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Transcriptome Analysis of Porcine Liver Reveals Hepatoprotective Mechanisms of Dietary Curcuma Longa Extract Supplementation against Aflatoxin B1: A Single-Cell RNA Sequencing Perspective

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Abstract

Aflatoxin B1 (AFB1), a potent mycotoxin produced by *Aspergillus* species, poses significant threats to livestock health and food safety, particularly in swine production. AFB1-induced hepatotoxicity involves complex molecular mechanisms including cytochrome P450-mediated bioactivation, oxidative stress, inflammatory responses, and disrupted cellular homeostasis. Dietary supplementation with *Curcuma longa* extract (curcumin) has emerged as a promising hepatoprotective intervention, demonstrating efficacy through multiple molecular pathways including inhibition of phase-I bioactivation, activation of Nrf2-driven antioxidant responses, anti-inflammatory effects, and restoration of autophagy. While bulk transcriptomic studies in porcine models have revealed broad gene expression changes, single-cell RNA sequencing (scRNA-seq) offers unprecedented resolution to dissect cell-type-specific responses in the heterogeneous liver microenvironment. This review synthesizes current evidence on AFB1 toxicity mechanisms, curcumin's hepatoprotective pathways, and the transformative potential of scRNA-seq technology to resolve cellular heterogeneity in porcine liver responses to mycotoxin exposure and dietary intervention. We highlight the critical role of porcine-specific cytochrome P450 2A19 in AFB1 bioactivation, intercellular communication via extracellular vesicles in fibrogenesis, and the methodological advances enabling single-cell transcriptomics in primary porcine hepatocytes. This synthesis provides a roadmap for future scRNA-seq studies to definitively map cell-type-specific protective mechanisms of curcumin against AFB1 hepatotoxicity in swine.

Keywords: aflatoxin B1; curcumin; single-cell RNA sequencing; porcine liver; hepatoprotection; mycotoxin.

1. Introduction

Aflatoxin B1 (AFB1), produced by *Aspergillus flavus* and *Aspergillus parasiticus*, stands as one of nature's most dangerous hepatotoxins and potent carcinogens [1], [8]. It poses a serious threat to livestock production worldwide, particularly in swine production, where feed grain contamination causes real damage: reduced growth, weakened immune responses, and increased vulnerability to infections [1]. The liver bears the brunt of this toxic assault. As the organ primarily responsible for AFB1 metabolism and detoxification, it faces a complex barrage of damage involving bioactivation of the toxin, overwhelming oxidative stress, inflammatory reactions, and disruption of critical cellular functions [8].

To combat this challenge, researchers have turned to natural compounds as practical dietary interventions. *Curcuma longa* (turmeric), particularly its active ingredient curcumin, has shown genuine promise in protecting the liver across multiple experimental systems [5], [2]. What makes curcumin so effective is its versatility—it works through multiple pathways simultaneously: it modulates how the body processes foreign chemicals, boosts built-in antioxidant defenses, dampens inflammatory signals, and restores the cellular quality control systems that keep hepatocytes healthy [5]. Porcine feeding studies have demonstrated that curcumin supplementation can reduce AFB1-induced growth losses and normalize the dramatic changes in liver gene expression that AFB1 causes, highlighting its potential for practical application in swine production [1].

However, our current understanding relies heavily on traditional bulk transcriptomic methods—microarray and RNA-sequencing—which have provided valuable glimpses into how AFB1 and curcumin alter gene expression in porcine liver tissue [1]. These approaches, though informative, come with a fundamental limitation: they measure only the average gene expression across an entire tissue sample, obscuring the nuances of individual cell types. The reality of the liver is far more complex: it's home to multiple cell types, each with its own distinct role—hepatocytes (the main working cells, comprising roughly 80% of liver mass), Kupffer cells (resident immune sentinels), hepatic stellate cells that store vitamin A and activate during injury, liver sinusoidal endothelial cells forming the liver's unique vascular system, and cholangiocytes lining the bile ducts. Each cell type responds differently to both toxins and protective interventions, but traditional bulk transcriptomics obscures these critical differences, potentially hiding the very cell-specific mechanisms that explain how protection works.

Single-cell RNA sequencing (scRNA-seq) represents a genuine breakthrough, offering unprecedented resolution by capturing the complete transcriptome of individual cells [7]. By separating tissue into individual cells and sequencing each one independently, scRNA-seq can reveal which cells are responding to toxins and which are benefiting from curcumin protection. It uncovers hidden cell populations, tracks how cells change over time, and reconstructs the communication networks between different cell types. Recent technical advances have successfully overcome the substantial challenges of applying scRNA-seq to primary porcine hepatocytes, finally opening the door to understanding cell-by-cell how AFB1 damages the liver and how curcumin works its protective magic in swine [7].

This review synthesizes current evidence on how AFB1 damages liver tissue at the molecular level, identifies the specific pathways through which curcumin protects hepatocytes, and explores how scRNA-seq can resolve these protective mechanisms at cellular resolution in porcine liver. We emphasize species-specific factors in pigs—particularly the outsized role of cytochrome P450 2A19 in AFB1 activation—and discuss how scRNA-seq can fill critical gaps in our knowledge regarding hepatocyte variations across the liver lobule, macrophage activation states, stellate cell responses, and how different cell types communicate in the face of mycotoxin exposure and dietary protection. Our synthesis offers both a conceptual framework and practical roadmap for future scRNA-seq studies aimed at definitively mapping how curcumin protects individual liver cells against AFB1 damage in swine.

2. Mechanisms of Aflatoxin B1 Hepatotoxicity

2.1 Cytochrome P450-Mediated Bioactivation

The hepatotoxicity of AFB1 is fundamentally dependent on its metabolic bioactivation by cytochrome P450 (CYP) enzymes in hepatocytes. AFB1 itself is relatively inert; however, oxidation by CYP enzymes generates the highly reactive AFB1-8,9-epoxide (AFBO), which forms covalent adducts with DNA, particularly at

guanine residues, producing the mutagenic AFB1-N7-guanine adduct. This DNA damage initiates a cascade of genotoxic stress responses, cell cycle arrest, and potentially carcinogenesis.

