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Presentation of the levels of Gene Expression of Titanium Dioxide Nanoparticles in Caco-2/HT29-MTX, SAE, and THP-1 cells, using Bioinformatics tools (in silico)

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Abstract

The evolution of studies and learning about the functioning of the human genome made gene expression techniques increasingly explored and applied in recent years' research. There is a need for a deeper understanding of these materials' toxicology in bioinformatics and nanotechnology. Therefore, the present work aims to present the gene expression levels of three studies using three different human cell lines (co-culture of Caco-2/HT29-MTX, SAE, and THP-1 cells), submitted to NPs of carbon dioxide of titanium, via free bioinformatics tools, such as the Limma package (Bioconductor) and Reactome. The data were prepared and standardized by executing the Limma package (Bioconductor), and the results of the most and least expressed genes were used in Reactome. Results show the activation of some genes in common to the experiments, highlighting the co-culture samples of Caco-2/HT29-MTX and THP-1 cells treated with titanium dioxide Nps.

Keywords: Bioinformatics, Metallic nanoparticles, Nanotoxicology, Database.

1. Introduction

Nanotechnology emerged as a promise of rapid advances and significant impacts on future human life. It results from the interaction of quantum physics, molecular biology, electronics, and materials engineering. The new properties that arise from the exploration and manipulation of materials at the atomic and molecular levels are an object of interest in several areas (PYRRHO and SCHRAMM, 2012).

There is considerable investment in research on nanotechnology's technical and scientific aspects,

where some products have already reached the consumer. Nowadays, several fields of activity such as agriculture, food science, biomedicine, pharmaceutical industry, and advanced materials are researched. Morais et al. (2015) cite some examples, such as the creation of stain-resistant fabrics that do not wrinkle; household items with antimicrobial features; scratch- and salinity-proof car paint; fast-penetrating sunscreens; cosmetics with excellent absorption; glass that does not retain water; packaging with controlled release of active compounds; biosensors, composites, microprocessors, and electronic equipment with better performance, among many other possibilities.

It should be noted that nanomaterials, during their manufacturing process by human action, undergo some type of structuring or manipulation at the nanometer level (PYRRHO and SCHRAMM, 2012). Exposure to these nanomaterials to humans and the environment is an object of study in nanotoxicology. Several studies report the toxicity imposed by nanoparticles. However, these are still not enough to accurately assess the toxicity caused due to the diversity and distribution of nanoparticles (KUMAR et al., 2020).

It is necessary to study and apply techniques that help investigate nanotoxicology from the above. One of them is the DNA microarray technique, which is based on the hybridization of nucleic acids, being a system capable of detecting the expression of many genes in parallel (PACHECO et al., 2019). The authors also emphasize that it is possible that certain physiological states can be characterized through patterns of gene expression. It corroborates the study by Reisner (2016), where there is a recognition that gene expression patterns in certain types of lesions, for example, cancer, can influence the clinical behavior of this lesion and its response to therapy.

As the detection of genomic diseases evolves, large databases containing various information regarding studies of metabolic pathways, protein structures, transcriptional bases, among other databases, are available for research. Bioinformatics has brought essential baggage for the in silico analysis of these databases, providing access and agility in the search for information.

This study uses “in silico” tools and free online DNA microarrays databases referring to three human cell lines (co-culture of Caco-2/HT29-MTX, SAE, and THP-1 cells) subjected to carbon dioxide nanoparticles titanium (TiO_2) in order to analyze the gene expression of these strains.

The three cell lines used represent those most likely to be affected by exposure to NPs (TILTON et al., 2014). SAE cells are commercially available human cells and represent the respiratory tract's epithelial cells. THP-1 cells are of human origin and are often used as a macrophage model in toxicology studies (HAMILTON et al., 2012; VERMA et al., 2012; XIA et al., 2013 apud TILTON et al., 2014), referring to acute leukemia. Caco-2 cells are a human colon cancer cell line, dependent on epithelial growth factor (ATCC Number:HTB37[1]) (ALPAY et al., 2019). Co-culture HT29-MTX, a goblet cell line (*in vitro*), is very similar to the intestinal function *in vivo* (WALTER et al., 1996; WIKMAN-LARHED; ARTURSSON, 1995 apud TILTON et al., 2014). Here, in this study, the co-culture of CACO-2/HT29-MX cells, an *in vitro* model of the human intestinal epithelium, was analyzed (DORIER et al., 2019).

Tilton et al. (2014) comment that TiO_2 NPs are considered relatively harmless and are widely used as a component of sunscreens, toothpaste, and cosmetic pigments, but several studies report the toxicity of these NPs, mainly *in vitro* cellular medium. Still, the authors clarify that exposure to NPs brings responses

[1] ATCC:HTB37: https:www.atcc.org/products/all/htb-37.aspx.

that are related to their exposure conditions and protocols used.

