# Network Pharmacology Approach Reveals Potential Mechanisms of Quercetin in Treating Liver Cancer Through Inhibition of PIK3CG, IGF1R, and MET Gene Expression

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**Abstract:**

**Aim and objective:**

Liver cancer is a major health concern with high mortality rates. Quercetin, a flavonoid found in Ginkgo biloba leaves, has potential in liver cancer therapy. It inhibits cancer cell growth, induces apoptosis, and possesses anti-inflammatory and antioxidant properties. Further research is needed to explore quercetin's network pharmacological effects and its use in liver cancer treatment.

**Materials and methods:** Liver cancer a major health concern worldwide and effective treatment options are limited. The study presented here utilized a novel network-pharmacology approach to investigate the potential of quercetin as a treatment for liver cancer. By combining target fishing, Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis, and compound-target-pathway network structured analysis, the researchers were able to gain insights into the mechanisms by which quercetin exerts its therapeutic effects. The results suggest that quercetin may act through multiple pathways, including those involved in cell cycle regulation, apoptosis, and inflammation. Furthermore, the compound-target-pathway network analysis revealed potential targets for quercetin in liver cancer cells. Overall, these findings provide important

insights into the potential use of quercetin as a therapeutic agent for liver cancer and highlight the value of network pharmacology approaches in drug discovery research.

**Results:** DisGeNET identified 11 differential co-expression genes associated with liver cancer, enriched in biological processes and enzyme inhibitor activity. Five hub genes (PIK3CG, IGF1R, MET, TOP1, and ACHE) were found in a PPI network, suggesting quercetin may be useful in the management of liver cancer. Recent studies have shown that the genes PIK3CG, IGF1R, and MET are associated with liver cancer. Quercetin, a natural compound found in fruits & vegetables, has been shown to inhibit the expression of these genes and have anti-cancer properties. More research is needed to understand its potential benefits.

**Keywords:** Network pharmacology, quercetin, liver cancer, pharmacological mechanism, pharmacokinetic, signaling pathway. .

**Introduction:**

One of the main fatal liver illnesses is liver cancer. According to the most recent estimates from WHO, the global burden of liver cancer (LC) in a span of twenty years would rise by 55 percent by 2040. By 2020, 830,200 people will die from liver cancer, and 905,700 will be diagnosed with it globally. 1.3 million individuals will die from liver cancer by 2040, as per the estimations made by scientists using current incidence and mortality rates. The projections indicate an urgent need for increased efforts towards prevention, early detection, and treatment of liver cancer.

In most cases, liver cancer is diagnosed in advanced stages, and the patients face several problems, such as: a) losing the opportunity for surgical resection; b) most chemotherapeutic drugs used in advanced LC having toxic or side effects. c) Few treatments have been able to effectively improve the prognosis of advanced LC. Due to these limitations, alternative therapies are being used to improve the quality of life. A combination of both allopathic and flavonoids, such as quercetin, has been found to improve the survival of patients.

Quercetin is 3,5,7,3′,4′-pentahydroxyflavone, the main member of flavonoid subclass of flavanol’s. In numerous cancer models, quercetin has been widely researched as a

chemoprevention drug because of its anti-oxidant, anti-tumor, and anti-inflammatory activities [Jeong, J.H., et al., 1993]. Many malignancies, including prostate, cervical, lung, breast, and colon cancers, have been shown to be inhibited by quercetin's effect. According to recent studies [Yang et al., 2005; Lee, T.J et al., 2006], quercetin suppresses cell proliferation via inducing apoptosis and/or cell cycle arrest. [Choi, J.A., et al.,2001, Ong, C.S., et al., 2004, Beniston, and Campo's 2003] study found that quercetin consumption causes cell cycle apprehension in a variety of cell types, including G2/M and G1 seizure. Furthermore, cytochrome c release, caspase activation, stress protein stimulation, disruption of microtubules and mitochondria, and the disruption of microtubules and mitochondria may all contribute to quercetin-mediated apoptosis [Yoshizumi, M., et al., 2001]. Based on the findings of quercetin The purpose of the current study was to identify a mechanistic approach and determine a network pharmacology approach for quercetin's liver activity.