Species-specific differences in CYP enzyme expression and activity profoundly influence AFB1 susceptibility. In pigs, cytochrome P450 2A19 (CYP2A19) has been identified as the dominant enzyme responsible for AFB1 bioactivation. Wang et al. demonstrated that porcine CYP2A19 exhibits high catalytic efficiency in converting AFB1 to AFBO, resulting in substantial DNA adduct formation and phosphorylation of histone H2AX (γH2AX), a marker of DNA double-strand breaks [4]. Comparative studies showed that CYP2A19 confers greater AFB1 sensitivity to porcine hepatocytes compared to other porcine CYP isoforms, identifying a hepatocyte-intrinsic metabolic vulnerability specific to swine. This species-specific bioactivation pathway underscores the importance of using porcine models to study AFB1 toxicity and protective interventions relevant to swine production.

Modulation of CYP expression and activity represents a central mechanism by which both AFB1 exerts toxicity and protective compounds exert their effects. Transcriptomic studies in pigs fed AFB1-contaminated diets have documented altered expression of multiple CYP genes, reflecting adaptive and maladaptive responses to xenobiotic stress [1]. The balance between phase-I bioactivation (CYP-mediated) and phase-II detoxification (conjugation-mediated) determines the net toxicological outcome of AFB1 exposure.

2.2 Oxidative Stress and Lipid Peroxidation

AFB1 exposure induces profound oxidative stress in hepatocytes through multiple mechanisms. The formation of AFBO and subsequent redox cycling generate reactive oxygen species (ROS), including superoxide anions, hydrogen peroxide, and hydroxyl radicals. These ROS overwhelm endogenous antioxidant defenses, leading to oxidative damage to lipids, proteins, and nucleic acids. Lipid peroxidation, measured by elevated malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) levels, is a hallmark of AFB1-induced hepatotoxicity across multiple animal models [4], [5].

Oxidative stress not only causes direct cellular damage but also activates stress-responsive signaling pathways that can amplify toxicity. ROS-mediated activation of mitogen-activated protein kinases (MAPKs), nuclear factor-κB (NF-κB), and p53 pathways contributes to inflammatory responses, cell cycle arrest, and apoptosis. The mitochondria, as both major sources and targets of ROS, undergo structural and functional impairment during AFB1 exposure, including loss of membrane potential, cytochrome c release, and activation of intrinsic apoptotic pathways.

The oxidative stress response is heterogeneous across liver cell types. Hepatocytes, which express high levels of CYP enzymes and are the primary site of AFB1 bioactivation, experience the most severe oxidative burden. Kupffer cells, the resident liver macrophages, respond to hepatocyte injury signals and oxidized cellular debris by producing additional ROS and inflammatory mediators, potentially amplifying oxidative damage. Single-cell approaches are needed to resolve these cell-type-specific oxidative stress signatures and their temporal dynamics.

2.3 Inflammatory Signaling and Inflammasome Activation

AFB1-induced hepatocyte injury triggers robust inflammatory responses involving both parenchymal and non-parenchymal liver cells. Damaged hepatocytes release damage-associated molecular patterns (DAMPs), including high-mobility group box 1 (HMGB1), ATP, and oxidized DNA, which activate pattern recognition receptors on Kupffer cells and other immune cells. This innate immune activation leads to production of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) and chemokines that recruit additional immune cells and amplify tissue inflammation.

The NLRP3 inflammasome, a multiprotein complex that activates caspase-1 and promotes maturation of IL-1β and IL-18, plays a critical role in AFB1-induced liver inflammation. Studies have demonstrated that AFB1 exposure activates the NLRP3 inflammasome through ROS generation and mitochondrial dysfunction, leading to pyroptotic cell death characterized by plasma membrane rupture and release of inflammatory cellular contents [5]. Curcumin has been shown to suppress NLRP3 inflammasome activation, representing one mechanism of its anti-inflammatory hepatoprotective effects.

NF-κB signaling serves as a master regulator of inflammatory gene expression in AFB1 hepatotoxicity. Activation of NF-κB in both hepatocytes and Kupffer cells drives transcription of pro-inflammatory cytokines, chemokines, and adhesion molecules. The inflammatory milieu not only contributes to acute hepatocyte injury but also promotes chronic pathological processes including fibrosis through activation of hepatic stellate cells. Understanding the cell-type-specific patterns of inflammatory signaling and their intercellular coordination requires single-cell resolution approaches.

2.4 Impaired Detoxification and Phase-II Enzyme Dysregulation

While phase-I CYP enzymes bioactivate AFB1 to toxic metabolites, phase-II conjugation enzymes—particularly glutathione S-transferases (GSTs)—play critical protective roles by conjugating AFBO with glutathione, forming less reactive and more readily excretable metabolites. The balance between phase-I bioactivation and phase-II detoxification is a key determinant of AFB1 toxicity.

Transcriptomic studies in pigs fed AFB1-contaminated diets have revealed dysregulation of multiple GST family members and related detoxification pathways [1]. Specifically, altered expression of GSTT1, GSTA3, and GSTM2 has been documented, indicating compromised conjugation capacity. This impairment may result from direct toxic effects on hepatocyte protein synthesis, oxidative inactivation of GST enzymes, or depletion of glutathione pools due to excessive ROS generation and conjugation demands.

The nuclear factor erythroid 2-related factor 2 (Nrf2) pathway serves as the master regulator of phase-II detoxification enzyme expression. Under basal conditions, Nrf2 is sequestered in the cytoplasm by Kelch-like ECH-associated protein 1 (Keap1) and targeted for proteasomal degradation. Oxidative stress or electrophilic compounds modify Keap1 cysteine residues, leading to Nrf2 stabilization, nuclear translocation, and transcriptional activation of antioxidant response element (ARE)-containing genes, including GSTs, NAD(P)H quinone oxidoreductase 1 (NQO1), and heme oxygenase-1 (HO-1).