2. Methodology

For the present study's analysis, the microarray data from the GSE42069 research were extracted from Geo Datasets. In turn, the study is divided into three types of human cell lines (co-culture of Caco-2/HT29-MTX, SAE, and THP-1 cells) submitted to 10 and 100 μg/mL for 1 and 24h, identified as GSE42066, GSE42067, and GSE42068, respectively. Table 1 describes the studies and their characteristics. Each one initially contains 30 samples related to two types of nanoparticles (carbon nanotubes and titanium dioxide). In this study, only data referring to TiO_2 samples were analyzed.

Table 1. Description of studies related to experiments with TiO_2 in CACO-2/HT29-MTX cells (co-culture), SAE, and THP-1.

Serie	Sample	Cell type	Plataform
GSE42066	18	Caco-2/HT29-MTX *cells*	*[HG-U133A_2] Affymetrix Human Genome U133A 2.0 Array*
GSE42067	18	SAE *cells*	*[HG-U133A_2] Affymetrix Human Genome U133A 2.0 Array*
GSE42068	18	THP-1 *cells*	*[HG-U133A_2] Affymetrix Human Genome U133A 2.0 Array*

Source author's own

2.1 Data preparation

Each study requires a combination of samples for data to be generated; that is, the most and least expressed genes of each sample compared to the control group are listed. All studies contain the control group and the experiment group, where the cell lines were submitted to TiO_2 NPs, at concentrations of 10 and 100 μg/mL between 1 and 24h. Tables 2 to 4 represent the basic information about the combinations between the samples and their respective terminologies.

Table 2. Clustering between samples from control groups and samples from co-culture of CACO-2/HT29-MTX cells subjected to TiO_2 NPs (GSE42066). The control groups were named COU (control referring to the 1-hour experiments group) and COV (control referring to the 24-hour experiments group). The groups of samples with cells were named: CDU (group of cells submitted to 10 μg/mL of TiO_2 NP for 1 hour), CCU (group of cells submitted to 100 μg/mL of TiO_2 NP for 1 hour), CDV (group of cells submitted to 10 μg/mL of TiO_2 NP for 24 hours) and CCV (group of cells submitted to 100 μg/mL of TiO_2 NP for 24 hours).

Combinations	Description
COU x CDU	Control (1 hour group) x Caco-2/ HT29-MTX cells with TiO_2 (10 μg/mL) for 1 hour
COU x CCU	Control (1 hour group) x Caco-2/ HT29-MTX cells with TiO_2 (100 μg/mL) for 1 hour
COV x CDV	Control (24-hour group) x Caco-2/ HT29-MTX cells with TiO_2 (10 μg/mL) for 24 hours
COV x CCV	Control (24-hour group) x Caco-2/ HT29-MTX cells with TiO_2 (100 μg/mL) for 24 hours

Source author's own

Table 3. Clustering between samples from control groups and samples from SAE cells subjected to TiO_2 NPs (GSE42067). The control groups were named COU (control referring to the 1-hour experiments group) and COV (control referring to the 24-hour experiments group). The groups of samples with cells were named as SDU (group of cells subjected to 10 μg/mL of TiO2 NP for 1 hour), SCU (group of cells subjected to 100 μg/mL of TiO_2 NP for 1 hour), SDV (group of cells submitted to 10 μg/mL of TiO_2 NP for 24 hours) and SCV (group of cells submitted to 100 μg/mL of TiO_2 NP for 24 hours).

Combinations	Description
COU x SDU	Control (1 hour group) x SAE cells with TiO_2 (10 μg/mL) for 1 hour
COU x SCU	Control (1 hour group) x SAE cells with TiO_2 (100 μg/mL) for 1 hour
COV x SDV	Control (24-hour group) x SAE cells with TiO_2 (10 μg/mL) for 24 hours
COV x SCV	Control (24-hour group) x SAE cells with TiO_2 (100 μg/mL) for 24 hours

Source author's own

Table 4. Clustering between samples from control groups and samples from THP-1 cells subjected to TiO_2 NPs (GSE42068). The control groups were named COU (control referring to the 1-hour experiments group) and COV (control referring to the 24-hour experiments group). The groups of samples with cells were named as TDU (group of cells submitted to 10 μg/mL of TiO_2 NP for 1 hour), TCU (group of cells submitted to 100 μg/mL of TiO_2 NP for 1 hour), TDV (group of cells submitted to 10 μg/mL of TiO_2 NP for 24 hours) and TCV (group of cells submitted to 100 μg/mL of TiO_2 NP for 24 hours).

Combination	Description
COU x TDU	Control (1 hour group) x THP-1 cells with TiO_2 (10 μg/mL) for 1 hour
COU x TCU	Control (1 hour group) x THP-1 cells with TiO_2 (100 μg/mL) for 1 hour
COV x TDV	Control (24-hour group) x THP-1 cells with TiO_2 (10 μg/mL) for 24 hours
COV x TCV	Control (24-hour group) x THP-1 cells with TiO_2 (100 μg/mL) for 24 hours

Source author's own

2.2 Statistical analysis

After data preparation, the Limma package was executed, considering the necessary adaptations in the algorithms and libraries. This package analyzes linear models for microarray data in control versus

experiment, using significant statistics involving Bayesian and T-Student methods, which compare different groups. After preparation for analysis (normalization), the package produces a result that will be analyzed by the logarithm of the expression change (log_2FC– logarithm of fold-change) to determine the expressed and significantly altered genes (GEO2R, 2021)[2].

