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Review Article

Functional Interpretation of Differential Gene Expression in Cancer: From Lists to Mechanisms

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Abstract

High-throughput transcriptomic technologies have made differential gene expression analysis a cornerstone of cancer research by generating extensive lists of differentially expressed genes across tumor types and conditions. However, such lists provide limited biological insight without functional and mechanistic interpretation. This review examines the transition from descriptive gene expression profiles to a mechanistic understanding of cancer biology. We discuss integrative approaches that place expression changes within signaling pathways, transcriptional regulatory networks, and protein–protein interaction networks, thereby helping to identify functional modules and candidate upstream regulators. We emphasize the context-dependent nature of gene expression, which is shaped by genetic alterations, epigenetic landscapes, microenvironmental signals, and cellular heterogeneity. We also examine methodological advances, including gene set enrichment analysis, network-based modeling, and integration of genomic, epigenomic, proteomic, metabolomic, and single-cell transcriptomic data. Case studies across cancer types illustrate how mechanistic analyses can reveal context-specific transcriptional programs associated with oncogenic signaling, tumor suppression, metabolic reprogramming, epithelial–mesenchymal transition, and tumor–immune interactions. We highlight potential translational applications, including candidate biomarker discovery, prioritization of druggable targets, rational design of combination therapies, and investigation of therapeutic resistance. Finally, we discuss current challenges and emerging technologies, such as spatial transcriptomics and clustered regularly interspaced short palindromic repeats (CRISPR)-based perturbation screens, that are advancing the field toward dynamic, systems-level models of tumor biology. Integrating computational analyses with experimental validation can help translate transcriptomic data into clinically relevant hypotheses for precision oncology and personalized cancer therapy.

Introduction

The advent of high-throughput genomic technologies has revolutionized cancer research, generating an unprecedented volume of gene expression data. Microarrays and, more recently, RNA sequencing have enabled simultaneous profiling of thousands of genes, producing lists of differentially expressed genes (DEGs) across tumor subtypes, stages, and treatment conditions. Although these lists provide valuable initial observations, they offer limited functional insight without further interpretation. The central challenge is to translate differential expression into mechanistic insight by determining how changes in transcript abundance propagate through regulatory networks, alter cellular phenotypes, and may contribute to oncogenesis, metastasis, and therapeutic response.

Differential gene expression analysis has traditionally been used to identify candidate biomarkers and classify tumors into molecular subtypes. For example, early microarray studies in breast cancer identified gene sets that distinguish luminal from basal-like subtypes, providing prognostic information and informing treatment strategies.[1,2] Similar approaches have been applied to colorectal, lung, and hematologic malignancies. Despite their utility, gene lists have important limitations: they are context dependent, sensitive to sample heterogeneity, and generally do not establish causality or functional relationships. These limitations highlight the need for methods that move beyond enumeration toward functional and mechanistic interpretation.

Functional interpretation integrates gene expression data with

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complementary biological knowledge, including signaling pathways, transcriptional regulatory networks, protein–protein interactions, and epigenetic landscapes. Pathway enrichment analysis, Gene Ontology mapping, and network-based approaches have become standard tools for contextualizing differential expression and identifying coherent biological processes affected by transcriptional changes.[3-9] For instance, upregulation of glycolytic genes in a tumor subtype may suggest metabolic reprogramming, but mechanistic insight emerges only when these genes are linked to upstream regulators, interacting proteins, and changes in metabolic flux.[10-12] Similarly, identifying transcription factors whose target genes are overrepresented among DEGs can support inference of candidate upstream regulators and generate testable mechanistic hypotheses.

The complexity of cancer biology necessitates a multi-layered approach. Gene expression changes often reflect interactions among genetic mutations, epigenetic modifications, microenvironmental signals, and feedback from cellular signaling networks. For example, activation of the *KRAS* oncogene does not uniformly induce a fixed set of genes; instead, the downstream transcriptional response varies with tissue type, chromatin accessibility, and coexisting mutations. Likewise, stress responses, such as hypoxia or DNA damage, induce context-dependent transcriptional programs that may overlap with developmental or differentiation pathways.[13-16] Functional interpretation must therefore account for both intrinsic cellular context and extrinsic influences, requiring integrative, systems-level analyses.

Methods for functional interpretation have evolved substantially. Gene set enrichment analysis and related techniques map DEGs to curated pathway databases, revealing overrepresented biological processes.[3] Curated resources, including the Molecular Signatures Database, Gene Ontology, and Reactome, provide structured biological frameworks for enrichment analyses.[4-7] Network-based approaches, including coexpression networks, transcription factor–target networks, and protein–protein interaction networks, help identify modules of functionally related genes and support inference of regulatory relationships.[8,9] Single-cell RNA sequencing has further refined functional interpretation by resolving cellular heterogeneity and revealing context-specific gene programs that may be obscured in bulk analyses. Integration of genomic, transcriptomic, epigenomic, proteomic, and metabolomic data can provide a more comprehensive view of the regulatory architecture underlying differential expression and cancer phenotypes.[17-21]

Mechanistic interpretation of differential expression has important translational implications. Functional annotation of DEGs can help prioritize candidate drug targets, support prediction of therapeutic response, and suggest potential mechanisms of resistance. For example, enrichment of DNA repair pathway genes may indicate potential sensitivity to PARP inhibitors, whereas upregulation of immunosuppressive cytokine genes may suggest avenues for immunotherapy research. Network-based approaches can also identify hub genes and regulatory bottlenecks whose perturbation may have substantial effects, thereby informing rational drug design and combination-therapy strategies.

However, the functional relevance of many DEGs is context dependent and influenced by tissue of origin, tumor stage, the microenvironment, and cellular heterogeneity. Static pathway databases may not capture novel interactions, rewired networks, or noncanonical regulatory mechanisms active in specific cancer contexts. Moreover, statistical overrepresentation does not establish biological causality; genes may covary because of shared upstream regulators or stochastic transcriptional noise rather than functional interdependence. Addressing these challenges requires iterative cycles of computational prediction, experimental validation, and integration of multidimensional data to build models that more accurately represent the functional effects of gene expression changes. An overview of this workflow is presented in Figure 1.

While numerous reviews have described tools for differential gene expression analysis, this work specifically focuses on the critical transition from gene lists to biological mechanisms. We provide an integrative framework that connects differential expression results with pathway analysis, network modeling, multi-omics integration, and experimental validation, emphasizing their combined role in mechanistic interpretation and clinical translation.[17-19,22,23] This review highlights current limitations, proposes best practices, and outlines emerging strategies to improve the functional interpretation of transcriptomic data.

Mechanistic dissection and pathway interpretation of differential gene expression in cancer

Translating lists of DEGs into mechanistic insight requires a detailed understanding of the molecular and cellular pathways that govern tumor behavior.[3] Mechanistic interpretation integrates pathway knowledge, regulatory hierarchies, and systems-level interactions to move from descriptive enumeration toward mechanistically informed understanding.[24,25]

One foundational approach is to map DEGs onto well-characterized signaling pathways. Curated pathway databases, such as the Kyoto Encyclopedia of Genes and Genomes (KEGG), Reactome, Gene Ontology, and BioCarta, provide structured frameworks that link genes to canonical molecular cascades, thereby enabling identification of enriched biological processes.[4,6,7] For example, in colorectal cancer, a list of upregulated genes may show enrichment of WNT signaling components, suggesting a potential role in tumor initiation and stemness.[26-28] However, enrichment alone is insufficient for mechanistic interpretation; pathway directionality, regulatory dependencies, and context-specific modulation must also be considered. Pathways rarely operate in isolation. Crosstalk among the MAPK, PI3K–AKT, and JAK–STAT pathways, for example, can modulate transcriptional outcomes in ways that depend on tumor type, mutational background, and microenvironmental conditions.[29-31] Mechanistic dissection therefore requires integration of differential expression with functional interactions, kinetic parameters, and regulatory constraints.[24,25,32-34]

Transcription factor analysis is a critical step in mechanistic interpretation. DEGs often cluster as targets of specific transcription factors whose activity governs phenotypic outcomes.[35,36] Computational tools, such as motif enrichment analysis and regulon inference, allow the identification of transcriptional regulators that may be driving observed gene expression changes. In cancers driven by MYC or *TP53* dysregulation, for example, differential expression often

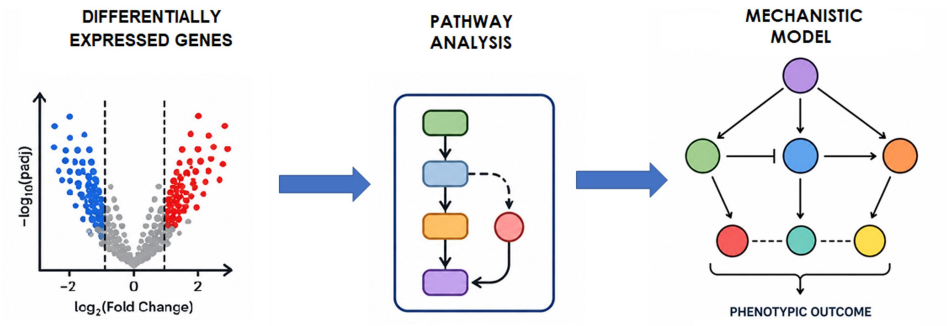

Fig. 1. Workflow from differential gene expression to mechanistic insight. This figure summarizes the analytical workflow used to transform differentially expressed gene (DEG) data into biologically meaningful mechanistic insights. The process begins with transcriptomic profiling and identification of DEGs between experimental conditions, followed by pathway enrichment and gene set analyses to identify dysregulated biological processes. Network-based approaches are subsequently applied to uncover gene interactions and key regulatory hubs associated with the observed phenotype. Integration with complementary omics datasets, including proteomics and epigenomics, further refines biological interpretation and improves mechanistic resolution. Finally, candidate pathways and regulatory mechanisms are experimentally validated and assessed for their translational and clinical relevance.

converges on transcriptional modules that reflect the downstream regulatory influence of these oncogenes or tumor suppressors.[37] Mechanistic insight emerges by connecting the observed DEGs to the activity state of these transcription factors, integrating cofactor availability, chromatin accessibility, and post-translational modifications that modulate DNA-binding affinity and transcriptional output.

Network-based approaches provide a complementary perspective, emphasizing the modular and interconnected nature of cellular regulation.[8,9] Co-expression networks, constructed from correlation patterns among genes across samples, reveal clusters or modules that often correspond to functionally coherent processes such as cell cycle regulation, DNA repair, or immune signaling. Mapping DEGs onto these networks can identify modules disproportionately affected in specific tumor contexts, highlighting mechanistic hubs and regulatory bottlenecks. Protein–protein interaction networks further refine this analysis by providing physical interaction context, identifying key nodes whose perturbation may disproportionately affect network stability and cellular phenotypes. Mechanistic inference is enhanced when network topology is combined with differential expression magnitude and directionality, allowing prioritization of candidate driver genes within broader functional modules.[38]

Functional interpretation also benefits from integrating temporal and spatial dimensions of gene expression.[39-42] Cancer progression and therapy response are dynamic processes; transcriptional programs evolve over time in response to intrinsic genetic changes and extrinsic stimuli.[43,44] Longitudinal sampling, coupled with differential expression analysis at multiple time points, can identify early and late response genes, revealing temporal order in regulatory cascades. Spatially resolved transcriptomics adds another layer, showing how local microenvironment modulates transcriptional programs in a context-dependent manner.[39,41,42] Genes upregulated only in hypoxic tumor regions, for instance, may reflect activation of HIF-driven metabolic reprogramming, whereas genes expressed at tumor–stroma interfaces may mediate invasive or immunomodulatory programs.[45,46] Mechanistic dissection therefore requires not only pathway and network mapping but also careful consideration of the temporal and spatial context in which gene expression changes occur.