AFB1 exposure can paradoxically both activate Nrf2 (as an adaptive stress response) and impair its function (through oxidative damage to signaling components). The net effect depends on dose, duration, and cellular context. Importantly, pharmacological or dietary activation of Nrf2 represents a major mechanism of hepatoprotection against AFB1, as discussed in Section 3.

2.5 Autophagy Dysregulation and Cell Death Pathways

Autophagy, the cellular process of degrading and recycling damaged organelles and protein aggregates, plays a complex and context-dependent role in AFB1 hepatotoxicity. Under moderate stress, autophagy serves a protective function by removing damaged mitochondria (mitophagy) and protein aggregates, thereby limiting ROS generation and maintaining cellular homeostasis. However, excessive or dysregulated autophagy can contribute to cell death.

Studies have demonstrated that AFB1 exposure disrupts autophagic flux through multiple mechanisms. Yang et al. showed that AFB1 exposure increased hepatocyte PINK1/Parkin-dependent mitophagy via a p53→PARK2 (Parkin) signaling axis [10]. While mitophagy initially serves to remove damaged mitochondria, excessive mitophagy can deplete the mitochondrial pool and compromise cellular energy metabolism.

Importantly, this study revealed that AFB1-induced mitophagy in hepatocytes promoted the release of extracellular vesicles (EVs) that activated hepatic stellate cells, initiating fibrogenic responses. Genetic or pharmacological blockade of PARK2 or p53 reduced both mitophagy and EV-mediated HSC activation, highlighting a novel intercellular communication pathway in AFB1 hepatotoxicity [10].

Curcumin has been shown to restore autophagy homeostasis in AFB1-exposed cells. Zhang et al. demonstrated that curcumin reactivated autophagy-related factors including beclin-1, ATG5, and LC3 variants, and counteracted p53/mTOR-mediated autophagy suppression in chicken liver exposed to AFB1 [3]. This restoration of autophagic flux reduced organelle damage and inflammatory responses, contributing to hepatoprotection.

AFB1 exposure activates multiple cell death pathways including apoptosis, necroptosis, and pyroptosis. The intrinsic (mitochondrial) apoptotic pathway is activated by cytochrome c release and caspase-9/caspase-3 activation. Necroptosis, a regulated form of necrotic cell death mediated by receptor-interacting protein kinases (RIPK1/RIPK3) and mixed lineage kinase domain-like protein (MLKL), has been implicated in AFB1 toxicity. Zhang et al. identified a long non-coding RNA (LOC769044)/miR-1679/STAT1 regulatory axis that mediates AFB1-induced liver necroptosis in chickens, suggesting that similar mechanisms may operate in porcine liver [3]. Pyroptosis, mediated by inflammasome activation and gasdermin D cleavage, contributes to inflammatory cell death and cytokine release.

The relative contribution of different cell death modalities likely varies across liver cell types and depends on the severity and duration of AFB1 exposure. Single-cell transcriptomics can resolve these cell-type-specific death signatures and identify which populations are most vulnerable to AFB1-induced cell death and most responsive to curcumin protection.

3. Hepatoprotective Pathways of Curcuma Longa Extract

3.1 Inhibition of Phase-I Bioactivation Enzymes

A primary mechanism by which curcumin mitigates AFB1 hepatotoxicity is through suppression of CYP enzymes responsible for bioactivation. By reducing the conversion of AFB1 to the reactive AFBO epoxide, curcumin decreases the formation of DNA adducts and the initiation of genotoxic stress cascades [2], [3].

Multiple studies have demonstrated that curcumin downregulates CYP expression and/or inhibits CYP enzymatic activity. In porcine feeding studies, curcumin supplementation altered the expression of CYP2A19, the dominant AFB1-bioactivating enzyme in pigs, along with other CYP family members [1]. The mechanisms of CYP suppression by curcumin are multifactorial and may include: (1) transcriptional repression through modulation of nuclear receptors such as the aryl hydrocarbon receptor (AhR) and constitutive androstane receptor (CAR), which regulate CYP gene expression; (2) direct inhibition of CYP enzyme activity through competitive or non-competitive binding; and (3) post-translational modifications that reduce CYP protein stability.

The inhibition of phase-I bioactivation represents a "first-line" defense mechanism that prevents the generation of toxic metabolites. This upstream intervention is particularly effective because it reduces the burden on downstream detoxification and antioxidant systems. However, the extent of CYP inhibition must be carefully balanced, as excessive suppression could impair the metabolism of other xenobiotics and endogenous compounds.

3.2 Activation of Nrf2 and Phase-II Detoxification

Complementing its inhibition of phase-I enzymes, curcumin potently activates the Nrf2 pathway, enhancing the expression of phase-II detoxification enzymes and antioxidant proteins. Curcumin is an electrophilic compound that modifies Keap1 cysteine residues, leading to Nrf2 stabilization and nuclear translocation [2].

Zhang et al. demonstrated that curcumin supplementation in AFB1-exposed broiler chickens upregulated Nrf2 signaling and downstream conjugating enzymes including GSTA3 and GSTM2, resulting in increased GST activity and enhanced AFB1 detoxification capacity [23]. Similar Nrf2 activation has been documented in multiple animal models and cell systems exposed to curcumin. The Nrf2-driven transcriptional program includes not only GSTs but also other critical detoxification and antioxidant enzymes such as NQO1, HO-1, glutamate-cysteine ligase (GCL, the rate-limiting enzyme in glutathione synthesis), and various glutathione peroxidases and reductases.

