GMR
The Original

MOLECULAR PROFILING OF SIGNAL TRANSDUCTION ALTERATIONS IN DISEASE STATES

Dr. Dhanalakshmi S[1], Eswar S[2], Dr. N. Kannan[3]

[1.] Professor, Pharmacognosy Meenakshi College of Pharmacy, Meenakshi Academy of Higher Education and Research, India, Email: sdhanalakshmi@maher.ac.in
[2.] Assistant Professor Meenakshi College of Allied Health Sciences, Meenakshi Medical College Hospital & Research Institute, Meenakshi Academy of Higher Education and Research, India, Email: eswarsahs@maher.ac.in
[3.] Associate Professor, Anaesthesiology Sree Balaji Medical College and Hospital, Bharath Institute of Higher Education and Research, India, ORCID: https://orcid.org/0000-0002-5273-0166

ABSTRACT

Signal transduction pathways are essential in controlling cellular functions including proliferation, survival, differentiation and stress response. These pathways are dysregulated in a characteristic of many disease conditions, such as cancer, metabolic disorders, and inflammatory conditions. The molecular changes within signaling networks are important to understand to determine key disease progression drivers and potential therapeutic targets. The current research is expected to systematically profile signal transduction changes and elicit important regulatory elements in disease-linked molecular malfunction.

It used a detailed analytical method, which combined the use of gene expression profiling and protein-level signaling analysis. The differentially expressed genes were detected by RNA sequencing (RNA-seq) and RT-qPCR, and the activation status of the main signaling proteins was measured by the Western blot analysis. Analysis of functional enrichment with Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) data sets was performed with the aim of identifying signaling pathways significantly changed.

The outcomes showed there was a notable dysregulation of key signaling pathways, such as PI3K-Akt, MAPK, NF-kB, and p53. Third, a number of signaling molecules were differentially expressed showing an upregulation of survival and proliferation-associated and downregulation regulatory and apoptotic signaling. Analysis of the network revealed that these pathways are extensively networked and also play a role in the development of diseases.

Conclusively, the paper indicates that the role of disease pathogenesis is played by disruption of signaling networks and not solitary pathway changes. The discovery of important regulatory pathways and hub signaling molecules offers meaningful information into the molecular pathways of disease states and has arbitered where therapeutic intervention could be directed.

KEYWORDS: Signal transduction, PI3K-Akt pathway, MAPK signaling, NF-KB, p53 pathway, molecular profiling, pathway enrichment, disease mechanisms, cellular signaling networks.

1. INTRODUCTION

Signal transduction is a basic biologic process by which cells sense and react to internal and external signals by transforming biochemical signals into particular cellular actions. These signaling pathways control vital cellular functions such as proliferation, differentiation, survival, apoptosis and stress response. Signal transduction pathways have to operate properly to ensure cellular homeostasis, and their disruption may cause the emergence of a variety of diseases (Allen et al., 2014).

The survival of the cell and adaptation to stress are regulated through the interplay of signaling pathways that include phosphoinositide 3-kinase/protein kinase B (PI3K-Akt), mitogen-activated protein kinase (MAPK), nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB), and p53 pathways. Previous reports have shown that Akt stimulation by insulin and growth factor signaling is a key contributor to cell survival and metabolism (Alessi et al., 1996; Alessi et al., 1997). In the same way, transcriptional regulation mediated by p53 is critical to regulating cell cycle arrest and cell death (Allen et al., 2014). The integrated action of these pathways is required to achieve a balanced response of cells and can result in the uncontrolled increase in the number of cells, inflammation, and the development of diseases in case of disconnection.

Maladaptation in signaling pathways has been implicated strongly in the diseases cancer, inflammatory disorders, and neural diseases. Increased PI3K-Akt and MAPK signaling leads to increased tumor growth and resistance to apoptosis, and compromised p53 signaling results in genomic instability and cancer onset (Ablain et al., 2014; Alexandrov et al.,

2016). Continuous stimulation of inflammatory signaling pathways, such as NF-KB is also associated with extreme inflammatory conditions like sepsis (Cohen et al., 2015; Liu et al., 2014). Moreover, signaling mechanisms have been suggested to be changed with synaptic malfunction and psychiatric conditions, indicating the expanded role of signaling maladjustment in human illness (Abdallah et al., 2015).

