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# METAL-BASED COMPLEXES IN BIOLOGICAL SYSTEMS: IMPLICATIONS FOR THERAPEUTIC APPLICATIONS

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## ABSTRACT
Metal-based complexes, and especially copper-containing systems, have been drawing growing attention due to their capacity to cause cellular homeostasis and regulated cell death; the transcriptomic consequences of copper-induced stress in cancer cells are largely uncharacterized, though. The present work investigated gene expression changes and pathway-level responses associated with copper exposure using high-throughput RNA sequencing data from CAL-27 cells treated with an elesclomol–copper complex and untreated controls. Following data normalization and quality assessment, differential expression analysis was performed with adjusted $p < 0.05$ and $|\log2FC| \geq 1$ as thresholds, and functional interpretation was conducted using Gene Ontology, pathway enrichment, and gene set enrichment analyses. Copper treatment produced a clear transcriptomic separation between conditions and resulted in 2,462 significantly differentially expressed genes, comprising 324 upregulated and 2,138 downregulated genes. Notably, stress- and metal-response genes such as HMOX1, MT1X, and HSPA1A were strongly induced, whereas proliferation-associated genes including PLK1, VIM, and FZD2 were suppressed. Enrichment analyses revealed significant involvement of p53 signaling, cell cycle regulation, apoptosis, mineral absorption, endoplasmic reticulum stress, and ferroptosis-related pathways, indicating coordinated disruption of cellular homeostasis. These findings demonstrate that copper-based treatment induces extensive transcriptomic reprogramming characterized by activation of stress-response mechanisms and inhibition of proliferative processes, supporting the therapeutic relevance of metal-based complexes in cancer biology.

**KEYWORDS:** copper-based complexes, cuproptosis, transcriptomic analysis, differential gene expression, cancer therapeutics

## 1. INTRODUCTION
Metal ions are essential elements of the biological system, and they are critical in the preservation of cells and their functions. Enzymatic catalysis, redox reactions, and intracellular signaling processes are biogenic metals like copper, iron, zinc, and manganese involved in cellular homeostasis (Zoroddu et al., 2019). The biological action of these metals is mostly mediated by their capacity to develop a complex with proteins and other biomolecules, thus controlling some important physiological pathways. Here, biogenic element-based metal complexes are part of various cell functions, affecting processes such as enzyme activity to molecular signaling (Kostova, 2023). Homeostasis of key metals is closely controlled, where a lack of them and their excess may disrupt the homeostasis of cells and lead to the development of diseases. Oxidative stress, protein misfolding, and disrupted signaling pathways resulting in a defective cellular integrity have been linked to the imbalances in metal levels (Jomova et al., 2022). Copper is of particular interest because it is a redox-active metal that can be involved in electron transfer reactions, as well as facilitate the formation of reactive oxygen species in dysregulated states (Jomova et al., 2025). These two characteristics underscore the precarious state of homeostasis that is needed in copper. To this end, the growing area of metallomics has offered useful information on the complicated functions of metal ions in the control of genes, cellular signals, and the pathogenesis of diseases (Maret, 2021).
On the basis of this biological background, more focus has been given to the therapeutic value of metal-based complexes. Their different chemical properties, such as variable oxidation states and coordination geometries, enable them to react to biological systems differently than the traditional organic drugs (Karges et al., 2021). Consequently, metal-based therapeutics are being considered as promising candidates to attack key cellular processes like DNA replication, protein

activity and redox balance. Their capability of having many simultaneous pathways has placed them as possible agents to overcome drug resistance and improve the treatment efficacy (Castro-Ramírez & Barba-Behrens, 2025).

There are copper-based compounds that have shown significant potential in terms of cancer therapy. They may cause oxidative stress, disrupt mitochondrion, and selectively target cancer cells by taking advantage of their disturbed metabolic conditions (Abdolmaleki et al., 2024). Further, the recent progress in learning about the mechanisms of cell death influenced by transition metals has highlighted the expanded use of metal ions in controlling cell fate. The intricate interactions between metal-induced cytotoxicity and metabolic pathways, oxidative stress responses, and signaling networks are reflected in these processes (Song et al., 2025). One recent breakthrough in the area is the discovery of cuproptosis, a new copper-dependent programmed cell death. The description of this mechanism is the accumulation of copper in the cell, which results in the aggregation of lipoylated proteins in the tricarboxylic acid (TCA) cycle, and destabilization of the iron-sulfur cluster proteins (Li et al., 2022). Compared to other types of programmed cell death, cuproptosis is strongly interconnected with mitochondrial metabolism and a unique biological pathway with possible therapeutic implications. The copper homeostasis regulation is thus a key determinant of cell sensitivity to this process (Chen et al., 2022). Besides, cuproptosis has been suggested as a promising anti-cancer therapy modality, as it takes advantage of metabolic addictions, which tend to be typical of tumor cells (Wang et al., 2022). Cellular signal pathways also have a significant role in regulating the responses to copper-induced stress. One of the most important regulators of cell proliferation, differentiation, and stemness is the Wnt/β-catenin signaling pathway, which has been extensively involved in cancer progression and therapeutic resistance (Yu et al., 2021). However, at the same time, the use of copper-based systems, such as nanoparticles and coordination complexes, as targeted therapeutic interventions, has been broadened due to the progress in biomedical research (Woźniak-Budych et al., 2023). A combination of these advances underscores the increasing significance of copper as well as its complexes to both biology and medicine.