Post-transcriptional regulation represents another critical component in mechanistic interpretation. Differential gene expression is shaped not only by transcriptional control but also by RNA stability, splicing, and translation efficiency. MicroRNAs and RNA-binding proteins modulate mRNA abundance and stability, often in a context-dependent manner.[47] For example, overexpression of specific microRNAs in breast cancer can selectively repress tumor suppressor transcripts, amplifying oncogenic signaling pathways. Similarly, alternative splicing events can generate isoforms with distinct functional properties, altering signaling dynamics, metabolic flux, or interaction networks.[48]

Tumor cells frequently reprogram metabolic gene expression to meet the demands of rapid proliferation, resist oxidative stress, or adapt to nutrient-depleted microenvironments.[34,49] Differential expression of glycolytic enzymes, mitochondrial transporters, and lipid metabolism regulators often occurs in concert, reflecting coordinated transcriptional programs. Mechanistic interpretation involves linking these transcriptional changes to flux through metabolic pathways, regulatory feedback from signaling networks, and potential vulnerabilities that can be therapeutically exploited. In pancreatic and colorectal cancers, for example, differential expression of glutamine transporters and enzymes controlling anaplerotic reactions reflects context-specific metabolic adaptation driven by *KRAS* signaling and hypoxic stress, highlighting mechanistic connections between transcriptional changes and metabolic phenotypes.[50-52]

Finally, integrating mechanistic interpretation with experimental perturbation studies can validate functional relevance. RNA interference, CRISPR-mediated gene editing, CRISPR screens, and Perturb-seq allow hypotheses generated by differential expression and pathway mapping to be tested.[53-55] Genes that are highly connected within networks, enriched in

pathway modules, or identified as candidate upstream regulators of DEGs can be systematically perturbed to assess their roles in tumor phenotypes. Such iterative computational and experimental approaches refine mechanistic models and help distinguish passenger transcriptional changes from driver events that orchestrate phenotypic outcomes. Figure 2 illustrates the integration of multi-omic data for mechanistic interpretation.

In summary, mechanistic dissection and pathway interpretation transform differential gene expression data from descriptive lists into functional, mechanistically informed frameworks that help explain how tumors acquire and maintain malignant phenotypes (Table 1).

Case studies in oncogenic processes and tumor subtypes

The following case studies highlight how functional dissection transforms gene lists into mechanistic understanding, revealing both common principles and context-specific regulatory features across tumor types. This section explores representative examples, including oncogene-driven transcriptional programs, tumor suppressor networks, metabolic reprogramming, epithelial–mesenchymal transition, and tumor–immune interactions across diverse cancer subtypes.

KRAS-driven transcriptional programs in pancreatic and colorectal cancer

KRAS mutations are highly prevalent in pancreatic ductal adenocarcinoma and a subset of colorectal cancers, yet their downstream transcriptional consequences are highly context dependent. In pancreatic ductal adenocarcinoma, differential expression analysis reveals marked upregulation of genes controlling glycolysis, nucleotide biosynthesis, and macropinocytosis, consistent with KRAS-driven metabolic reprogramming that supports proliferation under hypoxic and nutrient-limited conditions.[56] Mechanistic interpretation links these transcriptional changes to MAPK and PI3K–AKT signaling cascades and to MYC and HIF-1α transcription factors, which regulate metabolic enzymes such as HK2, LDHA, and GLS.[57]

By contrast, in colorectal cancer, *KRAS* mutations alone may be insufficient to activate the full metabolic transcriptional program observed in pancreatic tumors. Additional co-occurring mutations in *APC* or *TP53* are typically required to induce comparable proliferative and metabolic adaptation.[58] Network analysis suggests that KRAS-driven transcriptional modules in colorectal tumors show substantial crosstalk with WNT signaling and immunomodulatory pathways, illustrating how context-specific co-occurring mutations can reshape transcriptional networks and functional outcomes.[59] These observations underscore the need for mechanistic dissection: the same oncogenic mutation can produce divergent transcriptional landscapes depending on tumor context, lineage, and coexisting genetic alterations.[60] Context-dependent *KRAS* signaling is illustrated in Figure 3.

TP53 loss and context-dependent regulatory effects

The tumor suppressor *TP53* exemplifies how differential gene expression integrates with context-specific regulatory programs. *TP53* loss alters hundreds of target genes, but the functional consequences vary dramatically across tissue types.[61] In epithelial tumors, *TP53* inactivation often leads to upregulation of survival and antiapoptotic genes, such as *BCL2* and *MCL1*, contributing to evasion of programmed cell death.[62] In hematopoietic malignancies, *TP53* loss preferentially impacts differentiation-associated transcriptional programs, resulting in accumulation of immature progenitor cells and leukemogenesis.[63]

Mechanistic dissection of TP53-dependent expression integrates transcriptional networks with epigenetic states and cofactor availability. Mutant p53 proteins may acquire gain-of-function activities, engaging noncanonical transcriptional targets and reprogramming chromatin landscapes to promote invasion, stemness, or metabolic adaptation.[64] For instance, in breast cancer, mutant p53 interacts with ETS2 and NF-Y transcription factors to drive genes involved in proliferation and extracellular matrix remodeling, illustrating the power of mechanistic interpretation to distinguish canonical tumor suppressor effects from context-specific oncogenic rewiring.[65]

MYC and tissue-specific transcriptional dependencies

MYC is a pleiotropic oncogenic transcription factor that controls cell cycle progression, metabolism, and ribosome biogenesis.[66,67] Differential expression analyses reveal that MYC-induced gene programs vary with tissue origin and differentiation state.[68] In neural progenitor-derived tumors, MYC preferentially activates cell cycle and neuronal differentiation genes, whereas in hematopoietic malignancies, MYC promotes metabolic gene networks and suppresses differentiation signals.[68,69] Mechanistic interpretation incorporates network topology, cofactor interactions, and chromatin accessibility, allowing identification of context-specific MYC target modules that are critical for proliferation and survival.[67] Such insights have direct translational relevance: therapies that disrupt MYC-driven transcriptional hubs can be tailored to the specific context of the tumor, maximizing efficacy while minimizing systemic toxicity.[66]

Metabolic reprogramming in hypoxic tumor microenvironments

Tumor hypoxia drives context-dependent transcriptional adaptation, prominently through HIF-1α and HIF-2α transcription factors.[70,71] Differential gene expression under hypoxic conditions reveals coordinated upregulation of glycolytic enzymes, glucose transporters, and angiogenic factors.[72] Mechanistic interpretation links these DEGs to functional outcomes: increased glycolytic flux, lactate production, and vascular remodeling, which collectively enhance tumor survival and growth under low-oxygen conditions.[71,73] Importantly, metabolic transcriptional programs vary by tissue context; renal cell carcinomas, for example, exhibit stronger HIF-2α-driven angiogenic signatures, whereas glioblastomas preferentially activate HIF-1α-mediated glycolytic pathways.[71,74] Integrating pathway analysis with flux modeling and epigenetic context reveals mechanistic principles governing metabolic adaptation and identifies potential metabolic vulnerabilities for therapeutic exploitation.[72]

Epithelial-mesenchymal transition and invasion

Epithelial–mesenchymal transition (EMT) exemplifies the complexity of context-dependent gene expression.[75,76] EMT-associated DEGs vary widely across tumor types and environmental conditions, yet they converge functionally on enhanced motility, invasion, and stemness.[77,78] Mechanistic dissection links key transcription factors such as SNAIL, ZEB1,

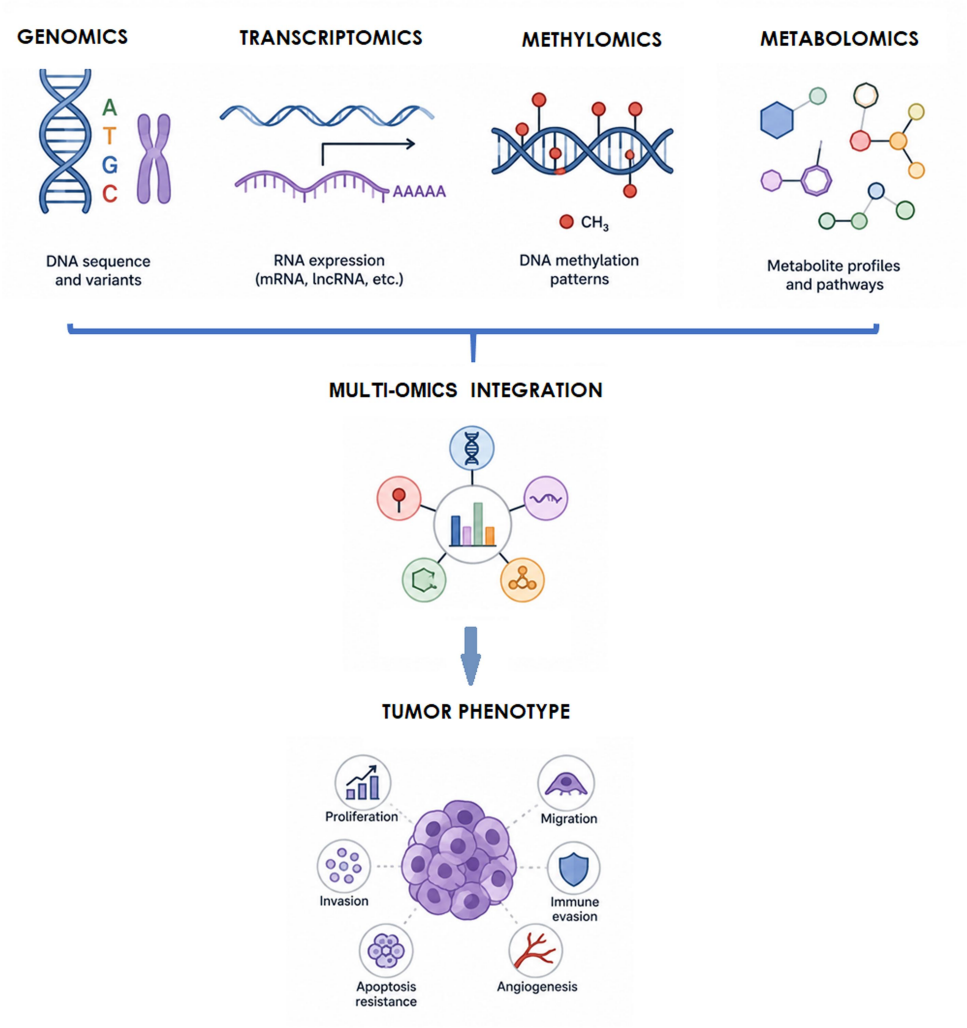

Fig. 2. Integration of multi-omics data for mechanistic understanding. This figure illustrates the integration of transcriptomic data with additional omics layers, including proteomics, epigenomics, and metabolomics, to enhance mechanistic interpretation of biological systems. Each omics platform provides complementary information regarding cellular regulation and functional states, enabling a more comprehensive reconstruction of molecular networks. The figure highlights key integration points, such as correlations between gene expression and protein abundance or the relationship between epigenetic modifications and transcriptional regulation. It also emphasizes analytical challenges associated with multi-omics integration, including data heterogeneity and the need for computational frameworks capable of harmonizing diverse datasets. The figure underscores the importance of multi-omics approaches for achieving systems-level biological understanding. lncRNA, long non-coding RNA; mRNA, messenger RNA.

and TWIST to downstream target genes governing cytoskeletal reorganization, extracellular matrix remodeling, and cell adhesion.[79,80] In breast cancer, EMT programs are spatially heterogeneous, with cells at the invasive front expressing distinct transcriptional modules compared to those in the tumor core.[81,82] Network modeling further reveals that EMT is not a single linear pathway but a modular and reversible process regulated by context-specific signaling inputs, including TGF-β, WNT, and NOTCH, highlighting opportunities for targeted therapeutic intervention.[83,84]

Tumor-immune interactions and contextual gene programs

Differential gene expression also informs mechanistic understanding of tumor–immune interactions.[85-87] Tumors exhibit context-specific expression of cytokines, chemokines, and immune checkpoint ligands, shaping local immune landscapes.[88-90] For instance, melanoma subtypes with high expression of interferon-stimulated genes demonstrate enhanced T-cell infiltration and increased responsiveness to checkpoint blockade,[91,92] whereas tumors expressing immunosuppressive factors such as *IDO1* or *TGFB1* create a hostile microenvironment.[93,94] Integrating differential expression with pathway and network analyses identifies key regulatory nodes that modulate immune evasion, providing mechanistic insight into therapeutic response and resistance.