The recent developments in omics technologies and massive genomic initiatives have now made it possible to analyse signaling pathways at the system level. Other projects, like the Genotype-Tissue Expression (GTEx) one, have given an insightful picture of how genes regulate tissues (GTEx Consortium et al., 2015). Moreover, combined multi-omics analyses have enhanced the discovery of disease-related molecular signatures and pathway modifications (Das et al., 2020). Nevertheless, there are still difficulties with assimilating heterogeneous datasets and understanding sophisticated molecular interactions, which is especially evident in multi-omics studies (Tarazona et al., 2021).

Despite the major advances in the study of individual signaling pathways, research continues to concentrate on individual molecular parts instead of the network behavior of signaling systems. This constraint decreases the capability to gain a complete picture of cross-talk of pathways and global signaling dynamics in the course of disease progression. The current research thus seeks to conduct a combined molecular profiling of signal transduction changes in disease conditions. Using a combination of gene expression, pathway enrichment, and network-based methods, this research aims to determine key regulatory pathways and molecular interplay in the context of disease etiology and potential future intervention points.

2. RELATED WORK

Signal transduction pathways have been a subject of wide study due to their important role in controlling cell activities and pathogenesis. Initial mechanistic research on the activation of protein kinase B (Akt) provided the molecular basis to the PI3K-Akt signaling pathway and showed the role of insulin and growth factor signaling in the regulation of cellular survival and metabolism (Alessi et al., 1996; Alessi et al., 1997). The significance of p53-mediated transcriptional regulation in the stabilization of genome and regulation of apoptosis was also emphasized by the research on tumor suppressor pathways (Allen et al., 2014). Moreover, research on the promyelocytic leukemia-p53 axis highlighted the importance of signaling interactions to therapeutic responses and cancer progression (Ablain et al., 2014).

Recent developments in transcriptomic and genomic technologies have greatly enhanced the capability to study signaling networks at the systems level. Genotype-Tissue Expression (GTEx) project and large-scale projects served as a source of sufficient information on the patterns of gene regulation and expression in tissues (GTEx Consortium et al., 2015). Combination of multi-omics methods have also facilitated the detection of disease-related molecular signatures and pathway dysregulation in cancer and other diseases (Das et al., 2020). Moreover, the mutational signature analysis demonstrated the role of environmental and molecular factors in signaling abnormalities in the process of cancer development (Alexandrov et al., 2016). Regardless of these advancements, combing heterogeneous omics data and deriving biologically significant insights continue to be key challenges to multi-omics research (Tarazona et al., 2021).

The role of signaling dysregulation in both inflammatory and neurological diseases has also been studied in a number of studies. Sepsis studies have shown that the dysregulation of immune signaling pathways is an important factor in disease severity and mortality (Cohen et al., 2015; Liu et al., 2014). In neurology, the studies on the use of ketamine as a treatment option gave a new understanding of the ketamine signaling that may be involved in the process of synaptic plasticity and mood regulation (Abdallah et al., 2015). Moreover, new treatment methods, including engineered extracellular vesicles, have demonstrated prospects in treating disease by targeting dysregulated signaling pathways (Ma et al., 2025).

Although significant advances have been made in the knowledge on the signaling mechanisms of an individual, the majority of current literature considers separate pathways or in disease states. This compartmentalized practice restrains the in-depth knowledge of interactions of signaling networks and cross-talk between pathways during disease progression. As such, this research seeks to fill this gap by combining gene expression profiling, pathway enrichment analysis, and molecular network analysis to gain a system-wide insight into the changes in signal transduction during disease states.

