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Multiaxial Biophysical Control of Oncogenic Phase Separation by Indoleamines: A Proof-of-Concept Synthesis of Landscape-Level Regulation

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ABSTRACT

Oncogenic condensates act as biophysical sanctuaries that stabilize malignant survival programs. However, a universal regulator capable of orchestrating the integrated biophysical axes governing cellular phase behavior has remained elusive. Here, we introduce a sovereign singularity framework, presenting a deductive biophysical model that positions the indoleamine melatonin as a master regulator of biological phase separation. A systematic synthesis and integrative bioinformatics analysis were performed to identify the intersection between melatonin-responsive genes and the phase-separation proteome. We identified a core 26-gene regulatory signature—including *AR*, *BCL2*, *CGAS*, *CTNNB1*, *EP300*, *EZH2*, *EGFR*, *IKBK*G (NEMO), *KEAP1*, *KDM1A* (LSD1), *LEF1*, *MYC*, *NANOG*, *PRNP* (PRP^c), *SMAD3*, *SOX9*, *SQSTM1*, *TFEB*, *TFAM*, *TP53*, *TWIST1*, *USP10*, *WWTR1* (TAZ), *VIM*, *YAP1*, and *YTHDF3*—at the intersection of melatonin signaling and condensate architecture. We propose that melatonin utilizes a tri-lever framework of redox tuning (Lever I), multivalent plasticization (Lever II), and dielectric recalibration (Lever III) to render oncogenic programs biophysically untenable. This model provides a mechanical basis for high-resolution regulatory outcomes that modulate the organizational logic of nuclear decision-making (Axis I), state-transition (Axis II), and stress-adaptation (Axis III) condensates. Our results define a strategic platform for disrupting condensate-driven malignancy through the systemic modulation of the cellular biophysical landscape.

1 | Introduction

Phase separation is an evolutionarily conserved thermodynamic process utilized by all tested living organisms across the three major domains of life to organize and sustain fundamental biological processes. Under physiological conditions, phase separation is a reversible process that forms dynamic, membraneless,

micron-scale, liquid-like compartments known as biomolecular condensates (BCs). These condensates can rapidly respond to fluctuating cellular environments. The formation and maintenance of BCs are driven by nonequilibrium thermodynamic conditions arising from the competition between entropy and enthalpy. BCs organize and reorganize cellular biochemistry through the selective inclusion or exclusion of substrates, thereby

Abbreviations: ADCs, antibody-drug conjugates; BCs, biomolecular condensates; CAT, catalase; EMT, epithelial-mesenchymal transition; GO, gene ontology; GSH, glutathione; ICIs, immune checkpoint inhibitors; IDRs, intrinsically disordered regions; LSD1, lysine-specific demethylase 1 A (KDM1A); NEMO, NF-kappa-B essential modulator (IKBK); PhaSepDB, Phase separation database; PPI, protein-protein interaction; PRISMA 2020, Preferred reporting items for systematic reviews and meta-analyses; SG, stress granule; TAZ, transcriptional coactivator with PDZ-binding motif (WWTR1); TF, transcription factor; TME, tumor microenvironment.

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tuning, promoting, or inhibiting cellular functions and reactions [1–3]. Aberrant phase separation that irreversibly transitions fluid condensates into solid aggregates, resulting in the loss of normal cellular functions that provides the biophysical sanctuary for cancer cells is now a major therapeutic target in oncology [4–7]. In addition to mutations, dysregulation of pH and redox balance in the tumor microenvironment (TME) promotes aberrant phase separation that can inhibit tumor-suppressor genes and enhance oncogene expression [8–10]. Intriguingly, cancer cells often sustain an abnormally reduced intracellular redox state to preserve the liquid-like integrity of redox-sensitive condensates that coordinate core transcriptional and epigenetic programs essential for malignancy [11–13]. Pro-oxidative interventions that selectively perturb these condensates can disrupt their oncogenic functions and relieve repression of tumor suppressor genes [14]. In parallel, recent advances show that phase separation modulates the folding and stability of redox-sensitive, non-canonical DNA structures, including G-quadruplexes and i-motifs, which in turn influence gene activation and repression [15–18]. Together, these insights strengthen the emerging rationale for developing cancer therapeutics that target aberrant phase separation.

The biosynthesis of melatonin (N-acetyl-5-methoxytryptamine) is evolutionarily conserved and has been detected in all tested representatives of Bacteria [19], Eukarya [20], and Archaea [21]. These three domains all originated under a largely anoxic atmosphere during early evolution [22]. The identification of the melatonin biosynthetic pathway in Archaea may suggest that the physiological roles of melatonin in early life forms extended beyond its classic antioxidant and pro-oxidant functions [23, 24]. First isolated from the bovine pineal gland in 1958 [25], melatonin is now recognized to be produced predominantly outside the pineal gland, with mitochondrial synthesis in vertebrates estimated to account for more than 95% of total organismal production [26, 27]. Since the first in vivo demonstration of melatonin's anti-tumor effects in 1973 [28], research on melatonin and cancer has expanded substantially across experimental, translational, and clinical domains. Melatonin has been reported to exert multifaceted, both direct and indirect, antitumor effects across a broad spectrum of molecular pathways, including antioxidant and pro-oxidant activities [29, 30], apoptosis and autophagy [31, 32], angiogenesis and metastasis [33, 34], regulation of cell proliferation and cell-cycle arrest [35, 36], epigenetic modification [29, 37], immunomodulation [38], metabolic reprogramming [39–41], modulation of signaling pathways [42–44], and enhanced sensitization to therapy [45, 46]. To date, a universal mechanism that can satisfactorily account for the remarkable versatility of melatonin's protective actions against tumorigenesis has not been elucidated.

The search for a unifying mechanism necessitates a shift toward the fundamental chemical architecture of the molecule. The indoleamine scaffold, of which melatonin is the prototypical representative, possesses unique biophysical attributes that allow it to interact with a vast array of macromolecular assemblies. The amphiphilic nature and “privileged structure” of the indole [47] are intrinsically linked to its ability to engage in redox, multivalent, and electrostatic interactions [48, 49]. By viewing melatonin through the lens of an indoleamine-mediated landscape regulator, we can begin to theorize how it might orchestrate cellular behavior at a scale beyond individual molecular pathways.

Guided by this biophysical perspective, the objective of this integrative systematic review and bioinformatics analysis is to identify genes implicated in human cancer that (1) have experimentally validated roles in biomolecular phase separation and (2) are independently reported to be regulated by melatonin, albeit without prior assessment of their condensate behavior. Substantial conceptual and experimental evidence indicates that melatonin modulates phase-separation dynamics across specific pathological contexts [50–58]. Building on this foundation, we investigate whether the intersection of melatonin-responsive and phase-separation gene sets reveals a potential biophysical basis for regulating condensate functions during tumorigenesis. We further evaluate the relevance of these genes to cancer initiation, progression, and metastasis. Collectively, this work aims to define and characterize genes jointly regulated by phase separation and melatonin and to assess the therapeutic potential of this landscape-level modulation in cancer processes. Accordingly, our integrative study addresses the following questions: which cancer-associated genes are jointly linked to biomolecular phase separation and melatonin regulation, and what functional and therapeutic significance does this convergence imply? The following defines some relevant biophysical concepts used during our investigation:

1.1 | Principles of Phase Separation

Liquid–liquid phase separation (LLPS)—An evolutionarily conserved thermodynamic process wherein a supersaturated macromolecular solution spontaneously demixes into a dense phase and a dilute phase, enabling cells to compartmentalize biochemical processes without lipid membranes.

Biomolecular condensates (BCs)—Fluid, micron-scale macromolecular hubs formed via LLPS that selectively concentrate or exclude specific proteins and nucleic acids to dynamically tune intracellular signaling pathways.

Intrinsically disordered regions (IDRs) — Structural segments of proteins lacking a fixed three-dimensional conformation, whose conformational plasticity and conformational sampling drive the multi-valent interactions required for LLPS.

Critical concentration (C_{crit})—The thermodynamic threshold concentration above which molecules spontaneously undergo phase separation; below (C_{crit}), the components remain homogeneously dissolved in the ambient cytosolic or nuclear fluid.

Active solubility—The capacity of energy-consuming, non-equilibrium cellular processes (such as ATP-dependent enzymatic remodeling) to maintain macromolecular components in a fluid, dissolved state, preventing pathological condensation even when concentrations exceed the baseline (C_{crit}).

1.2 | Structural and Thermodynamic Drivers (Levers I and II)

Thermodynamic pressure—The net driving force—compounded by high protein abundance, macromolecular crowding, and localized metabolic alterations—that shifts the cellular system toward spontaneous phase separation.

Molecular crowders—Inert or active macromolecules and highly polar metabolites that occupy physical solvent volume, reducing the available free space for other proteins and thereby thermodynamically favoring phase transitions.

Multivalent interactions—Cooperative, non-covalent intermolecular cross-linking between repetitive structural motifs that provides the structural integrity and network persistence of the dense phase.

π - π and cation- π interactions—Electrostatic interactions involving the delocalized electron clouds of aromatic amino acid residues (e.g., tyrosine, tryptophan, phenylalanine) that supply the transient, multivalent “stickiness” driving IDR-mediated gelation and scaffold maturation.

1.3 | Electrostatic and Microenvironmental Dynamics (Lever III)

Electrostatic shielding—A biophysical phenomenon wherein localized charge-neutralization inside a dense phase establishes an insulated bio-electrochemical microenvironment, shielding the condensate's interior from ambient ionic changes.

Solvation environment—The distinct local dielectric and hydration properties characterizing the interior of a condensate, which can fundamentally alter the ionization states and biochemical reactivity of partitioned biomolecules.

Proton trap—An active microenvironmental anomaly wherein a condensate maintains an autonomous internal pH gradient at steady state, effectively decoupling its internal chemistry from the increasingly acidic tumor microenvironment (TME).

Static dielectric constant (ϵ)—A macroscopic measure of a medium's relative permittivity or electrical polarizability. Shifting the local ϵ within a dense phase alters the Coulombic forces governing the electrochemical gradients that stabilize oncogenic sanctuaries.

1.4 | Methodological Framework

Mesoscale organization—The spatial arrangement of macromolecular assemblies at a scale intermediate between individual atomic structures and macroscopic cellular organelles (typically 10 to 1000 nm).

Bulk quantification—Standard analytical modalities (e.g., bulk transcriptomics or proteomics) that resolve net cellular abundance but remain fundamentally agnostic to spatial, mesoscale partitioning.

2 | Methods

This systematic review and bioinformatics analysis was conducted in accordance with the preferred reporting items for systematic reviews and meta-analyses (PRISMA) 2020 guidelines [59]. A comprehensive overview of the integrated systematic review and bioinformatics pipeline—including specific

objectives, software versions, and analytical parameters for each stage—is summarized sequentially in Table 1.

2.1 | Data Sources and Search Strategy

A systematic search of PubMed and Web of Science was conducted in April 2025 using predefined search terms (“melatonin AND cancer” and “phase separation AND cancer”). The initial search was carried out by one author, and all authors reviewed and approved the final set of studies included and excluded from the review.

2.2 | Data Collection, Study Screening, and Selection Criteria

All retrieved records were imported into Rayyan [60], a web-based application that facilitates efficient systematic and other literature reviews, for screening and deduplication. A total of 14,090 references were imported into Rayyan. Rayyan flagged 7099 potential duplicates; 3596 duplicate records were deleted prior to screening, 3492 duplicate clusters were resolved (retaining one record per cluster), and 11 flagged records were judged to be not duplicates. After deletion, 10,494 records remained and proceeded to title/abstract screening.

Studies were excluded if they were as follows: (i) review articles, (ii) clinical trials, (iii) studies involving adjuvants or co-treatments administered in addition to melatonin or (iv) studies with irrelevant topics. Studies were included if they met either of the following criteria: (i) investigation of phase separation of genes in cancer, or (ii) investigation of genes modulated by melatonin in cancer.