According to Rauber, Bugs, and Simão (2015), it is possible to observe the value of the expression change for each gene in a combination by calculating the logarithm of the expression of the cell subjected to an X concentration of a nanoparticle by its respective control (healthy cell or sick):

$$\log_2 FC = \left(\frac{ENP}{N}\right),$$

where ENP is gene expression of the cell sample submitted to an NP (experiment group) and N is the expression of the typical cell sample - healthy or untreated diseased (control group). If the log calculation generates an expression change value for each gene in a combination, it can be analyzed as follows: values close to 0 there is no expression change; values greater than 0, there is a change in expression in the experimental samples and values less than 0, there is a change in expression in the control samples (BAÚ, 2016).

The P-value (p-value) is a statistical measure used to measure the significance level equal to or more extreme than that observed in a sample. This statistical method is used to locate genes significantly expressed in gene expression samples (BAÚ, 2016). The value considered significant must be less than 0.05.

3. Results and Discussion

Each study (GSE) was processed, generating the log_2 fold-change results. The threshold adopted for the analysis of the identified genes was from 1 (value), considering a positive regulation, and less than 1 (value), assuming a negative regulation. The most and least expressed genes were represented in Figures 1 to 5, considering the TOP10.

Figure 1 illustrates the study of cells' most expressed genes (upregulation) from co-culture of Caco-2/HT29-MTX cells subjected to TiO_2 NPs (GSE42066). The genes listed were: *AP3D1*, *CDC14B*, *FAM13A*, *GATAD1*, *KANK1*, *KLHL24*, *MTUS1*, *MYO6*, *PCK1*, *PDPK1*, *PHACTR2*, *RARβ*, *SLC35A3*, *TOP1*, and *TPR*. The sample with the highest number of genes expressed had the highest concentration and exposure time (CCV – 100 μg/mL of TiO_2 NPs for 24h). The *TOP1* and *TPR* genes obtained the highest values of log_2*FC*, equivalent to 4.43 and 5.77 times higher, compared to the control group, respectively. The *FAM13A*, *KLHL24*, *PCK1*, and *KANK1* genes were expressed in the two samples in which the cells were exposed simultaneously (1h) but at different concentrations (10 and 100 μg/mL). The *TOP1* gene was expressed in samples in which cells were also exposed simultaneously (24h) and at different concentrations (10 and 100 μg/mL).

