In vivo effects of Cisplatin and titanium dioxide nanoparticles combined treatment

ABSTRACT

Cisplatin, the first metal-based chemotherapeutic drug, remains widely used in treating various solid cancers despite its side effects and drug resistance. To mitigate these issues, researchers have explored combining Cisplatin with different substances. Various carriers or associated nanoparticles have been studied with Cisplatin, primarily using in vitro models. In this context, *Drosophila melanogaster* serves as a valuable alternative model for evaluating the toxicity of numerous substances and studying diseases like cancer, Alzheimer's, and alcoholism. This work investigates the *in vivo* effects of orally administered Cisplatin (50 μg/ml) and titanium dioxide ($NPTiO_2$; 50 μg/ml), alone and in combination, in *D. melanogaster*. Using RNA-seq and bioinformatics studies, we analyzed gene modulation to assess the toxic effects in each administration group. We found that flies treated with either Cisplatin or $NPTiO_2$ alone exhibited gene modulation related to drug and non-toxic substance processing. However, the group treated with Cisplatin and TiO_2 nanoparticles showed decrease in expression levels, a small number of DEGs, and a disruption in cell division processes and DNA/RNA metabolism. Mortality rates remained statistically unchanged across all experimental groups. These findings suggest that co-exposure to Cisplatin and TiO_2 nanoparticles leads to transcriptional repression and disruption of essential cellular pathways, indicating potential impairments in cellular function.

INTRODUCTION

Cisplatin is the commercial name for the compound (SP-4-2)-diamminedichloridoplatinum (II), the first metal-based anticancer drug widely used to treat several cancers. Multiple *in vitro* and *in vivo* studies have shown its mechanism of action, which involves the generation of DNA lesions. These lesions trigger several signal transduction pathways that inhibit replication and transcription, cell cycle arrest, DNA repair, and apoptosis (Ghosh, 2019). The DNA damage results from Cisplatin's interaction with the purine bases on DNA, generating DNA interstrand adducts (G-G

60%, A-G 20%), and DNA/protein interaction. In humans, polymorphisms in genes responsible for DNA adduct repair, such as ERCC2 and XPC, as well as genes related to membrane pumps, drug efflux, or detoxifying genes (ACYP2, ABCC3, COMT, TPMT), are associated with ototoxicity (Karasawa; Steyger, 2015). A genotoxic study using the Comet assay indicates that in *D. melanogaster*, DNA damage detected has higher levels of G–G cross-links than G monoadducts. This way, G-G cross-links in *D. melanogaster* contribute to the induction of DNA strand-breaks, through both blockage of DNA replication and repair, and not all the Cisplatin-induced adducts contribute to generating DNA strand-breaks (García Sar *et al.*, 2012). Upregulated TIMELESS gene, a human homologue of a *D. melanogaster Tim* gene related to circadian rhythm and cell cycle checkpoint, was demonstrated *in vitro* (NPC cell lines) and *in vivo* to confer resistance to Cisplatin-induced apoptosis via the Wnt/β-catenin pathway (Liu *et al.*, 2017).

Groen *et al.,* performed a survivor assay demonstrating that Cisplatin has dose-dependent toxicity associated with reduced survival of *Drosophila*. In this assay, authors showed that *Drosophila* treated with Cisplatin at concentrations ranging from 10 to 50 μg/ml diluted in the culture medium, reported a relative increase in the activation of genes linked to ROS (reactive oxygen species) in axons' mitochondria. They also demonstrate that flies with the glutathione peroxidase gene silenced by RNA interference (RNAi) show reduced sensitivity in the presence of Cisplatin when compared to wild-type flies. It indicates the usefulness of the fruit fly as a model animal for *in vivo* studies of chemotherapy-induced peripheral neuropathy (CIPN) (GROEN, 2022). In this sense, previous data suggest phagocytosis metabolism is affected in Cisplatin-treated PC12 cells, which decreases membrane lipid content, affecting exocytosis and changing apoptosis metabolism (Mohammadi; Li; Ewing, 2018).

Different carriers have been used to reduce Cisplatin's side effects. In this context, the combined use of 50 mg/ml of NPTiO2 and 10 μg/ml of Cisplatin improved chemotherapy response *in vitro* in the F10 melanoma model and *in vivo* in C57BL/6 mice. The induction of autophagy induced by the nanoparticles played a crucial role in sensitizing F10 melanoma cells (Adibzadeh *et al.*, 2021).

Although largely used in food, pharmaceutical, and cosmetic industries, titanium dioxide nanoparticles ($NPTiO_2$) have *in vivo* side effects that are still unconfirmed. An *in vivo* test using the bivalve *Mytilus galloprovincialis* exposed to 1, 10, or 100μgL^{-1} of

$NPTiO_2$ shows lysosomal and oxidative stress biomarkers altered, and the transcription level of immune-related antioxidant genes decreases (Barmo *et al.*, 2013). Mice orally treated with $NPTiO_2$ at 5, 50, or 500 mg per kg body weight dosage for five consecutive days, and sacrificed 24h after treatment, showed persistent apoptotic DNA fragmentation and mutations in p53 exons at one or two weeks after treatment. They also exhibit persistent elevations in malondialdehyde and nitric oxide levels, decreases in the glutathione level, and catalase activity compared with the control mice in a dose and time-dependent manner.

$NPTiO_2$ administration is directly related to persistent inflammation, apoptosis, and oxidative stress, and induction of chronic gastritis (Mohamed, 2015). Also, it directly binds to proteins involved in cellular functions, generating primary genotoxic effects. The main mechanism of this $NPTiO_2$ genotoxic effects was demonstrated to be caused by reactive oxygen species (ROS) generated by an increase in H_2O_2, singlet oxygen, and lipid peroxide levels, which cause a reduction in glutathione (GSH) and antioxidant levels (Wani; Shadab, 2020). Also, a study considering 34 robust datasets does not support a direct DNA-damaging mechanism for TiO_2 in the nano or micro-form (Kirkland *et al.*, 2022).

Here, we investigate the *in vivo* effects of oral administration of Cisplatin (50μg/ml) and TiO_2 nanoparticles (50μg/ml) isolated and in combination in *Drosophila melanogaster*. Further, we analyze the gene modulation by identifying differentially expressed genes (DEGs) obtained by RNA-seq. Also, we performed downstream analysis to verify the ontology terms related to these different treatments in *Drosophila*. Our data show a specific expression pattern in flies treated with Cisplatin and TiO_2 nanoparticles simultaneously, presenting an intermediate transcript level with a very small number of upregulated DEGs.

MATERIALS AND METHODS

1. *Drosophila melanogaster* maintenance

Drosophila melanogaster strain Oregon (RRID:BDSC.5) was obtained from the Bloomington Drosophila Stock Center, Indiana University. The culture was performed in standard cornmeal-yeast-agar medium (BRENT; OSTER, 1974) and kept in an incubator at 25º Celsius and humidity between 70% - 80% with a 12-hour light cycle.

2. Treatments

1-to-4-day-old *D. melanogaster* strain Oregon flies were divided into four groups with 30 individuals each. Each group contains a 4:1 female/male ratio. The control group was treated with a standard medium enriched with a mixture of yeast, water, and a green food coloring dye. Treatment groups were treated as follows: CIS group – water was substituted by an aqueous Cisplatin solution (50μg/ml); NPTIO2 group - water solution was substituted by an aqueous TiO_2 solution (50μg/ml); CIS_NPTIO2 group – water was substituted by Cisplatin water solution (50μg/ml) and $NPTiO_2$ water solution (50μg/ml) concomitantly. All treatments were performed in triplicate. Flies were kept in this condition for 3 (three) days.

3. Prepare of Used Solutions

$NPTiO_2$ water solution was prepared using 50μg/ml of TiO_2 (Sigma-Aldrich, Cat. No.: 718467; nano powder, 21 nm primary particle size (TEM), ≥99.5% trace metals basis) was heated in an oven (200ºC) for 30 minutes. Then, TiO_2 was solubilized in deionized water by sonication (25ºC, 20 minutes). Cisplatin (Cat. No.: PHR1624) obtained from Merck was dissolved in Milli-Q water (18.2 MΩ cm distilled deionized water obtained from a Milli-Q system, Millipore, Bedford, MA, USA), forming a 50μg/ml Cisplatin water solution.

4. Survival analysis

The survival rate was then calculated by Kaplan-Meier, and survival analysis followed by the Log-rank (Mantel-Cox) test was performed in R Studio using the "survival package". After that, phenotypic analysis was made using a stereoscope to verify possible phenotypic alterations due to treatments.

