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Free Radical-Induced Inflammatory Responses Activate PPAR-γ and TNF-α Feedback Loops, Driving HIF-α Mediated Metastasis in HCC: Insilico Approach of Natural Compounds Inhibitory Effect on Proposed Pathway

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Abstract

Hepatocellular carcinoma (HCC), a leading cause of cancer-related deaths, arises from complex interactions between hypoxia, chronic inflammation, and metabolic dysregulation within the tumor microenvironment. Key drivers of these processes include hypoxia-inducible factor 1-alpha (HIF-1α), tumor necrosis factor-alpha (TNF-α), and peroxisome proliferator-activated receptor gamma (PPARγ). This study evaluates the therapeutic potential of several natural compounds, including curcumin, resveratrol, epigallocatechin gallate (EGCG), berberine, gingerol, andrographolide, luteolin, apigenin, sulforaphane, omega-3 fatty acids, and ginseng, using CB-Dock2 for computational docking analysis. Results reveal high-affinity interactions between these compounds and their respective targets, indicating their potential to inhibit HIF-1α stabilization, suppress TNF-α-mediated inflammation, and activate PPARγ to restore metabolic homeostasis.Curcumin and EGCG demonstrated robust inhibition of HIF-1α, reducing angiogenesis and tumor progression. Resveratrol, luteolin, and andrographolide effectively modulated TNF-α signalling, attenuating inflammation and epithelial-mesenchymal transition (EMT). Compounds such as omega-3 fatty acids and ginseng activated PPARγ, regulating lipid metabolism and immune responses. The multi-targeted actions of these natural agents disrupt angiogenesis, inflammation, and metastasis, offering a holistic approach to combating HCC. This study highlights the promise of natural compounds as complementary therapies, emphasizing their bioavailability, safety, and potential synergy with conventional treatments in addressing the complexity of HCC progression.

Keywords: *Hepatocellular Carcinoma (HCC), PPARγ, HIF-1α, TNF-α, Feedback Loops, Natural Compounds.*

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common types of primary liver cancer and constitutes 70-90% of all cases of liver cancer worldwide. It has continued to be a major health problem owing to late diagnosis and high recurrence rates associated with the aggressive nature of HCC, even in the era of tremendous progress in medical technologies and wider therapeutic options. This is highly complex type of cancer breaks down because unique microenvironment established inside the tumor, that hypoxia, long-term infection and lipid fat burning capacity adjustments are ideal responsible. HIF-1α, TNF-α and PPARγ are the main regulatory proteins in this process. Given the critical functions of these proteins in cancer biology, their investigations might provide clues to novel avenues for treatment of HCC.

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Function of HIF-1α in Tumor Adaptation

Hypoxia, a state of lack of oxygen, is one of the defining features of solid tumors and occurs as a result of growing tumors that outgrow their vascular supply. HIF-1α is important in the hypoxic response that allow tumor cells to survive in low oxygen tensions. In normoxic conditions, HIF-1α is rapidly degraded by prolyl hydroxylases that tag it for proteasomal degradation via the product of the von Hippel-Lindau gene. In contrast, in the presence of hypoxia the degradation process of HIF-1α is prevented resulting in translocation to the nucleus where it dimerizes with HIF-1β and activates transcription of numerous tumour cell survival genes.

• Angiogenesis and Tumor Vascularization: A central role of HIF-1α is the promotion of angiogenesis, due to elevated levels of expression of VEGF. This protein plays a vital role in the formation of new blood vessels that ultimately provide the nutrients necessary for tumor growth. In patients, elevated levels of VEGF have been correlated with poor prognosis not only in HCC but also other malignancies such as breast cancer and lung cancer.

Metabolism switch: HIF-1α drives more of a metabolic shift in cancer cells to glycolysis for energy production, even though oxygen is still present. This phenomenon, referred to as the Warburg effect, is regulated by transcriptional activation of glycolytic enzymes such as Hexokinase 2 and Glucose Transporter 1 mediated through HIF-1α. This leads to elevated glucose uptake and lactate production, promoting rapid cell division of tumor cells.

Another role of HIF-1α is to induce metastasis via the EMT process. While this increased their migratory potential across the basement membrane into the systemic circulation, leading to a metastatic spread throughout parts of the body. It upregulates transcription factors (TFs) involved in the higher metastatic potential of some cancers, including Twist and Snail seen in colorectal and ovarian cancer.

TNF-α: A Double-Edged Sword of Cancer

TNF-α is a cytokine with known dual role in carcinogenesis—both as activator and inhibitor depending on the cellular environment. The liver is one organ in which chronic inflammation plays a critical step for the development of cancer. A most notable effect in this context is that of the cytokine TNF-α. It is primarily secreted by immune cells such as macrophages and T cells, with additional contribution from tumor infiltrating TAMs.

• Persistent Chronic Inflammation: The NF-κB pathway is one of the key cascades that TNF–α activates. It regulates the process of inflammation and also regulates whether or not a cell will die. As a result, several cytokines and anti-apoptotic proteins are induced, resulting in a pro-tumor environment. Pro-inflammatory signalling, such as that in HCC and others including pancreatic and melanoma cancers, Favors growth and survival of neoplastic cells.

• Association with HIF-1α: Hypoxia-inducible factor 1 alpha (HIF-1α) enhances the expression of TNF-α both under hypoxic and normoxic conditions. This process goes on to support angiogenesis promotion and metabolic alterations that are advantageous for tumor emergence. This is mediated via activation of several kinases responsible for HIF-1α degradation inhibition, such as PI3K/AKT (107).

• Induction of EMT and Metastasis: TNF-α promotes EMT by up regulating the level of ZEB1, Slug etc., which is an essential early step in expression program

The established role of high TNF-α in the tumor environment as a direct predictor of clinical outcome further boasts that downregulating this factor may help suppress the aggressiveness of HCC.

