Transcriptome-based prediction of the toxicity of the marine biotoxin okadaic acid in human vascular endothelial cells

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ABSTRACTS

Okadaic acid (OA), a marine toxin generated by dinoflagellates, poses considerable health risks to humans by causing diarrhetic shellfish poisoning through the consumption of contaminated bivalve mollusks. This study examined the cytotoxic impact of OA on the EA.hy926 human vascular endothelial cell line. MTT assays revealed dose-dependent inhibition of cell viability. Transcriptomic analysis based on RNA-seq revealed significant differential gene expression associated with disrupted cell growth, proliferation, DNA damage, angiogenesis, and DNA replication via GO and KEGG pathway enrichment analyses. In conjunction with the findings of ingenuity pathway analysis, the activation of inflammatory pathways, including the osteoarthritis and cachexia signaling pathways, is highlighted. Further validation via RT-qPCR analysis confirmed the upregulation of cell cycle-related genes (*CDKN2D*, *CDKN1A*, *SOD2*, *SIRT1*, and *PLK1*). Furthermore, flow cytometry showed an increase in the number of cells in the G2/M- and S-phases, suggesting cell cycle arrest. These findings indicate that exposure to OA leads to inflammation, cell cycle disruption, and impaired DNA repair mechanisms in vascular endothelial cells. Our findings highlight the toxicological impacts of OA and emphasize the need for stringent monitoring and regulatory measures to mitigate the health risks associated with marine biotoxins, particularly in the context of ongoing climate change.

INTRODUCTION

The warming of oceanic waters due to climate change has increased the frequency of harmful algal blooms, leading to the production of marine biotoxins like okadaic acid (OA) that accumulate in bivalve mollusks and pose a significant threat to human health through seafood consumption. OA, produced by dinoflagellate algae, is the primary cause of diarrheal shellfish poisoning and exhibits a range of toxic effects, including cytotoxic, neurotoxic, immunotoxic, hepatotoxic, and genotoxic. These effects highlight the need for comprehensive toxicological studies.

OA inhibits protein serine/threonine phosphatases, leading to growth inhibition or cell death in various cell types, but its impact on vascular endothelial cells remains unexplored. Endothelial cells are crucial in maintaining vascular function, and their dysfunction can result in oxidative stress, inflammation, and acute arterial thrombosis. This study focused on the EA.hy926 human vascular endothelial cell line, conducting transcriptome profiling to assess OA's effects on these cells and identify differentially expressed genes. Through bioinformatics analyses, including Gene Ontology classification, KEGG pathway mapping, and Ingenuity Pathway Analysis, the study identified several biological processes regulated by OA, offering new insights into its toxic effects on vascular endothelial cells.

