Coumarins in Cancer: Mechanistic Links Between Mitochondrial DNA Signaling, Oxidative Stress, and Innate Immunity

Yasser Fakri Mustafa*

Department of Pharmaceutical Chemistry, College of Pharmacy, University of Mosul, Mosul, 41001, Iraq

Abstract: Mitochondrial DNA (mtDNA) release connects mitochondrial dysfunction and oxidative stress to innate immune signaling in cancer. Although coumarins influence mitochondrial homeostasis and inflammatory pathways, their direct involvement in mtDNA-dependent signaling remains incompletely established. This narrative review critically evaluates the mechanistic relationships between coumarin-mediated mitochondrial regulation, mtDNA signaling, and innate immune responses in cancer. Published mechanistic, cellular, and preclinical evidence was synthesized to examine coumarin-induced changes in mitochondrial function, reactive oxygen species production, mtDNA integrity, and innate immune signaling. Available findings indicate that coumarins exert context-dependent effects on mitochondrial redox balance, autophagy, mitophagy, and inflammatory responses involving cGAS–STING, TLR9, and NLRP3. These activities vary according to chemical structure, concentration, cellular characteristics, and experimental conditions. Although several studies demonstrate modulation of mitochondrial function or downstream inflammatory pathways, direct evidence establishing coumarin-induced mtDNA release as the causal mediator of these responses remains limited. Consequently, the mitochondrial and immunological effects of coumarins cannot consistently be attributed to a unified mtDNA-dependent mechanism. Coumarins may therefore regulate mitochondrial–immune interactions in cancer, but their therapeutic relevance requires further experimental validation. Future investigations should prioritize direct measurements of mtDNA release, pathway-specific functional studies, and comparative analyses of malignant and immune-cell populations to establish mechanistic causality and translational significance.

Keywords: Coumarins, Mitochondrial DNA, Oxidative stress, Innate immunity, Cancer immunotherapy.

1. INTRODUCTION

Mitochondria play fundamental roles in cellular energy production, metabolic regulation, and intracellular signaling. Beyond its classical function in encoding components of the oxidative phosphorylation machinery, mitochondrial DNA (mtDNA) has emerged as an important regulator of cellular stress responses and immune signaling. In cancer, mitochondrial dysfunction, oxidative stress (OS), and metabolic reprogramming frequently compromise mtDNA integrity, leading to its release into the cytoplasm or extracellular environment. Once displaced from mitochondria, mtDNA can function as a damage-associated molecular pattern (DAMP), triggering innate immune responses through pattern-recognition receptors such as Toll-like receptor 9 (TLR9) and the cyclic GMP–AMP synthase–stimulator of interferon genes (cGAS–STING) pathway. Activation of these signaling networks links mitochondrial damage to inflammation, redox imbalance, and antitumor immunity, thereby influencing multiple aspects of tumor development and progression [1].

Coumarins constitute a large family of naturally occurring phytochemicals widely distributed in medicinal plants, dietary sources, and traditional herbal remedies. These compounds have attracted considerable attention because of their diverse pharmacological activities, including antioxidant [2], anti-inflammatory [3], antimicrobial [4], anti-obesity [5], anti-hyperlipidemia [6], and anticancer [7] effects. Increasing evidence indicates that coumarins can modulate mitochondrial function and cellular redox homeostasis, suggesting a potential role in regulating mtDNA-mediated signaling pathways. Through their ability to influence OS, mitochondrial integrity, and immune activation, coumarins may affect the generation, release, and downstream signaling of mtDNA in cancer cells and within the tumor microenvironment (TME) [8]. Understanding the molecular mechanisms underlying these interactions may provide new opportunities for the development of mitochondria-targeted therapeutic strategies.

The anticancer properties of coumarins have been extensively investigated in both preclinical and clinical settings. In addition to their direct effects on tumor cell proliferation, survival, and metastasis, coumarins can modulate the activity of immune cells infiltrating the TME. However, their immunological actions appear to be context-dependent and may vary according to the cellular and molecular landscape of individual tumors. Notably, the influence of coumarins on innate immune signaling remains incompletely understood [9]. Emerging studies suggest that these compounds can regulate key inflammatory and immune pathways, including the NLRP3 inflammasome, the cGAS–STING

*Address correspondence to this author at the Department of Pharmaceutical Chemistry, College of Pharmacy, University of Mosul, Mosul, 41001, Iraq;
E-mail: Dr.yassermustafa@uomosul.edu.iq

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axis, and type I interferon signaling. Depending on the biological context, coumarins may either enhance or suppress these pathways, highlighting the complexity of their immunomodulatory effects [10].

Despite growing evidence that coumarins influence mitochondrial function, redox homeostasis, and inflammatory signaling, a critical mechanistic gap remains: whether these effects are directly mediated by changes in mtDNA integrity or release and subsequent activation of innate immune pathways in cancer. Most available findings describe mitochondrial or inflammatory responses without establishing a causal sequence linking individual coumarins to mtDNA-dependent cGAS–STING, TLR9, or NLRP3 signaling. Moreover, the extent to which these relationships differ between malignant and immune cells remains insufficiently characterized. Resolving these uncertainties is essential for distinguishing experimentally supported mechanisms from proposed molecular interactions and for evaluating their potential therapeutic significance. Accordingly, the available evidence is considered at four distinct levels: (i) direct measurements of cytosolic or extracellular mtDNA following coumarin exposure; (ii) experimentally demonstrated mitochondrial dysfunction or OS without direct assessment of mtDNA release; (iii) modulation of cGAS–STING, TLR9, or NLRP3 signaling without establishing mtDNA involvement; and (iv) proposed mechanistic relationships inferred from indirect observations or non-cancer experimental models. This distinction is essential because mitochondrial alterations and inflammatory pathway modulation do not independently demonstrate mtDNA-dependent signaling.

This narrative review critically examines the mechanistic relationships among coumarin-mediated mitochondrial regulation, mtDNA signaling, and innate immune responses in cancer. Its distinctive contribution lies in integrating these interconnected processes within an evidence-based framework rather than discussing the antioxidant, mitochondrial, and immunomodulatory properties of coumarins independently. Particular attention is given to evaluating whether experimentally observed changes in mitochondrial function, ROS production, and inflammatory signaling provide direct evidence of mtDNA-dependent activation of cGAS–STING, TLR9, or NLRP3. The review also examines how differences in coumarin structure, experimental conditions, cancer models, and the TME may account for contrasting biological outcomes. By distinguishing established observations from mechanistic hypotheses, this analysis identifies the limitations of current evidence and prioritizes experimentally testable questions concerning the potential involvement of coumarins in mitochondrial–immune interactions. Accordingly, the novelty of this review resides in its critical integration and evaluation of existing evidence rather than the assertion of a previously demonstrated therapeutic mechanism.

2. LITERATURE SEARCH STRATEGY AND STUDY SELECTION

A structured literature search was conducted using PubMed/MEDLINE, Scopus, and Web of Science to identify publications examining the relationships among coumarins, mitochondrial dysfunction, mtDNA signaling, OS, innate immunity, and cancer. Search terms included combinations of “coumarin,” “coumarin derivatives,” “mitochondrial DNA,” “mtDNA release,” “mitochondrial dysfunction,” “oxidative stress,” “reactive oxygen species,” “cGAS–STING,” “TLR9,” “NLRP3 inflammasome,” “innate immunity,” “cancer,” and “tumor microenvironment.” Boolean operators (AND and OR) were used to combine relevant terms and broaden or refine the searches. The reference lists of relevant publications were also considered to identify additional studies.

Eligible publications included original experimental investigations examining the effects of natural or synthetic coumarins on mitochondrial function, redox homeostasis, mtDNA integrity or release, and innate immune signaling. Particular emphasis was placed on studies involving cancer cell lines, animal tumor models, and molecular pathways associated with cancer progression or antitumor immunity. Mechanistic investigations conducted in non-cancer experimental systems were also considered when they provided relevant insights into mitochondrial quality control, mtDNA-associated inflammatory responses, or coumarin-mediated regulation of cGAS–STING, TLR9, and NLRP3. Relevant review articles were included to establish the broader biological context and identify established mechanistic relationships. Publications without a clear relationship to the principal review objectives, insufficiently characterized experimental findings, or unsupported mechanistic interpretations were not prioritized for inclusion.

The selected literature was critically evaluated according to experimental model, investigated compound, molecular targets, reported biological outcomes, and strength of mechanistic evidence.

Particular attention was given to distinguishing studies directly demonstrating cytosolic or extracellular mtDNA release from those reporting mitochondrial dysfunction, ROS production, or inflammatory pathway modulation without establishing mtDNA involvement. Evidence obtained from non-cancer models was interpreted as mechanistic support rather than direct confirmation of anticancer activity. Likewise, experimentally demonstrated effects were distinguished from proposed molecular interactions and therapeutic applications requiring further validation.

Given the heterogeneity of experimental models, coumarin structures, and investigated signaling pathways, the findings were integrated through a qualitative narrative synthesis rather than quantitative meta-analysis. The synthesis focused on identifying consistent mechanistic observations, context-dependent biological responses, conflicting findings, and unresolved questions concerning the potential contribution of coumarins to mtDNA-associated innate immune signaling in cancer.

3. MTDNA SIGNALING IN CANCER

mtDNA resides within mitochondria, where it encodes essential components required for oxidative phosphorylation and cellular energy production. Beyond its classical role in bioenergetics, mtDNA has emerged as an important regulator of cellular signaling and immune responses. Under conditions of mitochondrial stress or dysfunction, mtDNA can escape from mitochondria and accumulate in the cytoplasm or be released into the extracellular space. In these locations, it is recognized as a DAMP, triggering innate immune pathways that normally respond to microbial nucleic acids. Activation of these signaling networks stimulates inflammatory mediators, including nuclear factor-κB (NF-κB) and type I interferons, which influence host defense, tissue repair, and cancer-related processes [11].

Despite its small size, mtDNA contains 37 genes that are indispensable for mitochondrial function, including 13 protein-coding genes involved in oxidative phosphorylation, 22 tRNA genes, and two rRNA genes. The encoded proteins contribute directly to the respiratory chain complexes responsible for cellular ATP generation. In addition to these coding regions, mtDNA contains a regulatory control region that governs genome replication and transcription [12]. Owing to its close proximity to the electron transport chain and the absence of many protective features present in nuclear DNA, mtDNA is highly vulnerable to oxidative injury. Excessive production of reactive oxygen species (ROS) can induce mtDNA damage, resulting in mutations, fragmentation, or leakage of mtDNA into the cytoplasm and extracellular milieu. Once released from mitochondria, mtDNA is recognized by the cell as a danger signal and can initiate innate immune responses through DNA-sensing pathways such as cGAS–STING and TLR9. These events establish a mechanistic link between mitochondrial dysfunction, OS, inflammation, and cancer progression [13].

Several mechanisms contribute to mtDNA release, including defective mtDNA repair, excessive mitochondrial biogenesis, OS, and increased mitochondrial membrane permeability. Although cancer cells frequently suppress innate immune sensing pathways to evade immune surveillance, they can still respond to mtDNA-derived signals. Consequently, the biological effects of mtDNA release in cancer appear to be highly context dependent [14]. In immunosuppressed TME, extracellular or cytosolic mtDNA may promote chronic inflammation, tumor growth, and disease progression [15]. In contrast, in tumors with intact immune surveillance, mtDNA release can enhance antitumor immunity by activating immune cells and stimulating inflammatory pathways that restrict tumor development [16].