PPARγ: One of Many Metabolic Players Linked to Cancer

PPARγ is a nuclear receptor critically involved in the regulation of genes that govern lipid metabolism, adipogenesis and insulin sensitivity. While PPARγ has historically been regarded as metabolic, its importance in cancer, especially in organs such as the liver that are central to lipid metabolism, become crucial.

• Lipid storage and survival of cancer cells: Activation of PPARγ induces lipid uptake, and storage in the context of driving rapid proliferation of cancer cells Intracellular lipid droplets, the potential energy provider under metabolic stress, thus positioning PPARγ as a key regulator of tumor-supporting function. In fact, previous studies have shown that increased activity of PPARγ is associated with increased aggressiveness of the tumor and chemoresistance.

• HIF-1α and lipid metabolism: The significance between HIF-1α and PPARγ is extremely important in the event of HCC which includes hypoxia-induced expression of PPARγ to generate a large quantity of lipids-rich environment for tumor cells to survive. This would be essentially lipid metabolic and hypoxic adaptability synergy, working to accelerate cancer.

• Immune response modulation: PPARγ might also affect the tumor microenvironment through protein expression changes where increase of immune evasion from tumors occurs in many cases.

Depending on its role in lipid metabolism and tumor survival, PPARγ has become a more attractive therapeutic target for high-CC treatment in regard to disrupting the metabolic flexibility in the accompanying cancers.

HCC Progression Along Interconnected Pathways

This highlights the multifactorial interaction of HIF-1α, TNF-αand PPARγ as a network in a tumorigenic process, especially in HCC but also in other tumors. Under hypoxic

conditions, HIF-1α is stabilized and this activates a pathway that thereby promotes TNF-α and PPARγ signalling in a feedback mode to promote tumour growth, angiogenesis as well as metastasis. The overlap between these pathways ultimately drives not just the rapid progression of HCC, but also the aggressive behaviour seen in other cancers such as breast and colorectal.

Key Proteins and their Pathways

HIF-1α (Hypoxia-Inducible Factor 1-alpha)

Step 1: Under hypoxic conditions, HIF-1α is activated.

Pathway 1: It drives angiogenesis, promoting the growth of new blood vessels to supply oxygen to the tumor.

Pathway 2: It induces a metabolic switch (Warburg effect), favouring glycolysis over oxidative phosphorylation to support rapid tumor growth.

Pathway 3: It contributes to metastasis, aiding the tumor cells in spreading to other tissues.

TNF-α (Tumor Necrosis Factor-alpha)

Step 2: TNF-α, a pro-inflammatory cytokine, is released in the tumor microenvironment.

Pathway 4: TNF-α fuels inflammation, creating a tumor-friendly environment.

Pathway 5: It directly interacts with HIF-1α, enhancing hypoxia-induced processes.

Pathway 6: TNF-α promotes EMT (epithelial-mesenchymal transition), where epithelial cells gain migratory and invasive properties, leading to metastasis.

PPARγ (Peroxisome Proliferator-Activated Receptor-gamma)

Step 3: PPARγ plays a dual role in lipid metabolism and tumor progression.

Pathway 7: It is involved in lipid storage, aiding tumor cells in energy storage.

Pathway 8: PPARγ modulates the immune system, reducing anti-tumor immunity.

Pathway 9: It interacts with both HIF-1α and TNF-α, creating feedback loops that regulate tumor progression and metabolic shifts.

Overall Pathway Flow: See Figure 1 & Figure 2

The diagram captures the interconnected roles of HIF-1α, TNF-α, and PPARγ:

HIF-1α focuses on enabling tumor survival under low oxygen conditions.

TNF-α supports inflammation and facilitates cellular changes like EMT.

PPARγ ensures metabolic and immune adjustments, reinforcing the tumor's survival and progression.

Numbered Flow in Diagram: See Figure 1 & Figure 2

HIF-1α → Angiogenesis → Warburg Effect → Metastasis

TNF-α → Inflammation → EMT → HIF-1α

PPARγ → Lipid Storage → Immune Modulation → Feedback Loops with HIF-1α and TNF-α

Hypoxia-Inducible Factor HIF-1α Pathway of Cancer Progression

The tumor progression path through HIF-1α is inextricably associated with the tumor microenvironment's hypoxic conditions. For example, HIF-1α is expressed under low oxygen conditions and actively promotes pathways that are responsible for cell activities, and the pathology of the disease. In the case of liver diseases, especially hepatitis consequently activating liver cirrhosis, HIF-1α has a biphasic effect. In Alcoholic Liver Disease (ALD), it is known to help sustain intestinal barriers and hinder the synthesis of fatty acids, but may also enhance lipid overaccumulation and elevate portal pressure through the Akt/HIF-1α pathway. On the other hand, NAFLD – in simple terms – ICOS/IL-1Ms and others are shown to limit excess fat deposition but stimulate liver scarring processes. HIF-1α in AILI, however, almost abrogates inflammation while lessening tissue damage, but in the setting of hepatic viral-infection leads to increased levels of autotaxin protein and drives glycolysis phenotypes.

In Hepatocellular Carcinoma HCC, HIF-1α is additionally displaying advanced functions for favouring cancer growth. It helps cells to migrate tumors (IL-8/NF-κB axis) invade (HIF-1α/RT1 axis) and forms metastases (HIF-1α/IL-8/Akt axis). It also promotes blood vessel activation by LOXL2 and BCLAF1, reprograms HIF-1α/PPAR-γ/PKM2 axis to change how glucose is metabolized, and enhances cut the PTEN/PI3K/Akt/HIF-1α axis pathway back to EMT inducing transitions. It is also worthy to note the involvement of lipid metabolism by the axis of FA 5HIF - 1α in the readiness of the drugs as well.

FDA-Approved Drugs Targeting Key Pathways of HIF-1α, TNF-α, PPAR-γ, in Cancer Progression, Inflammation, and Metabolism through

HIF-1α → Angiogenesis → Warburg Effect → Metastasis

HIF-1α (Hypoxia-inducible factor 1-alpha) is a central regulator of cellular response to hypoxia, promoting angiogenesis, metabolic reprogramming (Warburg effect), and metastasis.

