





## Evaluation of Anticancer and Neuroprotective Properties of Curcumin: a Network Analysis

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### Abstract

**Background and objectives:** Curcumin as a medicinal substance has shown effective in different kinds of diseases especially cancer. To understand its underlying mechanism, molecular complementary study of differentially expressed microRNAs (DEMs) could assist. In this view, regulatory network analysis of DEMs of melanoma cancer treated with curcumin versus the untreated male *Mus musculus* was investigated in this study. **Methods:** Data was obtained from the database of Gene Expression Omnibus (GEO), <https://www.ncbi.nlm.nih.gov/geo/>. At first, the log fold change (FC)  $\geq 2$  was assigned for predicting a cut off for DEMs in the following study. GEO2R detected a number of 250 top significantly changed microRNAs based on the priority of the most statistically significant ones. These miRNAs were then explored for regulatory network analysis via Cytoscape software v.3.7.2 and its plug-ins. **Results:** The findings indicated that a number of 21 miRNAs were statistically significant with differential expression amounts. Regulatory network also identified important microRNAs of mmu-miR-199a, mmu-miR-199b, mmu-miR-21, mmu-miR-142-3p, mmu-miR-148a, mmu-miR-214 and genes of Pkp3, Usp19, Ercc4, Ttc25, Atp13a2, Akr1b7, Umod, Nup188, Imp3, and Tmem74b. The highest ranked hub was mmu-miR-199a, which had nine connections. **Conclusion:** The present study offers new insights into the molecular mechanism of curcumin health benefits in melanoma cancer.

**Keywords:** curcumin; melanoma; microRNA

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### Introduction

The biological activity of *Curcuma longa* L. (Zingiberaceae) known as turmeric has been

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attributed to curcuminoids comprising of curcumin and two related compounds demethoxy curcumin and bisdemethoxycurcumin [1]. Curcumin as the mainly effective substance of turmeric has been famous for its many medical properties including anti-hepatotoxic, anti-microbial, anti-inflammatory, and anti-tumor advantages [2-4]. P53 upregulation and NF- $\kappa$ B, Akt/PI3K, and MAPK pathways inhibitions are the most prominent anticancer mechanisms which have been suggested for the functions of curcumin. On the other hand, microRNAs as potential regulatory targets in medicine are small non coding RNAs that have been reported playing role in different types of therapeutic approaches such as neural regeneration, heart health and cancer treatment [5-7]. In this sense, curcumin could also induce epigenetic changes in diseases such as cancer; for instance, down-regulation of miR-21, miR-17-5p, miR-20a, and miR-27a, and likewise up-regulation of tumor suppressor agents, miR-34 a/c and EMT (mesenchymal-epithelial transition) [8-10]. In addition, other studies have also showed that curcumin regulates miR-1, miR-7, miR-9, miR-34a, miR-181, miR-21, and miR-19 expressions in cancer [11]. This natural polyphenol substance has been also reported effective on melanoma as one of the hazardous type of cancers as well [12]. This type of skin cancer which effects on melanocytes has been accounted for yearly death of 55500 cases [13]. To identify the therapeutic molecular mechanism of curcumin influence, microarray analysis could assist. In a study by Indra N. Dahmke et al. microRNA expression profiling of curcumin treated mouse with melanoma was considered for the following bioinformatics research [14]. In silico analysis as a complementary study could provide more knowledge in this field by means of examining the regulatory network of differentially expressed microRNAs and their hub target genes. In this way, more information related to mechanisms by which curcumin provides health benefits could be reached. For this purpose, a regulatory network of curcumin influence on melanoma has been assessed.

## Materials and Methods

### Ethical considerations

This study was originated from the project with ethical code: IR.SBMU.REC.1398.154 approved by the ethical committee of Shahid Beheshti University of medical sciences.

