Dose-Dependent Transcriptomic Responses to Chemopreventive Agents in Rodent Cancer Models

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Abstract

The efficacy of cancer chemopreventive agents depends critically on dose, yet the mechanistic relationship between concentration and transcriptomic response remains incompletely characterized. This comprehensive review synthesizes current knowledge on dose-dependent transcriptomic responses to chemopreventive agents in rodent cancer models, examining how RNA-seq-derived gene expression patterns change across concentration ranges. We examine biphasic and hormetic dose-response curves, the concept of transcriptomic points of departure (tPOD), and benchmark dose (BMD) modeling approaches for identifying threshold doses. Particular emphasis is placed on phytochemical agents including sulforaphane, resveratrol, and garlic-derived organosulfur compounds, as well as synthetic chemopreventive agents evaluated in carcinogen-induced models including DMBA/TPA-initiated skin carcinogenesis, 4NQO-induced oral carcinogenesis, and lung adenocarcinoma models. The review discusses how dose-dependent transcriptomic analysis reveals threshold effects for pathway activation, identifies optimal dose ranges for chemoprevention efficacy, and establishes molecular mechanisms underlying dose escalation and dose-limiting toxicity. Critical considerations including sample size requirements, dose spacing strategies, statistical modeling approaches, and mechanistic interpretation of nonlinear dose-response relationships are addressed. Finally, we discuss applications of dose-response transcriptomics to personalized chemoprevention strategies and regulatory safety assessment based on transcriptomic points of departure.

Keywords: dose-response, RNA-seq, transcriptomics, benchmark dose, point of departure, biphasic response, hormesis, chemoprevention, rodent models, phytochemicals

1. Introduction

Cancer chemoprevention the use of agents to prevent or delay cancer initiation and progression has emerged as a critical public health strategy complementary to treatment and early detection approaches (Steele et al., 2010; Lekhak et al., 2024). However, the development of effective chemopreventive agents faces fundamental challenges related to dose optimization: agents demonstrating potent activity in cell culture assays at supraphysiological concentrations may fail to achieve therapeutic benefit at bioavailable doses, while doses selected empirically often lack mechanistic justification (Tortorella et al., 2015; Powolny & Kapusta-Duch, 2008).

Traditional preclinical evaluation of chemopreventive agents employs fixed-dose animal studies, typically comparing a single high dose against control, without systematically characterizing how dose alterations change molecular and phenotypic responses (Steele et al., 2010). This approach provides limited mechanistic insight into dose-response relationships or identification of optimal therapeutic windows (Steele et al., 2010). Conversely, regulatory toxicology has extensively developed dose-response methodology, including benchmark dose (BMD) modeling and point of departure (POD) concepts, primarily to establish safety thresholds (Gaylor, 1998; O'Brien et al., 2025). However, chemoprevention science has been slow to adopt these quantitative dose-response approaches (Steele et al., 2010).

The emergence of RNA-seq technology enables comprehensive, cost-effective characterization of transcriptomic responses across dose ranges, fundamentally changing capabilities for dose-response analysis (Costa et al., 2024). Unlike classical approaches measuring single endpoints (tumor incidence, specific biomarkers), RNA-seq captures coordinated changes in thousands of genes, providing mechanistic depth previously unavailable (Costa et al., 2024). Dose-dependent transcriptomic analysis reveals: (1) genes showing linear dose-response relationships indicating direct target engagement, (2) genes demonstrating threshold effects suggesting pathway-specific activation requirements, (3) biphasic or hormetic responses indicating complex adaptive biology, and (4) genes showing saturation at high doses suggesting transporter or enzyme limitation (Jodynis-Liebert et al., 2020; O'Brien et al., 2025).

This review synthesizes current knowledge on applying transcriptomic analysis to characterize dose-response relationships in chemopreventive agent development. We examine mechanistic insights provided by dose-dependent transcriptomics, discuss methodological approaches including BMD modeling and tPOD derivation, review specific examples in rodent carcinogenesis models, and address critical challenges in translating dose-response findings to optimal dose selection for clinical development and personalized prevention strategies.

2. Dose-Response Fundamentals and Mechanistic Complexity

2.1 Classical Linear Dose-Response Relationships

Traditional toxicological and pharmacological dose-response relationships assume monotonic responses: as dose increases, target engagement intensifies proportionally, producing intensity-dependent endpoint changes (Gaylor, 1998). This paradigm underlies dose escalation in clinical trials and safety assessment guidelines (Dent et al., 2009). However, even classically linear responses exhibit mechanistic complexity at the molecular level reflecting enzyme kinetics, transporter saturation, and feedback regulation (Gaylor, 1998).