[2] https://www.ncbi.nlm.nih.gov/geo/info/geo2r.html

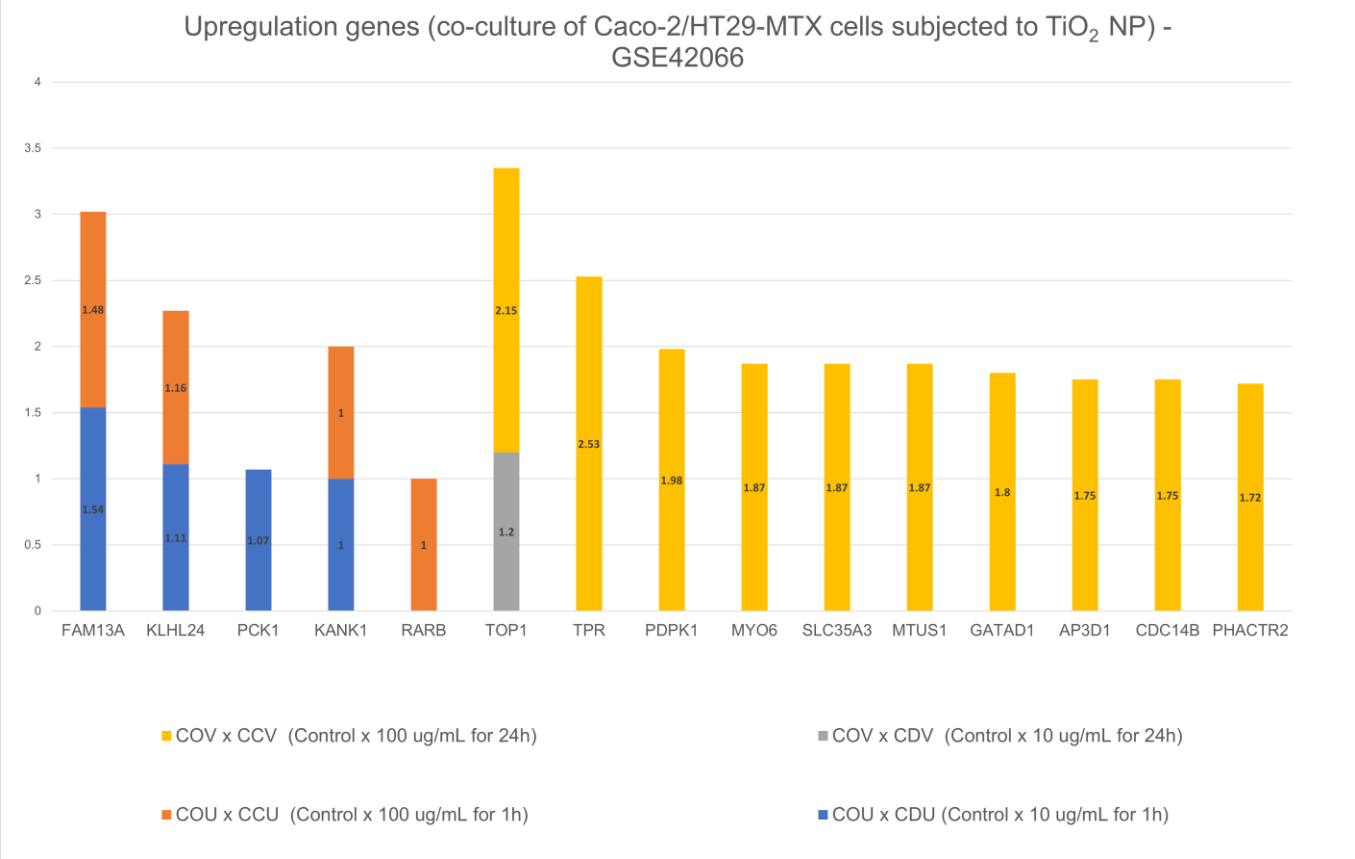

Figure 1. Genes most expressed among all clusters of the GSE42066 study of co-culture of Caco-2/HT29-MTX cells subjected to TiO_2 NPs. The *FAM13A*, *KLHL24*, and *KANK1* genes showed more expressed results in the samples between the control groups (COU) and groups with cells submitted to 10 µg/mL of TiO_2 NPs for 1 hour (CDU) and 100 µg/mL of TiO_2 NPs for 1h (CCU). The *TOP1* gene was more expressed in samples between the control group (COV) and groups with cells submitted to 10 µg/mL of TiO_2 NPs for 24 hours (CDV) and 100 µg/mL of TiO_2 NPs for 24 hours (CCV). Source author's own.

The *TOP1* gene (DNA Topoisomerase I) encodes a DNA topoisomerase, an enzyme that controls and alters the topological states of DNA during transcription. This enzyme catalyzes the transient breakage and reintegration of a single strand of DNA, allowing strands to pass through each other, thus altering the topology of the DNA (REFSEQ, 2008)[3]. It is also known as *TOPI*. The detrimental effect of *TOP1*-induced DNA damage on cell survival has made it a prime target for cancer therapies and a focus on developing potential new treatments for a subtype of autism spectrum disorder (LI and LIU, 2016). In their study, Liu et al. (2018a) present the role of *TOP1* and TOP2A in liver cancer and investigate the inhibitory effect of nitidine chloride on the two topoisomerases. Tests such as Real-Time Polymerase Chain Reaction (RT-qPCR), immunohistochemical staining (IHC), and molecular docking were used to determine the inhibitory effects of nitidine on *TOP1* and *TOP2A*. Two groups of cancer patients were named as high and low risk.

High levels of gene expression were detected in the study, compared to normal liver tissues, with the level of *TOP2A* being much higher than *TOP1* in the high-risk group. As for the treatment with nitidine, the study showed that it reduced the expression of *TOP1* and *TOP2A*, and as for the molecular docking,

[3] RefSeq (Jul/2008): https://www.ncbi.nlm.nih.gov/gene/7150.

there was a direct confirmation of nitidine with the genes. Therefore, the study points to an oncogenic role for genes in liver cancer cases and indicates their potential use as biomarkers to predict the diagnosis of this disease and identify high-risk cases. Furthermore, treatment with nitidine has revealed a possible therapeutic agent for treating liver cancer. Ogino et al. (2020) evaluated in their study the importance of topoisomerases (*TOPs*) expression as prognostic markers of breast cancer. The authors evaluated tumor and non-tumor mRNA levels of TOP1 and *TOP2A* using RT-qPCR, where the expressions of cancerous and non-cancerous tissues were calculated. In the results, of the 60 eligible patients, 46 showed positive expressions of TOP1 and TOP2A; that is, the expression levels of cancerous tissue were higher than normal tissue. As for the 14 patients who presented negative expressions, they did not have recurrent disease. Therefore, the authors suggest that *TOP1*/*TOP2A* expression may be indicators of disease recurrence in operable breast cancer cases, and negative expression cases may have a lower risk of recurrence, bringing an effective method for prognostic accuracy of breast cancer.

