but one smoker were males and 6 out of 29 male participants were smokers), moreover there are more DEGs associated with Smoking than with Sex so Smoking was selected during the NBSA. Further, we performed the sex-stratified analysis by splitting the participants by sex and applied the factor optimization process, DEA, and pathway analysis for each group separately (**Additional file 1:** Fig. S4**, B-C**). When examining the MRR male group, we find only CD8+ T memory cells as an obligatory factor and B memory, erythrocytes, CD14+ monocytes, and RNA concentration as backtracking factors (**Additional file 1:** Fig. S4**, B**). In the female MRR group, we found a larger set of obligatory factors consisting of CD14+ monocytes, CD4+ T memory cells, MAIT, NK, platelets, and B intermediate cells. Backtracking adds erythrocytes as an additional factor (**Additional file 1:** Fig. S4**, C**). The maximum number of potential DEGs was larger than the number of genes when both sexes were analyzed jointly.

Additionally, we applied the NBSA to the cumulative Z-Score of non-essential metals (As, Sb, Ni, Tl, Be) in the MRR group as MFI using the DTC Factors for optimization (**Additional file 1:** Fig. S4**, D**). The obligatory factors were CD14+ monocytes, cDC2, CD16+ monocytes, T-reg cells, platelet cells, and NK cells. The backtrack factors were: CD8+ naive cells, CD4+ naive cells, B-memory cells, RNA RIN value, MAIT cells, Smoking, B-naive cell, library molarity, B-intermediate cell, library length, RNA concentration, BMI, Erythrocytes, CD4+ TCM cells, Age, and Gender.