After screening and full-text assessment, 462 articles remained for detailed review. Of these, 207 studies met all inclusion criteria and were retained for analysis, while 255 were excluded. No conflicts or unresolved decisions remained following screening. The study selection process is summarized in the PRISMA 2020 flow diagram (Figure 1).

2.3 | Bioinformatics Analysis

To check for shared genes between our manual search and those listed in the Phase Separation Database (<https://db.phasep.pro/>, accessed in October 2025), we used a Venn diagram [61]. Briefly, Phase Separation Database (PhaSepDB) provides detailed information on proteins and biomolecules involved in liquid-liquid phase separation, helping to advance the understanding of cellular organization and the role of LLPS in various biological processes and diseases [62].

Next, a protein-protein interaction (PPI) network was constructed utilizing the STRING database (Version 12.5; RRID:SCR_005223; <https://string-db.org/>, accessed October 2025). Functional enrichment analysis was performed within the STRING platform to identify significantly represented Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways and Gene Ontology (GO) terms (biological process, molecular function, and cellular component) associated with the shared

TABLE 1 | Comprehensive overview of the integrated systematic review and bioinformatics pipeline.

Pipeline stage	Objective	Software/database (version)	Inputs/key parameters
Systematic Literature Search	Identify relevant phase separation and melatonin-responsive genes in cancer	PubMed, Web of Science	Date: April 2025 Search terms: “melatonin AND cancer”; “phase separation AND cancer” Guidelines: PRISMA 2020
Study Screening & Selection	Deduplication and criteria-based screening	Rayyan web-based application	Inclusion criteria: Investigation of phase separation of genes in cancer OR investigation of genes modulated by melatonin in cancer
Phase Separation Validation	Confirm functional roles of identified genes in biomolecular condensates	PhaSepDB (Phase Separation Database)	Curated annotations of phase separation-prone molecules via Venn diagram overlap
Protein-Protein Interaction (PPI)	Construct molecular interaction network	STRING database (Version 12.5)	Input: Shared gene set Confidence Score: 0.900 (highest confidence) Sources: Text mining, experimental evidence, curated databases, co-expression
Functional Enrichment Analysis	Identify associated pathways and ontologies	STRING database, MSigDB, R Studio (clusterProfiler, Enrichr)	Pathways: KEGG, Reactome, WikiPathways Ontologies: Biological process, molecular function, cellular component Significance: FDR < 0.05
Network Visualization	Visualize PPI, miRNA-gene networks, and functional co-occurrence	Cytoscape (Version 3.10.4)	Customized mapping of regulatory nodes and functional clusters
Data Visualization (Plots/Enrichment)	Generate functional enrichment plots	R Studio (ggplot2 package, version 4.0.0)	Odds ratios and FDR-adjusted significance
Transcription Factor (TF) Mapping	Identify upstream TFs regulating the shared gene set	TRRUST v.2, X2Kweb	Direct mapping of TFs to target genes
Regulatory Axis Mapping	Illustrate connections between TFs, genes, functional effects, and cancer types	R Studio (ggalluvial package)	Generated multi-layered alluvial diagram
miRNA-Gene Regulatory Network	Predict microRNAs targeting core cancer-related genes	R Studio (multiMiR package)	Parameters: target = genes, org = “hsa”, table = “validated”, predicted.cutoff.type = “p”, predicted.cutoff = 20 (top 20%), predicted.site = “conserved”, summary = T Filter: Only miRNAs validated by Luciferase Reporter Assay
Clinical Survival Analysis	Evaluate prognostic significance of the 26-gene signature	GEPIA repository (TCGA datasets)	Evaluation: Overall survival rates correlating high versus low gene expression risks

gene set. To analyze and visualize the underlying biological mechanisms, we utilized the STRING database to construct a protein-protein interaction network. To isolate a robust regulatory core, a stringency threshold of 0.900 (highest confidence) was applied across a comprehensive set of interaction sources, including text mining, experimental evidence, curated databases, and co-expression data [63]. Cytoscape (version 3.10.4; RRID:SCR_003032; cytoscape.org) [64], was used to visualize the PPI and microRNA–gene regulatory networks, and functional co-occurrence.

GO and pathway enrichment analyses were visualized using the ggplot2 package in R (version 4.0.0) [65]. Transcription factors

(TFs) associated with the gene set were identified using TRRUST v.2 (www.grnpedia.org/trrust/) [66] and X2Kweb (maayanlab.cloud, accessed October 2025) [67]. The relationships among TF, genes and types of cancer were illustrated through an alluvial diagram, generated with the ggalluvial R Studio package [68]. To search for miRNA-related genes involved in phase-separation, the multiMiR R studio package was used [69]. For this analysis, the parameters were as follows: target = genes, org = “hsa”, table = “validated”, predicted.cutoff.type = “p”, predicted.cutoff = 20 (representing the top 20% of predicted targets), predicted.site = “conserved”, summary = T. Only miRNAs validated by Luciferase Reporter Assay were selected for further analysis. The overall survival data were retrieved from the GEPIA

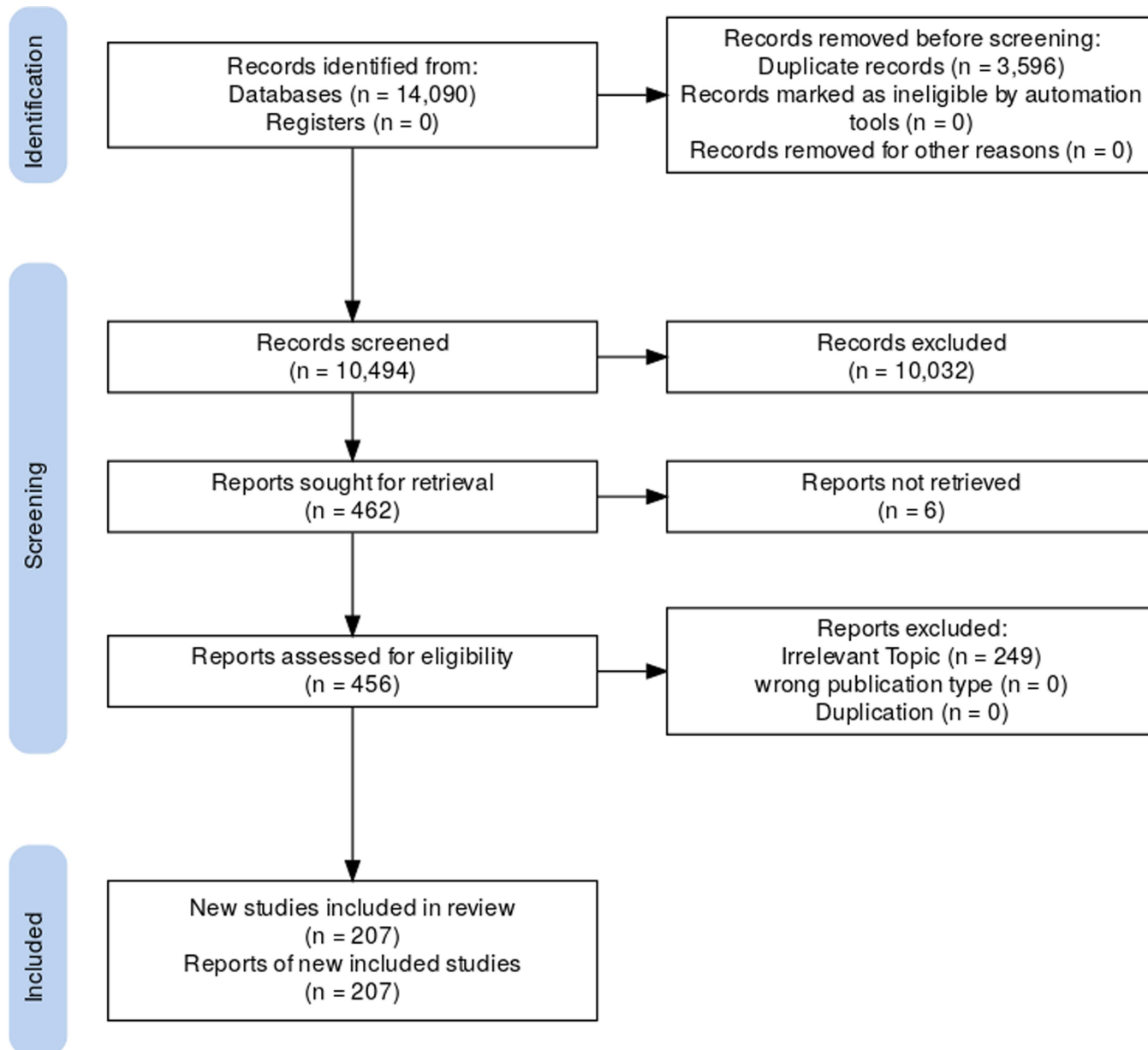


FIGURE 1 | PRISMA 2020 flow diagram for study selection.

repository (RRID:SCR_026154; <http://gepia2.cancer-pku.cn/>, accessed in November 2025).

3 | Results

3.1 | Melatonin Potentially Alters Phase Separation-Related and Cancer-Associated Genes, Suppressing Major Oncogenic Pathways

After a rigorous screening of the included studies, we identified a total of 121 phase separation-related genes across different biological contexts (Table 2). To better understand the roles of genes associated with phase separation, we first validated this gene set using PhaSepDB, a comprehensive repository providing curated annotations on phase separation-prone molecules including intrinsic determinants, functional relevance, and extrinsic regulatory factors. Of the 121 phase separation-

associated genes, 82 were confirmed in PhaSep database (Figure 2A). Pathway enrichment analysis revealed that these genes were predominantly associated with Wnt/ β -catenin signaling, Hippo signaling regulation, Extracellular vesicle-mediated signaling in recipient cells (Wiki pathway). Additional enriched pathways included transcriptional regulation by *RUNX3* and *SMAD2/3/4*-mediated transcriptional regulation (Reactome) (Figure 2B). Gene Ontology analysis further demonstrated enrichment of biological processes such as regulation of RNA biosynthesis and DNA-templated transcription, and regulation of epithelial-mesenchymal transition (EMT). The main molecular functions represented included transcription coregulator binding, cis-regulatory region binding, and nuclear receptor binding. These genes were notably localized to key cellular compartments involved in RNA and protein dynamics, including the nucleus, P-bodies, cytoplasmic stress granules, and intracellular membrane-bound organelle (Figure 2C).

TABLE 2 | Integrated bioinformatic synthesis: overlap analysis of phase separation, melatonin, and PhaSepDB-validated genes.