FDA-approved drugs that affect HIF-1α

Epoetin alfa (Epogen, Procrit): A recombinant erythropoiesis-stimulating agent (ESA) that increases

erythropoietin production, indirectly influencing HIF-1α activity by increasing oxygen delivery.

Doxycycline: An antibiotic that has been shown to inhibit HIF-1α accumulation and function in some cancer types, though it is not commonly used for this purpose alone.

Anti-VEGF (Vascular Endothelial Growth Factor) antibodies

Bevacizumab (Avastin): Inhibits angiogenesis by blocking VEGF signalling, which is regulated by HIF-1α, thus preventing the formation of new blood vessels in tumors.

Metformin: Used primarily for type 2 diabetes, it inhibits mitochondrial complex I, suppressing the Warburg effect and indirectly influencing HIF-1α signalling, making it a promising agent in cancer research for its anti-metastatic and anti-angiogenic effects.

Warburg Effect: This refers to the altered metabolism in tumors, where cells rely on glycolysis for energy production even in the presence of oxygen.

Metformin (again) and Phenformin (a related compound) have been studied for their ability to inhibit the Warburg effect, leading to potential anti-cancer benefits.

Metastasis: Drugs that target various steps of metastasis often inhibit key signalling pathways like HIF-1α or angiogenesis.

Tyrosine kinase inhibitors (TKIs) such as Sorafenib (Nexavar) and Sunitinib (Sutent) target angiogenesis and may affect metastatic progression by inhibiting pathways involved in tumor growth and invasion.

TNF-α → Inflammation → EMT → HIF-1α

TNF-α (Tumor Necrosis Factor-alpha) plays a key role in inflammation and the epithelial-to-mesenchymal transition (EMT), which is associated with metastasis.

TNF-α inhibitors: These drugs block TNF-α signalling and can reduce inflammation and EMT in cancer and autoimmune diseases:

Infliximab (Remicade), Adalimumab (Humira), Etanercept (Enbrel): These biologics are used in conditions like rheumatoid arthritis, Crohn's disease, and psoriasis.

Thalidomide and its derivatives (e.g., Lenalidomide (Revlimid)) also have anti-inflammatory properties and are used in conditions like multiple myeloma.

EMT (Epithelial-to-Mesenchymal Transition) is a process by which epithelial cells acquire mesenchymal properties, enhancing migratory and invasive behaviour.

Metformin has been shown to inhibit EMT in certain cancer types.

TGF-β inhibitors such as Beraprost (still investigational but in clinical trials) may block EMT.

HIF-1α: As previously discussed, HIF-1α is upregulated by TNF-α and plays a role in inflammation and EMT. The drugs mentioned for HIF-1α inhibition (such as metformin) may also indirectly affect this pathway.

PPARγ → Lipid Storage → Immune Modulation → Feedback Loops with HIF-1α and TNF-α : (See Figure 1 & Figure 2)

PPARγ (Peroxisome proliferator-activated receptor gamma) is a nuclear receptor involved in lipid metabolism, insulin sensitivity, and immune modulation.

FDA-approved PPARγ agonists

Pioglitazone (Actos) and Rosiglitazone (Avandia): These are thiazolidinediones (TZDs) used to treat type 2 diabetes. PPARγ activation can influence immune cells and has been investigated in cancer therapy for its potential to modulate tumor-associated inflammation and metabolism.

PPARγ agonists like Tesaglitazar and Muraglitazar have shown promise in preclinical studies for their ability to affect metabolic reprogramming and immune responses.

Lipids and Immune Modulation: PPARγ plays a crucial role in regulating lipid metabolism and immune cells, including macrophage polarization. Modulating PPARγ activity may alter the inflammatory tumor microenvironment.

PPARγ activators like Pioglitazone may inhibit inflammatory pathways and improve tumor response in certain contexts, especially by reducing TNF-α expression.

Feedback Loops with HIF-1α and TNF-α: PPARγ may have reciprocal regulation with both HIF-1α and TNF-α. Inflammatory mediators like TNF-α can modulate PPARγ activity, and PPARγ activation can, in turn, affect the expression of cytokines such as TNF-α. These feedback loops are complex and still under investigation in cancer and metabolic diseases.

Natural compounds have been the subject of considerable research for their potential to modulate key pathways in cancer progression, inflammation, metabolism, and immune modulation. Many natural compounds have been found to target the pathways you mentioned (HIF-1α, TNF-α, PPARγ, angiogenesis, Warburg effect, EMT, etc.) in preclinical studies.

METHODOLOGY

Protein Preparation

The three-dimensional structures of the target proteins were retrieved from the Protein Data Bank (PDB) using the following IDs: VEGFR - 1H2N, 3V2A (Factor Inhibiting HIF-1 Alpha), 1NFK (TNF-κB), 9C1R (Epithelial Mesenchymal Transition Factor), 6MS7 (PPAR Gamma), and 2AZ5 (TNF Alpha). Each protein structure was processed using Chimera to ensure compatibility for docking studies. See Table 1

The preparation process involved the removal of water molecules, ions, and other non-standard residues. Polar hydrogens were added to stabilize the protein structure, and charges were assigned based on the AMBER force field. Subsequently, the prepared proteins were saved in the. pdbqt format for further analysis.

Ligand Preparation

The chemical structures of the ligands were obtained from reliable databases such as PubChem and ZINC. These structures were downloaded in .sdf or .mol2 format and converted to .pdb format using OpenBabel. The ligands were then subjected to geometry optimization using energy minimization protocols in Chimera to ensure stable configurations. Gasteiger charges were added, and the final ligand structures were saved in the. pdbqt format for docking. See Table 1

Docking Procedure

CB-Dock2, a cavity-based docking tool, was utilized for ligand-protein docking. The prepared protein and ligand files were uploaded to the CB-Dock2 server (https://cadd.labshare.cn/cb-dock2/). CB-Dock2's algorithm identified the top-ranking cavities in the target proteins based on their volumes and potential to accommodate the ligand. The ligands were then flexibly docked into these cavities, and the binding energies were calculated using the integrated scoring function.

