Stratifying Tumors and Normal Tissues via MIR100HG-Centered Multi-Omics Signatures

Anshul Gupta
Department of Engineering Mathematics
University Of Bristol
Bristol, United Kingdom
uv24461@bristol.ac.uk

Lingchen Cai
Department of Engineering Mathematics
University Of Bristol
Bristol, United Kingdom
ry24050@bristol.ac.uk

Qizhong Wang
Department of Engineering Mathematics
University Of Bristol
Bristol, United Kingdom
fo24863@bristol.ac.uk

Sri Hari Khara Sudan Tanga Raaj
Department of Engineering Mathematics
University Of Bristol
Bristol, United Kingdom
mf24469@bristol.ac.uk

***Abstract*—*MIR100HG*, a long non-coding RNA implicated in cancer progression, orchestrates complex transcriptional and epigenetic programs that drive tumor biology. Here, we present a comprehensive, integrative multi-omics analysis across five adenocarcinomas—pancreatic (PAAD), melanoma (SKCM), lung (LUAD), prostate (PRAD), and stomach (STAD)—leveraging gene expression, transcription factor (TF) activity, and DNA methylation profiles. By stratifying TCGA samples into high and low *MIR100HG* expression groups, we uncover robust differential gene signatures, pinpoint key TF hubs, and reveal consistent methylation shifts at the *MIR100HG* locus. Functional enrichment highlights EMT, TGF-β signaling, and extracellular matrix remodeling as central pathways modulated by *MIR100HG*. Constructing an integrated feature matrix from shared DEGs and TFs, we achieve clear cancer–normal discrimination via PCA and demonstrate that select latent dimensions strongly correlate with patient survival in PAAD and LUAD. Notably, PC3 stratification separates high-risk and low-risk groups (log-rank $p < 0.01$), underscoring *MIR100HG*'s prognostic value. These findings position *MIR100HG* as a context-dependent master regulator of oncogenic networks and suggest its potential utility as both a biomarker for tumor stratification and a candidate target for therapeutic intervention.**

I. Introduction

Long non-coding RNAs (lncRNAs) have emerged as key regulators in cancer biology, influencing gene expression through transcriptional, post-transcriptional, and epigenetic mechanisms. Among these, *MIR100HG*, an lncRNA host gene for the *miR*-100/*let*-7a/*miR*-125b group, has gained attention for its multifaceted roles in tumor development, progression, and immune evasion [1]. Despite increasing evidence linking *MIR100HG* to oncogenic pathways such as TGF–β signaling, EMT, and chromatin remodeling, its broader impact on the cancer transcriptome and regulatory networks remains incompletely understood.

To address this, we investigate the systems-level influence of *MIR100HG* using a multiomics framework that integrates differential gene expression, transcription factor (TF) activity, and DNA methylation profiling across five adenocarcinomas: pancreatic (PAAD), skin (SKCM), lung (LUAD), prostate (PRAD) and stomach (STAD). We hypothesize that *MIR100HG* regulates tumor progression in a tissue-specific yet partially convergent manner, mediated through both transcriptional and epigenetic axes.

Our approach stratifies samples based on *MIR100HG* expression and explores the resulting molecular differences at multiple layers. We identify key DEGs and TFs, construct condition-specific regulatory networks, and examine differential methylation patterns within the *MIR100HG* locus. Furthermore, we build an integrated feature matrix to evaluate cancer–normal discrimination and assess prognostic value using survival models.

By contextualizing *MIR100HG* activity across diverse tumor types, our study reveals consistent molecular signatures and potential clinical relevance, laying the groundwork for future biomarker development and functional studies.

II. Literature Review

MIR100HG, a long non-coding RNA (lncRNA) located on chromosome 11, has emerged as a critical modulator of cancer progression. Several studies have reported its overexpression across diverse tumor types—including pancreatic, lung, prostate, stomach, and skin cancers—where it is consistently associated with poor prognosis and increased metastatic potential [1], [2]. Functional studies indicate that *MIR100HG* promotes cancer cell invasion and epithelial–mesenchymal transition (EMT) through the regulation of transcription factors such as *ZEB1*, *SMAD3*, and *SNAI1*, particularly within the TGF-β signaling axis [3]. These transcriptional regulators play key roles in tumor plasticity, stemness, and immune evasion.