Table 1. Comparison of approaches for functional interpretation of gene expression data

Method	Main use	Advantages	Limitations
Over-representation analysis	Identifies overrepresented biological pathways from DEG lists	Simple, fast, widely used; easy biological interpretation	Depends on arbitrary DEG thresholds; ignores gene–gene interactions; biased by database annotations
Gene set enrichment analysis	Detects coordinated changes in predefined gene sets without strict DEG cutoff	Uses full dataset; more sensitive to subtle changes; reduces threshold bias	Requires large sample sizes; dependent on gene set quality; may yield broad results
Network analysis (e.g., protein–protein interaction and coexpression networks)	Identifies key regulatory hubs and gene interactions	Captures system-level organization; highlights key drivers (hub genes)	Network quality depends on input data; may include false-positive interactions; complex interpretation
Transcription factor analysis	Identifies upstream regulators controlling gene expression changes	Provides mechanistic insight into gene regulation; links DEGs to regulatory programs	Limited by incomplete TF databases; indirect inference; context-dependent activity
Multi-omics integration	Combines transcriptomics with proteomics, epigenomics, metabolomics	Provides comprehensive biological insight; improves mechanistic understanding	Data integration is computationally complex; requires matched datasets; potential batch effects
Single-cell RNA sequencing analysis	Resolves gene expression at the individual cell level	Identifies cellular heterogeneity; detects rare cell populations; high resolution	Expensive; technical noise (dropouts); complex data processing
Spatial transcriptomics	Maps gene expression within tissue architecture	Preserves spatial context; links molecular data with histology	Lower resolution (in some platforms); high cost; emerging analytical tools
CRISPR-based functional validation	Experimentally tests gene function and causal roles	Provides direct evidence of gene function; supports target validation	Time-consuming; not scalable for all candidates; may not capture complex interactions

This table summarizes commonly used approaches for the functional interpretation of differential gene expression data, highlighting their primary applications, strengths, and current limitations. Integrating multiple methods is often necessary to achieve robust mechanistic insights. CRISPR, clustered regularly interspaced short palindromic repeats; DEG, differentially expressed gene; GSEA, gene set enrichment analysis; ORA, over-representation analysis; PPI, protein–protein interaction; TF, transcription factor.

Integration with multi-omic data and single-cell perspectives

Mechanistic interpretation of differential gene expression is strengthened by integrating complementary biological layers, including genomics, epigenomics, proteomics, metabolomics, and single-cell transcriptomics.[17-19] Multi-omic integration can help connect DEGs to upstream genetic and epigenetic drivers, post-transcriptional regulation, protein activity, and downstream phenotypic outputs, thereby supporting construction of comprehensive mechanistic models of cancer biology.[17,18,22,95,96]

Genomic integration

Genomic alterations, including single-nucleotide variants, copy-number changes, and structural rearrangements, influence transcriptional output.[97,98] Mapping DEGs onto mutational landscapes can help identify context-dependent transcriptional consequences of driver mutations.[99] For example, *TP53* or *KRAS* mutations produce distinct gene expression programs depending on tissue type, co-occurring mutations, and chromatin accessibility.[100,101] Integrating whole-genome or exome sequencing with expression data can support hypotheses about causal relationships and help distinguish direct effects of mutations from secondary transcriptional changes.[58,102] This approach can also facilitate identification of context-specific synthetic-lethal interactions in which combined perturbation of a mutated gene and a transcriptionally upregulated target selectively compromises tumor-cell survival.[103]

Epigenomic and chromatin context

Chromatin accessibility, DNA methylation, and histone modifications define the regulatory landscape that permits or restricts transcription.[104,105] Assay for transposase-accessible chromatin using sequencing, chromatin immunoprecipitation sequencing, and bisulfite sequencing provide detailed information about enhancers, promoters, and epigenetic modifications influencing gene expression.[106,107] Differential expression can therefore be interpreted mechanistically in the context of epigenetic states: genes upregulated in tumors often correspond to regions of open chromatin or hypomethylated promoters, whereas silenced genes are frequently associated with repressive histone marks.[108,109]

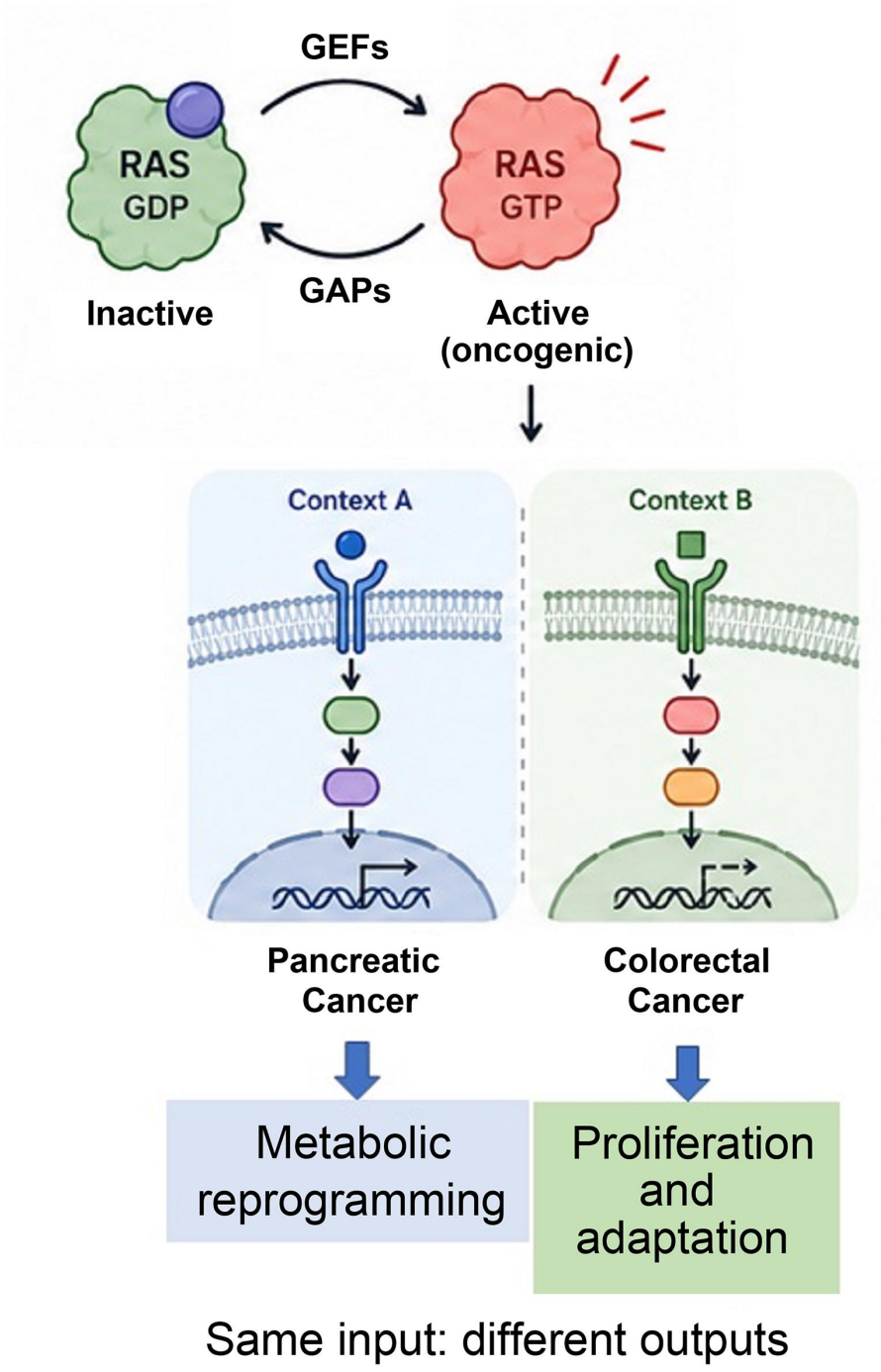

Fig. 3. Context-dependent signaling in KRAS-mutated cancers. This figure illustrates how oncogenic *KRAS* signaling produces distinct biological outcomes depending on tissue context and microenvironmental conditions. Activating *KRAS* mutations lead to constitutive engagement of downstream pathways, including the RAF–MEK–ERK (MAPK) and PI3K–AKT–mTOR signaling cascades. However, the intensity and functional consequences of these signaling outputs vary across tumor types and cellular environments. In pancreatic cancer, *KRAS* signaling cooperates with inflammatory and stromal cues to promote metabolic reprogramming and tumor progression. In colorectal cancer, feedback inhibition and lineage-specific transcriptional programs modulate WNT signaling and immune pathway activity, thereby influencing proliferation, differentiation, and tissue adaptation. The figure emphasizes that *KRAS* mutations do not generate a single uniform signaling state but instead produce context-dependent signaling programs that contribute to phenotypic

heterogeneity and variable therapeutic responses. AKT, protein kinase B; ERK, extracellular signal-regulated kinase; GAPs, GTPase-activating proteins; GDP, guanosine diphosphate; GEFs, guanine nucleotide exchange factors; GTP, guanosine triphosphate; KRAS, Kirsten rat sarcoma viral oncogene homolog; MAPK, mitogen-activated protein kinase; MEK, mitogen-activated protein kinase kinase; mTOR, mechanistic target of rapamycin; PI3K, phosphoinositide 3-kinase; RAF, rapidly accelerated fibrosarcoma kinase; RAS, rat sarcoma viral oncogene homolog; WNT, wingless-related integration site.

Integrating epigenomic data with differential expression enables identification of upstream regulators, enhancer networks, and transcription factor binding events that orchestrate context-dependent gene programs, providing mechanistic insight into both activation and repression of target genes.[110]

Proteomic integration

Transcript levels do not always correlate with protein abundance due to post-transcriptional regulation, translation efficiency, and protein stability.[111] Mass spectrometry–based proteomics, including quantitative and phosphoproteomics, complements transcriptomic data by revealing functional protein abundance, activation states, and signaling pathway engagement.[112,113] Integrating proteomic data with DEGs allows refinement of mechanistic models, highlighting which transcriptional changes result in functional protein alterations and downstream pathway activation.[114,115] For example, upregulation of mRNA encoding a kinase may only be functionally relevant if the kinase is phosphorylated or stabilized, information obtainable through proteomic profiling.[116,117]

Metabolomic context

Metabolic reprogramming is a hallmark of cancer, and transcriptional changes frequently reflect adaptations in nutrient utilization, energy production, and biosynthetic pathways.[118,119] Integrating differential expression with metabolomic profiling enables mechanistic interpretation of gene programs in functional terms.[120,121] For instance, upregulation of glycolytic genes in hypoxic tumors correlates with increased lactate production, acidification of the microenvironment, and altered metabolic flux.[60] Multi-omic integration allows identification of context-specific metabolic dependencies, informing therapeutic targeting of metabolic enzymes, transporters, or cofactor pathways that could be critical for tumor survival.[122-124]

Single-cell transcriptomics

Single-cell RNA sequencing (scRNA-seq) provides a transformative perspective on differential expression by resolving heterogeneity within tumors.[125-130] Bulk RNA sequencing averages gene expression across thousands of cells, obscuring rare but functionally important subpopulations.[131,132] Single-cell analyses uncover context-dependent gene programs associated with specific lineages, differentiation states, or microenvironmental niches.[126,127,133,134] For example, in glioblastoma, scRNA-seq reveals coexisting proliferative, hypoxic, and stem-like transcriptional programs within the same tumor, each exhibiting distinct regulatory networks and functional phenotypes.[135] Integrating scRNA-seq with pathway and network analysis may help identify context-specific drivers and potential therapeutic vulnerabilities in rare but clinically relevant subpopulations.[136]

Spatial transcriptomics and microenvironmental context

Spatially resolved transcriptomics complements single-cell approaches by preserving tissue architecture, revealing how local microenvironmental cues influence differential gene expression.[39-42] Genes upregulated at invasive fronts, hypoxic regions, or tumor–stroma interfaces often reflect context-dependent signaling influenced by neighboring cells and extracellular matrix components.[137,138] Mechanistic interpretation combines spatial localization with network and pathway analysis, connecting microenvironmental signals to transcriptional programs that regulate invasion, angiogenesis, or immune evasion.[139,140] For example, tumors with spatially segregated immunosuppressive and proliferative niches exhibit differential expression of chemokines, cytokines, and immune checkpoint molecules that govern local immune response and therapeutic sensitivity.[141,142]

