Texila International Journal of Public Health
Special Issue-2024
DOI: 10.21522/TIJPH.2013.SE.24.05.Art023

Histone Demethylase (KDM3A) Regulation and Its Impact on Estrogen Receptor-Positive Breast Cancer Progression

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Abstract

The histone demethylase-encoding gene KDM3A is a crucial modulator of estrogen receptor (ER) signaling, impacting the development of ER-positive breast cancer. Resistance develops despite the effectiveness of endocrine drugs that target ER signaling, necessitating a deeper understanding of the underlying molecular processes. This work investigated KDM3A depletion in ER-positive breast cancer cells to gain a better understanding of how it impacts estrogen-induced gene expression and its potential as a therapeutic target in endocrine-resistant breast cancer. We used the GEO dataset GSE68918, which contains gene expression profiles from MCF-7 cell lines treated with KDM3A RNA (siKDM3A) and scrambled RNA (siSCR), to find 845 differentially expressed genes (DEGs). Following KDM3A knockdown, 402 of these genes showed upregulation, and 444 showed downregulation. Significant downregulation was observed for BRCA1 and CCNB1, but a notable upregulation was observed for CEACAM6. This suggests that KDM3A is involved in signaling through estrogen receptors, repairing DNA, and regulating the cell cycle. A study of protein-protein interaction (PPI) networks showed that hub genes, including TOP2A, CCNA2, and CCNB1, are essential for KDM3A-related networks. Significant enrichment in the p53 signaling pathway, DNA replication, and cell division was found by Gene Ontology (GO) and KEGG pathway studies, highlighting the influence of KDM3A on important biological processes. These findings suggest that KDM3A could be a valuable therapeutic target in the management of breast cancer endocrine resistance. Future research should look at the therapeutic potential of KDM3A inhibitors to enhance the efficacy of treatment for endocrine-resistant breast cancer, particularly when combined with other forms of therapy.

Keywords: *Breast Cancer, Estrogen Receptor, Endocrine Resistance, Gene Expression, KDM3A, Therapeutic Target.*

Introduction

Breast cancer, which is caused by uncontrolled cell growth and proliferation, continues to be one of the most common and complicated diseases impacting women globally. Many of these malignancies are estrogen receptor (ER)-positive, indicating that ER signaling is essential for the development and spread of the tumor. Endocrine treatments that target ER signaling, including tamoxifen and aromatase inhibitors, are the mainstay of current treatment options for ER-positive breast tumors. These medications have proven to be highly effective in treating the disease [1,2]. The unavoidable emergence of resistance, which results in treatment failure

Received: 28.08.2024 ***Accepted: 14.10.2024*** ***Published on: 27.12.2024***
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and subsequent disease progression, is a significant drawback of this therapy [3]. This resistance highlights the need for further understanding of the molecular mechanisms behind this process and poses a significant challenge to the long-term therapy of breast cancer.

The utilization of gene expression profiling has become essential in comprehending the behavior and advancement of malignancies. Researchers can predict clinical outcomes, find possible treatment targets, and more precisely define cancers by comparing the gene expression patterns of malignant and normal cells [4,5]. The simultaneous analysis of hundreds of genes has been made possible by DNA microarrays and RNA sequencing, which offer a comprehensive picture of the molecular alterations linked to the development of cancer and resistance to treatment [6-8]. The role of KDM3A affects global gene expression and plays a part in the emergence of endocrine resistance is still unknown, despite the progress made in the study of ER signaling [9,10].

To better understand the function of KDM3A in ER-positive breast cancer cells, we aim to look at the changes in gene expression that occur when KDM3A is depleted. In the context of endocrine-resistant breast cancer, we specifically aim to comprehend how KDM3A affects estrogen-induced gene expression and its potential as a therapeutic target. This work illustrates the therapeutic potential of targeting histone demethylases, like KDM3A, to improve the effectiveness of breast cancer treatments, in addition to adding to our understanding of the molecular pathways behind the progression of breast cancer [11–13].