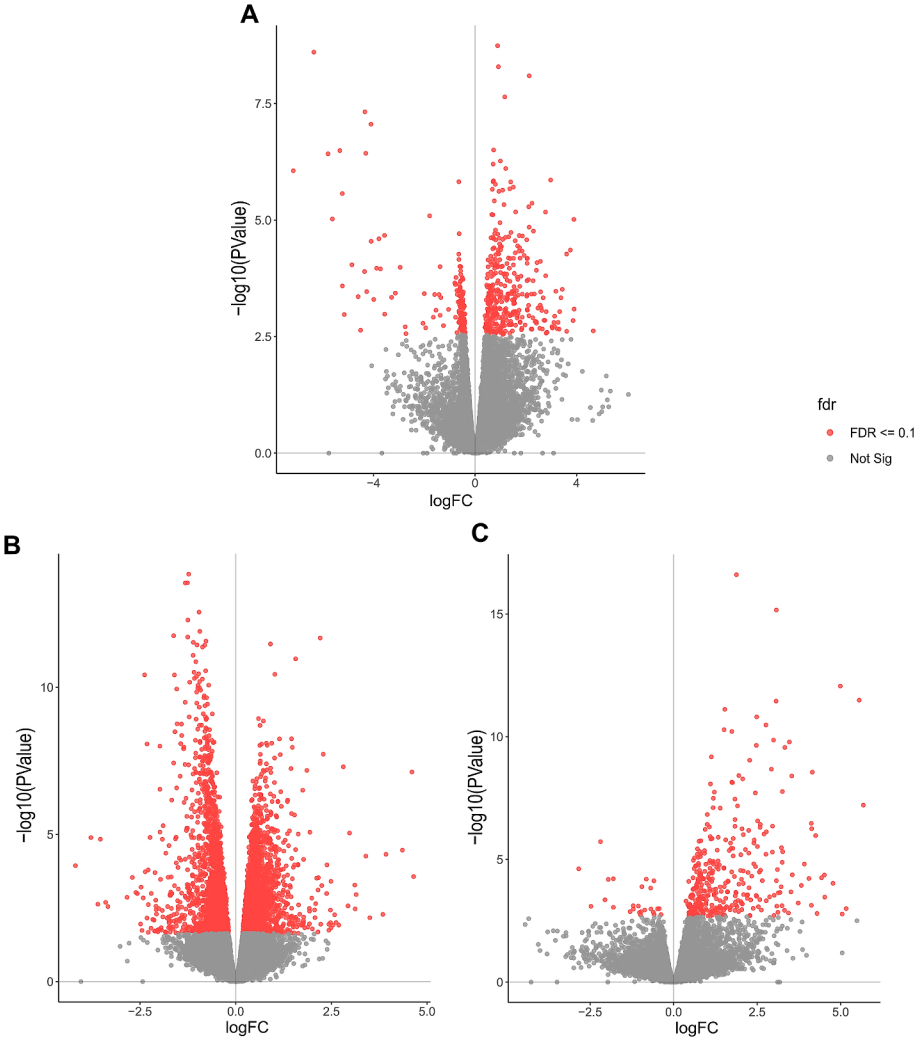

Fig. 3. Differentially expressed genes (DEGs) across study groups are represented as volcano plots. (A) MRR vs NMR, (B) MRR vs NMR males, (C) MRR vs NMR females. Volcano plot of data with log base 2-transformed fold change values plotted on the X-axis and negative log10 p values plotted on the Y-axis, representing the significance level.

3.4. Differential gene expression analysis

Next, we performed DEA to find DEGs associated with residency in the whole group as well as in each sex-stratified group. For each analysis we included respective obligatory and backtracking factors identified during optimization as covariates. First, to investigate gene expression changes related to long time residency in the mining region, we compared gene expression levels between the MRR and NMR groups. Out of 18204 genes, there were 471 significant DEGs (160 down- and 311 up-regulated) (FDR < 0.05) between MRR and NMR individuals when using the optimized DTC factors as covariates (Fig. 3**A, Additional file 2: Spreadsheet 1**).

To further understand the association of non-essential metal levels with the gene expression changes observed in MRR, we examined genes that may be enriched in relation to higher blood levels of As, Sb, Ni, Tl, Be. According to our results, 621 genes (471 down- and 150 up-regulated) were associated with cumulative Z-Score in MRR using the optimized DTC factors (**Additional file 2: Spreadsheet 2**).

Next, to identify potential sex-specific effects that could be overlooked in a combined analysis, we analyze males and females separately. According to the results 2827 genes were differentially expressed in the MRR male group when the optimized DTC factors were included as covariates (1388 down- and 1439 up-regulated) (Fig. 3**B, Additional file 2: Spreadsheet 3**). In female MRR group 167 genes, 39 down- and 127 up-regulated, were significantly differentially expressed compared to the NMR female group in DTC-adjusted DEA (Fig. 3**C, Additional file 2: Spreadsheet 4**).

3.5. Pathway analysis

In the MRR group, the upregulated pathways were predominantly related to immune response and inflammation. Notably, pathways involving interferon-alpha (IFN-α) and interferon-gamma (IFN-γ) responses, Tumor Necrosis Factor-α (TNF-α) signaling via Nuclear Factor kappa-light-chain-enhancer of activated B cells (NFκB), NOD-like receptor signaling, interleukin-10 (IL-10), IL1-IL1R- c-Jun N-terminal kinases (JNK) signaling, and chemokine activity were significantly upregulated. Meanwhile, pathways associated with translation initiation and elongation, cellular response to starvation, rRNA processing, ribosome biogenesis, and signaling by Robo receptors were downregulated in the MRR group (Fig. 4**A, Additional file 3: Spreadsheet 1**).

We also performed enrichment analysis to find pathways associated with high cumulative Z-Score of the metal mixture in the MRR group. Although the analysis revealed fewer enriched pathways, the trends in biological processes and the directions of changes were consistent with those observed in the MRR group compared to the NMR group. Thus, the translation and ribosome-related pathways were downregulated along with high Z-Score values, while the number of immune response pathways such as IFN-α and IFN-γ response, TNF-α signaling via NFκB, IL-6, and TNF superfamily cytokine production were upregulated. Additionally, we found the mammalian Target Of Rapamycin Complex 1 (mTORC1), behavior, lipid localization, and negative regulation of Mitogen-Activated Protein Kinase (MAPK) cascade to be associated with cumulative Z-Score in the MRR group (Fig. 4**B, Additional file 3: Spreadsheet 2**).