Although these strides have been made, there are still some critical gaps in the knowledge of the impact of copper-induced stress on cellular systems on a global scale. Although the molecular pathways of cuproptosis have become more and more enlightened, little is known about the overall transcriptomic alterations that accompany copper exposure to cancer cells. Specifically, the degree to which copper stresses the reorganization of networks of gene expression and signaling pathways is not adequately described. Moreover, little is known about the interaction between metal homeostasis, stress-response pathways, and possible resistance pathways, studied thoroughly through integrative transcriptomic methods.

The current research examines global alterations in gene expression in response to copper-induced stress in cancer cells via high-throughput gene expression data. Through the analysis of the patterns of differential gene expression and pathway enrichment, the research plans to describe the biological processes and signaling networks that are impacted by these conditions. This method gives a systems-level view of the effects of copper-based complexes on cellular responses, thus helping in understanding their role in biological systems and their possible applicability in therapeutic use.

## 2. METHODOLOGY

### 2.1 Dataset acquisition

The dataset of gene expression (GSE248083) employed in this study was obtained in the Gene Expression Omnibus (GEO) database and is based on an earlier study of copper-induced cell death pathways in cancer cells (Liu et al., 2024). The dataset includes high-throughput RNA sequencing data of cells (Homo sapiens CAL-27), which were untreated (control) and treated with an elesclomol copper (ES-Cu) complex. The experiment was conducted on the BGISEQ-500 (GPL23227) platform, and it has 6 samples, 3 biological replicates in each condition (control and ES-Cu-treated). Such an experimental design allows conducting a strong comparative transcriptome to study molecular reactions in relation to cuproptosis and copper homeostasis.

### 2.2 Identification of differentially expressed genes

The level of expression of the genes in the control and ES-Cu-treated groups were compared to identify the difference in the genes that were differentially expressed (DEGs). The expression values were initially converted to log2(TPM + 1) to equalize the data distribution. The average expression of the two groups (per gene) was computed, and the log2 fold change (log2FC) was obtained. Welch's t-test was used to determine statistical significance. Adjusting p-values with the Benjamini-Hochberg method was done to control against multiple testing errors. Genes satisfying the criteria of adjusted $p < 0.05$ and $|\log2FC| \geq 1$ were considered significantly differentially expressed.

### 2.3 Functional enrichment analysis

Gene Ontology (GO) enrichment analysis was conducted to find the overrepresented biological processes to examine the biological importance of DEGs. Moreover, pathway enrichment analysis was performed based on curated databases of signaling pathways to identify significantly related signaling pathways. The results of the enrichment were assessed by the adjusted p-values and the most significant biological processes and pathways were chosen to be interpreted. Such analyses made it possible to classify genes into biological categories that are relevant.

### 2.4 Gene set enrichment analysis

Gene Set Enrichment Analysis (GSEA) was conducted to determine the coordinated alterations of pre-defined gene sets. All the genes were ranked according to log2 fold change, and enrichment scores were computed to check whether any particular pathway was enriched in one or the other condition. Permutation testing was used to determine statistical significance, and false discovery rate (FDR) correction was employed. Biologically relevant were the pathways that had high scores on enrichment.

### 2.5 Quality assessment and validation

Principal component analysis (PCA) was used to analyze data to visualize global transcriptomic variation across samples to assess data quality and sample consistency. Further, Pearson correlation was done to determine the reproducibility of biological replicates. Such analyses guaranteed the trustworthiness of the dataset and the appropriateness of the dataset to downstream differences in expression and enrichment studies.

## 3. RESULTS

### 3.1 Global transcriptomic alterations and differential expression overview

Principal component analysis (PCA) was done to evaluate global transcriptional change between the control (CON) and elesclomol-copper-treated (ES-Cu) samples. The first principal component (PC1) was clearly distinct between the two groups and explained 68.08% of the total variance, so the exposure to copper is the major factor of transcriptional differences (Figure 1).