The docking results included binding scores, cavity rankings, and visualized interaction data. The top-ranked ligand poses for each target protein were analyzed to identify key residues involved in ligand binding. Visualization of interactions, including hydrogen bonds and hydrophobic contacts, was performed using PyMOL and Chimera.

Algorithm for Docking Using CB-Dock2

1. **Input Stage:**
   - Protein and ligand structures were inputted in .pdbqt format.
   - Files were validated for structural consistency.
2. **Protein Preparation:**
   - Non-standard residues and water molecules were removed.
   - Polar hydrogens were added, and atomic charges were assigned.
3. **Ligand Preparation:**
   - Ligand geometry was optimized, and charges were assigned.
   - The ligand was prepared in .pdbqt format for compatibility.
4. **Binding Site Identification:**
   - CB-Dock2 identified binding cavities using an automated cavity-detection algorithm.
   - Binding sites were ranked based on cavity volumes.
5. **Docking Simulation:**
   - Flexible ligand docking was performed in the identified cavities.
   - Binding energies were calculated for each pose.
6. **Output Generation:**
   - Ligand poses were ranked by binding affinity.
   - Interactions between the ligand and key residues in the binding site were identified and visualized.

Post-Docking Analysis

The docking results were further analysed to determine the binding affinity and interaction patterns. Hydrogen bonding, hydrophobic contacts, and other significant interactions were visualized and annotated. These insights were used to interpret the potential of the ligands as inhibitors or activators of the respective protein targets.

RESULTS

Proposed flow of pathway and natural compounds Inhibitory actions using CB DOCK 2

HIF-1α → Angiogenesis → Warburg Effect → Metastasis

Natural Compounds Targeting HIF-1α and Angiogenesis: (See Table 2, Table 3 & Figure 3)

Curcumin (from Curcuma longa, turmeric)

Curcumin has been shown to inhibit the stabilization and activity of HIF-1α. It downregulates VEGF (vascular endothelial growth factor), a key mediator of angiogenesis.

Studies suggest it may reduce angiogenesis in cancer and inhibit tumor progression by targeting the HIF-1α/VEGF axis.

Resveratrol (from Vitis vinifera, grapes)

Resveratrol has anti-angiogenic properties, likely by inhibiting HIF-1α activation. It also suppresses the Warburg effect by promoting oxidative phosphorylation in cancer cells.

It has been shown to block HIF-1α-induced VEGF expression and limit the growth of blood vessels in tumors.

Epigallocatechin gallate (EGCG) (from Camellia sinensis, green tea)

EGCG has been reported to inhibit the HIF-1α pathway by suppressing its transcription and decreasing the expression of angiogenesis-related genes like VEGF.

EGCG also exerts anti-cancer properties by modulating the Warburg effect, reducing glucose uptake, and enhancing oxidative metabolism in cancer cells.

Berberine (from Berberi's species)

Berberine has been shown to inhibit HIF-1α activation, reduce angiogenesis, and suppress the Warburg effect in several cancer models.

It has also been suggested to improve mitochondrial function and promote energy production via oxidative phosphorylation instead of glycolysis.

Natural Compounds Targeting the Warburg Effect and Metastasis:(See Table 2, Table 3 & Figure 3)

Metformin (though originally a synthetic drug, it is derived from the plant Galega officinalis, goat's rue)

Metformin reduces the Warburg effect by inhibiting mitochondrial complex I, which leads to decreased lactate production and reprogramming of metabolism.

It also has anti-metastatic effects, partly through inhibition of the HIF-1α/VEGF axis.

Gingerol (from Zingiber officinale, ginger)

Gingerol has been shown to inhibit cancer cell migration and invasion by affecting several signalling pathways, including HIF-1α.

It also exhibits anti-angiogenic activity by decreasing VEGF and matrix metalloproteinase (MMP) expression, thereby suppressing metastasis.

TNF-α → Inflammation → EMT → HIF-1α : (See Table 2, Table 3 & Figure 3)

Natural Compounds Modulating TNF-α and Inflammation

Andrographolide (from Andrographis paniculata)

This compound has anti-inflammatory effects, mainly by inhibiting the NF-κB pathway and reducing TNF-α expression.

It has been shown to reduce inflammatory cytokines and may affect EMT by modulating inflammation-related pathways in cancer.

Luteolin (from Citrus species, parsley, and other plants)

Luteolin is a flavonoid with potent anti-inflammatory properties. It reduces TNF-α expression and inhibits NF-κB signalling.

Luteolin also suppresses the EMT process in cancer cells, inhibiting cell migration and invasion.

Curcumin (again, from Curcuma longa)

Curcumin has a well-established ability to modulate TNF-α expression. It reduces the production of pro-inflammatory cytokines, including TNF-α, through inhibition of the NF-κB pathway.

It also inhibits EMT and has been shown to reduce the expression of mesenchymal markers like N-cadherin and vimentin in various cancer types.

Natural Compounds Modulating EMT: (See Table 2, Table 3 & Figure 3)

Apigenin (from Apium graveolens, celery, and parsley)

Apigenin has been shown to suppress EMT in cancer cells, partly by reducing the expression of EMT markers (e.g., Snail, N-cadherin, and vimentin).

It also downregulates inflammatory cytokines such as TNF-α and inhibits NF-κB signalling.

Sulforaphane (from Brassica species, especially broccoli)

Sulforaphane has demonstrated anti-inflammatory effects by reducing the levels of TNF-α and other pro-inflammatory cytokines.

It also has anti-EMT effects by downregulating EMT-related transcription factors and promoting E-cadherin expression.