Disturbances in mitochondrial redox homeostasis further strengthen the link between mtDNA and cancer. Impaired repair mechanisms allow damaged and oxidized mtDNA to accumulate, leading to persistent OS and disruption of cellular redox balance. These alterations have been associated with malignant transformation, tumor progression, and metastatic dissemination [17]. Experimental studies have shown that restoring mitochondrial redox equilibrium through interventions targeting mtDNA maintenance and repair pathways can reduce tumor development in preclinical models [18]. In addition, OS influences several signaling cascades involved in carcinogenesis, including the redox-sensitive NLRP3 inflammasome and the SIRT1/p53 regulatory axis, both of which play critical roles in controlling cellular survival, inflammation, genomic stability, and tumor initiation [19].

3.1. OS and mtDNA Release

OS is one of the principal factors connecting mitochondrial dysfunction with the initiation of mtDNA-mediated signaling. Mitochondria continuously generate ROS as by-products of oxidative

phosphorylation, primarily at complexes I and III of the electron transport chain. Under physiological conditions, mitochondrial antioxidant systems, including superoxide dismutase, glutathione, and peroxiredoxins, maintain redox homeostasis by neutralizing excessive ROS. However, persistent metabolic stress, oncogenic signaling, hypoxia, and mitochondrial abnormalities frequently observed in cancer cells disrupt this balance, resulting in excessive ROS accumulation [20]. Because mtDNA is located in close proximity to the electron transport chain and lacks the protective histones present in nuclear DNA, it is particularly vulnerable to oxidative injury. Elevated ROS levels induce base modifications, strand breaks, deletions, and mutations within the mitochondrial genome. Among the most common oxidative lesions is 8-hydroxy-2′-deoxyguanosine, which serves as a marker of mtDNA oxidation and mitochondrial damage. Accumulation of such lesions compromises mitochondrial function and may impair the fidelity of mtDNA replication and repair, thereby amplifying OS in a self-perpetuating cycle [21].

OS also affects mitochondrial membrane integrity, where excessive ROS promote lipid peroxidation, destabilization of mitochondrial membranes, and opening of the mitochondrial permeability transition pore [22]. In parallel, activation of pro-apoptotic proteins such as BAX and BAK can induce mitochondrial outer membrane permeabilization, facilitating the escape of mitochondrial constituents into the cytoplasm. These structural alterations enable fragmented or oxidized mtDNA molecules to translocate from the mitochondrial matrix into the cytosol [23]. Under conditions of extensive cellular injury, mtDNA may additionally be released into the extracellular environment through necrosis, apoptosis-associated vesicles, extracellular vesicles, or active secretion mechanisms [24]. The biological consequences of mtDNA release depend not only on the amount of this type of DNA liberated but also on its oxidation status. Oxidized mtDNA exhibits enhanced immune-stimulatory activity compared with non-modified mtDNA and is more efficiently recognized by intracellular DNA-sensing pathways [25]. Cytosolic mtDNA activates innate immune sensors, including cGAS and TLR9, leading to downstream signaling through STING, NF-κB, and interferon regulatory factors. Activation of these pathways promotes the production of type I interferons, inflammatory cytokines, and chemokines that shape both local and systemic immune responses [26].

In cancer, the relationship between OS and mtDNA release is particularly complex. Moderate ROS production can support tumor cell proliferation, metabolic adaptation, angiogenesis, and metastatic dissemination. Conversely, excessive oxidative damage may trigger mtDNA-dependent immune activation capable of enhancing antitumor immunity. Consequently, OS functions as both a driver of mitochondrial genome instability and a critical regulator of mtDNA-mediated inflammatory signaling [27]. Understanding the molecular mechanisms governing ROS generation, mtDNA damage, and mitochondrial membrane permeabilization is therefore essential for developing therapeutic strategies aimed at controlling mtDNA signaling in the TME.

3.2. mtDNA as a DAMP and Innate Immune Activation

mtDNA shares several structural features with bacterial DNA, including its circular organization and relatively high frequency of unmethylated CpG motifs. Because of these evolutionary features, mtDNA is normally confined within mitochondria and remains inaccessible to cellular immune sensors. However, when mitochondrial integrity is compromised by OS, metabolic dysfunction, hypoxia, or anticancer therapies, mtDNA can accumulate in the cytosol or be released into the extracellular environment [28]. Under these conditions, mtDNA functions as DAMP, signaling the presence of cellular injury and initiating innate immune responses. This process establishes a direct mechanistic connection between mitochondrial dysfunction, inflammation, and cancer progression [29].

One of the principal pathways involved in mtDNA sensing is mediated by TLR9, an endosomal pattern-recognition receptor specialized in the detection of CpG-rich DNA sequences. Following cellular uptake of extracellular mtDNA or endosomal trafficking of intracellular mtDNA fragments, TLR9 activation promotes recruitment of the adaptor protein MyD88, leading to activation of NF-κB and mitogen-activated protein kinase signaling pathways. The resulting transcriptional program induces the production of inflammatory cytokines, chemokines, and survival factors that can profoundly influence the TME [30]. In several malignancies, persistent TLR9 stimulation has been associated with chronic inflammation, enhanced tumor cell proliferation, angiogenesis, and metastatic dissemination. Nevertheless, TLR9 activation may also facilitate antitumor immune responses through activation of dendritic cells and promotion of adaptive immune priming, highlighting the context-dependent nature of mtDNA-mediated signaling [31].

A second major mechanism of mtDNA recognition involves cGAS–STING pathway. Cytosolic mtDNA released from damaged mitochondria is detected by cGAS, which catalyzes the synthesis of cyclic GMP–AMP (cGAMP). This second messenger subsequently activates STING on the endoplasmic reticulum membrane, triggering phosphorylation of TANK-binding kinase 1 and interferon regulatory factor 3. Activation of this signaling cascade results in robust production of type I interferons and interferon-stimulated genes (ISGs) that coordinate antiviral and antitumor immune responses [32]. In cancer, cGAS–STING signaling exhibits dual biological functions. Acute activation can enhance immune surveillance, stimulate dendritic-cell maturation, and promote cytotoxic T-cell responses against malignant cells. In contrast, prolonged or dysregulated activation may contribute to chronic inflammation, immune exhaustion, and tumor adaptation, particularly in advanced-stage cancers [33].

Oxidized mtDNA also contributes to inflammasome activation; among the various inflammasome complexes, the NLRP3 inflammasome is particularly sensitive to mitochondrial dysfunction. Increased mitochondrial ROS production, together with the accumulation of oxidized mtDNA in the cytosol, promotes assembly of the NLRP3 inflammasome complex, leading to activation of caspase-1 and subsequent maturation of interleukin(IL)-1β (IL-1β) and IL-18 [34]. These cytokines amplify inflammatory responses and influence multiple aspects of tumor biology, including immune-cell recruitment, angiogenesis, and metastatic progression. The interaction between mtDNA release, ROS generation, and inflammasome activation therefore represents a critical regulatory axis linking mitochondrial stress to innate immune signaling [35].

These findings indicate that mtDNA can connect mitochondrial dysfunction with innate immune activation through several mechanistically distinct pathways. Released mtDNA can be recognized by cGAS and TLR9, whereas NLRP3 activation is associated with mitochondrial dysfunction, ROS production, ionic disturbances, and other cellular danger signals. Oxidized mtDNA may contribute to NLRP3 inflammasome activation under particular experimental conditions, but NLRP3 should not be classified as a direct DNA-sensing receptor. Whether these responses ultimately suppress or promote tumor growth depends on factors such as the extent of mtDNA release, the degree of OS, the immune status of the host, and the molecular characteristics of the tumor. Consequently, therapeutic strategies capable of modulating mtDNA sensing pathways have emerged as promising approaches for enhancing anticancer immunity while limiting chronic inflammation-associated tumor progression.

4. CHEMICAL PROPERTIES OF COUMARINS RELEVANT TO MITOCHONDRIAL INTERACTION

A comprehensive evaluation of the redox behavior of coumarins indicates that these compounds exhibit diverse activities, ranging from single-electron reactions to more complex multi-electron transfer processes. Depending on their chemical structure and biological context, coumarins can either suppress excessive ROS generation by acting as mitochondrial antioxidants or influence intracellular redox balance through modulation of oxidative signaling pathways [36]. Mitochondrial ROS are now recognized not merely as damaging byproducts of metabolism but also as important signaling molecules that regulate numerous cellular functions, including proliferation, differentiation, migration, survival, and activation of innate immune responses [37]. Notably, many coumarins possess physicochemical and structural characteristics favorable for mitochondrial targeting, such as sufficient lipophilicity, the ability to interact with mitochondrial membranes, and molecular motifs that facilitate accumulation within mitochondrial compartments [38].

These properties place coumarins at the intersection of OS regulation and innate immune signaling, making them particularly relevant to mtDNA-mediated pathways. Experimental evidence suggests that coumarins can influence mtDNA stability, mitochondrial dynamics, and the release of mtDNA into the cytosol or extracellular space. However, these effects are highly dependent on factors such as concentration, cell type, and pathological conditions, resulting in either enhancement or suppression of mtDNA release [39]. Similarly, the impact of coumarins on mtDNA-driven innate immune signaling varies considerably. They may modulate pathways associated with cGAS–STING, TLR9, or the NLRP3 inflammasome, leading to either activation or inhibition of downstream inflammatory responses. Differences observed among cancer models and TMEs further highlight the complexity of these interactions [40]. The occasionally contradictory findings reported within the same cancer type suggest that additional determinants, including mitochondrial status, immune-cell composition, metabolic conditions, and the specific coumarin derivative under investigation, play important roles in shaping the overall effect of coumarins on mtDNA-dependent innate immunity [41].

4.1. Redox Activity and Generation of ROS

Coumarins are redox-active compounds capable of modulating intracellular oxidative balance and, consequently, influencing a wide range of physiological and pathological processes [42], as recorded in Table **1**. Depending on their chemical structure and the surrounding redox environment, these molecules may function either as antioxidants by neutralizing ROS or as pro-oxidants by promoting their formation. Their ability to participate in both single-electron and multi-electron oxidation–reduction reactions underlies these dual biological effects [43]. The redox behavior of coumarins is strongly influenced by the nature and position of substituent groups on the benzopyrone scaffold. In particular, coumarins bearing a free hydroxyl group at the C-7 position readily undergo one-electron oxidation, generating phenoxyl radicals. These reactive intermediates may subsequently combine with nucleophilic molecules to form stable adducts or react with molecular oxygen, leading to the production of secondary radical species [44]. Experimental studies using cell-free redox systems have demonstrated that several 7-hydroxycoumarins can generate superoxide anions under appropriate conditions. A similar phenomenon has been observed with 7-acetoxycoumarin derivatives, whose acetoxy groups are rapidly hydrolyzed in aqueous environments, yielding hydroxylated metabolites capable of participating in redox cycling [45].