5. RNA isolation

We used Trizol™ reagent (ThermoFisher™ Scientific, Waltham, Massachusetts, USA) to isolate total RNA from two female flies from each treated group (CIS group, NPTIO2 group, and CIS_NPTIO2 group) and control group, according to manufacturer's instruction. The RNA isolation was performed using the whole animal. To avoid DNA contamination, we used RNase-Free DNase Set (QIAGEN, Cat. No.: 79254) according to manual instructions. Then, RNA was collected after precipitation in ethanol. RNA quality was analyzed using agarose gel visualized in Molecular Imager® Gel Doc™ XR+ Imaging system (Bio-Rad Laboratories, Inc.) and quantified

with NanoDrop 2000 spectrophotometer (ThermoFisher™ Scientific) and a QuBit 3.0 Fluorometer (ThermoFisher™ Scientific).

6. RNAseq

RNAseq ran according to manufacturer's instructions into LNCC (Laboratório Nacional de Computação Científica, Brazil) facilities in an Illumina Genome Analyzer HiSeq 2000mRNA. Libraries preparation was done according to the manufacturer's instructions (IDT ILMN RNA UDI A Lig 96 Idx 96 Spl index kit).

RNAseq was performed according to the manufacturer's instructions (IILMN Strnd mRNA Prep Lig Kit (16 Spl) and sequencing according to manufacturer's instructions (Sequencing (reads): NSQ 500/550 Hi Output KT v2.5 kit (150 CYS)). Only mRNA was considered in this work.

7. Sample processing

The quality of the sequenced libraries was analyzed using the FastQC software (Wingett and Andrews, 2018). Adapters sequences and low-quality regions were filtered using the Trimmomatic tool Version 0.36 (Bolger; Lohse; Usadel, 2014). The remaining readings were aligned to the reference genome of *Drosophila melanogaster* ASM2977511v1, date: April 17Th, 2023, using the BWA mapper (Li; Durbin, 2009). The alignments' quality was analyzed using the Samtools tool (Li *et al.*, 2009), and the count of mapped reads were performed with the HT-seq Count tool (Anders; Pyl; Huber, 2015).

All codes are available in: https://github.com/CristianoNCosta/CristianoNCosta.git

8. Quantification and analysis of differential gene expressions

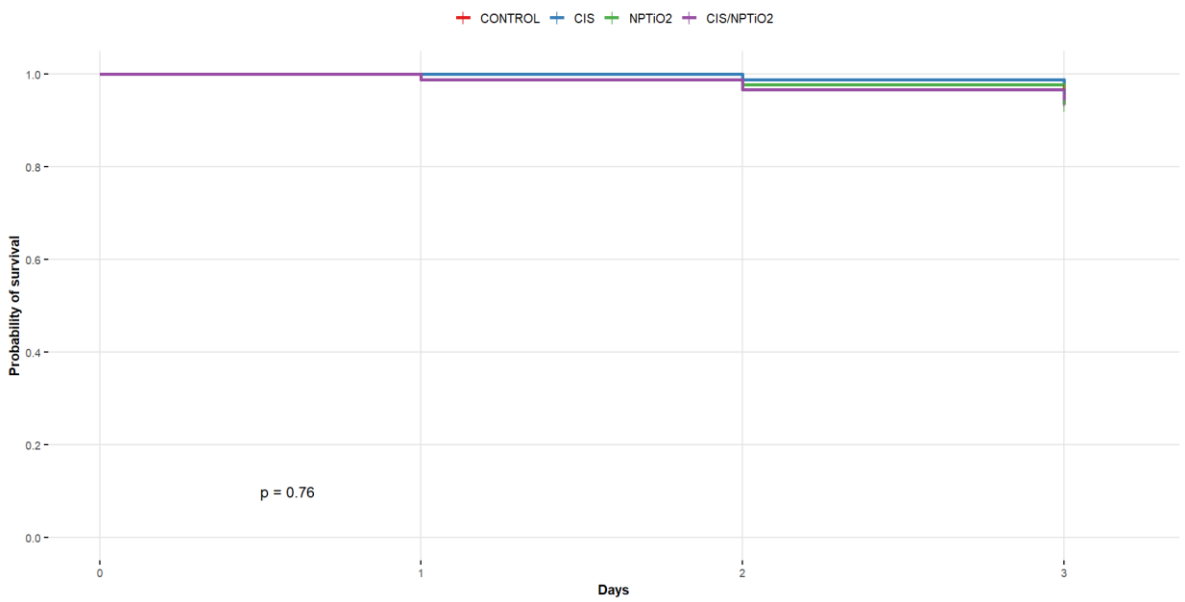
We used R environment tools to run the edgeR tool for read count normalization and differential gene expression analysis (Robinson; McCarthy; and Smyth, 2010). Tools from BiocManager package were used: DESeq2 tool (Love; Huber; and Anders, 2014) was used to compare the results using > $\log^2$ FC cutoff |1,0| and p-value adjusted (p_{adj}) < 0,05. RNA-seq expression values are in Log 2-fold change (Log2fc); the control was set as 1, and the treatment at the Log2fc respective value greater than 1 (Log2fc values and standard errors are supplied (*Supplementary Table 1*).

9. Data downstream analysis

Downstream analysis of differentially expressed genes (DEGs) obtained in the previous step. Then, a functional enrichment analysis of DEGs was performed. R statistical package environment was used, also databases like FlyBase (Öztürk-Çolak *et al.*, 2024), FlyEnrichr (Kuleshov *et al.*, 2019), Gene Ontology (GO) (The Gene Ontology Consortium, 2019), among others. Data supplied in annexed *Supplementary Data*.

RESULTS AND DISCUSSION

To evaluate the effects of individual and combined administration of Cisplatin (50 μg/ml) and TiO_2 nanoparticles (50 μg/ml) *in vivo*, we measure the mortality rates of *Drosophila melanogaster* using the described experimental protocol (materials and methods). The survival rate was calculated by comparing flies from each treated group against the control group (CG) using the Kaplan-Meier method followed by the Log-rank (Mantel-Cox) test (Figure 1). We found no significant variation among all considered groups (*p* = 0,76).



Kaplan–Meier survival curves of Drosophila melanogaster exposed to different treatments: control, Cisplatin (50 μg/ml CIS), titanium dioxide nanoparticles (50 μg/ml NPTIO2), and the combination CIS/NPTIO2 (50 μg/ml/50 μg/ml). Survival rates were similar across all experimental groups (p = 0.76). The Log-rank (Mantel–Cox) test showed no statistically significant differences among the groups; (Events: 18; *p*-value: 0.75347) indicating that none of the treatments significantly affected fly survival over the 3-day observation period.

To check possible variance among the data obtained in DESeq, we performed a Principal Component Analysis (PCA) related to the gene expression levels of each

group. PCA is widely used to analyze variance in data. This analysis suggests that CIS_NPTIO22 is an outlier sample (Figure 2).

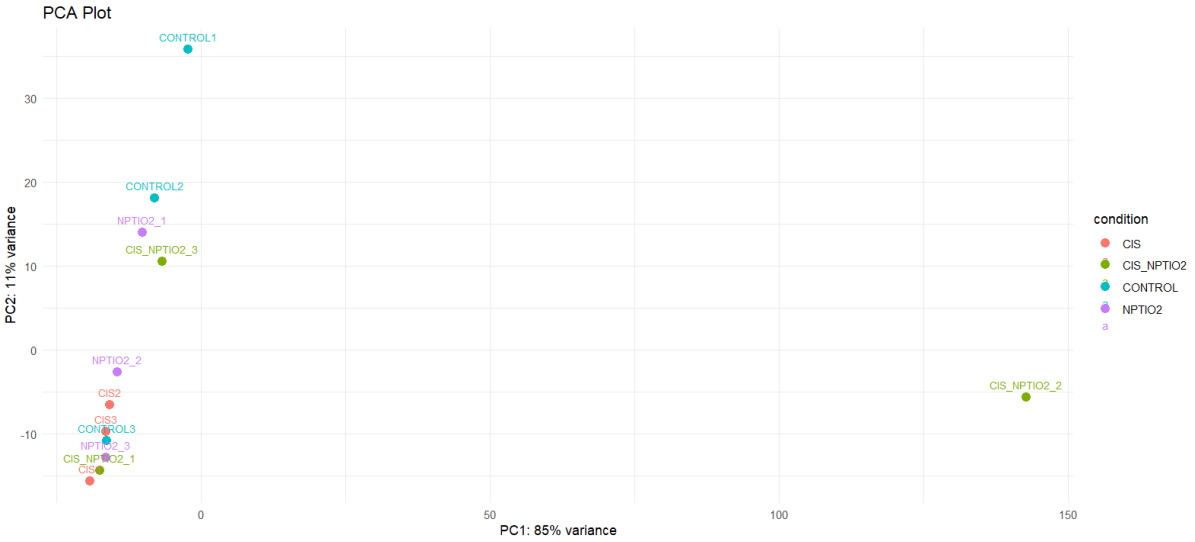


Figure 2. PCA plot of RNA-seq data (rlog-normalized) showing sample separation by treatment: controls (black) cluster on the left, while CIS (red), CIS_NPTIO2 (green), and NPTIO2 (purple) form distinct groups along PC1 (85% variance). PC2 explains minimal variation (11%). CIS_NPTIO22 exhibits an intermediate profile among CIS and NPTIO2.