RESULTS

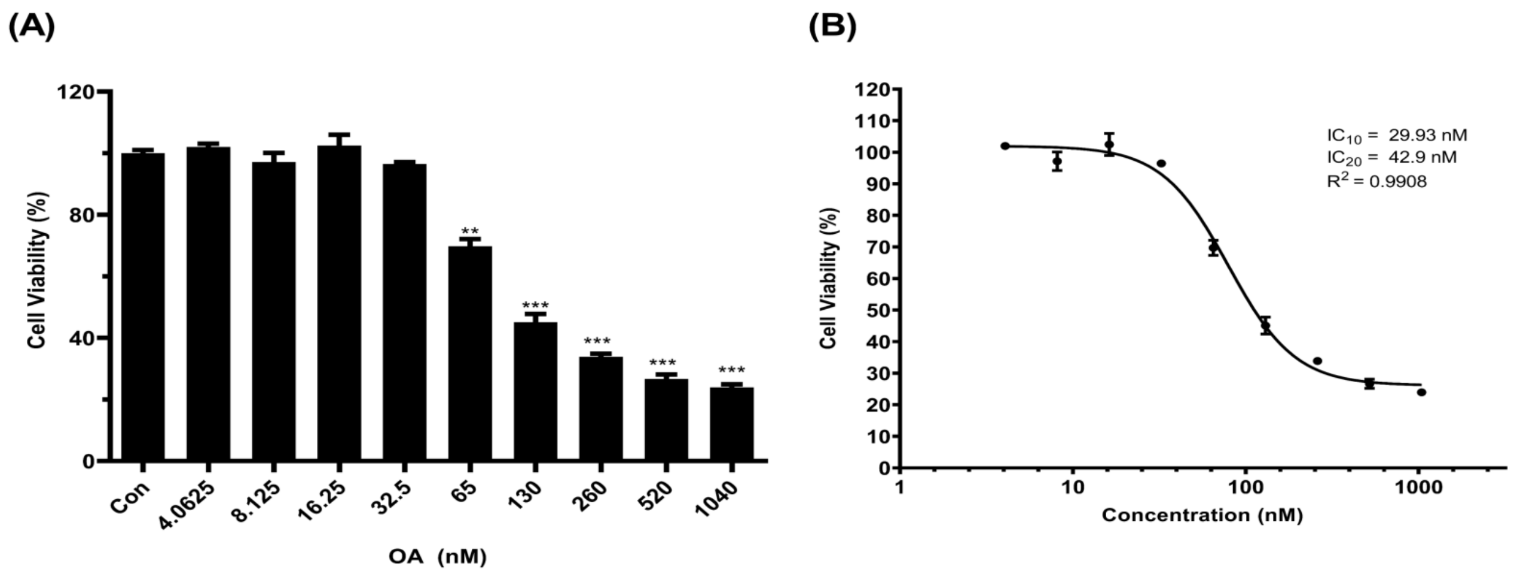

Fig. 1. Cytotoxicity of OA in EA.hy926 cells. The viability of EA.hy926 cells was determined using an MTT assay after 24 h exposure to OA. Values represent the mean ± SD (n = 5). The *p* values are determined using one-way ANOVA with Tukey's multiple comparisons. ** p < 0.01, and *** p < 0.001 compared with control.