3. MATERIALS AND METHODS

3.1 Data Acquisition and Sample Selection

The transcriptomic and protein-level data were used to explore changes in signal transduction in disease states. Available datasets were obtained publicly and included repositories like The Cancer Genome Atlas (TCGA) and the Gene Expression Omnibus (GEO), which included both disease and control samples. Selection criteria consisted of

the completeness of the data and the presence of both gene expression and clinical metadata, as well as being relevant to signaling pathway dysregulation.

Besides the publicly available datasets, experimental validation was conducted with the use of the established human cell lines, both of disease-representative cells and normal control cells. To replicate the conditions associated with the disease, the cells were grown under the usual laboratory conditions and exposed to controlled treatments. This integrated strategy guaranteed the extensive data integration as well as the test of signaling changes.

Table 1: Dataset and Sample Details

Dataset Source	Sample Type	Number of Samples	Description
TCGA	Disease	312	Tumor samples
GEO	Control	58	Normal samples
Cell Lines	Experimental	6	Validation experiments

3.2 Molecular Profiling

RNA sequencing (RNA-seq) was used to perform transcriptomic profiling, and reverse transcription quantitative polymerase chain reaction (RT-qPCR) to validate. Raw RNA-seq data were quality controlled to eliminate low-quality reads and sequencing artifacts, then aligned to the reference human genome using standard tools, like HISAT2 or STAR. The abundance of gene expression was measured and downstream analysis normalized.

Western blotting was conducted to analyze protein level signalling analysis to determine the activation status of key signaling molecules. The activation of pathways was specifically examined through phosphorylation of proteins such as p-AKT, p-ERK and NF-. Standardized procedures were used to extract proteins, quantify and run them in electrophoresis and transfer them to membranes, and then to detect them with chemiluminescence.

3.3 Differential Expression Analysis

To determine the significantly up regulated and down regulated genes between disease and control samples, differential gene expression analysis was carried out. To analyze the statistical significance, they employed statistical tools like DESeq2, and used a threshold of |human| of log 2 fold change > 1 and adjusted p-value < 0.05 to identify significance.

Genes that fit these parameters were divided into upregulated and downregulated, which are the activation or inhibition of signaling pathways. This comparison formed the foundation of detection of important molecular changes linked to the development of diseases.

3.4 Pathway Enrichment Analysis

To understand the biological meaning of the differentially expressed genes, the functional enrichment analysis was performed with the help of the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases. GO analysis revealed the enriched biological processes, molecular functions, and cellular components, and KEGG pathway analysis identified major signaling pathways in disease states.

A special focus was on the pathways including PI3K-Akt signaling, MAPK signaling and apoptosis-related ones that are known to be critical in cellular regulation. The statistical significance and enrichment scores were determined in order to select the most pertinent pathways that lead to dysregulation of signaling.

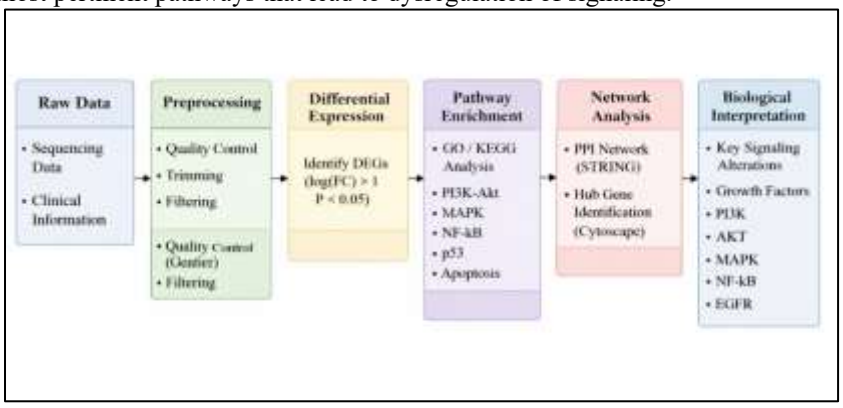

Fig 1: Signaling Analysis Pipeline

3.5 Network Analysis

To determine central regulatory hubs in the signaling network, protein protein interaction (PPI) network analysis was carried out. The data on the interactions were retrieved in the STRING database and visualized with Cytoscape software. The degree centrality and betweenness centrality parameters were applied to detect the hub genes that are high connectivity and regulatory nodes in the network.