Gene symbol	Phase separation curated (<i>n</i> = 121)	Melatonin curated (<i>n</i> = 134)	PhaSepDB supported (<i>n</i> = 82)	PhaSepDB + melatonin overlap (<i>n</i> = 26)
<i>ABCB1</i>	N	Y	N	N
<i>ABCB5</i>	N	Y	N	N
<i>ABCC1</i>	N	Y	N	N
<i>ABCC5</i>	N	Y	N	N
<i>ADAM10</i>	N	Y	N	N
<i>ADGRL4</i>	N	Y	N	N
<i>AHNAK</i>	Y	N	N	N
<i>AIF1</i>	N	Y	N	N
<i>AKAP8</i>	Y	N	Y	N
<i>AKAP95</i>	Y	N	N	N
<i>AKR1C1</i>	N	Y	N	N
<i>AKR1C2</i>	N	Y	N	N
<i>AKT</i>	N	Y	N	N
<i>ALK</i>	Y	N	Y	N
<i>ALKBH5</i>	Y	N	Y	N
<i>APOD</i>	N	Y	N	N
<i>AR</i>	Y	Y	Y	Y
<i>ARID1A</i>	Y	N	Y	N
<i>ARV7</i>	Y	N	N	N
<i>ASPM</i>	Y	N	Y	N
<i>ATG4B</i>	Y	N	N	N
<i>ATM</i>	Y	N	N	N
<i>AXIN</i>	Y	N	N	N
<i>BACE1</i>	N	Y	N	N
<i>BAX</i>	N	Y	N	N
<i>BCL2</i>	Y	Y	N	Y
<i>BMAL1</i>	N	Y	N	N
<i>BRD1</i>	Y	N	Y	N
<i>BRD4</i>	Y	N	Y	N
<i>C9orf153</i>	N	Y	N	N
<i>CAPRIN1</i>	Y	N	Y	N
<i>CCL12</i>	N	Y	N	N
<i>CCL24</i>	N	Y	N	N
<i>CCNB1</i>	N	Y	N	N
<i>CCND1</i>	N	Y	N	N
<i>CD274</i>	N	Y	N	N
<i>CDK1</i>	N	Y	N	N
<i>CDK2</i>	Y	Y	N	N
<i>CDK4</i>	N	Y	N	N
<i>CDKI</i>	Y	N	N	N
<i>CEBPA</i>	Y	N	Y	N
<i>CES1</i>	N	Y	N	N
<i>CGAS</i>	Y	Y	Y	Y
<i>CKAP4</i>	Y	N	Y	N
<i>CNBP</i>	Y	N	Y	N

(Continues)

TABLE 2 | (Continued)

Gene symbol	Phase separation curated (n = 121)	Melatonin curated (n = 134)	PhaseSepDB supported (n = 82)	PhaseSepDB + melatonin overlap (n = 26)
<i>CPSF6</i>	Y	N	Y	N
<i>CREBBP</i>	N	Y	N	N
<i>CRY1</i>	N	Y	N	N
<i>CRY2</i>	N	Y	N	N
<i>CTLA2A</i>	N	Y	N	N
<i>CTNNB1</i>	Y	Y	Y	Y
<i>CXCL2</i>	N	Y	N	N
<i>CYBA</i>	N	Y	N	N
<i>CYP11A1</i>	N	Y	N	N
<i>DACT1</i>	Y	N	Y	N
<i>DAZAP1</i>	Y	N	N	N
<i>DDX21</i>	Y	N	Y	N
<i>DKFZp566F0947</i>	N	Y	N	N
<i>DNAH12</i>	N	Y	N	N
<i>DNMT1</i>	N	Y	N	N
<i>DSG2</i>	N	Y	N	N
<i>EEF1E1</i>	Y	N	Y	N
<i>EGFL7</i>	N	Y	N	N
<i>EGFR</i>	Y	Y	Y	Y
<i>EML4</i>	Y	N	Y	N
<i>EP300</i>	Y	Y	Y	Y
<i>EPHA2</i>	Y	N	Y	N
<i>EREG</i>	N	Y	N	N
<i>ESM1</i>	N	Y	N	N
<i>EWS</i>	Y	N	N	N
<i>EZH2</i>	Y	Y	Y	Y
<i>F2RL1</i>	N	Y	N	N
<i>FGF19</i>	N	Y	N	N
<i>FGFR4</i>	N	Y	N	N
<i>FOSL1</i>	Y	Y	N	N
<i>FOXM1</i>	Y	N	Y	N
<i>FOXO1</i>	N	Y	N	N
<i>FOXP1</i>	Y	N	Y	N
<i>FSP1</i>	Y	N	N	N
<i>FUBP3</i>	Y	N	Y	N
<i>FUS</i>	Y	N	Y	N
<i>GAGE12D</i>	N	Y	N	N
<i>GALR3</i>	N	Y	N	N
<i>GATA2</i>	N	Y	N	N
<i>GIRGL</i>	Y	N	N	N
<i>HAS3</i>	N	Y	N	N
<i>HDAC4</i>	N	Y	N	N
<i>HDAC6</i>	Y	N	Y	N
<i>HDAC9</i>	N	Y	N	N
<i>HIF1</i>	N	Y	N	N

(Continues)

TABLE 2 | (Continued)

Gene symbol	Phase separation curated (<i>n</i> = 121)	Melatonin curated (<i>n</i> = 134)	PhaseSepDB supported (<i>n</i> = 82)	PhaseSepDB + melatonin overlap (<i>n</i> = 26)
<i>HIF1A</i>	N	Y	N	N
<i>HIST1H2AB</i>	N	Y	N	N
<i>HIST1H2BM</i>	N	Y	N	N
<i>HNRNPK</i>	Y	N	Y	N
<i>HOXA9</i>	Y	N	Y	N
<i>HSD17B3</i>	N	Y	N	N
<i>HSPB3</i>	N	Y	N	N
<i>IFIT3</i>	N	Y	N	N
<i>IGF2BP1</i>	Y	N	Y	N
<i>IKBK*G</i>	N	Y	N	Y
<i>IL1F6</i>	N	Y	N	N
<i>IRF1</i>	Y	N	Y	N
<i>KAT6A</i>	Y	N	Y	N
<i>KAT8</i>	Y	N	Y	N
<i>KDM1A (LSD1)</i>	Y	Y	N	Y
<i>KEAP1</i>	Y	Y	Y	Y
<i>KISS1</i>	N	Y	N	N
<i>KMT2D</i>	Y	N	Y	N
<i>KRAS</i>	Y	N	Y	N
<i>KRT1</i>	N	Y	N	N
<i>KRT23</i>	N	Y	N	N
<i>LAMB3</i>	N	Y	N	N
<i>LATS1</i>	Y	N	Y	N
<i>LEF1</i>	Y	Y	Y	Y
<i>LHB</i>	N	Y	N	N
<i>LRRC10B</i>	N	Y	N	N
<i>LRRC32</i>	N	Y	N	N
<i>LRRN4</i>	N	Y	N	N
<i>MAZ</i>	Y	N	Y	N
<i>MDK</i>	N	Y	N	N
<i>MET</i>	Y	N	N	N
<i>MLLT1</i>	Y	N	Y	N
<i>MMP13</i>	N	Y	N	N
<i>MMP2</i>	N	Y	N	N
<i>MMP3</i>	N	Y	N	N
<i>MMP9</i>	N	Y	N	N
<i>MOAP1</i>	Y	N	Y	N
<i>MST1</i>	Y	N	N	N
<i>MST2</i>	Y	N	N	N
<i>MTHFD1L</i>	N	Y	N	N
<i>MTN3</i>	Y	N	N	N
<i>MTNR1A</i>	N	Y	N	N
<i>MTNR1B</i>	N	Y	N	N
<i>MYC</i>	Y	Y	Y	Y
<i>MYL4</i>	N	Y	N	N

(Continues)

TABLE 2 | (Continued)

Gene symbol	Phase separation curated (<i>n</i> = 121)	Melatonin curated (<i>n</i> = 134)	PhaseSepDB supported (<i>n</i> = 82)	PhaseSepDB + melatonin overlap (<i>n</i> = 26)
<i>NANOG</i>	Y	Y	Y	Y
<i>NAT1</i>	Y	N	N	N
<i>NBR1</i>	Y	N	Y	N
<i>NEMO</i>	Y	Y	N	N
<i>NF2</i>	Y	N	Y	N
<i>NONO</i>	Y	N	Y	N
<i>NOP53</i>	Y	N	Y	N
<i>NOTCH1</i>	N	Y	N	N
<i>NR4A1</i>	Y	N	Y	N
<i>NRAS</i>	Y	N	N	N
<i>NTRK</i>	Y	N	N	N
<i>NUMB</i>	N	Y	N	N
<i>NUP98</i>	Y	N	Y	N
<i>OCLN</i>	N	Y	N	N
<i>OCT4</i>	N	Y	N	N
<i>PABPN1</i>	Y	N	Y	N
<i>PCYT1B</i>	N	Y	N	N
<i>PENK</i>	N	Y	N	N
<i>PER1</i>	N	Y	N	N
<i>PER2</i>	N	Y	N	N
<i>PIK3CA</i>	N	Y	N	N
<i>PML</i>	Y	N	Y	N
<i>PRDX1</i>	Y	N	N	N
<i>PRKAR1A</i>	Y	N	Y	N
<i>PRKCA</i>	N	Y	N	N
<i>PRMT6</i>	Y	N	N	N
<i>PRNP (PRP^C)</i>	Y	Y	N	Y
<i>PS1</i>	N	Y	N	N
<i>PTK6</i>	Y	N	N	N
<i>RAD51</i>	N	Y	N	N
<i>RAD51-AS1</i>	N	Y	N	N
<i>RAD52</i>	Y	N	Y	N
<i>RAP80</i>	Y	N	N	N
<i>RARA</i>	Y	N	Y	N
<i>RB1CC1</i>	Y	N	Y	N
<i>RBM14</i>	Y	N	Y	N
<i>RGS20</i>	N	Y	N	N
<i>RNF168</i>	Y	N	N	N
<i>ROCK1</i>	N	Y	N	N
<i>ROCK2</i>	N	Y	N	N
<i>RORA</i>	N	Y	N	N
<i>RUNX1-IT1</i>	Y	N	N	N
<i>SENS2</i>	Y	N	N	N
<i>SERPINB7</i>	N	Y	N	N
<i>SFPQ</i>	Y	N	Y	N

(Continues)

TABLE 2 | (Continued)

Gene symbol	Phase separation curated (<i>n</i> = 121)	Melatonin curated (<i>n</i> = 134)	PhaseSepDB supported (<i>n</i> = 82)	PhaseSepDB + melatonin overlap (<i>n</i> = 26)
<i>SHP2</i>	Y	N	N	N
<i>SIRT1</i>	N	Y	N	N
<i>SIRT3</i>	N	Y	N	N
<i>SMAD2</i>	Y	N	Y	N
<i>SMAD3</i>	Y	Y	Y	Y
<i>SMAD4</i>	Y	N	Y	N
<i>SNAI1</i>	N	Y	N	N
<i>SOX2</i>	N	Y	N	N
<i>SOX9</i>	Y	Y	N	Y
<i>SP1</i>	Y	N	Y	N
<i>SPIN1</i>	Y	N	Y	N
<i>SQSTM1</i>	Y	Y	Y	Y
<i>SQSTM2</i>	Y	N	N	N
<i>SRC-3</i>	Y	N	N	N
<i>SRSF9</i>	Y	N	N	N
<i>STARD4</i>	N	Y	N	N
<i>STAT3</i>	N	Y	N	N
<i>STK31</i>	N	Y	N	N
<i>SYT8</i>	N	Y	N	N
<i>TAF15</i>	Y	N	Y	N
<i>TFAM</i>	Y	Y	Y	Y
<i>TFEB</i>	Y	Y	Y	Y
<i>TGFB1</i>	N	Y	N	N
<i>TNFAIP8L2</i>	N	Y	N	N
<i>TP53</i>	Y	Y	Y	Y
<i>TP53BP1</i>	Y	N	Y	N
<i>TRIM26</i>	N	Y	N	N
<i>TWIST1</i>	Y	Y	Y	Y
<i>USP10</i>	Y	Y	Y	Y
<i>USP39</i>	Y	N	Y	N
<i>USP42</i>	Y	N	Y	N
<i>UTX</i>	Y	N	N	N
<i>VEGF</i>	N	Y	N	N
<i>VEGFA</i>	N	Y	N	N
<i>VIM</i>	Y	Y	Y	Y
<i>WWTR1 (TAZ)</i>	Y	Y	Y	Y
<i>YAP1</i>	Y	Y	Y	Y
<i>YBX1</i>	Y	N	Y	N
<i>YTHDC1</i>	Y	N	Y	N
<i>YTHDF2</i>	Y	N	Y	N
<i>YTHDF3</i>	Y	Y	Y	Y
<i>YY1</i>	Y	N	Y	N
<i>ZHX2</i>	Y	N	N	N
<i>ZMAT2</i>	Y	N	Y	N
<i>ZNF252P</i>	Y	N	N	N

(Continues)

TABLE 2 | (Continued)

Gene symbol	Phase separation curated (n = 121)	Melatonin curated (n = 134)	PhaseSepDB supported (n = 82)	PhaseSepDB + melatonin overlap (n = 26)
<i>ZNF503-AS1</i>	N	Y	N	N
<i>ZO-1</i>	N	Y	N	N

*“Y” indicates the gene is present in the specified dataset; “N” indicates it was not identified in that specific category. Overlap (n = 26) denotes genes validated by PhaSepDB that are also modulated by phase separation and melatonin. *Note on *IKBK*G (NEMO) regulation: While the majority of the oncogenic core is downregulated, the observed upregulation of *IKBK*G reflects a precision shift in alternative splicing favoring the tumor-suppressive NEMO-L isoform (exon 5 inclusion), as detailed in Section 4.5.3.