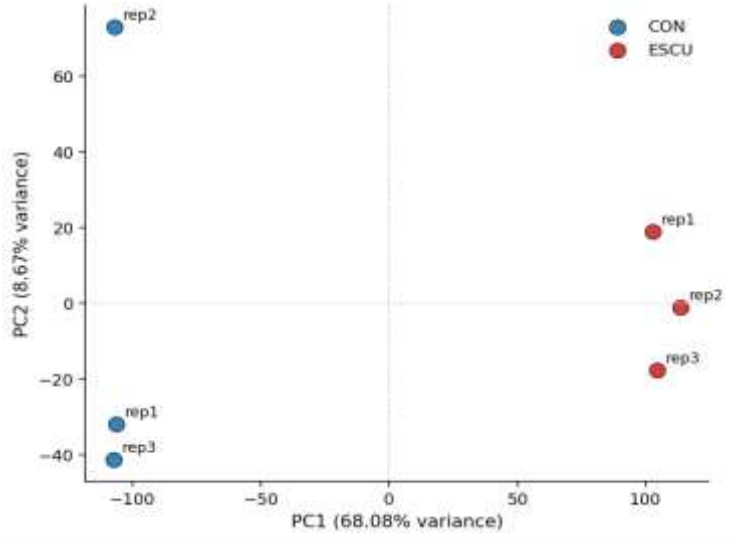


**Figure 1. Principal Component Analysis (PCA) of transcriptomic profiles in control and ES-Cu-treated CAL-27 cells.**

This pronounced separation was accompanied by extensive transcriptional remodeling. In total, 2,462 genes were identified as significantly differentially expressed, comprising 324 upregulated and 2,138 downregulated genes (adjusted $p < 0.05$, $|\log2FC| \geq 1$). The strong overrepresentation of suppressed genes indicates that there was a large-scale repression of gene expression programs to cope with copper-induced stress. Intra-group variability and reproducibility: Replicates within each condition were tightly clustered, indicating a high reproducibility and minimal variation within each group. The clear separation of the CON and ES-Cu groups, along with the many differentially expressed genes, supports a strong and biologically relevant transcriptomic reaction to copper-induced perturbation. Pearson correlation analysis was done on all the samples to further assess sample consistency. There was a high correlation coefficient (0.996-1.000) between replicates within each condition, and a relatively low correlation coefficient (0.964-0.966) between inter-group correlations, which supports the notion of high technical reproducibility and condition-specific transcriptional divergence, respectively (Figure 2).

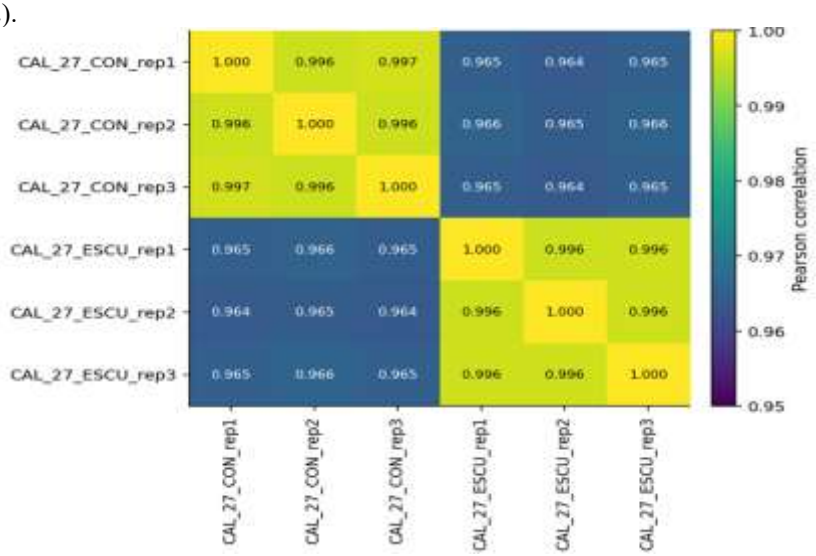


**Figure 2. Sample-to-sample Pearson correlation heatmap across all experimental conditions.**

Collectively, these analyses indicate that the data set is solid and that copper treatment causes a global transcriptional change that can be replicated.

### 3.2 Differential gene expression landscape

The analysis of differential expression revealed significant dysregulation of many genes after ES-Cu treatment. The general pattern of these genes is shown in the volcano plot, which both indicates the magnitude and statistical significance of changes in gene expression (Figure 3).