In the stratified analysis, we observed both common and sex-specific changes in transcriptome responses between males and females. Male MRR exhibits upregulation of complement and coagulation cascades, circulatory system process, regulation of blood pressure, lysosome, TNF-JNK signaling, circadian clock, oxidative stress-induced senescence, and activation of phospholipase C activity. Moreover, pathways related to ion transport were significantly upregulated in the PBMCs of male MRR (e.g., Inorganic ion homeostasis, active monatomic ion transmembrane transporter activity, inorganic molecular entity transmembrane transporter activity, monatomic cation transmembrane transporter activity). Additionally, our analysis revealed upregulation of several pathways related to epigenetic modifications such as DNA methylation, histone deacetylases (HDACs) deacetylate histones, arginine methyltransferases (RMTs) methylate histone arginines (Fig. 5**A, Additional file 3: Spreadsheet 3**).

Finally, in the female MRR, we found significant enrichment of acute inflammatory response, cytokine-cytokine receptor interaction, IL-10 signaling, CXCR chemokine receptor binding, P53 signaling, and prostaglandin secretion-related pathways (Fig. 5**B, Additional file 3: Spreadsheet 4**).

Since we identified numerous immune response pathways enriched with sex-specific differences, we examined the DEGs in the male and female MRR groups, referencing 159 known sex-specific immune response genes (Bongen et al., 2019; Stein et al., 2021; Gal-Oz and Immune, 2022). For this purpose, we examined the occurrence of reported sex-specific immune response genes among the DEGs identified in the MRR female and male groups (**Additional file 2: Spreadsheet 5**)

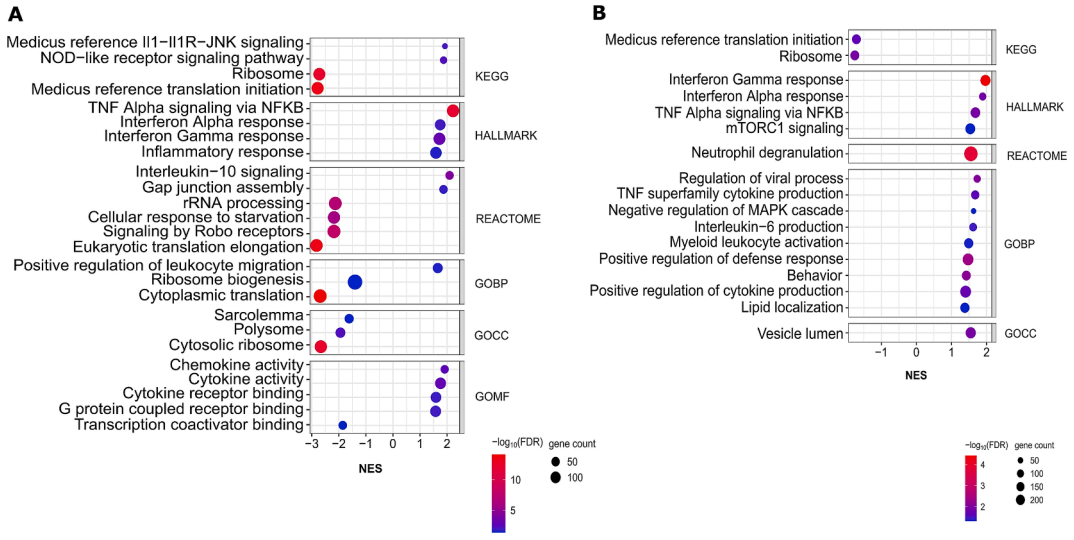

Fig. 4. Pathways and processes dysregulated in the MRR group compared to NMR (A) and enriched for high ZScore in the MRR group (B). Top terms from HALLMARK, KEGG, and REACTOME, Gene Ontology (GO) terms (Biological Process (GOBP), Cellular Component (GOCC), Molecular Function (GOMF)) are shown. The Y-axis represents the target term, and the X-axis indicates the Normalized Enrichment Scores. Bubble color and size correspond to the negative log10 transformed FDR and the number of genes enriched in the pathway, respectively.