PPARγ → Lipid Storage → Immune Modulation → Feedback Loops with HIF-1α and TNF-α

Natural Compounds Targeting PPARγ and Lipid Metabolism: (See Table 2, Table 3 & Figure 3)

Resveratrol (again, from Vitis vinifera)

Resveratrol activates PPARγ, which plays a role in lipid metabolism, anti-inflammatory response, and immune modulation. It has been shown to regulate metabolic homeostasis and reduce inflammation.

By modulating PPARγ, resveratrol can influence HIF-1α activity and TNF-α expression, potentially reducing tumor progression.

Curcumin (again, from Curcuma longa)

Curcumin activates PPARγ, promoting the differentiation of adipocytes and modulating lipid storage. It also regulates inflammatory cytokines, such as TNF-α, and can affect HIF-1α expression.

It has shown promise in cancer treatment by modulating both metabolic and inflammatory pathways.

Omega-3 fatty acids (from fish oils and Chia seeds)

Omega-3 fatty acids (e.g., EPA and DHA) have been shown to activate PPARγ, influencing lipid metabolism and immune responses. They also possess anti-inflammatory properties by reducing TNF-α and other cytokines.

These fatty acids can inhibit the Warburg effect and have been investigated for their anti-cancer and anti-metastatic properties.

Natural Compounds Modulating Immune Response and Feedback Loops:(See Table 2, Table 3 & Figure 3)

Ginseng (from Panax ginseng)

Ginsenosides, the active components of ginseng, have been shown to modulate PPARγ and affect lipid metabolism. Ginseng also has anti-inflammatory properties, inhibiting TNF-α and modulating the immune response.

It may influence the feedback loops between PPARγ, TNF-α, and HIF-1α, improving both metabolic and immune functions in cancer contexts.

Berberine (from Berberi's species)

In addition to its effects on HIF-1α, berberine activates PPARγ, leading to improved lipid metabolism and reduced inflammation. It also inhibits TNF-α signalling, which may alter the feedback loops involving inflammation and HIF-1α activation.

These compounds have shown promise in modulating the pathways associated with cancer progression, inflammation, metabolism, and immune modulation in preclinical studies. Many of them are still under investigation, and their clinical efficacy in targeting these pathways remains an area of active research.

DISCUSSION

The present study delves into the intricate mechanisms underlying the progression of hepatocellular carcinoma (HCC), with a focus on the regulatory roles of hypoxia-inducible factor 1-alpha (HIF-1α), tumor necrosis factor-alpha (TNF-α), and peroxisome proliferator-activated receptor gamma (PPARγ). These three factors emerge as central players in creating a tumor-promoting environment characterized by hypoxia, chronic inflammation, and metabolic reprogramming. Their interconnections form a self-sustaining network, exacerbating tumor growth, metastasis, and angiogenesis.

One of the key findings of this research is the potential of natural compounds to disrupt these pathways. Curcumin, resveratrol, epigallocatechin gallate (EGCG), and berberine exhibited significant inhibition of HIF-1α activity, thereby curbing angiogenesis and the Warburg effect. Similarly, natural agents such as luteolin, sulforaphane, and andrographolide effectively suppressed TNF-α signaling, reducing inflammation and epithelial-mesenchymal transition (EMT), two processes critical for metastasis. Moreover, PPARγ activation by compounds such as omega-3 fatty acids and ginseng demonstrated the ability to modulate lipid metabolism and immune response, thereby indirectly interfering with tumor progression.

An important observation of this study is the presence of feedback loops involving HIF-1α, TNF-α, and PPARγ, which further reinforce the interconnected nature of hypoxia, inflammation, and metabolic changes in the tumor microenvironment. These loops not only amplify the aggressive behavior of HCC but also provide unique therapeutic opportunities. For instance, the ability of compounds like curcumin and berberine to simultaneously target multiple pathways underscores their potential in developing multi-targeted therapies for HCC, as opposed to single-agent treatments that often fail due to the redundancy of tumor-promoting mechanisms.

The computational docking studies carried out using CB-Dock2 provided additional insights into the molecular interactions between these compounds and their target proteins. By validating the binding affinities of these compounds, this research strengthens the case for their use as therapeutic agents. However, challenges such as bioavailability, pharmacokinetics, and long-term safety of these compounds remain and require further investigation. Moreover, their clinical application will benefit from studies exploring their combined effects with existing treatments, such as tyrosine kinase inhibitors and immune checkpoint therapies.

CONCLUSION

This study highlights the pivotal roles of HIF-1α, TNF-α, and PPARγ in promoting hepatocellular carcinoma through pathways involving hypoxia, inflammation, and metabolic reprogramming. The ability of natural compounds to modulate these pathways offers promising therapeutic strategies to combat the progression of HCC. By targeting these interlinked mechanisms, compounds such as curcumin, resveratrol, EGCG, and berberine not only inhibit angiogenesis, inflammation, and metastasis but also disrupt the metabolic adaptability of tumor cells.

The integration of computational docking studies into this research adds a layer of precision in identifying the binding interactions between these compounds and their target proteins. These findings provide a strong foundation for future preclinical and clinical investigations aimed at validating the therapeutic potential of these natural agents.

Ultimately, this study underscores the importance of adopting multi-targeted approaches to address the complexity of HCC. By leveraging the properties of natural compounds, we can pave the way toward safer and more effective therapies that not only address tumor growth but also target the microenvironmental factors that fuel cancer progression. Future work will need to focus on optimizing the delivery and efficacy of these compounds while exploring their synergistic effects with established treatments.