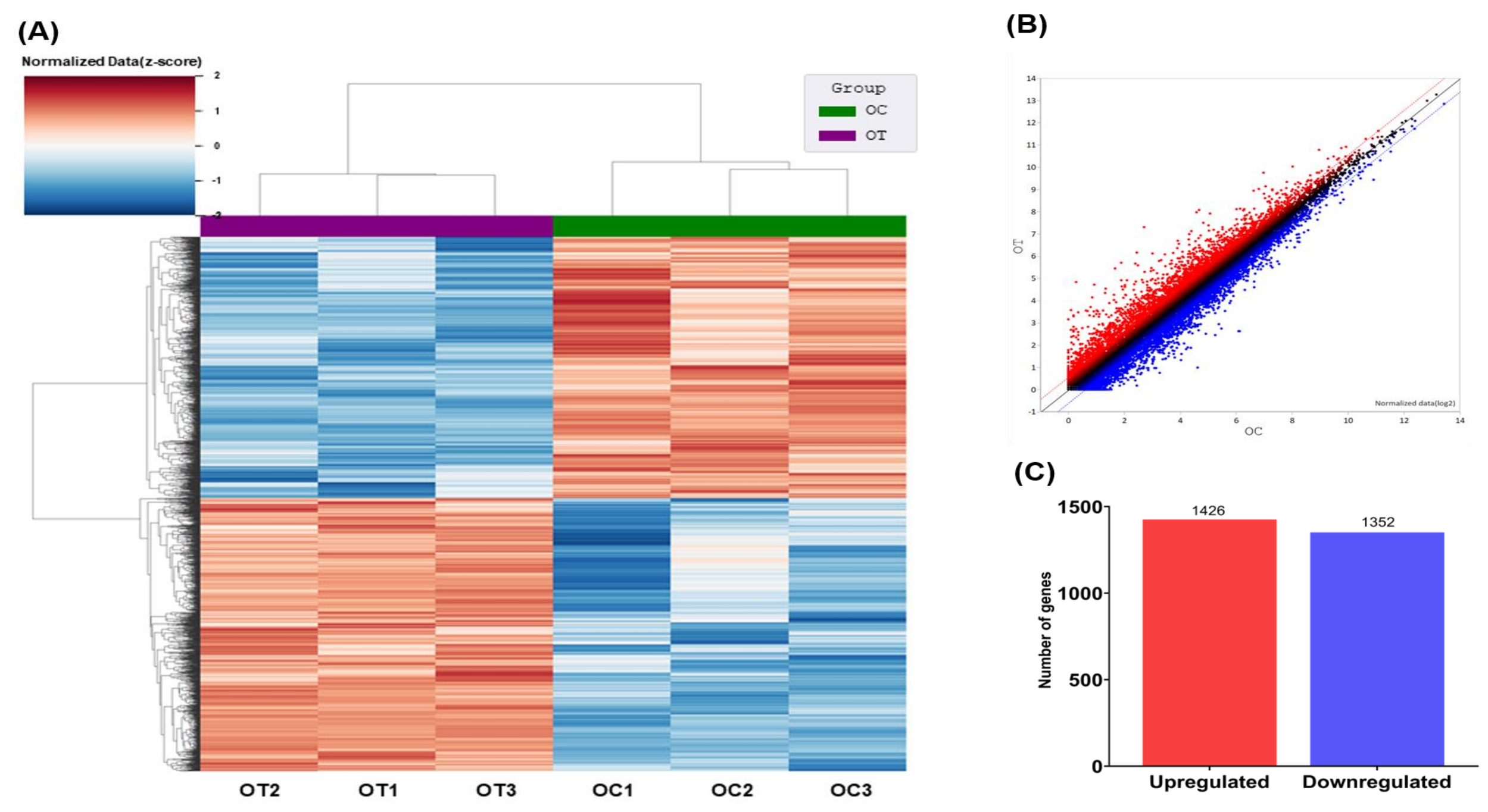

Fig. 2. RNA-seq profile of DEGs (fold change ≥ 1.5, p value < 0.05) in EA.hy926 cell cell line. (a) Hierarchical clustering heatmap of DEGs in EA.hy926 cells exposed to OA. Gene expression was shown as normalized data (z-score). The red denotes genes with high expression levels, and the blue denotes genes with low expression levels. (b) Scatter plots of EA.hy926 cell exposed to OA. The red dots indicate upregulated genes and the green dots indicate downregulated genes. (c) The number of upregulated and downregulated DEGs in EA.hy926 cells exposed to OA.