The hub genes were believed to be some of the main drivers of changing the signaling since they affect various downstream processes. Transcriptomics and network-level data integration allowed identifying key nodes of the signaling system that play a role in disease mechanisms.

Table 2: Analytical Methods and Tools

Step	Method	Tool/Software	Purpose
RNA-seq QC	Preprocessing	FastQC	Data cleaning
Alignment	Mapping	HISAT2/STAR	Genome alignment
DE Analysis	Statistical	DESeq2	Identify DEGs
Enrichment	GO/KEGG	DAVID / KEGG	Pathway analysis
Network	PPI	STRING	Interaction mapping
Visualization	Network	Cytoscape	Hub gene analysis

4. RESULTS AND DISCUSSION

4.1 Dataset Overview

Integrated dataset consisted of disease and associated control samples that were chosen to undergo the process of molecular profiling of signal transduction changes. Following screening 312 disease samples and 58 normal control were retained to be analysed. The low-quality reads, incomplete expression profiles, and samples lacking clinical metadata were filtered out using quality. Quality-control criteria after preprocessing indicated that around 96.5% of reads passed through this quality-control step, which is a strong indicator of sequencing reliability and low technical noise.

Normalization has been carried out to minimize batch effects and enhance comparability of disease and control groups. Normalization led to more stable expression distributions across samples, which implies that downstream differential expression, pathway enrichment, and network analyses are reliable. The sharp distinction of the disease and control profiles indicated that the disease states were linked to specific changes in the molecular signaling.

4.2 Differential Gene Expression

The analysis of the results of the differential expression revealed that the number of significantly dysregulated genes is 2,176 based on the criterion of using the value of the log 2 Fc with the threshold of 1 and the adjusted p-value with the threshold of 0.05. Of these, 1,248 genes were found to be up-regulated with 928 genes found to be down-regulated in disease samples relative to controls. The genes which were mainly up-regulated were related to cell survival, inflammatory signaling, proliferation and stress-response pathways.

There were some significant changes in expression of a number of important signaling-related genes. AKT1, MAPK1, RELA/NF-kB, JUN and EGFR were significantly increased indicating activation of survival, inflammatory and proliferative signals. Conversely, the expression levels of TP53, PTEN, and pro-apoptotic regulatory genes were lower, which means the loss of the checkpoint control and apoptotic control. This evidence indicates that conditions of disease are marked by activation of pro-survival signaling and inhibition of regulatory signaling.

Table 2: Top Differentially Expressed Signaling Genes

Gene	$\log_2$FC	Regulation	Associated Pathway
AKT1	2.3	Upregulated	PI3K-Akt
MAPK1	2.1	Upregulated	MAPK
RELA (NF-κB)	2.5	Upregulated	NF-κB
EGFR	2.2	Upregulated	Growth signaling

TP53	-1.8	Downregulated	p53/apoptosis
PTEN	-2.0	Downregulated	PI3K-Akt regulation

4.3 Heatmap of Signaling Genes

The heatmap of the differentially expressed signaling genes was evidently clustering between disease and control samples. The disease samples were found to cluster together due to enhanced expression of survival and inflammatory signaling genes whilst the control samples exhibited a relatively greater expression of the regulatory and homeostatic genes. This partitioning validates that the identified signaling genes are robust enough to be able to differentiate between disease states that are altered and normal physiological conditions.

PI3K-Akt, MAPK and NF- and - genes were more highly expressing in disease groups, indicating the participation of these pathways in disease. Conversely, there was a lower expression of genes associated with p53-mediated checkpoint control and apoptosis. Clustering pattern shows that the signal transduction changes are not isolated gene changes but at a coordinated pathway level.