Multi-omic network modeling

Integrating diverse omic layers into network models provides a systems-level view of context-dependent gene expression.[8,9,19] Regulatory networks that incorporate transcription factors, epigenetic modifiers, protein–protein interactions, and metabolic nodes provide a framework for examining how perturbations propagate through a system.[143] Differential expression can be mapped onto these networks to identify functional modules, hub regulators, and context-specific vulnerabilities.[144] Computational methods, including Bayesian networks, dynamic simulations, and machine learning, can support inference of putative causal relationships, identification of candidate driver genes, and characterization of compensatory pathways that may mediate therapy resistance.[145,146] Multi-omic integration thus transforms DEGs from static observations into dynamic mechanistic models that can guide experimental validation and prioritize therapeutic hypotheses.[9,17,19,22,40]

Translational implications

Context-specific DEGs linked to genetic, epigenetic, and proteomic features may help identify candidate biomarkers predictive of drug response, resistance, or disease progression.[147-149] For example, integrating transcriptional and epigenomic profiles can reveal transcription factor dependencies that predict sensitivity to targeted inhibitors, while metabolomic and proteomic integration may identify context-specific metabolic vulnerabilities.[114,123] Single-cell and spatial analyses can further help identify rare, therapy-resistant subpopulations, guiding rational combination therapies designed to preempt adaptive responses.[135] Mechanistic insights derived from multi-omic integration thus enable personalized treatment strategies that account for both intrinsic tumor heterogeneity and extrinsic microenvironmental influences.[150,151]

Translational and therapeutic implications of functional interpretation of differential gene expression

Functional interpretation of differential gene expression is both a

fundamental scientific endeavor and an important component of translational oncology.[152] By linking transcriptomic alterations to mechanistic pathways, regulatory networks, and cellular phenotypes, researchers can prioritize candidate actionable targets, support prediction of therapeutic responses, and develop personalized intervention strategies.[8] Mechanistic dissection can inform drug discovery, combination-therapy design, biomarker development, and adaptive treatment planning while accounting for the context-dependent nature of gene regulation in cancer.[17] Figure 4 summarizes the steps required for clinical translation.

Biomarker discovery and patient stratification

When integrated with pathway and network analyses, differential expression analysis can help identify candidate biomarkers associated with prognosis, therapeutic response, or disease progression.[3,153] Traditional gene expression signatures often rely on static DEG lists; mechanistic integration may instead reveal functionally relevant modules that more accurately represent tumor biology.[144,147] For instance, enrichment of DNA damage response genes may indicate potential susceptibility to PARP inhibitors,[154] whereas activation of immunosuppressive cytokine networks may be associated with a poor response to immune checkpoint blockade.[85,86,94] Multi-omic integration may improve biomarker reliability by combining transcriptomic, epigenomic, and proteomic profiles to capture the regulatory context that determines whether a DEG is functionally consequential.[149] Consideration of context-specific transcriptional programs may therefore improve patient stratification and help identify subgroups more likely to benefit from targeted therapy or immunotherapy.[147]

Identification of druggable targets

Mechanistic interpretation of differential gene expression can facilitate identification of druggable nodes within regulatory and signaling networks.[146] Core genes, transcription factors, or pathway bottlenecks that coordinate context-specific programs could represent high-value therapeutic targets.[8,144] For example, in KRAS-driven tumors, network analysis may reveal dependence on downstream effectors such as MEK or PI3K, suggesting combination therapies that exploit pathway vulnerabilities.[101,155] Similarly, transcription factors regulating metabolic reprogramming or EMT can be targeted indirectly through inhibitors of cofactors, epigenetic regulators, or upstream signaling pathways.[75,123] By prioritizing targets based on mechanistic and context-specific relevance rather than mere differential expression, researchers can improve the likelihood of therapeutic efficacy and reduce off-target toxicity.[9]

Rational design of combination therapies

Cancer cells frequently exhibit adaptive plasticity, activating compensatory pathways that circumvent single-agent therapies.[156] Functional interpretation of DEGs provides insight into these adaptive mechanisms by revealing context-dependent regulatory modules.[8,9] Mechanistic integration enables rational design of combination therapies that simultaneously target primary oncogenic pathways and adaptive resistance modules. In breast cancer, co-targeting HER2 signaling and downstream PI3K/AKT effectors, informed by transcriptomic and proteomic profiles, exemplifies a context-specific combination strategy grounded in mechanistic interpretation.[114,157]

Predicting and overcoming resistance

Therapeutic resistance often emerges from context-dependent transcriptional reprogramming. Longitudinal differential expression analysis, combined with mechanistic dissection, can identify genes and pathways activated in response to therapy, revealing early indicators of resistance.[135,136] For example, upregulation of epithelial-mesenchymal transition genes, anti-apoptotic factors, or drug efflux transporters can signal impending resistance to chemotherapeutic or targeted agents.[75] By mapping these DEGs onto regulatory networks, researchers may be able to predict compensatory pathways and design preemptive interventions.[144] Integration with single-cell and spatial transcriptomics further elucidates heterogeneity in resistance mechanisms, enabling targeted strategies against rare, therapy-refractory subpopulations.[141]

Precision oncology and personalized medicine

Mechanistic interpretation may enable differential expression profiles to inform individualized therapeutic decisions within precision oncology frameworks.[152,158,159] Context-dependent gene programs, informed by multi-omic integration, delineate tumor vulnerabilities unique to a patient's tumor profile.[115,127] For example, a tumor exhibiting high transcriptional activity in DNA damage response and cell cycle checkpoints may respond to inhibitors of ATR or CHK1,[154] whereas a tumor with strong EMT and invasion signatures may benefit from anti-metastatic or epigenetic interventions.[75] Spatial and single-cell analyses provide additional resolution, identifying niche-specific vulnerabilities that can inform localized or combination therapies.[135] This systems-level understanding could transform DEGs from descriptive markers into actionable mechanistic guides for personalized treatment.[160]

Integration with clinical trial design

Mechanistic insights from differential expression analysis can inform patient selection, trial stratification, and endpoint determination in clinical studies.[152,161] Identifying transcriptional modules or network dependencies linked to drug sensitivity allows enrichment of trial populations most likely to benefit, enhancing statistical power and therapeutic relevance.[3] Furthermore, monitoring context-dependent transcriptional changes during treatment provides dynamic biomarkers for adaptive trial designs, enabling real-time adjustments based on emergent resistance programs.[156] This approach bridges basic mechanistic understanding with translational application, ensuring that functional interpretation directly impacts clinical decision-making.[127]

Challenges and opportunities

While the translational potential of mechanistic interpretation is vast, challenges remain.[162,163] Tumor heterogeneity, both interpatient and intrapatient, complicates identification of universally actionable targets.[135] Context-specific transcriptional programs can differ dramatically between tissues, subtypes, or microenvironmental niches, necessitating integration of multi-omic, spatial, and single-cell data.[39] Despite these challenges, advances in computational modeling, high-throughput perturbation platforms, and precision profiling technologies

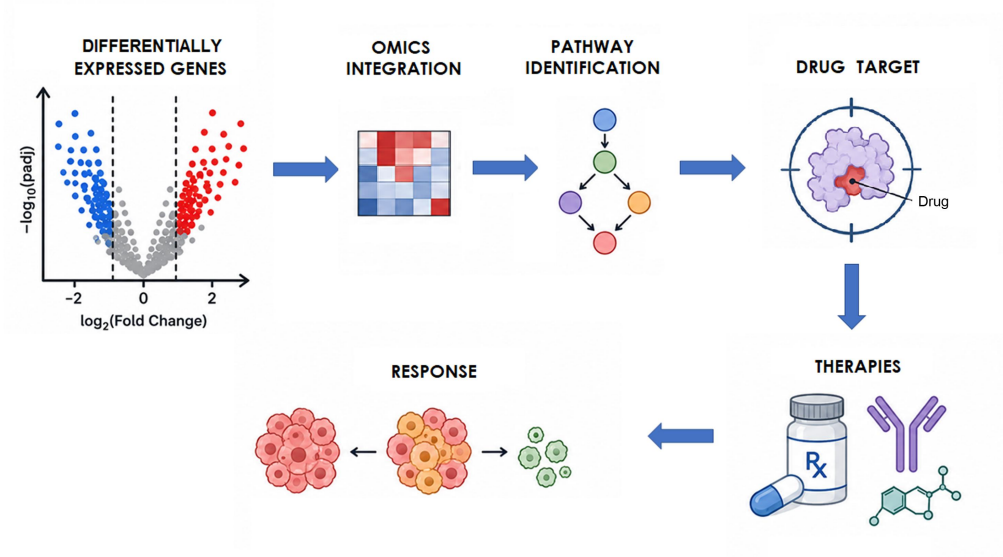

Fig. 4. Clinical translation of functional genomics findings. This figure summarizes the translational pathway through which functional genomics discoveries may contribute to clinical applications. Mechanistic insights derived from analyses of differentially expressed genes (DEGs) and integrative multi-omics approaches can support biomarker discovery, patient stratification, and identification of therapeutic targets. The figure also highlights the intermediate stages required for clinical implementation, including validation in independent patient cohorts, preclinical functional studies, and prospective clinical trials. In addition, it emphasizes that although functional genomics approaches hold considerable promise for precision medicine, successful clinical translation requires rigorous validation and careful consideration of disease-specific clinical contexts. Abbreviation: DEG, differentially expressed gene.

provide unprecedented opportunities to exploit mechanistic insights from differential expression for therapeutic gain.[148]

Limitations

Despite substantial advances in the functional interpretation of differential gene expression data, several limitations continue to affect the robustness, reproducibility, and biological relevance of current approaches. One major limitation is the reliance on arbitrary thresholds for defining DEGs. Common cutoffs based on fold change and statistical significance may exclude biologically relevant genes with modest expression changes while including false positives driven by technical variability.[164,165] Because downstream analyses such as pathway enrichment and network construction depend heavily on DEG selection, these threshold choices can significantly influence biological interpretation. In addition, pathway and gene set databases remain incomplete and biased toward well-characterized biological processes. Resources such as KEGG, Reactome, and Gene Ontology are continuously evolving and require ongoing curation and updating.[6,7]

Network-based approaches also present important limitations. Many protein–protein interaction and regulatory networks rely on predicted or aggregated interactions that may not accurately reflect the biological context under investigation. Consequently, inferred hub genes do not always correspond to true functional drivers, particularly in heterogeneous systems.[166] The integration of multiple omics layers introduces additional analytical complexity. Although multi-omics approaches provide a broader systems-level perspective, differences in data structure, scale, and quality complicate integration and interpretation. Common challenges include batch effects, missing data, and the lack of standardized computational frameworks.[17]

Limited reproducibility and validation are additional concerns. Many computational findings are not sufficiently validated in independent datasets or experimental systems, and variation among analytical pipelines frequently contributes to inconsistent results across studies.[167]

Future directions

Emerging technologies and methodological advances are expected to substantially improve the functional interpretation of gene expression data and its translation into biological and clinical applications. Artificial intelligence and machine learning approaches represent particularly promising developments for functional genomics and computational oncology.[168] These methods enable the integration of large-scale datasets and facilitate the identification of complex biological relationships that may not be detected using conventional analytical strategies.[168-170]

Advances in single-cell and spatial transcriptomics are also transforming the field by providing higher-resolution insights into gene expression patterns and cellular heterogeneity.[39,40,42,125-127] Single-cell RNA sequencing enables characterization of distinct cellular populations,[128,134] while spatial transcriptomics preserves tissue architecture and molecular context.[39,41,42]

The continued development of multi-omics integration strategies will further enhance mechanistic understanding of biological systems and translational systems oncology.[17,150,151] Future efforts will likely focus on improving computational frameworks, data standardization, and reproducibility across platforms.[17] In parallel, increasing emphasis is being placed on functional validation. Genome-editing technologies such as

CRISPR-Cas9 and Perturb-seq provide powerful tools for directly testing gene function and causal relationships beyond correlative analyses.[53-55]

Finally, the translation of functional genomics into clinical practice remains a major objective. Integrative and systems-level approaches may support biomarker discovery and precision medicine strategies, although successful implementation will require rigorous validation, standardized methodologies, and closer integration between computational and clinical research.[171,172]

Methodological limitations of this review

This review has several methodological limitations that should be considered when interpreting its conclusions and proposed perspectives. First, the literature discussed was selected based on conceptual relevance and representative examples rather than through a predefined systematic search strategy with formal inclusion and exclusion criteria. Consequently, the review may reflect selection bias toward studies that more clearly illustrate mechanistic interpretation of differential gene expression, potentially underrepresenting conflicting findings or alternative analytical frameworks.