Materials and Methods

Data Collection

The Gene Expression Omnibus (GEO) database, maintained by the National Center for Biotechnology Information (NCBI), was the source of the gene expression data. For this study, the GEO dataset GSE68918 was used. The MCF-7 breast cancer cell line, a well-known ER-positive breast cancer research model, has gene expression profiles included in this dataset. Ten samples in total are included in the dataset, which is split into two groups: five samples treated with scrambled RNA (siSCR) as a control and five samples treated with KDM3A RNA (siKDM3A) to disrupt the ER signaling pathway by knocking down KDM3A expression [9]. The selection of the dataset was based on its relevance to the study's focus on KDM3A's modulation of ER signaling, providing a comprehensive framework for investigating differentially expressed genes (DEGs) upon KDM3A depletion.

Finding Significant Genes

The GEO2R tool (https://www.ncbi.nlm.nih.gov/geo/geo2r/) was utilized to perform differential gene expression analysis. This tool facilitates the comparison of two or more groups of samples within a GEO series. GEO2R uses the Bioconductor project's 'limma' (Linear Models for Microarray Data) software, which is well-known for its strong statistical skills in the analysis of gene expression data [14]. The MCF-7 cell lines treated with scrambled RNA (siSCR) and those treated with KDM3A RNA (siKDM3A) were compared in this work. A noteworthy variation in gene expression levels was indicated by a log2 fold change > 1, and genes were considered differentially expressed if they satisfied the following criteria: an adjusted p-value < 0.05, which ensures statistical significance after multiple testing corrections [15].

Building a Network of Protein-Protein Interactions and Naming the Hub Gene, the STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) database (https://string-db.org/) was used to build a protein-protein interaction (PPI) network to

further investigate the functional links among the DEGs. Public text collections, computer prediction techniques, experimental data, and predictions about known and expected protein-protein interactions are all integrated into the STRING database [16]. Using empirically established interactions as a focus to assure dependability, the list of DEGs found in the GEO dataset was fed into STRING to create a PPI network.

After the PPI network was built, it was examined and displayed using the industry-leading platform for network biology analysis, Cytoscape software (version 3.8.0) (https://cytoscape.org/). The cytoHubba plug-in information, which are accessible by Chin et al. [17], was used in Cytoscape to find hub genes, or the most connected genes in the network. The top 10% of genes with the greatest connections were designated as hub genes using the degree approach, which ranks genes according to the number of direct interactions (edges) they have with other genes (nodes) in the network.

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis were used to further evaluate the hub genes found by cytoHubba for functional annotation. Using the SR Plot tool (https://www.biocloud.net/srplot/), which offers a thorough platform for assessing the biological importance of gene sets, this analysis was carried out. A p-value threshold of less than 0.05 was utilized to identify highly enriched items in the GO analysis, which was centered around three key categories: Molecular Functions (MF), Cellular Components (CC), and Biological Processes (BP) [18]. KEGG pathway analysis was used to map the hub genes for established biological pathways [19]. The hub genes with the greatest enrichment scores and possible links to endocrine signaling and breast cancer were highlighted.

Results

An Identification of DEGs in siKDM3A

Within the GSE68918 dataset, 845 DEGs ERGs) were found, comprising 402 upregulated and 444 downregulated genes when comparing MCF-7 cells treated with KDM3A RNA (siKDM3A) to those treated with scrambled RNA (siSCR). To verify the biological significance of the identified genes, the identification was conducted below the cutoffs of p-value < 0.05 and $|\log2FC| > 1.5$. This visual representation of DEGs is provided by the volcano plot (Figure 1), where blue dots denote downregulated genes and red dots upregulated genes.

With a robust differential expression indicated by a highly significant p-value of logP = 9.655 and the largest fold change of log2 (FC) = 3.480, CEACAM6 was one of the most significantly elevated genes. On the other hand, a log2FC = -1.007 and matching p-value indicated a significant downregulation of ARSG, suggesting that it may be involved in the molecular pathways affected by KDM3A knockdown.