In contrast, the presence of electron-withdrawing substituents adjacent to the hydroxyl group markedly reduces the tendency of coumarins to generate superoxide. Likewise, simple alkoxy-substituted coumarins generally lack the capacity to produce superoxide through direct redox reactions. Nevertheless, pro-oxidant activity is not exclusively restricted to hydroxylated derivatives [46]. Certain coumarins lacking free hydroxyl groups have been reported to stimulate superoxide generation in isolated cardiac and brain mitochondria, suggesting alternative mechanisms of radical formation [47]. Consistent with these observations, several investigations have shown that coumarins containing a methoxy substituent at the C-7 position are typically not associated with significant ROS production, highlighting the critical role of structural features in determining the redox properties and biological activities of this class of compounds [48].

4.2. Mitochondrial Targeting Potential of Coumarins

Recent efforts in medicinal chemistry have increasingly focused on designing molecules capable of selectively targeting mitochondria, given the central role of these organelles in cellular metabolism, redox regulation, and apoptosis [53]. Coumarins have attracted interest as potential mitochondria-directed compounds because certain derivatives can influence mitochondrial function and redox homeostasis, as summarized in Table **2**. However, their lipophilicity and mitochondrial biological effects do not necessarily

Table 1: Structural Characteristics, Redox-Related Activities, and Experimental Evidence for Representative Coumarins

Coumarin derivative	Key structural features	Redox-related activity	Mechanistic interpretation	Experimental model / evidence basis	Ref.
Esculetin	6,7-Dihydroxycoumarin	Antioxidant activity; context-dependent pro-oxidant responses have been discussed	Phenolic radical scavenging and modulation of redox-related pathways	Literature synthesis of experimental investigations (model varies by study)	[49]
Daphnetin	7,8-Dihydroxycoumarin	Reported antioxidant and OS-modulating activities	Redox modulation and regulation of oxidative injury-associated pathways	Literature synthesis of experimental investigations (model varies by study)	[50]
Herniarin	7-Methoxycoumarin	Antioxidant-related effects reported for a herniarin-containing nanocomposite	Observed effects cannot be attributed exclusively to free herniarin	Chitosan-coated herniarin–graphene oxide nanoparticle formulation; experimental investigation	[51]
Scopoletin	6-Methoxy-7-hydroxycoumarin	Associated with antioxidant properties of investigated plant materials	Direct compound-specific cellular redox mechanisms were not established by the cited study	Edible plant samples; scopoletin content and antioxidant-property assessment	[52]

Note: The redox-related activities of coumarins are influenced by their structural features, concentrations, and experimental conditions. The experimental models indicate the systems in which the reported effects were investigated. Findings from cell-free assays should not be directly extrapolated to cellular or in vivo conditions. Furthermore, antioxidant or pro-oxidant activity does not independently establish mitochondrial targeting, mtDNA release, or activation of innate immune signaling pathways.

Table 2: Reported Mitochondrial and Redox-Related Activities of Representative Coumarins in Defined Experimental Systems

Coumarin derivative	Structural feature	Reported observation / scope of evidence	Experimental model or evidence source	Ref.
Esculetin	6,7-Dihydroxycoumarin	Antioxidant and mitochondrial-protective effects have been discussed in pharmacological studies; specific outcomes depend on the underlying model.	Pharmacological review encompassing multiple experimental models	[59]
Umbelliferone	7-Hydroxycoumarin	Modulation of oxidative metabolism and related neutrophil functions; these findings do not establish direct mitochondrial targeting.	Isolated human neutrophils (ex vivo)	[60]
Daphnetin	7,8-Dihydroxycoumarin	Antioxidant and cytoprotective effects are summarized across preclinical investigations; direct mtDNA signaling is not established.	Pharmacological review of preclinical studies	[61]
Osthole	7-Methoxy-8-isopentenylcoumarin	Impairment of mitochondrial metabolism and autophagic flux in colorectal cancer models; redox effects should not be inferred without corresponding measurements.	Colorectal cancer experimental models (primary study)	[62]

Note: The mitochondrial and redox-related effects of coumarins depend on their chemical structures, concentrations, experimental models, and exposure conditions. The reported findings reflect changes in mitochondrial function, oxidative balance, or associated signaling pathways and should not be interpreted as evidence of selective mitochondrial targeting. Furthermore, alterations in mitochondrial membrane potential, ROS production, or apoptosis do not independently establish mtDNA release or activation of mtDNA-dependent innate immune signaling. Findings from non-malignant experimental systems should not be directly extrapolated to cancer model.

establish selective accumulation within this organelle. Enhanced lipophilicity often improves mitochondrial uptake, allowing these compounds to interact with the mitochondrial compartment and influence mitochondrial function. Certain structural motifs, particularly those that mimic the spatial and electronic characteristics of lipophilic cations such as triphenylphosphonium, can further improve mitochondrial targeting while preserving mitochondrial membrane potential [54]. In parallel, membrane-active agents, including selected nonionic surfactants, have demonstrated the ability to traverse the inner mitochondrial membrane and increase membrane permeability without causing substantial depolarization [55]. Other mitochondria-directed compounds are being developed to modulate mitochondrial permeability transition, selectively alter respiratory-chain activity, or regulate mitochondrial ROS production [56]. Consequently, effective mitochondrial-targeting molecules generally combine favorable lipophilicity, selective organelle accumulation, membrane-interacting properties, and the capacity to influence mitochondrial bioenergetics and redox signaling.

Beyond mitochondrial localization, the biological activities of coumarin derivatives within this organelle warrant detailed investigation. Important characteristics include their redox behavior, electron-transfer capabilities, ROS-scavenging potential, and interactions with components of the mitochondrial electron transport chain. For example, the diphenyl ether fragment present in hymenoxin can undergo oxidative transformation to reactive dioxetane intermediates, which subsequently participate in one- or two-electron transfer reactions with cellular targets. These processes initiate complex oxidation–reduction cascades that may substantially influence intracellular redox balance [57]. Likewise, coumarins containing a 3,4-dihydroxy-3-oxobutanoate pharmacophore have demonstrated the ability to exert either cytoprotective antioxidant effects or pro-oxidant actions, depending on the cellular environment and redox conditions [58]. Investigating these mechanisms is essential for understanding how coumarins and related molecules regulate cellular and mitochondrial redox homeostasis, particularly through their effects on ROS generation, detoxification, and signaling pathways. Such insights may facilitate the development of novel mitochondria-directed therapeutics with applications in diseases characterized by OS and mitochondrial dysfunction.

Despite these pharmacological characteristics, mitochondrial interactions should not be interpreted as evidence of selective mitochondrial accumulation or direct regulation of mtDNA signaling. Lipophilicity alone does not establish organelle-specific targeting, and antioxidant activity measured in cell-free systems may not accurately predict mitochondrial responses in intact cells. Moreover, the effects of individual coumarins depend on their structural features, concentrations,

metabolic stability, and experimental conditions. The available evidence therefore requires careful distinction between demonstrated mitochondrial effects, indirect indicators of mitochondrial involvement, and proposed mechanisms that remain experimentally unverified. Quantitative assessment of mitochondrial accumulation, concentration-dependent activity, and pathway-specific responses will be necessary to establish the mechanistic and translational significance of these compounds.

5. COUMARINS AS MODULATORS OF MTDNA SIGNALING

Both activation and inhibition of mtDNA-dependent signaling pathways have been implicated in processes that contribute to cancer development and progression. Emerging evidence indicates that coumarins can influence both the release and degradation of mtDNA, while also regulating key innate immune pathways associated with mtDNA sensing, including cGAS–STING, TLR9, and NLRP3 inflammasome. In several experimental models, these effects have been linked to enhanced expression of ISGs, activation of the NLRP3 inflammasome, and subsequent secretion of the pro-inflammatory cytokines IL-1β and IL-18 [63]. Understanding how structural modifications of coumarins influence OS and mtDNA-driven innate immune responses requires a comprehensive, context-specific approach. This need is particularly important because the precise roles of mtDNA-sensing pathways in cancer remain incompletely characterized and may vary depending on tumor type, cellular environment, and disease stage. Given the remarkable structural diversity of coumarin-based molecules and the close interplay between redox homeostasis and mtDNA dynamics, even subtle chemical modifications can produce markedly different biological effects [64]. Consequently, distinct coumarins may differentially regulate OS, mitochondrial function, and immune signaling, ultimately leading to diverse cellular and therapeutic outcomes.

5.1. Effects on mtDNA Release and Integrity

Evaluating the involvement of coumarins in mtDNA signaling requires distinguishing direct measurements of mitochondrial genome integrity and release from indirect indicators of mitochondrial dysfunction. Although changes in ROS production, mitochondrial membrane potential, and mitophagy provide information about mitochondrial responses to coumarins, these parameters do not independently establish mtDNA-dependent immune activation. Direct assessment of cytosolic or extracellular mtDNA, together with functional analysis of downstream DNA-sensing pathways, is therefore essential for determining mechanistic involvement. Published investigations suggest that coumarins can influence mitochondrial quality control, redox homeostasis, and inflammatory signaling, but the extent to which these effects depend specifically on mtDNA release remains uncertain [65, 66]. Accordingly, the available evidence must be interpreted according to the biological relevance and mechanistic specificity of the parameters investigated.

Current findings on the relationship between coumarins and mtDNA signaling reveal a common mechanistic framework but also highlight considerable biological variability. Several studies have shown that exposure to coumarins can activate the NLRP3 inflammasome, a multiprotein complex that may be stimulated by mitochondrial danger signals, including released mtDNA. Notably, several coumarin–chalcone derivatives have been reported to induce NLRP3 activation at concentrations ranging from 25 to 50 μM [67]. In some experimental models, this response appears to involve increased OS rather than direct mitochondrial damage, as inflammasome activation occurred without significant SH-SY5Y cell death. Furthermore, pharmacological or genetic inhibition of NLRP3 reduced the secretion of the pro-inflammatory cytokines IL-1β and IL-18, supporting the functional involvement of this pathway [68]. Nevertheless, NLRP3 activation following coumarin exposure does not establish mtDNA-dependent signaling. The observed response may arise from ROS accumulation, mitochondrial dysfunction, or other inflammasome-activating stimuli. Direct assessment of mtDNA release and appropriate functional interventions are necessary to determine whether mtDNA contributes to inflammasome activation in these experimental models. Collectively, these observations underscore the complexity of coumarin-mediated regulation of mtDNA-associated signaling and emphasize the importance of evaluating such effects within well-defined cellular and molecular settings.

A central challenge in evaluating coumarin-mediated mtDNA signaling is distinguishing mitochondrial stress responses from causally established activation of DNA-sensing pathways. Measurements of ROS production, mitochondrial membrane potential, cell viability, or inflammatory cytokines provide valuable information about cellular responses but cannot independently demonstrate mtDNA release or its functional involvement in innate immune activation. Stronger mechanistic evidence

requires complementary assessment of cytosolic mtDNA abundance, mitochondrial integrity, and downstream signaling, supported by pathway-specific genetic or pharmacological interventions. In particular, determining whether inhibition of mtDNA release or disruption of cGAS–STING, TLR9, or NLRP3 signaling alters the observed response would help establish causal relationships. This distinction is especially important because coumarins may influence inflammatory pathways independently of mtDNA. Consequently, the available findings should be interpreted according to the strength of their experimental validation rather than assuming that mitochondrial or inflammatory changes necessarily reflect mtDNA-dependent mechanisms.