### Data collection

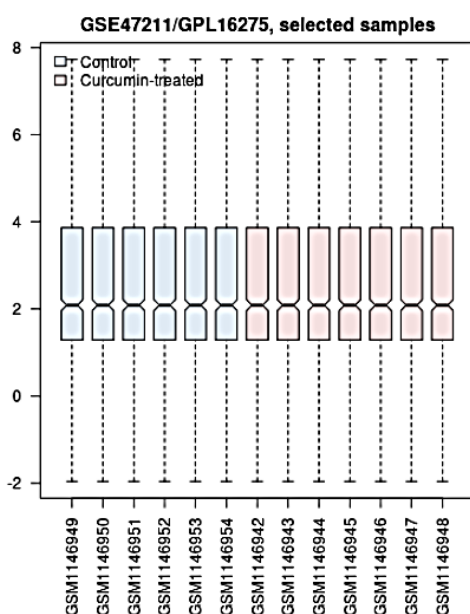
The keywords, Curcumin + Melanoma were searched in GEO database and the corresponding data; the microRNA expression profiles which were included in GSE47211 were downloaded. The platform for this analysis is GPL16275 with 13 samples of controls and curcumin-treated individuals. The investigated organism is male *Mus musculus* in which the sampling was carried out from tumors for analyzing non-coding RNA profiling by array. Curcumin prescribed in this analysis was C3 complex that contains 77% curcumin, 17% demethoxycurcumin, and 3% bisdemethoxycurcumin. The samples were either dieting standard or with curcumin 4%= 160 mg daily. The comparison of the two samples was handled by GEO2R, the online analyzer (<http://www.ncbi.nlm.nih.gov/geo/geo2r/>).

Through this analysis, a list of 250 statistically significant expressed microRNAs were found and among them a cut off with  $\log_2 FC \geq 2$  and adjusted  $p\text{-value} \leq 0.05$  were designated to detect the DEMs. These candidates were then selected for further analysis known as “regulatory network analysis”. Cytoscape software 3.7.2 was used to construct and analyze the DEMs network and CluePedia presents a regulatory network of combined hub microRNAs and hub target genes [15,16]. There are certain criteria for statistical analysis of regulatory network, including number of hub genes that should be inserted in the regulatory network, which was 10; the miRanda score cut off that was assigned to miRanda-SCORE-v5  $\geq 0.6$ . The regulatory network analysis source was miRanda (miRNA-V5-2012-07-19.txt.gz). To get a better visualizations, the expression profiles of microRNA data, Heatmapper (<http://heatmapper.ca/expression/>) presentation was provided [17]. Furthermore, to get a better knowledge of corresponding hub genes, enrichment analysis was carried out by ClueGO [18]. The statistical criteria for biological process study were kappa score cut off  $\geq 0.5$  for grouping and gene number and percentage per term were assigned as 1 and 1, respectively. Bonferroni step down was the p-value correction method.

### Results and Discussion

To understand whether the groups are comparable in terms of microRNA expression profile, box plotting is carried out to get more insight. As it is shown in the figure 1, microRNA

expression profiles of controls and treated samples are median centered therefore are matched statistically.



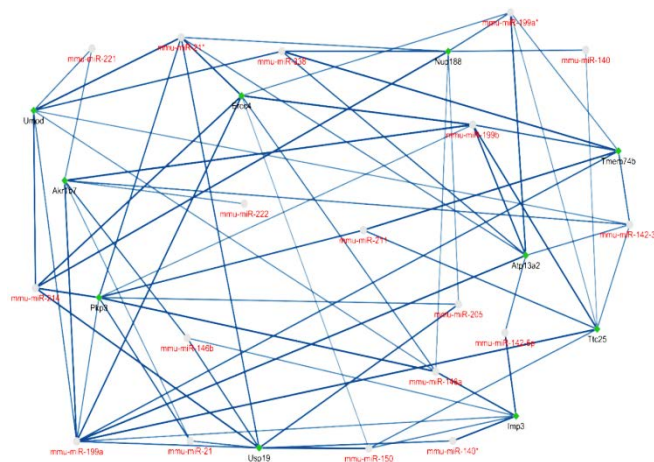
**Figure 1.** Distribution of DEM profiles are presented via box plot presentation; the blue and pink groups refer to control and curcumin-treated samples. The pattern of expression comparison is median-centered.

Considering the  $\log_{2}FC \geq 2$  and adjusted p-value  $\leq 0.01$ , 21 DEMs were obtained in this analysis that 18 microRNAs are up-regulated while 3 individuals are down-regulated.