4.4 Signaling Pathway Alterations

GO and KEGG pathway enrichment analyses showed that the analysis of the significant disease-related signaling pathways was highly enriched. The most enriched pathways were PI3K-Akt signaling, MAPK signaling, NF- -B signaling, apoptosis and p53 signaling. There were increased activation patterns of PI3K-Akt and MAPK-pathways demonstrating increased cell survival, growth, and proliferation. NF-kB signaling was also enhanced, which suggests transcriptional regulation of inflammatory and stress-linked activities.

Conversely, the activity of p53 pathways was decreased indicating the impairment of the DNA damage response and the weakening of the apoptotic control. This disproportion in activated survival pathways and suppressed regulatory pathways show that there is coordinated signaling disruption that drives the disease progression. The findings indicate that alteration of single pathway is not associated with occurrence of disease states but overall dysregulation of the network.

Table 4: Key Altered Signaling Pathways

Pathway	Trend	Functional Role
PI3K-Akt	↑	Cell survival and growth
MAPK	↑	Proliferation and stress response
NF-κB	↑	Inflammation and immune signaling
p53	↓	DNA damage response and apoptosis
Apoptosis	Altered	Cell death regulation

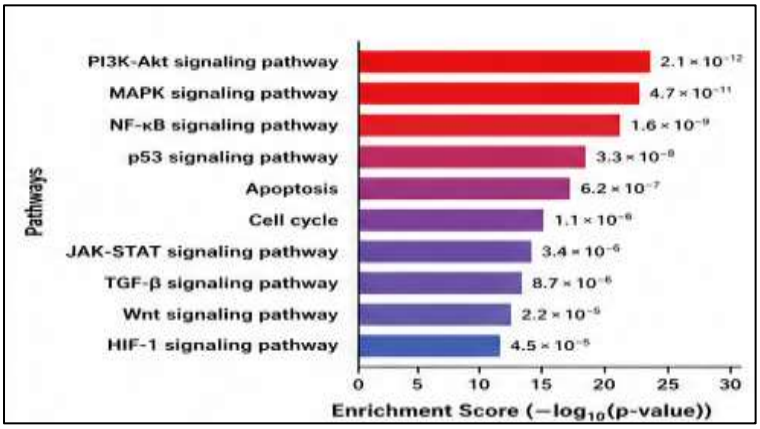

Fig 2: Pathway Enrichment Bar Graph

4.5 Mutation / Activation Profile

Protein-level signaling revealed that the transcriptional changes were observed at the activation level. Profiling by Western blot revealed enhanced phosphorylation of AKT, ERK/MAPK and NF- 0, suggesting survival, proliferation and inflammatory signaling pathway activation. By comparison, the down-regulation or deregulation of p53 and PTEN indicated the deregulation or breakdown of tumor-suppressive/regulating signals.

The activation profile revealed that a number of pathways were simultaneously altered. Greater p-AKT and p-ERK were an indicator of increased growth and survival signaling, and greater NF-B was an indicator of activation of inflammatory pathways. The decreased level of p53 indicated defective apoptotic response. Collectively, these results support that disease states entail transcriptional disorders as well as protein-level disorders.