5.2. Influence on mtDNA-Triggered Innate Immune pathways

Available studies indicate that selected coumarins can influence innate immune pathways associated with mtDNA sensing, although direct evidence establishing mtDNA-dependent mechanisms remains limited. For example, esculetin has been shown to attenuate mitochondrial ROS production and JNK-mediated OS in primary rat hepatocytes [69]. Although these findings demonstrate effects on mitochondrial redox regulation, they do not establish mtDNA release or activation of the cGAS–STING pathway. Similarly, certain coumarin derivatives have been reported to enhance NLRP3 inflammasome activation, accompanied by caspase-1 activation, IL-1β and IL-18 maturation, and pyroptosis-associated signaling [70]. Conversely, KAS-08 has demonstrated inhibitory effects on inflammasome activation under specific experimental conditions [71]. Reported effects on TLR9-associated inflammatory responses also suggest potential immunomodulatory activity, but do not necessarily establish direct regulation of mtDNA recognition [72]. Importantly, modulation of these pathways may occur independently of mtDNA release through changes in ROS production, mitochondrial function, or other upstream regulators. Therefore, the available findings support context-dependent immunomodulatory effects of selected coumarins more strongly than a unified mechanism involving direct control of mtDNA-triggered innate immune signaling. Establishing such causality requires direct measurements of mtDNA release combined with pathway-specific functional validation.

5.3. Context-Dependent Outcomes in Cancer Models

Tumor responses to coumarin-based interventions are strongly influenced by the characteristics of the TME, particularly metabolic status and innate immune signaling. Several factors, including mtDNA abundance, activation of the NLRP3 inflammasome or the cGAS–STING pathway, and the degree of tumor vascularization, can modify the biological effects of coumarins. Experimental conditions such as hypoxia, acidosis, and nutrient availability also affect mtDNA release and the subsequent activation of innate immune pathways [73]. In addition, normalization of the tumor vasculature may improve the therapeutic efficacy of coumarins when used alongside immune checkpoint blockade [74], whereas corticosteroids such as dexamethasone have been reported to attenuate the antitumor activities of coumarins including esculetin, scopoletin, and umbelliferone in both spontaneous and experimentally induced cancer models [75].

An important limitation in interpreting these findings is the biological heterogeneity of *in vitro* experimental systems. Cancer cell lines differ in mitochondrial metabolism, mtDNA abundance, redox regulation, and the functional integrity of innate immune pathways, potentially producing divergent responses to the same coumarin derivative. Moreover, monocultures cannot adequately reproduce the interactions among malignant cells, stromal components, and immune populations within the TME. Consequently, changes in ROS production, mitochondrial membrane potential, or inflammatory mediator expression should not independently be interpreted as evidence of mtDNA-dependent signaling. Establishing such a relationship requires direct assessment of mtDNA release and appropriate functional validation of the implicated signaling pathways. These considerations limit comparisons across experimental models and highlight the need for complementary investigations using biologically relevant systems.

The biological consequences of coumarin-mediated mitochondrial and inflammatory regulation may also differ among cancer types and cellular populations within the TME. Malignant cells exhibit substantial variation in mitochondrial metabolism, mtDNA content, redox homeostasis, and the functional integrity of innate immune pathways, potentially influencing their responses to individual coumarins. Furthermore, activation of cGAS–STING or NLRP3 signaling in cancer cells may produce different outcomes from activation in macrophages, dendritic cells, or other immune populations. In malignant cells, these pathways may influence cellular survival, inflammatory signaling, and interactions with the surrounding microenvironment, whereas their activation in immune

cells can affect cytokine production, antigen presentation, and immune-cell recruitment. Importantly, the consequences of these responses cannot be generalized across cancer types because they depend on tumor-associated molecular characteristics, immune composition, and the duration of pathway activation. Although these distinctions provide a plausible explanation for divergent experimental findings, direct comparative evidence demonstrating cancer type-specific and cell-specific mtDNA-dependent effects of individual coumarins remains limited. Future investigations should therefore evaluate matched malignant and immune-cell populations using defined coumarin derivatives and pathway-specific functional assays.

The dynamic interaction between tumor metabolism and innate immunity is therefore a key determinant of the therapeutic outcome of coumarin treatment. Beyond influencing established tumors, coumarins may also affect early stages of carcinogenesis. Esculetin, for example, has been shown to stimulate antitumor immune responses through activation of the cGAS–STING signaling cascade while simultaneously suppressing breast tumor initiation, potentially through modulation of steroid biosynthetic pathways [76]. Likewise, esculetin has demonstrated protective effects against inflammation-driven carcinogenesis through suppression of NLRP3 inflammasome activation and the consequent reduction of pro-inflammatory cytokine production, thereby limiting the tumor-promoting effects of chronic inflammation [77]. Evidence also suggests that coumarin derivatives like scopoletin can attenuate ultraviolet-induced skin carcinogenesis by reducing OS and inflammatory signaling pathways, including those associated with excessive NLRP3 activation. However, the relationship between coumarins and innate immune pathways appears to be highly context dependent and may vary according to the disease model, tissue type, and microenvironmental conditions [78]. So, these observations underscore the potential of coumarins to regulate mtDNA-mediated immune signaling during cancer development and progression. Nevertheless, important knowledge gaps remain, particularly regarding tissue-specific and disease-dependent responses that may explain the inconsistent findings reported across different experimental settings.

Overall, these observations indicate that the biological consequences of coumarin treatment cannot be predicted solely from antioxidant activity or mitochondrial protection. The relationship between mitochondrial alterations and innate immune responses depends on whether mtDNA is released, which sensing pathways remain functional, and how the resulting inflammatory signals influence malignant and immune cells. The following section therefore examines the mechanistic evidence connecting coumarin-mediated mitochondrial regulation with NLRP3, cGAS–STING, and mitochondrial quality-control pathways, while distinguishing experimentally supported interactions from proposed mechanisms. The proposed relationships among coumarin-mediated oxidative stress, mitochondrial dysfunction, mtDNA-associated innate immune signaling, and context-dependent cancer outcomes are illustrated in Figure **1**,

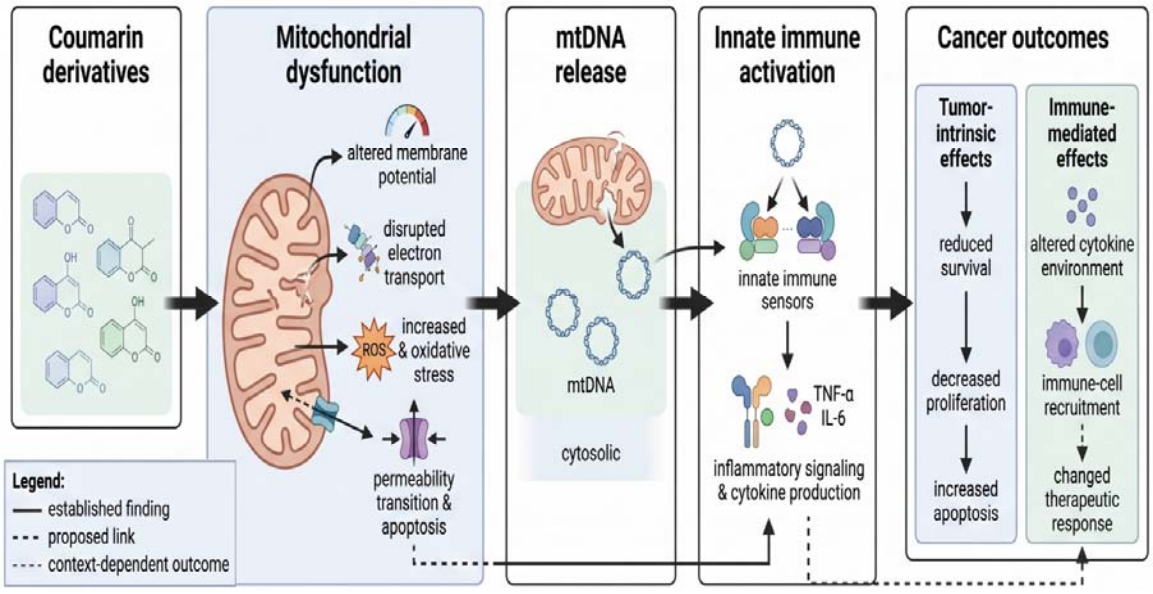


Figure 1: Proposed mechanistic links between coumarin-mediated mitochondrial regulation, mtDNA signaling, innate immunity, and cancer outcomes.

distinguishing experimentally supported observations from mechanistic connections that require further validation.

6. MECHANISTIC PATHWAYS LINKING COUMARINS TO OS AND IMMUNITY

The NLRP3 inflammasome is one of the key intracellular signaling platforms influenced by coumarin derivatives, although their effects are highly dependent on the specific compound, concentration, and biological setting [79]. Many coumarins, including esculetin, scopoletin, and osthole, suppress NLRP3 activation by limiting OS and reducing excessive ROS production, thereby preventing the assembly of the inflammasome complex and the subsequent inflammatory cascade [80]. Evidence from non-malignant experimental models indicates that certain natural coumarins, including pteryxin, can suppress NLRP3 inflammasome activation through modulation of upstream inflammatory signaling pathways [81]. However, these findings do not establish that coumarin-mediated NLRP3 regulation induces pyroptotic death in malignant cells, and the potential relevance of this mechanism to anticancer activity requires direct experimental validation.

Another innate immune pathway regulated by coumarins is the cGAS–STING axis, which plays a central role in sensing cytosolic DNA and initiating type I interferon responses. Screening studies of small-molecule libraries have identified several coumarin derivatives capable of enhancing STING signaling, partly through activation of activating transcription factor 3 and downstream interferon-dependent pathways. This activation promotes the expression of ISGs and strengthens innate immune responses [82]. Conversely, other coumarin compounds have been reported to suppress cGAS–STING signaling, highly-ghting the bidirectional nature of their immunomo-dulatory actions. Such regulation has attracted considerable interest in oncology, where modulation of the cGAS–STING pathway has been investigated in experimental models of breast, hepatocellular, and oral squamous cell carcinomas [83].

The relationship between autophagy, mitophagy, and mtDNA signaling provides an additional mechanism through which coumarins can influence innate immunity. Autophagy and its mitochondria-selective form, mitophagy, work together to preserve mitochondrial integrity and maintain mtDNA homeostasis [84]. By promoting the clearance of damaged mitochondria, certain coumarins reduce the release of mtDNA into the cytosol, thereby limiting the activation of DNA-sensing pathways such as cGAS–STING and the NLRP3 inflammasome. Enhanced removal of dysfunctional mitochondria also prevents excessive accumulation of inflammatory mtDNA fragments and attenuates chronic inflammatory responses *in vivo*. Through this coordinated regulation of mitochondrial quality control and innate immune signaling, coumarins contribute to the maintenance of cellular homeostasis and protection against inflammation-associated tissue damage [85].

