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Apigenin as a Novel Epigenetic Modulator for Enhancing Chemotherapeutic Efficacy in Hepatocellular Carcinoma

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Abstract

Hepatocellular carcinoma (HCC) remains a therapeutically challenging malignancy, as chemotherapy and targeted therapy are frequently limited by acquired resistance, tumor heterogeneity, and systemic toxicity. Emerging evidence positions apigenin, a dietary flavone, not merely as a cytotoxic phytochemical but as a multifunctional epigenetic regulator capable of reprogramming the molecular landscape of HCC. This narrative review explores current mechanistic evidence linking apigenin to modulation of DNA methylation, histone acetylation and methylation, and noncoding RNA networks, through which it restores tumor-suppressive gene expression and attenuates oncogenic transcriptional programs. It examines how apigenin-mediated inhibition of DNMT1, HDACs, EZH2, and KDM1A intersects with suppression of PI3K/Akt/mTOR, NF-κB, Wnt/β-catenin, and H19-dependent signaling to induce apoptosis,

autophagy, cell-cycle arrest, and inhibition of epithelial–mesenchymal transition. Particular emphasis is placed on apigenin's capacity to sensitize HCC cells to sorafenib, doxorubicin, and 5-fluorouracil by disrupting survival pathways, redox adaptation, cancer stem-like phenotypes, and drug-efflux mechanisms. The review further discusses the translational potential of apigenin-based combination regimens, including nanoformulation strategies designed to overcome its limited bioavailability and improve hepatic delivery. By framing apigenin as an epigenetic chemosensitizer rather than a conventional cytotoxic agent, this review proposes a precision-oriented model in which phytochemical modulation of chromatin and regulatory RNAs may restore chemotherapeutic vulnerability in HCC. Critical knowledge gaps, including target validation, dose-dependent epigenetic effects, pharmacokinetics, and clinical safety, are highlighted to guide future preclinical and clinical investigations.

Keywords: apigenin; hepatocellular carcinoma; chemosensitization; DNA methylation; histone acetylation; microRNA

1. Introduction

Hepatocellular carcinoma (HCC) accounts for the majority of primary liver cancers and is the sixth most common cancer and the third leading cause of cancer-related death worldwide [1]. Despite the expansion of the systemic therapeutic armamentarium from sorafenib to multikinase inhibitors and immune checkpoint inhibitors, the therapeutic window for advanced HCC remains narrow. Intrinsic and acquired drug resistance, hepatic dysfunction in the cirrhotic host, and the tumor's remarkable molecular heterogeneity conspire to limit durable responses. A recurring theme in HCC drug resistance is the reactivation of cytoprotective and survival programs, including antioxidant detoxification, autophagic cytoprotection, and epithelial plasticity, that cannot be fully suppressed by chemotherapeutic agents alone [1,2].

Many of these programs are established and maintained by epigenetic mechanisms, defined as heritable changes in gene expression that occur without alteration in the DNA sequence and that principally involve DNA methylation, histone modification, and non-coding RNA regulation [3,4]. Unlike genetic lesions, epigenetic modifications are potentially reversible, and pharmacological modulation of these aberrant processes has already made a significant impact on the treatment of hematological malignancies, with DNA methyltransferase and histone deacetylase inhibitors demonstrating remarkable efficacy in clinical trials [3,5]. In solid tumors, however, single-agent epigenetic therapy has generally been disappointing, and current

approaches increasingly favor epigenetic priming, in which epigenetic modulators are used to re-sensitize tumors to conventional cytotoxic or targeted agents [5,6].

Against this background, dietary flavonoids have attracted considerable interest as multi-target, low-toxicity modulators of the cancer epigenome [4,7]. Apigenin (4′,5,7-trihydroxyflavone) is a flavone found in a variety of foods, including parsley, celery, chamomile, and citrus, and has demonstrated anticancer potential by targeting multiple cellular processes, including apoptosis, cell-cycle regulation, angiogenesis, inflammation, and, notably, epigenetic regulation [7–9]. Preclinical studies in HCC have shown that apigenin can modulate key cancer-related pathways and potentially overcome resistance to doxorubicin, 5-fluorouracil, and sorafenib through the modulation of NRF2, miRNA, histone demethylases, and long non-coding RNA pathways [10–14].

Against this background, this narrative review examines the literature from an integrative perspective, proposing that apigenin is best understood not as a stand-alone cytotoxin but as a multi-modal epigenetic adjuvant for HCC chemotherapy. Specifically, we (i) map the epigenetic dysregulation that characterizes HCC, (ii) catalog the direct and indirect epigenetic targets documented across tumor models and, specifically, in liver cancer, (iii) analyze in detail the combination studies demonstrating chemosensitization in HCC cells and xenograft models, and (iv) identify the major translational barriers, particularly limited bioavailability and the absence of clinical evidence, that must be addressed before the preclinical promise of apigenin can be evaluated in patients. We conclude by highlighting the mechanistic questions and clinical trial designs most likely to advance this field.

2. Methodology

The purpose of this study was to review the literature on the potential role of apigenin as an epigenetic modifier that enhances the efficacy of chemotherapy for treatment of HCC. Because this area of research remains in its infancy, the review focuses on the concept of flavonoid-mediated modulation of epigenetic processes associated with increased chemosensitivity in HCC. The narrative design of this study allows flexibility in including mechanistic findings from in silico, in vitro, and preclinical studies. Nevertheless, a clearly defined literature search strategy was employed to maintain a balance between flexibility and precision in conducting this review.

2.1 Search Strategy

A search strategy comprising four databases was used in this study, including PubMed/MEDLINE, Scopus, Web of Science Core Collection, and Cochrane Library, as these databases cover the literature most comprehensively. The reason for including Cochrane Library, which focuses on systematic reviews, lies in the possibility that apigenin might have been subject to such studies, although the evidence on its mechanism of action remains largely preclinical. The search strategy was developed according to standard recommendations for narrative reviews aiming to identify preclinical mechanistic evidence. Three concepts were included in the search strategy: (1) apigenin, (2) epigenetics, and (3) hepatocellular carcinoma/chemotherapy. Controlled vocabulary (Medical Subject Headings) was combined with free-text terms for better sensitivity:

(Apigenin [MeSH] OR apigenin OR 4′,5,7-trihydroxyflavone OR apigenin flavone) AND ('Epigenesis, Genetic'[MeSH] OR epigenetic OR 'DNA methylation'[MeSH] OR methylation OR 'histone deacetylase' OR 'histone demethylase' OR 'KDM1A' OR 'LSD1' OR 'miRNA' OR 'microRNA' OR 'non-coding RNA') AND ('Carcinoma, Hepatocellular'[MeSH] OR 'hepatocellular carcinoma' OR 'liver cancer' OR 'HCC' OR 'HepG2' OR 'Huh7' OR 'BEL-7402') AND (chemotherapy OR chemosensitization OR 'drug resistance' OR 'multidrug resistance' OR doxorubicin OR sorafenib OR '5-fluorouracil' OR cisplatin)

2.2 Search Execution and Screening Process

The search strategy was executed iteratively between October and December 2025. In addition to database searches, manual reference screening of all included articles and pertinent review papers was conducted to identify sources not captured by the electronic queries, a critical step for narrative reviews addressing emerging mechanistic topics where terminology may not yet be standardized. Screening proceeded in two phases. First, titles and abstracts were reviewed against predefined eligibility criteria. Second, full-text articles were assessed for inclusion. The eligibility criteria were: **Inclusion criteria:** (i) Original research articles, including in vitro, in vivo, and in silico studies; (ii) Studies investigating apigenin in HCC cell lines or animal models; (iii) Studies examining epigenetic mechanisms (DNA methylation, histone modification, non-coding RNA regulation) OR chemosensitization effects; (iv) Studies evaluating apigenin either as a single agent or in combination with conventional chemotherapeutic or anticancer agents. **Exclusion criteria:** (i) Conference abstracts, editorials, commentaries, and letters without original full-text data; (ii) Studies on flavonoids other than

apigenin without comparative apigenin data; (iii) Non-human studies not utilizing HCC models; (iv) Articles lacking full-text availability.

2.3 Data Extraction and Synthesis

Data from included articles were extracted into a standardized matrix capturing: study design, HCC model system (cell line or animal), apigenin concentration/dosage, chemotherapeutic agent and dosage, epigenetic target(s) investigated, and key mechanistic findings. Given the heterogeneity of study designs and outcome measures, a qualitative narrative synthesis was performed, organizing findings thematically around: (1) apigenin's direct epigenetic targets in HCC, (2) evidence for chemosensitization via epigenetic modulation, and (3) mechanistic pathways linking epigenetic changes to enhanced drug efficacy. This synthesis approach prioritizes conceptual integration over statistical aggregation, consistent with the narrative review methodology's suitability for generating hypotheses and identifying research gaps.