In BiocManager package, standard PCA held by the DESeq2 package considers *regularized logarithm* (rlog) as the normalization method. rlog applies a variance-stabilizing log transformation tailored for count data and focuses on biological interpretability. Robust Scaler is another normalization method which standardizes data using median and interquartile range (IQR), making it resistant to and of general applicability (not RNA-seq-specific) prioritizes statistical robustness. For this reason, we perform another PCA analysis combining both normalization methods (rlog first, then Robust scaling) to optimize PCA results by balancing variance stabilization and outlier resilience.

Figure 3 and Table 1 present the data obtained from the robust PCA. Figure 3 is a heatmap with a dendrogram on the top side. Dendrogram reveals gene expression profiles of the samples based on the similarity of their expression patterns across the top 10,000 most differentially expressed genes. The clearest observation of Figure 3 reveals some variance between samples from the CONTROL group, also there is variance in samples from the treated groups (CIS and NPTIO2). But there is an enormous difference in samples of CIS_NPTIO2 group, specifically, CIS_NPTIO22 from other samples in the same group.

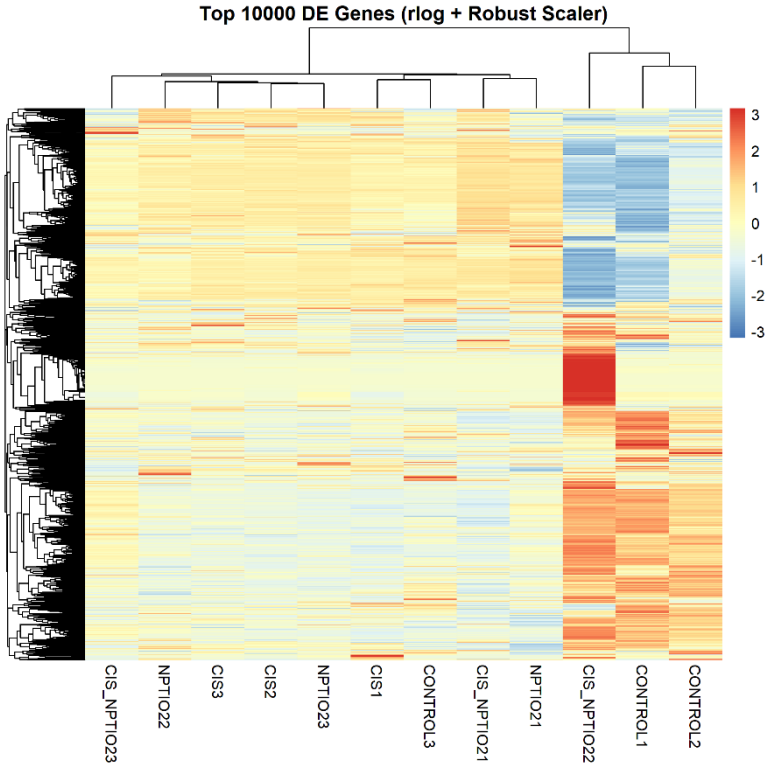


Figure 3: Heatmap from Principal Component Analysis (PCA) of rlog + Robust Scaler normalized RNA-seq data showing the transcriptome pattern of samples from the different experimental groups (CONTROL, CIS, $NPTiO_2$, CIS_$NPTiO_2$) of biological triplicates (n=3/group).

Table 1: Euclidean Distance between CIS_NPTIO22 sample and other samples. Scale units in standard deviation (Robust Scaler).

CIS1	CIS2	CIS3	CIS_NPTIO21	CIS_NPTIO23	
4974.11	4949.75	4944.15	4950.60	4940.42	
CONTROL1	**CONTROL2**	**CONTROL3**	**NPTIO21**	**NPTIO22**	**NPTIO23**
4919.46	4926.30	4950.49	4948.30	4943.52	4933.49

Table 1 shows the Euclidian distance between CIS_NPTIO22 and all other samples. Figure 3 indicates transcriptomic profile changing in response to the CIS_NPTIO2 treatment, suggesting that the combination treatment (CIS + $NPTiO_2$) produces a very distinct and consistent gene expression signature.

We then performed a quantitative analysis to verify the Euclidean distance among the replicates. Figure 4 shows that CIS_NPTIO22 has a distance greater than 4000 Standard Deviation (SD) among other CIS_NPTIO2 replicates, and similar distances are observed for all samples from any other treatment group. These data are consistent with the outlier condition of CIS_NPTIO22, indicating that this sample should be excluded from further analysis to maintain data integrity. Data from Figure 3 and Table 1 together support the hypothesis that CIS_NPTIO22 is an outlier.

After outlier removal, we again perform a PCA and a quantitative analysis to verify the Euclidean distance among the replicates. Figure 4 shows a 70% variance in PC1 and an 8% variance in PC2. Distances in PC2 among samples from the same group suggest potential minor biological variations among samples of same group. Notwithstanding it, across the PC1 axis, the most variable group is CONTROL group. CONTROL3 which is approximately 40% distant from CONTROL1 and CONTROL2 which are compatible with biological variance. Nonetheless, CONTROL group are still useful to compare transcription variance in in all treatment groups. Even, Figure 4 points out that the most homogeneous samples are from the CIS group, which are closer one from each other both in PC1 and PC2, suggesting these sample forms a defined cluster. In the meantime, PC1 shows a heterogeneous response among different samples from the same treatment groups. NPTIO21 is about 15% distant from NPTIO22 and NPTIO23, and CIS_NPTIO21 is distant from CIS_NPTIO23. These data demonstrate a biological variation among samples of those groups, but these variations are not drastic enough to discard these samples. Besides distances in PC1 demonstrate a biggest biological variance between sample in CONTROL group a treatment effect with a primary separation among CIS, NPTIO2, and CIS_NPTIO2 groups from the CONTROL group.

To confirm the abovementioned, we computed the Euclidean distance among the replicates after outlier removal, and neither the control nor the treated group has a distance greater than 750 from other replicates, confirming that there is no other outlier (*Supplemental material 01*). This data points to a possible synergistic effect from CIS_NPTIO2 treatment in relation to the administration of CIS and NPTIO2 separately.

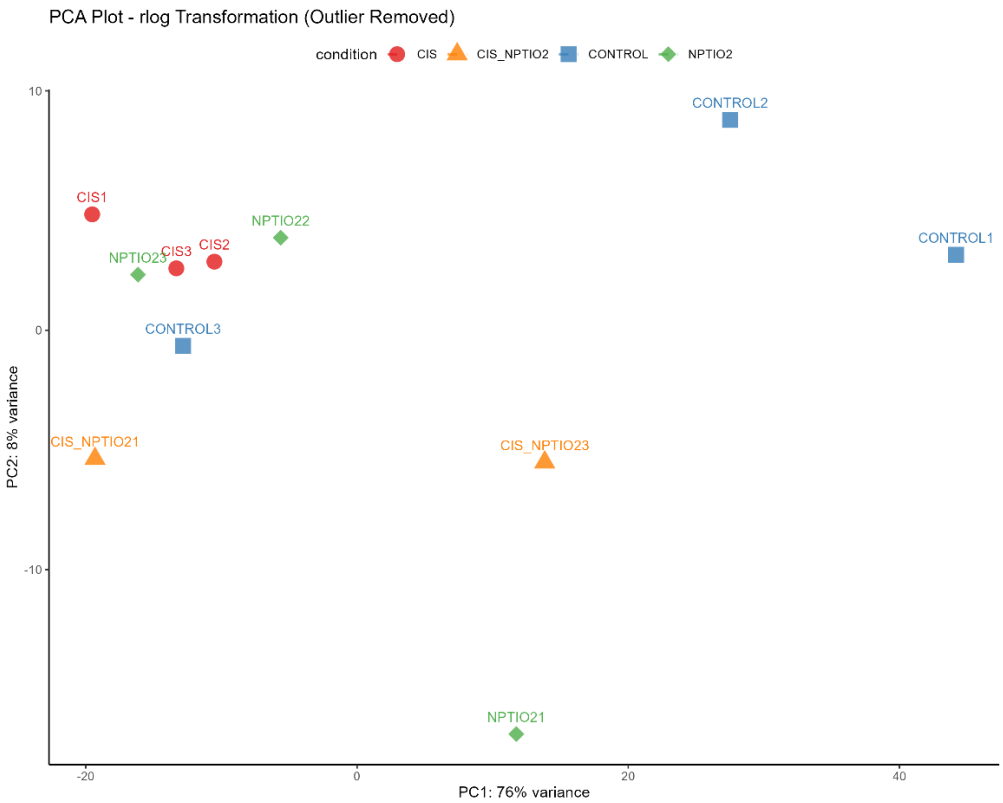


Figure 4: Two-dimensional PCA projection of rlog-transformed count data following quality control and outlier removal. Variance explained: PC1 (76%), PC2 (8%). Ellipses represent 95% confidence intervals for each experimental group. The improved cluster compactness and inter-group separation compared to pre-filtered data confirm the effectiveness of outlier removal in enhancing data quality and analytical robustness.