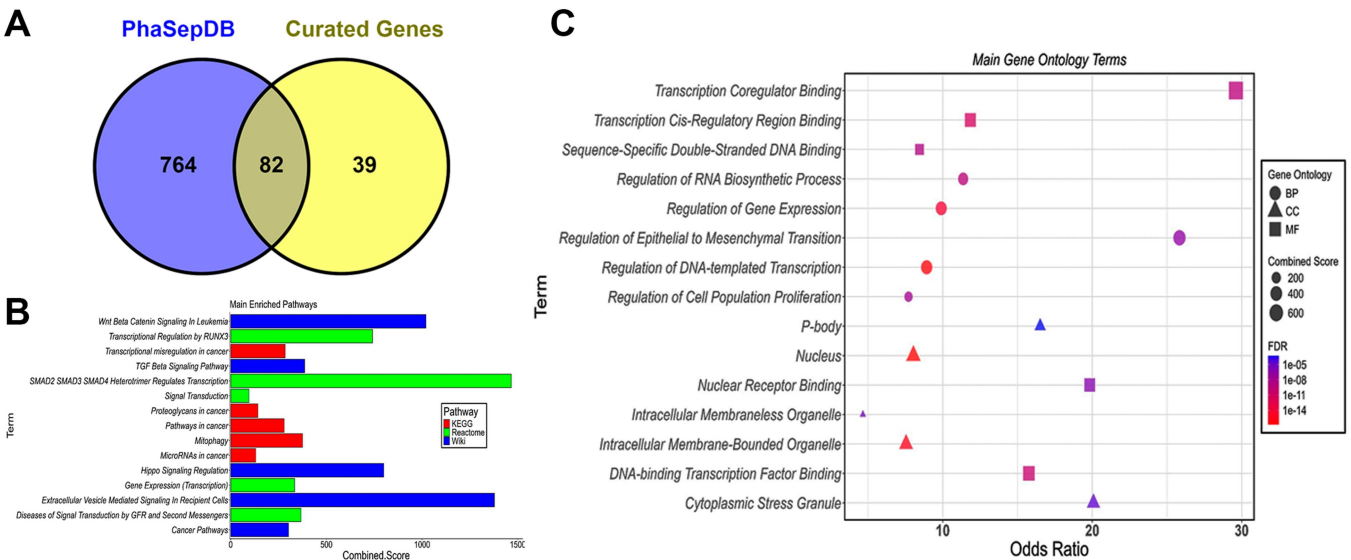


FIGURE 2 | Specific genes involved with phase-separation and functional pathway enrichment. (A) Venn diagram showing the overlap between curated genes and PhaSepDB entries. (B) Pathway enrichment analysis of the overlapping gene set using KEGG, Reactome, and WikiPathways. Combined scores indicate the strength of pathway enrichment across databases. (C) Gene Ontology (GO) enrichment analysis highlighting major biological processes (BP), cellular components (CC), and molecular functions (MF) associated with the intersecting genes. Terms with the highest odds ratios include transcriptional regulation, epithelial-to-mesenchymal transition, RNA biosynthetic processes, DNA-binding transcription factor activity, and localization to intracellular membrane-bounded organelles, P-bodies, and stress granules. Dot size represents combined enrichment score, while color indicates FDR significance. Functional enrichment was performed using the R package “clusterProfiler” or “Enrichr”, with p-values adjusted for multiple testing using the Benjamini-Hochberg False Discovery Rate (FDR) method ($q < 0.05$).

Through a systematic synthesis of high-confidence differentially expressed genes (DEGs) across diverse neoplastic models, we identified a consensus set of 134 conserved regulatory nodes significantly modulated by melatonin. Among them, 33 genes were upregulated in at least ten cancer models, predominantly linked to apoptosis, the p53 pathway, Wnt/ β -catenin signaling, and TNF- α signaling (MSigDB [70]; FDR < 0.05). In contrast, 103 genes were downregulated, and these were associated with apoptosis, the G2-M checkpoint, KRAS signaling, PI3K/AKT/mTOR signaling, apical junctions, hypoxia, EMT transition, and glycolysis (MSigDB; FDR < 0.05) (Table 2). Notably, two genes within the 134-gene set (*MTNR1A* and *MTNR1B*) did not exhibit standard directional regulation in the context of these oncogenic models, but were retained due to their foundational role in melatonin signaling. Network analysis revealed 23 genes with high-confidence molecular interactions (score = 0.9) (Figure 3A). These genes were mostly localized to the nucleoplasm, nucleus, organelle lumen, membrane-bounded and non-membrane bounded organelles, cytosol, chromosome, survivin complex, extracellular vesicles, among others. Our network analysis revealed a subset of high-confidence interacting genes,

localized to key cellular compartments, that are significantly modulated by melatonin.

3.2 | Integration of Melatonin-Responsive and Phase-Separation Genes Reveals Key Regulatory Networks in Cancer

By independently analyzing molecules involved in phase separation and those modulated by melatonin, we identified genes at the intersection of both processes to uncover potential functional links in cancer. A total of 26 genes—including *AR*, *BCL2*, *CGAS*, *EGFR*, *KEAP1*, *EZH2*, *KDM1A* (LSD1), *YAP1*, *SQSTM1*, *SMAD3*, *LEF1*, *MYC*, *IKBK*G (NEMO), *TP53*, *PRNP* (PRP^C), *SOX9*, *WWTR1* (TAZ), *NANOG*, *TFAM*, *TFEB*, *TWIST1*, *EP300*, *USP10*, *VIM*, *YTHDF3*, and *CTNBN1*—were shared between the two datasets, with most being downregulated by melatonin in cancer. Importantly, the vast majority of these shared oncogenic drivers are significantly downregulated by melatonin in cancer models, with the notable exception of the overexpressed tumor suppressor *TP53* and the adapter *IKBK*G

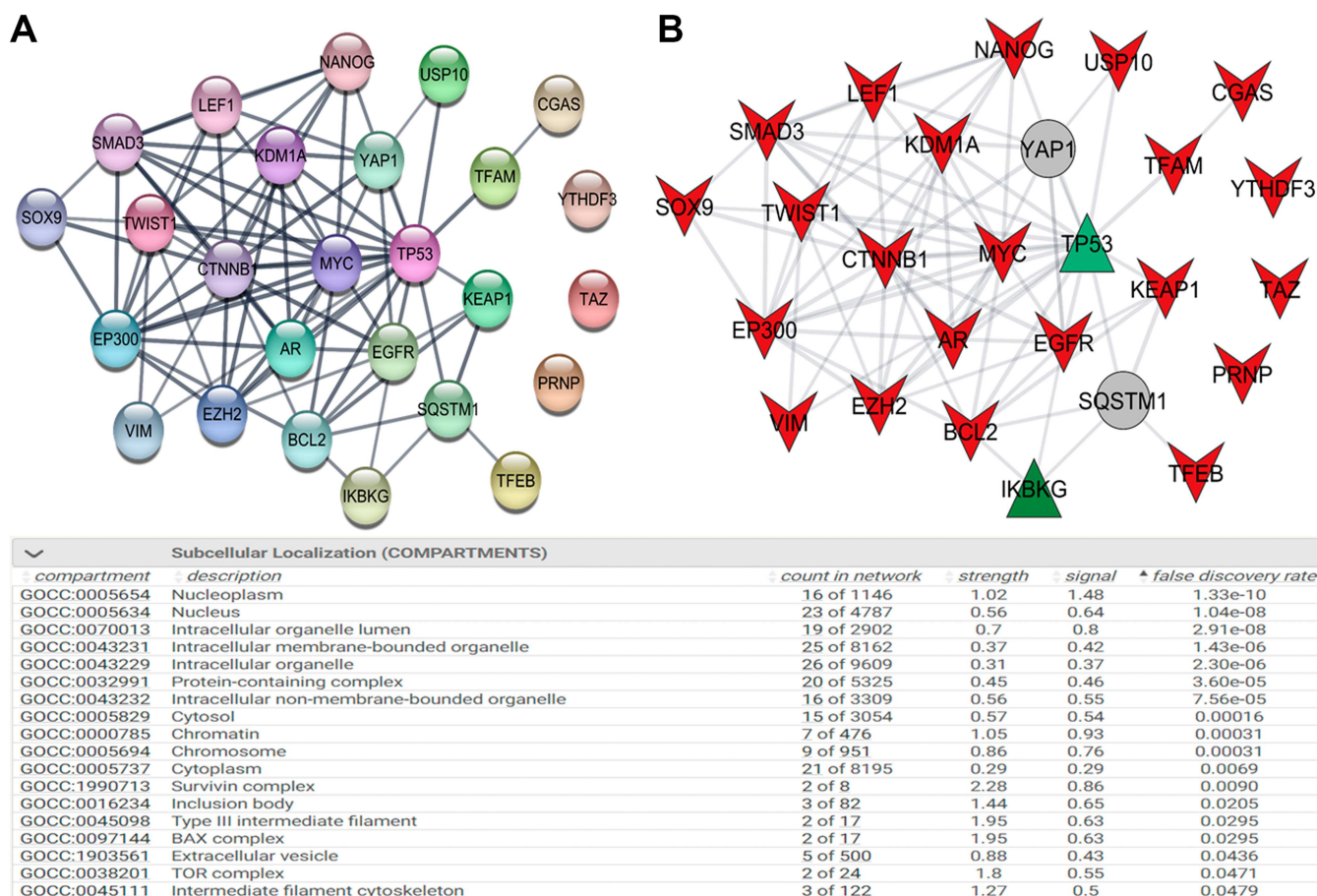


FIGURE 3 | Interaction networks and subcellular enrichment analysis of phase-separation- and melatonin-related genes in cancer (A) STRING-derived interaction network showing the connectivity among selected genes involved in phase separation and responsive to melatonin. Node colors represent distinct functional clusters, and edge thickness indicates interaction confidence. (B) Network highlighting common genes and its expression direction. Red and green nodes indicate respectively downregulation and upregulation by melatonin, and gray nodes denote absence of expression modulation. Lower panel: Enrichment table listing the most significantly overrepresented subcellular compartments among the gene set, including nucleoplasm, cytosol, chromatin, and intracellular organelles, with counts, enrichment strength, signal values, and false discovery rates.

(NEMO)—the latter correlating with a biophysical steering of RNA processing toward the protective NEMO-L isoform rather than broad-spectrum NF- κ B activation. We further mapped these genes to their corresponding transcription factors (TFs), cellular effects, and associated cancer types. As shown in Figure 4A, 18 TFs were directly linked to these targets. Notably, *MYC*-, *TP53*-, *BCL2*-, *EGFR*-, and *VIM*-related regulatory networks were prominently represented, connecting to antitumor effects, apoptosis, proliferation, chemoresistance, and metastatic processes in breast, gastric, and liver cancers, as well as glioblastoma.