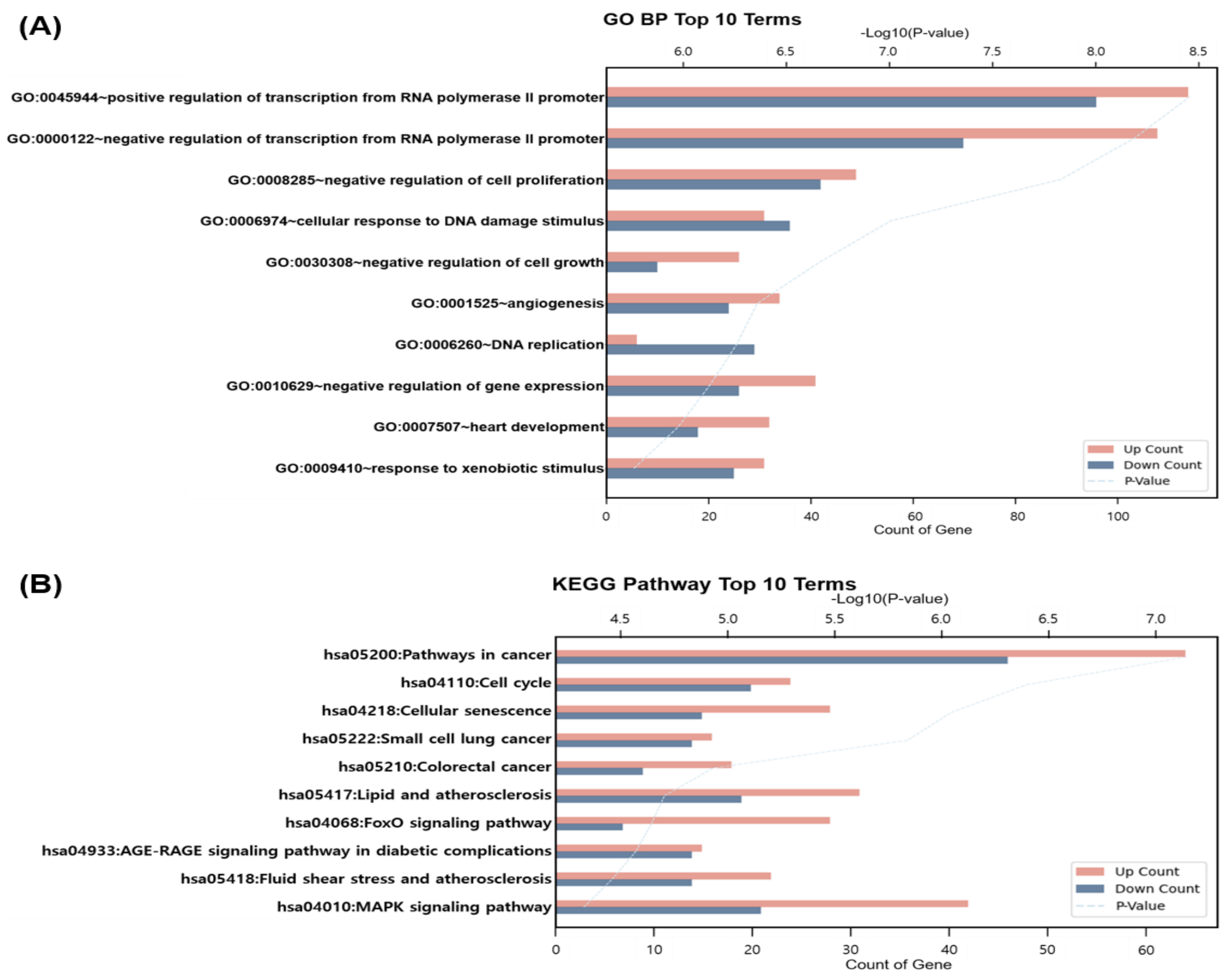

Fig. 3. The top 10 most significant (*p* value < 0.05) (A) GO BP and (B) KEGG pathway enrichment analysis of DEGs in EA.hy926 cells exposed to OA. The dotted line represents the –Log10 (*p* value) of the classification.