Samples are colored by experimental condition: CONTROL (blue), CIS (red), CIS_NPTIO2 (orange), and NPTIO2 (green). The analysis demonstrates clear separation between treatment groups following outlier exclusion.

Taken together, these statistical analyses demonstrate that the removal of the CIS_NPTIO22 outlier leads to greater consistency across biological replicates and supports robust inference about treatment effects and allows for more reliable downstream comparisons and interpretation.

Expanding upon these findings, we next examined the global patterns of gene expression and differential regulation elicited by each treatment group.

Figure 5 shows Volcano plots with the distribution of differentially expressed genes (DEGs) of each challenged group (CIS, NPTIO2, and CIS_NPTIO2) compared with the control. Each dot represents a gene; black denotes non-significant changes, and red denotes DEGs with significant fold changes. ($\log_2 fc > |1.0|$ and $p_{adj} < 0.05$), and Figure 6 shows the total volume of transcripts by each control or treatment group.

Comparing the treatment groups with the CONTROL group, our data shows that both the CIS and CIS_NPTIO2 groups significantly increased the total transcript count. However, the NPTIO2 treatment alone appears to have a less pronounced effect but still presents a higher total transcript count than the control group, as demonstrated by the slightly higher median than the control group. Although it presented a lower number of upregulated and downregulated DEGs, the CIS_NPTIO2 group had a marginally higher transcript count than the CIS group alone and, more importantly, showed much less variability, suggesting CIS_NPTIO2 has more stable or robust effect of the combination of Cisplatin (50 μg/ml) and TiO_2 nanoparticles (50 μg/ml) causes an intermediate effects in animals treated in this way.

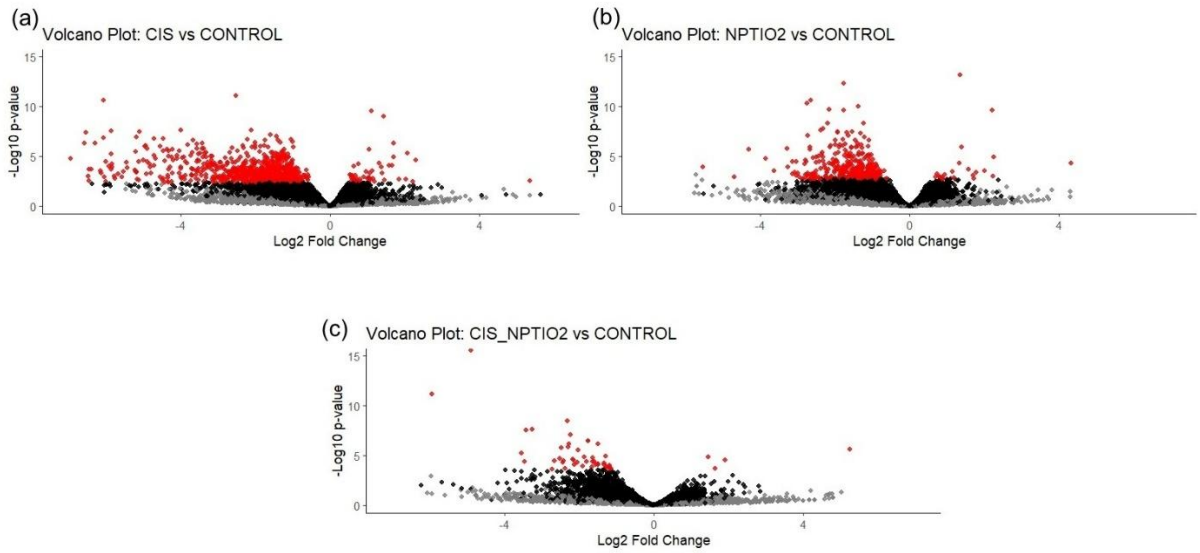


Figure 5: Differential gene expression analysis. Volcano plots display the relationship between statistical significance (-$\log_{10}$ p-value) and the magnitude of change ($\log_2$ fold change) for all measured genes. (a) CIS treatment versus CONTROL, (b) NPTIO2 treatment versus CONTROL, (c) CIS_NPTIO2 treatment versus CONTROL. DEGS with differentiated expression (fold change (log2fc > |1.0| and p-Value <0.05): Significant (•), Not significant (•).

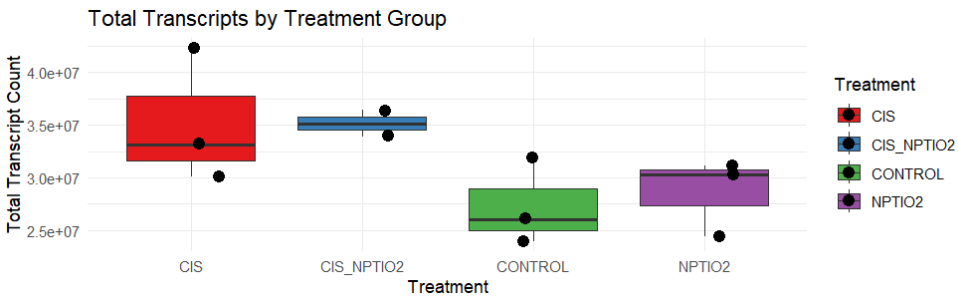


Figure 6 - Total Transcript Counts by Treatment Group: Box plots show the total transcript counts for each of the four treatment groups (CIS, CIS_NPTIO2, CONTROL, and NPTIO2). The line within each box indicates the median value, with the box representing the interquartile range (IQR). Black dots represent individual sample data points. Total transcript counts are highest in the CIS and CIS_NPTIO2 treatment groups, while the CONTROL group shows the lowest counts.

After confirming the statistical data and outlier removal, we began to analyze the DEGs in each group. The Venn diagram (Figure 7) indicates the total number of DEGs obtained by each group isolated and those DEGs found in two or more groups. It shows that there are two upregulated DEGs detected in all groups (*CG10013*, *aqz*). CIS and NPTIO2 groups have 4 upregulated DEGs (*CG11263*, *CG18088*, *Tango6*, *inaF-D*) and one long non-coding RNA (lncRNA:CR43836). One upregulated DEG was presented in CIS and CIS_NPTIO2 groups (CG5568), and NPTIO2 and CIS_NPTIO2 groups have one upregulated DEG (*CG3213*). Conversely, treatment conditions generate a different profile of downregulated DEGs in each group. Besides a larger number of DEGs were downregulated, only a few DEGs were downregulated both by CIS_NPTIO2 group and NPTIO2 or CIS groups separately or by all treated groups at same time. Additionally, we identified an expressive number of DEGs simultaneously downregulated by both CIS and NPTIO2 groups.

The reduced number of up and downregulated DEGs, specifically in the CIS_NPTIO2 group suggests a possible synergistic effect of cisplatin and TiO_2 nanoparticles administered at same time to the *Drosophila*. This observation is according to previously described by Adibzadeh and Cols. (2021) in murine melanoma models, and probably, is caused by a reduction in cell signaling processes due to joint administration of these substances in *Drosophila*.

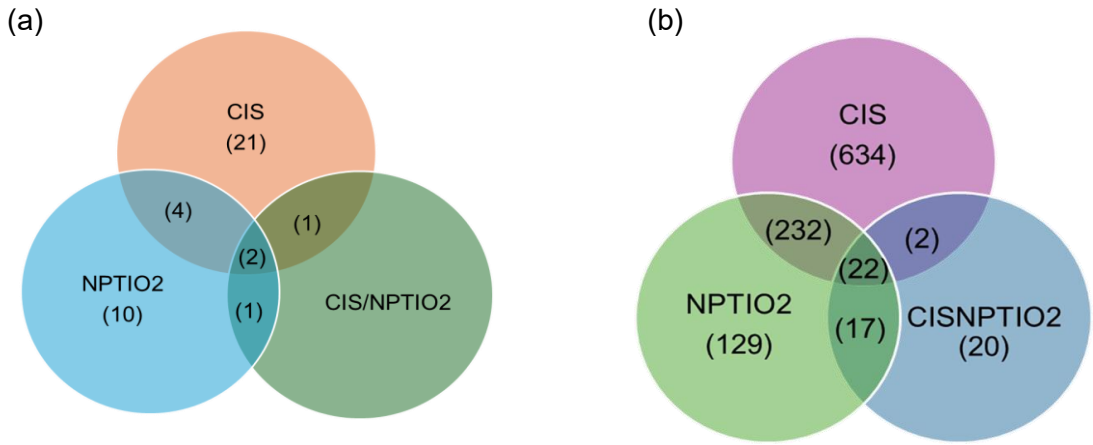


Figure 7: Venn diagram relative to the number of DEGs obtained in (a) upregulated DEGs and (b) downregulated DEGs in the challenged groups.

