Targeting Colorectal Cancer with Curcumin: Identification of Hub Genes and Survival Significance through Computational Approaches

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ABSTRACT

Colorectal cancer (CRC) is the top contributor for cancer mortality worldwide. The current therapies are not effective as cancer emerge to be drug resistant. Curcumin, a phytochemical has revealed to be a promising drug for CRC therapy due to its anticancer, anti-inflammatory and antioxidant properties. Recent *in vitro* and *in vivo* studies have validated curcumin as a promising therapeutic agent for CRC. By applying bioinformatics, the CRC-associated hub genes were explored to study their relations with curcumin. This study performs an integrated computational approach to identify the hub targets for CRC and analyse them with curcumin. This study aimed to retrieve the differently expressed genes of CRC by GEO2R analysis and intersect the set with curcumin target genes set to identify the hub genes. The hub genes were further analyzed by molecular docking and survival analysis by Kaplan-Meier method. The integration of docking and survival analyses highlighted MAOA followed by EGFR as key prognostic and therapeutic targets for CRC therapy. Further studies in biological systems are essential to fully comprehend their mechanisms.

Keywords- Colorectal cancer, Phytochemical, Molecular Docking, Kaplan Meier Plot

1. INTRODUCTION

Colorectal cancer (CRC) is ranked as one of the topmost leads for cancer-related mortality and most diagnosed cancer worldwide [1]. Despite developments in therapeutics in surgery, radiotherapy, chemotherapy and pharmacological approaches, the survival rate for CRC remains low. Processes like cell proliferation, migration and invasion and apoptosis resistance are featured in the development of CRC. Cancer stem cells contribute to the initiation of cancer and builds resistance to chemotherapies [2]. The challenges demand a necessity for a new, accessible and affordable therapeutics.

Curcumin, a polyphenol derived from the plant Curcuma longa, as a therapeutic candidate for cancer treatment [3]. It has therapeutic traits such as anti-inflammatory, antiangiogenic, antioxidant and anticancer [4]. This phytochemical has exhibited to inhibit the proliferation of CRC and inducing apoptotic activities. Preclinical and clinical studies have displayed the efficacy of curcumin to promote apoptosis in various cancers and a safe phytochemical for complementary therapy with chemotherapy [5,6].

Recent studies have validated curcumin as a promising therapeutic agent for CRC therapy in both *in vitro* and *in vivo* experiments [1]. It supresses several oncogenic signalling pathways such as Wnt/β-catenin (Wingless-related integration site/Beta-catenin) and EGFR/MAPK (Epidermal Growth Factor Receptor/ Mitogen-Activated Protein Kinase) pathway which causes proliferation in CRC, breast and pancreatic cancer [7,8]. Induction of apoptosis in CRC is modulated through targeting P13K/Akt (Phosphoinositide-3-Kinase/Protein Kinase B) pathway as curcumin eliminates the survival signal by inhibiting Akt [9]. It also supresses NF-κB (Nuclear Factor Kappa-light-chain-enhancer of activated B cells) and STAT3 (Signal Transducer and activator of Transcription 3) signalling pathways by overriding the apoptotic mediators in CRC like Bcl-xL (B-cell lymphoma-x-Long), Bcl-2 (B-cell lymphoma 2) and survivin [9,10].

In this study, the differently expressed genes (DEGs) retrieved by GEO2R analysis was intersected with curcumin target genes obtained from SwissTargetPrediction to identify the hub genes. The hub genes were further analyzed by molecular docking to predict the binding affinity and molecular interactions between the hub genes and curcumin. Survival analysis was performed by Kaplan-Meier method to evaluate their prognostic potentials.

2. METHODOLOGY

2.1 GEO2R Analysis

For the detection of DEGs in normal colon mucosa cell line (NCM460) and CRC cell lines (HT29 and DLD1), GEO2R analysis was conducted. The samples were collected from GEO (https://www.ncbi.nlm.nih.gov/geo/) series dataset with accession number GSE68468 [11]. This dataset was chosen based on the relevance to the study and the number of samples available for each group. Two groups were made for comparison, normal and CRC. Normal group had 1 sample for NCM460 cell lines while the CRC group had 4 samples. The CRC group had 2 different cancer cell line, 1 sample of HT29 cell lines and 3 samples of DLD1 cell lines. Both the groups were analyzed and the DEGs were retrieved from the dataset.

2.2 Retrieval of Curcumin Targets

The Simplified Molecular Input Line Entry System (SMILES) notation for curcumin was obtained from PubChem database (https://pubchem.ncbi.nlm.nih.gov/) (CID- 969516) [12] and was used in SwissTargetPrediction (https://www.swisstargetprediction.ch/) to obtain the target genes of the ligand [13].

2.3 Hub Genes Selection

A Venn diagram was constructed using an online tool, Venny 2.1 (https://bioinfogp.cnb.csic.es/tools/venny/) to identify the overlapping gene between DEGs and curcumin targets set [14].