2.4 Methodological Considerations and Limitations

The systematic search strategy used in this review enhances transparency compared with purely opportunistic narrative reviews. Nevertheless, some limitations need to be noted. First, language bias can be introduced through reliance on English language publications. Second, the lack of dual independent screening is a departure from systematic review standards; this was a pragmatic concession to the narrative review format. Third, the lack of clinical trials testing apigenin as an epigenetic modulator in HCC required reliance on preclinical evidence, which may not directly translate to clinical efficacy. Limitations are inherent to the scope of the review and should guide interpretation of its findings.

3. The Epigenetic Landscape of HCC: Why Modulation Is an Adjuvant Strategy

3.1 DNA Methylation and Histone Modification

HCC genomes are characterized by global DNA hypomethylation, focal CpG-island hypermethylation at tumor-suppressor loci, copy-number-independent silencing of regulatory genes, and recurrent alterations in histone-modifying enzymes [2,3]. DNA methyltransferases (DNMTs) and histone methyltransferases (HMTs) act in concert: DNMT-mediated promoter methylation and HMT-driven histone methylation (e.g., repressive H3K9 and H3K27 marks) condense chromatin and lock tumor suppressors, including miRNA clusters and detoxification genes, into a silent state that promotes proliferation, immune escape, and drug resistance [3,15]. The reversibility of these marks is the conceptual foundation of epigenetic therapy in solid

tumors [3,5]. A further layer of complexity arises from methylation-dependent regulation of drug handling. Epigenetic silencing of antioxidant response and xenobiotic clearance genes, together with NRF2-driven overexpression of cytoprotective enzymes, can alter the intracellular concentrations and toxicity of chemotherapeutic agents in liver cancer [1,10]. Thus, the epigenetic state is a determinant of chemosensitivity, rather than merely of tumorigenesis.

3.2 Non-coding RNAs and lncRNAs

MicroRNAs (miRNAs) are post-transcriptional regulators whose clusters are frequent targets of epigenetic silencing in HCC; their deregulation affects proliferation, invasion, autophagy, and drug response [1,5,16]. Long non-coding RNAs such as H19 can scaffold oncogenic signaling, notably through the H19–Wnt/β-catenin axis, and may constitute druggable regulatory nodes upstream of classical pathways [17]. Recent work has extended the epigenetic landscape to RNA modifications and chromatin-associated readers involved in HCC therapy resistance [1]. Because miRNAs and lncRNAs are regulated by the same DNA methylation and histone-modifying machinery that regulates protein-coding genes, agents targeting chromatin-modifying enzymes may also reshape the non-coding transcriptome, a consideration that is central to interpreting the effects of apigenin discussed below.

3.3 Limitations of Classical Epi-drugs in Solid Tumors

First-generation DNMT inhibitors are nucleoside analogues requiring incorporation into DNA, and HDAC inhibitors show limited single-agent activity in solid tumors, in part because of pharmacokinetic shortfalls and pathway redundancy [5,18]. This has redirected interest toward orally available, pleiotropic dietary compounds that combine weak multi-target epigenetic enzyme inhibition with concurrent modulation of signaling pathways, a strategy termed "combinatorial epigenetic intervention", of which apigenin is a leading example [4,7,19].

4. Apigenin: Chemical, Pharmacological, and Safety Profile

Subsequent studies identified apigenin and analogous dietary flavones as dual inhibitors of DNA methyltransferases (DNMTs) and histone methyltransferases (HMTs), indicating their capacity to influence both DNA methylation and repressive histone methylation [15,20,21]. Beyond chromatin enzymes, apigenin modulates a broad signaling network involving PI3K/Akt/mTOR, p38 MAPK, NF-κB, STAT3, NRF2, that intersects multiple hallmarks of cancer, and it induces apoptosis, autophagy, cell-cycle arrest, and anti-angiogenic responses

across tumor types [7–9,22]. Its intrinsic toxicity appears to be relatively low, and studies have consistently identified a favorable safety profile as a potential advantage for an adjuvant role [7,9]. The translational caveat is equally consistent: apigenin's oral bioavailability is limited by poor aqueous solubility and rapid metabolism, which motivates both the nanodelivery strategies discussed in Section 8 and the sub-toxic dosing designs used in combination studies [7,9,13].

5. Direct Epigenetic Targets of Apigenin

5.1 DNMT and HMT Inhibition

The most direct evidence that apigenin is a bona fide epigenetic enzyme modulator comes from the Zwergel et al. study: Apigenin binds GC-rich DNA and inhibits DNA methylation [20], and dietary flavones including apigenin act as dual inhibitors of DNMTs and HMTs, with docking and DNA-binding data supporting direct engagement of both enzyme classes [15,21]. Combinatorial studies of polyphenol epigenetics place apigenin among the flavonoids with documented activity against DNA methylation, histone acetylation, and histone methylation marks across cancer models [4,19,23]. Functionally, this class of activity explains how a single molecule can reactivate silenced tumor-suppressor miRNAs while concurrently reducing repressive histone marks (Figure 1).

5.2 Histone Deacetylation and Acetylation

Apigenin efficiently targets HDAC1 and HDAC3 in tumor models, and apigenin-mediated HDAC inhibition reverses aberrant epigenetic events in cancer cells, with the prostate cancer system providing the cleanest pharmacological characterization [19,24]. Conversely, in a non-malignant setting, apigenin inhibits CBP/p300-mediated histone acetylation, demonstrating that its net effect on the acetylome is context- and cofactor-dependent rather than a simple pan-HDAC effect [25]. In HCC-relevant literature, apigenin is repeatedly cited among flavonoids that promote histone H3 acetylation and reshape the acetylation landscape in liver-derived cells [23,26]. The therapeutic implication is therefore context-dependent: apigenin's acetylation effects favor re-expression of differentiation and tumor-suppressor programs in cancer cells while damping pathological acetylation-driven transcription in inflamed tissue (Figure 1).

5.3 Histone Demethylation: KDM1A in HCC

A 2024 Phytomedicine study provides the most direct HCC-specific epigenetic-enzyme evidence: apigenin inhibits lipid metabolism of HCC cells by targeting the histone demethylase

KDM1A (lysine-specific demethylase 1), directly linking chromatin-modifying enzyme engagement to metabolic reprogramming of hepatoma cells [14]. This finding is mechanistically important for two reasons. First, it demonstrates on-target epigenetic activity in HCC cells themselves, not by extrapolation from other tumor types. Second, KDM1A-mediated lipid-metabolic reprogramming connects apigenin's chromatin activity to a metabolic dependency that is itself a chemosensitization lever, since metabolic plasticity is a recognized component of HCC drug resistance [1,2].

5.4 Epigenetic Regulation of the NRF2/KEAP1 Axis

The NRF2 transcription factor governs the antioxidant response and is a double-edged sword in cancer: cytoprotective in normal hepatocytes, pro-survival and pro-chemoresistance when constitutively activated in tumors [10,27]. Studies of NRF2/KEAP1 epigenetic regulation by phytochemicals document that apigenin modulates the NRF2 axis through epigenetic mechanisms and, critically, that this epigenetic NRF2 regulation leads to chemosensitization of adriamycin-resistant hepatocellular carcinoma cells [27]. The downstream consequence is the normalization expression of Nrf2-driven detoxification genes such as NQO1, which reduces the drug-extruding and drug-inactivating capacity of resistant hepatoma cells [10]. This places apigenin in the unusual position of being simultaneously an antioxidant chemopreventive agent in normal tissue and an NRF2-suppressive chemosensitizer in tumor tissue (Figure 1), a context-dependence that studies now treat as a defining feature of its pharmacology [7,9,27].

5.5 EZH2, Sirtuins, and Chromatin-associated Metabolism

Enhancer of zeste homolog 2 (EZH2), the catalytic methyltransferase of Polycomb repressive complex 2, is frequently overexpressed in HCC and promotes tumor progression through trimethylation of histone H3 lysine 27 (H3K27me3), silencing of tumor-suppressive microRNAs, and repression of genes governing apoptosis, cell-cycle control, immune recognition, and metastasis [28–30]. EZH2-mediated silencing of miR-200c, for example, supports BMI1-dependent hepatocarcinogenesis, whereas pharmacologic or genetic EZH2 inhibition can restore NK-cell ligand expression and enhance immune-mediated tumor-cell elimination. Apigenin has been shown to inhibit EZH2 under hypoxic conditions and trigger ER-stress-associated autophagic cell death in gastric cancer, providing a mechanistic precedent for testing whether it similarly destabilizes the EZH2–H3K27me3 axis in HCC. If validated in liver cancer models, apigenin could complement EZH2 inhibitors by reducing the epigenetic

plasticity that enables HCC cells to evade sorafenib, immunotherapy, and other first-line treatments [31-34].