Network pharmacology is a tool to analyze and evaluate pharmacological targets, a method that combines network analysis, computational biology, systems biology, multidirectional pharmacology, and other cutting-edge ideas and techniques [Zhang, W., et al 2016, Chandran, U., et al., 2017, Sakle, N.S., et al 2020, Wei, C., et al., 2022, Zhang, Q., et al 2021]. The major application techniques of contemporary medicine for the diagnosis and treatment of ailments involve the use of flavonoids [Davis, J.M., et al 2009, Li, Y., Yao, et al 2016,]. By applying network pharmacology techniques in the study of the relationship between flavonoid quercetin and the complicated system of biological organisms, it is possible to determine the molecular mechanism of drug prevention and treatment as well as to elucidate the mechanism of action of flavonoid quercetin from a molecular perspective [David, A.V.A., et al 2016, Yang, D., et al 2020, Salehi, B., et al 2020]. The functional analysis of pharmaceuticals has made extensive use of network pharmacology [Zu, G. et al 2021, Kodam, P., et al 2023, Yang, L., et al 2020, Wei, C., et al 2022, Zhang, M., 2021]. Network pharmacology was employed in the current study to look into the molecular pathways underlying quercetin's anticancer property to treat liver cancer.

**Materials and Methods:**

**Quercetin:**

The PubChem database was used to find the molecular structure of quercetin. The 2D and 3D structures of quercetin are illustrated in Fig.1.

**Quercetin target gene identification**

In addition to the PubChem database, various other tools were utilized in this network pharmacology study. The database and analysis tools used were carefully selected to ensure the accuracy and reliability of the results. For instance, Cytoscape was used to construct and visualize the network of quercetin target genes. STRING database was employed to obtain protein-protein interaction data, which was then integrated with gene expression data from the GEO and TCGA databases. Furthermore, pathway enrichment analysis was conducted using the DAVID tool to identify enriched pathways associated with quercetin target genes. Overall, a comprehensive approach was taken in this study to identify potential therapeutic targets of quercetin through network pharmacology analysis. The findings of this investigation offer significant perspectives into molecular mechanisms underlying the therapeutic effects of quercetin and may aid in the development of novel treatments for various diseases.

**Network Establishment**

The primary quercetin targets for liver cancer therapy were determined by examining the overlap between quercetin targets and genes linked to liver cancer. We used the software Cytoscape 3.8.0 to visualize the interactions between quercetin, genes, and their targets (www.cytoscape.org). The disease, significant targets, and quercetin were represented by the nodes of the interaction network for quercetin therapy of liver cancer in Microsoft Excel. The network was finally loaded into Cytoscape 3.8.0 for additional analysis. Fig.2 depicts the interaction.

**GO and KEGG Enrichment Analysis:**

The DAVID database, which is a database for annotation, visualization, and integrated discovery, was used in this work to perform GO and KEGG enrichment analyses. David, an online biological knowledge library and analytical tool, was used to map the predicted target genes and discover the relevant biological processes, cellular components, molecular activities, and KEGG pathways. The KEGG database was also used to create the map of the KEGG signaling pathways and shown in Fig.3.

**Results and Discussion:**

**Prediction of the Quercetin targets:**

The chemical structure of quercetin is shown in Figure 1A, which was retrieved from the PubChem database. The Binding DB and Swiss Target Prediction database systems were used to estimate probable quercetin target genes in accordance with its structure. Following the screening of common target genes, 42 targets were chosen for quercetin from the 187 probable target genes collected from the databases. The targets are represented in Fig. 4 & 5.

**Network Analysis: Cytospace**

Cytospace helps identify the interaction between quercetin, genes, and diseases and shown in Fig.6. The Cytohubb plugin of Cytoscape was employed to identify five hubb genes (PIK3CG, IGF1R, MET, TOP1, and ACHE) in a protein-protein interaction (PPI) network with 11 nodes and 4 edges. The hub genes and their interactions were illustrated in Table.1.