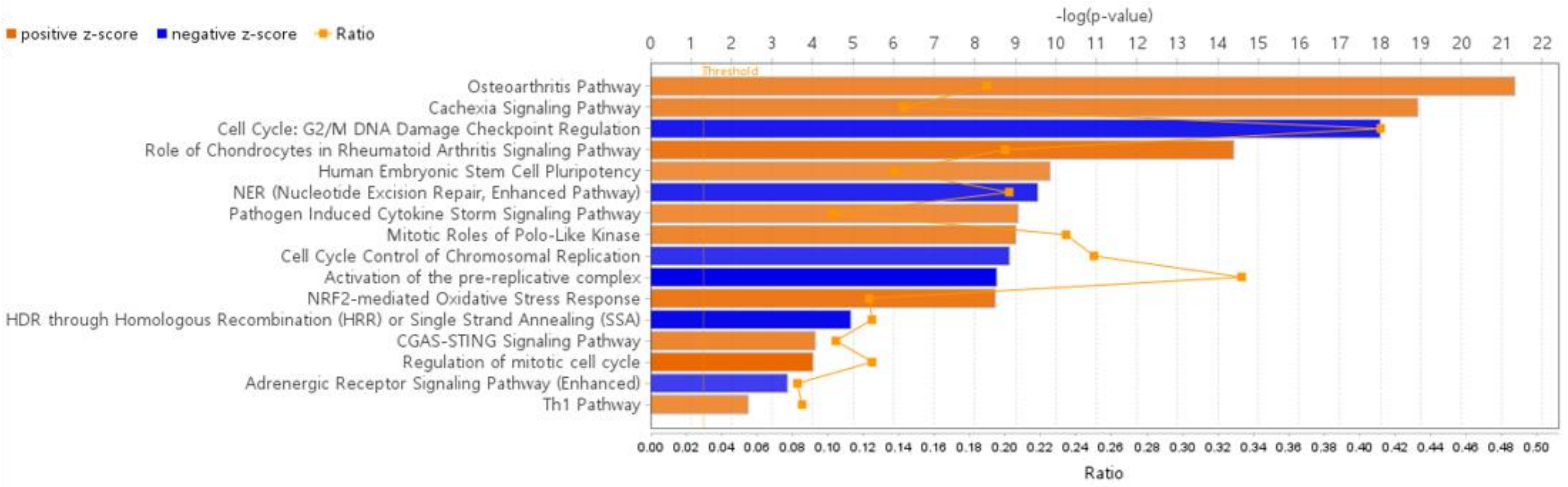

Fig. 4. Canonical pathway through IPA analysis of 680 DEGs identified from the GO BP and KEGG pathway enrichment analyses. The results were sorted by –log p value (p value < 0.05) and an activation absolute z-score greater than 2.5. Orange and blue bars indicate the predicted pathway activation or inhibition, respectively. The orange line connecting square points in each bar indicates the ratio of DEGs to the total molecules in the pathway.