Sirtuins constitute a second, metabolically sensitive epigenetic node through which apigenin may influence HCC. These NAD^+-dependent deacetylases couple cellular NAD^+ availability to histone acetylation, p53 activity, inflammatory signaling, autophagy, and mitochondrial homeostasis; consequently, their activity is intrinsically responsive to the metabolic state of the tumor. Apigenin has been reported to activate the SIRT1–p53 axis in liver injury models, promoting deacetylation of p53 and autophagy while restraining oxidative and inflammatory injury [35,36]. However, in established HCC, SIRT1 can display context-dependent oncogenic functions, with elevated expression linked to advanced disease, excessive autophagy, macrophage inflammatory polarization, and poor prognosis [37,38]. This apparent paradox underscores the need for a tumor-stage–resolved model: apigenin may be hepatoprotective in early metabolic liver disease by supporting adaptive SIRT1 signaling, yet may need to be evaluated carefully in advanced HCC, where SIRT1-dependent chromatin states could support survival and immune evasion.

A particularly innovative aspect of apigenin's proposed action is its capacity to link chromatin regulation with hepatocellular lipid metabolism. Apigenin suppresses proliferation, migration, invasion, and lipid accumulation in HCC cells by downregulating the H3K4 demethylase KDM1A/LSD1; restoration of KDM1A partially rescues these metabolic and malignant phenotypes. Because KDM1A coordinates histone methylation with transcriptional control of lipid-handling genes, apigenin may therefore interrupt a feed-forward loop in which aberrant chromatin remodeling sustains de novo lipogenesis, membrane synthesis, and energetic flexibility in HCC. This mechanism is especially relevant to HCC arising in nonalcoholic fatty liver disease and metabolic syndrome, where lipid-driven inflammation and epigenetic reprogramming jointly shape tumor initiation and progression [39-42].

Collectively, apigenin may be conceptualized as a multi-node epigenetic modulator that simultaneously targets EZH2-dependent Polycomb repression, sirtuin/NAD^+-linked deacetylation, and KDM1A-associated chromatin control of lipid metabolism. Rather than producing a uniform "epigenetic inhibition" effect, its activity may be network-dependent: EZH2 suppression could reactivate tumor-suppressive and immune-recognition programs, whereas SIRT1 modulation could recalibrate autophagy and inflammatory chromatin states according to tumor context [37,43-45]. Future studies should therefore combine apigenin

treatment with CUT&Tag/CUT&RUN mapping of H3K27me3 and H3K4 methylation, ChIP-seq for EZH2 and KDM1A occupancy, ATAC-seq for chromatin accessibility, NAD^+/NADH and acetyl-proteomic profiling, and lipidomics in genetically diverse HCC models. Such an integrated approach would determine whether apigenin can be developed as an adjuvant epigenetic-metabolic therapy capable of sensitizing HCC to EZH2 inhibitors, immune checkpoint blockade, or tyrosine kinase inhibitors [46-48].

6. Modulation of Non-Coding RNA Networks

6.1 miRNA Transcriptome Remodeling in HCC

The clearest HCC-specific non-coding RNA evidence comes from Wang and colleagues, who used next-generation miRNA transcriptomic sequencing of Huh7 cells to show that apigenin broadly reshapes the miRNA landscape: differential miRNA expression was validated by qRT-PCR, targets were curated against miRTarBase, and pathway analysis mapped the regulated genes to proliferation, cell-cycle, and invasion circuits (Figure 2). Apigenin inhibited Huh7 proliferation, colony formation, and invasion concentration-dependently, and reduced xenograft growth with reduced Ki67 and increased Bax expression [11]. Conceptually, this study shows that in HCC cells apigenin's anti-tumor phenotype is executed substantially through miRNA-mediated post-transcriptional reprogramming, i.e., it is an epigenetic-scale effect, not a single-target effect.

6.2 miR-520b/ATG7: From Transcriptome to Chemosensitization

The mechanistic link between apigenin's miRNA activity and drug response is provided by the miR-520b/ATG7 axis. In doxorubicin-treated HCC cells, apigenin upregulates miR-520b, which silences ATG7, a key autophagy regulator; suppression of drug-induced cytoprotective autophagy enhances doxorubicin's cytotoxicity [12,49,50]. Studies of flavonoid-mediated epigenetic modulation of drug resistance illustrate this relationship with an even more specific example, namely, that a flavonoid restores a tumor suppressor miRNA, which in turn removes an autophagic survival shield that otherwise blunts chemotherapy [16,51]. This is a paradigmatic example of epigenetic modulation that converts a non-responding tumor cell into a chemotherapy-sensitive one (Figure 2).

6.3 lncRNA and Wnt-axis Regulation

Complementing the miRNA data, apigenin suppresses HCC tumor growth through the H19 lncRNA–Wnt/β-catenin regulatory axis, indicating that its non-coding RNA reach extends to

long non-coding scaffolds of oncogenic signaling [17]. In colorectal cancer, apigenin inhibits growth through miR-215-5p-mediated down-regulation of E2F1/3 [31], and studies document additional apigenin-responsive miRNAs (e.g., miR-138) across tumor systems [52]. The consistent pattern that apigenin re-equilibrates miRNA/lncRNA networks that cancer has pathologically rewired, supports classifying it as a non-coding RNA modulator in its own right (Figure 2).

7. Apigenin as a Chemosensitizer in HCC: Combination Evidence

7.1 Doxorubicin: Dual Mechanistic Proof in the Same Tumor

The most rigorous chemosensitization evidence in HCC comes from two complementary studies of doxorubicin (ADM) resistance. First, in doxorubicin-resistant BEL-7402/ADM cells, a non-toxic dose of apigenin significantly sensitized cells to doxorubicin and increased intracellular ADM concentration. Mechanistically, apigenin reduced NRF2 at both mRNA and protein levels through downregulation of the PI3K/Akt pathway, shrinking the NRF2-dependent cytoprotective gene program; in BEL-7402/ADM xenografts, combined treatment inhibited tumor growth, reduced proliferation, and induced apoptosis more substantially than doxorubicin alone [10]. Second, as described above, apigenin also sensitizes HCC cells to doxorubicin through miR-520b/ATG7-mediated suppression of protective autophagy [12]. The importance of this pairing is that the same flavone attacks two independent resistance circuits, antioxidant/detoxification programming and autophagic cytoprotection, from different mechanistic angles (signaling-pathway suppression and miRNA restoration, respectively) (Figure 3). This multi-hit profile expected of an epigenetic modulator and rarely achievable with a single-target adjuvant [10,12,16].

7.2 5-Fluorouracil: Mitochondrial Apoptosis in vitro and in vivo

Hu and colleagues showed that subtoxic apigenin (4 μmol/L) enhanced the cytotoxicity of 5-FU (100 μg/mL) in HCC cells, and that, in HCC xenografts, the combination of apigenin (20 mg/kg, five times weekly for three weeks) plus 5-FU significantly inhibited tumor growth. Annexin V/PI staining, TUNEL assays, and western blotting confirmed synergy: the combination increased reactive oxygen species, collapsed mitochondrial membrane potential ($\Delta\Psi m$), decreased Bcl-2, and triggered the mitochondrial apoptotic pathway [13]. Here apigenin's contribution is the lowering of the apoptotic threshold, a downstream consequence of its signaling and epigenetic remodeling, rather than any change in 5-FU metabolism per se (Figure 3).

7.3 Sorafenib: Bridging the Flavonoid Adjuvant Concept to Standard Care

Because sorafenib remains the archetype of HCC systemic therapy, the 2024 Translational Oncology study by Singh and colleagues is a strategically important bridge. Using in silico docking, HepG2 and Huh7 exposure experiments, and animals bearing diethylnitrosamine (DEN)-induced tumors treated with apigenin (50 mg/kg), sorafenib (10 mg/kg), or the combination, the authors found good apigenin bioavailability (UPLC-verified) and demonstrated that apigenin enhances sorafenib's anti-tumor efficacy in HCC [53]. Given that sorafenib's own resistance involves epigenetically regulated survival and angiogenic programs [1,2], apigenin's chromatin- and miRNA-level modulation provides a coherent mechanistic scaffold for this combination, although the sorafenib study did not dissect a specific epigenetic mediator (Figure 3).