The *TPR* gene (Translocated Promoter Region, Nuclear Basket Protein) encodes a sizeable coiled-coil protein (superhelix) that forms intranuclear filaments attached to the inner surface of nuclear pore complexes (NPCs). The protein directly interacts with various components of the NPC. It is necessary for the nuclear export of mRNAs and some proteins. In some neoplasms, oncogenic fusions of the 5' end of this gene with several different kinase genes occur (REFSEQ, 2008)[4].

Figure 2 illustrates the study of less expressed genes (downregulation) of cells from co-culture of CACO-2/HT29-MTX cells subjected to TiO_2 NPs (GSE42066). The genes listed were: *BHLHE40*, *DDIT4*, and *UTS2*.

The *BHLHE40* gene showed the least expressed value for CCU (-1.26 – control x 100 μg/mL of NPs for 1h). Downregulated genes occur due to a process in which mRNA transcription was inhibited; that is, protein synthesis was not completed, and the gene lost expression compared to the control group. The *BHLHE40* gene (Basic Helix-Loop-Helix Family Member e40) is also known as *DEC1*, *HLHB2*, *BHLHB2*, *Clast5*, *SHARP2*, *STRA13*, *Stra14*, and *SHARP-2*. This gene encodes a basic helix-loop-helix protein that is expressed in various tissues. This gene is believed to be involved in circadian rhythm control and cell differentiation (REFSEQ, 2018)[5]. The expression patterns of *BHLHE40* and its impact on tumor development are tumor type-specific; that is, it is upregulated in some and downregulated in others. Some examples: compared to some non-tumor controls, *BHLHE40* expression is upregulated in the thyroid, gastric, breast, and brain tumors, while it is downregulated in colorectal, esophageal, pancreatic, and non-small cell lung tumors. MUDRYJ and GHOSH, 2020). These genes can still have a bimodal function; they can progress or decrease their expression in the same population (MOODY et al., 2019; KISS, Mudryj and GHOSH, 2020).

[4] RefSeq (Jul/2008): https://www.ncbi.nlm.nih.gov/gene/7175.

[5] RefSeq (Mai/2018): https://www.ncbi.nlm.nih.gov/gene/8553.

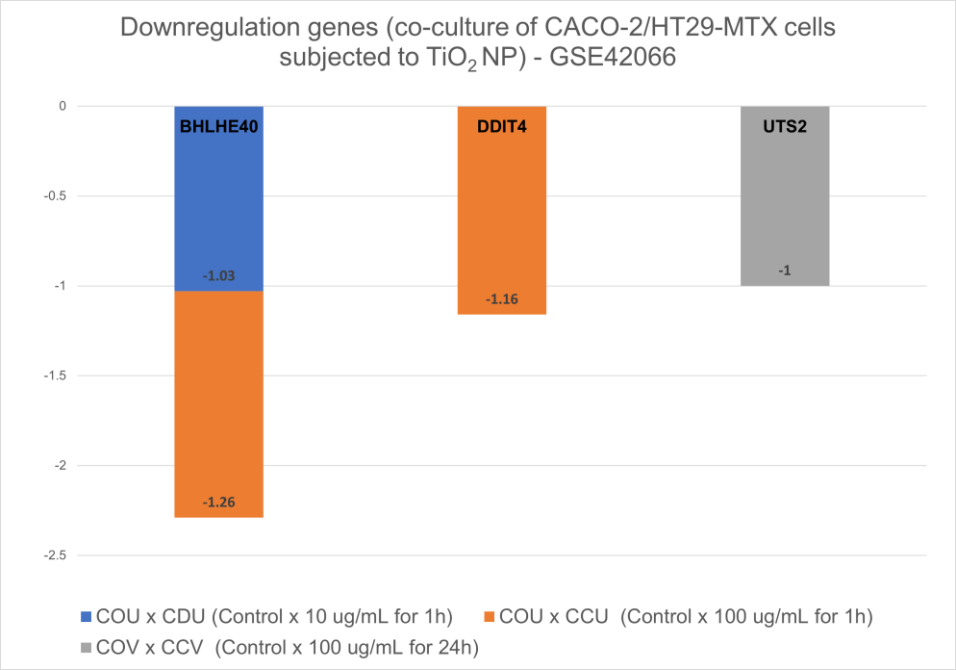

Figure 2. Downregulated genes among all clusters of the GSE42066 study of co-culture of Caco-2/HT29-MTX cells subjected to TiO_2 NPs. The *BHLHE40* gene appeared in two samples where cells were subjected to 10 µg/mL of NPs for 1h (CDU) and 100 µg/mL of NPs for 1 hour (CCU). Source author's own.