**String Analysis:**

The identification of top hub genes is a vital step in understanding the biological activity of quercetin treatment. In this present study, in fig.7 six hub genes were indicated in red, including IGF1R, PIK3CG, LCK, GSK3B, ACHE, and MET. These genes play a critical role in the network and are likely to be essential for the biological activity of quercetin treatment. Additionally, we found that other genes associated with these hubb genes were displayed as purple nodes in the network. This observation suggests that these genes may also be involved in the quercetin treatment process. By identifying these key genes and their associations with other genes in the network, we can gain a better understanding of how quercetin works at a molecular level.

**Functional Enrichment Analysis for Quercetin Targets**

To gain a complete knowledge of these target genes, GO and KEGG enrichment analyses were done utilizing the DAVID database. There were 22 biological activities and 9 chemical functions among the enriched GO keywords. Figure 1 shows the top five GO phrases. Negative modulation of programmed cell death, cellular response to oxygen-containing

compounds, protein self-phosphorylation and positively regulated protein kinase B signaling were all detected. Carbonate dehydratase activity, phosphatidylinositol 3-kinase binding, protein tyrosine enzyme activity, and activity of kinase were found to be enriched, suggesting that they may be involved in the physiological functioning of the quercetin treatment procedure. There were also 13 enhanced KEGG pathways Fig.8.

The significant genes found in hepatocellular carcinoma suggest that quercetin may target frequently altered genes involved in critical driver signaling processes, such as telomere maintenance level, TP53 protein, the cell cycle regulation, the Wnt/beta-catenin path (CTNNB1 with AXIN1), and phosphatidylinositol-3 kinase (PI3K). Furthermore, recurrent mutations have been found in new driver genes implicated in chromatin remodeling (ARID1A and ARID2) and oxidative stress (NFE2L2) pathways (discovered by whole-exome sequencing).

**Conclusion:**

Liver cancer is a growing concern, with the number of new cases and deaths predicted to rise significantly in the coming years. While traditional treatments such as chemotherapy and radiation remain the standard of care, researchers are exploring alternative options such as natural supplements like quercetin.

Studies have shown that quercetin may have anti-cancer effects in liver cancer by inhibiting tumor growth and inducing cell death. One such study found that quercetin may exert its anticancer effects by targeting several key pathways involved in liver cancer development, including the PI3K/Akt/mTOR, the MAPK/ERK, and the JAK/STAT pathways. Quercetin has antioxidant & anti-inflammatory properties, which may contribute to its potential anticancer effects in liver cancer. However, more research is needed to fully understand its potential benefits and limitations. Additionally, efforts should be made to raise awareness about liver cancer and improve access to screening and treatment options in order to address this growing public health concern in India and around the world.

**References:**

Beniston, R.G. and Campo, M.S., 2003. Quercetin elevates p27Kip1 and arrests both primary and HPV16 E6/E7 transformed human keratinocytes in G1. *Oncogene*, *22*(35), pp.5504-5514.

Chandran, U., Mehendale, N., Patil, S., Chaguturu, R. and Patwardhan, B., 2017. Network pharmacology. *Innovative approaches in drug discovery*, p.127.

Choi, J.A., Kim, J.Y., Lee, J.Y., Kang, C.M., Kwon, H.J., Yoo, Y.D., Kim, T.W., Lee, Y.S. and Lee, S.J., 2001. Induction of cell cycle arrest and apoptosis in human breast cancer cells by quercetin. *International journal of oncology*, *19*(4), pp.837-844.

David, A.V.A., Arulmoli, R. and Parasuraman, S., 2016. Overviews of biological importance of quercetin: A bioactive flavonoid. *Pharmacognosy reviews*, *10*(20), p.84.

Davis, J.M., Murphy, E.A. and Carmichael, M.D., 2009. Effects of the dietary flavonoid quercetin upon performance and health. *Current sports medicine reports*, *8*(4), pp.206-213.