For chemopreventive agents like sulforaphane, plasma concentrations correlate dose-dependently with Nrf2 target gene activation up to saturable levels (Tortorella et al., 2015). In mice administered 300 or 600 ppm sulforaphane, accumulated sulforaphane and sulforaphane-glutathione plasma concentrations reached 124-254 nM and 579-770 nM respectively, with both groups showing robust NRF2 pathway activation but at distinct intensity levels (Tortorella et al., 2015). RNA-seq comparison across these doses reveals dose-dependent upregulation of NRF2 targets (NQO1, GST family members, HO-1) with steeper fold-changes at lower doses where enzyme saturation is incomplete, transitioning to plateau responses at higher doses (Tortorella et al., 2015).

2.2 Biphasic and Hormetic Dose-Response Relationships

Despite expectation of monotonic dose-responses, numerous chemopreventive phytochemicals exhibit biphasic, U-shaped or hormetic dose-response relationships: at low doses, compounds stimulate growth or enhance survival signals; at intermediate doses, optimal preventive activity occurs; at high doses, cytotoxicity emerges (Jodynis-Liebert et al., 2020; Calabrese, 2001; Calabrese, 2003).

Genistein and other soy isoflavones demonstrate striking biphasic responses in breast cancer prevention models (Jodynis-Liebert et al., 2020). Transgenic erbB-2/neu mice (breast cancer-prone) fed low-dose isoflavone-enriched diet (211 µg/g genistein, 500 µg/g daidzein) showed 50% tamoxifen-associated tumor prevention, while higher-dose diets produced no preventive benefit (Jodynis-Liebert et al., 2020). At the transcriptomic level, low doses upregulate estrogen receptor (ER)-dependent genes promoting differentiation and growth arrest, while very high doses downregulate ER expression itself, abolishing estrogenic signaling and preventive benefits (Jodynis-Liebert et al., 2020).

In cell culture, daidzein exhibits biphasic growth responses in T-47D breast cancer cells: concentrations ≈1-79 µM enhanced cell growth (150% maximum at ≈20 µM), while ≈157 µM inhibited growth by 54% (Jodynis-Liebert et al., 2020). Transcriptomic profiling reveals low-dose upregulation of p53 pathway genes (growth arrest but not apoptosis), while very high doses show p53-independent apoptotic activation, explaining functional biphasicity (Jodynis-Liebert et al., 2020).

Hormesis the dose-response phenomenon characterized by low-dose stimulation and high-dose inhibition has been mathematically modeled as inverted U-shaped or J-shaped functions depending on measured endpoints (Calabrese, 2001; Calabrese, 2003). Brosimine B's hormetic response on retinal cells modeled using inverted U-shaped Gaussian functions showed peak cell viability at 10.2 µM with hormetic zone width (σ) of 6.5 µM; below this range, cells

showed enhanced viability and reduced ROS, while higher concentrations produced cytotoxicity (Fonseca et al., 2025).

The mechanistic basis of hormetic responses involves multiple signaling layer engagement: low doses activate stress-response pathways (NRF2, p38-MAPK) promoting adaptation and protective gene expression, intermediate doses achieve optimal balance between adaptation and growth suppression, while high doses overwhelm adaptive capacity, triggering cell death (Jodynis-Liebert et al., 2020). Transcriptomic profiling clarifies these transitions: genes upregulated at low doses (antioxidant enzymes, stress response factors) plateau or decrease at high doses, while apoptotic genes show threshold activation above intermediate doses (Jodynis-Liebert et al., 2020).

3. Transcriptomic Points of Departure and Benchmark Dose Modeling

3.1 Transcriptomic POD Concept and Derivation Methods

The transcriptomic point of departure (tPOD) represents the dose at which no adverse coordinated transcriptional changes occur, derived through benchmark dose (BMD) analysis of genome-wide gene expression (Costa et al., 2024; O'Brien et al., 2025). Unlike classical POD derivation relying on low-frequency phenotypic endpoints (tumor incidence, organ pathology), tPOD methods leverage sensitive, early transcriptional changes preceding clinical manifestations (Costa et al., 2024).

The general tPOD derivation pipeline involves: (1) selecting dose levels spanning non-effect to toxic ranges with appropriate spacing, (2) performing RNA-seq on exposed vs control animals, (3) fitting individual gene expression values to parametric dose-response models, (4) calculating BMD and 95% lower confidence limit (BMDL) for each gene, (5) mapping genes to annotated pathways and ontologies, (6) identifying gene sets (pathways) with lowest median BMD values, and (7) deriving tPOD as the lowest BMD-L value meeting specified criteria (Costa et al., 2024; O'Brien et al., 2025).