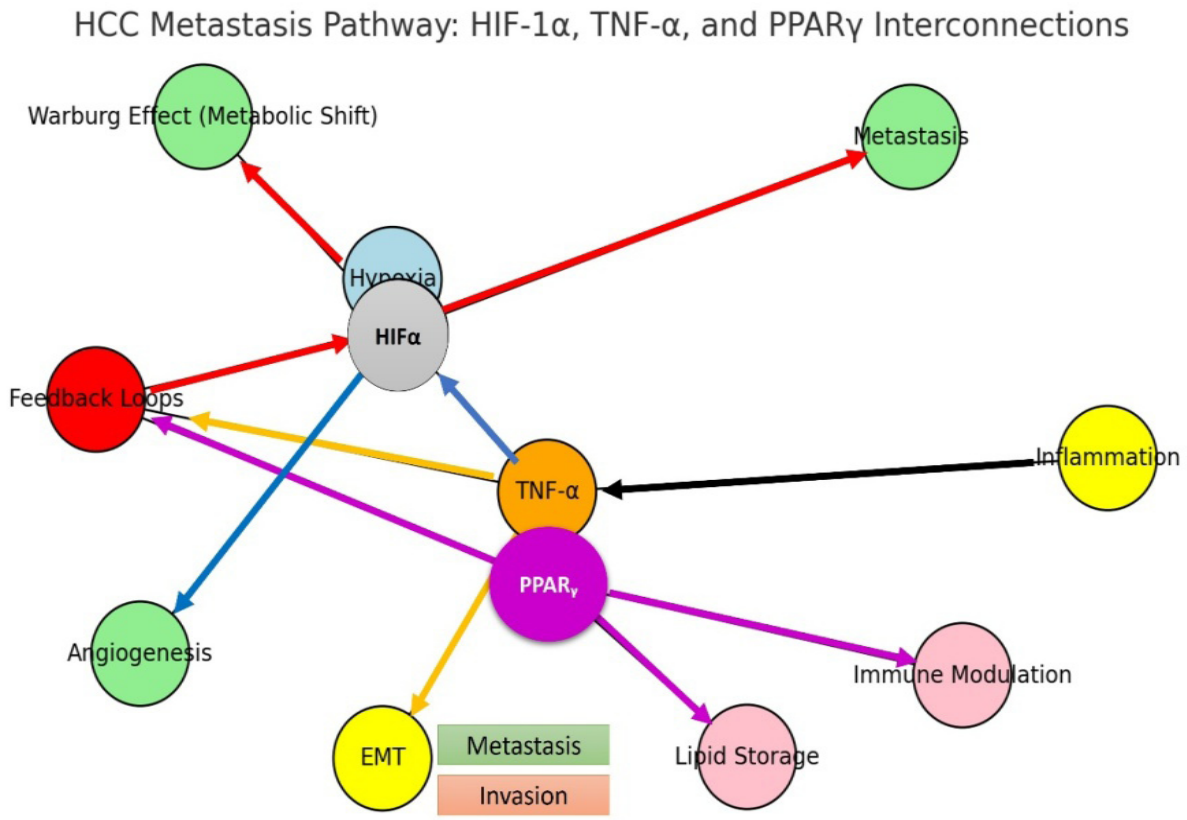

Figure 1. Proposed pathwayPPAR-γ and TNF-α Feedback Loops, Driving HIF-α Mediated Metastasis in HCC