Jeong, J.H., An, J.Y., Kwon, Y.T., Rhee, J.G. and Lee, Y.J., 2009. Effects of low dose quercetin: Cancer cell- specific inhibition of cell cycle progression. *Journal of cellular biochemistry*, *106*(1), pp.73-82.

Kodam, P., Sai Swaroop, R., Pradhan, S.S., Sivaramakrishnan, V. and Vadrevu, R., 2023. Integrated multi-omics analysis of Alzheimer's disease shows molecular signatures associated with disease progression and potential therapeutic targets. *Scientific Reports*, *13*(1), p.3695.

Lee, T.J., Kim, O.H., Kim, Y.H., Lim, J.H., Kim, S., Park, J.W. and Kwon, T.K., 2006. Quercetin arrests G2/M phase and induces caspase-dependent cell death in U937 cells. *Cancer letters*, *240*(2), pp.234-242.

Li, Y., Yao, J., Han, C., Yang, J., Chaudhry, M.T., Wang, S., Liu, H. and Yin, Y., 2016. Quercetin, inflammation and immunity. *Nutrients*, *8*(3), p.167.

Ong, C.S., Tran, E., Nguyen, T.T., Ong, C.K., Lee, S.K., Lee, J.J., Ng, C.P., Leong, C. and Huynh, H., 2004. Quercetin-induced growth inhibition and cell death in nasopharyngeal carcinoma cells are associated with increase in Bad and hypophosphorylated retinoblastoma expressions. *Oncology reports*, *11*(3), pp.727-733.

Sakle, N.S., More, S.A. and Mokale, S.N., 2020. A network pharmacology-based approach to explore potential targets of Caesalpinia pulcherima: An updated prototype in drug discovery. *Scientific reports*, *10*(1), p.17217.

Salehi, B., Machin, L., Monzote, L., Sharifi-Rad, J., Ezzat, S.M., Salem, M.A., Merghany, R.M., El Mahdy, N.M., Kılıç, C.S., Sytar, O. and Sharifi-Rad, M., 2020. Therapeutic potential of quercetin: New insights and perspectives for human health. *Acs Omega*, *5*(20), pp.11849-11872.

Wei, C., Li, S., Zhu, Y., Chen, W., Li, C. and Xu, R., 2022. Network pharmacology identify intersection genes of quercetin and Alzheimer's disease as potential therapeutic targets. *Frontiers in Aging Neuroscience*.

Wei, C., Li, S., Zhu, Y., Chen, W., Li, C. and Xu, R., 2022. Network pharmacology identify intersection genes of quercetin and Alzheimer's disease as potential therapeutic targets. *Frontiers in Aging Neuroscience*.

Yang, D., Wang, T., Long, M. and Li, P., 2020. Quercetin: its main pharmacological activity and potential application in clinical medicine. *Oxidative Medicine and Cellular Longevity*, *2020*.

Yang, L., Hu, Z., Zhu, J., Liang, Q., Zhou, H., Li, J., Fan, X., Zhao, Z., Pan, H. and Fei, B., 2020. Systematic elucidation of the mechanism of quercetin against gastric cancer via network pharmacology approach. *BioMed research international*, *2020*.

Yoshizumi, M., Tsuchiya, K., Kirima, K., Kyaw, M., Suzaki, Y. and Tamaki, T., 2001. Quercetin inhibits Shc-and phosphatidylinositol 3-kinase-mediated c-Jun N-terminal kinase activation by angiotensin II in cultured rat aortic smooth muscle cells. *Molecular Pharmacology*, *60*(4), pp.656-665.

Zhang, M., Yang, J., Zhao, X., Zhao, Y. and Zhu, S., 2021. Network pharmacology and molecular docking study on the active ingredients of qidengmingmu capsule for the treatment of diabetic retinopathy. *Scientific Reports*, *11*(1), pp.1-11.

Zhang, Q., Wen, F., Sun, F., Xu, Z., Liu, Y., Tao, C., Sun, F., Jiang, M., Yang, M. and Yao, J., 2022. Efficacy and Mechanism of Quercetin in the Treatment of Experimental Colitis Using Network Pharmacology Analysis. *Molecules*, *28*(1), p.146.