Epigenetic modulation of *MIR100HG* has also been highlighted as a driver of oncogenic expression. Hypomethylation of promoter-proximal CpG sites has been shown to enhance *MIR100HG* transcriptional activity, further contributing to cancer progression [4]. Additionally, transcription factors such

as *STAT3* and *EP300*, which are well-known mediators of inflammation, chromatin remodeling, and metastasis, have been identified as downstream targets or potential regulators of *MIR100HG* [5].

Given its consistent upregulation in tumors compared to normal tissues, *MIR100HG* has been proposed as both a diagnostic and prognostic biomarker [6]. However, most existing studies focus on isolated cancer types or specific molecular axes. Our study addresses this gap by employing a multi-omics, pan-cancer framework to explore *MIR100HG*–associated gene regulation, transcription factor activity, methylation, and clinical outcomes across five major tumor types.

III. Methodology

Fig. 1 provides an overview of the methodological workflow we followed throughout our study.

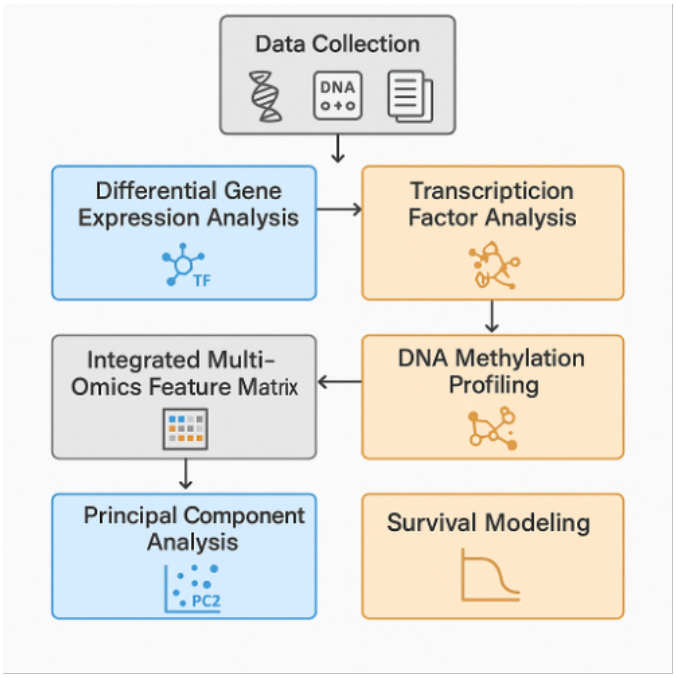

Fig. 1. Methodology Workflow

A. Data Collection

First, assembling the diverse multi-omics datasets for *MIR100HG* across five cancer types posed a significant challenge due to their volume and heterogeneity. To impose structure, we developed a logical model that categorizes data sources into four levels: Level -1 comprises the core *MIR100HG* centric datasets—TCGA gene expression ($\log_2$-TPM), Illumina 450 K methylation arrays, and ENCODE transcription factor–target associations; Level -2 includes directly supportive data such as GTEx normal tissue expression, TCGA clinical survival and staging metadata, and curated transcription factor lists; Level -3 contains essential annotation files, including hg19 gene-region annotations and the Illumina probe mapping resource; and Level -4 captures derived datasets—stratified high/low *MIR100HG* groups, differential expression lists, TF–target networks, and the integrated feature matrix—serving as inputs for all downstream statistical modeling and visualization.

B. Differential Gene Expression Analysis

To identify genes whose expression is modulated by *MIR100HG*, we stratified samples into "high" and "low" expression groups via a median split of $\log_2(\mathrm{TPM} + 0.001)$ values. We then applied two-sample Student's *t*-tests—with Benjamini–Hochberg correction (FDR < 0.01)—to each gene, selecting those with the most extreme *t*-statistics as differentially expressed. The *t*-test is a straightforward, well-established statistical test for comparing means between two groups; its parametric assumptions are satisfied by our log-transformed, normalized RNA-seq data, ensuring sensitivity to true expression differences while controlling false discovery. Ranking by absolute *t*-statistic allowed consistent selection of the top 100 up- and down-regulated genes in each cancer and normal cohort, setting the stage for downstream regulatory and functional analyses.

C. Transcription Factor Analysis and Network Construction

To uncover the upstream regulators driving these expression changes, we intersected our DEG lists with a curated ENCODE transcription factor–target database. We first performed two-sample Student's *t*-tests—with Benjamini–Hochberg correction (FDR < 0.01)—on each transcription factor (TF) to identify those significantly upregulated in high *MIR100HG* samples, reasoning that active TFs should show elevated expression. Next, we computed Pearson correlation coefficients, r, between each candidate TF and all DEGs, retaining TF–gene pairs with $|r| > 0.5$ and $p < 0.01$. Pearson correlation is ideal for detecting linear co-variation and thus plausible regulatory associations. Constructing tissue-specific networks from these high-confidence links enabled calculation of node degrees ("hubness"), highlighting TFs most central to *MIR100HG*-associated regulatory programs.