Another limitation arises from the rapidly evolving nature of transcriptomic and multi-omic methods. Technologies such as single-cell RNA sequencing, spatial transcriptomics, and artificial intelligence–driven analytical approaches are developing quickly and may rapidly outpace current conceptual models and computational tools.[134,168-170] Consequently, some methods or datasets discussed in this review may soon be superseded by more advanced analytical strategies, limiting the long-term generalizability of certain conclusions.

The review also relies heavily on published datasets and previously reported computational analyses, which themselves are subject to methodological variability. Differences in sequencing platforms, normalization procedures, statistical thresholds, batch correction methods, and bioinformatic pipelines can substantially influence differential expression results and downstream functional interpretation. Because many studies use distinct analytical models, direct comparison between datasets and conclusions across studies remains challenging.

In addition, many mechanistic interpretations discussed throughout this review are based primarily on correlative analyses rather than direct experimental validation. Although pathway enrichment, network modeling, and multi-omic integration provide valuable mechanistic hypotheses, they do not necessarily establish causality.[3-7] Consequently, some proposed regulatory relationships or functional dependencies may not fully reflect true biological drivers in specific tumor contexts.

Another important limitation is the dependence on existing pathway and interaction databases, including KEGG, Reactome, Gene Ontology, and protein–protein interaction repositories. These resources are inherently incomplete and biased toward well-characterized genes and signaling pathways, potentially limiting the identification of novel or context-specific regulatory mechanisms. Moreover, static pathway representations may not adequately capture the dynamic and adaptive nature of cancer signaling networks.

Tumor heterogeneity also represents a significant challenge for the interpretation of transcriptomic data. Many studies discussed in this review are based on bulk RNA sequencing approaches that average gene expression across heterogeneous cellular populations, potentially obscuring biologically relevant subpopulations and spatially restricted transcriptional programs. Although single-cell and spatial transcriptomic approaches partially address these limitations, these technologies still face important technical and analytical constraints, including noise, dropout effects, limited spatial resolution, and difficulties in data integration.

Finally, the translational implications discussed in this review should be interpreted cautiously. While functional interpretation of differential gene expression holds considerable promise for biomarker discovery and precision oncology, many proposed applications remain exploratory or preclinical.[167] The successful translation of transcriptomic insights into clinically actionable strategies will require rigorous experimental validation, standardized analytical methodologies, and prospective clinical investigation.

Conclusions

Functional interpretation of differential gene expression in cancer represents a shift from descriptive gene lists toward mechanistic, systems-level understanding. By integrating pathway analysis, network modeling, multi-omic data, single-cell and spatial resolution, and experimental validation, researchers can construct dynamic models that clarify how context-dependent transcriptional programs may contribute to tumor initiation, progression, and therapeutic response. These frameworks can support precision oncology by informing biomarker development, target prioritization, combination-therapy design, and investigation of resistance mechanisms. As technologies and datasets continue to advance, integrating functional interpretation into clinical research may improve cancer diagnosis, treatment selection, and personalized care by translating transcriptomic observations into testable and clinically relevant hypotheses.

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Conflict of interest

Amancio Carnero is Editor-in-Chief of *Gene Expression*. The author has no other conflicts of interest to declare.

Author contributions

AC is the sole author of the manuscript.

References

[1] Garber K. Genomic medicine. Gene expression tests foretell breast cancer's future. Science 2004;303(5665):1754–1755. DOI: 10.1126/science.303.5665.1754, PMID: 15031473.

[2] Marx J. Medicine. DNA arrays reveal cancer in its many forms. Science 2000;289(5485):1670–1672. DOI: 10.1126/science.289.5485.1670, PMID: 11001727.

[3] Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, *et al*. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci U S A 2005;102(43):15545–15550. DOI: 10.1073/pnas.0506580102, PMID: 16199517.

[4] Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, *et al*. Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. Nat Genet 2000;25(1):25–29. DOI: 10.1038/75556, PMID: 10802651.

[5] Liberzon A, Birger C, Thorvaldsdóttir H, Ghandi M, Mesirov JP, Tamayo P. The Molecular Signatures Database (MSigDB) hallmark gene set collection. Cell Syst 2015;1(6):417–425. DOI: 10.1016/j.cels.2015.12.004, PMID: 26771021.

[6] Gillespie M, Jassal B, Stephan R, Milacic M, Rothfels K, Senff-Ribeiro A, *et al*. The reactome pathway knowledgebase 2022. Nucleic Acids Res 2022;50(D1):D687–D692. DOI: 10.1093/nar/gkab1028, PMID: 34788843.

[7] Aleksander SA, Balhoff J, Carbon S, Cherry JM, Drabkin HJ, Ebert D, *et al*. The Gene Ontology knowledgebase in 2023. Genetics 2023;224(1):iyad031. DOI: 10.1093/genetics/iyad031, PMID: 36866529.

[8] Barabási AL, Gulbahce N, Loscalzo J. Network medicine: a network-based approach to human disease. Nat Rev Genet 2011;12(1):56–68. DOI: 10.1038/nrg2918, PMID: 21164525.

[9] Ideker T, Krogan NJ. Differential network biology. Mol Syst Biol 2012;8:565. DOI: 10.1038/msb.2011.99, PMID: 22252388.

[10] Liang MZ, Huang XF, Zhu JC, Bao JX, Chen CL, Wang XW, *et al*. A machine learning-based glycolysis and fatty acid metabolism-related prognostic signature is constructed and identified ACSL5 as a novel marker inhibiting the proliferation of breast cancer. Comput Biol Chem 2025;119:108507. DOI: 10.1016/j.compbiolchem.2025.108507, PMID: 40403353.

[11] Simon M, Benary U, Baum K, Schramm A, Wolf J. A computational model elucidates the effects of oncogene-induced expression alterations on the energy metabolism of neuroblastoma. Sci Rep 2025;15(1):32708. DOI: 10.1038/s41598-025-18656-w, PMID: 40993267.

[12] Sun H, Zhou Y, Skaro MF, Wu Y, Qu Z, Mao F, *et al*. Metabolic Reprogramming in Cancer Is Induced to Increase Proton Production. Cancer Res 2020;80(5):1143–1155. DOI: 10.1158/0008-5472.CAN-19-3392, PMID: 31932456.

[13] Dengler VL, Galbraith M, Espinosa JM. Transcriptional regulation by hypoxia inducible factors. Crit Rev Biochem Mol Biol 2014;49(1):1–15. DOI: 10.3109/10409238.2013.838205, PMID: 24099156.

[14] Dehne N, Brüne B. Sensors, transmitters, and targets in mitochondrial oxygen shortage-a hypoxia-inducible factor relay story. Antioxid Redox Signal 2014;20(2):339–352. DOI: 10.1089/ars.2012.4776, PMID: 22794181.

[15] Carnero A, Lleonart M. The hypoxic microenvironment: A determinant of cancer stem cell evolution. Bioessays 2016;38 Suppl 1:S65–S74. DOI: 10.1002/bies.201670911, PMID: 27417124.

[16] Clemente-González C, Carnero A. Role of the Hypoxic-Secretome in Seed and Soil Metastatic Preparation. Cancers (Basel) 2022;14(23):5930. DOI: 10.3390/cancers14235930, PMID: 36497411.

[17] Hasin Y, Seldin M, Lusis A. Multi-omics approaches to disease. Genome Biol 2017;18(1):83. DOI: 10.1186/s13059-017-1215-1, PMID: 28476144.

[18] Huang Y, Duanmu J, Liu Y, Yan M, Li T, Jiang Q. Analysis of multi-omics differences in left-side and right-side colon cancer. PeerJ 2021;9:e11433. DOI: 10.7717/peerj.11433, PMID: 34026368.

[19] Noecker C, Eng A, Muller E, Borenstein E. MIMOSA2: a metabolic network-based tool for inferring mechanism-supported relationships in microbiome-metabolome data. Bioinformatics 2022;38(6):1615–1623. DOI: 10.1093/bioinformatics/btac003, PMID: 34999748.

[20] Chakraborty S, Hosen MI, Ahmed M, Shekhar HU. Onco-Multi-OMICS Approach: A New Frontier in Cancer Research. Biomed Res Int 2018;2018:9836256. DOI: 10.1155/2018/9836256, PMID: 30402498.

[21] Subramanian I, Verma S, Kumar S, Jere A, Anamika K. Multi-omics Data Integration, Interpretation, and Its Application. Bioinform Biol Insights 2020;14:1177932219899051. DOI: 10.1177/1177932219899051, PMID: 32076369.

[22] Argelaguet R, Velten B, Arnol D, Dietrich S, Zenz T, Marioni JC, *et al*. Multi-Omics Factor Analysis-a framework for unsupervised integration of multi-omics data sets. Mol Syst Biol 2018;14(6):e8124. DOI: 10.15252/msb.20178124, PMID: 29925568.

[23] Noecker C, Eng A, Srinivasan S, Theriot CM, Young VB, Jansson JK, *et al*. Metabolic Model-Based Integration of Microbiome Taxonomic and Metabolomic Profiles Elucidates Mechanistic Links between Ecological and Metabolic Variation. mSystems 2016;1(1):e00013–15. DOI: 10.1128/mSystems.00013-15, PMID: 27239563.

[24] Di Filippo M, Pescini D, Galuzzi BG, Bonanomi M, Gaglio D, Mangano E, *et al*. INTEGRATE: Model-based multi-omics data integration to characterize multi-level metabolic regulation. PLoS Comput Biol 2022;18(2):e1009337. DOI: 10.1371/journal.pcbi.1009337, PMID: 35130273.

[25] Badia-I-Mompel P, Wessels L, Müller-Dott S, Trimbour R, Ramirez Flores RO, Argelaguet R, *et al*. Gene regulatory network inference in the era of single-cell multi-omics. Nat Rev Genet 2023;24(11):739–754. DOI: 10.1038/s41576-023-00618-5, PMID: 37365273.

[26] Zhou M, Niu H, Cui D, Xu M, Li J, Huang G, *et al*. Cancer stem cell-driven drug resistance in colorectal carcinoma: molecular aspects and therapeutic potentials. Mol Cancer 2026;25(1):54. DOI: 10.1186/s12943-025-02557-7, PMID: 41566465.

[27] Okotel M, Huang C. Review Article: β-Catenin-Driven Cancer Stemness in Colorectal Cancer: Molecular Targets and Therapeutic Opportunities. Crit Rev Oncog 2025;30(4):1–20. DOI: 10.1615/CritRevOncog.2025060830, PMID: 41662634.

[28] Rocha MR, Castillo-Medina YK, de Lima Coelho BM, Rios LLL, Morgado-Diaz JA. Wnt/β-catenin pathway as a link between therapy resistance-driven epithelial-mesenchymal transition and stemness in colorectal cancer. Cell Biol Int 2025;49(2):154–160. DOI: 10.1002/cbin.12270, PMID: 39707719.

[29] Samanta S, Mahata R, Santra MK. The Cross-Talk between Epigenetic Gene Regulation and Signaling Pathways Regulates Cancer Pathogenesis. Subcell Biochem 2022;100:427–472. DOI: 10.1007/978-3-031-07634-3_13, PMID: 36301502.

[30] Slobodyanyuk M, Bahcheli AT, Klein ZP, Bayati M, Strug LJ, Reimand J. Directional integration and pathway enrichment analysis for multi-omics data. Nat Commun 2024;15(1):5690. DOI: 10.1038/s41467-024-49986-4, PMID: 38971800.

[31] Turlej E, Domaradzka A, Radzka J, Drulis-Fajdasz D, Kulbacka J, Gizak A. Cross-Talk Between Cancer and Its Cellular Environment-A Role in Cancer Progression. Cells 2025;14(6):403. DOI: 10.3390/cells14060403, PMID: 40136652.

[32] Wang L, Trasanidis N, Wu T, Dong G, Hu M, Bauer DE, *et al*. Dictys: dynamic gene regulatory network dissects developmental continuum with single-cell multiomics. Nat Methods 2023;20(9):1368–1378. DOI: 10.1038/s41592-023-01971-3, PMID: 37537351.