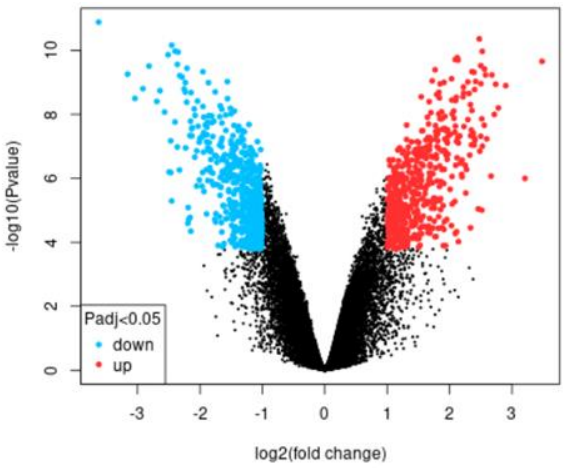
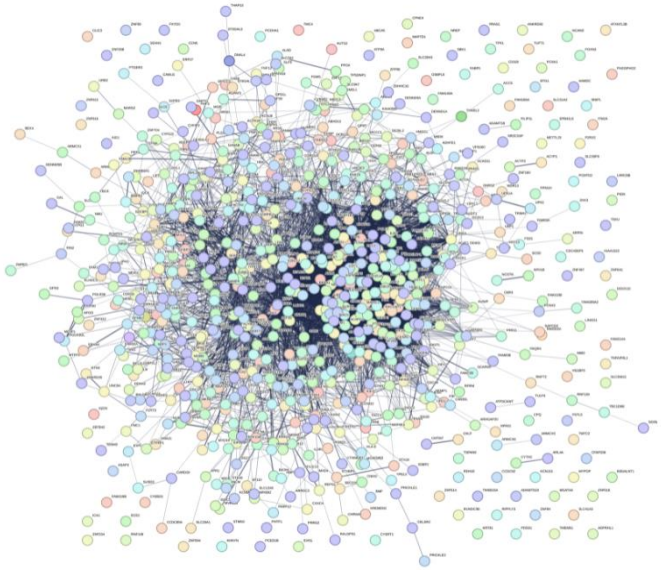


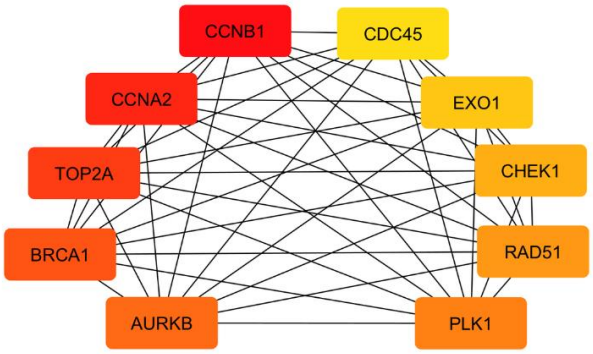
Figure 1. Volcano (Plot GSE68918) Represents the DEGs: siSCR vs siKMD3A. The Red Dot and Blue Dot Indicate Upregulated and Downregulated Genes, Respectively.

Identification of the Hub Gene and the Protein-Protein Interaction (PPI) Network

Using the STRING database, the PPI network's complex network illustrates the vast protein interaction involved in KDM3A expression (Figure 2a). The network density indicates that the system is highly interconnected, with a few central hub proteins functioning as critical proteins. Hub genes are crucial for comprehending molecular functions, cellular components, and biological processes. Additionally, hub genes on degree centrality measures have been examined in pathways impacted by KDM3A in the PPI network that was provided. Degree centrality analysis revealed that CCNB1, CCNA2, TOP2A, BRCA1, AURKB, PLK1, RAD51, CHEK1, EXO1, and CDC45 are the top genes (Table 1). These findings highlight their crucial functions in the network by showing a large overlap with those found by degree centrality (Figure 2b). It has been demonstrated that KDM3A knockdown results in lower cell proliferation and compromised DNA repair mechanisms due to reduced expression of CCNB1, CCNA2, and BRCA1.



A)



B)

Figure 2. Interaction Network of 845 Genes (A), Cytoscape Identified the Top 10 Hub Genes by Degree of Centrality (B).

Table 1. Cytohubba Methods Rank Hub Genes.