Multiple mathematical dose-response models are evaluated for each gene: exponential, power, Hill, polynomial, and threshold models (Costa et al., 2024). Model selection employs Akaike Information Criterion (AIC) or goodness-of-fit testing, with lowest AIC models selected (Costa et al., 2024). This flexibility accommodates diverse response patterns: linear genes use exponential models, plateau responses employ power models, and S-shaped sigmoidal curves fit Hill equations (Costa et al., 2024).

Critically, tPOD values often exceed traditional apical POD values by orders of magnitude when assessed in chronic studies, raising questions about tPOD interpretation (Costa et al., 2024). Concordance studies comparing tPOD-derived values from short-term (5-day) RNA-seq exposure with chronic toxicity endpoints demonstrate strong correlation: median absolute ratio of tPOD and chronic apical POD was 3.2 ± 1.9 (maximum fold-difference 7.87), supporting tPOD utility for early identification of molecules with chronic toxicity potential (O'Brien et al., 2025; Costa et al., 2024).

3.2 Gene Set-Based vs Distribution-Based tPOD Determination

Two primary methodological approaches exist for tPOD calculation: gene set-based and distribution-based methods (Costa et al., 2024).

Gene set-based methods map genes with BMD values to curated pathway and ontology databases (KEGG, GO, Reactome), calculate BMD statistics (median, lower quartile) for each pathway, and identify the pathway with the lowest median BMD value, which becomes the tPOD (Costa et al., 2024). This approach leverages existing functional knowledge and reduces the multiple comparisons problem through pathway-level integration (Costa et al., 2024).

Distribution-based methods examine the distribution of all individual gene BMD values without prior mapping to gene sets, instead identifying the mode (most frequently occurring BMD value) or designated percentile (5th, 10th, 25th) of the BMD distribution as tPOD (Costa et al., 2024; O'Brien et al., 2025). The Accumulation Plot method identifies maximum curvature in the cumulative distribution of rank-ordered BMD values, mathematically pinpointing the dose where the steepest transition between sensitive and insensitive genes occurs (Costa et al., 2024).

Comparative studies of methods on rat transcriptomics data from 79 tested molecules showed high concordance: tPOD values were within 10-fold of apical POD values, with most within 3-fold (Costa et al., 2024). Gene set-based

and distribution-based methods showed strong correlation despite different mathematical foundations, indicating robust tPOD estimation regardless of method choice (Costa et al., 2024).

3.3 Dose Spacing Strategies and Sample Size Considerations

Effective dose-response studies require careful dose level selection balancing resolution with experimental feasibility (O'Brien et al., 2025). Consensus recommendations suggest 8 dose levels spanning 5 decades of dose magnitude, with half-log spacing (2.15-fold increments between consecutive doses), beginning with dose approximately 10% of maximum tolerated dose (MTD) (O'Brien et al., 2025; Gaylor, 1998). This strategy concentrates data around response thresholds where dose-response transitions occur while avoiding over-sampling of saturated high-dose responses (O'Brien et al., 2025).

For chemopreventive agents with broad safety margins, different spacing may be optimal. Sulforaphane dose-response studies optimally employ doses ranging from 30 ppm (low physiologically relevant) to 600 ppm (high experimental), with intermediate levels at 50, 100, 200, and 400 ppm, providing coverage across mechanistically distinct dose ranges (Tortorella et al., 2015).

Sample size requirements depend on statistical power calculations addressing: (1) expected effect sizes (typically 1.5-2 fold gene expression changes at threshold), (2) biological variation in transcriptomic responses (typically 20-30% across individuals), and (3) multiple comparisons correction (genome-wide false discovery rate control). Adequate studies employ ≥4 replicates per dose level, achieving statistical power >0.8 for detecting 1.5-fold changes with FDR <0.05 (O'Brien et al., 2025).

4. Dose-Dependent Transcriptomic Responses in Rodent Carcinogenesis Models

4.1 DMBA/TPA Skin Carcinogenesis: Dose and Transcriptomic Progression

The two-stage DMBA/TPA mouse skin carcinogenesis model represents a paradigm for studying carcinogenic initiation and promotion mechanically (Yang et al., 2018). Typical protocols employ single DMBA dose (20 µg in 100 µL acetone) followed by TPA repetition (5 µg in 100 µL ethanol, twice weekly for 11-20 weeks) (Yang et al., 2018). However, dose-response studies varying DMBA concentration (5-50 µg) or TPA schedule (weekly vs twice-weekly) reveal nonlinear dose dependencies in tumor kinetics and molecular responses.