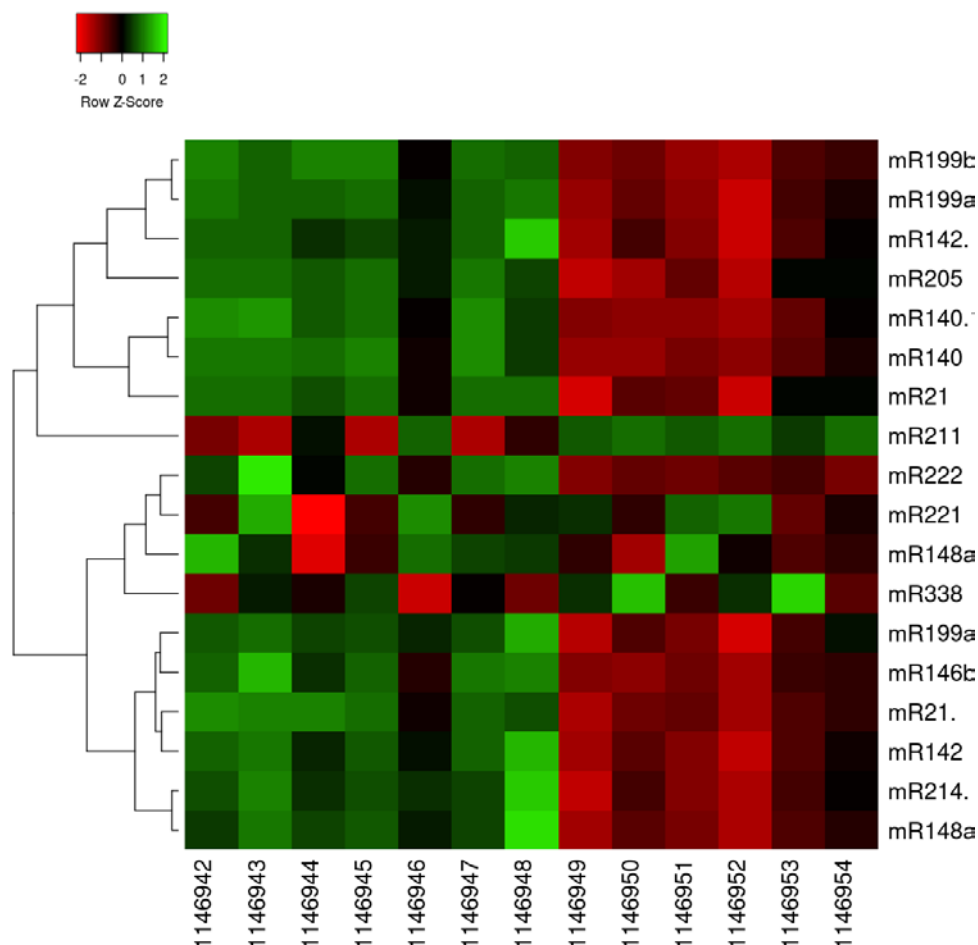
All 21 DEMs were searched and recognized by CluePedia to form regulatory network. After adding 10 hub genes (Pkp3, Usp19, Ercc4, Ttc25, Atp13a2, Akrlb7, Umod, Nup188, Imp3, and

Tmem74b) to the 21 miRNAs, a main connected component including 28 elements (18 microRNAs+10 genes) was constructed. The other 3 microRNAs (mmu-miR-1199-5p, mmu-miR-3096-5p, and mmu-miR-205-5p) were remained as separated elements and did not come in the regulatory network. The first two microRNAs are down-regulated and mmu-miR-205-5p is up-regulated one. The main connected component is shown in the figure 2. Expression amounts of 18 microRNA that are included in the main connected component in the control and treated samples are shown in figure 3. Clustering of 18 hub microRNAs of the constructed regulatory network is presented via heat map. Hub genes that were included in the network are Pkp3, Usp19, Ercc4, Ttc25, Atp13a2, Akrlb7, Umod, Nup188, Imp3, and Tmem74b. Hub genes and their relationships to the microRNAs are shown in the table 1.