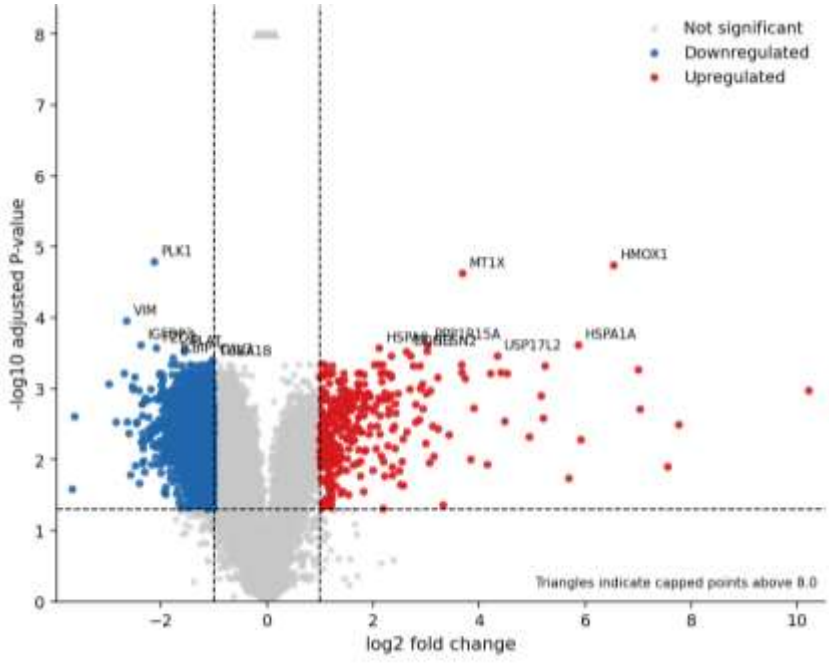


**Figure 3. Volcano plot depicting differentially expressed genes between control and ES-Cu-treated cells.**

There was a clear cluster of highly expressed genes on the positive axis and downregulated genes on the negative axis. Stress response and metal homeostasis genes (HMOX1, MT1X, and HSPA1A) were highly upregulated. Conversely, cell proliferation and signaling genes, such as PLK1, VIM, and FZD2, were significantly reduced. Table 1 displays a summary of the most altered genes significantly.

**Table 1. Top differentially expressed genes identified between control and ES-Cu-treated samples.**

| Gene | log2FC | P-value | Adjusted P-value | Regulation |
|---|---|---|---|---|
| PLK1 | -2.120 | 1.073e-08 | 1.639e-05 | Downregulated |
| HMOX1 | 6.542 | 1.308e-08 | 1.832e-05 | Upregulated |
| MT1X | 3.692 | 1.840e-08 | 2.380e-05 | Upregulated |
| VIM | -2.643 | 9.302e-08 | 1.117e-04 | Downregulated |
| HSPA1A | 5.878 | 2.758e-07 | 2.440e-04 | Upregulated |
| PPP1R15A | 3.036 | 2.710e-07 | 2.440e-04 | Upregulated |
| IGFBP3 | -2.373 | 2.668e-07 | 2.440e-04 | Downregulated |
| HSPA8 | 2.119 | 3.511e-07 | 2.690e-04 | Upregulated |
| FZD2 | -2.082 | 3.521e-07 | 2.690e-04 | Downregulated |
| SESN2 | 3.023 | 4.066e-07 | 2.972e-04 | Upregulated |

These findings suggest a concerted transcriptional response that involves activation of stress-related pathways and inhibition of proliferative signals.

### 3.3 Clustering of differentially expressed genes

Hierarchical clustering of the top differentially expressed genes was further done to explore more about the gene expression pattern. This heatmap showed the presence of clear segregation of control and treated samples, as well as discrete clusters of upregulated and downregulated genes (Figure 4).

