Int J Adv Life Sci Res. Volume 3(1)16-23
doi: 10.31632/ijalsr.2020v03i01.003

International Journal of Advancement in Life Sciences Research

Online ISSN: 2581-4877

journal homepage http://ijalsr.org

Original Article

Effect of Flavonoid Quercetin in MicroRNA and Transcription Factor Regulatory Network in Hepatocellular Carcinoma

Anamika Basu

Lecturer, Department of Biochemistry, Gurudas College, Kolkata, India

Correspondence E-mail : basuanamikaami@gmail.com*

Abstract

Hepatocellular carcinoma is the second most common cause of death due to cancer. Flavonoids have the ability to control cell division and proliferation in cell biology. In medical science, flavonoids show important effects in cancer prevention and therapy. In this research paper, the effect of flavonoid quercetin on the microRNAs and transcription factors related with hepatocellular carcinoma has been investigated. From microarray dataset, regulatory relationship among miRNAs, TFs and target genes from various databases, are analyzed for hepatocellular carcinoma using CMTCN, a web tool. Regulatory interactions of transcription factors and miRNAs and their target genes play important role in cancer science. In presence of flavonoid quercetin, the up and down regulated genes with their regulators are analyzed in details. Gene network with 500 upregulated genes, shows 109 nodes and 132 edges. Result of gene enrichment analysis with KEGG pathway shows that among 24 genes, 7 genes are related with cancer. Among them P53, MDM2 and PTEN are related with p53 signaling pathway. Gene network with top 500 downregulated genes, illustrates network topology with 146 nodes and 177 edges. Gene enrichment analysis using KEGG pathways, reveals that among 36 genes 9 genes are related to cancer. These genes are APC, CTNNB1, PML, TP53, FOS, JUN, CEBPA, PTEN and Bcl 2. Target gene B-cell lymphoma 2 (Bcl 2), which is down regulated in presence of flavonoid quercetin, has been identified. Here quercetin binds to the BH3 domain of Bcl 2 protein and inhibits its activity which leads to cancer cell apoptosis. Several miRNAs can post-transcriptionally regulates the gene expression of Bcl2 gene. Gene network shows that miRNA has-mir-590 inhibits Bcl 2 target gene. NR112 transcription factor can regulate both target gene and its inhibitor miRNA. By analyzing transcription factor- target gene and miRNA- target gene binding the role of flavonoid quercetin in hepatocellular carcinoma can be elucidated.

Keywords: *Gene regulatory network, miRNA, Transcription factor, flavonoid, quercetin, B-cell lymphoma 2 gene*

Introduction

Cancer is a heterogeneous disease generated by permanent damage of cellular homeostasis and function. Cancer development is a result of uncontrolled cell growth and differentiation accompanied by loss of apoptotic functions of cells leading to a massive enlargement in neoplastic cells mass. Natural phenolic compounds are considered as one of the most important phytochemicals for their chemopreventive and chemotherapeutic