Zhang, W., 2016. Network pharmacology: A further description. *Network Pharmacology*, *1*(1), pp.1-14.

Zu, G., Sun, K., Li, L., Zu, X., Han, T. and Huang, H., 2021. Mechanism of quercetin therapeutic targets for Alzheimer disease and type 2 diabetes mellitus. *Scientific Reports*, *11*(1), p.22959.

**Tables:**

**Table.1: The hub genes and their interactions**

| Top 10 in network Merged Network ranked by MCC method | | |
|---|---|---|
| Rank | Name | Score |
| 1 | Liver carcinoma | 11 |
| 1 | Quercitin | 11 |
| 3 | Liver and Intrahepatic Biliary Tract Carcinoma | 6 |
| 3 | Adult Liver Carcinoma | 6 |
| 5 | PIK3CG | 5 |
| 5 | IGF1R | 5 |
| 5 | MET | 5 |
| 5 | Cholangiocarcinoma | 5 |
| 9 | TOP1 | 4 |
| 9 | ACHE | 4 |

**Figures:**

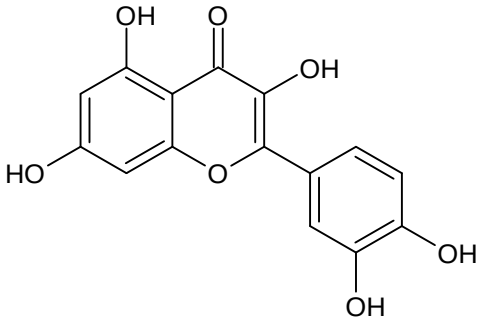


**Figure1: Quercetin Chemical Structure**

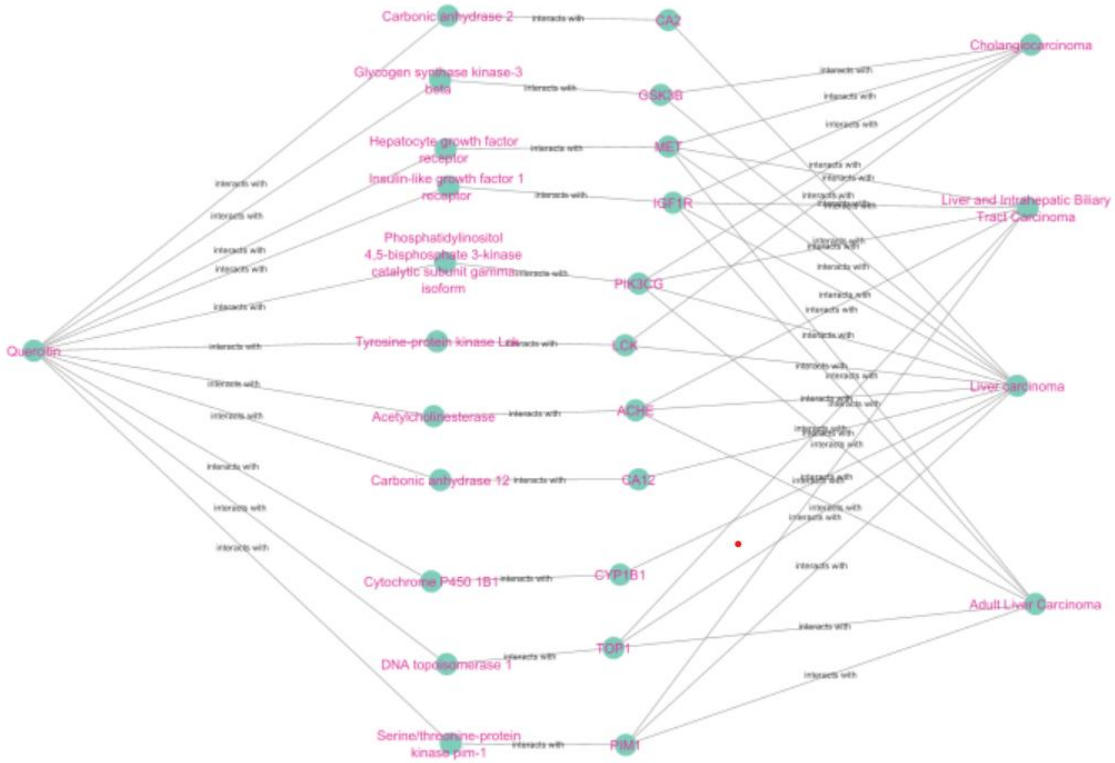


**Figure 2: Network of interactions between quercetin, genes, and their targets**

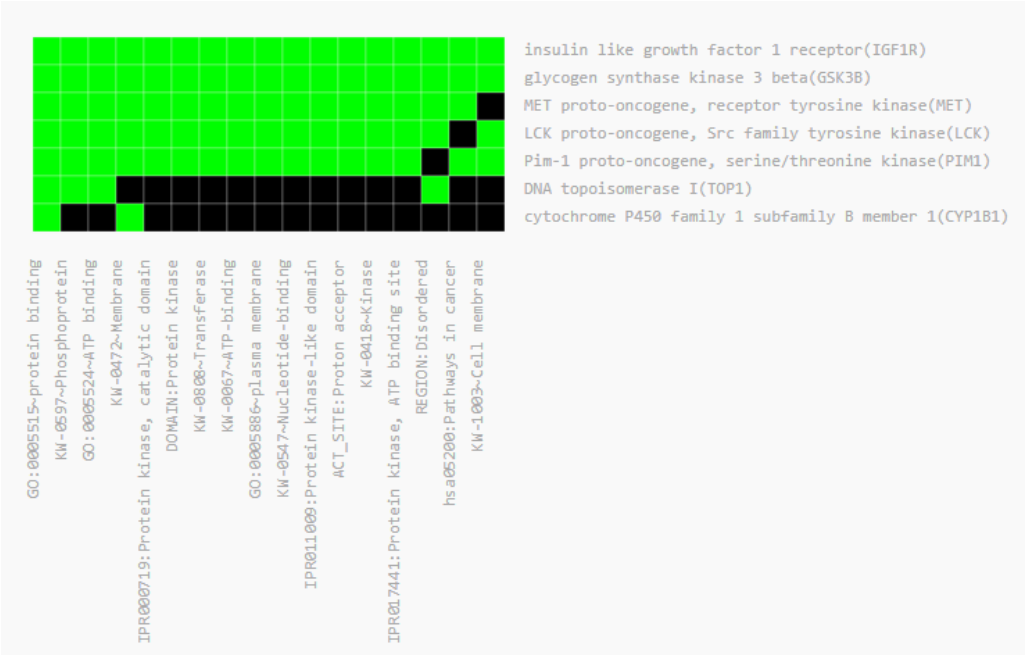


**Figure 3: GO-Enrichment analyses**

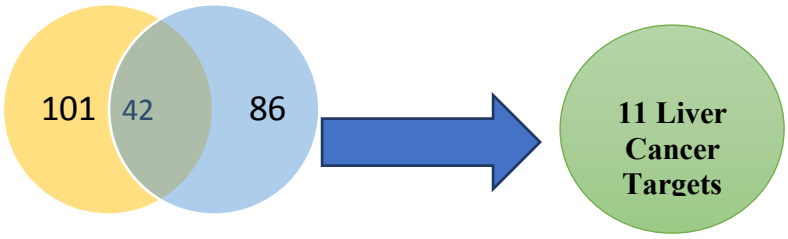