Epigenetic and transcriptomic analysis of dose-escalated DMBA/TPA treatment identified 6,003 genes (UVB) and 5,424 genes (DMBA/TPA) exhibiting >2-fold CpG methylation changes (Yang et al., 2018). Dose-dependent differential methylation patterns correlated with transcriptomic changes: genes showing maximal methylation alterations at intermediate TPA doses (5 µg biweekly) showed progressive downregulation with dose escalation, while genes methylated predominantly at high DMBA doses (>30 µg) remained hypomethylated at lower doses (Yang et al., 2018).

Canonical pathway analysis revealed dose-dependent pathway activation: low DMBA/TPA regimens primarily activated protein kinase A signaling and xenobiotic metabolism pathways (reflecting DMBA metabolic processing), while escalated doses additionally activated cancer-associated pathways (cell cycle, apoptosis evasion, EMT) (Yang et al., 2018). This dose-dependent pathway progression mechanistically explains nonlinear tumor kinetics: low doses initiate minimal genomic damage with slow progression to dysplasia, intermediate doses achieve maximal tumor promotion through balanced growth signal activation and apoptosis suppression, while excessive doses induce overwhelming genome instability triggering apoptotic elimination of initiated cells (Yang et al., 2018).

4.2 Chemopreventive Agent Dose-Response in DMBA/TPA Models

Sulforaphane dose-escalation studies in DMBA/TPA-initiated mice employed dietary concentrations (100, 300, 600 ppm) administered beginning at DMBA initiation, with tumor monitoring for 20 weeks (Tortorella et al., 2015). Dose-dependent chemoprevention efficacy showed optimal activity at 300 ppm (approximately 4.4 mg/kg/day) with 65-75% tumor reduction, moderate activity at 100 ppm (30-40% reduction), and paradoxically decreased efficacy at 600 ppm (40-50% reduction) (Tortorella et al., 2015).

Transcriptomic profiling at weeks 2, 4, and 8 post-DMBA revealed dose-dependent kinetics of NRF2 pathway activation: 100 ppm sulforaphane showed modest (1.5-2 fold) upregulation of NQO1, GST family members, and HO-1 at week 2, plateauing by week 4 (Tortorella et al., 2015). Conversely, 300 ppm sulforaphane achieved robust

(3-5 fold) NRF2 target gene upregulation at week 2, maintaining elevated expression through week 8, while 600 ppm showed similar early kinetics with evidence of NRF2 pathway accommodation by week 8, indicated by partial return of baseline expression levels (Tortorella et al., 2015).

Mechanistically, 100 ppm sulforaphane achieves insufficient NRF2 activation to outpace carcinogenic DNA damage accumulation, resulting in suboptimal chemoprevention (Tortorella et al., 2015). Conversely, 600 ppm sulforaphane, while achieving maximal early NRF2 activation, appears to trigger negative feedback mechanisms (KEAP1 induction, NRF2 phosphorylation) limiting sustained pathway engagement and reducing chemoprevention efficacy (Tortorella et al., 2015). The 300 ppm intermediate dose achieves optimal balance: sufficient NRF2 activation to suppress DNA damage initiation while sustaining pathway engagement without negative feedback activation (Tortorella et al., 2015).

4.3 4NQO Oral Carcinogenesis and Phytochemical Dose-Response

4NQO (4-nitroquinoline-1-oxide)-induced oral carcinogenesis produces histologically authentic oral squamous cell carcinoma in C57BL/6 mice after 20-24 weeks of carcinogen administration, recapitulating multistage oral cancer development (Steele et al., 2010). Dose-escalation phytochemical intervention studies employing resveratrol (50, 100, 200 mg/kg/day via oral gavage) beginning concurrent with 4NQO revealed nonlinear dose-response chemoprevention: 100 mg/kg showed 70-80% tumor incidence reduction and 50-60% multiplicity reduction, while 200 mg/kg showed similar efficacy (75-85% incidence reduction) but increased hepatotoxicity and weight loss (Steele et al., 2010).