Downstream analysis of upregulated DEGs detected in flies treated with 50μg/ml Cisplatin, hereinafter referred to as the CIS group, shows no significative KEGG pathway, but presented 24 upregulated DEGs and 3 long non-coding RNA (Figure 8). The upregulated DEGs in the CIS group have GO terms for biological processes (BP)

related to response to stimulus, development, biogenesis, DNA metabolism and immune response. GO terms for molecular function (MF) corresponding to enzyme, metal ion binding, RNA binding, carbohydrate binding. Still, these DEGs have cellular component (CC) GO terms related to membrane, cell periphery, cytosol, nucleus and others. (*Supplementary Table 1)*. This data indicates that *Drosophila* treated with 50μg/ml Cisplatin activates metabolism to the general response a stimulus and DNA repair machinery. It is distinct from previous data, which identify DEGs upregulated only at higher cisplatin concentrations (Mombach *et al.*, 2022).

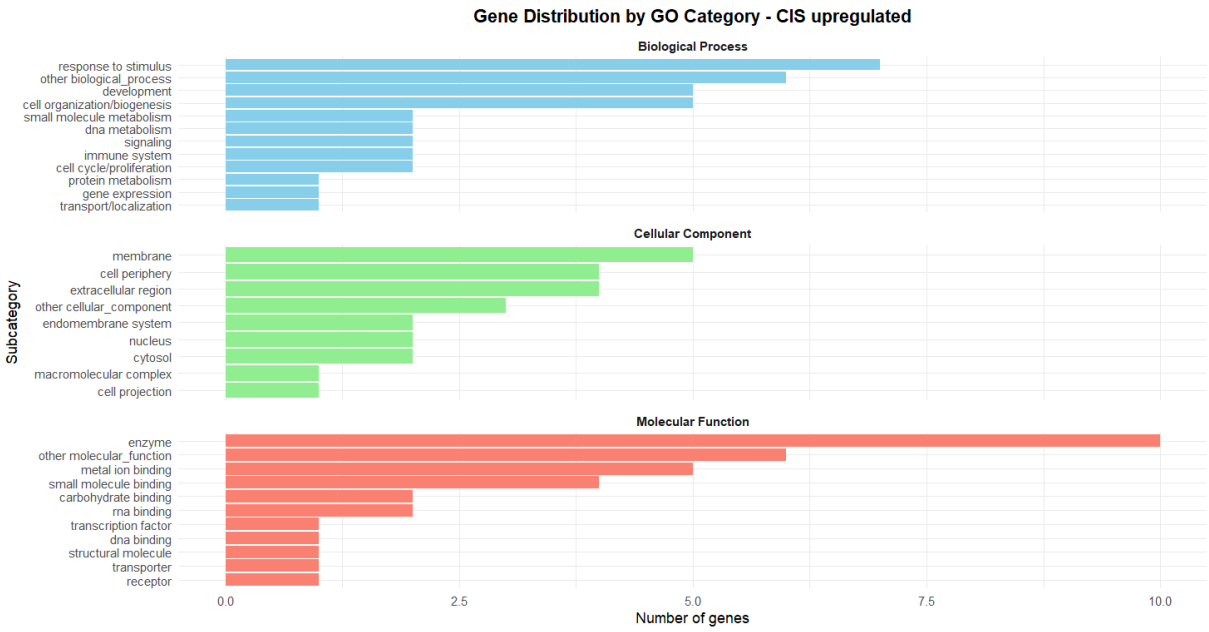


Figure 8 - GO enrichment analysis of upregulated genes under CIS treatment: (■) Biological Process, (■) Cellular Component, and (■) Molecular Function. Full results are in Supplementary Table 1. Data source GO Summary Ribbon (Fly Base).

Flies treated with 50μg/ml of $NPTiO_2$ (NPTIO2 group) exhibit upregulation in 16 DEGs and one long non-coding RNA. 2 DEGs are also upregulated by all treatment groups, 4 DEGs are shared with CIS group, and one DEG is at the same time upregulated by CIS_NPTIO2. These DEGs are related to 10 biological processes (gene expression, protein metabolism, development, signaling, response to stimulus, etc.); 6 molecular functions (small molecule binding, RNA binding, transport, enzyme, receptor binding), and nine cellular components (macromolecular binding, nucleus, membrane, chromosome, endomembrane system) (Figure 9). This NPTIO2 group, our data is in accordance with previous data, showing that gene expression is not affected by $NPTiO_2$ uptake, and occurs activation in DEGs related to response to stimulus and transport/cell localization (Jovanović; Cvetković; Mitrović, 2016).

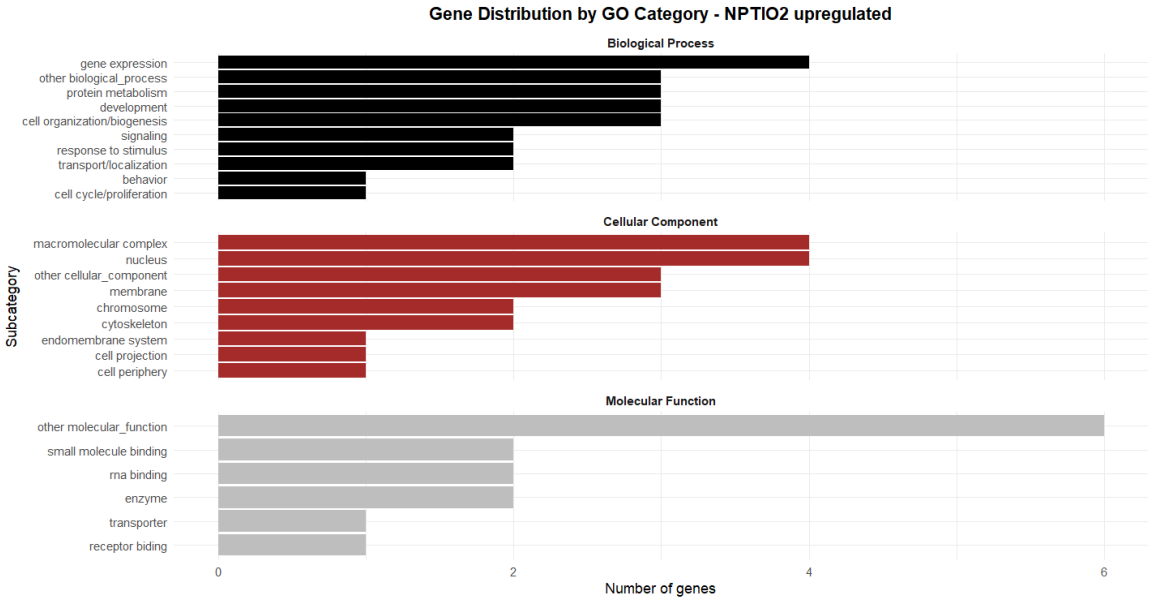


Figure 9: **GO enrichment analysis of upregulated genes under NPTIO2 treatment**: (■) Biological Process, (■) Cellular Component, and (■) Molecular Function. Full results are in Supplementary Table 1. Data source GO Summary Ribbon (Fly Base).

Finally, Table 2 shows observed four upregulated DEGs in the CIS_NPTIO2 group (flies treated with 50µg/ml of Cisplatin and 50µg/ml of $NPTiO_2$ concomitantly). We demonstrate that $NPTiO_2$ activate cytoskeleton and lipid metabolism genes, which are normally repressed in flies treated only with Cisplatin, but are active in flies treated with nanoparticles in general (Adibzadeh et al., 2021; González-López et al., 2020; Mohammadi; Li; Ewing, 2018). As these genes are related to endocytosis, it suggests a possible increase in the incorporation of Cisplatin due to the effect of $NPTiO_2$ jointly administered.

Table 2: Gene Ontology (GO) enrichment analysis of CIS_NPTIO2-upregulated genes

GO Category	Subcategory	Nº Genes
Cellular Component	cytoskeleton	1
	nucleus	1
Biological Process	fatty-acyl-CoA biosynthetic process	1
	long-chain fatty acid metabolic process	1
	biological_process	1
Molecular Function	enzyme	1

Table 2: DEGs from CIS_NPTIO2 group classified using GO Summary Ribbon (Fly Base). Only the most specific terms are shown. Full data available in Supplementary Table 1.