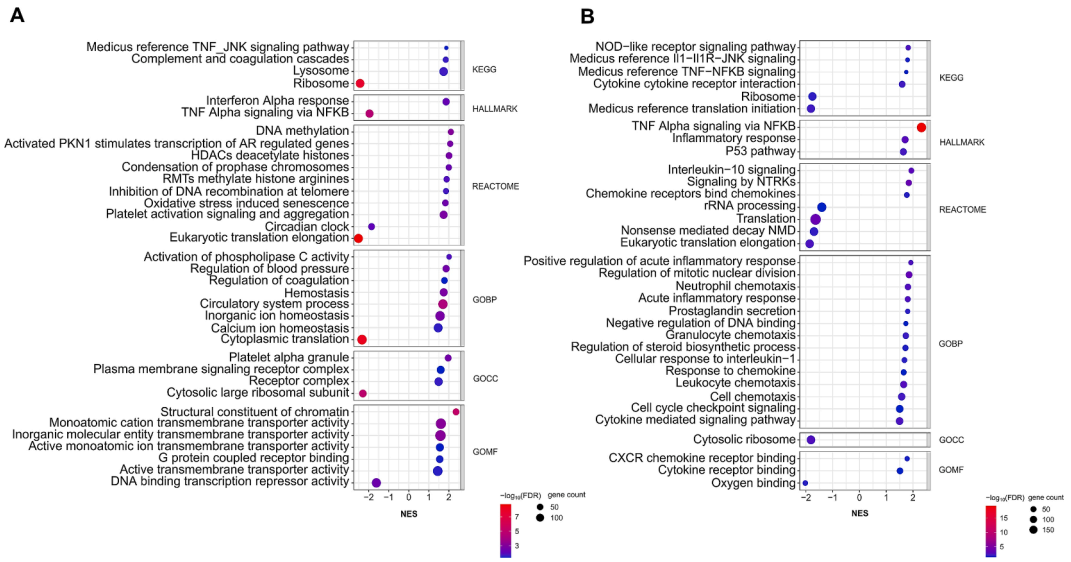

Fig. 5. Pathways and processes dysregulated in the male MRR group (A) and in the female MRR group (B). Top terms from HALLMARK, KEGG, and REACTOME, Gene Ontology (GO) terms (Biological Process (GOBP), Cellular Component (GOCC), Molecular Function (GOMF)) are shown. The Y-axis represents the target term, and the X-axis indicates the Normalized Enrichment Scores. Bubble color and size correspond to the negative log10 transformed FDR and the number of genes enriched in the pathway, respectively.

(Bongen et al., 2019; Stein et al., 2021; Gal-Oz and Immune, 2022). According to our results, among the 159 sex-specific immune response genes, 42 DEGs were identified in male MRR (33 down- and 9 upregulated), and 5 DEGs were identified in female MRR (1 down- and 4 upregulated) (**Additional file 1:** Fig. S5).

4. Discussion

The present study revealed that long-term residence in a mining area is correlated with alterations of the blood transcriptome in immune system responses, ion transport, ribosomal function, protein translation, and epigenetic modifications, with responses varying by sex.