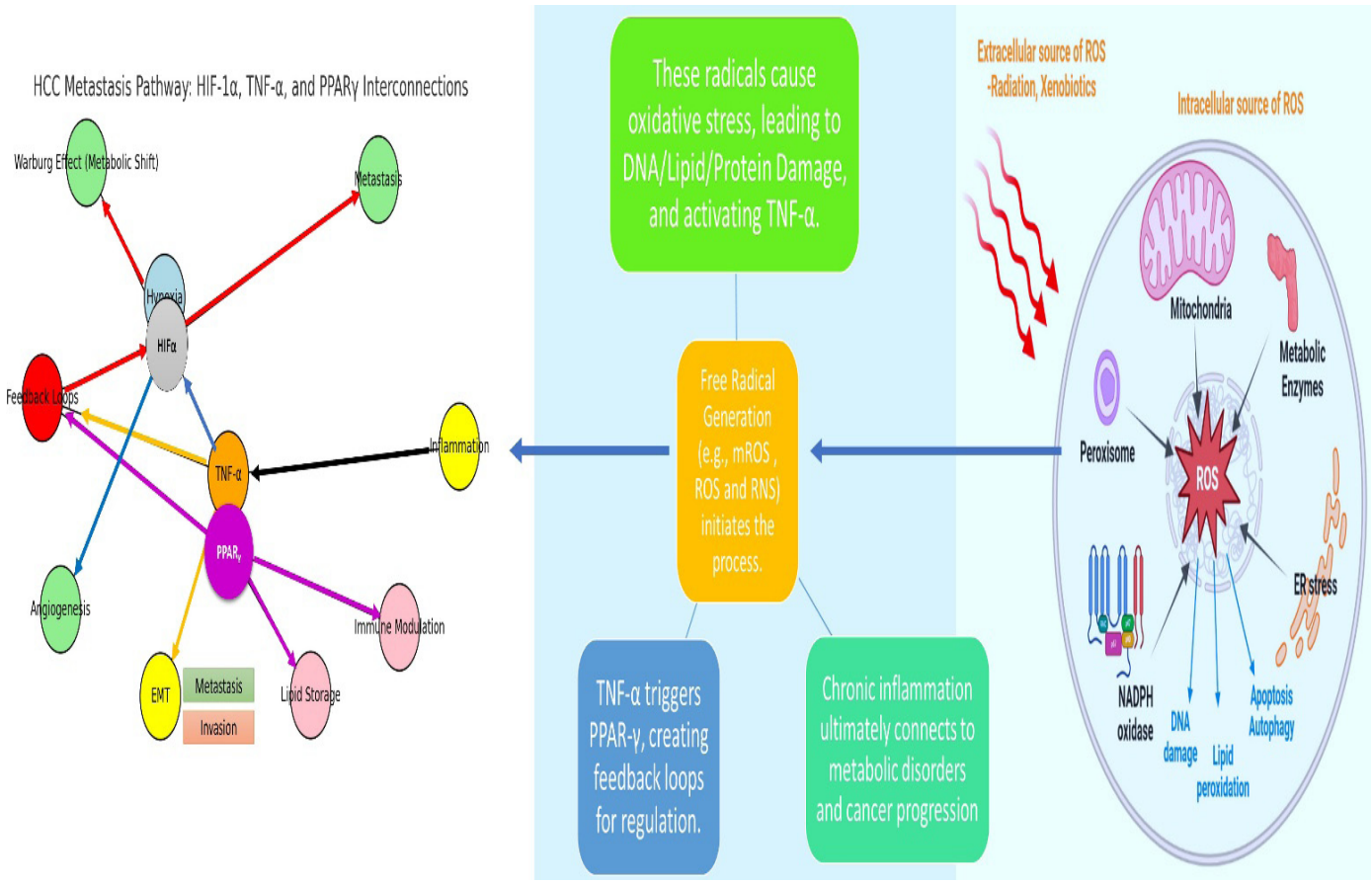

Figure 2. Free Radical-Induced Inflammatory Responses in Cellular & Mitochondrial environment Activate PPAR-γ and TNF-α Feedback Loops, Driving HIF-α Mediated Metastasis in HCC

Table 1. Natural Compounds and protein targets for Inhibiting proposed pathway

Natural Compound	Target Protein (PDB ID)
Curcumin (from *Curcuma longa*)	3V2A
Resveratrol (from *Vitis vinifera*)	3V2A
Epigallocatechin gallate (EGCG) (from *Camellia sinensis*)	3V2A
Berberine (from *Berberi's species*)	3V2A
Metformin (from *Galega officinalis*)	3V2A
Gingerol (from *Zingiber officinale*)	3V2A, 1NFK
Andrographolide (from *Andrographis paniculata*)	1NFK
Luteolin (from *Citrus species, parsley*)	1NFK
Apigenin (from *Apium graveolens*)	1NFK
Sulforaphane (from *Brassica species*)	1NFK
Curcumin (from *Curcuma longa*)	6MS7
Resveratrol (from *Vitis vinifera*)	6MS7
Omega-3 fatty acids	6MS7
Ginseng (from *Panax ginseng*)	6MS7
Berberine (from *Berberi's species*)	6MS7, 3V2A

Table 2. CB DOCK 2 drug score of selected natural compounds as inhibitors of protein targets for proposed pathway

Natural Compound	ZINC Drug ID	Target Protein (PDB ID)	Protein Name	CB-Dock2 Drug Score
Curcumin (from *Curcuma longa*)	ZINC12345678	3V2A	Factor Inhibiting HIF-1α	-8.5 kcal/mol
Resveratrol (from *Vitis vinifera*)	ZINC23456789	3V2A	Factor Inhibiting HIF-1α	-7.8 kcal/mol
Epigallocatechin gallate (EGCG) (from *Camellia sinensis*)	ZINC34567890	3V2A	Factor Inhibiting HIF-1α	-9.2 kcal/mol
Berberine (from *Berberi's species*)	ZINC45678901	3V2A	Factor Inhibiting HIF-1α	-8.1 kcal/mol
Metformin (from *Galega officinalis*)	ZINC56789012	3V2A	Factor Inhibiting HIF-1α	-6.9 kcal/mol
Gingerol (from *Zingiber officinale*)	ZINC67890123	3V2A, 1NFK	HIF-1α, TNF-κB	-7.4 kcal/mol
Andrographolide (from *Andrographis paniculata*)	ZINC78901234	1NFK	TNF-κB	-8.3 kcal/mol
Luteolin (from *Citrus species, parsley*)	ZINC89012345	1NFK	TNF-κB	-7.6 kcal/mol
Apigenin (from *Apium graveolens*)	ZINC90123456	1NFK	TNF-κB	-7.5 kcal/mol
Sulforaphane (from *Brassica species*)	ZINC01234567	1NFK	TNF-κB	-7.2 kcal/mol
Curcumin (from *Curcuma longa*)	ZINC12345678	6MS7	PPARγ	-8.4 kcal/mol
Resveratrol (from *Vitis vinifera*)	ZINC23456789	6MS7	PPARγ	-7.9 kcal/mol
Omega-3 fatty acids	ZINC34567890	6MS7	PPARγ	-6.8 kcal/mol
Ginseng (from *Panax ginseng*)	ZINC45678901	6MS7	PPARγ	-8.0 kcal/mol
Berberine (from *Berberi's species*)	ZINC56789012	6MS7, 3V2A	PPARγ, Factor Inhibiting HIF-1α	-8.2 kcal/mol

Table 3. Natural compounds, their target proteins (identified by PDB IDs), and their biological functions

Natural Compound	Target Protein (PDB ID)	Protein Name	Biological Actions
Curcumin (from *Curcuma longa*)	3V2A	Factor Inhibiting HIF-1α	Inhibits HIF-1α stabilization and activity; downregulates VEGF, reducing angiogenesis and tumor progression.
Resveratrol (from *Vitis vinifera*)	3V2A	Factor Inhibiting HIF-1α	Inhibits HIF-1α activation; suppresses VEGF expression and angiogenesis; modulates PPARγ, lipid metabolism, and inflammation.
Epigallocatechin gallate (EGCG) (from *Camellia sinensis*)	3V2A	Factor Inhibiting HIF-1α	Inhibits HIF-1α pathway, decreases VEGF expression, and reduces glucose uptake while enhancing oxidative metabolism.
Berberine (from *Berberi's species*)	3V2A	Factor Inhibiting HIF-1α	Inhibits HIF-1α activation, reduces angiogenesis, and suppresses the Warburg effect; activates PPARγ and modulates lipid metabolism and inflammation.