7.4 Death-receptor Sensitization: TRAIL

Kim and Kim showed that apigenin synergizes with TNF-related apoptosis-inducing ligand (TRAIL) in Huh-7 cells: the combination markedly inhibited growth, induced chromatin condensation and PARP cleavage, and activated both intrinsic and extrinsic caspase cascades with an increased Bax/Bcl-2 ratio [54]. TRAIL resistance is transcriptionally and epigenetically regulated in HCC [1], so this result extends the priming concept to death-receptor therapeutics. Related combination logic documented in the broader literature includes apigenin–paclitaxel cooperation in other tumor systems [19], and studies of apigenin's chemoprotective and chemosensitizing roles explicitly flag BEL-7402-lineage HCC cells as a validated setting for sensitization [51].

7.5 Synthesis: Why "Epigenetic Adjuvant" is the Right Frame

Across these combinations, three consistent patterns emerge. (1) Dose-sparing: in every HCC combination study, apigenin was used at sub-toxic concentrations, its role was to reconfigure cell state, not to add cytotoxic load [10,13]. (2) Resistance-circuit targeting: the mechanisms identified (NRF2 downregulation, ATG7 silencing via miR-520b, apoptotic-threshold lowering) are precisely the circuits that epigenetic dysregulation had weaponized for resistance [1,10,12]. (3) Multimodal action: apigenin effects HDAC/p300, KDM1A, EZH2, as well as miRNA-, and lncRNA-mediated regulatory networks, concurrently [11,12,14,15,20], so its adjuvant effect is best modeled as landscape-level chromatin and transcriptome remodeling rather than single-enzyme inhibition (Figure 3). Tables 1 and 2 summarize the HCC combination evidence.

8. Beyond Sensitization: Chemoprevention, Anti-EMT, and Delivery

8.1 Upstream and Microenvironmental Effects

Apigenin's HCC-relevant activity extends beyond the treated tumor cell. It inhibits NF-κB and Snail signaling to suppress EMT and metastasis in human HCC [55,56], represses YAP-driven migration and invasion [57], blocks angiogenesis and migration of hepatoma cells [58,59], and induces apoptosis/autophagy through PI3K/Akt/mTOR inhibition [22]. Regarding chemoprevention, apigenin-loaded nanoparticles delayed hepatocarcinogenesis in rats [60], and studies of apigenin's hepatoprotective profile document anti-inflammatory, antioxidative, and anti-steatotic actions that could plausibly modify the cirrhotic field in which HCC arises [61]. Indirect genomic-indirect support comes from network-guided analyses that rank apigenin among the top phytochemical candidates against HCC-associated kinases such as MARK4 using TCGA-derived mutated-gene networks [62].

8.2 Nanodelivery

Two independent nanotechnology studies directly address the bioavailability limitation: apigenin-loaded nanoparticles delayed HCC development in a rat model [60], and galactose-tailored PLGA nanoparticles achieved liver-targeted delivery of apigenin for HCC treatment [63], consistent with broader studies of apigenin nanodelivery in cancer [9]. For an adjuvant intended to be co-administered with chemotherapy, hepatotropic delivery is especially attractive because it concentrates the epigenetic modulator at the target organ while limiting systemic exposure.

8.3 Apigetrin and Metabolite Considerations

Apigenin's 7-glucoside (apigetrin) shows anti-HCC activity, with integrated bioinformatic, in vitro, and transcriptomic analyses of liver cancer cells mapping its effects onto HCC gene-expression programs [64,65]. Consideration of metabolite-level activity is important for translational development because orally administered apigenin reaches the liver predominantly as glucuronidated and sulfated conjugates. The available data on apigetrin therefore raise the possibility that conjugated or metabolite forms may retain biological activity, although the extent and clinical relevance of this activity remain unquantified [66,67].

9. Critical Barriers and Open Questions

The concentrations producing epigenetic effects in vitro (typically low micromolar; e.g., 4 μmol/L in the 5-FU combination [13]) exceed the free plasma levels achievable with oral apigenin in humans [7,9], although the DEN rat model reported measurable bioavailability at 50 mg/kg in the sorafenib study [53]. This exposure gap represents an important translational limitation, as the concentrations associated with efficacy in preclinical models may not be readily achievable through conventional oral administration in humans. Improving apigenin bioavailability and tumor exposure through optimized formulations or delivery strategies may therefore be necessary before its preclinical epigenetic and chemosensitizing effects can be evaluated clinically. Nanodelivery partly mitigates this [60,63], but a formal pharmacokinetic/pharmacodynamic linkage demonstrating that hepatocyte chromatin marks actually change at tolerated exposures has not been established. Establishing this relationship represents a major translational priority. Studies of apigenin in cancer consistently note the lack of clinical trial evidence, and the chemosensitization concept in HCC remains entirely preclinical [7,9,51,61]. Since HCC patients are typically treated on modern systemic backbones (immunotherapy combinations), the historically relevant doxorubicin and 5-FU combinations would need to be reframed, e.g., as transarterial chemoembolization regimens (doxorubicin is the standard TACE drug) or as adjuncts to TKI/IO therapy, before clinical testing is rational.

Apigenin's epigenetic effects are bidirectional depending on context (HDAC inhibition in cancer vs CBP/p300 HAT inhibition in retinal cells [19,25]), and most evidence derives from cell-line studies without target-rescue experiments. Whether the miRNA and chromatin changes are primary (causal) or downstream of signaling suppression cannot be resolved from the existing data; the strongest causal claims remain the direct DNMT/HMT binding work [15,20,21] and the KDM1A target study [14]. No study has yet connected apigenin's epigenetic enzyme engagement to its chemosensitization outcomes within a single HCC model, i.e., shown that KDM1A or DNMT inhibition is required for the NRF2 or miR-520b effects. Resolving this would require epigenome-wide mapping (e.g., methylome or ChIP-seq before and after apigenin) paired with pharmacological or genetic blockade of the candidate enzyme, an experiment that would substantially upgrade the field's causal story. NRF2 suppression in tumor cells is desirable, but systemic NRF2 attenuation could interact with hepatic oxidative-stress management in cirrhotic patients; the hepatoprotective literature suggests net benefit [61,73-76], yet this tension is untested clinically.

10. Docking and Bioinformatics Studies

The central innovation of this review lies in coupling structure-based molecular docking with integrative bioinformatics to position apigenin as a rational epigenetic chemosensitizer rather than merely a complementary phytochemical. Molecular docking against epigenetic effector proteins, including DNA methyltransferases, histone deacetylases, histone methyltransferases/demethylases, bromodomain proteins, and chromatin-remodeling components, can identify high-affinity binding modes, conserved interaction fingerprints, and allosteric vulnerabilities that may underlie apigenin's capacity to restore tumor-suppressive gene-expression programs (Table 3). Subsequent network pharmacology, target enrichment, protein–protein interaction, gene ontology, and pathway analyses can then delineate how these predicted epigenetic targets converge on apoptosis, cell-cycle arrest, ferroptosis, immune recognition, lipid metabolism, and DNA-damage response pathways relevant to HCC [77-81].

This computational framework is particularly valuable for understanding chemotherapy sensitization, where epigenetic plasticity frequently enables HCC cells to survive cytotoxic pressure through activation of detoxification, anti-apoptotic, autophagic, and stem-like survival programs. Prior evidence indicates that apigenin can potentiate doxorubicin activity in chemoresistant HCC cells by suppressing the PI3K/Akt/Nrf2 axis, while also modulating autophagy and miRNA-mediated regulatory networks; these observations support the hypothesis that epigenetic targeting by apigenin may lower the apoptotic and adaptive threshold required for conventional chemotherapy to eliminate malignant hepatocytes. Docking-derived binding poses can therefore be interpreted not as isolated ligand–protein events, but as entry points into a broader regulatory architecture that determines chemotherapeutic response [10,12,22,81-83].

11. Combination Therapeutic Applications

Table 4, presents preclinical evidences demonstrating that apigenin acts as a epigenetic modulator to enhance the efficacy of standard chemotherapeutic agents in HCC. Across multiple cell lines and in vivo models, apigenin consistently produces synergistic or additive anticancer effects, reverses chemoresistance, promotes apoptosis and cell-cycle arrest, suppresses stemness and invasion, and mitigates drug toxicity. These benefits are mechanistically linked to miRNA upregulation, inhibition of Nrf2/PI3K/Akt and oncogenic transcription factors, dual molecular docking occupancy, and the established ability of apigenin

to inhibit HDACs, DNMTs, and related epigenetic enzymes, thereby restoring tumor-suppressor networks and overcoming resistance pathways.