Elevated environmental metal levels may have harmful effects on mining region residents. Several studies reported increased accumulation of toxic metals in hair, blood, and urine samples of mining region residents (Dai et al., 2023; Jiang et al., 2024; Li et al., 2012; Mafulul et al., 2022; Wang et al., 2019; Yang et al., 2015). A limited number of studies have investigated blood transcriptome and epigenome changes in human populations residing near mining areas for a long time (Korashy et al., 2017; Cai et al., 2022; Stepanyan et al., 2023). In the study of Korashy et al, transcriptome analysis was performed in the whole blood cells of 40 healthy male volunteers living near a mining area with significant contamination of heavy metals. The study revealed 425 dysregulated genes involved in protein synthesis, RNA post-transcriptional modification, cell cycle, DNA replication, energy metabolism, and cellular movement processes (Korashy et al., 2017). Another study demonstrated epigenetic changes among the mother–child pairs from active and historical mining sites in Montana polluted with heavy metals (Cai et al., 2022). The methylation changes in 200 genes enriched for actin cytoskeleton regulation, ABC transporters, leukocyte trans-endothelial migration, focal adhesion, and adherens junction were observed in that study (Cai et al., 2022). Our findings also suggest that the primary factor affecting the observed transcriptome alterations in mining region residents may be attributed to the heavy metals in the environment. This is evidenced by transcriptomic changes associated with the blood levels of five non-essential metals overlapping with the observed dysregulation in the MRR group in our study. However, it should be noted that our study design does not address whether the exposure originates from human activities or natural geological sources.

Several studies have identified gene alterations associated with translation repression in response to heavy metal exposure (Korashy et al., 2017; Krauskopf et al., 2020; Prat et al., 2005). The Pb exposure-induced downregulation of the human eukaryotic translation initiation factor 5 (eIF5) was observed in a recent transcriptome-wide metal exposure association study (Krauskopf et al., 2020). The genes involved in the eukaryotic translation initiation factors 2 (EIF2) signaling pathway were found to be associated with human environmental Pb, Cd, and Hg exposure (Korashy et al., 2017). Transcriptional downregulation of two eukaryotic translation elongation factors (*EEF1A1* and *EEF1G*) was observed in human renal cells in response to uranium toxicity (Prat et al., 2005). Our results are in line with these findings, as we similarly observed the downregulation of pathways linked to translation initiation and elongation, ribosome biogenesis, and rRNA processing in the MRR group. This was evidenced by a significant decrease in transcript levels of genes encoding translation initiation and elongation factors (*EIF3E, EEF1A1, EEF1B2, EEF1G, EEF2*, and *EIF3L*). A large body of evidence has demonstrated the key role of these genes in translational reprogramming and global repression of translation during cellular stress response (Liu et al., 2008; Liu and Qian, 2014; Guan BJ, 2014; Wu et al., 2019). Indeed, we observed significant upregulation of transcripts encoding 70kD stress-inducible heat shock proteins HSPA1A and HSPA6, previously recognized for their involvement in cellular stress response and adaptation mechanisms induced by heavy metals (Koizumi et al., 2013; Lee et al., 2013; Bilog et al., 2019). Additionally, the translation initiation and ribosome pathways were downregulated along with the increase of Z-Score values of blood plasma metal mixtures (As, Be, Ni, Sb, Tl) in MRR. The general inhibition of translation and increased expression of some chaperone transcripts observed in our study may be associated with PBMCs adaptive response to stress caused by chronic toxic metal exposure. The changes in the expression of genes related to ribosomal biogenesis and translation have been reported in several studies investigating transcriptomic signatures of aging (Peters MJ, 2015; Schmidt et al., 2020). This suggests that long-term residence in mining areas may impact some biological pathways associated with aging. This observation opens up potential avenues for further research into how chronic environmental exposure might contribute to age-related molecular changes and accelerated aging.

Extensive research suggests toxic metal occupational and environmental exposure triggered activation of transcripts associated with inflammatory processes, mediated by alterations in DNA methylation