**Figure 4: Venn diagram**

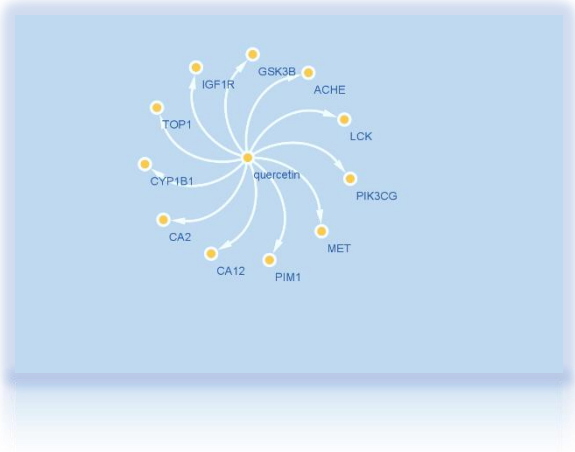


**Figure 5: Interaction of Quercetin with targets**

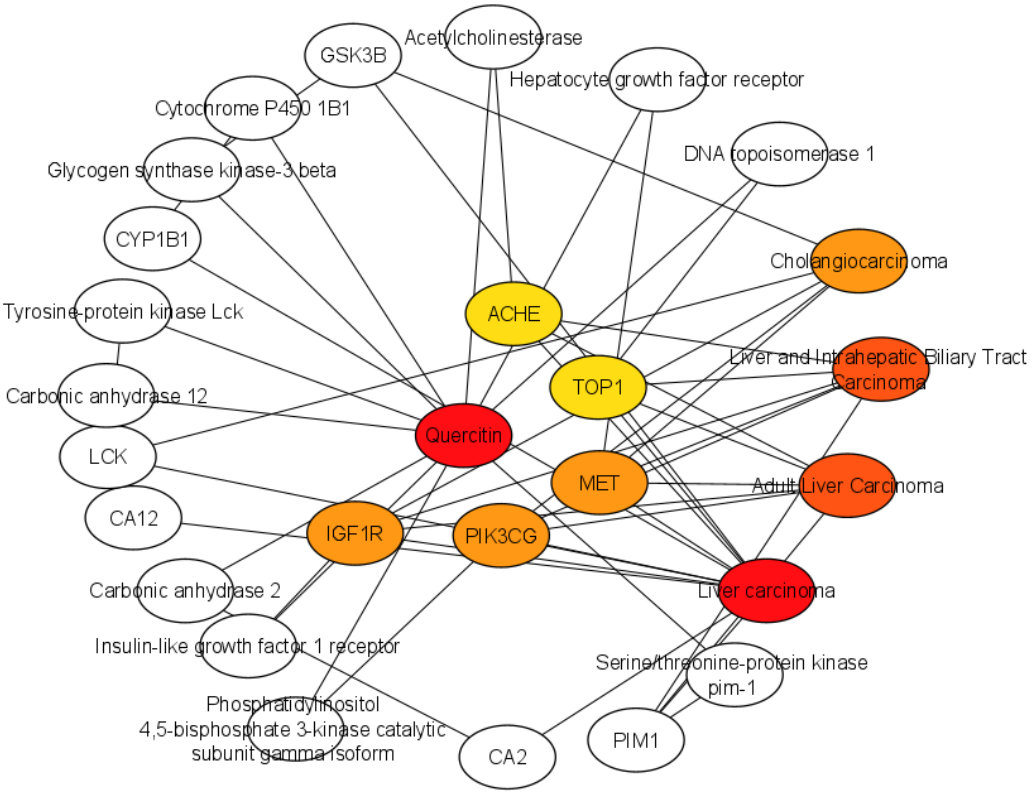


**Figure 6: Network Pharmacology by Cytospace**

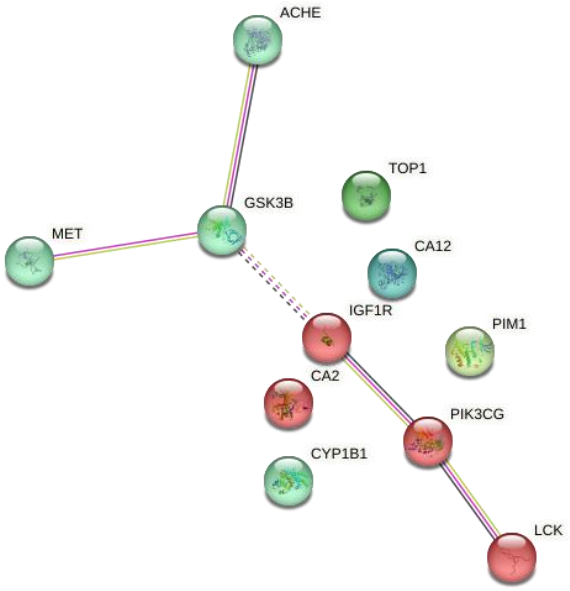


**Figure 7: Protein-protein interaction (PPI)**

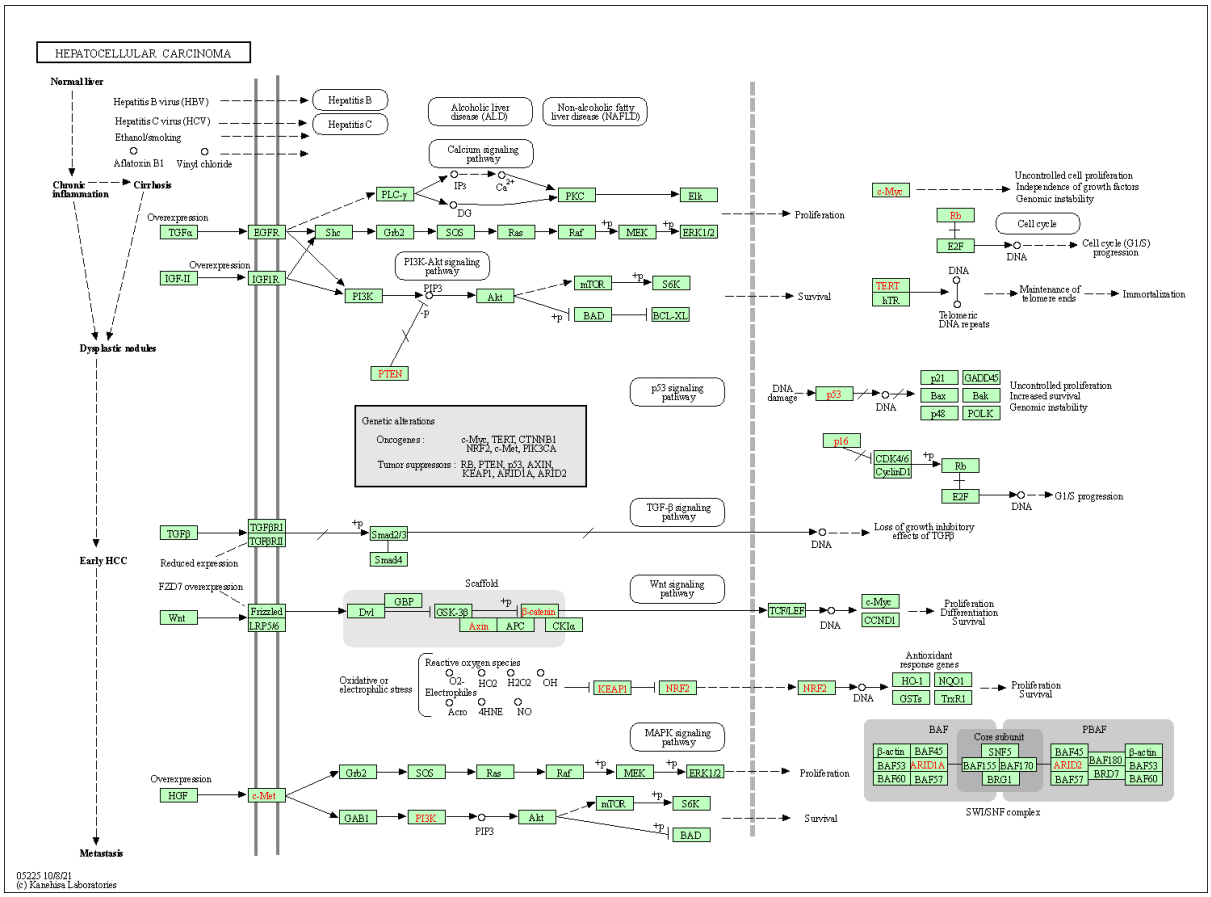


**Figure 8: KEGG Pathway**