We found two genes upregulated in all three treatment groups: *CG10013* and *aqz*. *CG10013* codes for a ubiquitin-conjugating enzyme E2-binding protein which accept ubiquitin from specific E2 ubiquitin-conjugating enzymes, and transfers it to substrates, generally promoting their degradation by the proteasome. CG10013 contributes to genome structural dynamics in *Drosophila*. Also, (Bastide , 2016). The gene *aqz* is predicted to be active in the nucleus and codes for the Aaquetzalli protein isoform A. *aqz* (Mendoza-Ortíz; Murillo-Maldonado; Riesgo-Escovar, 2018). Together *CG10013* and *aqz* plays a role in responses to oxidative stress and other conditions that affect the cell and are expected to be upregulated in flies of all treated groups.

CG5568 is regulated in flies from both CIS and CIS_NPTIO2 groups. This gene is involved in the transcriptional regulation of the cell cycle, acting as a zinc finger protein that binds to DNA and has a role in eye development (Morrison *et al.*, 2006). Also, *CG5568* is highly expressed in female germline cells and involved in fatty-acyl-CoA biosynthetic process (Li *et al.*, 2022). Upregulation in *CG5588* is expected due to the protein rich diet which leads to mitosis activation, despite the Cisplatin treatment.

CG3213 is upregulated in flies from both NPTIO2 and CIS_NPTIO2 groups; this gene is involved in ribosomal and protein translation (Pospisilik *et al.*, 2010). Also, *CG3213* was identified in a genome-wide screen for obesity-related genes in *Drosophila* and a single-nucleotide polymorphism (SNP) in *CG3213* is associated with variation in starvation resistance (Mackay *et al.*, 2012). A screening performed in adult flies, including both sexes, demonstrates that triglyceride levels, a direct measure of adiposity, were determined in adult flies following RNAi induction. Inhibition of *CG3213* in muscle cells was shown to regulate triglyceride levels,(Garlovsky; Sandler; Karr, 2022). Yet, *CG3213* is related to distinct processes in the germline stem cell lineage(Matsuoka et al., 2017). Upregulation in DEGs related to fatty-acid and lipids metabolisms in flies treated with TiO2 nanoparticles ($NPTiO_2$) is expected.

DEGs upregulated by the CIS_NPTIO2 group alone or simultaneously in one or the other two treated groups are related to biological processes described in Table 2. This data strongly suggests a possible increase in the incorporation of Cisplatin due to the increase of endocytosis mediated by the $NPTiO_2$ jointly administration.

Drosophila from both CIS and NPTIO2 groups have 3 upregulated DEGs in common (*CG11263*, *CG18088* and *InaF-D*) and an upregulated long non-coding RNA (lncRNA:CR43836). Recent data suggest that long non-coding RNAs (lncRNAs) may have important regulatory functions in the genome (Zhong *et al.*, 2020). Specifically about the lncRNA:CR43836, data show this lncRNA is expressed in many embryonic tissues, larval visceral muscle cell, and adult female (Matthews *et al.*, 2015). *CG11263* is an RNA-binding protein related to lipid binding, cilium assembly, and intracellular transport, expressed in *Drosophila* embryos, prepupa and pupa stages and in adult females (ADAMS, 2000). Upregulation in *CG11263* could explain the presence of lncRNAs in treated flies in these groups, due to its role in transcription regulation. *CG18088* is related to *Drosophila's* immune response to *Wolbachia sp.*. affecting transduction related genes. *InaF-D* binds to transient receptor potential (TRP) calcium channel and affects metal absorption (Chen; Montell, 2020). The last to DEGs are also related to immune response and metal response and are expected to be upregulated in flies treated with those metal compounds.

All flies were kept for three days, fed on food supplemented with wet yeast paste containing Cisplatin, $NPTiO_2$, or both substances concomitantly, without previous starvation. This can positively affect cell proliferation in the flies, regardless of the group they are part of, i.e., the control group or the treated groups. Several studies demonstrate that the protein intake affects tumor growth and oogenesis in female ovaries, so, the observation of upregulated DEGs of genes related to this function is expected (Ables *et al.*, 2016; Ables; Laws; Drummond-.

Previous studies have demonstrated that mated females can store sperm transcripts in their spermatheca, and the RNAs of sperm related to many genes, including those of the accessory gland and seminal fluid proteins, can be detected (Cridland; Begun, 2023; Prokupek *et al.*, 2008). Our treatment conditions, time of feeding, food supplementation provided to flies, and use of mated females could explain the difference among our results and the previous study in flies treated with the same Cisplatin concentration (Mombach *et al.*, 2022). Also, it could explain the identification of DEGs primarily related to *Drosophila* males but also expressed in females in our data.

CIS and NPTIO2 groups presented a response whose enrichment has the GO terms (Figures 8 and 9) related to response to stimulus, gene expression, protein metabolism and development (BP); and enzyme, nucleic acid binding and small

molecule binding (MF); DEGs in these groups are located in different cellular components but enrichment GO terms related to the membrane, endomembrane system, and macromolecular complex has found in both. Our data is partially in accordance with a previous study indicating that, independently of the treatment to which Drosophila are subjected, upregulated genes have GO terms related to response to a stimulus, and those coding lysozymes, cytochrome, and mitochondrial components (BROWN *et al.*, 2014).

Our data shows upregulated DEGs related to different Response to stimulus GO terms. According to GO Summary Ribbons (FlyBase (*FB2025_03, released July 24, 2025*)), the CIS group has six up-regulated DEGs with Response to stimulus GO Term: *FANCI, St4, Ir7c, PPO1, out and CG10738*. A more detailed analysis of the FlyEnrichr database shows that this set of DEGs is related to the following GO biological processes, including defense response to bacteria, oogenesis, regulation of cell proliferation, wound healing, among others (*Supplementary Table 2*). The molecular functions of this set of DEGs are related to sulfotransferase activity, DNA polymerase binding, and hydrolase (Supplementary Table *2*).

CIS group flies also present three upregulated lncRNA (lncRNA:CR43836, lncRNA:CR44707, lncRNA:CR45052). lncRNA:CR43836, as mentioned before, and all knowledge regarding these lncRNAs is related to regulation in transcription. Our data is according to the state of the art, which indicates that Cisplatin treatment induces lncRNAs-mediated responses (Brown *et al*., 2014; Zhang *et al*).

The CIS group also has DEGS related to development: *CG9503, dec, Cad88C, Cad74A, out*. The FlyEnrichr database shows that this set of DEGS is associated with the following GO biological processes: cell-cell adhesion, female germline sex determination, regulation of DNA endoreduplication, and ecdysteroid metabolic process, among others (Supplementary Table 1). These molecular functions are related to hydrolase activity and ionic binding (*Supplementary Table 2*). The simultaneous super expression of all these genes suggests that the organism is undergoing an intense phase of development, differentiation, and tissue reorganization. They appear to be working together to coordinate the complex morphological changes that occur in *Drosophila* body.

In the NPTIO2 group, we found upregulation in *CG1503*, *l(2)03659,* and *CG44838*. Its upregulation suggests *Drosophila* treated with 50μg/ml of TiO_2 needs to

activate transport machinery to metabolize TiO_2. In case, CG44838 is a transcription factor known for signal transduction and nervous system development (Olson *et al.*, 2019) and activate *CG1503* which is a protein regulator having hydrolase activity and are related to decreased carbohydrate metabolism (Hu *et al.*, 2021), finally *l(2)03659* which is related to response to xenobiotics or metabolites mitochondrial stress-relief mechanism associated with multidrug resistance (Yeh *et al.*, 2017) due to activation in transmembrane transport mechanism.

CG11263, tut, and *CG18088* DEGs were upregulated by $NPTiO_2$. These DEGs are related to the negative gene expression regulation and piRNA metabolic process. *CG11263* is co-expressed and functions as part of the transcription machinery that enables the generation of piRNA precursors from heterochromatin while maintaining the suppression of transposon-encoded promoters and enhancers. This is also related to pupal and adult female viability (Matthews *et al.*, 2015). *CG10013* and *ord* are co-expressed and promote normal formation and maintenance of the synaptonemal complex and centromere clustering (Das *et al.*, 2016). Also, *ord* is essential for proper maintenance of sister-chromatid cohesion in both male and female meiosis. Yet, mutations in this gene cause premature separation of the sister chromatids in meiosis I and random segregation in both meiotic divisions and reduce recombination in female meiosis (Miyazaki; Orr-Weaver, 1990).

Previous data indicate that *D. melanogaster* ovaries infected with *Wolbachia sp*. present upregulation in *tut, Mal-A7*, and *dany* genes, which are involved in biological processes such as cell division and recombination processes, and these genes are also related to infection-associated differential expression (FRANTZ *et al.*, 2023). Our data demonstrate that Drosophila from NPTiO2 group presents upregulation only in *tut* but develops the same responses of flies treated with *Wolbachia sp…*, so *tut* should have a key role in *Drosophila* metabolism against $NPTiO_2$ repressing other genes.