Figure 3 illustrates the study of the least expressed genes (downregulated only) in SAE cells subjected to TiO_2 NPs (GSE42067). The genes listed were: *ANKRD1*, *CALD1*, *CDC42BPA*, *CYP1B1*, *DDX17*, *DESI2*, *EGR2*, *EGR3*, *ETV1*, *ETV5*, *FERMT2*, *GBP1*, *GLIPR1*, and *IER3*. The *TOP1* and *DDX17* genes obtained greater sharing between samples. However, it is worth noting that *TOP1* and *GLIPR1* have the lowest values for SCU (-2.12 and -2.55, respectively – control x 100 µg/mL of NPs for 1h).

The *DDX17* gene (DEAD-box helicase 17) is also known as *P72* and *RH70*. This protein is a supposed RNA helicase, which implies a series of cellular processes that alter the RNA secondary structure, such as translation initiation, nuclear and mitochondrial splicing, ribosome, and spliceosome assembly. Some family members are believed to be involved in embryogenesis, spermatogenesis, and cell growth and division (REFSEQ, 2011)[6]. Lin, Cai, and Wang (2020) presented a study to determine the expression of *circDDX17* between tissue samples affected with prostate cancer and unaffected samples and the role of *circDDX17* in metastasis epithelial-mesenchymal transition (TEM) in cancer cells. The results showed that *circDDX17* repressed metastasis and TEM progression in cells affected by prostate cancer, abolishing the effect of miR-346, which acted to increase the expression of *LHPP*. This enzyme protein acts as a tumor suppressor. The researchers suggested that the regulatory network of the *cicDDX17*/*miR-346*/*LHPP* signaling pathway may be a potential prognostic and therapeutic target to aid in the improvement of diagnoses and treatments for prostate cancer. Sun et al. (2021) pointed to the *DDX17* gene as a potential therapeutic target for acquired chemotherapy resistance in patients with gastric cancer. The authors' study analyzed bioinformatics tools' data, which needed to be validated *in vitro* and *in vivo* experiments.

[6] RefSeq (Abr/2011): https://www.ncbi.nlm.nih.gov/gene/10521.

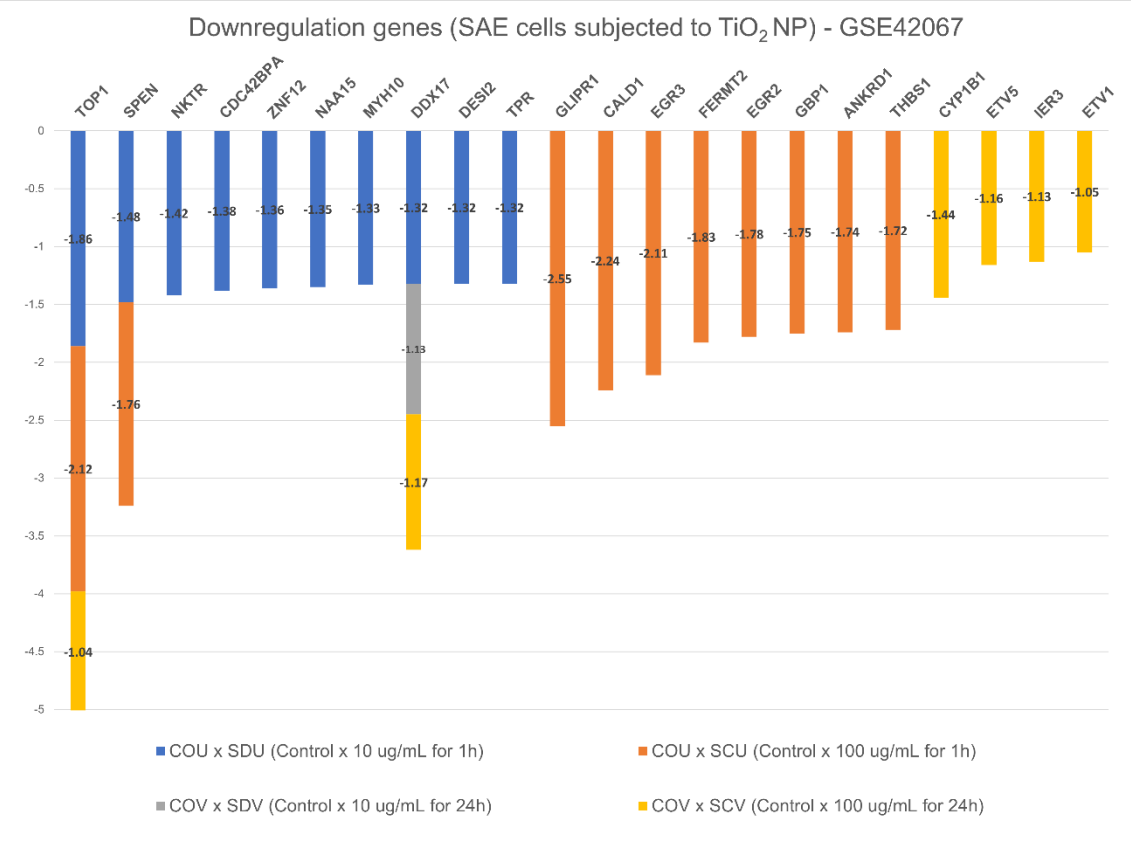

Figure 3. Downregulated genes among all clusters of the GSE42067 study of SAE cells subjected to TiO_2 NPs. The genes that share the most are *TOP1* and *DDX17*, with *GLIPR1* having the lowest log2FC of -2.55 for SCU (control x 100 µg/mL of NPs for 1h). Source author's own.