We next clustered melatonin-regulated phase separation genes based on functional co-occurrence, revealing their involvement across interconnected pathways (Figure 4B). Among these, *LSD1*, *EP300*, *CGAS*, and *EZH2* genes were enriched in epigenetic regulation, while *TFAM* was associated with mitochondrial functions and *WWTR1* (*TAZ*) with Hippo-mediated transcriptional regulation. To examine microRNA-gene regulatory interactions, we integrated predictions from three databases, identifying miRNAs targeting core cancer-related genes suppressed by melatonin. For instance, miR-34a-5p, miR-15a-5p, miR-15b-5p uniquely targeted *BCL2* gene; miR-16-5p

targeted both *BCL2* and *TP53* genes; miR-7-5p targeted *EGFR* and *BCL2* genes; and miR-17-5p targeted *VIM* and *BCL2*. In addition, *EZH2* gene was linked to several miRNAs (miR-101-3p, -26a-5p, and -124-3p) whereas *MYC* was associated with miR-145-5p and miR-24-3p (Figure 4C).

To assess clinical significance, we evaluated overall survival of patients using TCGA datasets [71] for high and low risks considering melatonin- and phase separation-related genes. Higher expression levels of specific genes correlated with poorer survival in breast cancer ($p = 0.038$, HR = 1.4) and gastric cancer ($p = 0.028$, HR = 1.4), whereas low *MYC* expression was associated with reduced survival rate in low-grade glioma, underscoring the prognostic relevance of these regulatory genes.

4 | Discussion

Recent advances in the study of phase separation have revealed fundamental organizing principles of biological systems. This rapidly expanding literature has developed largely independently of melatonin [72–74]. Here, we present a synthesis of the extensive evidence documenting the pleiotropic anticancer

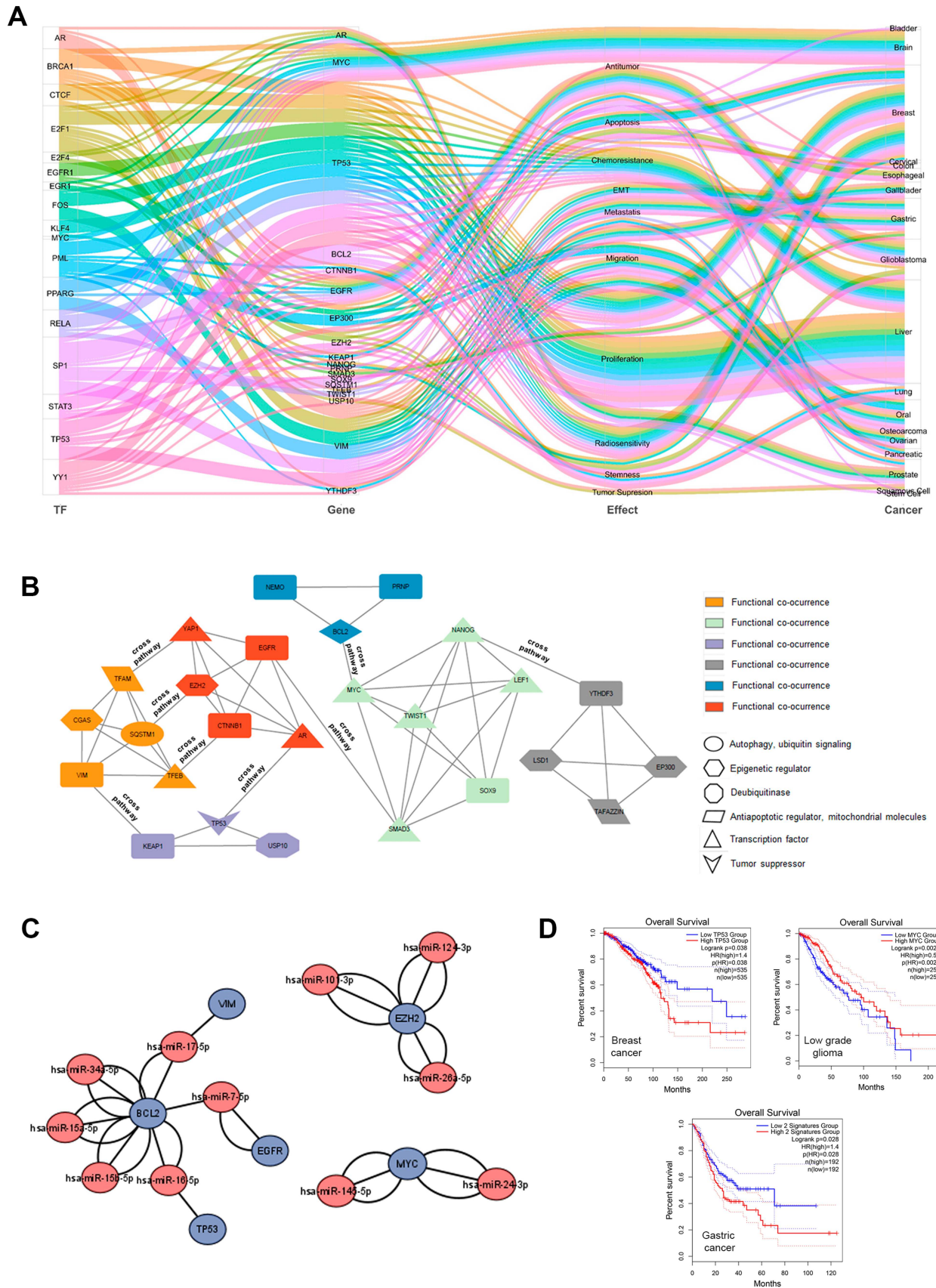


FIGURE 4 | Legend on next page.

effects of melatonin on an integrative framework in which phase separation provides a physical basis for melatonin-regulated cancer networks.

To facilitate the interpretation of our results, the 26 identified genes are organized into three functional phase-separation axes that reflect predominant cellular roles rather than exclusive molecular functions. Each gene is assigned to a single phase-separation axis based on its primary condensate-associated role. Importantly, all axes assignments reflect the primary organizational logic of the condensate, not the full spectrum of downstream cellular outcomes. Notably, miRNA regulation converged on all three axes, preferentially targeting *MYC*, *EZH2*, *BCL2*, *EGFR*, *TP53*, and *VIM*—genes that anchor phase-separated hubs across nuclear, signaling, and stress-response condensates.

Conceptual Overview (Sections 4.1-4.3): Cancer cells exploit biomolecular condensates—fluid, droplet-like hubs—to ensure their survival across three distinct functional arenas, driven by the 26-gene signature identified in our analysis. Axis I condensates act as nuclear command centers, orchestrating gene expression to drive cancer progression. Axis II condensates function as signaling relay stations, helping cells transition between different states to invade or resist treatment. Finally, Axis III condensates serve as emergency bunkers, buffering the cancer cell against intense environmental stress and preventing irreversible collapse.

4.1 | Axis I: Transcriptional Condensates That Drive Cellular Decision-Making

Nuclear decision-making condensates facilitate genome organization and gene-expression regulation by concentrating specific proteins and nucleic acids within biomolecular condensates. These condensates function as nuclear decision-making hubs that govern fundamental cellular choices—such as proliferation, differentiation, and cell-fate commitment—by dynamically enhancing or suppressing transcriptional programs. The assembly and dissolution of these condensates are tightly coupled to key cellular decisions during development and in response to environmental or genotoxic cues [75, 76].

All genes assigned to Axis I—including *MYC*, *TP53*, *AR*, *KDM1A* (LSD1), *NANOG*, *SOX9*, *EP300*, *EZH2*, *LEF1*, *SMAD3*, and *YTHDF3*—contain intrinsically disordered regions (IDRs)

that enable the formation of phase-separated condensate hubs within the nucleus. These hubs often localize to specific genomic regions, such as super-enhancers, where they coordinate transcriptional output. Within the nucleus, proteins such as *EZH2*, *LEF1*, *SMAD3*, and *YTHDF3* are frequently recruited into Axis I condensates as regulatory or signaling intermediates. This recruitment enables the integration of upstream signaling inputs into transcriptional outcomes that determine cellular responses, including DNA repair or programmed cell death [13, 77–80].

4.2 | Axis II: Signal Integration Condensates That Orchestrate Cell Reprogramming

Signal integration and state transition condensates comprise assemblies that integrate mechanical, biochemical, and spatial signals to drive discrete transitions between cellular states. Canonical examples include YAP/TAZ-, β -catenin-, and EGFR-associated condensates, which act as molecular decision hubs that convert graded extracellular and intracellular inputs into switch-like transcriptional and phenotypic programs such as proliferation, differentiation, epithelial–mesenchymal transition (EMT), or quiescence [5, 81]. Structural and transcriptional modulators such as *VIM* and *TWIST1* have been reported to couple cytoskeletal mechanics and lineage-defining transcriptional programs to condensate-associated signaling thresholds, thereby reinforcing commitment to transitional cell states. *VIM* can assemble into dynamic non-filamentous states, including biomolecular condensates, enabling rapid reconfiguration in response to mechanical and biochemical cues [82]. In cancer, these adaptable *VIM* assemblies contribute to epithelial–mesenchymal transition, invasion, and metabolic rewiring, reinforcing commitment to transitional cellular states [83]. Notably, *TWIST1* forms phase-separated condensates with YY1 and p300 at super-enhancers to drive oncogenic transcriptional reprogramming in hepatocellular carcinoma [84]. In parallel, NEMO (*IKBKG*)-containing nuclear signaling condensates integrate inflammatory and stress-associated cues to bias NF- κ B-dependent transcription toward state reprogramming outcomes [85]. Crucially, the regulatory valence of these NEMO-associated condensates is determined by isoform-specific stoichiometry; whereas standard isoforms facilitate oncogenic signaling, the alternative splicing of *IKBKG* toward the NEMO-L variant inhibits NF- κ B-mediated state transitions in cancer cells [86]. Although these condensates can secondarily promote stress tolerance through transcriptional rewiring, their defining feature is the orchestration of context-dependent state transitions rather than the direct buffering of cellular stress.

FIGURE 4 | Integrated functional landscape of key regulatory networks in cancer. (A) Sankey diagram illustrating the connections between transcription factors (TFs), target genes, their functional effects, and associated cancer types. The visualization highlights major regulatory axes, including TF–gene interactions linked to apoptosis, proliferation, migration, EMT, metabolism, and radioresistance across multiple tumor types such as breast, lung, colorectal, liver, and glioblastoma. (B) Functional co-occurrence network showing molecular interactions grouped by biological categories, including autophagy/ubiquitin signaling (yellow), epigenetic regulators (orange), deubiquitinases (blue), autophagic/mitochondrial molecules (green), transcription factors (red), and tumor suppressors (gray). Node clustering emphasizes cooperative functional modules participating in tumor progression. (C) MicroRNA–gene regulatory networks showing miRNAs predicted to target core cancer-related genes. Red nodes represent miRNAs and blue nodes represent genes affected by melatonin. (D) Kaplan–Meier survival analyses evaluating the prognostic significance of selected gene signatures across different cancer types.

Whereas Axis II condensates mediate the decision to enter a particular cellular state, Axis III condensates stabilize survival within that state under adverse conditions. Notably, YAP/TAZ can also participate in stress-induced condensates that enhance cellular persistence under mechanical or metabolic constraint; such assemblies are classified here under Axis III when their dominant function shifts from state transition to stress endurance.

4.3 | Axis III: Stress-Responsive Condensates That Buffer Cellular Collapse

Stress adaptation and survival condensates are distinguished by their switch-like sensitivity to environmental stress, enabling rapid transitions between cellular preservation and collapse. Axis III condensates primarily function as reactive storage and protective hubs that buffer oxidative, metabolic, and proteotoxic stress [87]. In contrast to Axis I condensates, which support continuous information flow and transcriptional regulation, Axis III condensates prioritize immediate cell survival, determining whether cancer cells tolerate stress or undergo irreversible collapse.