6.1. NLRP3 inflammasome activation and suppression

Several naturally occurring and synthetic coumarins, including esculetin, osthole, scopoletin, imperatorin, and fraxetin, have been reported to influence the activity of the NLRP3 inflammasome, a key regulator of innate immune responses, as illustrated in Figure **2**. Interestingly, the effects of coumarins on NLRP3 signaling appear to be highly context-dependent. In some experimental settings, coumarins suppress inflammasome activation by reducing OS, limiting mitochondrial dysfunction, and attenuating the production of ROS [86]. Although most coumarins suppress NLRP3 inflammasome activation, excessive concentrations or prolonged exposure to certain coumarin derivatives may theoretically favor inflammasome activation by inducing mitochondrial dysfunction, increasing mitochondrial ROS production, disrupting mitochondrial dynamics, or promoting the release of mitochondrial DAMPs. However, direct experimental evidence for this effect remains limited [87]. Importantly, NLRP3 is not a direct DNA-sensing receptor. Its activation involves diverse upstream signals, including mitochondrial stress, ROS production, potassium efflux, and lysosomal damage. Although oxidized mtDNA may contribute to inflammasome activation under specific conditions, the involvement of mtDNA cannot be inferred solely from increased NLRP3 expression, caspase-1 activation, or IL-1β and IL-18 maturation.

The divergent outcomes observed across studies likely reflect differences in the chemical structure and concentration of individual coumarins, the genetic background of the target cells, and the characteristics of the surrounding TME. Moreover, the contribution of downstream inflammasome events, including caspase-1 activation, maturation of IL-1β and IL-18, and induction of pyroptotic cell death, remains incompletely

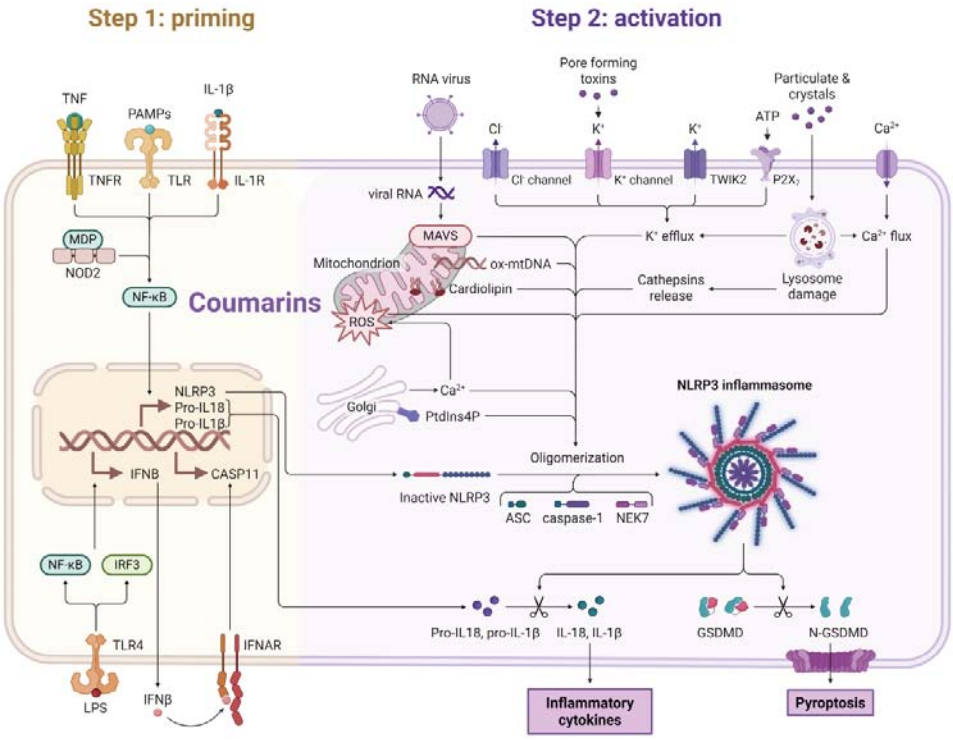


Figure 2: The modulation role of coumarins in the NLRP3 inflammasome pathway.

understood and warrants further investigation [88]. Additional research is also needed to clarify how coumarin-mediated redox modulation intersects with upstream regulators of NLRP3 signaling, including NF-κB, interferon regulatory factor 3, and hypoxia-inducible factor-1.

The ability of coumarins to either suppress or activate NLRP3 signaling may offer unique opportunities for cancer therapy. In tumors where chronic NLRP3 activation promotes inflammation, immune evasion, and disease progression, inhibition of the inflammasome by coumarins could help restore antitumor immune surveillance and limit tumor-supportive inflammation. Conversely, controlled activation of NLRP3 may be advantageous in selected settings by enhancing local immune responses and increasing tumor immunogenicity. Such effects could potentially improve the efficacy of immune checkpoint inhibitors, radiotherapy, or conventional chemotherapeutic agents [89]. Nevertheless, the therapeutic exploitation of NLRP3 modulation requires careful consideration because the inflammasome can exert either tumor-promoting or tumor-suppressive functions depending on cancer type and disease stage. So, the biological effects observed following coumarin treatment may not be attributable solely to NLRP3 regulation but may also involve interconnected pathways governing OS, inflammation, metabolism, and immune signaling [90]. A deeper understanding of these interactions may be essential for the rational development of coumarin-based combination therapies in oncology.

The apparently opposing immunostimulatory and immunosuppressive activities of coumarins should be interpreted in relation to the biological conditions under which they occur. Differences in chemical structure, concentration, exposure duration, and mitochondrial redox status may alter the balance between inflammatory pathway activation and suppression. Cellular identity is equally important because inflammatory signaling in malignant cells may have different consequences from signaling in macrophages, dendritic cells, or other immune populations within the TME. Furthermore, transient activation of innate immune pathways may support antitumor immune surveillance, whereas persistent inflammatory signaling can contribute to tissue injury, immunosuppression, or tumor progression. Consequently, suppression of NLRP3 or cGAS–STING signaling should not automatically be interpreted as therapeutically beneficial, nor should pathway activation invariably be regarded as advantageous. Importantly, these context-dependent explanations remain mechanistic interpretations unless supported by direct comparative experiments involving defined coumarin derivatives, exposure conditions, and immune-cell populations. Future studies should therefore evaluate both the

magnitude and duration of pathway activation alongside tumor-specific and immune-mediated outcomes.

6.2. cGAS–STING Axis Modulation by Coumarins

The activation of innate immune signaling depends on the ability of host cells to detect foreign or misplaced nucleic acids and initiate appropriate defensive responses. Among the key sensors involved in this process is cGAS, which recognizes cytosolic DNA originating from invading pathogens, damaged mitochondria, or genomic instability. Upon activation, as shown in Figure **3**, cGAS generates cGAMP, which subsequently activates the stimulator of interferon genes (STING), leading to the induction of type I interferons (IFNs) and other pro-inflammatory mediators [91]. Emerging evidence suggests that coumarins like auraptene may influence this signaling cascade, thereby modulating ISG expression, macrophage polarization, and broader immune responses [92].

Evidence from structurally unrelated natural products demonstrates that pharmacological modulation of the mtDNA–cGAS–STING axis can produce distinct biological outcomes. In a cisplatin-induced rat model of gastrointestinal toxicity, 6-shogaol, a phenolic constituent of ginger, attenuated inflammatory injury and suppressed mtDNA-associated cGAS–STING signaling, accompanied by increased OGG1 and decreased FEN1 expression [93]. Conversely, a separate investigation demonstrated that 6-shogaol promoted hepatic stellate cell senescence through activation of the cGAS–STING–NF-κB axis, contributing to reduced liver fibrosis [94]. These findings highlight the context-dependent consequences of modulating this pathway. Importantly, 6-shogaol is not a coumarin, and neither study establishes coumarin-mediated regulation of mtDNA signaling. Nevertheless, these investigations provide comparative mechanistic evidence supporting the need to evaluate mitochondrial DNA release, pathway activation, and downstream inflammatory responses independently when investigating coumarin derivatives. Establishing whether individual coumarins exert comparable effects requires compound-specific experimental validation in appropriate cancer models.

Natural products have likewise demonstrated the capacity to modulate inflammatory responses. For instance, bearberry (*Arctostaphylos uva-ursi*) extract exhibits anti-inflammatory and antioxidant activities that may benefit inflammatory skin disorders, although direct modulation of type I IFN signaling has not yet been clearly established . Coumarins may exert similar effects by directly targeting the cGAS–STING–IFN pathway or by influencing downstream signaling components. In human bladder cancer cells, the cGAS–STING pathway exhibits context-dependent functions, acting as either a tumor-suppressive or tumor-promoting signaling axis depending on the cellular and tumor microenvironment. Pharmacological modulation of this pathway has therefore emerged as a

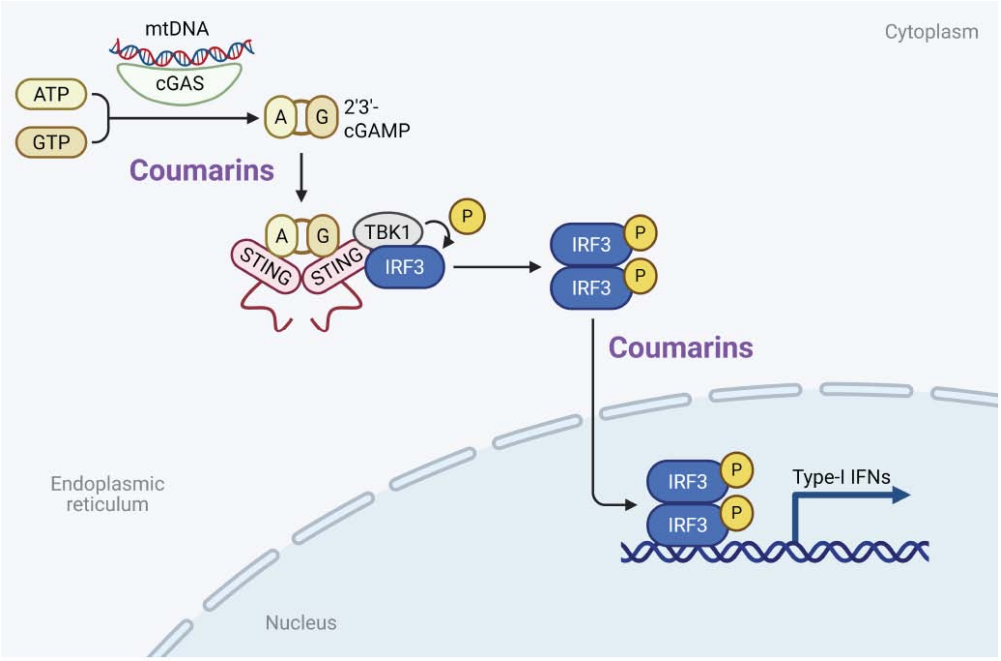


Figure 3: The role of coumarins in the modulation of cGAS–STING axis.