effects in different types of cancers. These compounds show strong activities for cancer prevention and its treatment. There are many phenolic compounds present in medicinal and edible plants such as, flavonoids, bioflavonoids etc (Veeramuthu et al, 2017). Kaempferol is present in apple, grapes, tomato and green tea is flavonoid in nature, is proved to be effective in breast cancer, cervical cancer, human osteosarcoma, lung cancer, pancreatic cancer (López Lázaro et al, 2013) by regulating p53 protein. Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer (Balogh et al, 2016). Hepatocellular carcinoma occurs most often in people with chronic liver diseases, such as cirrhosis caused by hepatitis B or hepatitis C infection. Quercetin (3,30 ,40 ,5,7-pentahydroxy flavone) is a phytochemical belonging to the family of flavonoids. Quercetin has various biological and therapeutic effects such as anti-tumor, hepatoprotective, antioxidant, anti-inflammatory, cardioprotective and anti-diabetic (Ashrafizadeh et al, 2019). Quercetin exerts different antitumor activities such as cell viability reduction, apoptosis induction, cell cycle arrest at S and G2/M phases, autophagy induction, cell migration and invasion suppression by JAK2/STAT3 signaling pathway inhibition in HCC (Fernández-Palanca,et al, 2019). There are a number of genes involved in hepatocellular carcinoma progression, which have been reported to be downregulated in some studies and overexpressed in other studies (e.g. p53) (Niu et al, 2016). A large number of studies are based on a reductionist approach to confirm the role of one or another gene or signaling pathway such as Wnt/β-catenin signaling pathway, PI3K-AKT-mTOR pathway, as a key player in HCC metastasis (Niu et al, 2016). MicroRNAs (MiRNAs) are a class of small (22 nucleotide), non-coding RNAs that control gene expression in a sequence-specific manner and are involved in almost all cellular processes. MiRNAs are able to regulate gene expression at post-transcriptional level by binding to partially complementary mRNA sequences and mediating degradation of that mRNA. MiRNA dysregulation often results in cancer progression as a cause for tumor suppressor gene repression or oncogene de-repression or both (Peng and Crece, 2016),(Zaheer et al, 2019). Many transcription factors (TFs) play crucial very role in the regulation of prostate cancer metastases. For example, p53 is an important TF that is responsible for the progression of HCC by affecting a wide variety of pathways involved in cell proliferation and differentiation, apoptosis, tumor suppressing and various cell signaling pathway (Niu et al, 2016). TFs and miRNAs are two key regulators that control (directly or indirectly) their own genes expression and as well as, the expression of their mutual target genes in the form of feedback and feed-forward loops (FFLs) in gene regulatory network (Peng et al, 2013). TF regulatory network and miRNA regulatory network analyses can help to identify key genomic factors and, importantly, also their interactions, which may deliver insights into the causes of cancer such as HCC (Mohamed et al, 2019). By using these types of FFLs, the effect of quercetin as anticancer drug on target gene in cancer treatment, can be elaborated. Identification of molecular targets for the active constituents present in nutraceuticals, gene network construction with oncogenes and documentation of synergistic effect can be executed with the help of computational techniques. Different types of cancers can be prevented and treated by nutraceuticals and their molecular mechanism of action can be explained and visualized by using computational study.

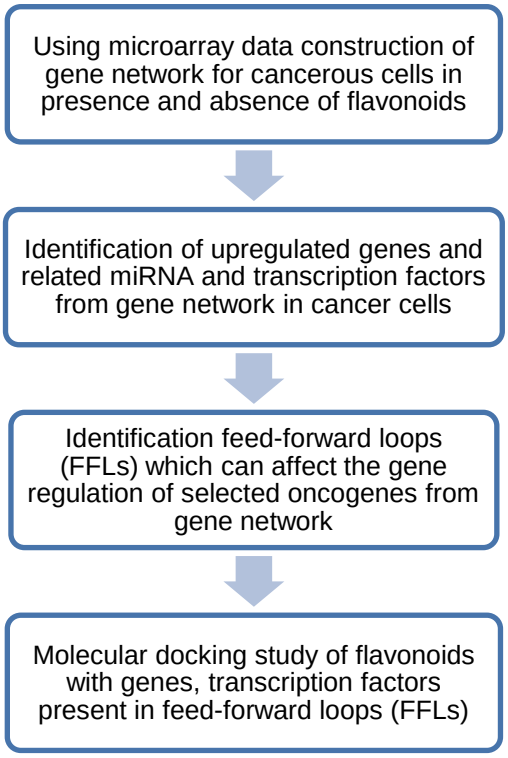
Materials and Methods

1. Analysis of microarray network from NCBI

1.1. *Dataset retrieval*

Microarray dataset for cancer patients is retrieved and downloaded from the National Center for Biotechnology Information (NCBI) GEO database (http://www.ncbi.nlmNih.gov/geo) using Gene expression data of human hepatocellular carcinoma (HCC) with GSE 14520. In this dataset gene expression profiles of different groups are analyzed. Normal individual and cancer patients is selected as control and test

samples. Notas et al, 2012 describe the effect of quercetin on cells of HCC and compare with normal liver cell.



1.2. *Selection of differentially expressed genes*

A web-based tool, GEO2R (http://www.ncbi.nlm.nih. gov/geo/geo2r/) is used to analyze sophisticated R-based analysis of GEO datasets as control group and cancer patients. This tool is based on a t-test (ANOVA) for analysis of variance. Comparison of these two groups of samples across the same experimental conditions is carried out to characterize differentially expressed microRNAs or genes.