Rank	Name	Score
1	CCNB1	176
2	CCNA2	174
3	TOP2A	172
4	BRCA1	170
5	AURKB	169
6	PLK1	168
7	RAD51	167
8	CHEX1	165
9	EXO1	164
10	CDC45	159

Gene Ontology and KEGG Pathway Analysis of DEGs

The biological implications of the DEGs found in the siKDM3A condition were further characterized by performing Gene Ontology (GO) analysis and KEGG pathway enrichment analysis. The biological mechanisms, cellular elements, and molecular roles connected to the DEGs were revealed by the GO analysis. These genes have an important role in biological processes, especially in cell division and genome stability, as demonstrated by the cnet plot [18,20] (Figure 3). Some of the significant enrichment mechanisms that have been identified include mitotic nuclear division, organelle fission, chromosomal segregation, sister chromatid segregation, DNA replication, and microtubule cytoskeleton organization. The significance of KDM3A on cell cycle regulation is highlighted by these mechanisms, which are essential for the correct conduct of mitosis and preservation of genomic integrity [21] (Figure 4a).

Significant enrichment was seen in cellular components related to cell division, including spindles, kinetochores, and the centromere region of chromosomes (Figure 4b). Significant enrichment in activities such single-stranded DNA binding, DNA-dependent ATPase activity, and catalytic activity on DNA was revealed by the molecular function analysis, suggesting that KDM3A influences genes implicated in crucial DNA processing processes [22] (Figure 4c). Additional crucial pathways affected by KDM3A knockdown were found by KEGG pathway analysis, with notable enrichment found in the p53 signaling, DNA replication, and cell cycle pathways (Figure 5). The regulation of apoptosis and cell proliferation depends on these pathways, indicating that

KDM3A knockdown may result in gene changes that affect important processes for cellular homeostasis and DNA damage response. The pathway enrichment scores highlight the possible function of KDM3A in preserving cellular integrity and reacting to genomic stress.

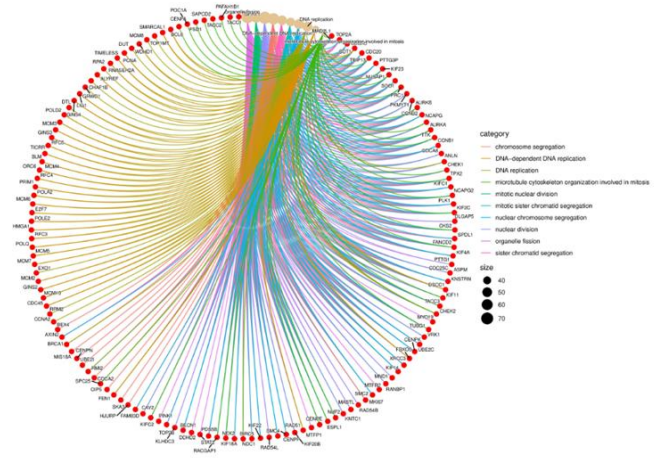


Figure 3. Biological Process Cnet Plot.

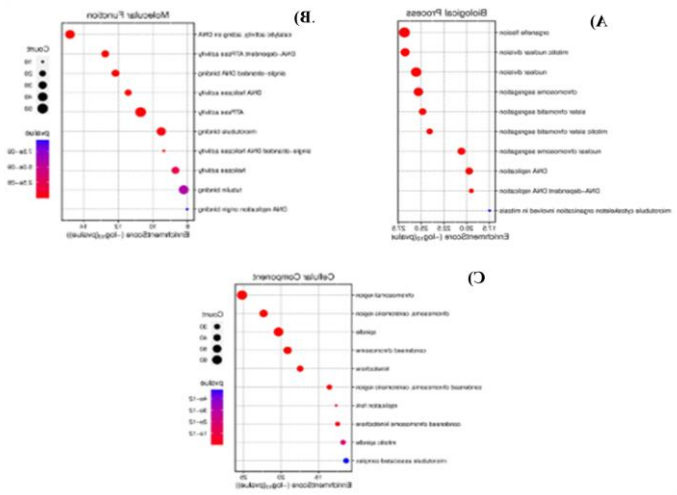


Figure 4. A) Biological Process Dot plot, B) Molecular Function Dot Plot, C) Cellular Component Dot Plot.