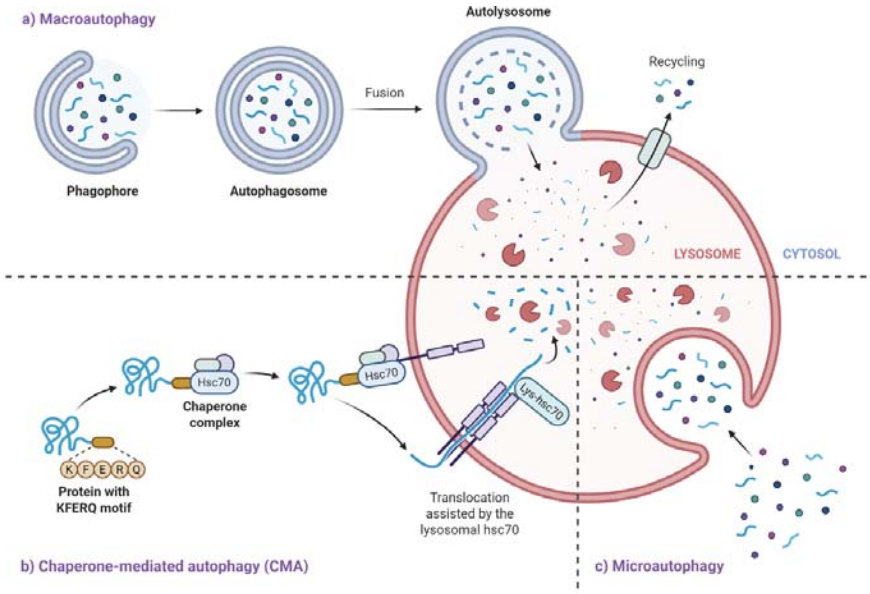


Figure 4: Cellular autophagy pathways governing mitochondrial quality control.

promising therapeutic strategy [95]. Furthermore, medicine-based evidence suggests that KLF6 may influence innate inflammatory responses, whereas STING, TLR9, and the NLRP3 inflammasome constitute an interconnected DNA-sensing network. Together, these pathways may represent complementary therapeutic targets that could be modulated by coumarin-based compounds.

6.3. Autophagy and Mitophagy Interplay

Autophagy, in its three types (Figure **4**), is an evolutionarily conserved cellular quality-control process that degrades and recycles damaged organelles, misfolded proteins, and other unnecessary intracellular components through lysosomal pathways. Mitophagy, a specialized form of autophagy, selectively targets dysfunctional mitochondria for degradation, thereby preserving mitochondrial integrity and cellular homeostasis [96]. Impairment of mitophagy results in the accumulation of damaged mitochondria, which can release mtDNA into the cytosol and extracellular environment, promoting sterile inflammatory responses. In contrast, efficient mitochondrial clearance limits mtDNA leakage and helps restrain mtDNA-driven inflammation [97]. Because of this close relationship between mitochondrial quality control and innate immune signaling, increasing attention has been directed toward understanding how coumarins influence autophagy- and mitophagy-dependent regulation of mitochondrial turnover [98].

Evidence from non-cancer experimental models indicates that pharmacological regulation of autophagy may protect against mitochondrial injury and inflammatory responses. For example, pioglitazone has demonstrated renoprotective effects in experimental renal ischemia–reperfusion injury, partly through mechanisms involving antioxidant activity, inflammatory regulation, and autophagy-associated signaling [99]. Although these findings support the broader importance of mitochondrial quality-control mechanisms in tissue protection, they do not establish comparable effects for coumarindione or demonstrate coumarin-dependent regulation of mtDNA release, TLR9, or NLRP3 signaling.

Additional studies have demonstrated that coumarin-mediated activation of autophagy and mitophagy facilitates the clearance of damaged mitochondria. Conversely, pharmacological or genetic inhibition of autophagy impairs mitochondrial quality control, leading to the accumulation of dysfunctional mitochondria, increased cytosolic mtDNA release, and amplification of mtDNA-dependent inflammatory signaling [100]. The medicine-based evidence indicates that the neuroprotective effects of coumarins are partly mediated through regulation of mitochondrial quality control. By modulating mitophagy, coumarins can influence mitochondrial homeostasis and NLRP3 inflammasome activation, thereby altering neuroinflammatory responses in cultured microglia. Because mitophagy–NLRP3 signaling also contributes to the pathogenesis of vascular dementia, particularly in disease associated with large-artery atherosclerosis, modulation of this pathway may represent an important mechanism underlying the neuroprotective potential of coumarins [101]. These findings collectively suggest

that the ability of coumarins to regulate autophagy and mitophagy may represent an important mechanism through which they control mtDNA release and downstream inflammatory signaling.

7. THERAPEUTIC IMPLICATIONS AND CHALLENGES

The therapeutic relevance of coumarins in cancer extends beyond their reported effects on malignant cell viability to potential modulation of mitochondrial function and innate immune signaling. Experimental studies indicate that selected derivatives influence redox homeostasis, NLRP3 activity, and pathways associated with mtDNA sensing [102]. However, evidence directly linking these effects to improved responses to chemotherapy, radiotherapy, or immune checkpoint blockade remains limited. Moreover, the biological consequences of mitochondrial and inflammatory modulation depend on compound structure, concentration, tumor characteristics, and the surrounding TME. Consequently, the therapeutic value of coumarin-mediated regulation of mtDNA-associated pathways remains a hypothesis requiring further mechanistic and translational validation [103].

Despite their broad pharmacological versatility, current knowledge regarding the safety, selectivity, and immunological consequences of coumarin-mediated modulation of mtDNA-associated pathways remains limited. Most studies evaluating coumarin derivatives as anticancer agents focus primarily on their cytotoxic activity, whereas their influence on inflammatory signaling and antitumor immunity is often insufficiently characterized [104]. This knowledge gap is further compounded by an incomplete understanding of their molecular mechanisms of action, which restricts the rational design of derivatives with improved therapeutic specificity. In addition, unintended interactions with non-target signaling pathways may compromise their clinical utility. Because alterations in innate immune signaling can profoundly affect the immune landscape of tumors, comprehensive pharmacological characterization of coumarin derivatives is essential before their therapeutic application can be fully realized [105].

Future investigations should prioritize systematic evaluation of how coumarins influence mtDNA-mediated immune responses and determine whether these effects can be exploited to improve the efficacy of immune checkpoint inhibitors and other anticancer therapies. Detailed pharmacokinetic and pharmacodynamic studies of promising analogues are also required to identify compounds with favorable efficacy and safety profiles [106]. Furthermore, the assessment of biomarkers associated with mtDNA signaling may provide valuable insight into treatment responses. Potential biomarkers include circulating mtDNA levels, components of the NLRP3 inflammasome, indicators of cGAS–STING pathway activation, and transcriptional signatures associated with type I interferon responses. Monitoring these parameters could facilitate the development of coumarin-based therapeutic strategies and support their translation from experimental models to clinical oncology [107].

7.1. Potential for Combination Therapies

The potential application of coumarins alongside immune checkpoint inhibitors, radiotherapy, and conventional chemotherapeutic agents remains an area of preclinical investigation. Although selected studies have reported favorable anticancer effects involving coumarin derivatives, these findings do not establish that modulation of mtDNA-associated innate immune signaling improves the efficacy of combination treatments [8]. Furthermore, the direction and magnitude of pharmacological interactions may depend on the specific derivative, treatment concentration, tumor characteristics, and immune status of the experimental model. Evidence obtained from cultured cancer cells cannot adequately predict therapeutic responses within the complex TME, while immunodeficient xenograft models provide limited information about immune-mediated treatment effects. Consequently, the available findings should be regarded as preliminary support for further investigation rather than confirmation of therapeutic synergy or clinical benefit. Establishing the relevance of coumarin-based combinations requires appropriately controlled studies evaluating treatment interactions, mitochondrial and immune responses, pharmacokinetic compatibility, and potential toxicity [108].

The translational interpretation of *in vivo* findings requires careful consideration of experimental design and model-specific limitations. Immunodeficient xenograft models may demonstrate direct antitumor activity but cannot adequately establish the contribution of adaptive immunity or fully reproduce interactions within the TME. Conversely, immunocompetent models provide a more informative setting for investigating immune-mediated responses, although differences in tumor biology and host physiology may limit their clinical relevance. Variations in treatment allocation, dosing schedules, administration routes, and outcome measurements further complicate comparisons among

published studies. Accordingly, preclinical evidence should be interpreted in relation to the characteristics of each experimental model, and changes in tumor growth or inflammatory markers should not be regarded as direct confirmation of mtDNA-dependent immune modulation without appropriate mechanistic validation. These limitations highlight the need for pharmacokinetic characterization, standardized experimental reporting, and complementary immunologically relevant models before clinical translation can be considered.

Potential safety concerns must also be considered, particularly when coumarins are administered at high doses or over extended periods. Although many coumarins, including osthole, suppress NLRP3 inflammasome activation under pathological conditions, their immunomodulatory effects are context-dependent. Consequently, excessive or prolonged modulation of inflammasome signaling could theoretically disturb immune homeostasis and warrants careful evaluation during long-term therapeutic use [109]. In addition, some compounds within this class, such as dicoumarol, can impair mitochondrial function through inhibition of mitochondrial electron transport and increased oxidative stress. Although these effects contribute to their antitumor activity, excessive or prolonged mitochondrial dysfunction may also compromise the viability of non-malignant cells, emphasizing the importance of careful dose optimization and appropriate therapeutic settings [110]. Combination strategies with coumarin treatment involving immune checkpoint blockade, radiotherapy, or chemotherapeutic agents that stimulate interferon-β and interferon-γ signaling pathways are particularly attractive, although their effectiveness is likely to vary according to tumor type, disease stage, and the characteristics of the TME [111].

A critical step in evaluating these therapeutic combinations is the assessment of mtDNA-associated signaling pathways. Measurements of circulating mtDNA in serum or plasma, together with quantification of NLRP3 inflammasome components and biomarkers related to the cGAS–STING pathway, including cytokine production, downstream target expression, and pathway activation status, could provide valuable information on whether coumarins enhance or suppress mtDNA-driven immune responses [112]. Future preclinical studies should employ experimental models that preserve mtDNA release and downstream immune signaling, preferably within immunocompetent systems that more closely reflect the physiological TME [113]. Furthermore, advanced multicellular platforms, such as integrated organoid-based bioreactor systems capable of reproducing interactions among multiple tissues and organs, may offer powerful tools for investigating the efficacy and safety of these combination therapies before clinical translation [114].

7.2. Safety and Specificity Considerations

Coumarins represent a promising class of compounds for modulating mtDNA-driven innate immune responses in cancer; however, their successful therapeutic application depends on overcoming challenges related to safety, selectivity, and pharmacokinetic performance. Factors such as limited bioavailability, off-target biological effects, and the highly dynamic nature of the TME can substantially influence treatment outcomes. Moreover, inconsistent findings across experimental models suggest that coumarins may exert context-dependent effects, potentially reducing therapeutic efficacy in certain settings or leading to the loss of beneficial responses after treatment discontinuation. Continued mechanistic and translational research may be essential to define the conditions under which these compounds can be used safely and effectively [115].