1.3. *Pathway enrichment analysis of DEGs*

The Database for Annotation, Visualization and Integrated Discovery (DAVID) v6.8 (Haung et al, 2009) is a compilation web-accessible programs which is used to investigate functional annotation for differently expressed genes according to the KEGG pathway, in which they belong to. Similar tool is applied for functional annotation of genes which are present in gene regulatory network.

1.4. *Construction of gene regulatory network using microRNAs and transcription factors*

CMTCN is a freely available web tool (Li et al, 2018) that provides a comprehensive and intensive analysis of combinatorial regulatory interactions between transcription factors, microRNAs, and target genes. For each type of cancer, CMTCN identifies four types of 3-nodes TF-miRNA co-regulatory motifs (three FFLs and one co-regulation motif) consisting of a TF, a miRNA and their co-targeted gene. CMTCN can construct and depict cancer-specific co-regulatory networks in various tumor types, and meanwhile, it uncovers miRNA and TF co-regulatory relationships. On top of all that, CMTCN screens out important nodes in the co-regulatory network based on network topology analysis and provides genes/TFs/miRNAs enrichment according to different annotated datasets.

2. *Gene regulatory network construction with downregulated genes in cancer cells*

Significantly down-regulated genes ($P < 0.05$) from GEO datasets has been selected. Combinatorial regulatory interrelations between miRNAs, TFs and genes using CMTCN software is executed. The following R packages are utilized in CMTCN are Shiny (>=0.14.2), shinyjs (>=0.8), shinydashboard (>=0.7.0), shinythemes (>=1.1.1), data.table (>=1.10.4-3), DT (>=0.4), RSQLite (>=2.1.0),DBI (>=0.8),ggplot2 (>=2.2.1), ggthemes (>=3.4.2), networkD3 (>=0.4), dplyr (>=0.7.4), markdown (>=0.8), shinycssloaders (>=0.2.0), clusterProfiler (>=3.8.0), GSEABase (>=1.42.0), igraph (>=1.2.1), RTCGAToolbox (>=2.10.0). CMTCN curated cancer-related genes/miRNAs manually for 33 types of cancer by referring to cancer gene/miRNA databases, including TissGDB, SEGreg, IntOGen, HMDD v2.0, miR2Disease, PhenomiR, and miRCancer. CMTCN identified FFLs and co-regulatory pairs from the combinatorial network using the network motif detection algorithm FANMOD and constructed the co-regulatory network and incorporated expression data from The Cancer Genome Atlas (TCGA).

3. Transcription factors, miRNAs and oncogenes identification from gene regulatory network

miRNAs and TFs have been shown to regulate shared target genes in feed-forward-loops (FFLs) and co-regulating pairs. At the network level, miRNA-TF FFLs and co-regulating pairs are major network motifs (i.e., genetic interconnection patterns that occur more often by chance in biological networks), serving as basic building blocks of a complex regulatory system. In the complex cancer-related gene expression regulation networks, miRNAs and TFs can work cooperatively as oncogenes or tumor suppressors.

4. PDB structures extraction for known proteins

The key nodes in a co-regulation network have biological significance because they are signal convergence sites with pronounced control and influence over the network; accordingly, they represent potential candidates for biomarker prediction, clinical prognosis, and treatment. A node with a high hub score contains a large number of outgoing links, and a node with a high authority score is pointed to by many other nodes with high hub scores. Key node is selected according to node degree, hub score, and authority score from network. PDB structures are extracted for proteins and transcription factors with X- ray crystal structures.

5. Homology modeling for unknown protein, DNA

For proteins with unknown crystal structures and three-dimensional structures of mRNAs are modelled with SWISSMODEL (Waterhouse et, al 2018), 3D-DART (van Dijk et al, 2009) respectively.

6. Molecular docking study with protein-DNA-ligand binding

Precise molecular mechanism for anticancer activity of flavonoids can be studied with protein- DNA-ligand binding structures using molecular docking software e.g. AutoDock (Morris et, al 2009) etc.

Results

1. Analysis of microarray network from NCBI

1.1. Dataset retrieval

Gene expression data of human hepatocellular carcinoma (HCC) with dataset GSE 14520 is analyzed by comparing between healthy donor liver (control) and tumor cell for sub-datasets (GSM363450, GSM363451) and (GSM363420, GSM363422) for liver tissue of six healthy donors and liver tumor tissue LCG-333A cells respectively. Using 4 sets down regulated genes are identified as GEO2R result where, T value (-) means up regulated genes in tumor.