The coordinated upregulation of these enzymes creates a comprehensive cellular defense system. GSTs conjugate AFBO with glutathione, NQO1 reduces quinone metabolites, HO-1 degrades pro-oxidant heme and generates antioxidant bilirubin, and GCL maintains glutathione pools. This multi-layered protection is more effective than targeting any single enzyme or pathway.

Importantly, Nrf2 activation by curcumin appears to be dose-dependent and context-specific. At physiologically relevant concentrations, curcumin activates Nrf2 without causing excessive oxidative stress. The ability of curcumin to simultaneously inhibit phase-I bioactivation and enhance phase-II detoxification represents a synergistic dual mechanism that shifts the metabolic balance toward detoxification and away from bioactivation.

3.3 Antioxidant and Anti-Inflammatory Effects

Beyond its effects on xenobiotic metabolism, curcumin exerts direct antioxidant and anti-inflammatory activities that contribute to hepatoprotection. Curcumin's chemical structure, featuring conjugated double bonds and phenolic hydroxyl groups, enables it to scavenge free radicals and chelate metal ions that catalyze ROS generation [4], [5].

Multiple studies have demonstrated that curcumin supplementation reduces oxidative stress markers in AFB1-exposed animals. Kang et al. showed that curcumin (along with astaxanthin) attenuated AFB1-induced hepatotoxicity by activating Nrf2 and inhibiting NF-κB signaling, resulting in reduced lipid peroxidation and inflammatory cytokine production [15]. The antioxidant effects of curcumin are mediated both through direct ROS scavenging and through upregulation of endogenous antioxidant enzymes via Nrf2 activation.

Curcumin's anti-inflammatory effects involve multiple mechanisms. At the transcriptional level, curcumin inhibits NF-κB activation by preventing IκB degradation and p65 nuclear translocation, thereby suppressing the expression of pro-inflammatory genes [15]. Curcumin also inhibits the activation of the NLRP3 inflammasome, reducing caspase-1 activation and IL-1β maturation [5]. Additionally, curcumin modulates the activity of inflammatory enzymes such as cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS).

The anti-inflammatory effects of curcumin are particularly relevant in the context of AFB1 hepatotoxicity, where inflammatory signaling amplifies hepatocyte injury and promotes fibrogenesis. By dampening inflammatory responses in both hepatocytes and Kupffer cells, curcumin may interrupt the positive feedback loops that drive chronic liver pathology. Single-cell studies could reveal whether curcumin shifts Kupffer cells from pro-inflammatory (M1-like) to anti-inflammatory/reparative (M2-like) phenotypes, a hypothesis that remains to be tested in porcine liver.

3.4 Restoration of Autophagy and Cell Survival

Curcumin has been shown to restore autophagy homeostasis in cells exposed to AFB1 and other stressors. Zhang et al. demonstrated that curcumin reactivated autophagy-related factors including beclin-1, ATG5, and LC3 variants in AFB1-exposed chicken liver [3]. Curcumin counteracted p53-mediated autophagy suppression and mTOR dysregulation, restoring autophagic flux and enabling efficient clearance of damaged organelles and protein aggregates.

The restoration of autophagy by curcumin serves multiple protective functions: (1) removal of damaged mitochondria reduces ROS generation and prevents activation of intrinsic apoptotic pathways; (2) clearance of protein aggregates prevents endoplasmic reticulum (ER) stress and unfolded protein response (UPR) activation; (3) recycling of cellular components provides metabolic substrates during stress; and (4) regulation of inflammatory signaling, as autophagy can degrade inflammasome components and limit cytokine production.

Curcumin also modulates cell survival signaling pathways. By activating Nrf2 and inhibiting excessive p53 activation, curcumin promotes cell survival under stress conditions. The balance between autophagy (cell survival) and apoptosis (cell death) is tightly regulated, and curcumin appears to shift this balance toward survival in the context of AFB1 exposure.

Sethi et al. reviewed the therapeutic potential of nutraceuticals including curcumin against drug-induced liver injury, noting that curcumin can modulate oxidative stress, inflammation, autophagy, and UPR pathways across multiple liver cell types including hepatocytes, Kupffer cells, and hepatic stellate cells [8]. This multi-cellular action suggests that curcumin's hepatoprotective effects involve coordinated responses across the liver microenvironment, a hypothesis that scRNA-seq is uniquely positioned to test.

3.5 Phenotypic Outcomes in Porcine Models

The molecular mechanisms described above translate into measurable phenotypic improvements in porcine feeding studies. In weanling pigs fed AFB1-contaminated diets, curcumin supplementation improved feed efficiency and attenuated the broad hepatic transcriptomic alterations induced by AFB1 [1]. Specifically, curcumin modulated pathways related to growth, coagulation, immune function, metabolism, and detoxification. Expression of key genes including GSTT1 and CYP2A19 was shifted by curcumin addition, consistent with the mechanisms of enhanced detoxification and reduced bioactivation.

Beyond growth performance, curcumin supplementation has been shown to reduce liver histopathology, decrease serum markers of hepatocellular injury (e.g., alanine aminotransferase, aspartate aminotransferase), and improve antioxidant status in AFB1-exposed animals across multiple species [5], [23]. These phenotypic improvements validate the translational relevance of the molecular mechanisms and support the practical application of curcumin supplementation in swine nutrition.

Importantly, the effective dose of curcumin, its bioavailability, and potential interactions with other dietary components remain active areas of investigation. Curcumin has relatively poor oral bioavailability due to limited absorption, rapid metabolism, and rapid elimination. Formulation strategies including nanoparticles, liposomes, and cyclodextrin complexes have been developed to enhance curcumin bioavailability [18]. Understanding the tissue distribution and cellular uptake of curcumin in porcine liver, and how these factors influence cell-type-specific responses, represents an important direction for future research.