One of the principal concerns associated with coumarin-based therapies is their capacity to induce OS. Although moderate increases in ROS may contribute to antitumor activity, excessive ROS accumulation can amplify inflammatory signaling, promote macrophage polarization toward a pro-inflammatory M1 phenotype, and enhance free-radical production. These potential adverse effects may be mitigated through careful selection of coumarin derivatives, optimization of dosing regimens, and, where appropriate, the incorporation of antioxidant strategies to preserve therapeutic efficacy while minimizing off-target toxicity [116]. In this regard, regulation of the NLRP3 inflammasome appears particularly important. Numerous studies have demonstrated that suppression of NLRP3 signaling can alleviate inflammation and improve outcomes in a variety of pathological conditions [117]. Nevertheless, some coumarins like esculetin may disrupt cellular redox homeostasis in a manner that favors tumor cell survival or proliferation under specific circumstances. Conversely, these same redox-modulating properties could be exploited therapeutically when integrated with appropriately selected immunotherapeutic strategies [118]. Therefore, a detailed understanding of the biological activities of individual coumarins, together

with careful patient and treatment selection, could be critical for maximizing therapeutic benefit while minimizing adverse effects.

7.3. Biomarkers for Monitoring mtDNA Signaling Modulation

Successful evaluation of the biological effects of coumarins on mtDNA signaling requires robust and clinically informative biomarkers. Among the most promising candidates are circulating cell-free mtDNA and biomarkers reflecting NLRP3 inflammasome activation, both of which may provide insight into the extent of mitochondrial stress and innate immune activation induced by coumarin treatment [119]. Additional indicators include components of the cGAS–STING signaling pathway, such as cGAMP, phosphorylated STING, type I interferons, and ISGs, whose expression may be altered following exposure to specific coumarin derivatives [120].

In clinical and preclinical settings, quantification of circulating cell-free mtDNA offers a minimally invasive strategy for evaluating cancer progression and therapeutic efficacy. Elevated plasma or serum mtDNA concentrations have been associated with immunosuppressive TMEs and reduced patient survival. Likewise, measurement of IL-1β in tumor tissues or circulation provides insight into inflammasome activity and the composition of inflammatory cell infiltrates, both of which are linked to unfavorable clinical outcomes [121]. Consequently, monitoring cell-free mtDNA, IL-1β, and selected ISGs represents a rational approach for determining whether coumarins influence mtDNA release, inflammasome activation, and downstream innate immune signaling pathways in cancer.

8. INVESTIGATING COUMARIN MODULATION OF MTDNA SIGNALING

A rigorous experimental framework is essential for elucidating how coumarins influence mtDNA-dependent innate immune signaling in cancer. Because mtDNA release and sensing are closely linked to mitochondrial function, OS, and immune activation, experimental models should enable simultaneous assessment of mitochondrial integrity, mtDNA dynamics, and downstream inflammatory responses. Studies should evaluate both the release of mtDNA and the activation of key sensing pathways, including TLR9, cGAS–STING, and the NLRP3 inflammasome [122]. In addition, because modulation of cellular redox homeostasis represents a major mechanism through which many coumarins exert their biological effects, changes in oxidative stress should be carefully considered when interpreting experimental findings and their impact on mtDNA-dependent signaling [123].

8.1. *In Vitro* Systems for mtDNA Signaling Studies

Cell-based models provide controlled experimental settings for investigating the potential effects of coumarins on mitochondrial function and mtDNA-associated innate immune signaling, as summarized in Table **3**. However, their suitability depends on cellular origin, mitochondrial characteristics, and the functional integrity of the relevant DNA-sensing pathways. Accordingly, the proposed models should be considered experimental platforms for mechanistic investigation rather than evidence of established coumarin-mediated mtDNA signaling. Comparisons across cellular systems require careful consideration of differences in experimental conditions, pathway expression, and biological responses. Cancer cell lines with documented mitochondrial dysfunction or constitutive activation of innate immune pathways are particularly useful for investigating the relationship between coumarin exposure and mtDNA-mediated immune responses. The selection of cellular models should consider the expression of critical signaling components such as TLR9, cGAS, STING, NLRP3, and downstream inflammatory mediators. Mechanistic studies may be strengthened by employing genetically modified experimental systems, including cells deficient in cGAS, STING, TLR9, or NLRP3, together with models that selectively alter mtDNA integrity, maintenance, or release. These approaches would facilitate the establishment of causal relationships between coumarin exposure and activation of specific innate immune signaling pathways [124]. Quantification of cytosolic or extracellular mtDNA by quantitative PCR (qPCR), assessment of mitochondrial membrane potential using fluorescent probes, and measurement of mitochondrial permeability transition are valuable tools for monitoring mitochondrial responses [125]. Furthermore, analyses of cytokine production, inflammasome activation, and ISG expression can provide insights into the downstream consequences of mtDNA release [126].

8.2. *In Vivo* Models and Translational Relevance

Animal models provide an important framework for investigating the physiological relevance of coumarin-mediated mitochondrial regulation and its potential

Table 3: Experimental Evidence and Proposed *In Vitro* Models For Investigating Coumarin-Mediated Mitochondrial Dysfunction and Mtdna-Associated Innate Immune Signaling

Coumarin derivative	*In vitro* system	Experimental evidence or proposed application	Evidence status	Ref.
Esculetin–furoxan–DEAC hybrid A11	MDA-MB-231 breast cancer cells	Reported mitochondrial ROS accumulation, increased CypD expression, apoptosis, and G_2/M arrest. mtDNA release and cGAS–STING involvement were not established.	Reported mitochondrial effects; mtDNA involvement unestablished	[127]
Esculetin	THP-1-derived macrophages	Proposed assessment of NLRP3 activation, IL-1β and IL-18 production, and the possible contribution of mtDNA release.	Proposed investigation	[128]
Isoscopoletin	HaCaT keratinocytes	Proposed evaluation of ROS production, mitochondrial integrity, and inflammatory responses, with direct mtDNA measurements required to establish involvement.	Proposed investigation	[129]
Scopoletin	BV2 microglial cells	Proposed investigation of mitochondrial stress, NLRP3 activation, and potential mtDNA-associated sterile inflammation.	Proposed investigation	[130]
Osthole	HepG2 hepatocellular carcinoma cells	Proposed examination of mitochondrial dysfunction, ROS generation, mtDNA integrity, and cytosolic mtDNA accumulation.	Proposed investigation	[131]
Synthetic osthole-based derivatives	RAW264.7 macrophages	Proposed assessment of macrophage inflammatory responses, mitochondrial injury, and possible mtDNA-associated immune signaling.	Proposed investigation	[132]
Imperatorin	A549 lung adenocarcinoma cells	Proposed evaluation of mitochondrial ROS production, mtDNA release, and cGAS–STING activation using pathway-specific functional assays.	Proposed investigation	[133]
Fraxetin	HCT116 colorectal cancer cells	Proposed investigation of mitochondrial injury, mtDNA release, and downstream innate immune responses.	Proposed investigation	[134]
Daphnetin	SH-SY5Y neuroblastoma cells	Proposed assessment of mitochondrial injury, mtDNA leakage, and NLRP3 activation without presuming mtDNA-dependent inflammasome signaling.	Proposed investigation	[135]
Umbelliferone and its synthetic derivatives	PC3 prostate cancer cells	Proposed examination of mitochondrial redox regulation, mtDNA integrity, and potential mtDNA-associated signaling.	Proposed investigation	[136]
Coumarindione	HK-2 renal tubular epithelial cells	Proposed investigation of autophagy, mitophagy, mtDNA clearance, and TLR9/NLRP3 responses.	Proposed investigation	[137]
7-Hydroxycoumarin-3-carboxylic acid	T24 bladder cancer cells	Proposed evaluation of cytosolic mtDNA accumulation, cGAS–STING signaling, and downstream IFN responses.	Proposed investigation	[138]

Note: The table distinguishes reported experimental findings from proposed applications of cellular models. Reference [127] supports experimentally observed mitochondrial effects of the esculetin-derived hybrid A11, without establishing mtDNA-dependent signaling. The remaining coumarin–model combinations are proposed research directions and should not be interpreted as experimentally validated combinations. The other references provide varying degrees of contextual support and do not necessarily establish that the specified coumarin was investigated in the corresponding cellular system. Direct mtDNA measurements, appropriate functional controls, and pathway-specific interventions are required to establish mtDNA-dependent mechanism.

relationship with mtDNA-associated immune signaling, as summarized in Table **4**. Immunocompetent tumor-bearing models are particularly informative because they preserve interactions among malignant cells, stromal components, and immune populations within the TME [139]. Nevertheless, the suitability of individual models depends on their biological characteristics and the mechanisms under investigation. Syngeneic cancer models may facilitate evaluation of immune-mediated antitumor responses, whereas non-malignant inflammatory models can provide complementary mechanistic information without directly establishing anticancer efficacy. Functional manipulation of cGAS–STING, TLR9, or NLRP3 signaling, combined with direct assessment of mtDNA release, would help determine whether these pathways mediate the

Table 4: Proposed *In Vivo* Models for Investigating Coumarin-Mediated Mitochondrial Dysfunction and Mtdna-Associated Immune Signaling

Coumarin derivative	Proposed *in vivo* model	Research objective	Ref.
Esculetin	4T1 syngeneic breast cancer (BALB/c mice)	Examine mtDNA release, cGAS–STING signaling, and antitumor immune-cell responses.	[142]
Esculetin	AOM/DSS-induced colorectal carcinogenesis	Assess mitochondrial injury, mtDNA release, NLRP3 responses, and inflammation-associated tumorigenesis.	[143]
Scopoletin	UVB-induced skin carcinogenesis	Investigate OS, mitochondrial damage, and possible mtDNA-associated inflammatory responses.	[144]
Scopoletin	APP/PS1 transgenic mice	Investigate neuroinflammation, mitochondrial dysfunction, and potential contribution of mtDNA to inflammasome signaling.	[145]
Osthole	Hepa1-6 syngeneic hepatocellular carcinoma	Evaluate mitochondrial stress, mtDNA release, and immune-mediated antitumor responses.	[146]
Osthole	LPS-induced systemic inflammation	Assess inflammatory cytokines and NLRP3 activation with direct tests for mtDNA involvement.	[147]
Imperatorin	Lewis lung carcinoma (C57BL/6 mice)	Assess mitochondrial injury, cGAS–STING activity, and tumor-associated immune responses.	[148]
Fraxetin	ApcMin/+ intestinal tumor model	Investigate mitochondrial dysfunction and possible mtDNA-related immune signaling in intestinal tumorigenesis.	[149]
Daphnetin	Orthotopic glioma model	Evaluate mitochondrial stress and potential mtDNA-associated neuroimmune responses.	[150]
Umbelliprenin	Prostate cancer xenograft	Investigate tumor growth, mitochondrial redox effects, and mtDNA-related outcomes; interpret immunity cautiously.	[151]
Coumarindione	Renal ischemia–reperfusion injury	Test mitophagy, mtDNA release, and TLR9/NLRP3 responses in non-cancer injury.	[152]
7-Hydroxycoumarin-3-carboxylic acid	Syngeneic bladder cancer	Examine mtDNA release, cGAS–STING activation, and immune-mediated antitumor responses.	[153]

Note: The coumarin–animal model combinations presented in this table are proposed experimental approaches unless otherwise supported by compound-specific primary studies. The inclusion of a particular model does not indicate that the corresponding coumarin has been evaluated in that system. Similarly, evidence of mitochondrial dysfunction, ROS production, or inflammatory regulation does not independently establish mtDNA-dependent immune signaling. Non-cancer models may provide complementary mechanistic information but cannot demonstrate anticancer efficacy. Future studies should incorporate direct measurements of mtDNA release, pathway-specific functional interventions, and appropriate controls to distinguish mitochondrial effects from mtDNA-mediated signaling.

observed effects of coumarins [140]. Differences in species, experimental conditions, treatment exposure, and immune competence must also be considered when interpreting preclinical findings and their potential clinical relevance [141].