1.2. Selection of differentially expressed genes

For differentially expressed genes in GSE14520 in excel file, 197 duplicate genes are removed and 2042 unique genes has been identified.

1.3. Pathway enrichment analysis of DEGs

The Database for Annotation, Visualization and Integrated Discovery (DAVID) v6.8 is a compilation web-accessible programs which is used to investigate functional annotation for differently expressed genes according to the KEGG pathway, shows that among 2042 DEGs, 1285 genes have DAVID IDs.

2. Construction of gene regulatory network using microRNAs and transcription factors

CMTCN is used to provide a comprehensive and intensive analysis of combinatorial regulatory interactions between transcription factors, microRNAs, and target genes for top 500 downregulated genes. Network topology analysis shows that number of nodes present in network in 146 and edges 177 with network density 0.00836 (as shown in Figure 1).

miRNAs and TFs have been shown to regulate shared target genes in feed-forward-loops (FFLs) and co-regulatory pairs

Table 1 Analysis of Top 500 down regulated genes in presence of quercetin

TFs in Co-regulatory Network	Genes in Co-regulatory Network
APC,CTNNB1,ARID1B,CNOT2,NFIB,CREB1,ASB10,BRCA1,MBD1,NR1I2,PML,TP53,FOS,MYCN,JUND,JUNB,PPARA,SMAD1,NR5A2,HNF4A,MYBL2,MYB,TFAP2A,JUN,CEBPA,ESR1,ESR2,GATA3,YBX1,GATA2,FOXA1,GATA1	USP34,CEP68,UBE2D3,MACF1,CLU,SERPING1,PTEN,HHLA1,PRKAB2,HNRNPC,APOH,ITCH,NF1,APOC3,APOB,CXCL2,THBS1,BCL2,ABCG5,PCK1,CYP4A11,FGB,UGT2B4,MYH10,IGFBP1,TAT,HMGCS1,CYP2C19,COL9A2,CCL2