Central to this axis are stress-responsive condensates organized around USP10, SQSTM1 (p62), and stress granules (SGs) that sequester signaling components to bias cellular programs toward survival. While transient formation of SGs is protective under acute stress, their persistent stabilization and accumulation under chronic stress conditions—such as those encountered in tumors—can promote pathological survival states [88]. A defining feature of Axis III regulation is the sequestration of KEAP1 into SQSTM1-containing condensates, enabling NRF2-dependent cytoprotective gene expression and antioxidant adaptation [89]. In addition to buffering metabolic and proteotoxic stress, Axis III condensates modulate immune and apoptotic thresholds. Phase separation of cGAS serves as a switch leveraged by cancer cells to drive metastasis (on) or evade immune detection (off) [90]; while condensate-associated interactions involving PrP^c (PRNP) and BCL2 raise apoptotic thresholds under stress [91]. In parallel, phase separation-linked regulation of TFEB and TFAM supports lysosomal and mitochondrial programs that reinforce metabolic flexibility and stress tolerance [92–94]. Collectively, Axis III condensates function as stress-responsive decision nodes, enabling cancer cells to survive hostile microenvironments through dynamic molecular buffering and survival prioritization.

The assembly of phase-separated condensates in all three axes are evolutionarily conserved, transient, physiological responses to acute stress. Under chronic stress conditions, including cancer, phase separation transitions into an aberrant phase, becoming drivers and promoters of oncogenesis [95]. The tumor microenvironment can modulate biophysical parameters that affect condensate behavior, leading to aberrant phase separation. Condensates in the three phase separation axes are extremely sensitive to several biophysical axes of control that regulate condensate behavior [96]. Accordingly, the 26 genes all exhibit sensitivity to multiple biophysical levers, not surprisingly, known to be modulated by melatonin. For clarity, we refer to these biophysical axes as control levers when discussing their modulatory effects on genes and condensates.

4.4 | Beyond Bulk Expression: Identifying the Biophysical Levers of Phase Separation

Conceptual overview: *The formation and stability of cancer-driving condensates are controlled by three fundamental biophysical levers. Lever I (Redox Tuning) acts as the initial trigger, sensing oxidative stress to flip the switch for condensation. Lever II (Multivalent Interactions) provides the molecular “stickiness” that scaffolds these dense droplets together. Lever III (Electrostatic Control) acts as an electrochemical shield and proton trap, allowing the condensates to maintain a safe internal environment despite the hostile, acidic conditions of the tumor. Crucially, standard laboratory techniques that only measure total protein amounts often fail to detect these critical physical changes.*

To accurately interpret melatonin-regulated gene expression in cancer, the limitations of bulk quantification approaches must be carefully considered. While these dominant techniques successfully measure net transcript or protein abundance, they remain inherently agnostic to the mesoscale organization of molecules assembled via phase separation. Consequently, the observed melatonin-induced up- or downregulation of phase separation-associated proteins neither precludes their simultaneous partitioning into biomolecular condensates, nor excludes melatonin-mediated modulation of the phase separation process itself. While bulk measurements capture changes in total transcript or protein abundance, phase separation has been increasingly implicated in modulating gene expression through the formation of transcriptional condensates that concentrate or sequester regulatory factors and thus influence transcriptional outputs in a dynamic, context-dependent manner [75, 97]. In this context, changes in bulk expression may reflect downstream consequences of altered condensate formation, stability, or material properties, as well as feedback regulation arising from condensate-dependent transcriptional or post-transcriptional control.

In this framework, the directionality of regulation observed in our study does not merely reflect changes in bulk abundance, but serves as a proxy for shifts in system flux and active solubility. While traditional models prioritize the critical concentration (c_{crit}) for phase separation, the anti-concentration factor in non-equilibrium systems demonstrates that energy-consuming active processes—such as those modulated by melatonin’s metabolic recalibration—can maintain cellular fluidity even when protein levels remain high [98]. Thus, melatonin-mediated downregulation likely functions by lowering molecular connectivity and reducing the thermodynamic pressure toward pathological condensation.

The identification of 26 genes jointly associated with phase separation and melatonin regulation, therefore, supports a model in which melatonin influences not only protein abundance but also the biophysical state and functional compartmentalization of key cancer-relevant regulators—effects that are not resolvable by bulk measurements alone. Notably, the three phase-separation axes are defined not only by biological

function, but also by distinct biophysical sensitivities—redox state, multivalent interaction strength, and electrostatic balance—known to be influenced by melatonin. The existence of a sovereign singularity, where phase-separation architecture and melatonin biology converge to govern health and disease, warrants further elucidation.

4.4.1 | *Lever I: Redox Tuning of Condensate Stability*

Since the origins of life, redox (reduction-oxidation) chemistry and phase separation have been intrinsically linked in cellular organization and stress response mechanisms [99]. Redox-controlled phase separation acts as a molecular switch that enables cells to sense, respond, and adapt to environmental and metabolic fluctuations by governing the rapid formation and dissolution of BCs [100–102]. In the pursuit of survival and proliferation in a hostile environment of persistent oxidative stress and a reversed pH gradient, cancer cells rebalances cellular redox homeostasis [103], upregulating antioxidant defenses by flipping protein redox switches to cause the aberrant phase separation of oncogenic condensates.

This redox-first assembly initiates the formation of functional hubs across the cell's architecture: from nuclear decision-making (Axis I) driven by LSD1, EP300, MYC, and EZH2, to the signal integration (Axis II) of EGFR, and the stress adaptation (Axis III) of KEAP1 and BCL2. While redox reactions trigger the initial molecular switch—often mediated by redox-sensitive post-translational modifications [104]—the ultimate stability and material properties of these condensates are dictated by an intricate interplay with multivalent scaffolding (Lever II) and electrostatic control (Lever III).

4.4.2 | *Lever II: Multivalent Interactions As the Structural Reinforcement of Condensates*

While redox reactions flip the initial molecular switch, the structural persistence and material properties of oncogenic condensates are maintained by multivalent interactions. Lever II governs the cooperative cross-linking of the dense phase, utilizing a diverse chemical toolkit—including hydrophobic effects, cation- π interactions, π - π stacking, and hydrogen bonding—to create interconnected molecular networks [105]. Notably, while many condensates rely on IDRs, multivalent scaffolding in several key oncogenic drivers occurs independently of classic disorder. For instance, the structural integrity of β -catenin (*CTNNB1*) and SQSTM1 is mediated primarily through folded domains and repetitive binding interfaces rather than disordered motifs [106, 107].

In the cancer TME, Lever II is exploited to stabilize nuclear decision-making (Axis I) through the recruitment of AR, LEF1, SMAD3, and SOX9, forming high-density transcriptional hubs. Similarly, signal integration (Axis II) is amplified via the multivalent assembly of the Wnt and Hippo pathways—specifically through β -catenin (*CTNNB1*), TAZ (*WWTR1*), YAP1, and TWIST1. Furthermore, in stress adaptation (Axis III), proteins like PrP^c (*PRNP*) and SQSTM1 leverage their domain-based multivalency to sequester vital cellular components, providing a physical shield against apoptotic signals [108]. This layer of structural reinforcement ensures that these aberrant

condensates remain resilient to transient environmental fluctuations, representing a critical therapeutic vulnerability in tumorigenesis [5].

4.4.3 | *Lever III: Electrostatic Shielding and the Tumor Microenvironment Proton Trap*

While multivalency provides the structural scaffold, electrostatic and stoichiometric control dictate the selectivity and internal environment of the dense phase of condensates. Lever III regulates the electrical environment of the condensate by controlling ion partitioning and charge-density matching [109]. This localized bio-electrochemical field enables these hubs to maintain a stable internal pH through charge neutralization. By sustaining this electrochemical equilibrium, the hubs effectively decouple internal protonation dynamics from the increasingly acidic TME [110]. In the cancer TME, the reversed pH gradient acts as a biophysical catalyst for these interactions, promoting a proton-trap mechanism. Recent all-atom continuous constant pH molecular dynamics simulations demonstrate that condensate microenvironments universally favor protonated states by acting as unique solvation environments that reshape the pK_a values of amino acid side chains. This shift stabilizes the charged forms of cationic residues (HIS, LYS) and the neutral forms of anionic residues (ASP, GLU), fundamentally altering the solvation properties that govern biochemical function [111, 112].

These physicochemical shifts drive aberrant phase transitions, an emerging hallmark of cancer [5]. While metabolites universally shift the thermodynamic equilibria of biomolecular condensates [113], the unique metabolic profile of the cancer TME—characterized by high interstitial fluid pressure and a surplus of molecular crowders and nucleic acids—exploits this physical sensitivity. These conditions drive aberrant phase transitions, effectively strengthening the electrostatic forces that stabilize oncogenic hubs. This electrostatic shield is exploited across all functional axes: nuclear regulators (Axis I) such as NANOG, EZH2, and YTHDF3 utilize charge-density matching to lock in transcriptional programs [114]; while signal integration (Axis II) hubs like EGFR, NEMO (*IKBKG*), and VIM leverage increased positive charge to amplify pro-survival signaling [85]. Most critically, for stress adaptation (Axis III), the formation of cGAS, TFEB, USP10, and BCL2 condensates is stabilized by this charge-dependent insulation, as exemplified by TFAM, which protects mitochondrial genomic integrity [115, 116]. By exploiting the proton trap, cancer cells ensure that oncogenic survival programs remain biophysically viable despite the hostile microenvironmental, thermodynamic landscape.

4.5 | **The Sovereign Singularity: How Melatonin Dismantles Oncogenic Condensate Networks**

Conceptual overview: Grounded in our deductive framework, melatonin operates as a master regulator—a sovereign singularity—that simultaneously disrupts all three biophysical levers sustaining cancer condensates.

Melatonin fundamentally alters the physical environment of the cell by resetting the redox trigger (Lever I), acting as a molecular plasticizer to break apart sticky scaffolds (Lever II), and functioning as a dielectric tuner to collapse protective electrostatic shields (Lever III). By physically dismantling these sanctuaries, melatonin leaves the cancer cell entirely exposed and vulnerable.

The complexity of the 26-gene oncogenic network—spanning nuclear hubs (Axis I), signaling cascades (Axis II), and survival adaptations (Axis III)—suggests that current clinical strategies must evolve to match the scale of cancer's biophysical adaptability. Modern oncology has made significant strides with a combination lock approach, utilizing synergistic pairings such as antibody-drug conjugates (ADCs) and immune checkpoint inhibitors (ICIs) to address multiple, distinct facets of tumor biology simultaneously [117]. While current advanced therapies target comprehensive downstream effects, they frequently find themselves perpetually chasing an evolving oncogenic landscape. We propose that melatonin represents a higher-order intervention—a sovereign singularity—that circumvents the need to target individual molecular pathways. By regulating the fundamental biophysical parameters that govern the 26-gene survival network, melatonin systematically alters the cellular environment to render the oncogenic program biophysically unviable, effectively stabilizing the landscape before subsequent evolutionary evasion can occur.

While the sovereign singularity remains a theoretical synthesis, it is grounded in emerging experimental evidence suggesting that melatonin does not merely inhibit proteins, but fundamentally alters the physical state of the oncogenic niche [29, 41, 58]. By bridging the gap between traditional pharmacology and systems biophysics, our proposed framework offers a strategic roadmap for future experimental validation of melatonin as an evolutionarily conserved, universal regulator of cellular phase behavior (Figure 5).

4.5.1 | *Lever I: Melatonin Recalibrates Redox Homeostasis As the Ultimate Phase-Transition Switch*

The traditional view of melatonin as a monolithic antioxidant is increasingly challenged by its context-dependent pro-oxidant effects in malignant cells. Recent evidence demonstrates that melatonin can paradoxically reduce glutathione (GSH) and catalase (CAT) levels in ovarian cancer, promoting cytotoxicity while stripping the cell of its oxidative defenses [118]. Within the sovereign singularity framework, this effect is not a contradiction but a strategic biophysical recalibration. In the TME, cancer cells utilize redox-driven phase separation to sequester and stabilize antioxidant machinery—such as the NRF2/KEAP1 and MCM5 hubs—effectively creating a redox shield that permits survival under high oxidative stress [58]. The paradoxical pro-oxidant and cytotoxic effects of melatonin represent a systemic phase reset rather than mere chemical reactions. When melatonin disrupts these aberrant thermodynamic conditions—as characterized within our biophysical model—it effectively deprives the cancer cell of this protective condensate architecture, exposing vulnerabilities that lead to the demise of the malignant cell. This

recalibration systematically drives the redox landscape back toward a physiological baseline, rendering the 26-gene survival network biophysically exposed.