4. The Role of Single-Cell RNA Sequencing in Resolving Cell-Type-Specific Responses

4.1 Limitations of Bulk Transcriptomics

Bulk transcriptomic approaches, including microarray and RNA-seq, have provided foundational insights into the molecular responses to AFB1 exposure and curcumin intervention in porcine liver [1]. These studies have identified differentially expressed genes, enriched pathways, and potential biomarkers of toxicity and protection. However, bulk transcriptomics suffers from a fundamental limitation: it measures the average gene expression across all cells in a tissue sample, obscuring cellular heterogeneity.

The liver is composed of multiple cell types with distinct functions: hepatocytes (parenchymal cells, ~80% of liver mass) perform most metabolic and detoxification functions; Kupffer cells (resident macrophages, ~10% of liver cells) mediate innate immune responses; hepatic stellate cells (~5-8%) store vitamin A and, when activated, produce extracellular matrix in fibrosis; liver sinusoidal endothelial cells form the fenestrated endothelium of liver sinusoids; and cholangiocytes line the bile ducts. Each cell type expresses a distinct transcriptional program and responds differently to toxicological insults and protective interventions.

When bulk RNA-seq is performed on whole liver tissue, the resulting gene expression profile is a weighted average dominated by the most abundant cell type (hepatocytes). Changes in less abundant but functionally critical cell types (e.g., Kupffer cells, HSCs) may be diluted below detection thresholds. Moreover, even within a single cell type, there can be substantial heterogeneity. Hepatocytes, for example, exhibit zonal heterogeneity along the porto-central axis of the liver lobule, with periportal hepatocytes specializing in oxidative metabolism and pericentral hepatocytes expressing higher levels of certain CYP enzymes and glutamine synthetase.

Bulk transcriptomics also cannot resolve intercellular communication networks. AFB1 toxicity and curcumin protection likely involve coordinated responses across multiple cell types, mediated by secreted factors (cytokines, chemokines, growth factors), extracellular vesicles, and direct cell-cell contacts. These intercellular interactions are invisible to bulk approaches but can be inferred from single-cell data using computational tools that predict ligand-receptor interactions.

4.2 Methodological Advances in Porcine Hepatocyte scRNA-seq

Single-cell RNA sequencing has revolutionized our understanding of tissue biology by enabling unbiased, genome-wide transcriptional profiling of individual cells. However, applying scRNA-seq to primary hepatocytes, particularly from large animal models like pigs, has been technically challenging due to hepatocyte size, fragility, high RNA content, and the need for gentle dissociation protocols that preserve cell viability and transcriptional integrity.

Recent methodological advances have overcome these challenges for porcine liver. Sharma and colleagues developed a robust pipeline for scRNA-seq of primary porcine hepatocytes, optimizing tissue dissociation, cell isolation, and library preparation protocols [1]. This species-optimized pipeline enables high-quality single-cell transcriptomes from porcine liver, opening new opportunities to dissect cellular heterogeneity in swine hepatotoxicology and nutrition studies.

The porcine scRNA-seq pipeline typically involves: (1) perfusion of liver tissue to remove blood and facilitate dissociation; (2) enzymatic digestion using collagenase and other proteases to dissociate cells while maintaining viability; (3) enrichment or depletion steps to adjust cell-type proportions if needed (e.g., enriching for non-parenchymal cells); (4) single-cell capture using droplet-based platforms (e.g., 10x Genomics) or plate-based methods; (5) cDNA synthesis and library preparation; (6) high-throughput sequencing; and (7) computational analysis including quality control, normalization, dimensionality reduction, clustering, cell-type annotation, differential expression analysis, and trajectory inference.

Quality control is critical in scRNA-seq experiments. Cells with low gene counts (indicating poor capture or dead cells), high mitochondrial gene percentages (indicating stressed or dying cells), or high doublet scores (indicating capture of multiple cells) are typically filtered out. For hepatocytes, which have high metabolic activity and large cell size, careful optimization of dissociation and capture protocols is needed to minimize stress-induced transcriptional artifacts.

Cell-type annotation in porcine liver scRNA-seq data relies on known marker genes: hepatocytes express albumin (ALB), cytochrome P450 enzymes (CYP2A19, CYP3A29), and metabolic enzymes; Kupffer cells express CD68, CD163, and MARCO; hepatic stellate cells express desmin (DES), vitamin A storage genes (LRAT), and when activated, alpha-smooth muscle actin (ACTA2); liver sinusoidal endothelial cells express PECAM1 (CD31) and stabilin-2 (STAB2); and cholangiocytes express cytokeratin 19 (KRT19) and aquaporin 1 (AQP1). Computational clustering algorithms group cells with similar transcriptional profiles, and these clusters are annotated based on marker gene expression.

4.3 Cell-Type-Specific Hypotheses for AFB1 and Curcumin Responses

The mechanistic insights from bulk transcriptomic and biochemical studies, combined with the capabilities of scRNA-seq, enable the formulation of specific, testable hypotheses regarding cell-type-specific responses to AFB1 and curcumin in porcine liver.

Hepatocytes: As the primary site of AFB1 bioactivation, hepatocytes are expected to show the most pronounced transcriptional responses to AFB1 exposure. Key hypotheses include:

1. Zonal heterogeneity in AFB1 bioactivation: Pericentral hepatocytes, which express higher levels of certain CYP enzymes, may exhibit greater CYP2A19 expression and consequently higher AFB1 bioactivation, DNA damage (γH2AX), and cell death signatures compared to periportal hepatocytes [4]. scRNA-seq can map the spatial distribution of CYP2A19 expression and correlate it with DNA damage response gene expression within individual cells.

2. Nrf2 activation by curcumin: Curcumin treatment is expected to upregulate Nrf2 target genes (GSTs, NQO1, HO-1, GCL) in hepatocytes [2], [23]. scRNA-seq can quantify the proportion of hepatocytes that activate Nrf2 signaling and identify whether certain hepatocyte subpopulations are more responsive to curcumin.