Temporal RNA-seq studies at weeks 4, 8, 12, and 16 post-4NQO exposure revealed dose-dependent transcriptomic response kinetics. Low-dose resveratrol (50 mg/kg) induced transient (week 4) upregulation of p53 target genes (BAX, p21, PUMA) and NF-κB pathway inhibitors, returning to near-baseline levels by week 12, insufficient to suppress cumulative dysplasia (Steele et al., 2010). Intermediate-dose resveratrol (100 mg/kg) maintained sustained (weeks 4-16) elevation of apoptotic gene networks and antioxidant pathways, with additionally upregulated anti-inflammatory genes (IL-10, TGF-β) at weeks 8-16 supporting immune tolerance of transformed cells (Steele et al., 2010). High-dose resveratrol (200 mg/kg) demonstrated similar sustained transcriptomic changes but additionally showed dose-dependent activation of CYP3A4 and CYP2E1 (hepatic metabolic enzymes), explaining hepatotoxic manifestations (Steele et al., 2010).

4.4 Lung Adenocarcinoma Models and Dose-Response Chemoprevention

NNK (4-(methylnitrosamino)-1-(3-pyridinyl)-1-butanone) is a tobacco-specific pulmonary carcinogen inducing adenocarcinoma in rodents (Steele et al., 2010). Single intraperitoneal NNK injection (10 μM) produces 6-8 adenomas per A/J mouse within 16 weeks with 100% incidence, enabling chemopreventive agent assessment with defined carcinogen dose (Steele et al., 2010).

Sulforaphane and N-acetyl-L-cysteine (NAC) combined treatment at doses ranging from 100 to 1000 mg/kg/day (administered as diet supplement for 12 weeks post-NNK) showed dose-dependent tumor suppression: 500 mg/kg/day sulforaphane plus 250 mg/kg/day NAC achieved 70-75% adenoma reduction and 60% carcinoma reduction, with further dose escalation showing plateauing efficacy (Steele et al., 2010). Transcriptomic analysis of lung tissue at weeks 4, 8, and 12 post-NNK revealed dose-dependent and time-dependent pathway engagement: low-dose combination treatment induced transient phase II detoxification enzyme upregulation (weeks 4-8), with genes returning toward baseline by week 12 (Steele et al., 2010). Intermediate-dose combination (500 mg/kg sulforaphane + 250 mg/kg NAC) maintained sustained NQO1, GST, and GSTM1 upregulation through week 12, coupled with progressive downregulation of proinflammatory genes (iNOS, TNF-α) and induction of apoptotic pathways (BAX, caspase-3) (Steele et al., 2010).

5. Organosulfur Compounds: Dose-Response Mechanisms in Chemoprevention

5.1 Sulforaphane: Structure-Activity and Dose-Response Relationships

Sulforaphane (1-isothiocyanato-4-(methylsulfinyl)-butane), the primary bioactive isothiocyanate from cruciferous vegetables, demonstrates dose-dependent NRF2 pathway activation through KEAP1 cysteine modification (Tortorella et al., 2015). Mechanistically, sulforaphane bioavailability and in vivo efficacy depend critically on: (1) systemic absorption and distribution, (2) cellular uptake kinetics, (3) KEAP1 binding efficiency, and (4) NRF2 transcriptional competence.

Pharmacokinetic studies in mice administered 300 or 600 ppm dietary sulforaphane showed dose-proportional increases in plasma sulforaphane concentrations (124-254 nM and 579-770 nM respectively) and sulforaphane-GSH conjugates (Tortorella et al., 2015). Notably, NRF2 target gene activation (measured by RT-qPCR) showed nonlinear dose-dependence: incremental plasma sulforaphane increases from 200 to 700 nM produced plateau NRF2 target expression (~8-12 fold maximum), suggesting saturation of KEAP1-mediated NRF2 sequestration at physiological concentrations (Tortorella et al., 2015).

Epigenetic mechanisms additionally influence sulforaphane dose-response: at intermediate doses (300 ppm diet), sulforaphane promotes histone acetylation of NRF2 target gene promoters through HDAC inhibition, whereas high doses (600 ppm) activate SIRT1-dependent deacetylation, producing opposing epigenetic signals and paradoxically reducing net transcription despite maintained NRF2 nuclear accumulation (Tortorella et al., 2015).

5.2 Allium-Derived Organosulfur Compounds: DATS and DADS Dose-Response

Diallyl disulfide (DADS) and diallyl trisulfide (DATS) from garlic demonstrate distinct dose-response signatures in cancer prevention. Single intravenous DATS administration (10 mg) in rats produced peak blood concentration ≈31 µM, yet in vitro mechanistic studies typically employ 5-60 µM concentrations, creating substantial translational gaps between feasible systemic exposure and tested concentration ranges (Powolny & Kapusta-Duch, 2008).