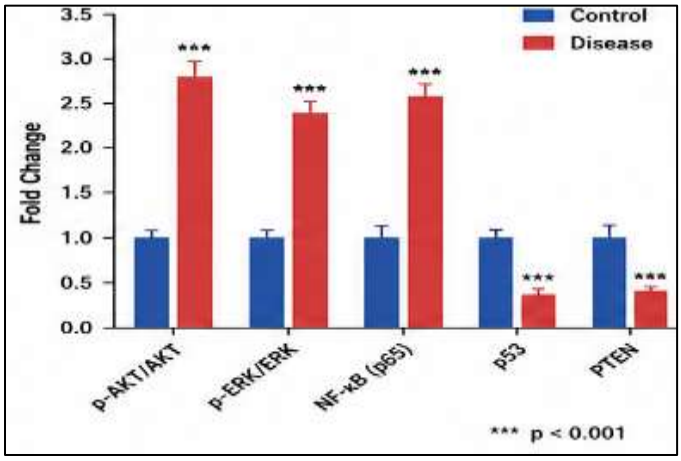

Fig 3: Signaling Activation Profile

4.6 Network Analysis and Hub Regulators

The protein protein interaction network analysis indicated that there were a number of hub regulators which had many connections that were highly linked with altered signal transduction. The most outstanding hub genes were AKT1, TP53, MAPK1, RELA/NF-kB, JUN, and EGFR. The degree of centrality of these genes and their interaction with several downstream signaling components were very high, and it implies the significance of these genes in the regulation of disease-related pathways.

Two of these, AKT1 and MAPK1, were linked to signaling survival, proliferation, and stress response, and RELA/NF-kB and TP53 were tumor suppressors and key checkpoints and regulators of apoptosis, respectively. These regulators were found to be not individual actors but constitute an interlocking core of signaling. This serves to suggest that the development of disease occurs because coordinated network perturbation, and not single molecular events drives disease progression.

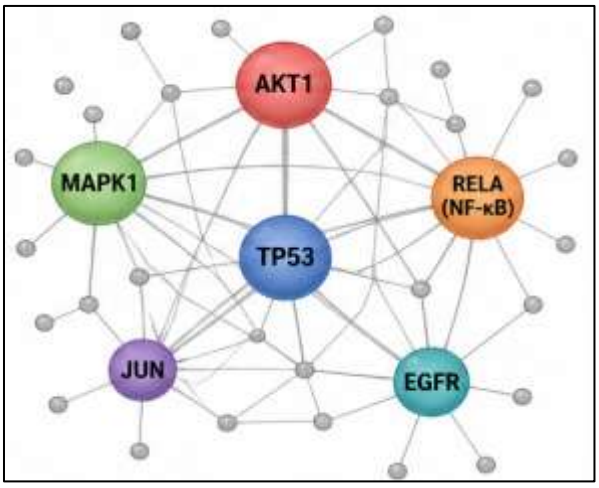

Fig 4: PPI Network of Hub Regulators

4.7 Discussion

The current research exposes variations in signal transduction pathways that are significant in representation of the disease states, both in terms of gene expression and protein activation. The completely separated disease and control samples and the identified high number of differentially expressed genes is evidence of the fact that distinct molecular signatures in signal-dysregulation is present. The upregulation of genes implicated in PI3K-Akt, MAPK and NF- 9B pathways suggests that cells are able to grow faster, survive, and respond to inflammation whereas the downregulation of p53 and additional control genes suggests a loss of checkpoint control and apoptosis.

The pathway enrichment and network analyses go even further to show that these signaling pathways do not work in isolation but are a connected regulatory network. The discovery of hub genes like AKT1, MAPK1, RELA (NF-KB), TP53, JUN, and EGFR underlines their pivotal position in the organization of several downstream events. The fact that the observed activation of survival pathways are accompanied by suppression of apoptotic signaling, is likely an indication of a transition to unregulated growth and cell death resistance, which is characteristic of most disease states. This synchronized malfunction assists the principle of the network-level changes instead of solitary molecular incidences governing the disease advancement.

5. CONCLUSION

This paper details a global molecular analysis of signal transduction changes in disease conditions through a combination of gene expression analysis, pathway enrichment and network-based analysis. These findings indicate that major signaling pathways such as PI3K-Akt, MAPK, and NF-kB are stimulated and regulatory pathways such as p53 are inhibited, resulting in cell survival and proliferation and apoptosis imbalance. These results affirm that pathophysiological changes are closely correlated with the co-ordinated dysregulation of several signaling pathways and not single molecular events in the progression of the disease.