3. Autophagy and mitophagy signatures: AFB1 exposure is expected to increase expression of autophagy-related genes (ATG5, BECN1, LC3/MAP1LC3 variants) and mitophagy-specific genes (PINK1, PARK2) in hepatocytes [10]. Curcumin treatment may normalize these signatures, and scRNA-seq can reveal whether autophagy modulation occurs uniformly across hepatocytes or in specific subpopulations.

Kupffer Cells (Macrophages): Kupffer cells respond to hepatocyte injury signals and play critical roles in inflammation and tissue repair. Key hypotheses include:

1. Inflammatory activation by AFB1: AFB1-induced hepatocyte injury is expected to activate Kupffer cells, leading to upregulation of pro-inflammatory cytokines (TNF, IL1B, IL6), NF-κB pathway genes, and oxidative stress response genes [5], [15]. scRNA-seq can identify pro-inflammatory (M1-like) Kupffer cell subpopulations and quantify their abundance.

2. Anti-inflammatory effects of curcumin: Curcumin treatment is expected to reduce pro-inflammatory gene expression in Kupffer cells and potentially shift them toward anti-inflammatory/reparative (M2-like) phenotypes characterized by expression of IL10, ARG1, and CD163 [8]. scRNA-seq can test whether curcumin alters the balance of Kupffer cell subpopulations.

3. Inflammasome activation: AFB1 exposure may activate the NLRP3 inflammasome in Kupffer cells, leading to increased expression of NLRP3, CASP1, and IL1B [5]. Curcumin's suppression of inflammasome activation can be assessed at single-cell resolution.

Hepatic Stellate Cells (HSCs): HSCs are the primary fibrogenic cells in the liver and are activated by chronic injury signals. Key hypotheses include:

1. Activation by hepatocyte-derived signals: AFB1-induced hepatocyte injury, particularly through the p53→PARK2 mitophagy pathway and extracellular vesicle release, is expected to activate HSCs, leading to upregulation of fibrogenic markers including ACTA2 (α-SMA), COL1A1, and TGF-β pathway genes [10]. scRNA-seq can identify activated HSC subpopulations and quantify their abundance.

2. Curcumin's anti-fibrotic effects: Curcumin treatment may reduce HSC activation by dampening hepatocyte injury signals and directly modulating HSC signaling pathways [8]. scRNA-seq can test whether curcumin reduces the proportion of activated HSCs or shifts them toward quiescent phenotypes.

Liver Sinusoidal Endothelial Cells (LSECs) and Cholangiocytes: These cell types are less studied in the context of AFB1 toxicity but may contribute to overall liver responses. Hypotheses include:

1. Stress responses in LSECs: AFB1-induced oxidative stress and inflammation may affect LSEC function, potentially impairing their fenestration and scavenging functions. Curcumin's antioxidant effects may protect LSECs [8].

2. Cholangiocyte responses: While AFB1 primarily targets hepatocytes, cholangiocytes may respond to inflammatory signals and contribute to bile duct pathology in chronic exposure scenarios.

4.4 Intercellular Communication and Extracellular Vesicle Signaling

One of the most powerful applications of scRNA-seq is the reconstruction of intercellular communication networks. Computational tools such as CellPhoneDB, NicheNet, and CellChat use scRNA-seq data to predict ligand-receptor interactions between cell types based on the expression of ligands in sender cells and receptors in receiver cells.

Yang et al. provided compelling evidence that AFB1-induced hepatocyte injury involves intercellular communication via extracellular vesicles (EVs) [10]. AFB1 exposure increased hepatocyte PINK1/Parkin-dependent mitophagy via a p53→PARK2 axis, and this mitophagy promoted the release of hepatocyte-derived EVs. These EVs were taken up by hepatic stellate cells and activated fibrogenic signaling pathways. Genetic or pharmacological blockade of PARK2, p53, or EV secretion reduced HSC activation, demonstrating a causal role for this intercellular communication pathway in AFB1-induced fibrogenesis [10].

scRNA-seq can extend these findings by:

1. Mapping EV cargo signatures: By analyzing the transcriptomes of hepatocytes with high mitophagy/EV secretion signatures, researchers can predict the molecular cargo of AFB1-induced EVs and identify potential fibrogenic signals.

2. Identifying HSC receptors: scRNA-seq of HSCs can reveal which receptors or uptake mechanisms are upregulated in response to AFB1 exposure, providing targets for therapeutic intervention.

3. Testing curcumin's effects on intercellular communication: If curcumin reduces hepatocyte mitophagy and EV secretion, scRNA-seq should reveal reduced expression of EV biogenesis genes in hepatocytes and reduced activation signatures in HSCs. Ligand-receptor analysis can quantify changes in predicted intercellular interactions.

Beyond EV signaling, scRNA-seq can reveal other intercellular communication pathways relevant to AFB1 toxicity and curcumin protection, such as:

- Hepatocyte-Kupffer cell interactions: Injured hepatocytes release DAMPs and cytokines that activate Kupffer cells; activated Kupffer cells release inflammatory mediators that amplify hepatocyte injury. Curcumin may interrupt these positive feedback loops.
- Kupffer cell-HSC interactions: Activated Kupffer cells release TGF-β and other profibrogenic factors that activate HSCs. Curcumin's anti-inflammatory effects on Kupffer cells may indirectly reduce HSC activation.
- LSEC-HSC interactions: LSECs regulate HSC quiescence through secretion of factors such as nitric oxide. AFB1-induced LSEC dysfunction may contribute to HSC activation, and curcumin may preserve LSEC function.

Reconstructing these intercellular communication networks from scRNA-seq data will provide a systems-level understanding of how AFB1 toxicity propagates across liver cell types and how curcumin's pleiotropic mechanisms coordinate to achieve hepatoprotection.

5. Comparative Analysis of Transcriptomic Studies

To contextualize the potential of scRNA-seq in porcine liver, it is instructive to compare existing transcriptomic studies that have investigated AFB1 toxicity and curcumin intervention across different species and methodological approaches. Table 1 summarizes key studies from the literature.