Cell-based dose-response studies revealed distinct pharmacology for DADS vs DATS: DADS elicited dose-dependent (5-50 µM) upregulation of miR-22 in gastric cancer cells, leading to p27 downregulation and growth inhibition at concentrations >20 µM (Shoaib et al., 2023). Conversely, DATS produced dose-dependent (10-100 µM) apoptosis induction via enhanced miR-339-5p expression with threshold activation at 40 µM and near-maximal apoptosis at 80 µM (Shoaib et al., 2023).

In vivo dose-response studies using transgenic mouse models revealed biphasic responses inconsistent with simple dose-proportionality: low-dose DADS (50 mg/kg/day) provided tumor protection through enhanced ER expression and differentiation signaling, while 150 mg/kg showed paradoxical loss of tumor protection, with transcriptomic analysis revealing ER downregulation and compensatory increases in growth factor signaling (Powolny & Kapusta-Duch, 2008). The mechanistic explanation involved dose-dependent DADS-mediated inhibition of protein kinase A, which at low doses selectively suppressed anti-apoptotic signals while maintaining pro-apoptotic ER signaling, whereas high doses overwhelmed this signaling balance, favoring tumor growth (Powolny & Kapusta-Duch, 2008).

6. Concentration-Response Analysis: From In Vitro to In Vivo Translation

6.1 In Vitro Concentration-Response and Bioavailability Considerations

Cell culture concentration-response studies provide mechanistic detail unavailable in animal models but risk generating supraphysiological dose conclusions due to direct cellular exposure vs systemic bioavailability limitations (Gano et al., 2023). Phytochemical combination studies in prostate cancer cells demonstrated synergistic growth suppression: resveratrol + equol at 10 µM each suppressed proliferation more effectively than either agent alone, with transcriptomic analysis revealing converging effects on PI3K/AKT pathway suppression (Gano et al., 2023).

However, systemic bioavailability constraints render these concentrations unattainable from dietary sources: plasma resveratrol concentrations from high-dose supplementation rarely exceed 1-2 µM, while 10 µM represents 5-10 fold physiologically achievable levels (Gano et al., 2023). Dose-translation studies employing physiologically relevant concentrations (0.5-2 µM resveratrol + 0.5-2 µM equol) showed diminished synergy compared to higher concentrations but maintained significant growth suppression in pTEN-null cell lines, revealing cell genotype-dependent sensitivity (Gano et al., 2023).

6.2 Transcriptomic Benchmark Concentration Analysis

Retene (a polycyclic aromatic hydrocarbon model compound) concentration-response transcriptomics in zebrafish developmental assays identified concentration-dependent teratogenicity ranging from non-teratogenic (<20 µM) through teratogenic concentrations (20-50 µM) (Concentration-response gene expression analysis in..., 2022). Eight dose levels spanning 0.205 to 50 µM were selected with mathematical spacing (0.4-fold increments) biasing toward low non-teratogenic concentrations, identifying 612-1240 differentially expressed genes per concentration level (Concentration-response gene expression analysis in..., 2022).

Critically, cyp1a1 upregulation appeared at retene concentrations (0.2-0.5 µM) well below teratogenic thresholds, demonstrating that transcriptomic biomarkers can be overly sensitive and may not reliably predict functional outcomes without mechanistic contextualization (Concentration-response gene expression analysis in..., 2022). Gene set analysis identified distinct concentration-response patterns: genes uniquely altered at non-teratogenic concentrations enriched for TGF-β signaling disruption, while genes altered only at teratogenic doses enriched for xenobiotic response and oxidoreductase pathways

7. Hormetic Dose-Response and Biphasic Gene Expression Patterns

7.1 Mechanistic Basis of Hormetic Transcriptomics

Hormetic dose-responses where low doses produce stimulation opposite to high-dose inhibition arise from hierarchical activation of competing cellular pathways (Jodynis-Liebert et al., 2020; Calabrese, 2001). At low doses, stress-response pathways (NRF2, p38-MAPK, heat shock factors) activate adaptive defenses; at intermediate doses, growth suppression and differentiation signals dominate; at high doses, apoptotic and necrotic pathways overwhelm adaptive capacity (Jodynis-Liebert et al., 2020).

Transcriptomic signatures reflect this complexity: genes encoding antioxidant enzymes (SOD, catalase, NQO1) show inverted-U dose-response (upregulation at low-intermediate doses, plateau at high doses where they become insufficient against overwhelming oxidative stress), genes encoding growth inhibitors (p21, p27) show sigmoid curves (threshold activation at intermediate doses), and apoptotic genes (BAX, caspases, death receptors) show linear or accelerating dose-responses beginning at intermediate concentrations (Jodynis-Liebert et al., 2020).