[33] Schito L, Rey-Keim S. Transcriptional regulation of hypoxic cancer cell metabolism and artificial intelligence. Trends Cancer 2025;11(2):88–90. DOI: 10.1016/j.trecan.2024.10.003, PMID: 39482194.

[34] Li L, Sun M, Wang J, Wan S. Multi-omics based artificial intelligence for cancer research. Adv Cancer Res 2024;163:303–356. DOI: 10.1016/bs.acr.2024.06.005, PMID: 39271266.

[35] Chen C, Padi M. Flexible modeling of regulatory networks improves transcription factor activity estimation. NPJ Syst Biol Appl 2024;10(1):58. DOI: 10.1038/s41540-024-00386-w, PMID: 38806476.

[36] Thatikonda V, Supper V, Wachter J, Kaya O, Kombara A, Bilgilier C, *et al*. Genetic dependencies associated with transcription factor activities in human cancer cell lines. Cell Rep 2024;43(5):114175. DOI: 10.1016/j.celrep.2024.114175, PMID: 38691456.

[37] Grześ M, Jaiswar A, Grochowski M, Wojtyś W, Kaźmierczak W, Olesiński T, *et al*. A common druggable signature of oncogenic c-Myc, mutant KRAS and mutant p53 reveals functional redundancy and competition among oncogenes in cancer. Cell Death Dis 2024;15(8):638. DOI: 10.1038/s41419-024-06965-3, PMID: 39217152.

[38] Pashaei E, Liu S, Li K, Zang Y, Yang L, Lautenschlaeger T, *et al*. DiCE: differential centrality-ensemble analysis based on gene expression profiles and protein-protein interaction network. Nucleic Acids Res 2025;53(13):gkaf609. DOI: 10.1093/nar/gkaf609, PMID: 40626556.

[39] Ståhl PL, Salmén F, Vickovic S, Lundmark A, Navarro JF, Magnusson J, *et al*. Visualization and analysis of gene expression in tissue sections by spatial transcriptomics. Science 2016;353(6294):78–82. DOI: 10.1126/science.aaf2403, PMID: 27365449.

[40] Longo SK, Guo MG, Ji AL, Khavari PA. Integrating single-cell and spatial transcriptomics to elucidate intercellular tissue dynamics. Nat Rev Genet 2021;22(10):627–644. DOI: 10.1038/s41576-021-00370-8, PMID: 34145435.

[41] Marx V. Method of the Year: spatially resolved transcriptomics. Nat Methods 2021;18(1):9–14. DOI: 10.1038/s41592-020-01033-y, PMID: 33408395.

[42] Moses L, Pachter L. Museum of spatial transcriptomics. Nat Methods 2022;19(5):534–546. DOI: 10.1038/s41592-022-01409-2, PMID: 35273392.

[43] Carnero A. A Darwinian Perspective on Tumor Evolution. Int J Biol Sci 2026;22(8):3925–3949. DOI: 10.7150/ijbs.130014, PMID: 42088420.

[44] Espinosa-Sánchez A, Carnero A. Artificial Intelligence in Tumor Evolution: Understanding Cancer Complexity Through Multi-Modal Data Integration in Precision Oncology. Cells 2026;15(11):1031. DOI: 10.3390/cells15111031, PMID: 42274624.

[45] Oh MS, Abascal J, Rennels AK, Salehi-Rad R, Dubinett SM, Liu B. Tumor Heterogeneity and the Immune Response in Non-Small Cell Lung Cancer: Emerging Insights and Implications for Immunotherapy. Cancers (Basel) 2025;17(6):1027. DOI: 10.3390/cancers17061027, PMID: 40149360.

[46] Sheng S, Margarida Bernardo M, Dzinic SH, Chen K, Heath EI, Sakr WA. Tackling tumor heterogeneity and phenotypic plasticity in cancer precision medicine: our experience and a literature review. Cancer Metastasis Rev 2018;37(4):655–663. DOI: 10.1007/s10555-018-9767-4, PMID: 30484007.

[47] Aswathy R, Chalos VA, Suganya K, Sumathi S. Advancing miRNA cancer research through artificial intelligence: from biomarker discovery to therapeutic targeting. Med Oncol 2024;42(1):30. DOI: 10.1007/s12032-024-02579-z, PMID: 39688780.

[48] Peng Q, Zhou Y, Oyang L, Wu N, Tang Y, Su M, *et al*. Impacts and mechanisms of alternative mRNA splicing in cancer metabolism, immune response, and therapeutics. Mol Ther 2022;30(3):1018–1035. DOI: 10.1016/j.ymthe.2021.11.010, PMID: 34793975.

[49] Martínez-Reyes I, Chandel NS. Cancer metabolism: looking forward. Nat Rev Cancer 2021;21(10):669–680. DOI: 10.1038/s41568-021-00378-6, PMID: 34272515.

[50] Thakur R, Wang D, Hu T, He C, Zhao J, Suresh V, *et al*. KRAS Signaling Inhibition Induces a Targetable Metabolic Dependency on Lipophagy-Dependent Fatty Acid Oxidation in Pancreatic Cancer. Cancer Res 2026;86(14):3519–3536. DOI: 10.1158/0008-5472.CAN-24-1984, PMID: 42054558.

[51] Encarnación-Rosado J, Sohn ASW, Biancur DE, Lin EY, Osorio-Vasquez V, Rodrick T, *et al*. Targeting pancreatic cancer metabolic dependencies through glutamine antagonism. Nat Cancer 2024;5(1):85–99. DOI: 10.1038/s43018-023-00647-3, PMID: 37814010.

[52] Xiang L, Mou J, Shao B, Wei Y, Liang H, Takano N, *et al*. Glutaminase 1 expression in colorectal cancer cells is induced by hypoxia and required for tumor growth, invasion, and metastatic colonization. Cell Death Dis 2019;10(2):40. DOI: 10.1038/s41419-018-1291-5, PMID: 30674873.

[53] Shalem O, Sanjana NE, Zhang F. High-throughput functional genomics using CRISPR-Cas9. Nat Rev Genet 2015;16(5):299–311. DOI: 10.1038/nrg3899, PMID: 25854182.

[54] Dixit A, Parnas O, Li B, Chen J, Fulco CP, Jerby-Arnon L, *et al*. Perturb-Seq: Dissecting Molecular Circuits with Scalable Single-Cell RNA Profiling of Pooled Genetic Screens. Cell 2016;167(7):1853–1866.e17. DOI: 10.1016/j.cell.2016.11.038, PMID: 27984732.

[55] Frangieh CJ, Melms JC, Thakore PI, Geiger-Schuller KR, Ho P, Luoma AM, *et al*. Multimodal pooled Perturb-CITE-seq screens in patient models define mechanisms of cancer immune evasion. Nat Genet 2021;53(3):332–341. DOI: 10.1038/s41588-021-00779-1, PMID: 33649592.

[56] Ying H, Kimmelman AC, Lyssiotis CA, Hua S, Chu GC, Fletcher-Sananikone E, *et al*. Oncogenic Kras maintains pancreatic tumors through regulation of anabolic glucose metabolism. Cell 2012;149(3):656–670. DOI: 10.1016/j.cell.2012.01.058, PMID: 22541435.

[57] Santana-Codina N, Roeth AA, Zhang Y, Yang A, Mashadova O, Asara JM, *et al*. Oncogenic KRAS supports pancreatic cancer through regulation of nucleotide synthesis. Nat Commun 2018;9(1):4945. DOI: 10.1038/s41467-018-07472-8, PMID: 30470748.

[58] Cancer Genome Atlas Network. Comprehensive molecular characterization of human colon and rectal cancer. Nature 2012;487(7407):330–337. DOI: 10.1038/nature11252, PMID: 22810696.

[59] White M, Mills ML, Millett LM, Gilroy K, Hong Y, Zeiger LB, *et al*. MAPK-driven epithelial cell plasticity drives colorectal cancer therapeutic resistance. Nature 2026;650(8102):748–758. DOI: 10.1038/s41586-025-09916-w, PMID: 41286180.

[60] Semenza GL. HIF-1 mediates metabolic responses to intratumoral

hypoxia and oncogenic mutations. J Clin Invest 2013;123(9): 3664–3671. DOI: 10.1172/JCI67230, PMID: 23999440.

[61] Fischer M. Census and evaluation of p53 target genes. Oncogene 2017;36(28):3943–3956. DOI: 10.1038/onc.2016.502, PMID: 28288132.

[62] Vousden KH, Prives C. Blinded by the Light: The Growing Complexity of p53. Cell 2009;137(3):413–431. DOI: 10.1016/j.cell.2009.04.037, PMID: 19410540.

[63] Lu M, Hoffman R. p53 as a target in myeloproliferative neoplasms. Oncotarget 2012;3(10):1052–1053. DOI: 10.18632/oncotarget.719, PMID: 23165648.

[64] Di Agostino S, Strano S, Emiliozzi V, Zerbini V, Mottolese M, Sacchi A, *et al*. Gain of function of mutant p53: the mutant p53/NF-Y protein complex reveals an aberrant transcriptional mechanism of cell cycle regulation. Cancer Cell 2006;10(3):191–202. DOI: 10.1016/j.ccr.2006.08.013, PMID: 16959611.

[65] Freed-Pastor WA, Prives C. Mutant p53: one name, many proteins. Genes Dev 2012;26(12):1268–1286. DOI: 10.1101/gad.190678.112, PMID: 22713868.

[66] Dang CV. MYC on the path to cancer. Cell 2012;149(1):22–35. DOI: 10.1016/j.cell.2012.03.003, PMID: 22464321.

[67] Kress TR, Sabò A, Amati B. MYC: connecting selective transcriptional control to global RNA production. Nat Rev Cancer 2015;15(10):593–607. DOI: 10.1038/nrc3984, PMID: 26383138.

[68] Sabò A, Kress TR, Pelizzola M, de Pretis S, Gorski MM, Tesi A, *et al*. Selective transcriptional regulation by Myc in cellular growth control and lymphomagenesis. Nature 2014;511(7510):488–492. DOI: 10.1038/nature13537, PMID: 25043028.

[69] Laurenti E, Varnum-Finney B, Wilson A, Ferrero I, Blanco-Bose WE, Ehninger A, *et al*. Hematopoietic stem cell function and survival depend on c-Myc and N-Myc activity. Cell Stem Cell 2008;3(6): 611–624. DOI: 10.1016/j.stem.2008.09.005, PMID: 19041778.

[70] Semenza GL. Hypoxia-inducible factors in physiology and medicine. Cell 2012;148(3):399–408. DOI: 10.1016/j.cell.2012.01.021, PMID: 22304911.

[71] Keith B, Johnson RS, Simon MC. HIF1α and HIF2α: sibling rivalry in hypoxic tumour growth and progression. Nat Rev Cancer 2011; 12(1):9–22. DOI: 10.1038/nrc3183, PMID: 22169972.

[72] Semenza GL. Hypoxia-inducible factors: mediators of cancer progression and targets for cancer therapy. Trends Pharmacol Sci 2012;33(4):207–214. DOI: 10.1016/j.tips.2012.01.005, PMID: 22398146.

[73] Vaupel P, Mayer A. Hypoxia in cancer: significance and impact on clinical outcome. Cancer Metastasis Rev 2007;26(2):225–239. DOI: 10.1007/s10555-007-9055-1, PMID: 17440684.

[74] Schödel J, Oikonomopoulos S, Ragoussis J, Pugh CW, Ratcliffe PJ, Mole DR. High-resolution genome-wide mapping of HIF-binding sites by ChIP-seq. Blood 2011;117(23):e207–e217. DOI: 10.1182/blood-2010-10-314427, PMID: 21447827.

[75] Thiery JP, Acloque H, Huang RY, Nieto MA. Epithelial-mesenchymal transitions in development and disease. Cell 2009;139(5):871–890. DOI: 10.1016/j.cell.2009.11.007, PMID: 19945376.

[76] Nieto MA, Huang RY, Jackson RA, Thiery JP. EMT: 2016. Cell 2016; 166(1):21–45. DOI: 10.1016/j.cell.2016.06.028, PMID: 27368099.

[77] Lamouille S, Xu J, Derynck R. Molecular mechanisms of epithelial-mesenchymal transition. Nat Rev Mol Cell Biol 2014;15(3): 178–196. DOI: 10.1038/nrm3758, PMID: 24556840.