Figure 4 illustrates the study of the most expressed genes of THP-1 cells submitted to TiO_2 NPs (GSE42068). The genes listed were: *ANKRD11*, *DDX17*, *DICER1*, *EDEM3*, *MTUS1*, *MYO6*, *NKTR*, *PDGFA*, *PTPRC*, *SMC3*, *SON*, *SPEN*, *SRSF6*, and *TOP1*. The *TOP2A*, *TOP1*, *SPEN*, and *ANKRD11* genes had more shares among the samples. As in the first study (Co-culture cells of Caco-2/HT29-MTX cells submitted to TiO_2 NPs), THP-1 cells submitted to the same protocols showed the *TOP1* and *TPR* genes as the most expressed for TCV (2.48 and 2.61, respectively – control x 100 µg/mL of NPs for 24h). Some information about genes has been described earlier.

Figure 4. Upregulation genes expressed among all clusters of the GSE42068 study of THP-1 cells subjected to TiO_2 NPs – TOP10. The *TOP2A*, *TOP1*, *SPEN*, and *ANKRD11* genes had more shares among the samples. Source author's own.

Figure 5 illustrates the study of downregulated genes of THP-1 cells subjected to TiO_2 NPs (GSE42068). The genes listed were: *CCL4*, *CCR7*, *CXCL1*, *CXCL2*, *CXCL3*, *NR4A2*, *NR4A3*, *PTGS2*, *TNFAIP6*, and *ZBTB10*. Only the TCV sample (control x 100 µg/mL of NPs for 24h) showed downregulated genes, with *CCR7* having the lowest value (-3.34), representing 10.12 times lower than the standard control.

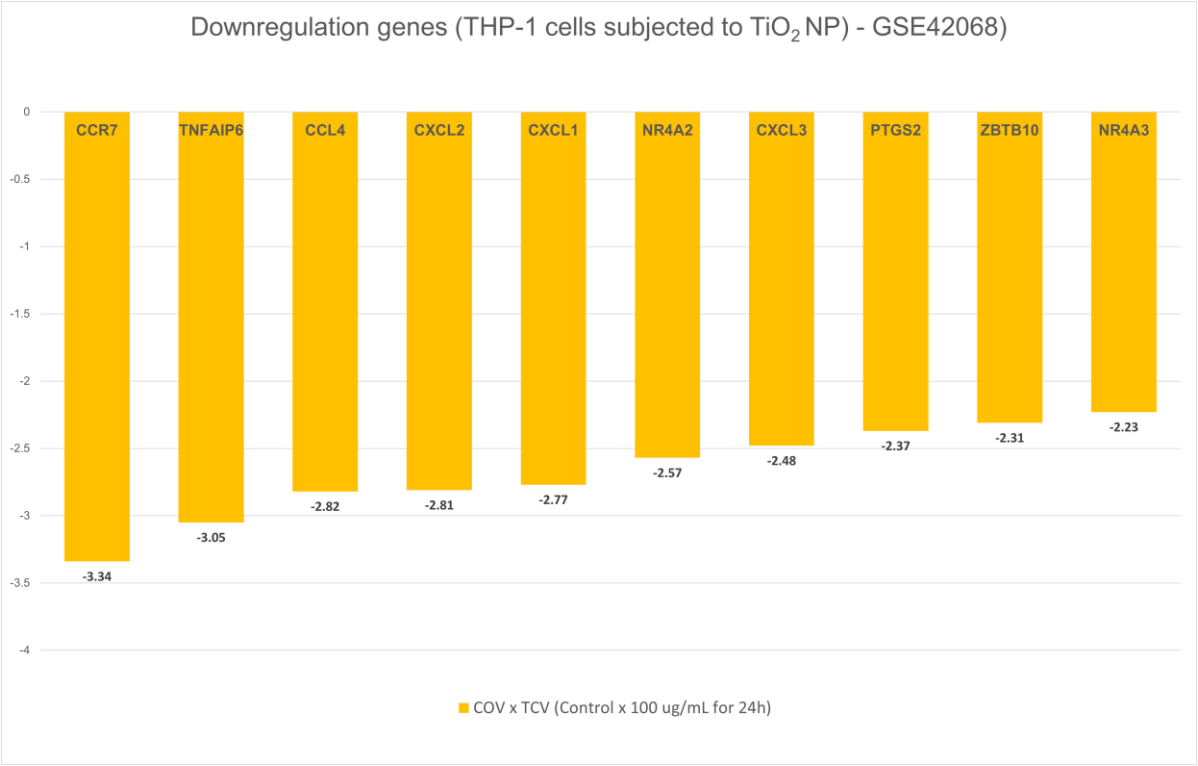

Figure 5. Downregulated genes among all clusters of the GSE42068 study of THP-1 cells subjected to TiO_2 NPs. Only the TCV sample (control x 100 µg/mL of NPs for 24h) showed the values. Source author's own.