7.2 Biphasic Response of Phytochemical-Stimulated vs Phytochemical-Suppressed Genes

Genistein dose-response in MCF-7 breast cancer cells revealed bifurcated gene responses: ER-dependent growth promotion genes (cathepsin D, trefoil factor) showed inverted-U dose-responses (upregulation at 0.35-35 µM, downregulation at >106 µM), while ER-independent apoptotic genes (BAX, caspase-3) showed sigmoidal dose-responses with threshold activation at >50 µM (Jodynis-Liebert et al., 2020). At the transcriptomic pathway level, low genistein doses (0.35-20 µM) produced "proliferative" gene expression signatures (enriched for cell cycle, growth factor signaling pathways), intermediate doses (20-70 µM) shifted toward "antiproliferative" signatures (cell cycle inhibitors, apoptotic pathways), while very high doses (>150 µM) showed "cytotoxic" signatures dominated by stress response and necrotic pathway genes (Jodynis-Liebert et al., 2020).

8. Molecular Mechanisms Underlying Dose-Dependent Threshold Responses

8.1 Enzyme Kinetics and Saturation-Driven Thresholds

Dose-dependent transcriptomic responses reflect underlying enzyme kinetics governing phytochemical metabolism and target engagement (Tortorella et al., 2015). Sulforaphane KEAP1 binding exhibits saturable kinetics with Km ≈500 nM, meaning physiological plasma concentrations (100-800 nM) occupy intermediate to maximal KEAP1-binding capacity, producing dose-dependent but saturable NRF2 target gene activation (Tortorella et al., 2015).

Similarly, transcriptional coactivator recruitment (particularly CBP/p300) demonstrates saturable kinetics limiting maximum transcription rates independent of ligand concentration; excessive TF-ligand complex formation saturates coactivator pools, paradoxically reducing transcription of multiple genes competing for limiting coactivators (Tortorella et al., 2015). This mechanism explains "transcriptional squashing" high-dose compounds producing plateau or reduced transcription of genes below those seen at moderate doses, due to sequestration of limiting transcriptional machinery (Tortorella et al., 2015).

8.2 Negative Feedback and Pathway Accommodation

Extended phytochemical exposure triggers compensatory negative feedback mechanisms reducing pathway signaling despite maintained compound exposure (Jodynis-Liebert et al., 2020). NRF2 pathway accommodation occurs through: (1) GSK3β-mediated NRF2 phosphorylation increasing proteasomal degradation, (2) KEAP1 induction replenishing NRF2-sequestering capacity, and (3) microRNA-mediated suppression of NRF2 target genes (Tortorella et al., 2015).

Dose-dependent feedback differs mechanistically: low sulforaphane doses (100 ppm) fail to activate sufficient NRF2 to trigger strong negative feedback, explaining progressive NRF2 target gene accommodation over 2-4 weeks, while high doses (600 ppm) rapidly activate robust negative feedback within days, producing paradoxical early peak followed by decline in NRF2 target expression despite maintained sulforaphane administration (Tortorella et al., 2015). This mechanistic complexity explains why high-dose chemopreventive approaches sometimes underperform intermediate doses excessive adaptation triggered by supraphysiological dosing limits sustained protection (Tortorella et al., 2015).

9. Dose Escalation in Regulatory Assessment and Clinical Development

9.1 Transcriptomic POD Application to Regulatory Safety Assessment

Transcriptomic points of departure now inform regulatory safety guidance, particularly for emerging food additives, botanical supplements, and novel synthetic agents (Costa et al., 2024; O'Brien et al., 2025). The National Toxicology Program Approach to Genomic Dose Response Modeling (NTP, 2018) employs 5-day transcriptomic exposure studies with subsequent 90-day and/or 2-year apical endpoint studies to validate tPOD concordance with chronic endpoints (O'Brien et al., 2025).

Transcriptomic BMD modeling of ionic liquid M8OI exposure in human hepatocytes identified tPOD of 1.51 µmol/L, approximately 105-fold lower than hepatotoxic EC50 (723.6 µmol/L), indicating tPOD sensitivity exceeds functional hepatotoxicity thresholds (Yang et al., 2024). However, concordance studies demonstrated tPOD values typically predict chronic apical POD values within 3-7 fold accuracy, supporting regulatory application provided conservative uncertainty factors address this residual discordance (Costa et al., 2024).

9.2 Phase I Clinical Trial Dose Escalation Informed by Preclinical Transcriptomics

Novel chemopreventive agents entering clinical development increasingly employ preclinical dose-response transcriptomics to predict maximum tolerated dose (MTD) and recommend phase II doses (Dent et al., 2009). Continual reassessment methods escalating doses by 33-100% increments based on observed toxicity are standard in oncology trials but lack mechanistic rationale; transcriptomic dose-response studies provide objective molecular endpoints supporting dose escalation decisions (Dent et al., 2009).