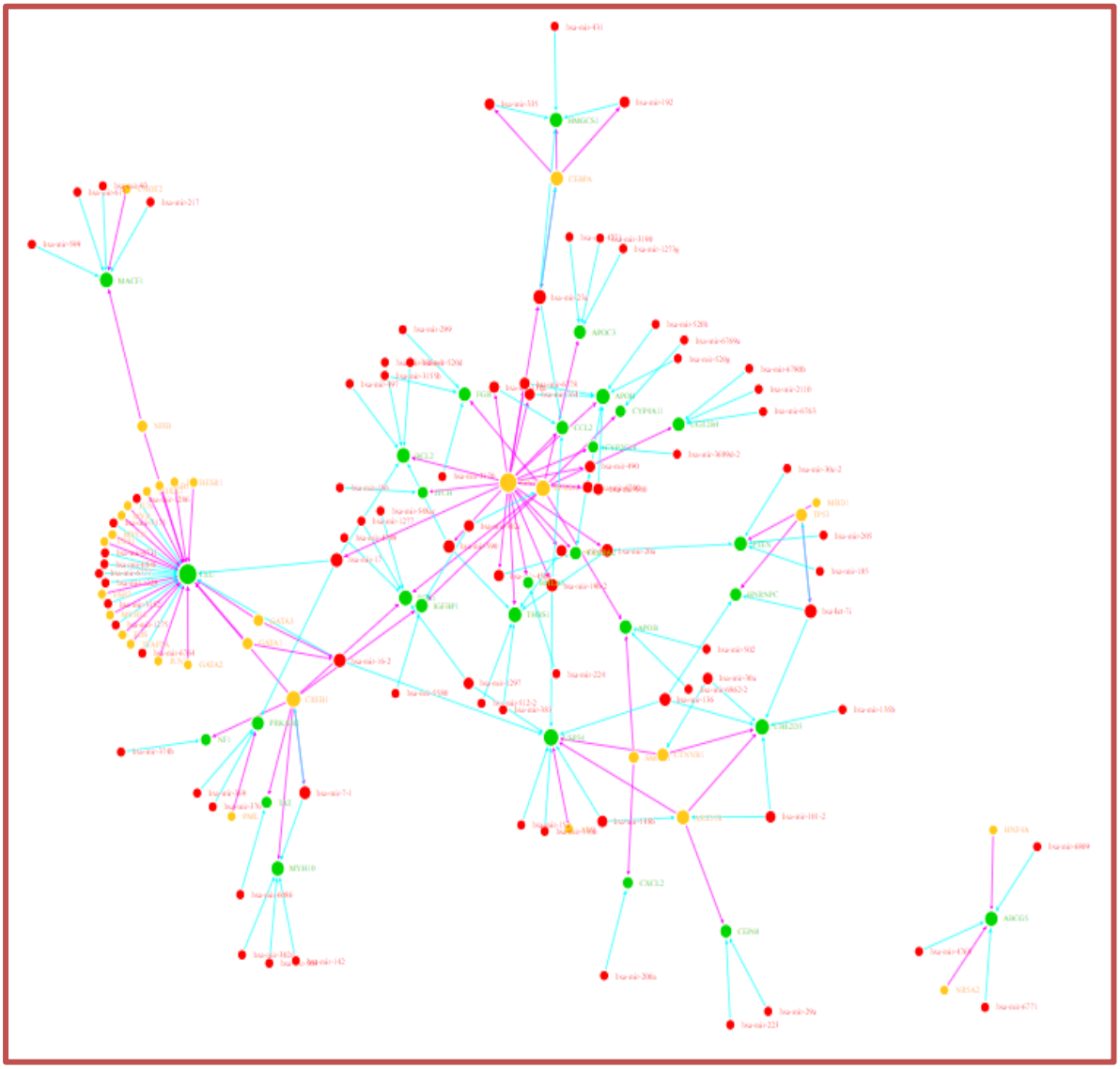


Figure1 Gene regulatory network in HCC with down regulated genes in presence of quercetin

3. Transcription factors, miRNAs and oncogenes identification from gene regulatory network

Functional annotation according to KEGG pathway, shows that genes related with cancer are APC, CTNNB1, PML, TP53, FOS, JUN, CEBPA, PTEN, BCL2. These genes are characterized considering their nodes, degree and group which they belong to in Table 1.

Table 1 Characteristics of genes related with cancer

Nodes	Degree	Group
APC	1	TF
CTNNB1	3	TF
PML	1	TF
TP53	3	TF
FOS	1	TF
JUN	1	TF
CEBPA	5	TF
PTEN	5	Gene
BCL2	6	Gene

So, BCL2 is selected as target gene which is connected with the highest number of nodes in gene regulatory network. Authority scores for BCL2 gene is 0.05003.

Considering the target gene Bcl2, a TF-FFLs network is selected for further analysis. In this network, NR1I2 acts as TF on Bcl2 gene. At the same time, two miRNAs hsa-mir-17 and hsa-mir-590 inhibit the activity of Bcl2 mRNA (as shown in Figure 2).

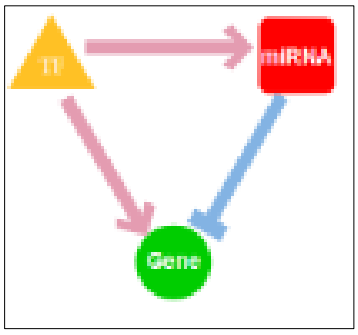


Figure 2 TF-FFLs network

Table 2 Nodes present in TF-FFLs network

Gene	miRNAs	TF
BCL2	hsa-mir-17	NR1I2
BCL2	hsa-mir-590	NR1I2

4. PDB structures extraction for known proteins

PDB structures for NR1I2 and Bcl2 are extracted and modified for further analysis.