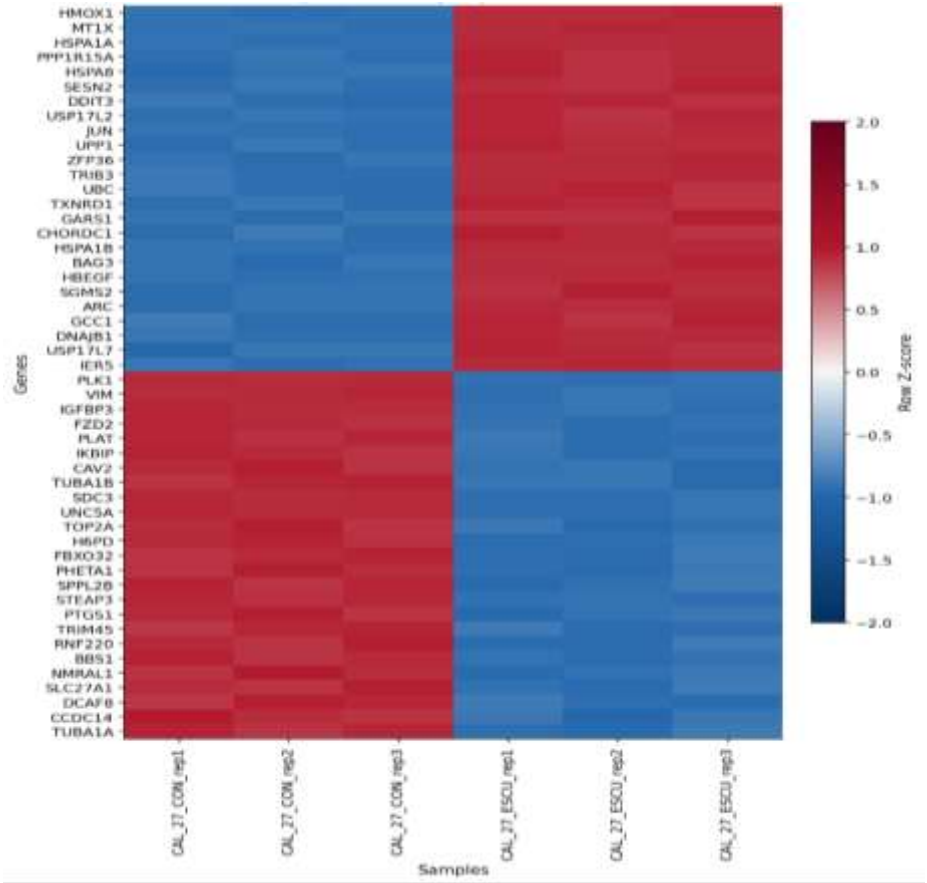


**Figure 4. Heatmap of top differentially expressed genes showing hierarchical clustering across samples.**

The genes that were upregulated in the ES-Cu-treated samples exhibited continually high expression in all treated replicates, and the downregulated genes had low expression compared to controls. This trend is an indication of a shift in a proliferative system to a stress-adaptive cellular program in the presence of copper conditions.

### 3.4 Functional enrichment of biological processes

To explore the biological implications of the observed transcriptional changes, Gene Ontology (GO) enrichment analysis was performed. Table 2 below summarizes the most enriched biological processes, and Figure 5 below visualizes them.

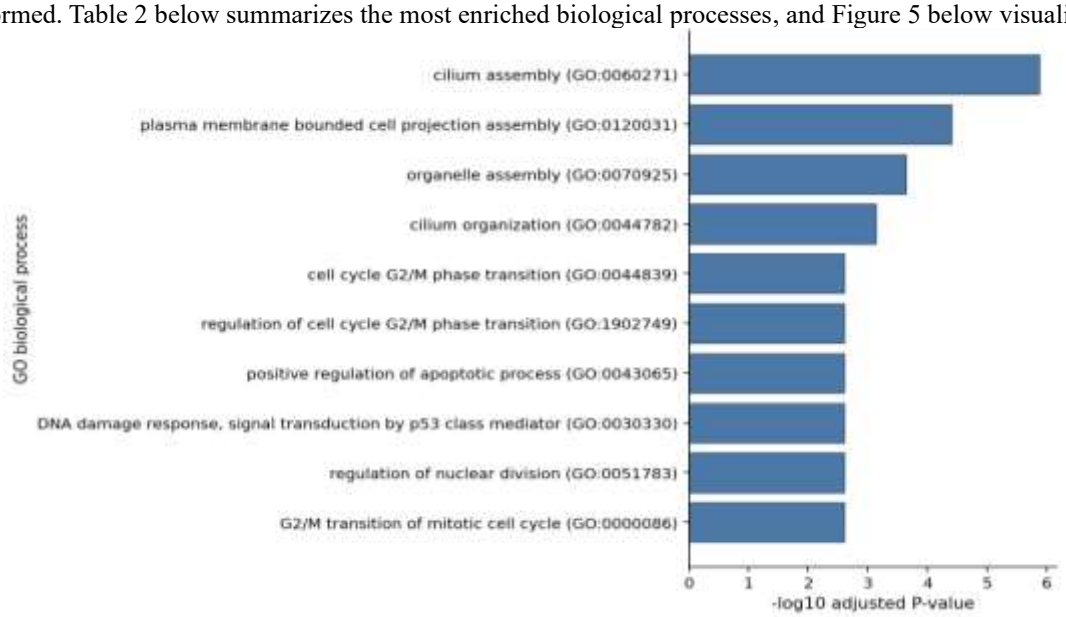


**Figure 5. Bar plot of top enriched GO biological processes based on –log10 adjusted P-values.**

**Table 2. Top enriched Gene Ontology (GO) biological processes associated with differentially expressed genes.**

| GO Term | Overlap | Adjusted P-value | Odds Ratio |
|---|---|---|---|
| cilium assembly | 79/314 | 1.315e-06 | 2.441 |
| plasma membrane bounded cell projection assembly | 68/278 | 3.869e-05 | 2.344 |