Table 1. Comparative Analysis of Transcriptomic Studies on AFB1 Toxicity and Curcumin Intervention

Study Model	Method	Key Findings	Curcumin Effect	Reference
Weanling pigs fed AFB1 ± curcumin	Liver microarray with RT-qPCR validation	Altered pathways for growth, coagulation, immune function, metabolism, detoxification; GSTT1 and CYP2A19 expression shifted	Improved feed efficiency; modulated detoxification gene expression	[1]
Broiler chickens exposed to AFB1 ± curcumin	Liver transcriptomics and biochemical assays	AFB1 induced oxidative stress, inflammation, and impaired detoxification; curcumin activated Nrf2 signaling and upregulated GSTA3, GSTM2	Reduced oxidative damage and inflammation; enhanced GST activity	[23]
Chicken liver exposed to AFB1 ± curcumin	Bulk lncRNA and mRNA sequencing	AFB1 induced liver necroptosis via LOC769044/miR-1679/STAT1 axis; curcumin showed anti-necroptotic effects	Reduced necroptosis markers	[3]
Porcine hepatocytes (in vitro)	CYP enzyme activity assays and DNA adduct analysis	Porcine CYP2A19 identified as dominant AFB1-bioactivating enzyme; high AFBO formation and γH2AX induction	Not tested	[4]
Mouse hepatocytes exposed to AFB1	In vitro and in vivo models with genetic/pharmacological interventions	AFB1 increased PINK1/Parkin-dependent mitophagy via p53→PARK2 axis; hepatocyte-derived EVs activated HSCs	Not tested, but PARK2/p53 blockade reduced fibrogenic activation	[10]
Multiple species (review)	Literature synthesis	Nutraceuticals including curcumin modulate oxidative stress, inflammation, autophagy, and UPR in hepatocytes, Kupffer cells, and HSCs	Multi-cellular hepatoprotective effects	[8]

This comparative analysis reveals several important patterns:

1. Species-specific mechanisms: The dominant role of CYP2A19 in porcine AFB1 bioactivation [4] highlights the importance of using porcine models for translational research relevant to swine production. Findings from rodent or avian models may not fully capture porcine-specific metabolic pathways.

2. Convergent protective mechanisms: Across multiple species and experimental systems, curcumin consistently activates Nrf2 signaling, enhances phase-II detoxification, reduces oxidative stress, and suppresses inflammatory pathways [2], [3], [23]. This convergence suggests that these mechanisms are fundamental and likely operate in porcine liver as well.

3. Intercellular complexity: The discovery of hepatocyte-HSC communication via EVs in AFB1 toxicity [10] underscores the importance of studying intercellular interactions. Bulk transcriptomics of whole liver tissue would not have revealed this cell-type-specific mechanism.

4. Methodological gap: Despite the wealth of bulk transcriptomic data, there is a notable absence of single-cell studies specifically addressing AFB1 toxicity and curcumin protection in porcine liver. The methodological advances in porcine hepatocyte scRNA-seq [1] now make such studies feasible.

5. Mechanistic hypotheses for scRNA-seq validation: The mechanistic insights from bulk studies provide a rich set of hypotheses that can be tested at single-cell resolution, including zonal heterogeneity in CYP2A19 expression [4], cell-type-specific Nrf2 activation [23], macrophage polarization states [8], and HSC activation signatures [10].

6. Future Directions and Recommendations

The integration of single-cell RNA sequencing with porcine models of AFB1 toxicity and curcumin intervention represents a transformative opportunity to advance our understanding of hepatoprotective mechanisms and inform practical strategies for mycotoxin mitigation in swine production. Based on the evidence synthesized in this review, we propose the following future directions and recommendations:

1. Conduct definitive scRNA-seq studies in porcine liver exposed to AFB1 and curcumin

A well-designed scRNA-seq experiment should include multiple treatment groups (control, AFB1 alone, curcumin alone, AFB1 + curcumin) with sufficient biological replicates to enable robust statistical analysis. Time-course experiments would reveal the temporal dynamics of cellular responses. The study should capture all major liver cell types (hepatocytes, Kupffer cells, HSCs, LSECs, cholangiocytes) and achieve sufficient sequencing depth to detect low-abundance transcripts and subtle transcriptional changes.

2. Integrate scRNA-seq with spatial transcriptomics

While scRNA-seq provides unparalleled cellular resolution, it loses spatial information during tissue dissociation. Spatial transcriptomics technologies (e.g., 10x Visium, MERFISH, seqFISH) enable transcriptional profiling while preserving tissue architecture. Integrating scRNA-seq (for cellular resolution) with spatial transcriptomics (for spatial context) would enable mapping of cell-type-specific responses to their anatomical locations within the liver lobule, revealing zonal patterns of AFB1 bioactivation, injury, and curcumin protection.

3. Perform multi-omics integration

Combining scRNA-seq with other single-cell omics technologies would provide a more complete picture of cellular responses. Single-cell ATAC-seq (assay for transposase-accessible chromatin sequencing) reveals

chromatin accessibility and transcription factor binding, enabling identification of the regulatory mechanisms underlying transcriptional changes. Single-cell proteomics, though still technically challenging, would reveal post-translational modifications and protein-level responses that may not be captured by transcriptomics alone. Metabolomics at the single-cell level remains aspirational but would directly measure the metabolic consequences of AFB1 bioactivation and curcumin intervention.

4. Develop computational models of intercellular communication

Leveraging scRNA-seq data to reconstruct intercellular communication networks will require sophisticated computational approaches. Beyond predicting ligand-receptor interactions, future studies should model the dynamics of intercellular signaling, including feedback loops, signal amplification, and temporal coordination. Agent-based models or ordinary differential equation models parameterized with scRNA-seq data could simulate how AFB1 toxicity propagates across cell types and how curcumin intervention interrupts these pathways.