4.5.2 | *Lever II: Melatonin As a Molecular Plasticizer to Prevent Scaffold Maturation*

While the redox switch initiates the transition, the persistence of oncogenic hubs depends on the multivalent stickiness of protein IDRs in Lever II. Proteins such as β -catenin (CTNNB1) and YAP1 rely on transient π - π and cation- π interactions to form dense, gel-like scaffolds. We propose that melatonin disrupts pro-survival signaling by acting as a molecular plasticizer within the condensate environment. Driven by its electron-rich indole ring and distinct dipole moment, melatonin partitions into proteins with IDRs, competing for the aromatic contacts that drive cross-linking [57, 119–121]. By interfering with this multivalent molecular grammar, melatonin prevents the 26-gene assembly from maturing into a rigid, therapy-resistant signaling haven, thereby maintaining the structural fluidity required for standard enzymatic degradation. By functioning as a molecular plasticizer, melatonin is predicted to prevent the rigidification of oncogenic scaffolds, thereby keeping them accessible to the physiological degradation machinery.

4.5.3 | *Lever III: Melatonin As a Dielectric Tuner to Neutralize the Proton Trap*

To complete the tri-lever triad, we move from the internal architecture of the condensate to its relationship with the surrounding environment. This is where the sovereign role of melatonin is most apparent, as it governs the very energetics of the cellular solvation and fluidity landscape. Cancer cells exploit the proton trap in Lever III in order to maintain aberrant condensates with internal pK_a values that are decoupled from the increasingly acidic TME. Recent evidence confirms that such condensates can sustain significant pH gradients relative to the bulk environment at equilibrium through a process of charge neutralization [110]. The decoupling of condensate internal pK_a from the increasingly acidic TME provides a biophysical sanctuary that renders Axis III survival programs resistant to extracellular stress [111].

Our model predicts that melatonin disrupts cancer cell Lever III defenses through a dual mechanism of metabolic recalibration and direct dielectric tuning. By shifting the cellular program from glycolytic fermentation to oxidative phosphorylation, melatonin reduces the accumulation of metabolic crowders that shift thermodynamic equilibria [113, 122]. Simultaneously, melatonin—particularly when stacked with ATP—modulates the local dielectric constant (ϵ) within the condensate environment [57]. Melatonin acts as a critical biophysical buffer by forming a high-dipole molecular complex via stacking between its indole ring and the adenine purine ring of ATP, neutralizing the localized charge gradients identified by Ausserwöger et al. [110]. Although directly measuring the internal dielectric constant of condensates remains a technical challenge, our model predicts that melatonin-mediated restoration of oxidative phosphorylation fundamentally reshapes this dielectric landscape via the thermodynamic coupling between metabolic flux and solvent polarizability, consequently

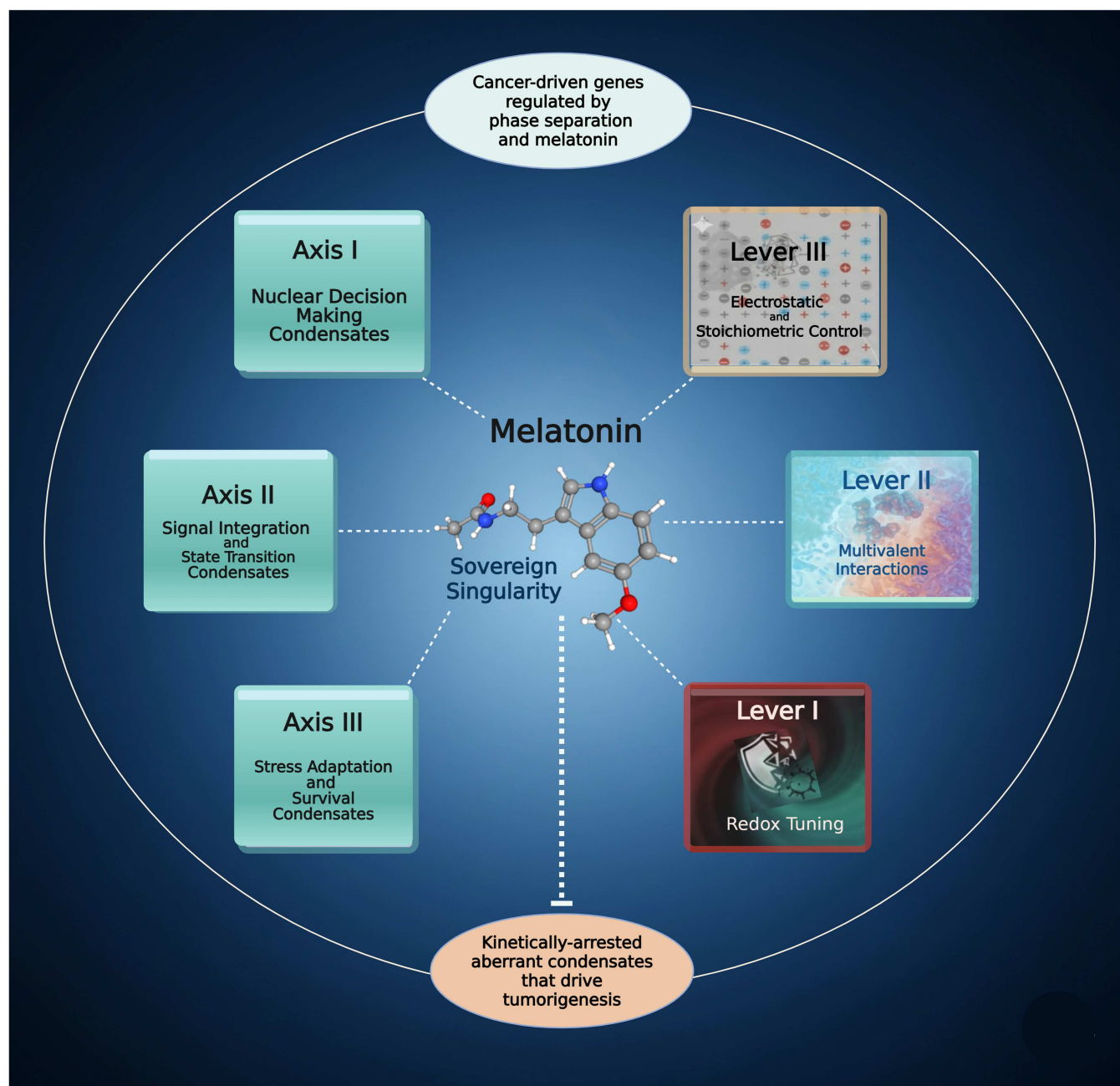


FIGURE 5 | Melatonin: a sovereign singularity that disrupts oncogenic phase-separated networks. Melatonin targets integrated biophysical axes (Axes 1–3) that stabilize oncogenic phase-separated condensates by modulating a tri-lever framework (Levers 1–3). Melatonin recalibrates redox homeostasis, disrupts multivalent interactions acting as a molecular plasticizer, and normalizes electrochemical energetics by functioning as a dielectric tuner. By exerting a field effect in the tumor environment, melatonin enforces fundamental physicochemical constraints that render the oncogenic survival program biophysically unviable.

neutralizing the underlying electrochemical gradients. By altering the concentration of highly polar metabolites and forming these synergistic dipole complexes, melatonin modulates the static dielectric constant (ϵ) of the dense phase. This shift effectively neutralizes the protective electrochemical gradients that sustain oncogenic sanctuaries. By dissipating these localized electrochemical fields, melatonin is predicted to drive the re-equilibration of oncogenic hubs with the physiological environment, consequently rendering the 26-gene and other potential cancer networks biophysically untenable and accessible to therapeutic intervention [29].

The functional impact of this dielectric shift is most profound within the nuclear landscape (Axis I). Recent findings demonstrate that charge-driven condensation acts as a physical switch for non-canonical DNA structures, stabilizing i-motifs (iM) while destabilizing G-quadruplexes (G4) [17]. By dissipating the localized electrochemical fields that sustain these proton traps, melatonin effectively interferes with the charge-driven environment required for such structural transitions. Given melatonin's demonstrated influence on the dynamic stability of the DNA structure [123] its role as a dielectric buffer potentially normalizes the folding equilibria of these non-canonical

structures. This structural normalization provides a direct biophysical mechanism for the reset of oncogenic nuclear decision-making programs in Axis I. A hallmark of this versatility is the melatonin-mediated upregulation of specific regulatory isoforms, such as the alternative splicing of *IKBKG* (NEMO). By biophysically steering transcriptional machinery at the DNA interface, melatonin promotes the inclusion of exon 5, favoring the production of the tumor-suppressive NEMO-L isoform [86]. Thus, melatonin does not merely act as a global inhibitor; it functions as a precision dielectric tuner that can selectively upregulate protective pathways by recalibrating the physical landscape of gene processing. Table 3 summarizes the 26 phase-separation cancer genes regulated by melatonin; it details their expression patterns (upregulation/downregulation), biophysical levers, functional axes, and roles in cancer progression. Please refer to Table 3 for synonyms and full names of the 26 genes.

5 | Limitations

While our proposed framework provides a novel unification of melatonin's pleiotropic effects, several conceptual boundaries must be noted. First, the three biophysical levers identified here are representative of the primary regulatory forces currently known to govern cellular organization. They are intended as a fundamental template for biophysical regulation rather than an exhaustive catalog of all potential force-fields involved in phase behavior. Second, the 26-gene survival network synthesized in this study provides strategic coverage of core oncogenic hubs across multiple functional axes. As the study of biomolecular condensates expands, the list of validated targets will undoubtedly grow. However, the consistent modulation of this representative core suggests that the underlying biophysical principles—the sovereign regulation of the cellular environment—remain the universal common denominator of melatonin's efficacy, regardless of the specific genes involved.

Furthermore, a critical epistemological limitation of our proof-of-concept synthesis lies in the data-model paradox inherent to contemporary cancer systems biology. While Section 4.4 rightly critiques traditional bulk quantification approaches for being agnostic to the mesoscale organization of phase-separated assemblies, the bioinformatic convergence analysis presented in Table 3 necessarily relies on published transcriptomic and proteomic datasets derived from those very same bulk methodologies. Consequently, our framework must infer shifts in system flux, thermodynamic pressure, and active solubility from macroscopic expression changes rather than from the direct, real-time visualization of phase boundaries or critical concentration (C_{crit}) fluctuations in vivo.

Additionally, while advanced computational pipelines—such as all-atom continuous constant pH molecular dynamics simulations—provide elegant, localized evidence for proton-trap mechanisms and side-chain pK_a shifts, these models operate in isolation from the full complexity of a living tumor microenvironment. In vivo, these biophysical levers must contend with intense macromolecular crowding, fractal tortuosity, and elevated interstitial fluid pressures that are rarely fully replicated in standard in vitro configurations. Finally, because the direct metrological tracking of local static dielectric

constants (ϵ) or localized electrochemical fields within intracellular dense phases remains a technical frontier, the precise energetic boundaries of the sovereign singularity framework must currently be treated as a robust theoretical blueprint rather than an empirically mapped landscape. Far from weakening the framework, however, candidly defining these current data mismatches provides the exact conceptual leverage required to understand historical bottlenecks in oncology—a breakdown explored directly in our translational perspectives below.