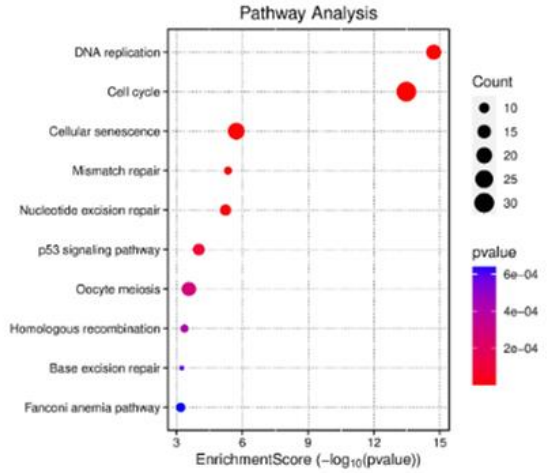


Figure 5. Pathway analysis Dot plot.

Discussion

The principal aim of the study was to examine the alterations in gene expression that transpired after KDM3A depletion in ER-positive breast cancer cells. The investigation specifically aimed to comprehend the influence of KDM3A on estrogen-induced gene expression and the feasibility of KDM3A as a therapeutic target in endocrine-resistant breast cancer. This study's findings directly address these goals by identifying 845 DEGs that have substantial alterations in important genes related to DNA repair, cell cycle regulation, and estrogen receptor signaling pathways. The discovery of these DEGs, in particular the downregulation of BRCA1 and CCNB1 and the overexpression of CEACAM6, emphasizes how important KDM3A is in regulating these pathways [16,23]. With evidence that KDM3A influences gene expression patterns necessary for cell proliferation and survival, this study supports the protein's potential as a therapeutic target and clarifies its involvement in ER-positive breast cancer.

Histone demethylases, such as KDM3A, have been linked to the regulation of gene expression and the advancement of cancer. As an illustration, prior studies linking CEACAM6 to enhanced tumor invasiveness and a poor prognosis in cancer patients [24] support the overexpression of this gene in a variety of malignancies, as seen in our work. The discovery that these genes exhibit differential expression after KDM3A knockdown validates previous findings and supports the concept that KDM3A is an essential modulator of genes linked to the advancement of cancer and the development of endocrine resistance [25]. Still, this work offers new perspectives that expand upon current understanding. To investigate the metabolic effects of KDM3A depletion, for instance, new pathways are suggested by the downregulation of ARSG, a gene not previously linked to KDM3A activity in the setting of breast cancer. This unexpected discovery could lead to more investigation into the metabolic pathways impacted by KDM3A and suggest new targets for treatment.

Further study is necessary to explore the therapeutic potential of targeting KDM3A in conjunction with existing medications. Examining how KDM3A inhibitors affect preclinical models, particularly when combined with other cell cycle inhibitors or drugs that damage DNA, could yield important information on how well this strategy works to overcome endocrine resistance [26].

Furthermore, new regulatory mechanisms and prospective biomarkers for the advancement of cancer may be discovered by mechanistic investigations focused on comprehending the direct interactions between KDM3A and the detected DEGs, particularly those connected to metabolic pathways. Potentially leading to the identification of new metabolic targets for therapy, additional research on the involvement of ARSG and other novel DEGs in metabolic reprogramming and their effect on breast cancer metabolism should be conducted [27,28].

Conclusion

By identifying 845 DEGs after KDM3A depletion, our study offers important new insights into the function of KDM3A in ER-positive breast cancer. Notably, alterations were observed in major DNA repair and cell cycle regulators, including CEACAM6, BRCA1, and CCNB1. These results demonstrate the potential of KDM3A as a therapeutic target, especially in endocrine-resistant patients, and imply the potential role of BRCA1 and CEACAM6 as biomarkers for aggressive cancer morphologies. In endocrine-resistant breast cancer, targeting KDM3A may enhance treatment outcomes. Additionally, the identified DEGs may develop targeted treatments and individualized treatment plans. The work accomplishes its goals by

demonstrating KDM3A's potential as a therapeutic target and clarifying its function in regulating gene expression in ER-positive breast cancer cells. Future research should focus on enhancing treatment results for patients with endocrine-resistant breast cancer by validating these findings and investigating the therapeutic consequences of targeting KDM3A.

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Acknowledgements

The authors thank the Centre for Global Health Research, Saveetha Medical College, and Hospital for providing the facilities and support for preparing this article.

Conflict of Interest

The authors hereby declare that there is no conflict of interest in this study.

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