5. Validate cell-type-specific mechanisms with targeted interventions

scRNA-seq can generate hypotheses about cell-type-specific mechanisms, but these hypotheses require validation through targeted interventions. Cell-type-specific gene knockout or knockdown (e.g., using CRISPR/Cas9 or RNA interference delivered via cell-type-specific promoters or viral vectors) can test the causal roles of specific genes or pathways. For example, hepatocyte-specific knockout of CYP2A19 should reduce AFB1 bioactivation and toxicity, while Kupffer cell-specific knockout of NLRP3 should reduce inflammatory responses.

6. Optimize curcumin formulation and delivery

The relatively poor bioavailability of curcumin limits its therapeutic efficacy. Future studies should evaluate advanced formulations (nanoparticles, liposomes, cyclodextrin complexes) that enhance curcumin absorption and tissue distribution [18]. scRNA-seq can be used to assess whether improved formulations achieve better cellular uptake and more robust transcriptional responses across liver cell types. Additionally, identifying the specific curcuminoids (curcumin, demethoxycurcumin, bisdemethoxycurcumin) or metabolites responsible for hepatoprotection would enable more targeted interventions.

7. Translate findings to practical feeding strategies

Ultimately, the goal of this research is to develop practical dietary strategies to protect swine from AFB1 toxicity. Field trials in commercial swine production settings should evaluate the efficacy of curcumin supplementation under realistic conditions of mycotoxin contamination. Dose-response studies should identify the minimum effective dose of curcumin that provides hepatoprotection without excessive cost. Economic analyses should assess the cost-benefit ratio of curcumin supplementation relative to other mycotoxin mitigation strategies (e.g., mycotoxin binders, improved grain storage).

8. Extend to other mycotoxins and hepatotoxins

While this review has focused on AFB1, swine are exposed to multiple mycotoxins in contaminated feed, including deoxynivalenol (DON), zearalenone (ZEN), fumonisin B1 (FB1), and ochratoxin A (OTA). These mycotoxins have distinct mechanisms of toxicity and may interact synergistically. scRNA-seq studies should investigate whether curcumin provides broad-spectrum protection against multiple mycotoxins or whether different mycotoxins require tailored interventions. Additionally, the hepatoprotective mechanisms of curcumin may be relevant to other forms of liver injury (e.g., drug-induced liver injury, alcohol-induced liver disease, non-alcoholic fatty liver disease), expanding the translational impact of this research [8], [11].

9. Investigate individual variation and precision nutrition

Genetic variation among individual pigs may influence susceptibility to AFB1 toxicity and responsiveness to curcumin intervention. Polymorphisms in CYP2A19, GST genes, or Nrf2 pathway components could create subpopulations with different risk profiles. scRNA-seq studies that include animals with diverse genetic backgrounds could identify genetic markers associated with AFB1 susceptibility or curcumin responsiveness, enabling precision nutrition strategies tailored to individual or population-specific needs.

10. Develop predictive biomarkers

scRNA-seq can identify cell-type-specific gene expression signatures that predict AFB1toxicity or curcumin efficacy. These signatures could be translated into practical biomarkers measurable in blood or liver biopsies, enabling early detection of mycotoxin exposure and monitoring of intervention efficacy. Machine learning approaches applied to scRNA-seq data could identify minimal gene sets with maximal predictive power, facilitating the development of cost-effective diagnostic assays.

7. Conclusion

Aflatoxin B1 represents a persistent threat to swine health and productivity, exerting hepatotoxicity through complex, multi-cellular mechanisms involving cytochrome P450-mediated bioactivation, oxidative stress, inflammatory signaling, impaired detoxification, and dysregulated autophagy. The identification of porcine CYP2A19 as the dominant AFB1-bioactivating enzyme highlights species-specific vulnerabilities that must be considered in translational research. Dietary supplementation with *Curcuma longa* extract (curcumin) has emerged as a promising hepatoprotective strategy, operating through pleiotropic mechanisms including inhibition of phase-I bioactivation, activation of Nrf2-driven phase-II detoxification and antioxidant responses, suppression of inflammatory signaling, and restoration of autophagy homeostasis. These molecular mechanisms translate into measurable phenotypic improvements in porcine feeding studies, including enhanced feed efficiency and reduced hepatic injury.

While bulk transcriptomic approaches have provided valuable insights into the global gene expression changes induced by AFB1 and curcumin, they cannot resolve the cellular heterogeneity and intercellular communication networks that underlie hepatotoxicity and hepatoprotection. Single-cell RNA sequencing represents a transformative technology that enables unbiased, genome-wide transcriptional profiling at single-cell resolution, revealing cell-type-specific responses, rare cell populations, developmental trajectories, and intercellular signaling networks. Recent methodological advances have overcome technical challenges in applying scRNA-seq to primary porcine hepatocytes, opening new opportunities to dissect the cellular landscape of AFB1 toxicity and curcumin protection in swine liver.

This review has synthesized current evidence on AFB1 toxicity mechanisms, curcumin's hepatoprotective pathways, and the potential of scRNA-seq to resolve cell-type-specific responses. We have formulated specific, testable hypotheses regarding hepatocyte zonal heterogeneity in AFB1 bioactivation, Kupffer cell inflammatory activation and polarization, hepatic stellate cell activation via extracellular vesicle signaling, and the coordinated multi-cellular effects of curcumin. These hypotheses provide a roadmap for future scRNA-seq studies that will definitively map the cellular mechanisms of curcumin-mediated hepatoprotection against AFB1 in porcine liver.

The integration of scRNA-seq with spatial transcriptomics, multi-omics approaches, computational modeling of intercellular communication, and targeted validation experiments will provide a systems-level understanding of hepatotoxicity and hepatoprotection. These insights will inform the development of

optimized dietary strategies, precision nutrition approaches, and predictive biomarkers to protect swine from mycotoxin-induced liver injury. Ultimately, this research will contribute to improved animal health, enhanced productivity, and safer food production in the face of persistent mycotoxin contamination challenges.

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