| | | | |
|---|---|---|---|
| organelle assembly | 90/425 | 2.305e-04 | 1.948 |
| cilium organization | 55/228 | 7.159e-04 | 2.294 |
| positive regulation of apoptotic process | 67/310 | 2.498e-03 | 1.991 |

The enrichment profile shows that structural organization, cell cycle regulation, and apoptotic processes are significantly involved. Interestingly, DNA damage response and G2/M transition processes are enriched, which indicates the activation of the checkpoint responses to copper-induced stress.

### 3.5 Pathway enrichment analysis

To further determine the important signaling pathways that were influenced by ES-Cu treatment, there was a KEGG pathway enrichment analysis. Table 3 shows the most enriched pathways and is shown in Figure 6.

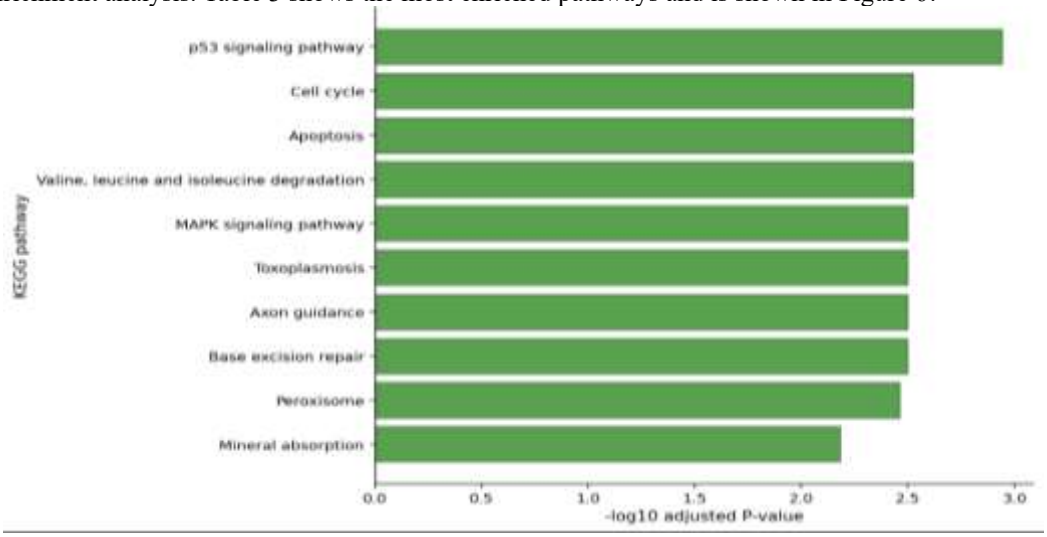


**Figure 6. Bar plot of top enriched KEGG pathways based on –log10 adjusted P-values.**

**Table 3. Top enriched KEGG pathways associated with differentially expressed genes.**

| KEGG Pathway | Overlap | Adjusted P-value | Odds Ratio |
|---|---|---|---|
| p53 signaling pathway | 24/73 | 1.140e-03 | 3.514 |
| Cell cycle | 32/124 | 2.987e-03 | 2.497 |
| Valine, leucine and isoleucine degradation | 17/48 | 2.987e-03 | 3.927 |
| Apoptosis | 35/142 | 2.987e-03 | 2.349 |
| MAPK signaling pathway | 60/294 | 3.172e-03 | 1.847 |

Activation of canonical stress-response pathways is reflected in the enrichment of pathways like p53 signaling, cell cycle regulation, and apoptosis. Also, the pathways associated with metabolism and metal management are enriched, which supports a lack of cellular homeostasis after copper exposure.

### 3.6 Gene set enrichment analysis reveals coordinated pathway shifts

Gene Set Enrichment Analysis (GSEA) was conducted to analyze coordinated transcriptional changes at a pathway level. These findings show that the metal ion regulation, stress response, and inflammatory signaling pathways are significantly enriched (Figure 7).