12. Conclusions and Future Perspectives

Apigenin emerges from the accumulated preclinical evidence as a promising natural epigenetic modulator capable of sensitizing HCC cells to conventional chemotherapeutic agents. By concurrently targeting DNA methylation machinery, histone-modifying enzymes (notably HDAC and HAT activity), and non-coding RNA networks, apigenin remodels the epigenetic landscape of HCC toward a more chemosensitive phenotype. This multi-layered interference disrupts key oncogenic pathways, including PI3K/Akt/mTOR, Wnt/β-catenin, and NF-κB signaling, while restoring expression of tumor-suppressor genes and attenuating epithelial–mesenchymal transition and cancer-stem-cell traits that underpin multidrug resistance. The net result is enhanced cytotoxic efficacy of agents such as doxorubicin, sorafenib, and 5-fluorouracil at lower doses, accompanied by reduced off-target toxicity in non-malignant hepatocytes, an especially attractive profile given the frequent underlying cirrhosis in patients with HCC.

Despite these encouraging findings, several critical gaps must be closed before apigenin can transition from a mechanistically compelling phytochemical to a clinically actionable adjuvant. First, the precise hierarchy and temporal dynamics of apigenin's epigenetic effects remain incompletely mapped; multi-omics approaches that integrate genome-wide DNA methylation, histone ChIP-seq, ATAC-seq, and single-cell transcriptomics in patient-derived organoids and orthotopic models are required to identify robust epigenetic signatures predictive of response. Second, pharmacokinetic limitations, rapid metabolism, modest oral bioavailability, and extensive phase-II conjugation necessitate rational formulation strategies. Lipid-based nanocarriers, polymeric nanoparticles, or pro-drug derivatives engineered for hepatic tropism could markedly improve intra-tumoral exposure while preserving the molecule's favorable safety margin. Third, the potential for context-dependent or even pro-tumorigenic epigenetic shifts under certain microenvironmental conditions (hypoxia, inflammation, microbiome-derived metabolites) warrants systematic evaluation in immunocompetent models that recapitulate the fibrotic, immune-exhausted niche of human HCC.

Looking forward, several innovative research avenues hold particular promise. Combinatorial epigenetic "priming" regimens in which low-dose apigenin is administered prior to or concurrently with immune-checkpoint inhibitors or next-generation tyrosine-kinase inhibitors

could exploit restored antigen presentation and reduced immunosuppressive cytokine networks. CRISPR-based epigenetic editing screens may further delineate the minimal set of apigenin-sensitive loci whose modulation is both necessary and sufficient for chemosensitization, thereby guiding the design of more selective synthetic analogs. In parallel, liquid-biopsy monitoring of circulating cell-free DNA methylation patterns and histone-modification marks could enable real-time assessment of epigenetic reprogramming in early-phase clinical trials. Ultimately, well-designed phase I/II studies that incorporate rigorous pharmacokinetic/pharmacodynamic endpoints, stratified biomarker analyses, and patient-reported outcome measures will be essential to determine whether apigenin's epigenetic activity can be harnessed to improve objective response rates, progression-free survival, and quality of life in patients with advanced HCC. By bridging natural-product pharmacology with precision epigenetic oncology, apigenin may help redefine the therapeutic index of existing chemotherapies and open new combinatorial paradigms for this still-devastating malignancy.

List of Abbreviations

Abbreviation	Full term
5-FU	5-Fluorouracil
ADM	Doxorubicin (adriamycin)
AFP	
Akt	Protein kinase B
ATG7	Autophagy-related protein 7
Bax	Bcl-2-associated X protein
Bcl-2	B-cell lymphoma 2
CBP	CREB-binding protein
CDDP	
CDK4	Cyclin-dependent kinase 4
DEN	Diethylnitrosamine
DNMT	DNA methyltransferase

EMT	Epithelial–mesenchymal transition
ER	
EZH2	Enhancer of zeste homolog 2
HAT	Histone acetyltransferase
HDAC	Histone deacetylase
HMT	Histone methyltransferase
HCC	Hepatocellular carcinoma
HSA	
KDM1A	Lysine demethylase 1A
KEAP1	Kelch-like ECH-associated protein 1
lncRNA	Long non-coding RNA
LSD1	Lysine-specific demethylase 1
MAPK	Mitogen-activated protein kinase
miRNA/miR	MicroRNA
MM-PBSA	Molecular Mechanics Poisson–Boltzmann Surface Area
mRNA	Messenger RNA
mTOR	Mechanistic target of rapamycin
NAD+	Nicotinamide adenine dinucleotide (oxidized form)
NADH	Nicotinamide adenine dinucleotide (reduced form)
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NQO1	NAD(P)H quinone dehydrogenase 1
NRF2	Nuclear factor erythroid 2-related factor 2
PARP	Poly(ADP-ribose) polymerase
PDB	Protein Data Bank
PI3K	Phosphoinositide 3-kinase
PLGA	Poly(lactic-co-glycolic acid)

PRC2	Polycomb Repressive Complex 2
RCT	Randomized controlled trial
qRT-PCR	Quantitative reverse-transcription polymerase chain reaction
ROS	Reactive oxygen species
SAHA	Suberoylanilide hydroxamic acid
SIRT1	Sirtuin 1
TACE	Tumor necrosis factor-alpha-converting enzyme
TCGA	The Cancer Genome Atlas
TKI	Tyrosine kinase inhibitor
TLR4	Toll-like receptor 4
TRAIL	TNF-related apoptosis-inducing ligand
UPLC	Ultra-performance liquid chromatography
YAP	Yes-associated protein
ΔΨm	Mitochondrial membrane potential

Conflicts of Interest

The authors declare no competing interests that may have influenced in this manuscript.

Author Contributions

YA: Conceptualization, Writing - Original Draft, Resources, Software; **FJD:** Conceptualization, Writing - Original Draft, Resources, Software; **AB:** Conceptualization, Writing - Original Draft, Resources, Software; **GG:** Conceptualization, Writing - Original Draft; **ST:** Conceptualization, Visualization, Writing - Original Draft, Writing – Review & Editing, Supervision, Project Administration, Validation. All authors have read and agreed to the published version of the manuscript.