D. melanogaster challenged with nanomaterials develops acute effects related to changes in pigmentation, survivor rate, life spam, and genotoxicity (Zhang *et al.*, 2023). Relative to $NPTiO_2$, a study using *D.melanogaster* chronically exposed to suspensions containing 5 mg/kg, 10 mg/kg, 15 mg/kg, and 20 mg/kg $NPTiO_2$ from the embryonic period of the P0 to F3 in adult stage, demonstrates a decrease in the total crawling distance of larvae and the total movement distance of adult males in F3. Results obtained in this study suggest disruption in the Impaired neuromuscular

junction (NMJ) due to an increase expression level of genes related to response to lithium-ion response (*List*) and neuron cellular homeostasis (*Gba1a* and *hll*), and a decrease in gene expression levels of thermosensory behavior gene (*Cyp6a17*) and axon target recognition gene (*frac*) (ZHANG *et al.*, 2023). Our data, which are based on prolonged but not chronic exposition, corroborate these observations regarding the reduction in the expression of *frac* and *Cyp6a17*.

Our results suggest that *aqz* and *CG10013, which* are DEGs upregulated by all challenged groups (CIS, NPTIO2 and CIS_NPTIO2)*,* should have a significant role in the fly response to Cisplatin and $NPTiO_2$. Also, the upregulation of these DEGs by all treated groups indicates that ribosomal production metabolism is affected by these treatments.

Our data suggests that changes in metabolism occur in the CIS_NPTIO2 group, mainly the cell division metabolism, which is greatly affected by the combined use of Cisplatin and $NPTiO_2$. Flies in this group have no statistical difference in survival (Figure 1), but despite the protein intake during the treatment, which leads to cell division and gametogenesis (Britton; Edgar, 1998), a strong quiescence in cell division metabolism is observed.

These results suggest that the combined use of Cisplatin and $NPTiO_2$ is effective in reducing the expression level and in promoting quiescence in dividing cells. Our *in vivo* study could indicate that the combination of these substances could affect cell division in *Drosophila* treated concomitantly with Cisplatin and NPTiO2, which may likely be useful in treating cancers for which Cisplatin alone does not have the desired effect.

References:

ABLES, Elizabeth T. *et al.* A Genetic Mosaic Screen Reveals Ecdysone-Responsive Genes Regulating Drosophila Oogenesis. **G3 Genes|Genomes|Genetics**, v. 6, n. 8, p. 2629–2642, 1 ago. 2016.

ABLES, Elizabeth T.; LAWS, Kaitlin M.; DRUMMOND-BARBOSA, Daniela. Control of adult stem cells *in vivo* by a dynamic physiological environment: diet-dependent systemic factors in *Drosophila* and beyond. **WIREs Developmental Biology**, v. 1, n. 5, p. 657–674, set. 2012.

ADAMS, MARK D. The Genome Sequence of Drosophila melanogaster. **Science**, v. 5461, p. 2185–2195, 2000.

ADIBZADEH, R. *et al.* Combination therapy with TiO2 nanoparticles and cisplatin enhances chemotherapy response in murine melanoma models. **Clinical and Translational Oncology**, v. 23, n. 4, p. 738–749, abr. 2021.

AHMED, Mohamed Ahmed Ibrahim; VOGEL, Christoph Franz Adam. Hazardous effects of octopamine receptor agonists on altering metabolism-related genes and behavior of Drosophila melanogaster. **Chemosphere**, v. 253, p. 126629, ago. 2020.

BARMO, Cristina *et al.* In vivo effects of n-TiO2 on digestive gland and immune function of the marine bivalve Mytilus galloprovincialis. **Aquatic Toxicology**, v. 132–133, p. 9–18, maio 2013.

BASTIDE, Héloïse *et al.* A Variable Genetic Architecture of Melanic Evolution in *Drosophila melanogaster*. **Genetics**, v. 204, n. 3, p. 1307–1319, 1 nov. 2016.

BOLGER, Anthony M.; LOHSE, Marc; USADEL, Bjoern. Trimmomatic: a flexible trimmer for Illumina sequence data. **Bioinformatics**, v. 30, n. 15, p. 2114–2120, 1 ago. 2014.

Brasil. Resolução Nº 58. 58. . 24 fev. 2023, Sec. 1, p. 8.

BRENT, M. M.; OSTER, I. I. Nutritional Substitution- a new approach to microbial control for Drosophila cultures. p. 155–157, 1974.

BRITTON, Jessica S.; EDGAR, Bruce A. Environmental control of the cell cycle in Drosophila: nutrition activates mitotic and endoreplicative cells by distinct mechanisms. **Development**, v. 125, n. 11, p. 2149–2158, 1 jun. 1998.

BROWN, James B. *et al.* Diversity and dynamics of the Drosophila transcriptome. **Nature**, v. 512, n. 7515, p. 393–399, 28 ago. 2014.

BUCHANAN, Mr. H. R. 2565. 15 abr. 2021.

CHEN, Zijing; MONTELL, Craig. A Family of Auxiliary Subunits of the TRP Cation Channel Encoded by the Complex InaF Locus. **Genetics**, v. 215, n. 3, p. 713–728, 1 jul. 2020.

CRIDLAND, Julie M.; BEGUN, David J. Male-derived transcripts isolated from the mated female reproductive tract in *Drosophila melanogaster*. **G3: Genes, Genomes, Genetics**, v. 13, n. 11, p. jkad202, 1 nov. 2023.

DAS, Arunika *et al.* Spindle Assembly and Chromosome Segregation Requires Central Spindle Proteins in Drosophila Oocytes. **Genetics**, v. 202, n. 1, p. 61–75, 1 jan. 2016.

FREIRES, I. A. *et al.* Alternative Animal and Non-Animal Models for Drug Discovery and Development: Bonus or Burden? **Pharm Res**, v. 34, p. 681–686, 2017.

GARCÍA SAR, Daniel *et al.* Relationships between cisplatin-induced adducts and DNA strand-breaks, mutation and recombination in vivo in somatic cells of Drosophila melanogaster, under different conditions of nucleotide excision repair. **Mutation Research/Genetic Toxicology and Environmental Mutagenesis**, v. 741, n. 1–2, p. 81–88, jan. 2012.

GARLOVSKY, Martin D.; SANDLER, Jessica A.; KARR, Timothy L. Functional Diversity and Evolution of the Drosophila Sperm Proteome. **Molecular & Cellular Proteomics**, v. 21, n. 10, p. 100281, out. 2022.

GHOSH, Sumit. Cisplatin: The first metal based anticancer drug. **Bioorganic Chemistry**, v. 88, p. 102925, jul. 2019.

GONZÁLEZ-LÓPEZ, M. A. *et al.* Reducing the effective dose of cisplatin using gold nanoparticles as carriers. **Cancer Nanotechnology**, v. 11, n. 1, p. 4, dez. 2020.

GRIMM, Herwig *et al.* Advancing the 3Rs: innovation, implementation, ethics and society. **Frontiers in Veterinary Science**, v. 10, 2023.

GROEN, Christopher M. *et al.* Genetic Reduction of Mitochondria Complex I Subunits is Protective against Cisplatin-Induced Neurotoxicity in Drosophila. **The Journal of Neuroscience**, v. 42, n. 5, p. 922, 2 fev. 2022.

HEREMANS, Isaac P. *et al.* Parkinson's disease protein PARK7 prevents metabolite and protein damage caused by a glycolytic metabolite. **Proceedings of the National Academy of Sciences**, v. 119, n. 4, p. e2111338119, 25 jan. 2022.

HU, Yanhui *et al.* FlyRNAi.org—the database of the Drosophila RNAi screening center and transgenic RNAi project: 2021 update. **Nucleic Acids Research**, v. 49, n. D1, p. D908–D915, 8 jan. 2021.

JOVANOVIĆ, Boris; CVETKOVIĆ, Vladimir J.; MITROVIĆ, Tatjana Lj. Effects of human food grade titanium dioxide nanoparticle dietary exposure on Drosophila melanogaster survival, fecundity, pupation and expression of antioxidant genes. **Chemosphere**, v. 144, p. 43–49, fev. 2016.

KARASAWA, Takatoshi; STEYGER, Peter S. An integrated view of cisplatin-induced nephrotoxicity and ototoxicity. **Toxicology Letters**, v. 237, n. 3, p. 219–227, set. 2015.

KATZ, Alan J. Genotoxic effects of cisplatin in somatic tissue of Drosophila melanogaster. **Environmental and Molecular Mutagenesis**, v. 10, n. 2, p. 197–203, jan. 1987.