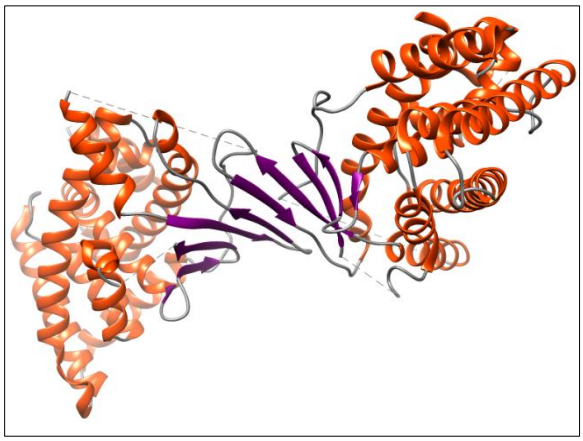


Figure 3 NR1I2 PDB structure

5. Homology modeling for unknown protein, DNA

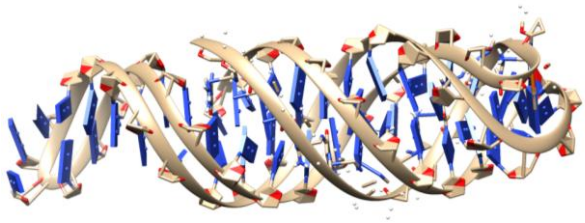


Figure 4 Quadruplex structure of Bcl2 P1 promoter

NR1I2 TF binds to the promoter region of Bcl2 gene. Homology modeling structure of this promoter region is shown in Figure 4.

6. Molecular docking study with protein-DNA-ligand binding

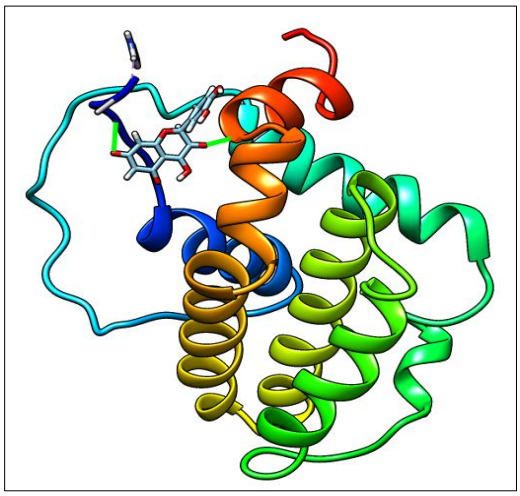


Figure 5 Quercetin binds with Bcl2 protein

Discussion

During tumor formation, regulation at different levels, by transcription factors (TFs) and microRNAs (miRNAs) has been identified at the transcriptional and post transcriptional stages. Specifically, the crosstalk between TFs and miRNAs, mainly affects the gene expression and as a result different cell signaling pathways, related to that gene. In gene regulatory network, TFs and miRNAs can regulate the expression of target gene in cancer through feed-forward loops (FFLs). In various types of cancer, for example in colorectal cancer, multiple myeloma and breast cancer, earlier scientist pointed out the involvement of different miRNAs and TFs in tumor growth and progression. In this biological network, a TF-FFLs network is constructed, where, Bcl2 is a protein which can regulate cell death (apoptosis) (Tsujimoto, 1998). Phytochemical quercetin can bind directly to the BH3 domain of Bcl2 protein (Primikyri et al, 2014) and thus it can inhibit the activity of this protein and results in promoting cancer cell apoptosis. Similarly, NR1I2 is a TF present in TF-FFLs network. Nuclear receptor subfamily 1 group I member 2 (NR1I2) protein has a DNA-binding domain, to exhibit DNA- binding transcription factor activity. Furthermore, this protein can bind with and activated by various plant metabolites e.g. quercetin etc (Avior et al, 2013). Quercetin can act as activator of human gene Nuclear receptor subfamily 1 group I member 2 (NR1I2). Quercetin also binds with target protein Bcl2 with -7.25 Kcal/ mole binding energy, forming H -bonding with ASN 11 and ASP196 amino acid residues. In presence of quercetin, the binding of NR1I2 with Bcl2 promoter region, would be affected. In future course of study, the docking study of transcription factor NR1I2 with this promoter region should be executed.

Conclusion

From this current work, from gene regulatory network for HCC patient, Bcl2 is identified as target gene in cancer treatment and effect of quercetin on this TF-FFLs network has been elucidated.

Acknowledgement:

Author is thankful to Dr. Achintya Saha and his research scholars of the Drug design laboratory, Department of Chemical Technology, under University of Calcutta, India for providing all the necessary facilities and support.

References

Ashrafizadeh, M., Ahmadi, Z., Farkhondeh, T., & Samarghandian, S. (2019). Autophagy as a molecular target of quercetin underlying its protective effects in human diseases. *Archives of Physiology and Biochemistry*, 1-9.

Avior, Y., Bomze, D., Ramon, O., & Nahmias, Y. (2013). Flavonoids as dietary regulators of nuclear receptor activity. *Food & function*, *4*(6), 831-844.