patterns or the dysregulation of miRNA expression (Vrijens et al., 2015; Martin and Fry, 2018; Wallace et al., 2020; Wu et al., 2023). Metals can inhibit enzymes and receptors by binding to them or disrupting cellular antioxidant homeostasis, thereby disrupting multiple molecular signaling pathways. This can result in the release of inflammatory cytokines and subsequently alter immune response (López-Vanegas et al., 2020; Goyal et al., 2021; Haidar et al., 2023; Wang et al., 2023; Huang et al., 2023). In our study, transcriptome analysis revealed dysregulation of key receptors/ligands and effector proteins involved in TNF-α regulation via NF-κB, IFN-γ response, NOD-like receptor, and interleukin-10 signaling in PBMCs of MRR. This was evidenced by the upregulation of transcripts encoding TNFα and its receptor superfamily member 1A, C-X-C chemokine receptors and their ligands, IFN-γ, IL-10 receptor, components of activator protein 1. The interferon response and TNF-α regulation pathways were enriched with higher Z-Score in the MRR group, highlighting the cumulative effect of studied metals in observed alterations.

Analysis of miRNA target gene sets enabled us to infer the dysregulation of several miRNAs. Pathway enrichment analysis revealed an abundance of gene targets for miR-548, miR-448, miR-144, miR-320 and miR-513. These miRNAs have been shown to play a role in the dysregulation of inflammatory responses induced by exposure to metals in epidemiological and *in vitro* studies (Li et al., 2013; Liang et al., 2012; Tahamtan et al., 2018; Wang et al., 2019). The predicted dysregulation of these miRNAs suggests potential post-transcriptional modifications influencing immune responses in PBMCs of mining region residents. However, future studies should focus on clarifying their role with a specific focus on non-coding RNA transcriptomes.

When we stratified our data by sex, differences in immune response-related key regulators and pathways were identified between males and females. The downregulation of the TNF-α signaling via NF-κB in GSEA of male MRR was described by decreased expression of the genes encoding receptor, signaling cascade components IκBα and TRAF6, as well as effector transcripts involved in the regulation of the pathway via negative feedback (A20, IκBα) (**Additional file 1:** Fig. S6). On the other hand, upregulation of TNF family ligands and their receptors as well as downstream effectors of this pathway, such as components of AP-1 (*FOS, JUN, JUNB*) transcription factor complex, chemokines and their receptors (*CCL3L1, CCL4L2, CCL8, CX3CR1, CXCL10, CXCL5, CXCR3*) were observed (**Additional file 2: Spreadsheet 3**). Moreover, other inflammation related pathways were found to be activated in males such as complement system, IFN-a, oxidative stress which could contribute to the observed transcriptomic changes.

Conversely, female MRR exhibited upregulated TNF-α signaling via NF-κB, indicated by increased expression primarily at the level of ligands (TNF-α, IL-1β) and effectors (IL-8, A20, IL-1β, IκBα,TNF-α). Moreover, the observed increase in cytokine transcripts in females may be a result of orchestrated activation of multiple pathways, including NOD-like receptor and IL-10 signaling. These findings indicate a stronger immune response in females and highlight molecular sex differences of changes related to the long-term residence in the mining area.

Sex bias in immune response is well documented in both healthy and diseased human conditions, yet the precise mechanisms remain to be elucidated (Bongen et al., 2019; Gade et al., 2021; Gal-Oz and Immune, 2022). Among the sex-specific immune response genes dysregulated in MRR females, *TNF* and X-inactive specific transcript (*XIST*) are of particular interest (**Additional file 1:** Fig. S5). The observed upregulation of TNF in females may result from hormonal differences between the sexes. Thus, estrogen has been shown to induce TNF-α upregulation, while testosterone is reported to downregulate it (Jaillon et al., 2019; Stovall et al., 2022). The downregulation of long non-coding RNA *XIST* among female MMRs in our study may partially explain the stronger inflammatory response in the latter as its silencing promotes the expression of pro-inflammatory cytokines and activation of NF-κB through a negative feedback loop (Gao et al., 2019; Ahmad et al., 2022). In male MRR, the majority of DEGs with immune sex expression signatures showed decreased expression (**Additional file 1:** Fig. S5). These genes are predominantly involved in histone demethylase activity (*ARID5B, KDM6A, UTY*) and various immunological diseases (*KDM6A*, *GPR174*) (Dimitrova et al., 2015; Gal-Oz and Immune, 2022). Additionally, we observed the upregulation of the X-linked chemokine receptor gene *CXCR3* in MRR males (Gal-Oz and Immune, 2022). The presence of different sets of sex-specific immune genes among the DEGs found in MRR male and female groups points to distinct molecular mechanisms of the immune response related to long-term residency in a mining region.