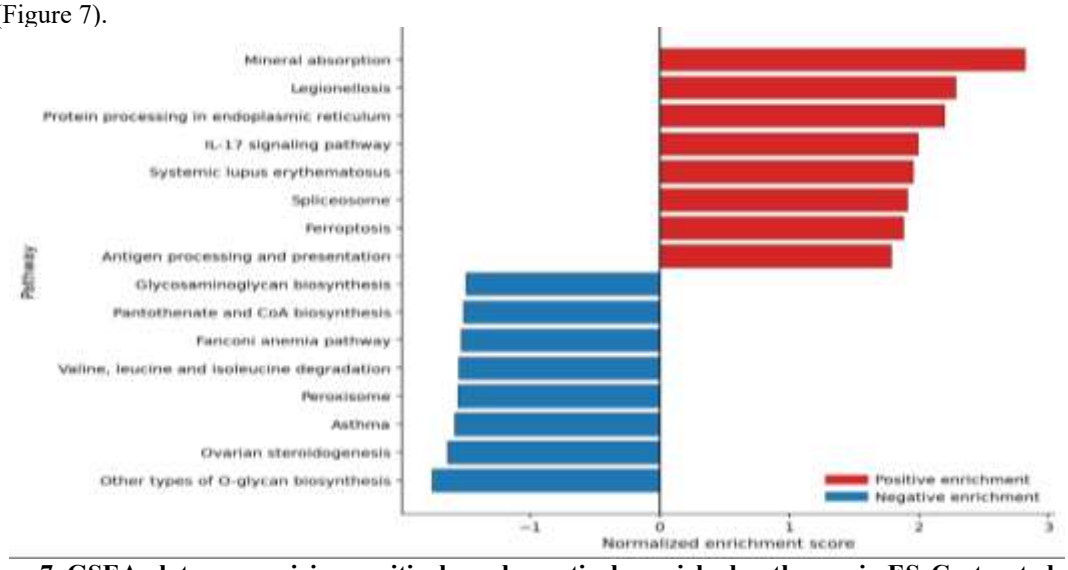


**Figure 7. GSEA plot summarizing positively and negatively enriched pathways in ES-Cu-treated cells.**

Of the positively enriched pathways, mineral absorption was most enriched, which implies the presence of the metal-handling mechanisms. Other enriched pathways were protein processing in the endoplasmic reticulum, IL-17 signaling, and ferroptosis, which are the responses to proteotoxic and oxidative stress. On the other hand, pathways that were negatively enriched were mainly linked to metabolic and biosynthetic processes, indicating inhibition of normal cellular metabolism in response to stresses. Together, these results indicate that copper exposure triggers the coordinated transcriptional reprogramming, which is accompanied by the activation of the stress-response pathways and suppression of proliferative and metabolic functions.

## 4. DISCUSSION

The copper exposure resulted in widespread and integrated transcriptional response in CAL-27 cells, as indicated by the distinct separation of experimental groups in the PCA and a high number of differentially expressed genes. The fact that most of the transcripts are downregulated indicates that copper stress does not simply induce a defense program, but rather it enforces a more generalized inhibition of proliferative and metabolic gene expression. The concomitant enhancement of cell-cycle, apoptotic and stress-related pathways confirms this interpretation. Specifically, PR regulators like PLK1 are downregulated, and the processes associated with G2/M transition are enriched, which shows that copper treatment alters the cells toward no longer growing and instead, toward checkpoint regulation, stress adaptation, and loss of proliferation potential. The findings at the pathway level also suggest that copper has a biological effect beyond the effect of oxidative damage. The p53 signaling pathway, apoptosis and MAPK signaling are enriched, pointing to the idea that copper-induced stress is incorporated into canonical damage-response networks. Meanwhile, the strong enrichment of mineral absorption and a significant induction of metallothionein-related genes, especially MT1X, demonstrates that the processing of copper and intracellular detoxification are the main characteristics of the response. HMOX1 upregulation and enrichment of ferroptosis in GSEA indicate an extra tier of redox-activated stress, which could overlap with other cell death signaling pathways. Taken together, these results suggest a model whereby copper-based treatment induces mitochondrial and proteotoxic stress, damage-response pathways, and a protective metal-buffering program and at the same time represses gene networks that maintain cell-cycle progression.