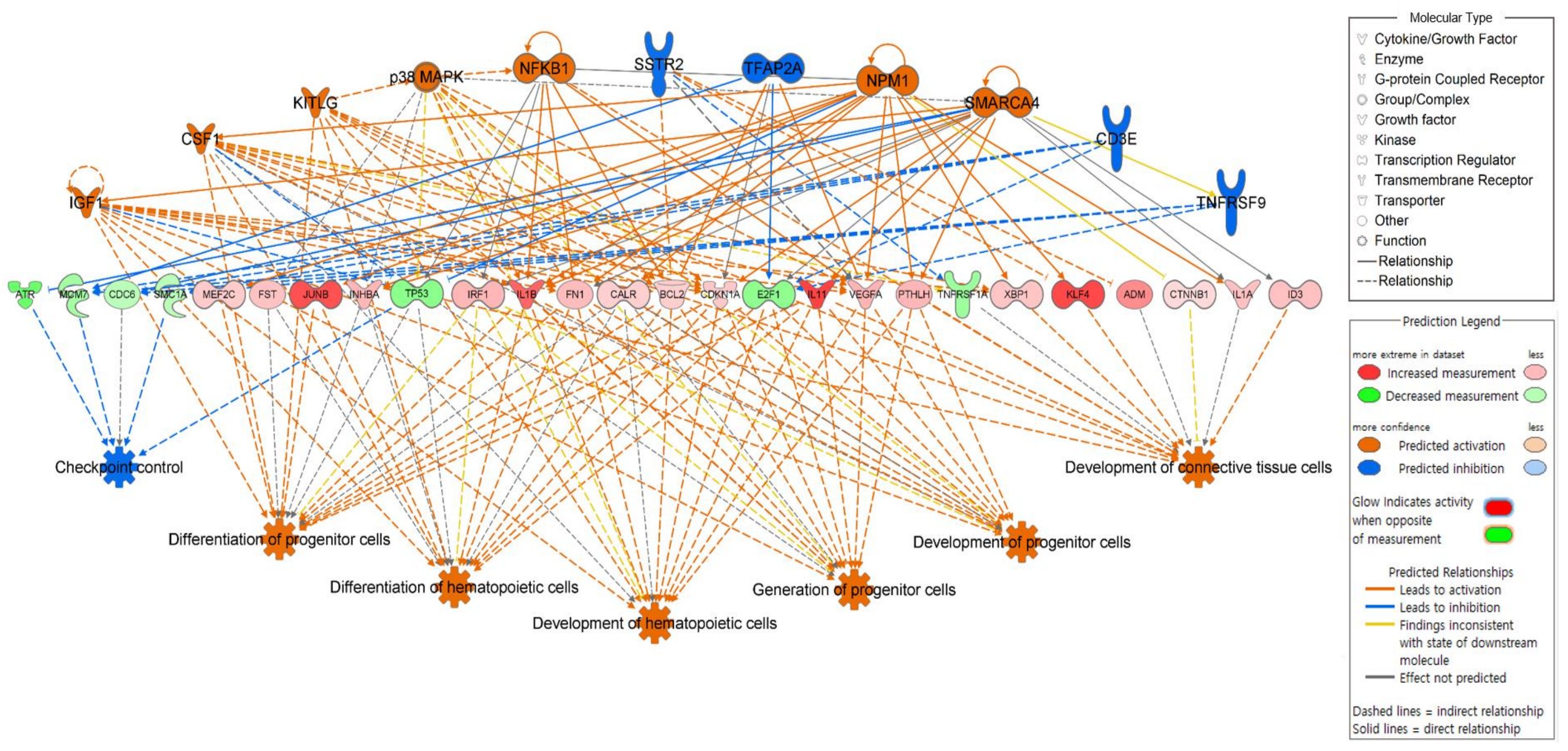

Fig. 5. Top regulator effect networks. This figure identifies which Upstream Regulator networks have the most consistent causal relationships (consistency score = 26.083) with specific Downstream Effects networks using IPA. Following OA treatment, it is hypothesized that the activation of seven upstream regulators and the inhibition of four upstream regulators drive expression changes in the DEGs (shown in the middle tier), leading to decreased checkpoint control and increased differentiation of progenitor cells, differentiation of hematopoietic cells, development of hematopoietic cells, generation of progenitor cells, development of progenitor cells, and development of connective tissue cells.