[78] Brabletz T, Kalluri R, Nieto MA, Weinberg RA. EMT in cancer. Nat Rev Cancer 2018;18(2):128–134. DOI: 10.1038/nrc.2017.118, PMID: 29326430.

[79] Peinado H, Olmeda D, Cano A. Snail, Zeb and bHLH factors in tumour progression: an alliance against the epithelial phenotype? Nat Rev Cancer 2007;7(6):415–428. DOI: 10.1038/nrc2131, PMID: 17508028.

[80] Stemmler MP, Eccles RL, Brabletz S, Brabletz T. Non-redundant functions of EMT transcription factors. Nat Cell Biol 2019;21(1): 102–112. DOI: 10.1038/s41556-018-0196-y, PMID: 30602760.

[81] Wu Y, Shi Y, Luo Z, Zhou X, Chen Y, Song X, *et al*. Spatial multi-omics analysis of tumor-stroma boundary cell features for predicting breast cancer progression and therapy response. Front Cell Dev Biol 2025;13:1570696. DOI: 10.3389/fcell.2025.1570696, PMID: 40206396.

[82] Pastushenko I, Brisebarre A, Sifrim A, Fioramonti M, Revenco T, Boumahdi S, *et al*. Identification of the tumour transition states occurring during EMT. Nature 2018;556(7702):463–468. DOI: 10.1038/s41586-018-0040-3, PMID: 29670281.

[83] Jolly MK, Jia D, Boareto M, Mani SA, Pienta KJ, Ben-Jacob E, *et al*. Coupling the modules of EMT and stemness: A tunable 'stemness window' model. Oncotarget 2015;6(28):25161–25174. DOI: 10.18632/oncotarget.4629, PMID: 26317796.

[84] Yang B, Bai H, Sa Y, Zhu P, Liu P. Inhibiting EMT, stemness and cell cycle involved in baicalin-induced growth inhibition and apoptosis in colorectal cancer cells. J Cancer 2020;11(8):2303–2317. DOI: 10.7150/jca.37242, PMID: 32127957.

[85] Spranger S, Gajewski TF. Tumor-intrinsic oncogene pathways mediating immune avoidance. Oncoimmunology 2016;5(3): e1086862. DOI: 10.1080/2162402X.2015.1086862, PMID: 27141343.

[86] Spranger S, Gajewski TF. A new paradigm for tumor immune escape: β-catenin-driven immune exclusion. J Immunother Cancer 2015;3:43. DOI: 10.1186/s40425-015-0089-6, PMID: 26380088.

[87] Chen DS, Mellman I. Elements of cancer immunity and the cancer-immune set point. Nature 2017;541(7637):321–330. DOI: 10.1038/nature21349, PMID: 28102259.

[88] Thorsson V, Gibbs DL, Brown SD, Wolf D, Bortone DS, Ou Yang TH, *et al*. The Immune Landscape of Cancer. Immunity 2018;48(4): 812–830.e14. DOI: 10.1016/j.immuni.2018.03.023, PMID: 29628290.

[89] Binnewies M, Roberts EW, Kersten K, Chan V, Fearon DF, Merad M, *et al*. Understanding the tumor immune microenvironment (TIME) for effective therapy. Nat Med 2018;24(5):541–550. DOI: 10.1038/s41591-018-0014-x, PMID: 29686425.

[90] Beylerli O, Musaev E, Wang C, Popova I, Gareev I. Tumor Microenvironment Dynamics: The Regulatory Influence of Long Non-coding RNAs. Gene Expr 2025;24(2):139–155. DOI: 10.14218/GE.2024.00069.

[91] Ayers M, Lunceford J, Nebozhyn M, Murphy E, Loboda A, Kaufman DR, *et al*. IFN-γ-related mRNA profile predicts clinical response to PD-1 blockade. J Clin Invest 2017;127(8):2930–2940. DOI: 10.1172/JCI91190, PMID: 28650338.

[92] Gide TN, Quek C, Menzies AM, Tasker AT, Shang P, Holst J, *et al*. Distinct Immune Cell Populations Define Response to Anti-PD-1 Monotherapy and Anti-PD-1/Anti-CTLA-4 Combined Therapy. Cancer Cell 2019;35(2):238–255.e6. DOI: 10.1016/j.ccell.2019.01.003, PMID: 30753825.

[93] Holmgaard RB, Zamarin D, Munn DH, Wolchok JD, Allison JP. Indoleamine 2,3-dioxygenase is a critical resistance mechanism in antitumor T cell immunotherapy targeting CTLA-4. J Exp Med 2013;

210(7):1389–1402. DOI: 10.1084/jem.20130066, PMID: 23752227.

[94] Mariathasan S, Turley SJ, Nickles D, Castiglioni A, Yuen K, Wang Y, *et al*. TGFβ attenuates tumour response to PD-L1 blockade by contributing to exclusion of T cells. Nature 2018;554(7693): 544–548. DOI: 10.1038/nature25501, PMID: 29443960.

[95] Rajalakshmi SG, Sreehari E, Viswanathan P. Artificial intelligence-driven multi-omics analysis of gut-kidney axis in chronic kidney disease. Prog Mol Biol Transl Sci 2026;222:85–110. DOI: 10.1016/bs.pmbts.2026.01.015, PMID: 42173637.

[96] Nikita, Akash, Upmanyu B, K SR, Bhalla P, Narula S, *et al*. Artificial intelligence in multi-omics analysis of heart diseases. Prog Mol Biol Transl Sci 2026;222:53–83. DOI: 10.1016/bs.pmbts.2026.03.011, PMID: 42173636.

[97] Vogelstein B, Papadopoulos N, Velculescu VE, Zhou S, Diaz LA Jr, Kinzler KW. Cancer genome landscapes. Science 2013;339(6127): 1546–1558. DOI: 10.1126/science.1235122, PMID: 23539594.

[98] Stratton MR, Campbell PJ, Futreal PA. The cancer genome. Nature 2009;458(7239):719–724. DOI: 10.1038/nature07943, PMID: 19360079.

[99] Sanchez-Vega F, Mina M, Armenia J, Chatila WK, Luna A, La KC, *et al*. Oncogenic Signaling Pathways in The Cancer Genome Atlas. Cell 2018;173(2):321–337.e10. DOI: 10.1016/j.cell.2018.03.035, PMID: 29625050.

[100] Donehower LA, Soussi T, Korkut A, Liu Y, Schultz A, Cardenas M, *et al*. Integrated Analysis of TP53 Gene and Pathway Alterations in The Cancer Genome Atlas. Cell Rep 2019;28(5):1370–1384.e5. DOI: 10.1016/j.celrep.2019.07.001, PMID: 31365877.

[101] Yuan TL, Amzallag A, Bagni R, Yi M, Afghani S, Burgan W, *et al*. Differential Effector Engagement by Oncogenic KRAS. Cell Rep 2018;22(7):1889–1902. DOI: 10.1016/j.celrep.2018.01.051, PMID: 29444439.

[102] Ciriello G, Miller ML, Aksoy BA, Senbabaoglu Y, Schultz N, Sander C. Emerging landscape of oncogenic signatures across human cancers. Nat Genet 2013;45(10):1127–1133. DOI: 10.1038/ng.2762, PMID: 24071851.

[103] Ashworth A, Lord CJ, Reis-Filho JS. Genetic interactions in cancer progression and treatment. Cell 2011;145(1):30–38. DOI: 10.1016/j.cell.2011.03.020, PMID: 21458666.

[104] Kundaje A, Meuleman W, Ernst J, Bilenky M, Yen A, Heravi-Moussavi A, *et al*. Integrative analysis of 111 reference human epigenomes. Nature 2015;518(7539):317–330. DOI: 10.1038/nature14248, PMID: 25693563.

[105] Allis CD, Jenuwein T. The molecular hallmarks of epigenetic control. Nat Rev Genet 2016;17(8):487–500. DOI: 10.1038/nrg.2016.59, PMID: 27346641.

[106] Buenrostro JD, Giresi PG, Zaba LC, Chang HY, Greenleaf WJ. Transposition of native chromatin for fast and sensitive epigenomic profiling of open chromatin, DNA-binding proteins and nucleosome position. Nat Methods 2013;10(12):1213–1218. DOI: 10.1038/nmeth.2688, PMID: 24097267.

[107] Lister R, Ecker JR. Finding the fifth base: genome-wide sequencing of cytosine methylation. Genome Res 2009;19(6):959–966. DOI: 10.1101/gr.083451.108, PMID: 19273618.

[108] Jones B. Cancer genomics: chromatin influence on cancer mutation rate. Nat Rev Genet 2012;13(9):596–597. DOI: 10.1038/nrg3312, PMID: 22868262.

[109] Cedar H, Bergman Y. Linking DNA methylation and histone modification: patterns and paradigms. Nat Rev Genet 2009;10(5): 295–304. DOI: 10.1038/nrg2540, PMID: 19308066.

[110] Spitz F, Furlong EE. Transcription factors: from enhancer binding to developmental control. Nat Rev Genet 2012;13(9):613–626. DOI: 10.1038/nrg3207, PMID: 22868264.

[111] Vogel C, Marcotte EM. Insights into the regulation of protein abundance from proteomic and transcriptomic analyses. Nat Rev Genet 2012;13(4):227–232. DOI: 10.1038/nrg3185, PMID: 22411467.

[112] Aebersold R, Mann M. Mass-spectrometric exploration of proteome structure and function. Nature 2016;537(7620): 347–355. DOI: 10.1038/nature19949, PMID: 27629641.

[113] Olsen JV, Mann M. Status of large-scale analysis of post-translational modifications by mass spectrometry. Mol Cell Proteomics 2013;12(12):3444–3452. DOI: 10.1074/mcp.O113.034181, PMID: 24187339.

[114] Mertins P, Mani DR, Ruggles KV, Gillette MA, Clauser KR, Wang P, *et al*. Proteogenomics connects somatic mutations to signalling in breast cancer. Nature 2016;534(7605):55–62. DOI: 10.1038/nature18003, PMID: 27251275.

[115] Zhang B, Wang J, Wang X, Zhu J, Liu Q, Shi Z, *et al*. Proteogenomic characterization of human colon and rectal cancer. Nature 2014; 513(7518):382–387. DOI: 10.1038/nature13438, PMID: 25043054.

[116] Humphrey SJ, Azimifar SB, Mann M. High-throughput phosphoproteomics reveals in vivo insulin signaling dynamics. Nat Biotechnol 2015;33(9):990–995. DOI: 10.1038/nbt.3327, PMID: 26280412.

[117] Humphrey SJ, James DE, Mann M. Protein Phosphorylation: A Major Switch Mechanism for Metabolic Regulation. Trends Endocrinol Metab 2015;26(12):676–687. DOI: 10.1016/j.tem.2015.09.013, PMID: 26498855.

[118] Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011;144(5):646–674. DOI: 10.1016/j.cell.2011.02.013, PMID: 21376230.

[119] Pavlova NN, Thompson CB. The Emerging Hallmarks of Cancer Metabolism. Cell Metab 2016;23(1):27–47. DOI: 10.1016/j.cmet.2015.12.006, PMID: 26771115.

[120] Wishart DS. Emerging applications of metabolomics in drug discovery and precision medicine. Nat Rev Drug Discov 2016;15(7): 473–484. DOI: 10.1038/nrd.2016.32, PMID: 26965202.

[121] Johnson CH, Ivanisevic J, Siuzdak G. Metabolomics: beyond biomarkers and towards mechanisms. Nat Rev Mol Cell Biol 2016; 17(7):451–459. DOI: 10.1038/nrm.2016.25, PMID: 26979502.

[122] DeBerardinis RJ. Proliferating Cells Conserve Nitrogen to Support Growth. Cell Metab 2016;23(6):957–958. DOI: 10.1016/j.cmet.2016.05.008, PMID: 27304492.

[123] DeBerardinis RJ, Chandel NS. Fundamentals of cancer metabolism. Sci Adv 2016;2(5):e1600200. DOI: 10.1126/sciadv.1600200, PMID: 27386546.

[124] Birsoy K, Wang T, Chen WW, Freinkman E, Abu-Remaileh M, Sabatini DM. An Essential Role of the Mitochondrial Electron Transport Chain in *Cell* Proliferation Is to Enable Aspartate Synthesis. Cell 2015;162(3):540–551. DOI: 10.1016/j.cell.2015.07.016, PMID: 26232224.