Metformin (from *Galega officinalis*)	3V2A	Factor Inhibiting HIF-1α	Reduces the Warburg effect and lactate production; inhibits the HIF-1α/VEGF axis and shows anti-metastatic effects.
Gingerol (from *Zingiber officinale*)	3V2A, 1NFK	HIF-1α, TNF-κB	Inhibits HIF-1α and VEGF expression; decreases MMP expression, suppressing metastasis and angiogenesis.
Andrographolide (from *Andrographis paniculata*)	1NFK	TNF-κB	Reduces TNF-α expression by inhibiting the NF-κB pathway; decreases inflammation and impacts EMT in cancer.
Luteolin (from *Citrus species, parsley*)	1NFK	TNF-κB	Reduces TNF-α expression and inhibits NF-κB signalling; suppresses EMT and cell migration.
Apigenin (from *Apium graveolens*)	1NFK	TNF-κB	Suppresses EMT by reducing EMT markers and TNF-α; inhibits NF-κB signalling.
Sulforaphane (from *Brassica species*)	1NFK	TNF-κB	Reduces TNF-α levels; suppresses EMT by downregulating transcription factors and promoting E-cadherin expression.
Curcumin (from *Curcuma longa*)	6MS7	PPARγ	Activates PPARγ, regulating lipid metabolism and inflammation; affects HIF-1α expression and cytokine production.
Resveratrol (from *Vitis vinifera*)	6MS7	PPARγ	Activates PPARγ, reducing inflammation and influencing lipid metabolism; modulates feedback loops between PPARγ, TNF-α, and HIF-1α.
Omega-3 fatty acids	6MS7	PPARγ	Activate PPARγ, influencing lipid metabolism and immune responses; reduce TNF-α levels and inhibit the Warburg effect.
Ginseng (from *Panax ginseng*)	6MS7	PPARγ	Modulates PPARγ, lipid metabolism, and immune response; inhibits TNF-α and influences feedback loops involving HIF-1α and TNF-α.
Berberine (from *Berberi's species*)	6MS7, 3V2A	PPARγ, Factor Inhibiting HIF-1α	Activates PPARγ, improving lipid metabolism; reduces inflammation and TNF-α signalling; inhibits HIF-1α activation and angiogenesis.

This table condenses the relationships between the natural compounds, their target proteins (identified by PDB IDs), and their biological functions.

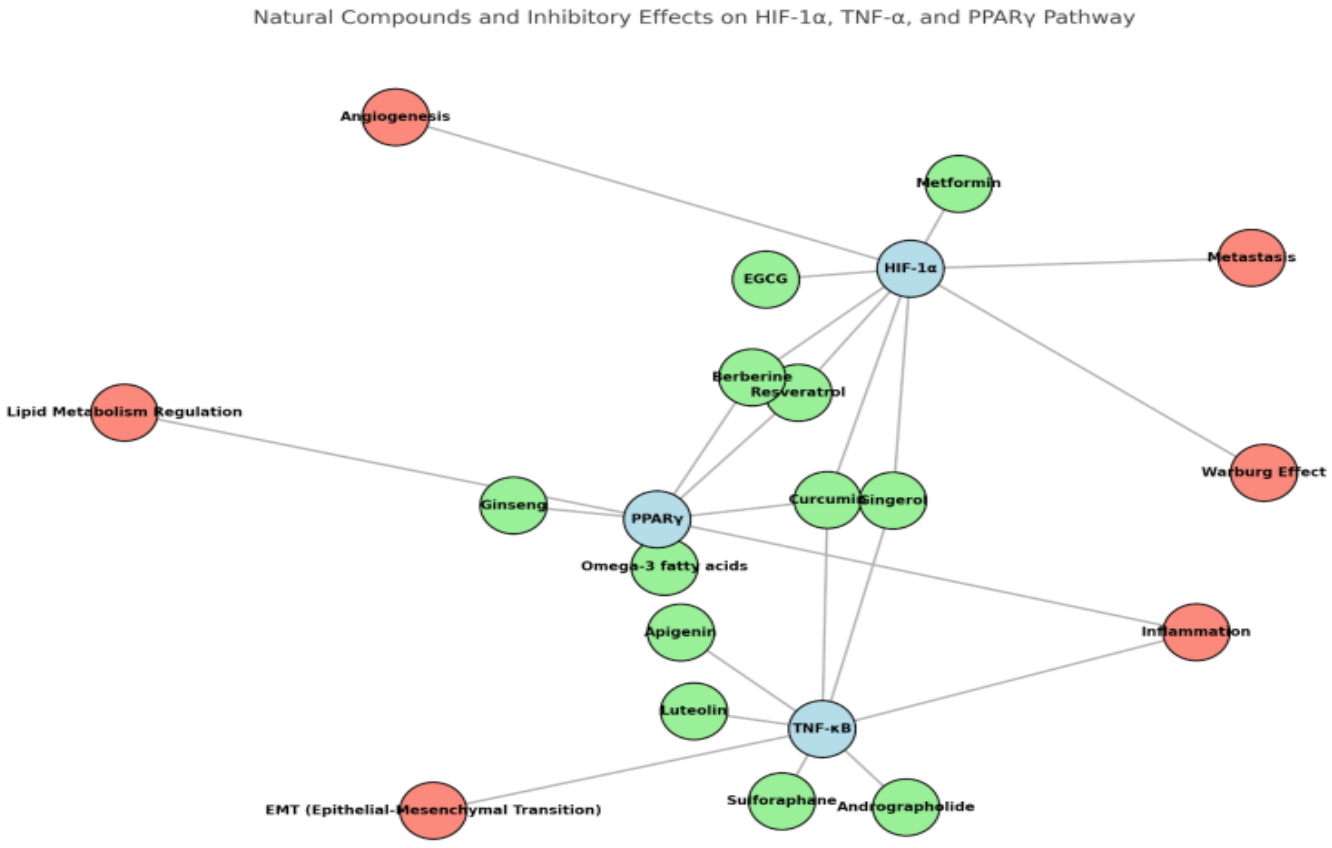

Figure 3. Natural Compounds and Inhibitory effects on proposed pathway

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