These findings are largely in line with previous evidence that copper can induce profound changes in signaling and metabolism of cancer cells. Copper is demonstrated to induce PDK1–AKT axis stimulation in a transporter-dependent fashion, thus establishing a connection between metal availability and tumor progression oncogenic signaling (Guo et al., 2021). The current results build upon the idea, demonstrating that the transcriptomic landscape, when treated with copper-complexes, is rather dominated by stress and inhibitory programs, meaning that the biological outcome of copper is context-dependent, and depending on the shape, concentration and cellular processing of the metal. The high expression of HMOX1 in the present study is also significant in the context of recent studies which found that copper overload can induce ferroptosis by upregulating HMOX1 (Zhao et al., 2025). On the same note, the significant upsurge of the expression of the MT1X corresponds with findings that copper overload activates the MTF1–MT1X regulatory module in cuproptotic responses (Wu et al., 2023). Collectively, these similarities indicate that the transcriptional signature that is evident in this case represents a biologically consistent copper-overload condition where the antioxidant defense and metal-sequestration processes are activated. The analytical approach adopted in this study is also supportive of the worthiness of combined transcriptomic apprehension. Expression profiling paired with a pathway-oriented analysis at systems level has been shown to be useful in solving molecular subtypes and biologically significant signatures of complex diseases (Huang et al., 2024). The overlap of DEG, GO, KEGG, and GSEA in the current setting reinforces the conclusion that exposure to copper disturbs several related processes instead of an individual pathway.

The cell-cycle arrest and stress-response programs enrichment was observed, which is consistent with the findings that copper complexes have the potential to suppress the growth of tumor cells by regulating checkpoints. Recently, a copper (II) complex was reported as causing G2/M arrest and suppressing the Wnt/β-catenin, JAK-STAT, and TGF-β signaling in lung cancer cells (Gałczyńska et al., 2025). This analogy is of special interest since the current findings also revealed suppression of growth and activation of damage-response, despite the absence of robust transcript-level enrichment of Wnt/β-catenin signaling. The emergence of ferroptosis-related enrichment provides further evidence that cell death pathways that depend on metals are not completely discrete, but likely interrelated. The recent reviews highlighted far-reaching crosstalk between ferroptosis and cuproptosis and other death pathways mediating metals, particularly via redox imbalance and mitochondrial dysfunction (Gu et al., 2024). The pattern of p53-mediation, seen here, can also be consistent with the reports of the metal-induced genotoxic stress as having the ability to cause DNA damage signaling, cell-cycle abnormalities, and p53 activation (Stannard et al., 2024). These findings are consistent with the perception that iron and copper are key implementers of controlled cell death by coming together to oxidative stress, metabolic susceptibility, and organelle malfunction (Li et al., 2023). They also correlate with the established role of stringently regulated copper trafficking, sequestration, and efflux in dictating cell tolerance or cell death to copper exposure (Herman et al., 2022).

These results have a number of implications for the therapeutic interpretation of metal-based complexes. First, the intense activation of metal-handling and stress-response pathways indicates that the copper-based agents may have an anti-cancer effect in multi-layered, but not in a single cytotoxic event. Second, overlapping vulnerabilities of cancer cells to mitochondrial functioning and redox imbalance are the potential targets of the concomitant activation of apoptosis, p53, and ferroptosis-related signatures, suggesting that copper complexes can be used to target overlapping vulnerabilities in cancer cells. Third, the gap between the established role of Wnt/β-catenin signaling in cuproptosis resistance and the relatively weak enrichment by the current transcriptomic analysis suggests that resistance is not necessarily manifested as a dominant transcriptional signature. Rather, it can be based on pathway activity, heterogeneity of cell-states or post-

transcriptional regulation. Translationally, this corroborates the notion that copper-based therapeutics can be optimally used as an adjunct to agents of stress-adaptation, metal transport or survival signaling.

There are some limitations also. The study was done on only one cell line and had a small sample size, which can affect the extrapolation of the findings. Also, the research relies on transcriptomic data and may not be comprehensive of regulatory events that occur at the protein or metabolic level. Since the analysis is based on in vitro data, it would be desirable to validate the biological relevance of the responses observed by further validation in in vivo models. Further development of the models and the complementary methods, like proteomic or metabolomic analyses, in the future would contribute to the insight into copper-induced cellular mechanisms and their applicability to therapeutic procedures.

## 5. CONCLUSION

The treatment with copper caused a significant and reproducible transcriptomic response in CAL-27 cells, with widespread differentially expressed genes and definite separation between it and untreated controls. The general pattern of expression suggested that there was a major suppression of proliferative programs and activating stress-response, apoptotic, and metal-handling pathways. Further enrichment analyses revealed copper exposure to be linked with p53 signaling, cell-cycle regulation, apoptosis, mineral absorption and responses to ferroptosis, which demonstrates the overall biological potential of copper-induced stress. The results obtained corroborate the idea that metal-based complexes can affect cellular systems via interrelated processes of redox imbalance, checkpoint activation, and metabolic homeostasis disruption. The current work contributes to illuminating the impact of copper-based complexes on cancer cell biology and supporting their applicability as possible therapeutic agents by offering a transcriptome-level viewpoint. The findings are relevant to the further comprehension of the biological and therapeutic importance of metal-based complexes, in the case of cuproptosis-related cellular reactions.

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