Seven clusters of biological processes (BP) were determined as the related BP to the hub genes (figure 4). The ranking of top biological process groups based on containing the highest number of terms are as “negative regulation of double-stranded telomeric DNA binding”, “polyamine transmembrane transport”, “negative regulation of proteolysis involved in cellular protein catabolic process”, “metanephric thick ascending limb development”, and “membrane protein intracellular domain proteolysis”, “desmosome assembly” and “alditol:NADP+ 1-oxidoreductase activity”.



**Figure 2.** Main connected component of regulatory network of 18 miRNAs (in red color) and 10 hub genes. In this network, 28 nodes and 70 links are presented. The miRNA score was 0.6 and the hub genes are shown in the green color.



**Figure 3.** The heat map presentation of the 18 microRNAs of regulatory network. The X axis refers to GSM series including GSM1146948-54 (7 rats as treated group) and GSM1146942-47 (6 rats as controls), right Y axis indicates microRNAs. Green and red colors code up-regulation and down-regulation respectively. Green to reds refers to the intermediate expression levels. Dendrogram is shown in the left Y axis which refers to the clustering of microRNAs.

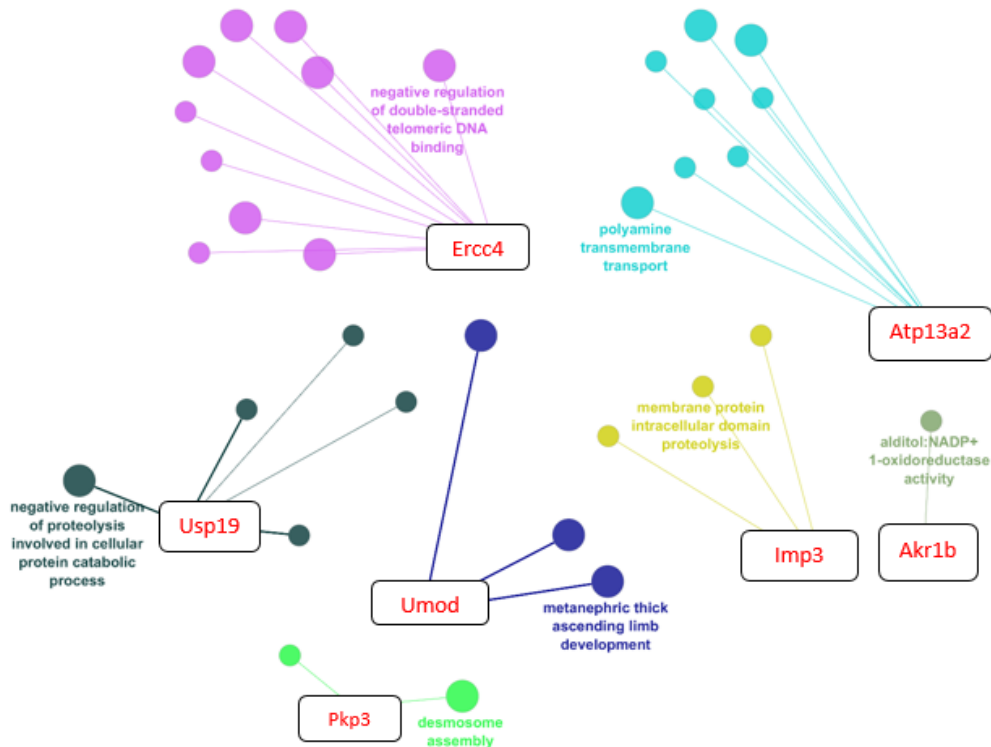
**Table 1.** The list of hub microRNAs and the corresponded hub genes

R	mi-RNA ID	RC	K	FC	Hub genes
1	mmu-miR-199a	Up	9	4.98	Imp3, Tmem74b, Akr1b7, Umod, Ercc4, Pkp3, Usp19, Ttc25, Atp13a2
2	mmu-miR-199b	Up	6	4.42	Tmem74b, Akr1b7, Ercc4, Pkp3, Ttc25, Atp13a2
3	mmu-miR-21	Up	6	7.34	Umod, Nup188, Ercc4, Pkp3, Usp19, Atp13a2
4	mmu-miR-142	Up	5	4.89	Tmem74b, Akr1b7, Umod, Ttc25, Atp13a2
5	mmu-miR-148a	Up	5	4.37	Imp3, Umod, Nup188, Ercc4, Pkp3
6	mmu-miR-214	Up	5	4.37	Umod, Nup188, Ercc4, Pkp3, Usp19