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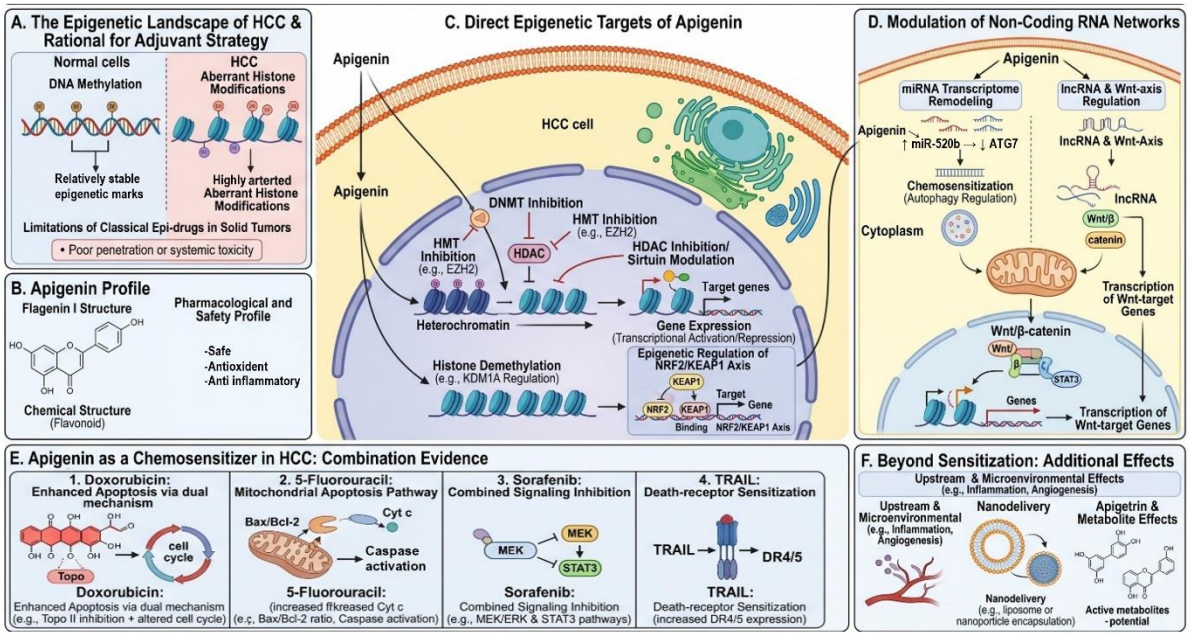
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Graphical Abstract. (A) HCC is depicted as an epigenetically dysregulated malignancy characterized by aberrant DNA methylation, histone modification, and non-coding RNA circuitry that promote tumor progression and influence therapy response. The panel also highlights why epigenetic modulation is attractive as an adjuvant strategy, given the limited performance of classical epigenetic drugs in solid tumors. **(B)** Apigenin is presented as a dietary flavonoid with a defined chemical scaffold, pleiotropic bioactivity, favorable translational profile, and compatibility with delivery optimization. **(C)** Direct epigenetic targets attributed to apigenin include DNMTs, histone methyltransferase-related repression, KDM1A/LSD1, HDACs, redox-linked chromatin signaling, the NRF2/KEAP1 axis, sirtuins, and metabolism-associated chromatin regulation. Across cancer models, apigenin has been reported to inhibit HDAC and DNMT activity, increase HAT activity, and modulate regulatory miRNAs. In HCC-relevant epigenetic contexts, KDM1A and EZH2 are notable nodes linked to malignant transcriptional control and sorafenib response. **(D)** Apigenin-mediated remodeling of miRNA and lncRNA networks is illustrated as a mechanism for suppressing oncogenic ncRNA programs, restoring tumor-suppressive signatures, and altering apoptosis, autophagy, and invasion. **(E)** Apigenin is framed as a chemosensitizer that may enhance apoptosis, mitochondrial dysfunction, cell-cycle arrest, ferroptosis- or death-receptor-linked responses, and overall antitumor efficacy when combined with doxorubicin, 5-fluorouracil, sorafenib, or TRAIL. This “epigenetic adjuvant” concept is consistent with evidence that chromatin regulators and ncRNA axes shape drug sensitivity in HCC. **(F)** Beyond chemosensitization, this section summarizes broader translational opportunities including anti-EMT activity, chemoprevention, tumor

microenvironment modulation, nanodelivery strategies, and metabolite-related considerations, supporting apigenin as a candidate precision-combination adjuvant in HCC.

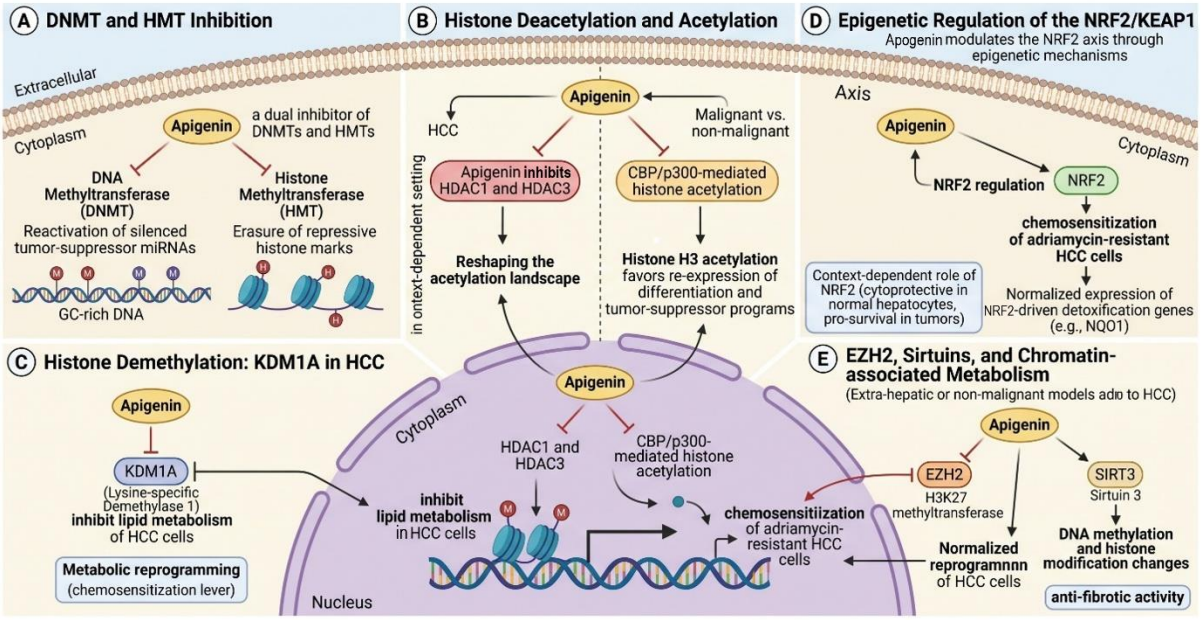


Figure 1: Apigenin is illustrated as a central flavone scaffold positioned within a hepatic context and linked to five epigenetic modules. **(A)** DNMT/HMT inhibition is represented by reduced DNA methylation at GC-rich regions and attenuation of PRC2/EZH2-associated H3K27me3, consistent with reversal of repressive chromatin states. **(B)** HDAC1/3 inhibition and acetylome remodeling are depicted as increased H3/H4 acetylation with context-dependent interaction with CBP/p300-linked histone acetylation machinery. **(C)** KDM1A inhibition in HCC is visualized as restoration of H3K4 methylation and suppression of lipid-metabolism–associated chromatin outputs, reflecting the importance of KDM1A-driven epigenetic control in liver cancer. **(D)** NRF2/KEAP1 axis rewiring is shown as epigenetic remodeling of NRF2-associated transcription with downstream modulation of antioxidant-response genes, including NQO1 and HO-1, leading to chemosensitization. **(E)** EZH2/sirtuin-linked chromatin metabolism integrates reduced PRC2-mediated H3K27me3 with autophagy, apoptosis, and improved therapeutic responsiveness; EZH2 is particularly relevant in HCC because its repression enhances ferroptosis and sorafenib sensitivity. Collectively, the schematic highlights how apigenin-centered chromatin modulation may couple transcriptional reprogramming to metabolic vulnerability and treatment response in HCC.

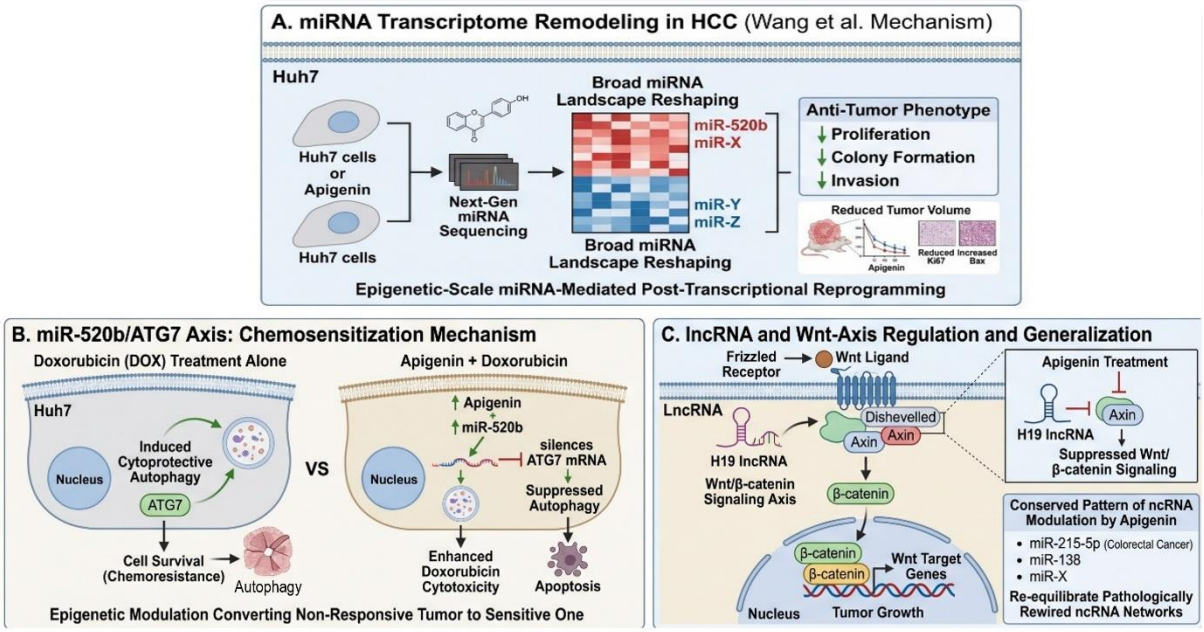


Figure 2: (A) Apigenin-induced miRNA transcriptome remodeling in HCC is illustrated as coordinated upregulation and downregulation of multiple miRNAs, resulting in global post-transcriptional network reprogramming that influences proliferation, cell-cycle progression, and invasion. **(B)** In doxorubicin-treated HCC cells, apigenin upregulates miR-520b, leading to repression of ATG7, attenuation of cytoprotective autophagy, and enhancement of doxorubicin-induced cell death; the inset summarizes the associated reduction in xenograft tumor growth and changes in Ki67 and Bax. **(C)** Apigenin suppresses the H19 lncRNA–Wnt/β-catenin axis, thereby reducing β-catenin-driven oncogenic transcription and inhibiting HCC proliferation and invasion. Together, the schematic presents apigenin as a multi-layer ncRNA modulator that acts through both miRNA and lncRNA-dependent regulatory circuits. NcRNA-mediated control of tumor signaling and Wnt/β-catenin output is highly relevant in HCC biology.