The discovery of central hub regulators, such as AKT1, MAPK1, RELA (NF- 2B), TP53, JUN and EGFR, underscores their importance in sustaining and disrupting signaling network integrity. These molecules are important control nodes that regulate several downstream signalling pathways, hence they are of great interest as potential biomarkers of disease onset and progression, and potential therapeutic targets. These findings are further supported by the incorporation of transcriptomic data with protein-level validation that, in turn, confirms that changes in gene expression observed are implemented into functional pathway regulation.

In sum, the present research paper provides a unified framework on the comprehension of disease-related modifications to signaling on a systems scale. The work has great potential in the development of multi-target therapeutic strategies because it shows how interdependent signaling networks lead to disease processes. The findings will need to be refined further with the use of multi-omics data, larger clinical datasets, and in vivo model experimental validation, which will be necessary in future research.

REFERENCES

[1] Ablain, J., Rice, K., Soilihi, H., De Reynies, A., Minucci, S., & de Thé, H. (2014). Activation of a promyelocytic leukemia–tumor protein 53 axis underlies acute promyelocytic leukemia cure. Nature medicine, 20(2), 167-174.

[2] Abdallah, C. G., Sanacora, G., Duman, R. S., & Krystal, J. H. (2015). Ketamine and rapid-acting antidepressants: a window into a new neurobiology for mood disorder therapeutics. Annual review of medicine, 66(1), 509-523.

[3] Alessi, D. R., Andjelkovic, M., Caudwell, B., Cron, P., Morrice, N., Cohen, P., & Hemmings, B. A. (1996). Mechanism of activation of protein kinase B by insulin and IGF-1. The EMBO journal, 15(23), 6541-6551.

[4] Alessi, D. R., James, S. R., Downes, C. P., Holmes, A. B., Gaffney, P. R., Reese, C. B., & Cohen, P. (1997). Characterization of a 3-phosphoinositide-dependent protein kinase which phosphorylates and activates protein kinase Bα. Current biology, 7(4), 261-269.

[5] Alexandrov, L. B., Ju, Y. S., Haase, K., Van Loo, P., Martincorena, I., Nik-Zainal, S., ... & Stratton, M. R. (2016). Mutational signatures associated with tobacco smoking in human cancer. Science, 354(6312), 618-622.

[6] Allen, M. A., Andrysik, Z., Dengler, V. L., Mellert, H. S., Guarnieri, A., Freeman, J. A., ... & Espinosa, J. M. (2014). Global analysis of p53-regulated transcription identifies its direct targets and unexpected regulatory mechanisms. Elife, 3, e02200.

[7] Cohen, J., Vincent, J. L., Adhikari, N. K., Machado, F. R., Angus, D. C., Calandra, T., ... & Pelfrene, E. (2015). Sepsis: a roadmap for future research. The Lancet infectious diseases, 15(5), 581-614.

[8] Das, T., Andrieux, G., Ahmed, M., & Chakraborty, S. (2020). Integration of online omics-data resources for cancer research. Frontiers in Genetics, 11, 578345.

[9] GTEx Consortium, Ardlie, K. G., Deluca, D. S., Segrè, A. V., Sullivan, T. J., Young, T. R., ... & Dermitzakis, E. T. (2015). The Genotype-Tissue Expression (GTEx) pilot analysis: multitissue gene regulation in humans. Science, 348(6235), 648-660.

[10] Liu, V., Escobar, G. J., Greene, J. D., Soule, J., Whippy, A., Angus, D. C., & Iwashyna, T. J. (2014). Hospital deaths in patients with sepsis from 2 independent cohorts. Jama, 312(1), 90-92.
[11] Ma, Y., Dong, S., Grippin, A. J., Teng, L., Lee, A. S., Kim, B. Y., & Jiang, W. (2025). Engineering therapeutical extracellular vesicles for clinical translation. Trends in Biotechnology, 43(1), 61-82.
[12] Tarazona, S., Arzalluz-Luque, A., & Conesa, A. (2021). Undisclosed, unmet and neglected challenges in multi-omics studies. Nature Computational Science, 1(6), 395-402.