Significant enrichment of genes encoding various transporter proteins in pathways related to ion transporter activity and ion homeostasis was found in the MRR male group but not in female MMRs. Leading edge genes were of different transporter classes: solute carrier (SLC) transporter, ion channel, ATP-binding cassette transporters (ABC), ATPases, and aquaporin. Zinc, calcium, and sodium ion transporters were particularly prevalent among upregulated genes. The upregulation of these transporters likely represents a compensatory effort by cells to enhance the efflux of heavy metals, thereby reducing intracellular concentrations or restoring channels blocked by the metals (Marchetti, 2013; Zhang et al., 2021; Wang et al., 2022). The expression and methylation levels of the genes encoding these transporters have been shown to change in response to various heavy metal exposures (Balali-Mood et al., 2021; Bozack et al., 2021; Dai et al., 2017). Additionally, several studies have identified sex-specific changes in transporter expression in response to metal exposure in both animal and human cells (Ljubojevic et al., 2004; Gade et al., 2021; Wang et al., 2022). The sex-specific differences in ion transport and immune response-related pathways among MMR subjects in our study may be influenced by variations in sex hormones, metabolic factors, or differential epigenetic regulation between males and females (Ljubojevic et al., 2004; Bongen et al., 2019; Gade et al., 2021).

In male MRRs we also identified dysregulation of transcripts involved in aberrant HDAC activity and DNA methylation. These epigenetic modifications may contribute to the silencing or activation of gene expression in male PBMCs, resulting in distinct expression patterns associated with long-term residence in the mining region (Dimitrova et al., 2015; Gažová et al., 2019).

Our study is one of the few exploratory investigations that demonstrates the effect of long-term residence in a mining region on the blood transcriptome. Although no significant differences in blood metal levels were found between the two groups, the mixture of non-essential metals had a cumulative effect, associated with changes in immune regulation and protein synthesis processes observed in the MRR group. Additionally, the differences in transcriptome alterations between male and female long-term residents of the mining region were revealed. These differences were reflected in molecular pathways leading to immune response changes and dysregulation in ion transport and epigenetic regulation in males. While numerous studies focus on the molecular mechanisms and diseases associated with metal exposure, our study highlights the need to expand knowledge about the changes resulting from long-term residence in mining regions among healthy people. These changes may result from an interplay of various factors, including environmental metal composition influenced by both human activities and natural factors, as well as the genetic and behavioral characteristics of local populations.

However, our study has several limitations. Firstly, the sample size is modest due to stringent criteria for selecting subjects with long-term residency in the mining area. Moreover, we investigated a limited number of metals; other metals and potentially toxic agents may have contributed to the observed changes and should be considered in future studies. Additionally, our evaluation of cell types relied exclusively on transcriptome data, and individual susceptibility to metal exposure was not considered.

5. Conclusions

Our data indicate that long-term residence in the mining region significantly impacts the PBMC transcriptome, altering immune response and translation processes, likely due to the cumulative effects of metal mixtures. These transcriptome changes show sex-specific patterns: females show a stronger immune response, while males exhibit changes in ion transport and epigenetic regulation. These findings contribute to understanding the transcriptome alterations related to long-term residence in mining regions and aid in developing more effective mitigation and risk assessment strategies in target populations.

CRediT authorship contribution statement

Ani Stepanyan: Writing – original draft, Visualization, Software, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Arsen Arakelyan:** Writing – review & editing, Visualization, Supervision, Software, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Jonathan Schug:** Writing – review & editing, Visualization, Supervision, Software, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2025.109344.

Data availability

The sequencing raw data is available at the dbGaP (Study Accession: phs003657.v1.p1)

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