2.4 Molecular Docking

PubChem and RCSB Protein Data Bank (https://www.rcsb.org/) was utilized to download the 3D structure of the ligand and proteins [15]. Maestro from Schrödinger suite was used to perform molecular docking. Protein Preparation Wizard was used to the protein structures while the ligand was prepared for docking using LigPrep. Ligand docking was conducted in extra precision mode using the glide module [16]. The 3D structures for the docked protein-ligand were visualized using PyMOL.

2.5 Kaplan-Meier Plot

Kaplan-Meier plotter (https://kmplot.com/analysis/) was used to perform survival analysis for hub genes [17]. This is an online tool that is used to analyse the prognosis relevance of genes in cancer studies.

3. RESULTS

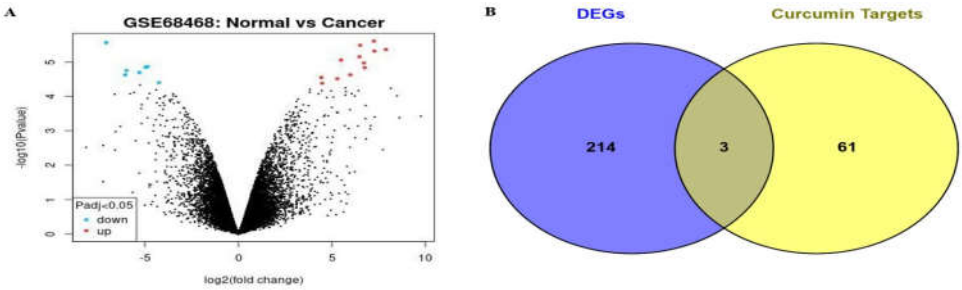

Figure 1: A. Volcano plot obtained from GEO2R analysis. B. Venn diagram constructed to identify the hub genes for CRC

3.1 GEO2R Analysis

The GEO2R analysis was performed for 2 groups with 1 sample in normal group and 4 samples in the CRC group. A total of 13297 DEGs were identified from with analysis, and they were presented with their P-value and log2 fold change threshold. The log2 fold change values were ranging from 7.25 to –7.07 and the top 218 DEGs were selected as target proteins.

3.2 Curcumin Targets

From SwissTargetPrediction, 100 possible targets for curcumin were identified out of which 64 genes with the probability score greater than zero were considered as curcumin target genes.

3.3 Hub Genes Selection

The overlapping targets were visualized by generating a Venn diagram to identify the hub genes. The Venn diagram was constructed for 218 DEGs and 64 curcumin targets which

showed 3 common genes- Monoamine oxidase A (MAOA), Arachidonate 5-lipoxygenase (ALOX5) and Epidermal growth factor receptor erbB1 (EGFR).

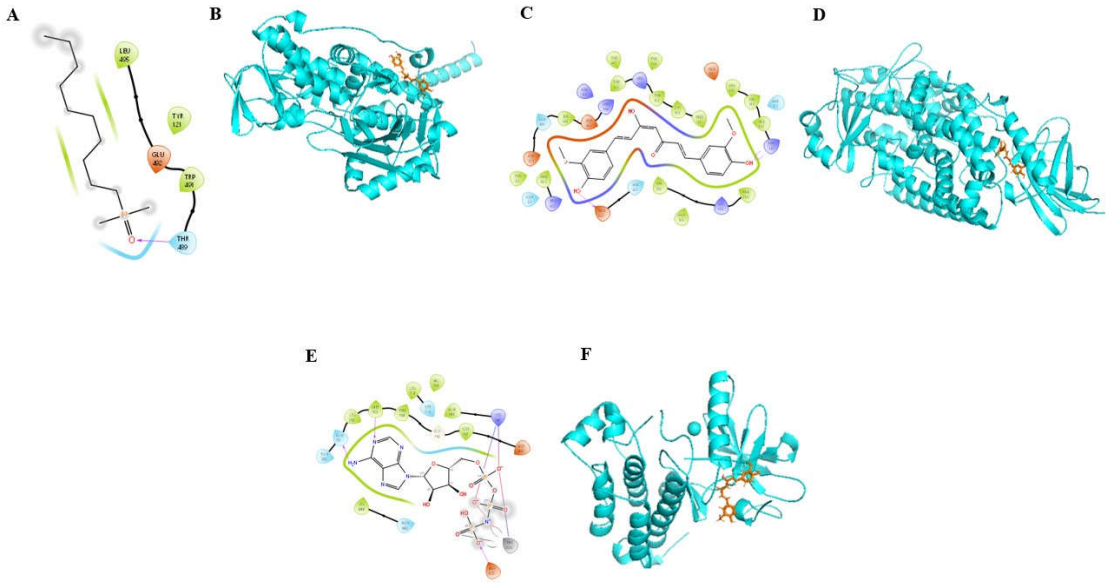

Figure 2: 2D structures of MAOA, ALOX5 and EGFR docked with curcumin are displayed in image **A, C** and **E** and their 3D structures are shown in image **B, D** and **F**

3.4 Molecular Docking

Hub Gene	Docking Score
MAOA	-6.48
ALOX5	-5.21567
EGFR	-5.9335

Table 1: Docking scores for hub genes with curcumin

Figure 2 consist of the 2D and 3D protein-ligand docking images of hub genes and curcumin and table 1 displays the docking score for each hub genes. The 2D image was obtained from protein-ligand interactions from glide module and the 3D structure was visualized using PyMOL. The highest docking score of MAOA implies to its stronger interaction and binding affinity towards curcumin compared to EGFR and ALOX5.