The cut off assigned for hub microRNAs is  $\geq 5$ . R: Row; RC: regulation condition; K: degree; FC: fold change; Up refers to up-regulation

MicroRNAs are the important regulators of gene expression that could play a role in disease pathogenesis. Treatments with natural polyphenols such as curcumin could have some effects on culprit microRNAs and leading them to the normal function [14,19]. Melanoma cancer as one of the most serious kinds of skin cancers has been examined for health benefits of curcumin via microarray study [14]. DEMs were detected in the original research and in our study,

a regulatory network of these elements was investigated. GEO2R analysis implied that the samples of control and treated ones were comparable in terms of expression profile. In the next step, DEMs were assigned as 18 up-regulated and 3 down-regulated ones. Among 21 DEMs, 18 individuals were included in the main connected component of the constructed network which accepts one microRNA the other were upregulated.



**Figure 4.** Biological process analysis of 10 hub genes considering gene per term; 1, gene percentage; 1, and kappa score cut off  $\geq 0.5$ . The related genes are presented in boxes. Ttc25, Nup188, and Tmem74b were not related to the biological processes.

Heatmap analysis showed expression pattern clearly separates samples into two classes of control and treated groups. Among 18 microRNA, mmu-miR-199a, mmu-miR-199b, mmu-miR-21, mmu-miR-142, mmu-miR-148a, and mmu-miR-214 were introduced as hub microRNA. As it is depicted in the table 1 the 6 introduced hub microRNA are associated to all 10 genes and mmu-miR-199a has maximum connections.

The potent hub; mmu-miR-199a is connected to all gene target except Nup188. J Zhou et al. reported that mmu-mir-199a plays a role in the regulation of metastatic genes in the melanoma cells [20]. They showed that in the miR-199a-5p-silenced versus overexpressing cells of studied mice, Cd44, Cdh1, Cxcr4, Etv4, Fxyd5, Rpsa, Mmp3, Myc, Rb1, Tcf20, Hprt1, Actb1 were up-regulate while Ctsk, Itga7, and Tnfsf10 were down-regulated. FC $>2$  was considered to determine the deregulated genes. Involvement in different types of pathological conditions is attributed to the mmu-miR-199 microRNA family such as; progress of liver fibrosis,

impaired mitochondria fatty acid oxidation, and oncogenic activity [21-23].

The changes of microRNAs could have some impact on biological processes via changing the regulation of target genes [24]. As it is shown in figure 4, among 10 target genes; 7 individuals including Imp3, Akr1b7, Umod, Ercc4, Pkp3, Usp19, and Atp13a2 are connected to the associated biological processes. Ercc4 is connected to the cluster of BP which includes most biological terms. There are several reports that Ercc4 mutation is associated to DNA repair defect in various types of diseases such as Fanconi anemia, xeroderma pigmentosum, and cancers [25-28].

ATP13a2 is the other important target gene that is connected to the “polyamine transmembrane transport” cluster of biological processes. Investigations have revealed neuroprotective role of ATP13a2. It is reported that ATP13a2 deficiency leads to neurotoxicity [29]. Prominent role of ATP13a2 deregulation in Parkinson disease is reported by A Di Fonzo et al. [30].

Umod and pcp3 are the two target genes that are related to the five hub microRNA. Highlighted role of Umod deregulation in the kidney disease and hypertension has been investigated by researchers [31,32]. It is emphasized that deregulation of Pcp3 leads to various types of cancers such as breast and gastric cancers [33,34]. As it is discussed, the gene expression study and also gene ontology evaluation revealed the anticancer and also neuroprotective properties of curcumin. It can be concluded that curcumin consumption is associated to the protection of body against important spectrum of diseases such as kidney, skin, vascular and neurodegenerative diseases, and cancer.

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### Author contributions

All of the authors participated in the design, performing the project, and editing of the manuscript. Mona Zamanian-Azodi provided the draft of the manuscript. Mostafa Rezaei-Tavirani edited the final version of the manuscript.

### Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

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### Abbreviations

DEMs: differentially expressed microRNAs; FC: fold change; GEO: Gene Expression Omnibus; RC: regulation condition; K: degree