8.3. Analytical Techniques for mtDNA and Immune Readouts

Investigating the potential involvement of coumarins in mtDNA-dependent immune signaling requires complementary analytical approaches capable of distinguishing mitochondrial dysfunction from direct activation of DNA-sensing pathways. Quantitative polymerase chain reaction (qPCR) can be used to assess mtDNA abundance in appropriately prepared cellular and extracellular samples, although rigorous controls are necessary to minimize contamination and ensure reliable interpretation [154]. Measurements of mitochondrial ROS, membrane potential, and mitochondrial integrity provide complementary information about organelle function but do not independently establish mtDNA release. Downstream immune responses can be investigated by assessing the activation of cGAS–STING, TLR9, and NLRP3 signaling, together with relevant inflammatory mediators [155, 156]. Importantly, pathway-specific inhibition or genetic manipulation is required to determine whether the observed responses depend on mtDNA-associated signaling. Table **5** summarizes potential analytical strategies for future mechanistic investigations rather than experimental procedures necessarily performed in the cited studies.

9. GAPS IN KNOWLEDGE AND FUTURE DIRECTIONS

Three mechanistic questions should be prioritized in future investigations. First, it remains necessary to establish whether individual coumarins directly alter mtDNA integrity or release, rather than merely influencing mitochondrial ROS production, membrane potential, or cellular viability. Second, the causal involvement of mtDNA in downstream cGAS–STING,

Table 5: Proposed Analytical Approaches for Investigating Mitochondrial and Mtdna-Associated Responses to Representative Coumarins

Coumarin derivative	Proposed analytical approach	Potential information obtained and interpretive limitations	Ref.
Esculetin	ELISA for IFN-β and CXCL10	Proposed measurement of interferon-associated and chemokine responses; pathway attribution requires complementary cGAS–STING analyses.	[157]
Scopoletin	MitoSOX and DCFH-DA fluorescence assays	Proposed assessment of mitochondrial superoxide-associated and broader intracellular oxidant signals; neither assay establishes mtDNA release.	[158]
Scopoletin	Caspase-1 activity assay and IL-1β ELISA	Proposed assessment of inflammasome-associated caspase-1 activation and cytokine release; NLRP3 dependence requires specific inhibition or genetic validation.	[159]
Osthole	Immunoblotting for NLRP3, ASC, and cleaved caspase-1	Proposed assessment of inflammasome components and caspase-1 processing; protein abundance alone does not demonstrate inflammasome assembly.	[109]
Osthole	JC-1 or TMRE mitochondrial membrane potential assay	Proposed evaluation of mitochondrial membrane potential; loss of potential is not direct evidence of mtDNA leakage.	[62]
Imperatorin	Immunoblotting for cGAS, STING, and phosphorylated TBK1/IRF3	Proposed evaluation of DNA-sensing pathway components and downstream phosphorylation; functional perturbation is required to establish pathway dependence.	[160]
Imperatorin	Immunofluorescence microscopy with mitochondrial and DNA markers	Proposed visualization of mitochondrial organization and possible extranuclear mtDNA localization; specificity requires appropriate DNA and organelle controls.	[161]
Daphnetin	Confocal microscopy with mitochondrial markers	Proposed assessment of mitochondrial morphology and fragmentation; mtDNA release requires separate, validated DNA-localization measurements.	[162]
Daphnetin	ELISA for IL-18 and IL-1β	Proposed quantification of inflammatory cytokines; cytokine abundance alone does not identify the upstream sensor or mtDNA involvement.	[163]
Umbelliferone	qPCR quantification of mitochondrial and nuclear DNA	Proposed assessment of relative cellular mtDNA abundance; cytosolic mtDNA release requires validated subcellular fractionation and contamination controls.	[164]
Umbelliferone	Flow cytometry for ROS-associated probes and apoptosis markers	Proposed assessment of oxidant-associated signals and apoptotic responses; mtDNA signaling requires independent analysis.	[165]
Coumarindione	Mitophagy markers: PINK1, Parkin, LC3-II, and p62	Proposed investigation of mitophagy-associated signaling; marker abundance alone cannot establish mitophagic flux or reduced mtDNA release.	[166]
Coumarindione	TLR9 expression by RT-qPCR and immunoblotting	Proposed assessment of TLR9 expression; receptor activation and mtDNA-dependent signaling require functional assays.	[167]
7-Hydroxycoumarin-3-carboxylic acid	Cellular 2′3′-cGAMP quantification	Proposed measurement of a cGAS product; cGAMP elevation does not by itself establish mtDNA as the activating DNA source.	[168]
7-Hydroxycoumarin-3-carboxylic acid	RT-qPCR for interferon-stimulated genes (ISGs)	Proposed assessment of interferon-responsive transcription; ISG induction is not specific to STING or mtDNA signaling.	[169]

Note: The listed analytical approaches are proposed for future investigations and do not imply that the corresponding coumarin–assay combinations have been experimentally validated. The cited references provide supporting methodological or biological context. Measurements of mitochondrial dysfunction, ROS production, and downstream inflammatory responses alone cannot establish mtDNA-dependent signaling. Mechanistic confirmation requires direct detection of released mtDNA, appropriate controls to exclude nuclear DNA contamination, and functional validation of the implicated signaling pathways.

TLR9, and NLRP3 responses must be determined through direct mtDNA measurements and pathway-specific functional interventions. Third, the molecular determinants governing immunostimulatory versus immunosuppressive outcomes require clarification, particularly the effects of compound structure, concentration, exposure duration, cancer type, and the immune composition of the TME. Addressing these questions in this order would provide a stronger mechanistic foundation for subsequent studies of biomarkers, therapeutic combinations, pharmacokinetics, and clinical translation.

These research priorities reflect important differences in the strength of the available evidence. Direct demonstration of coumarin-induced mtDNA release would provide a stronger basis for investigating

mtDNA-associated signaling than measurements of mitochondrial dysfunction or OS alone. Likewise, observed changes in cGAS–STING, TLR9, or NLRP3 activity establish pathway modulation but not necessarily mtDNA dependence. Findings derived from non-cancer models provide complementary mechanistic insights, although their relevance to malignant cells and antitumor immunity requires independent validation. Importantly, even direct detection of released mtDNA does not establish its causal involvement without appropriate functional experiments.

Evidence obtained from non-cancer experimental models provides valuable mechanistic information regarding the effects of coumarins on mitochondrial homeostasis, OS, and inflammatory signaling. Nevertheless, these observations should not be interpreted as direct evidence of anticancer efficacy or tumor-associated immune modulation. In particular, suppression of inflammatory cytokines or NLRP3 activation in non-malignant cells does not necessarily predict antitumor activity, because inflammatory signaling may exert context-dependent effects within the TME. Demonstrating anticancer activity mediated through innate immune pathways requires appropriate cancer models, assessment of tumor-associated immune responses, and functional evidence linking the relevant signaling pathway to therapeutic outcomes. Accordingly, findings from non-cancer systems should be considered supportive mechanistic evidence rather than direct validation of coumarin-mediated anticancer mechanisms.

The immunological effects of coumarins vary across cancer types, disease stages, and microenvironmental conditions. Both immunostimulatory and immunosuppressive activities have been reported, although their underlying determinants remain insufficiently characterized. Differences in experimental models, tumor biology, immune-cell composition, and local metabolic conditions may contribute to these divergent outcomes. Comparative investigations are therefore needed to distinguish compound-specific effects from responses determined primarily by the biological context. An additional challenge concerns the interactions among innate immune pathways and their cellular regulators. The relationship between NLRP3 inflammasome activity and cGAS–STING signaling, together with potential contributions from γδ T cells, requires further investigation in defined cancer models. Host-related variables, including age, sex, genetic background, and metabolic status, may also influence inflammatory responses. However, their specific contributions to coumarin-mediated mtDNA signaling remain largely unresolved.

The relationship between coumarin-associated redox modulation and mtDNA-dependent immune activation represents another important research priority. Although OS can influence mitochondrial integrity and inflammatory signaling, changes in ROS production alone do not establish mtDNA-mediated pathway activation. Future studies should therefore combine direct assessment of mtDNA release with measurements of mitochondrial function and pathway-specific interventions. Such approaches would help determine whether the observed immunological effects arise from mtDNA signaling or alternative mechanisms. From a translational perspective, the potential use of coumarins alongside immunotherapy, chemotherapy, or radiotherapy remains investigational. The contrasting consequences of transient and persistent inflammatory activation highlight the need to establish appropriate biological conditions before considering therapeutic combinations. Additional studies should examine cell type-specific responses, pharmacokinetic behavior, safety, and biomarkers of pathway engagement. These investigations may provide a more reliable foundation for evaluating the therapeutic relevance of coumarin-mediated mitochondrial and innate immune regulation in cancer.

10. CONCLUSION

Coumarins influence mitochondrial redox homeostasis, cellular stress responses, and innate immune pathways relevant to cancer biology. The principal contribution of this review is the critical integration of these interconnected processes within an evidence-based framework that distinguishes experimentally demonstrated effects from indirect mechanistic associations and proposed mtDNA-dependent pathways. However, direct evidence linking these effects to mtDNA release and subsequent activation of cGAS–STING, TLR9, or NLRP3 remains limited. The reported immunostimulatory and immunosuppressive activities vary with compound structure, concentration, cancer type, and the TME, preventing generalization across experimental models. Importantly, mitochondrial dysfunction or altered inflammatory signaling should not independently be interpreted as evidence of mtDNA-dependent mechanisms. Current evidence therefore supports distinct levels of mechanistic interpretation, ranging from direct mtDNA measurements to indirect

mitochondrial and inflammatory observations and hypotheses derived from non-cancer models. None of these findings should be interpreted as establishing a causal coumarin–mtDNA–innate immunity axis without appropriate experimental validation. Future investigations should prioritize direct assessment of mtDNA release, pathway-specific functional validation, and comparative studies involving malignant and immune cells. Establishing these mechanistic relationships, together with appropriate pharmacokinetic and safety evaluations, is essential before the potential therapeutic relevance of coumarin-mediated mtDNA signaling can be reliably determined.

AVAILABILITY OF DATA

This article does not contain any studies conducted by the author of this work.

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Not applicable.

CONSENT TO PARTICIPATE

The corresponding author is responsible for the overall work.

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DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

During the preparation of this work the author used ChatGPT (OpenAI) in order to improve readability and language. After using this tool/service, the author reviewed and edited the content as needed and took full responsibility for the content of the published article.

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