KAUFMAN, Thomas C. A Short History and Description of Drosophila melanogaster Classical Genetics: Chromosome Aberrations, Forward Genetic Screens, and the Nature of Mutations. **Genetics**, v. 206, n. 2, p. 665–689, 1 jun. 2017.

KIRKLAND, David *et al.* A weight of evidence review of the genotoxicity of titanium dioxide (TiO2). **Regulatory Toxicology and Pharmacology**, v. 136, p. 105263, dez. 2022.

KULESHOV, Maxim V. *et al.* modEnrichr: a suite of gene set enrichment analysis tools for model organisms. **Nucleic Acids Research**, v. 47, n. W1, p. W183–W190, 2 jul. 2019.

LI, Heng *et al.* The Sequence Alignment/Map format and SAMtools. **Bioinformatics**, v. 25, n. 16, p. 2078–2079, 15 ago. 2009.

LI, Heng; DURBIN, Richard. Fast and accurate short read alignment with Burrows–Wheeler transform. **Bioinformatics**, v. 25, n. 14, p. 1754–1760, 15 jul. 2009.

LI, Xiao *et al.* Cdk8 attenuates lipogenesis by inhibiting SREBP-dependent transcription in *Drosophila*. **Disease Models & Mechanisms**, v. 15, n. 11, p. dmm049650, 1 nov. 2022.

LIU, Sai-Lan *et al.* TIMELESS confers cisplatin resistance in nasopharyngeal carcinoma by activating the Wnt/β-catenin signaling pathway and promoting the epithelial mesenchymal transition. **Cancer Letters**, v. 402, p. 117–130, ago. 2017.

LOVE, Michael I.; HUBER, Wolfgang; ANDERS, Simon. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. **Genome Biology**, v. 15, n. 12, p. 550, dez. 2014.

MACKAY, Trudy F. C. *et al.* The Drosophila melanogaster Genetic Reference Panel. **Nature**, v. 482, n. 7384, p. 173–178, fev. 2012.

MATSUOKA, Shinya *et al.* Adipocyte Metabolic Pathways Regulated by Diet Control the Female Germline Stem Cell Lineage in <i>Drosophila melanogaster</i> **Genetics**, v. 206, n. 2, p. 953—971, jun. 2017.

MATTHEWS, Beverley B. *et al.* Gene Model Annotations for Drosophila melanogaster: Impact of High-Throughput Data. **G3 Genes|Genomes|Genetics**, v. 5, n. 8, p. 1721–1736, 1 ago. 2015.

MENDOZA-ORTÍZ, Miguel A.; MURILLO-MALDONADO, Juan M.; RIESGO-ESCOVAR, Juan R. aaquetzalli is required for epithelial cell polarity and neural tissue formation in Drosophila. **PeerJ**, v. 6, p. e5042, 21 jun. 2018.

MIRZOYAN, Zhasmine *et al.* Drosophila melanogaster: A Model Organism to Study Cancer. **Frontiers in Genetics**, v. 10, p. 51, 1 mar. 2019.

MISHRA, Pawan Kumar *et al.* Wood-Based Cellulose Nanofibrils: Haemocompatibility and Impact on the Development and Behaviour of Drosophila melanogaster. **Biomolecules**, v. 9, n. 8, p. 363, 13 ago. 2019.

MIYAZAKI, W.; ORR-WEAVER, T. Genetic and molecular analysis of orientation disrupter (ord). 1990.

MOHAMED, Hanan Ramadan Hamad. Estimation of TiO2 nanoparticle-induced genotoxicity persistence and possible chronic gastritis-induction in mice. **Food and Chemical Toxicology**, v. 83, p. 76–83, set. 2015.

MOHAMMADI, Amir Saeid; LI, Xianchan; EWING, Andrew G. Mass Spectrometry Imaging Suggests That Cisplatin Affects Exocytotic Release by Alteration of Cell Membrane Lipids. **Analytical Chemistry**, v. 90, n. 14, p. 8509–8516, 17 jul. 2018.

MOMBACH, Daniela Moreira *et al.* Molecular and biological effects of Cisplatin in Drosophila. **Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology**, v. 252, p. 109229, 2022.

MORRISON, Julie L. *et al.* A lock-and-key model for protein–protein interactions. **Bioinformatics**, v. 22, n. 16, p. 2012–2019, 15 ago. 2006.

NEGRE, Bárbara; RUIZ, Alfredo. HOM-C evolution in Drosophila: is there a need for Hox gene clustering? **Trends in Genetics**, v. 23, n. 2, p. 55–59, fev. 2007.

OLSON, John M. *et al.* Expression-Based Cell Lineage Analysis in Drosophila Through a Course-Based Research Experience for Early Undergraduates. **G3 (Bethesda, Md.)**, v. 9, n. 11, p. 3791–3800, 5 nov. 2019.

ÖZTÜRK-ÇOLAK, Arzu *et al.* FlyBase: updates to the Drosophila genes and genomes database. **Genetics**, v. 227, n. 1, p. iyad211, 1 maio 2024.

PANDEY, Udai Bhan; NICHOLS, Charles D. Human Disease Models in Drosophila melanogaster and the Role of the Fly in Therapeutic Drug Discovery. **Pharmacological Reviews**, v. 63, n. 2, p. 411–436, jun. 2011.

POSGAI, Ryan *et al.* Differential toxicity of silver and titanium dioxide nanoparticles on Drosophila melanogaster development, reproductive effort, and viability: Size, coatings and antioxidants matter. **Chemosphere**, v. 85, n. 1, p. 34–42, set. 2011.

POSPISILIK, J. Andrew *et al.* Drosophila Genome-wide Obesity Screen Reveals Hedgehog as a Determinant of Brown versus White Adipose Cell Fate. **Cell**, v. 140, n. 1, p. 148–160, jan. 2010.

PROKUPEK, Adrianne *et al.* AN EVOLUTIONARY EXPRESSED SEQUENCE TAG ANALYSIS OF DROSOPHILA SPERMATHECA GENES. **Evolution**, v. 62, n. 11, p. 2936–2947, nov. 2008.

RAND, Matthew D. *et al.* Developmental Toxicity Assays Using the *Drosophila* Model. **Current Protocols in Toxicology**, v. 59, n. 1, fev. 2014.

ROBINSON, Mark D.; MCCARTHY, Davis J.; SMYTH, Gordon K. edgeR : a Bioconductor package for differential expression analysis of digital gene expression data. **Bioinformatics**, v. 26, n. 1, p. 139–140, 1 jan. 2010.

SCCP, Scientific Committee on Consumer Products. **THE SCCP'S NOTES OF GUIDANCE FOR THE TESTING OF COSMETIC INGREDIENTS AND THEIR SAFETY EVALUATION 6TH REVISION**. European Commission, , 19 dez. 2006. Disponível em: <https://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_03j.pdf>. Acesso em: 30 maio. 2024

SCHMITT, Rebecca E. *et al.* Convergent Evidence From Humans and Drosophila melanogaster Implicates the Transcription Factor MEF2B /Mef2 in Alcohol Sensitivity. **Alcoholism: Clinical and Experimental Research**, v. 43, n. 9, p. 1872–1886, set. 2019.

SCOTT, Jeffrey G.; BUCHON, Nicolas. Drosophila melanogaster as a powerful tool for studying insect toxicology. **Pesticide Biochemistry and Physiology**, v. 161, p. 95–103, nov. 2019.

SZKLARCZYK, Damian *et al.* The STRING database in 2023: protein–protein association networks and functional enrichment analyses for any sequenced genome of interest. **Nucleic Acids Research**, v. 51, n. D1, p. D638–D646, 6 jan. 2023.

WANI, Mohammad Rafiq; SHADAB, Ggha. Titanium dioxide nanoparticle genotoxicity: A review of recent *in vivo* and *in vitro* studies. **Toxicology and Industrial Health**, v. 36, n. 7, p. 514–530, jul. 2020.

YEH, Johannes T. H. *et al.* eUnaG: a new ligand-inducible fluorescent reporter to detect drug transporter activity in live cells. **Scientific Reports**, v. 7, n. 1, p. 41619, 8 fev. 2017.

ZHANG, Liqin *et al.* lncRNA Sensing of a Viral Suppressor of RNAi Activates Non-canonical Innate Immune Signaling in Drosophila. **Cell Host & Microbe**, v. 27, n. 1, p. 115- 128.e8, jan. 2020.

ZHANG, Xing *et al.* Chronic exposure to titanium dioxide nanoparticles induces deficits of locomotor behavior by disrupting the development of NMJ in Drosophila. **Science of The Total Environment**, v. 888, p. 164076, ago. 2023.

ZHONG, Guocai *et al.* A reversible RNA on-switch that controls gene expression of AAV-delivered therapeutics in vivo. **Nature Biotechnology**, v. 38, n. 2, p. 169–175, fev. 2020.