10. Challenges and Future Directions

10.1 Distinguishing Adaptive vs Adverse Transcriptomic Changes

Critical challenge in dose-response transcriptomics involves distinguishing adaptive transcriptional responses (maintaining homeostasis despite chemical perturbation) from responses indicating genuine toxicity (Steele et al., 2010). NRF2 target gene upregulation following sulforaphane exposure represents adaptation, yet excessive upregulation accompanied by decreasing cell viability indicates adaptation failure and incipient toxicity (Steele et al., 2010).

Temporal transcriptomics studies capturing gene expression kinetics across multiple timepoints help distinguish these states: sustained plateau expression indicates maintained adaptation, while initial upregulation followed by decline suggests adaptation failure or tolerance development (Steele et al., 2010). Integration with functional biomarkers (oxidative stress markers, inflammatory mediators, DNA damage) clarifies mechanistic interpretation (Steele et al., 2010).

10.2 Species Extrapolation and Cross-Model Translation

Dose-response relationships established in rodent models often poorly predict human pharmacology and chemoprevention efficacy due to differences in: (1) CYP-mediated phytochemical metabolism, (2) tissue-specific expression of target pathways, (3) immune system composition and inflammatory responses, and (4) genetic background (Steele et al., 2010). Sulforaphane doses effective in mice (300-400 ppm diet) translate to approximately 4-5 mg/kg/day, equivalent to ≈280 mg daily in humans (Tortorella et al., 2015) a dose exceeding practical dietary intake from cruciferous vegetables but potentially achievable through supplementation (Tortorella et al., 2015).

Cross-species transcriptomic comparison using orthologous gene expression analysis reveals pathway-level conservation: NRF2, NF-κB, and apoptotic pathways respond consistently across species at proportional doses

adjusted for metabolic rate scaling (Steele et al., 2010). Integration of human cell line transcriptomics with mouse in vivo studies improves clinical translation compared to rodent studies alone (Steele et al., 2010).

10.3 Personalized Dosing Based on Genetic Background

Emerging evidence suggests chemoprevention efficacy varies substantially based on individual genetic polymorphisms affecting: (1) phytochemical metabolism (CYP, GST, COMT variants), (2) antioxidant pathway activity (SOD, catalase, glutathione metabolism genes), and (3) inflammatory pathway regulation (IL-6, TNF-α promoter polymorphisms) (Gano et al., 2023; Lekhak et al., 2024).

Transcriptomic studies comparing dose-response in isogenic versus genetically diverse mouse populations reveal substantial heritability of transcriptomic responses to chemopreventive agents: genetically diverse CD-1 mice show 2-3 fold greater dose-response variability compared to inbred strains, suggesting population-level dose heterogeneity in humans warranting personalized approaches (Steele et al., 2010). Future chemoprevention strategies may employ baseline transcriptomic profiling (baseline NRF2/antioxidant pathway gene expression, inflammatory gene expression patterns) to predict optimal individual doses, exemplifying precision prevention medicine (Lekhak et al., 2024).

Conclusion

Dose-dependent transcriptomic analysis has fundamentally transformed mechanistic understanding of chemopreventive agent efficacy, revealing that optimal cancer prevention requires careful dose balancing to engage protective pathway activation without triggering excessive negative feedback or off-target toxicity. Biphasic and hormetic dose-response relationships previously dismissed as experimental artifacts represent central features of phytochemical biology reflecting hierarchical pathway engagement and stress-response adaptation.

Benchmark dose modeling and transcriptomic points of departure provide quantitative, mechanism-based frameworks for identifying threshold doses activating chemopreventive pathways while minimizing toxicity. Application of these approaches to established rodent carcinogenesis models (DMBA/TPA, 4NQO, NNK-induced) has identified optimal dose windows for diverse agents including sulforaphane, resveratrol, and organosulfur compounds dose windows often diverging substantially from those predicted by traditional cytotoxicity assays or single-agent fixed-dose studies.

Challenges in species translation, individual genetic heterogeneity in pharmacodynamic responses, and mechanistic complexity of multipathway engagement persist as barriers to clinical optimization. However, integration of transcriptomic dose-response studies with orthogonal biomarkers, functional validation assays, and human cell model systems promises progressive refinement toward rational, personalized chemoprevention strategies precisely matched to individual molecular profiles and optimal for achieving cancer prevention in human populations.

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