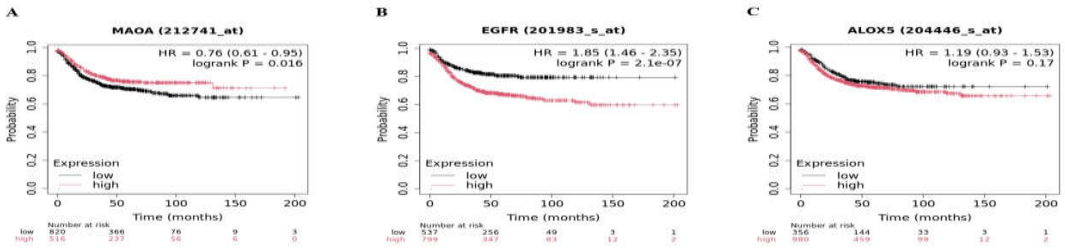

Figure 3: Kaplan-Meier survival plots of **A)** MAOA, **B)** EGFR and **C)** ALOX5

3.5 Survival Analysis

Kaplan-Meier survival analysis was performed to assess the prognostic potential of the hub genes. The associations between patient overall survival and the gene expressions are summarised in the generated plots. The overall survival analysis for each gene was performed in a default setting. High expression of MAOA showed significant survival benefit with the hazard ration (HR) of 0.76 while EGFR (HR= 1.85) with a high expression is a strong risk factor and ALOX5 (HR= 1.19) has failed to demonstrate significant survival association.

4. DISCUSSION

Through bioinformatics, the targets for CRC were investigated to assess their relations with curcumin. In this study, 217 DEGs were identified using GEO2R analysis of which 126 genes were upregulated, and 91 genes were downregulated. The hub genes were found by constructing a Venn diagram for the DEGs and curcumin target genes which are MAOA, ALOX5 and EGFR. The interaction and binding affinity of the hub genes with curcumin was examined by molecular docking.

The docking results confirms a strong binding affinity between curcumin and MAOA followed by EGFR while the docking score for curcumin with ALOX5 was observed to be relatively weak. MAOA promotes cancer by producing reactive oxygen species that stabilizes HIF-1α [18]. Recent studies have shown that the inhibition of MAOA has induced apoptosis and caused metastatic and angiogenic suppression in CRC therapy [19–21].

EGFR was observed to be overexpressed in about 25% to 82% of CRC and its inhibition was noted to stop the proliferation and survival of cancer cells [22]. EGFR inhibition using effective inhibitors have evidenced to induce apoptosis, suppressing tumor cells and hindering angiogenesis and metastasis in breast, pancreatic, oral and head and neck cancers [23,24].

The significant association of patient prognosis and the identified hub genes in CRC was evaluated using Kaplan-Meier survival analysis. This analysis clearly demonstrated that MAOA had favourable patient outcomes due to its high expression. Elevated EGFR expression was noticed which denotes poor survival and ALOX5 lacked the prognostic value. Some genetic experiment proposes some gene variants of ALOX5 may lower CRC risk yet higher expression of ALOX5 predicts worse prognosis [25,26].

In many studies, the expression of MAOA was unaffected or minimally decreased in CRC tissues compared to normal colon tissues, and MAOB (Monoanime Oxidase B) had higher prognostics value compared to MAOA [27,28]. The inhibition of MAOA in prostate cancer has exhibited to decrease the tumor growth [29]. Researchers found that the prognostic value of EGFR relies on the location of the tumor and its higher expression is associated to aggressive tumors [30,31]. Targeting MAOA and EGFR through curcumin has enhanced apoptosis, reduced epithelial-mesenchymal transition and invasion, and tumor growth [32].

5. CONCLUSION

The integrated computational approach analyzed the interaction with curcumin and the prognostic values of the CRC molecular targets. The molecular docking study explored the robust binding affinity of MAOA with curcumin followed by EGRF. Survival analysis evaluated MAOA as a potential prognostic marker and EGFR being associated with poorer prognosis. ALOX5 had the least binding affinity to curcumin and had no prognostic significance diminishing its therapeutic relevance. Therefore, with an *in-silico* groundwork this study positions MAOA as a promising target for CRC therapy. Further *in vitro* and *in vivo* studies are essential to understand their interactions and mechanisms in biological models.

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