6 | Conclusions and Future Perspectives

6.1 | Summary of the Sovereign Singularity

Our integrative bioinformatics proof-of-concept analysis reveals melatonin acting as a universal regulator of cellular fluid dynamics, ensuring biophysical and biochemical mechanisms that drive phase separation remain physiologically viable. By permeating and destabilizing the biophysical sanctuaries that enable cancer's evolutionary adaptability, melatonin effectively de-shields the malignant cell, rendering its survival machinery biophysically untenable. By modulating the fluid properties of cancer gene networks, this indoleamine strategically manages the diverse evasion toolkits used by malignant cells to avoid inhibition. Given its unique capacity to integrate redox tuning, multivalent interaction, and electrostatic balance, melatonin likely serves as the defining coordinate of this singularity—the point where the cell's physical architecture converges with its survival logic. Ultimately, the primary significance of this perspective lies in the transition from isolated molecular targeting to landscape-level biophysical intervention.

6.2 | Bridging the Translational Gap

Crucially, this biophysical lens directly addresses why promising in vitro and animal results have historically failed to translate into consistent clinical efficacy for human cancer patients. Traditional clinical trials have largely evaluated melatonin through standard pharmacological frameworks, which prioritize systemic dosing paradigms without accounting for the mesoscale spatial organization of intracellular condensates. By relying on bulk quantification to evaluate treatment efficacy, conventional methodologies cannot distinguish whether a therapeutic agent has successfully cleared an oncogenic target or inadvertently sequestered it into a highly fluid, condensed sanctuary.

If exogenous melatonin appears clinically inconsistent in human oncology to date, it is likely because standard clinical models fail to account for the robust thermodynamic and electrostatic forces shielding aberrant human condensates. Human oncology represents a highly chaotic, non-equilibrium system where survival programs are heavily insulated by proton-trap mechanisms. These mechanisms effectively decouple the internal protonation dynamics of survival condensates from the surrounding acidic microenvironment, creating an impenetrable evolutionary bunker against standard therapeutics. By acknowledging the data paradox of the current literature, we transform what appears to be clinical

TABLE 3 | Bioinformatic convergence analysis: Biophysical and functional profiles of genes regulated by melatonin and phase separation.

Gene symbol	Full name/synonym	Bio physical levers ^b			Role in phase separation (PS)	Regulation by melatonin ^c	Key pathways/functions	Associated cancers/effects
		PS Axes ^a	Lever II	Lever I, III				
<i>AR</i>	Androgen Receptor	Axis I	Lever II		Involved in nuclear condensates for transcriptional regulation	Down- regulated	Androgen receptor signaling; nuclear receptor binding; epigenetic modulation	Breast, prostate; promotes proliferation, chemoresistance
<i>BCL2</i>	B-cell lymphoma 2	Axis III	Lever I, III		Contributes to stress granule formation in anti-apoptotic contexts	Down- regulated	Apoptosis inhibition; targeted by miR-34a-5p, miR-15a-5p, miR-15b-5p, miR-16-5p, miR-7-5p	Breast, gastric, liver; anti-apoptotic oncogene; poorer survival with high expression
<i>CGAS</i>	Cyclic GMP-AMP synthase	Axis III	Lever III		PS in innate immune signaling condensates	Down- regulated	Epigenetic regulation; cGAS-STING pathway activation	Glioblastoma; enhances metastasis and immune evasion
<i>CTNNB1</i>	β -catenin	Axis II	Lever II		β -catenin core in Wnt PS condensates	Down- regulated	Wnt/ β -catenin signaling; nuclear translocation	Colorectal, liver; proliferation, EMT
<i>EGFR</i>	Epidermal growth factor receptor	Axis II	Lever I, III		Membrane-bound PS for receptor clustering	Down- regulated	Receptor tyrosine kinase signaling; targeted by miR-7-5p	Breast, gastric; drives proliferation, chemoresistance
<i>EP300</i>	Histone acetyltransferase p300	Axis I	Lever I, II		Acetyltransferase in enhancer PS	Down- regulated	Histone acetylation; epigenetic regulation	Multiple; coactivator in proliferation
<i>EZH2</i>	Enhancer of zeste homolog 2	Axis I	Lever I, III		Epigenetic writer in PS condensates for chromatin compaction	Down- regulated	Histone methylation; targeted by miR-101-3p, miR-26a-5p, miR-124-3p; epigenetic regulation	Breast, gastric; EMT promotion; poorer survival with high expression
<i>IKBK</i>	NEMO/NF- κ B essential modulator	Axis II	Lever III		Adapter in NF- κ B condensates	Up- regulated*	NF- κ B signaling; inflammatory response	General; pro-apoptotic in context
<i>KDMA</i>	LSD1/Lysine-specific demethylase 1	Axis I	Lever I		Demethylase in transcriptional condensates	Down- regulated	Epigenetic demethylation; coregulator binding	Multiple (e.g., breast); transcriptional activation of oncogenes
<i>KEAP1</i>	Kelch-like ECH-associated protein 1	Axis III	Lever I		Regulates phase-separated Nrf2 condensates	Down- regulated	Oxidative stress response; antioxidant pathway disruption	Liver, glioblastoma; promotes survival under hypoxia
<i>LEF1</i>	Lymphoid enhancer-binding factor 1	Axis I	Lever II		Co-factor in β -catenin condensates	Down- regulated	Wnt/ β -catenin signaling; cis-regulatory binding	Colorectal, breast; proliferation
<i>MYC</i>	c-Myc proto-oncogene	Axis I	Lever I, II		Super-enhancer PS for global transcription	Down- regulated	Transcriptional amplification; targeted by miR-145-5p, miR-24-3p; core TF network	Breast, gastric, glioma; proliferation, poorer survival (high expression in breast/gastric; low in glioma linked to reduced survival)

(Continues)

TABLE 3 | (Continued)

Gene symbol	Full name/synonym	Bio physical levers ^b		Role in phase separation (PS)	Regulation by melatonin ^c	Key pathways/functions	Associated cancers/effects
		PS Axes ^a	Lever				
<i>NANOG</i>	Nanog homeobox	Axis I	Lever III	Stemness factor in pluripotency condensates	Down- regulated	Stem cell maintenance; self-renewal	Glioblastoma; tumor initiation
<i>PRNP</i>	Prion protein	Axis III	Lever II	Prion protein in membrane PS	Down- regulated	Neurodegenerative/cancer crosstalk; vesicle-mediated signaling	Glioblastoma; invasion
<i>SMAD3</i>	SMAD family member 3	Axis I	Lever II	PS in TGF- β signaling condensates	Down- regulated	SMAD2/3/4-mediated transcription; EMT regulation	Gastric; breast; metastasis via EMT
<i>SOX9</i>	SRY-box transcription factor 9	Axis I	Lever II	Transcription factor in EMT condensates	Down- regulated	EMT and stemness; transcriptional regulation	Gastric; breast; metastasis
<i>SQSTM1</i>	p62/Sequestosome 1	Axis III	Levers II, III	Scaffold in autophagosomes and stress granules	Down- regulated (implied in shared set)	Autophagy; protein aggregation in PS	General; chemoresistance via survival signaling
<i>TFAM</i>	Mitochondrial transcription factor A	Axis III	Levers I, III	Mitochondrial transcription in nucleoid PS	Down- regulated (implied in shared set)	Mitochondrial biogenesis; anti-apoptotic	Liver; energy metabolism in cancer
<i>TFEB</i>	Transcription factor EB	Axis III	Lever III	Lysosomal biogenesis regulator in PS	Down- regulated	Autophagy-lysosome pathway	General; metabolic reprogramming
<i>TP53</i>	Tumor protein p53	Axis I	Levers I, III	PS in p53 tumor suppressor condensates	Up- regulated	p53 pathway; apoptosis; targeted by miR-16-5p	Multiple; antitumor effects, DNA repair
<i> Twist1</i>	Twist family bHLH transcription factor 1	Axis II	Lever II	EMT driver in nuclear condensates	Down- regulated	EMT transcription; mesenchymal transition	Breast; gastric; invasion and metastasis
<i>USP10</i>	Ubiquitin specific peptidase 10	Axis III	Lever III	Deubiquitinase stabilizing PS proteins	Down- regulated	Protein stability; p53 regulation	Glioblastoma; chemoresistance
<i>VIM</i>	Vimentin	Axis II	Lever III	Intermediate filament in cytoskeletal PS	Down- regulated	EMT marker; targeted by miR-17-5p	Breast; gastric; mesenchymal phenotype; metastasis
<i>WWTR1</i>	TAZ/Transcriptional coactivator	Axis II	Lever II	IDR-mediated multivalent scaffolding of transcriptional hubs	Down- regulated; potential structural intercalation	Suppression of EMT and Hippo-related survival programs	Metastasis; chemoresistance; stemness

(Continues)

TABLE 3 | (Continued)

Gene symbol	Bio physical levers ^b			Role in phase separation (PS)	Regulation by melatonin ^c	Key pathways/functions	Associated cancers/effects
	Full name/synonym	PS Axes ^a	Levers II, III				
<i>YAPI</i>	Yes-associated protein 1	Axis II	Levers II, III	Hippo effector in nuclear PS for TEAD interaction	Down- regulated (implied in shared set)	Hippo signaling; transcriptional coactivation	Liver; proliferation and metastasis
<i>YTHDF3</i>	YTH N ⁶ -methyladenosine RNA binding protein 3	Axis I	Lever III	m ⁶ A reader in RNA PS granules	Down- regulated	RNA metabolism; translation control	General; post-transcriptional oncogenesis

Abbreviations: EMT, epithelial-mesenchymal transition; IDR, intrinsically disordered region; MEL, melatonin; PS, phase separation.
^aPhase separation (PS) axes: Refers to the functional classification of oncogenic condensates as mapped in Figure 5. Axis I: Nuclear decision making condensates. Axis II: Signal integration and state transition condensates, Axis III: Stress adaptation and survival condensates.
^bBiophysical levers identifying the specific mechanisms of melatonin-mediated disruption: Lever I (redox tuning): Recalibrating redox homeostasis to trigger a systemic phase reset. Lever II (multivalent interactions): Acting as a molecular plasticizer to disrupt the molecular grammar of pro-survival scaffolds. Lever III (electrostatic/stoichiometric control): Functioning as a dielectric tuner to collapse the proton trap and normalize electrochemical energetics.
^cRegulation by melatonin: Represents the consensus directional change in gene expression/activity mediated by melatonin across the synthesized cancer models. This directionality serves as a proxy for shifts in the solvation landscape and molecular connectivity, rather than a static measurement of bulk protein abundance.*See note in Table 2 regarding *IKBK* splicing stoichiometry.

inconsistency into a predictable consequence of biophysical omission. Recognizing that the physical state of the oncogenic niche dictates therapeutic resistance offers a necessary baseline to evaluate past clinical shortcomings. Ultimately, these findings provide a conceptual foundation to design future clinical protocols that explicitly account for, and measure, these biophysical variables.

6.3 | Future Research Directions

To move this framework from a provocative hypothesis to a definitive biological principle, future studies must evolve beyond traditional expression profiling. Research integrating transcriptomic and proteomic data with spatially resolved or condensate-sensitive assays will be necessary to discern abundance-based effects from phase state-dependent regulation in melatonin-responsive cancer pathways. Importantly, future research designed with complex microenvironmental parameters in mind—such as high interstitial fluid pressure, molecular crowding, and extreme pH gradients—will be vital to elucidate existing clinical discrepancies. Examining these mechanisms will directly clarify how these biophysical levers modulate specific phase separation axes. Furthermore, investigating melatonin as a combinatorial adjuvant will determine how precise physiological or pharmacological parameters can optimize concurrent therapies by enforcing fundamental biophysical constraints within the tumor landscape.

Author Contributions

Doris Loh: conceptualization, methodology (systematic review), writing – original draft, writing – review and editing. **Luiz Gustavo de Almeida Chuffa:** methodology (bioinformatics), software, formal analysis, writing – original draft, writing – review and editing. **Fábio Rodrigues Ferreira Seiva:** methodology (bioinformatics), software, formal analysis, writing – original draft, writing – review and editing. **Russel J. Reiter:** writing – critical review and editing.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the Corresponding Author, Doris Loh, upon reasonable request.

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