[125] Regev A, Teichmann SA, Lander ES, Amit I, Benoist C, Birney E, *et al*. The Human Cell Atlas. eLife 2017;6:e27041. DOI: 10.7554/eLife.27041.

[126] Stuart T, Butler A, Hoffman P, Hafemeister C, Papalexi E, Mauck WM 3rd, *et al*. Comprehensive Integration of Single-Cell Data. Cell 2019;177(7):1888–1902.e21. DOI: 10.1016/j.cell.2019.05.031, PMID: 31178118.

[127] Stuart T, Satija R. Integrative single-cell analysis. Nat Rev Genet 2019;20(5):257–272. DOI: 10.1038/s41576-019-0093-7, PMID: 30696980.

[128] Lähnemann D, Köster J, Szczurek E, McCarthy DJ, Hicks SC, Robinson MD, *et al*. Eleven grand challenges in single-cell data science. Genome Biol 2020;21(1):31. DOI: 10.1186/s13059-020-1926-6, PMID: 32033589.

[129] Ren X, Kang B, Zhang Z. Understanding tumor ecosystems by single-cell sequencing: promises and limitations. Genome Biol 2018;19(1):211. DOI: 10.1186/s13059-018-1593-z, PMID: 30509292.

[130] Senra D, Guisoni N, Diambra L. Unraveling Tumor Heterogeneity: Quantitative Insights from Single-cell RNA Sequencing Analysis in Breast Cancer Subtypes. Gene Expr 2025;24(3):159–170. DOI: 10.14218/GE.2024.00071.

[131] Shalek AK, Benson M. Single-cell analyses to tailor treatments. Sci Transl Med 2017;9(408):eaan4730. DOI: 10.1126/scitranslmed.aan4730, PMID: 28931656.

[132] Wang Y, Navin NE. Advances and applications of single-cell sequencing technologies. Mol Cell 2015;58(4):598–609. DOI: 10.1016/j.molcel.2015.05.005, PMID: 26000845.

[133] Hao Y, Hao S, Andersen-Nissen E, Mauck WM 3rd, Zheng S, Butler A, *et al*. Integrated analysis of multimodal single-cell data. Cell 2021;184(13):3573–3587.e29. DOI: 10.1016/j.cell.2021.04.048, PMID: 34062119.

[134] Heumos L, Schaar AC, Lance C, Litinetskaya A, Drost F, Zappia L, *et al*. Best practices for single-cell analysis across modalities. Nat Rev Genet 2023;24(8):550–572. DOI: 10.1038/s41576-023-00586-w, PMID: 37002403.

[135] Neftel C, Laffy J, Filbin MG, Hara T, Shore ME, Rahme GJ, *et al*. An Integrative Model of Cellular States, Plasticity, and Genetics for Glioblastoma. Cell 2019;178(4):835–849.e21. DOI: 10.1016/j.cell.2019.06.024, PMID: 31327527.

[136] Kinker GS, Greenwald AC, Tal R, Orlova Z, Cuoco MS, McFarland JM, *et al*. Pan-cancer single-cell RNA-seq identifies recurring programs of cellular heterogeneity. Nat Genet 2020;52(11):1208–1218. DOI: 10.1038/s41588-020-00726-6, PMID: 33128048.

[137] Moncada R, Barkley D, Wagner F, Chiodin M, Devlin JC, Baron M, *et al*. Integrating microarray-based spatial transcriptomics and single-cell RNA-seq reveals tissue architecture in pancreatic ductal adenocarcinomas. Nat Biotechnol 2020;38(3):333–342. DOI: 10.1038/s41587-019-0392-8, PMID: 31932730.

[138] Thrane K, Eriksson H, Maaskola J, Hansson J, Lundeberg J. Spatially Resolved Transcriptomics Enables Dissection of Genetic Heterogeneity in Stage III Cutaneous Malignant Melanoma. Cancer Res 2018;78(20):5970–5979. DOI: 10.1158/0008-5472.CAN-18-0747, PMID: 30154148.

[139] Hunter MV, Moncada R, Weiss JM, Yanai I, White RM. Spatially resolved transcriptomics reveals the architecture of the tumor-microenvironment interface. Nat Commun 2021;12(1):6278. DOI: 10.1038/s41467-021-26614-z, PMID: 34725363.

[140] Wu SZ, Al-Eryani G, Roden DL, Junankar S, Harvey K, Andersson A, *et al*. A single-cell and spatially resolved atlas of human breast cancers. Nat Genet 2021;53(9):1334–1347. DOI: 10.1038/s41588-021-00911-1, PMID: 34493872.

[141] Pelka K, Hofree M, Chen JH, Sarkizova S, Pirl JD, Jorgji V, *et al*. Spatially organized multicellular immune hubs in human colorectal cancer. Cell 2021;184(18):4734–4752.e20. DOI: 10.1016/j.cell.2021.08.003, PMID: 34450029.

[142] Grünwald BT, Devisme A, Andrieux G, Vyas F, Aliar K, McCloskey CW, *et al*. Spatially confined sub-tumor microenvironments in pancreatic cancer. Cell 2021;184(22):5577–5592.e18. DOI: 10.1016/j.cell.2021.09.022, PMID: 34644529.

[143] Rolland T, Taşan M, Charloteaux B, Pevzner SJ, Zhong Q, Sahni N, *et al*. A proteome-scale map of the human interactome network. Cell 2014;159(5):1212–1226. DOI: 10.1016/j.cell.2014.10.050, PMID: 25416956.

[144] Chuang HY, Lee E, Liu YT, Lee D, Ideker T. Network-based classification of breast cancer metastasis. Mol Syst Biol 2007;3:140. DOI: 10.1038/msb4100180, PMID: 17940530.

[145] Friedman N, Linial M, Nachman I, Pe'er D. Using Bayesian networks to analyze expression data. J Comput Biol 2000;7(3-4):601–620. DOI: 10.1089/106652700750050961, PMID: 11108481.

[146] Sharan R, Ideker T. Modeling cellular machinery through biological network comparison. Nat Biotechnol 2006;24(4):427–433. DOI: 10.1038/nbt1196, PMID: 16601728.

[147] Huang S, Chaudhary K, Garmire LX. More Is Better: Recent Progress in Multi-Omics Data Integration Methods. Front Genet 2017;8:84. DOI: 10.3389/fgene.2017.00084, PMID: 28670325.

[148] Huang Y, Tao Y, Li X, Chang S, Jiang B, Li F, *et al*. Bioinformatics analysis of key genes and latent pathway interactions based on the anaplastic thyroid carcinoma gene expression profile. Oncol Lett 2017;13(1):167–176. DOI: 10.3892/ol.2016.5447, PMID: 28428828.

[149] Rodriguez H, Zenklusen JC, Staudt LM, Doroshow JH, Lowy DR. The next horizon in precision oncology: Proteogenomics to inform cancer diagnosis and treatment. Cell 2021;184(7):1661–1670. DOI: 10.1016/j.cell.2021.02.055, PMID: 33798439.

[150] Chen F, Wendl MC, Wyczalkowski MA, Bailey MH, Li Y, Ding L. Moving pan-cancer studies from basic research toward the clinic. Nat Cancer 2021;2(9):879–890. DOI: 10.1038/s43018-021-00250-4, PMID: 35121865.

[151] Zhao D, Qu Y, Gao N, Wu T. Integrated multi-omics characterization of SMAD4 mutant colorectal cancer. Discov Oncol 2024;15(1):386. DOI: 10.1007/s12672-024-01268-7, PMID: 39210191.

[152] Hood L, Friend SH. Predictive, personalized, preventive, participatory (P4) cancer medicine. Nat Rev Clin Oncol 2011;8(3):184–187. DOI: 10.1038/nrclinonc.2010.227, PMID: 21364692.

[153] Minina VI, Titov RA, Buslaev VY, Savchenko RR, Sleptcov AA, Gavrineva NA, *et al*. Microarray Analysis of Differential Expression of Long Non-coding RNAs in Peripheral Blood Mononuclear Cells in Luminal A Breast Cancer. Gene Expr 2025;24(4):e00021. DOI: 10.14218/GE.2025.00021.

[154] Lord CJ, Ashworth A. PARP inhibitors: Synthetic lethality in the clinic. Science 2017;355(6330):1152–1158. DOI: 10.1126/science.aam7344, PMID: 28302823.

[155] Samatar AA, Poulikakos PI. Targeting RAS-ERK signalling in cancer: promises and challenges. Nat Rev Drug Discov 2014;13(12):928–942. DOI: 10.1038/nrd4281, PMID: 25435214.

[156] Shaffer SM, Dunagin MC, Torborg SR, Torre EA, Emert B, Krepler C, *et al*. Rare cell variability and drug-induced reprogramming as a mode of cancer drug resistance. Nature 2017;546(7658):431–435. DOI: 10.1038/nature22794, PMID: 28607484.

[157] Sabatini DM. Twenty-five years of mTOR: Uncovering the link from nutrients to growth. Proc Natl Acad Sci U S A 2017;114(45):11818–11825. DOI: 10.1073/pnas.1716173114, PMID: 29078414.

[158] Ashley EA. Towards precision medicine. Nat Rev Genet 2016;17(9):507–522. DOI: 10.1038/nrg.2016.86, PMID: 27528417.

[159] Seyhan AA, Carini C. Are innovation and new technologies in precision medicine paving a new era in patients centric care? J Transl Med 2019;17(1):114. DOI: 10.1186/s12967-019-1864-9, PMID: 30953518.

[160] Karczewski KJ, Snyder MP. Integrative omics for health and disease. Nat Rev Genet 2018;19(5):299–310. DOI: 10.1038/nrg.2018.4, PMID: 29479082.

[161] Chen R, Snyder M. Promise of personalized omics to precision medicine. Wiley Interdiscip Rev Syst Biol Med 2013;5(1):73–82. DOI: 10.1002/wsbm.1198, PMID: 23184638.

[162] Malone ER, Oliva M, Sabatini PJB, Stockley TL, Siu LL. Molecular profiling for precision cancer therapies. Genome Med 2020;12(1):8. DOI: 10.1186/s13073-019-0703-1, PMID: 31937368.

[163] Bedard PL, Hansen AR, Ratain MJ, Siu LL. Tumour heterogeneity in the clinic. Nature 2013;501(7467):355–364. DOI: 10.1038/nature12627, PMID: 24048068.

[164] Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol 2014;15(12):550. DOI: 10.1186/s13059-014-0550-8, PMID: 25516281.

[165] Robinson MD, Oshlack A. A scaling normalization method for differential expression analysis of RNA-seq data. Genome Biol 2010;11(3):R25. DOI: 10.1186/gb-2010-11-3-r25, PMID: 20196867.

[166] Barabási AL, Oltvai ZN. Network biology: understanding the cell's functional organization. Nat Rev Genet 2004;5(2):101–113. DOI: 10.1038/nrg1272, PMID: 14735121.

[167] Ioannidis JP. Microarrays and molecular research: noise discovery? Lancet 2005;365(9458):454–455. DOI: 10.1016/S0140-6736(05)17878-7, PMID: 15705441.

[168] Eraslan G, Avsec Ž, Gagneur J, Theis FJ. Deep learning: new computational modelling techniques for genomics. Nat Rev Genet 2019;20(7):389–403. DOI: 10.1038/s41576-019-0122-6, PMID: 30971806.

[169] Kuenzi BM, Ideker T. A census of pathway maps in cancer systems biology. Nat Rev Cancer 2020;20(4):233–246. DOI: 10.1038/s41568-020-0240-7, PMID: 32066900.

[170] Kuenzi BM, Park J, Fong SH, Sanchez KS, Lee J, Kreisberg JF, *et al*. Predicting Drug Response and Synergy Using a Deep Learning Model of Human Cancer Cells. Cancer Cell 2020;38(5):672–684.e6. DOI: 10.1016/j.ccell.2020.09.014, PMID: 33096023.

[171] Topol EJ. A decade of digital medicine innovation. Sci Transl Med 2019;11(498):eaaw7610. DOI: 10.1126/scitranslmed.aaw7610, PMID: 31243153.

[172] Topol EJ. High-performance medicine: the convergence of human and artificial intelligence. Nat Med 2019;25(1):44–56. DOI: 10.1038/s41591-018-0300-7, PMID: 30617339.