The *CCR7* gene (C-C Motif Chemokine Receptor 7) is also known as *BLR2*, *EBI1*, *CCR*-7, *CD197*, *CDw197*, *CMKBR7*, and *CC-CKR-7*. The protein encoded by this gene is a G protein-coupled receptor family member. The receptor, identified as a gene induced by the Epstein-Barr virus (EBV), is considered a mediator of the effects of this virus on B lymphocytes; it is expressed in various lymphoid tissues and activates B and T lymphocytes; controls the migration of memory T cells to inflamed tissues, as well as stimulates the maturation of dendritic cells (REFSEQ, 2014)[7]. Brandum et al. (2021) present a review where they discuss the role of dendritic cells (DCs) and the central chemokine receptor *CCR7* in the initiation and maintenance of chronic inflammatory diseases, such as multiple sclerosis (MS), rheumatoid arthritis (RA), and psoriasis. The researchers conclude that the *CCR7* gene is a possible target for future drugs that can reduce inflammation in chronic inflammatory diseases and boost the reactivation of the immune system against cancer. The *CCR7* gene is also a potential marker for the prognosis of idiopathic pulmonary fibrosis (IPF) (LU et al., 2021).

Table 5 shows the number of genes (TOP10) up-and-down-regulated in descending order. Samples belonging to the co-culture of Caco-2/HT29-MTX cells subjected to 100 µg/mL of TiO_2 NPs for 24 hours (CCV+) and THP-1 cells subjected to 100 µg/mL of TiO_2 NPs for 24 h (TCV+) presented the highest number of positively expressed genes. In absolute values, the TCV+ sample showed the genes with the most expressed values, for *TPR* and *TOP1*, with log_2FC of 2.61 and 2.48, respectively.

[7] RefSeq (Set/2014): https://www.ncbi.nlm.nih.gov/gene/1236.

Table 5. Decreasing ratio of the number of genes represented in each sample, up- and down-regulated.

Upregulated samples and genes		Downregulated samples and genes	
CCV+	10	SDU-	10
TCV+	10	SDU-	10
TDV+	6	SCU-	10
CDU+	4	SCV-	6
CCU+	4	CCU-	2
TCU+	3	CDU-	1
CDV+	1	CCV-	1
TDU+	1	SDV-	1

Source author's own

Table 6 summarizes the typical behavior among the genes. The *TOP1*, *SPEN*, *DDX17*, and *TPR* genes showed the highest sharing among the identified samples.

Table 6. Genes and their typical samples. The highest number of shares: *TOP1*, *SPEN*, *DDX17*, and *TPR*.

Genes	Samples
ANKDR11	TCV+ e TDV+
BHLHE40	CDU- e CCU-
DDX17	TDV+, SDU-, SDV- e SCV-
FAM13A	CCU+ e CDU+
KANK1	CCU+ e CDU+
KLHL24	CCU+ e CDU+
MTUS1	TCV+ e CCV+
MYO6	TCV+ e CCV+
NKTR	TCV+ e SDU-
SPEN	TCV+, TDV+, SDU- e SCU-
TOP1	TCV+, TDV+, CCV+, CDV+, SDU-, SCU- e SCV-
TOP2A	TCU+ e TDU+
TPR	TCV+, CCV+ E SDU-

Source author's own

It can be seen that tumor cells (Caco-2 and THP-1) had the highest number of genes shared between them and their highest upregulated expression levels. The exposure time and PN distribution are factors to be considered. As for healthy cells (SAE), they only obtained the expression of their negatively regulated genes in all samples, mainly in SCU (control x 100 μg/mL of NPs for 1h). There must be an investigation into this behavior.

4. Final Considerations

The three studies presented, with different cell lines and, following the same protocols and procedures of concentration of the same type of NP, and the exposure time, were decisive for the initial analyses to be performed. One of the challenges for applying bioinformatics related to microarrays is the scarcity of resources with these characteristics.

The application of bioinformatics in this work occurred through the processing of algorithms already consolidated in the literature and, consequently, adapted to the characteristics of the studies carried out. The most and least expressed genes were initially observed by the *log_2 fold-change*, carrying out literary research on the genes and identifying the biological pathways.

When analyzing the behavior of the samples, the tumor cells (Caco-2 and THP-1) had the highest number of genes shared between them and their highest upregulated expression levels. The exposure time and PN distribution are factors to be considered. As for healthy cells (SAE), they only obtained the expression of their negatively regulated genes.

There must be an investigation into this behavior, as positive and negative regulation is directly linked to the function of the gene and its correlates.

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