Balogh, J., David Victor III, E. H. A., Burroughs, S. G., Boktour, M., Saharia, A., Li, X., ... & Monsour Jr, H. P. (2016). Hepatocellular carcinoma: a review. *Journal of hepatocellular carcinoma*, *3*, 41.

Fernández-Palanca, P., Fondevila, F., Méndez-Blanco, C., Tuñón, M. J., González-Gallego, J., & Mauriz, J. L. (2019). Antitumor Effects of Quercetin in Hepatocarcinoma In Vitro and In Vivo Models: A Systematic Review. *Nutrients*, *11*(12), 2875.

http://www.ncbi.nlm.nih. gov/geo/geo2r/

http://www.ncbi.nlmNih.gov/geo

Huang DW, Sherman BT, Lempicki RA. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. *Nucleic Acids Res. 2009;37(1):1-13.*

Li, R., Chen, H., Jiang, S., Li, W., Li, H., Zhang, Z., ... & Bo, X. (2018). CMTCN: a web tool for investigating cancer-specific microRNA and transcription factor co-regulatory networks. *PeerJ*, *6*, e5951.

López Lázaro, M., Burgos Morón, E., Tena, J. D., & Calderón Montaño, J. M. (2013). Consumption of the Dietary Flavonoids Quercetin, Luteolin and Kaempferol and

Overall Risk of Cancer-A Review and Meta-Analysis of the Epidemiological Data. *WebmedCentral, 4 (5)*.

Mohamed, R. H., Abu-Shahba, N., Mahmoud, M., Abdelfattah, A. M., Zakaria, W., & ElHefnawi, M. (2019). Co-regulatory Network of oncosuppressor miRNAs and transcription Factors for pathology of Human Hepatic Cancer stem Cells (HCsC). *Scientific reports*, *9*(1), 5564.

Niu, Z. S., Niu, X. J., & Wang, W. H. (2016). Genetic alterations in hepatocellular carcinoma: An update. *World journal of gastroenterology*, *22*(41), 9069.

Notas, G., Nifli, A. P., Kampa, M., Pelekanou, V., Alexaki, V. I., Theodoropoulos, P., ... & Castanas, E. (2012). Quercetin accumulates in nuclear structures and triggers specific gene expression in epithelial cells. *The Journal of nutritional biochemistry*, *23*(6), 656-666.

Peng, C., Wang, M., Shen, Y., Feng, H., & Li, A. (2013). Reconstruction and analysis of transcription factor–miRNA co-regulatory feed-forward loops in human cancers using filter-wrapper feature selection. *PloS one*, *8*(10), e78197.

Peng, Y., & Croce, C. M. (2016). The role of MicroRNAs in human cancer. *Signal transduction and targeted therapy*, *1*, 15004.

Primikyri, A., Chatziathanasiadou, M. V., Karali, E., Kostaras, E., Mantzaris, M. D., Hatzimichael, E., ... & Gerothanassis, I. P. (2014). Direct binding of Bcl-2 family proteins by quercetin triggers its pro-apoptotic activity. *ACS chemical biology*, *9*(12), 2737-2741.

Tsujimoto, Y. (1998). Role of Bcl-2 family proteins in apoptosis: apoptosomes or mitochondria?. *Genes to cells*, *3*(11), 697-707.

van Dijk, M., & Bonvin, A. M. (2009). 3D-DART: a DNA structure modelling server. *Nucleic acids research*, *37*(suppl_2), W235-W239.

Veeramuthu, D., Raja, W. R. T., Al-Dhabi, N. A., & Savarimuthu, I. (2017). Flavonoids: Anticancer Properties. *Flavonoids: From Biosynthesis to Human Health*, 287.

Waterhouse, A., Bertoni, M., Bienert, S., Studer, G., Tauriello, G., Gumienny, R., Heer, F.T., de Beer, T.A.P., Rempfer, C., Bordoli, L., Lepore, R., Schwede, T. SWISS-MODEL: homology modelling of protein structures and complexes. Nucleic Acids Res. 46, W296-W303 (2018)

Zaheer, U., Faheem, M., Qadri, I., Begum, N., Yassine, H. M., Al Thani, A. A., & Mathew, S. (2019). Expression profile of MicroRNA: An Emerging Hallmark of Cancer. *Current pharmaceutical design*, *25*(6), 642-653.