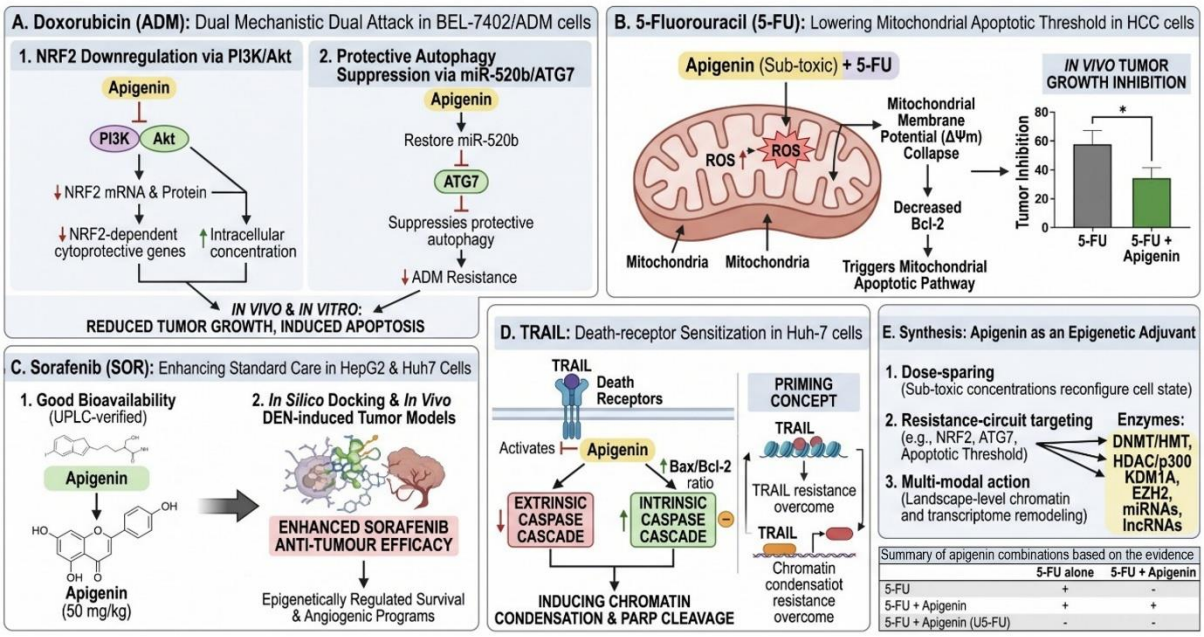


Figure 3: Schematic summary of the proposed chemosensitizing roles of apigenin across major therapeutic contexts in HCC. **(A)** In doxorubicin-resistant HCC, the figure depicts dual resistance-circuit targeting, including suppression of cytoprotective signaling and inhibition of protective autophagy; the miR-520b/ATG7 axis is directly supported in BEL-7402/ADM cells and xenografts, where apigenin increases doxorubicin sensitivity and suppresses tumor growth. **(B)** In the 5-fluorouracil setting, apigenin is illustrated as a mitochondrial apoptosis primer that promotes ROS accumulation, mitochondrial depolarization, cytochrome c release, caspase activation, and apoptotic cell death. **(C)** In the sorafenib setting, apigenin is shown as a translational adjuvant that enhances antitumor efficacy in vitro and in DEN-induced tumor-bearing models, with increased apoptosis/cell-cycle arrest and reduced stemness- and oncogenic-associated markers. **(D)** In the TRAIL setting, apigenin is visualized as a death-receptor sensitizer that promotes convergence of extrinsic and intrinsic apoptotic cascades. **(E)** The central panel synthesizes these observations into an epigenetic-adjuvant model, in which sub-toxic apigenin reprograms resistance circuits through multi-modal chromatin/transcriptomic and non-coding RNA-associated effects.

Table 1. Apigenin combination and mechanism studies in hepatocellular carcinoma.

	Model	Combination/agent	Key mechanism	Main outcome	Ref
Combination / Chemosensitization studies	BEL-7402/ADM cells; xenografts	Doxorubicin	PI3K/Akt downregulation → NRF2↓ → reduced detox genes; ↑ intracellular ADM	Sensitized resistant cells; greater tumor inhibition + apoptosis in vivo	[10]
	HCC cells	Doxorubicin	miR-520b↑ → ATG7 silencing → loss of protective autophagy	Enhanced doxorubicin cytotoxicity	[12]
	HCC cells; xenografts	5-FU	↑ROS, ΔΨm loss, Bcl-2↓, mitochondrial apoptosis	Sub-toxic apigenin synergized in vitro and in vivo	[13]
	HepG2/Huh7; DEN rat model	Sorafenib	Multi-target co-action; verified bioavailability	Enhanced anti-tumor efficacy	[53]
	Huh-7 cells	TRAIL	Caspase activation (intrinsic + extrinsic), Bax/Bcl-2↑	Synergistic apoptosis; TRAIL-resistance reversal	[54,74]
Monotherapy / Mechanistic studies	Huh7 cells; xenografts	(mono-therapy, miRNA basis)	miRNA transcriptome remodeling	Proliferation/invasion↓; tumor growth↓, Ki67↓, Bax↑	[11]
	HCC cells	(mono-therapy)	KDM1A histone demethylase inhibition	Lipid-metabolism suppression	[14]
	HCC cells	(mono-therapy)	PI3K/Akt/mTOR inhibition	Apoptosis + autophagy induction	[22]
	HCC cells	(mono-therapy)	NF-κB/Snail inhibition	EMT and metastasis suppression	[56]
	HCC cells	(mono-therapy)	H19–Wnt/β-catenin axis	Tumor growth suppression	[17]
	HCC cells	(mono-therapy)	YAP downregulation	Migration/invasion↓; autophagy↑	[57]
	HCC cells	(mono-therapy)	CyclinD1/CDK4 via p38 MAPK–p21	Cell-cycle arrest	[58]

Table 2. Studies are stratified from chromatin proof-of-concept to HCC-specific chemo-sensitization to human pharmacokinetic readiness (Arrows denote direction of change vs. appropriate control).

No.	Cancer type	Evidence tier / Model & regimen	Epigenetic target & modulation	Chemo partner & HCC efficacy	Mechanistic insight	Ref
A. PRECLINICAL — Chromatin and miRNA reprogramming						
P1	Prostate cancer	PC-3/22Rv1 + xenograft; 20–40 μM; 20–50	↓ HDAC1/3 + activity; ↑ H3/H4ac; ↑ H3ac at p21 promoter	Monotherapy: arrest + xenograft ↓	Class I HDACi → p21; Bax↑ Bcl-2↓	[72]