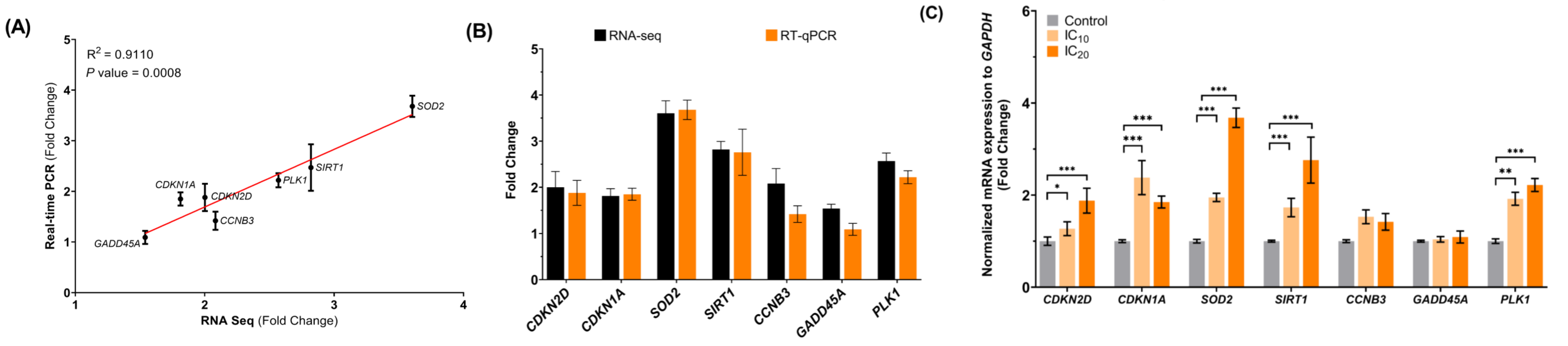

Fig. 6. (a) Correlation between the RT-qPCR and RNA-seq results. Correlation between differential expression inferred by bioinformatics predictions (Fold change by RNA-seq) and RT-qPCR analysis (Fold change by RT-qPCR). (b) Validation of the seven DEGs from RNA-Seq analysis by RT-qPCR. The direction and magnitude of the fold changes obtained using the qRT-PCR (orange bars) were similar to those of the RNA-Seq (black bars). (c) Relative quantification of selected DEGs in EA.hy926 cells exposed to IC10 and IC20 of OA. The data shows the average fold change in the experimental group (mean ± SE, n = 3). Each bar represents the mean. *p < 0.05, **p < 0.01 and ***p < 0.001 compared with control.

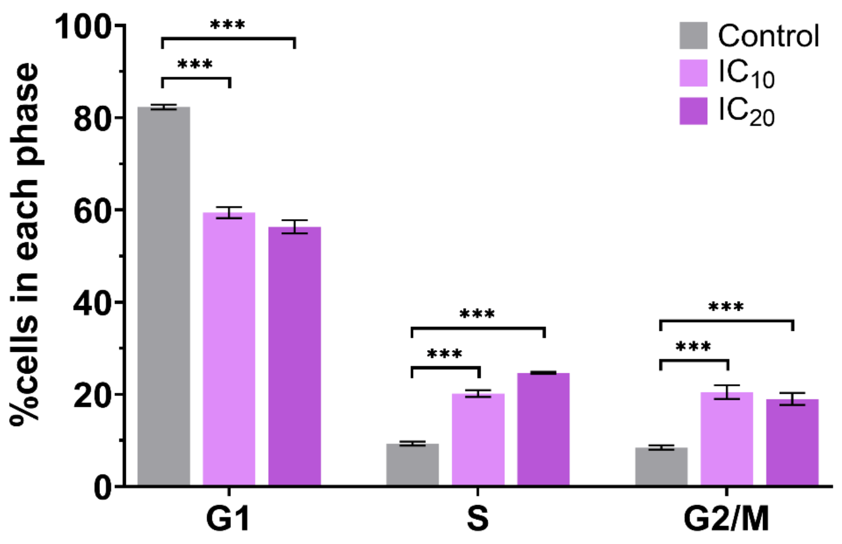

Fig. 7. Cell cycle distribution analysis of EA.hy926 cells treated with OA using flow cytometry. (a) Flow cytometry analysis of the cell cycle distribution of the cells with PI staining. (b) The bar graph shows the percentage of cells in the G1, S, and G2/M phases. Data are presented as mean ± SD; n = 3, ***p < 0.001 compared with control.

CONCLUSION

Our findings in this study provide novel insights into the toxic effects of OA on vascular endothelial cells. On the basis of our analyses of DEGs and pathways, we established that OA induces a strong inflammatory response and inhibits cell cycle progression in vascular endothelial cells, thereby impairing DNA repair mechanisms and potentially reducing the ability of cells to counter the detrimental effects of DNA damage. These findings are consistent with those reported previously, indicating that OA can exacerbate inflammatory conditions and interfere with cellular repair mechanisms. Additionally, we identified the potential role of OA in vascular inflammatory diseases such as osteoarthritis, rheumatoid arthritis, and cachexia. Collectively, our findings highlight the pronounced toxicological effects of OA on vascular endothelial cells and provide valuable insights into its potential mechanisms of action. These findings emphasize the importance of further research into the molecular pathways affected by OA, which could contribute to the development of strategies for mitigating the harmful effects of OA on human health, particularly in the context of an increasing likelihood of exposure to marine biotoxins as a consequence of climate change. Further studies should seek to establish the long-term effects of OA exposure and potential therapeutic interventions to counteract these deleterious effects.