		mg/mouse/d p.o. ×8 wk				
P2	Breast cancer	MDA-MB-231; apigenin ± vorinostat; docking/MD	↓ HDACs/DNMTs, ↑ HAT; miR-21↓ miR-200b↑	Combination: Synergy with vorinostat; apoptosis ↑	Dual blockade opens chromatin	[70]
P3	HCC	HCC cells; miRNA transcriptome-seq	Genome-wide miRNA reprogramming	Monotherapy: ↓ HCC growth	miR-network rewiring	[11]
P4	HCC	BEL-7402/ADM; non-toxic apigenin	↑ miR-101 → ↓ NRF2	Combination: + Doxorubicin: sensitivity restored	Antioxidant silencing	[73]
B. PRECLINICAL — HCC-specific chemo-sensitization						
P5	HCC	BEL-7402/ADM + xenograft; + doxorubicin	↓ PI3K/Akt → ↓ NRF2/NQO1/HO-1	Combination: ↑ Intracellular ADM; tumor ↓	Redox deprotection; MDR reversal	[10]
P6	HCC	BEL-7402/ADM + xenograft; + doxorubicin	↑ miR-520b → ↓ ATG7 → ↓ autophagy	Combination: Sensitization; xenograft ↓	Survival-to-death switch	[12]
P7	HCC	HepG2/Huh7 + xenograft; 48 μM + sorafenib 2 μM; HSA 21–24	↑ p53; ↓ NANOG/β-catenin/c-MYC/TLR4/AFP	Combination: G1 arrest; apoptosis 45.5% vs 31.9%; tumor ↓	Stemness collapse	[53]
C. TRANSLATIONAL–CLINICAL — Readiness and gaps						
T1	general evidence	Framework review; KYSE-510 extracts	~35% DNMT inhibition at 50 μM; HDAC/miR	Rationale for combos	Epi-drug scaffold	[75]
T2	HCC-related	Xenograft tolerability synthesis	Efficacy w/o overt toxicity	Tumor ↓; weight preserved	Adjuvant index favorable	[10,52]
T3	Human / clinical pharmacokinetic evidence	Human PK synthesis	Bioav. ~30%; Tmax 0.5–2.5 h; t1/2 ~2.5 h; sol. <0.001 mg/mL	Capsules/nano reach 1–5 μM	Need nano formulations	[76]
T4	Not applicable / research gap	Gap: no HCC apigenin–chemo RCTs	Biomarkers: HDAC, H3/H4ac, NRF2, miR-101/520b, ATG7, AFP	Phase I call	First-in-HCC path	[53,76]

Table 3. Molecular Docking Binding Affinities, Key Interactions, and Therapeutic Implications of Apigenin as an Epigenetic Modulator Enhancing Chemotherapeutic Efficacy in HCC.

Target Protein (PDB ID)	Binding Affinity (kcal/mol)	Key Interacting Residues / Interactions	Epigenetic / Oncogenic Implication in HCC	Reference
HDAC1 (e.g., 4BKX)	−6.8 to −9.3 (varies by protocol; focused docking often stronger than vorinostat)	Zn^{2+} coordination; H-bonds (Asn88, Asp92, His171, Tyr296); π–π stacking (Phe143, Phe198); hydrophobic contacts (Cys93, His134, etc.)	Class I HDAC inhibition → increased histone acetylation, reactivation of tumor-suppressor genes, apoptosis induction; supports chemo-sensitization	[81-83]
HDAC3 (e.g., 4A69)	−8.9 (AutoDock); MM-PBSA ΔG ≈ −92 kJ/mol	Zn^{2+} coordination; H-bonds and hydrophobic contacts in catalytic pocket comparable to SAHA (vorinostat)	HDAC downregulation modulates apoptotic regulators and miRNAs; synergistic with HDAC inhibitors for enhanced cytotoxicity	[71,81]
DNMT1	−6.38 (GlideScore); ≈ −7.8 (AutoDock)	H-bonds (Glu1168, Asn1267, Val1268, Cys1226); tethering at both ends of catalytic pocket	Non-nucleoside DNMT inhibition → DNA demethylation, reactivation of silenced	[15]

			tumor-suppressor genes; dual epigenetic reprogramming	
EZH2 (HMT)	−10.07 (GlideScore)	H-bonds (Tyr37, Ala83); non-polar and polar contacts (Arg81, Asp121, Arg123)	Inhibition of H3K27me3 → chromatin relaxation, reduced gene silencing; dual DNMT/HMT activity of flavones	[15]
p53	−7.0 (apigenin alone); −11.8 (with sorafenib)	Simultaneous docking occupies distinct sites; enhanced affinity in combination	Stabilization/activation of p53 pathway → apoptosis and cell-cycle arrest; synergistic with sorafenib	[35]
β-Catenin	−6.5 (alone); −13.7 (with sorafenib)	Dual-ligand engagement of active sites	Suppression of Wnt/β-catenin oncogenic signaling; reduced stemness and proliferation	[35]
NANOG	−6.5 (alone); −15.3 (with sorafenib)	Enhanced dual-ligand affinity	Downregulation of stemness marker; decreased self-renewal and chemoresistance	[35]
c-MYC	−5.7 (alone); −10.6 (with sorafenib)	Combined docking improves binding	Oncogene suppression → reduced proliferation and metabolic reprogramming	[35]
TLR4	−5.0 (alone); −9.5 (with sorafenib)	Dual-site engagement	Attenuation of inflammatory/TLR signaling linked to HCC progression	[35]
KDM1A (LSD1)	Predicted favorable interaction (molecular docking)	Interaction with histone demethylase catalytic site	Inhibition of lipid metabolism and proliferation in HCC cells via KDM1A downregulation	[14]

Caption notes: Binding energies are reported as published (more negative values indicate stronger affinity). Values vary with software (AutoDock, Glide, GOLD), scoring functions, and presence of water/Zn^{2+}. Simultaneous docking of apigenin + sorafenib consistently yields stronger affinities than either ligand alone, supporting chemosensitization. Apigenin exhibits dual epigenetic activity (HDAC/DNMT/HMT/KDM inhibition) that can reverse oncogenic silencing and enhance the efficacy of multikinase inhibitors such as sorafenib in HepG2/Huh7 models and DEN-induced HCC.

Table 4. Combination Studies of Apigenin with Chemotherapeutic Agents Demonstrating Enhanced Efficacy and Epigenetic Modulation in HCC.

Chemotherapeutic Agent	**Experimental Models**	**Key Combination Outcomes**	**Proposed Mechanistic / Epigenetic Contributions of Apigenin**	**Reference**
Sorafenib	In vitro: HepG2, Huh7 cells; In vivo: DEN-induced HCC in mice; Molecular docking	Dose-dependent synergy (HSA scores ~4.3–6.9); enhanced cytotoxicity, apoptosis induction, G0/G1 or G2/M arrest; significant tumor nodule reduction; upregulation of p53; downregulation of NANOG, β-catenin, c-MYC, AFP, CD10,	Dual occupancy of oncogenic protein active sites (p53, β-catenin, NANOG, c-MYC, TLR4) with superior combined binding affinities; potential HDAC/DNMT modulation restoring tumor-suppressor expression and overcoming stemness/resistance	[53,84]

		TLR4; reduced sorafenib-associated toxicity		
Cisplatin (CDDP)	In vitro: HepG2, Hep3B, Huh7 cells	Sensitization ranking HepG2 > Huh7 > Hep3B; synergistic or additive genotoxicity (sister chromatid exchange); cell-cycle delays; reduced clonogenicity; senescence-like phenotype (especially Hep3B/Huh7); strong anti-migratory and anti-invasive effects	Modulation of DNA repair/homologous recombination and genetic instability; cell-line-specific gene expression differences likely involving epigenetic reprogramming of EMT and DNA-damage responses	[85]
Doxorubicin (ADM)	In vitro: BEL-7402/ADM (resistant) cells; In vivo: xenograft models	Reversal of doxorubicin resistance; caspase-dependent apoptosis; increased intracellular drug accumulation; inhibition of ATG7-dependent autophagy; tumor growth suppression	Upregulation of miR-101 (targeting Nrf2 3′-UTR) and miR-520b (targeting ATG7); inhibition of PI3K/Akt/Nrf2 pathway; reduction of oxidative-stress defense and autophagic survival mechanisms linked to epigenetic miRNA regulation	[10,12,73]
5-Fluorouracil (5-FU)	In vitro: HCC cell lines (e.g., SK-Hep-1, BEL-7402); In vivo: HCC xenografts	Sub-toxic apigenin markedly potentiates 5-FU cytotoxicity; increased ROS, loss of mitochondrial membrane potential (ΔΨm); activation of mitochondrial apoptotic pathway (↓Bcl-2, ↑caspase-3/PARP); significant xenograft tumor inhibition	ROS-mediated disruption of drug resistance and concurrent mitochondrial apoptosis; possible contribution of apigenin-mediated epigenetic shifts in glycolytic and survival gene expression	[13]
Doxorubicin + Hesperidin (comparative)	In vitro: HepG2 cells	Strongest potentiation of doxorubicin toxicity among tested flavonoids; altered expression of glycolytic genes (HK2, LDHA); reduced oxidative DNA damage despite increased cytotoxicity	Dual flavonoid action on Warburg-effect genes and non-oxidative cytotoxic synergy; supports broader epigenetic influence of flavones on metabolic reprogramming	[86]