D. DNA Methylation Profiling and Correlation

To assess epigenetic regulation of *MIR100HG* itself, we focused on 31 Illumina 450 K probes spanning its promoter, gene body, and flanking regions. We used two-sided Student's *t*-tests—with Benjamini–Hochberg correction (FDR < 0.01)—to compare β-values between high and low *MIR100HG* groups, identifying consistently differentially methylated sites. A subsequent Pearson correlation analysis between probe methylation and *MIR100HG* expression distinguished putative activating (positive r) from repressive (negative r) CpGs. Combining differential testing with correlation ensures that identified probes are both statistically altered and functionally linked to transcript levels, providing robust candidates for epigenetic control points.

E. Integrated Multi-Omics Feature Matrix Construction

To capture the joint impact of transcriptional and regulatory changes, we merged DEGs and hub TFs common to cancer and normal samples into a single feature matrix. After standard scaling (zero mean, unit variance) to harmonize measurement scales, we applied an *Isolation Forest* outlier detector (2% contamination) to remove aberrant samples, improving robustness. Principal Component Analysis (PCA) then reduced dimensionality, summarizing the high-dimensional feature space into principal axes that explain the greatest variance. PCA is a widely used unsupervised technique that facilitates visualization and quantification of group separation; here, the first two PCs consistently delineated cancer from normal clusters, confirming that integrated *MIR100HG*-associated features carry strong discriminatory power.

F. Survival Modeling and Kaplan-Meier Analysis

For clinical relevance, we evaluated whether *MIR100HG*-driven features predict patient survival. We used the top 15 PCs from each tissue's PCA as covariates in penalized Cox proportional hazards models (L_2 penalty, $\lambda = 0.1$), which accommodate high-dimensional predictors while preventing overfitting. The CoxPH model is the standard for time-to-event analysis, estimating hazard ratios that quantify the effect of each covariate on survival risk. To validate model findings nonparametrically, we conducted Kaplan–Meier survival analysis by stratifying patients into PC3 "high" and "low" groups (median split) and assessed curve differences via the log-rank test. Kaplan–Meier estimation provides intuitive survival probability curves, while the log-rank test rigorously evaluates group-wise survival differences. Together, these complementary approaches demonstrate the prognostic value of *MIR100HG*-associated molecular signatures.

IV. DATA DESCRIPTION/ PREPARATION

A. Data Description

TCGA Gene Expression (TPM): Transcript-level expression data for PAAD, SKCM, LUAD, PRAD, and STAD were downloaded from the UCSC Xena "`tcga_RSEM_gene_tpm`" repository. Values are provided as $\log_2(\mathrm{TPM} + 0.001)$ and annotated with HGNC gene symbols. We retained only samples with complete matched methylation and clinical metadata, splitting each cancer type into its own matrix for downstream differential expression analysis.

GTEx Normal Tissue Expression: To provide a non-malignant reference, TPM data for pancreas, skin, whole blood, prostate, and stomach were obtained from the GTEx "`gtex_rsem_isoform_tpm`" and corresponding phenotype files on Xena. Isoform-level TPMs were collapsed to gene-level counts and converted to the same $\log_2(\mathrm{TPM} + 0.001)$ scale, then subset to the five tissues matching our TCGA cohorts.

ENCODE Transcription Factor–Target Associations: We downloaded the Harmonizome "ENCODE Transcription Factor Targets" dataset, which compiles ChIP-seq–derived TF→target relationships. This file served as the basis for identifying candidate regulators among our DEGs and constructing tissue-specific regulatory networks.

TCGA 450K DNA Methylation: Probe-level β-values for the Illumina HumanMethylation450 array were retrieved for each TCGA cohort via Xena sample maps. Each matrix contains up to 485 000 CpG sites. We also obtained the "`probeMap_IlluminaMethyl450_hg19_GPL16304_TCGAlegacy`" annotation file to map probes to genomic coordinates and gene contexts.

Probe and Gene Annotation: To contextualize methylation probes, we generated an hg19 gene-feature annotation (promoters, exons, UTRs) using the `annotatr` and `TxDb.Hsapiens.UCSC.hg19.knownGene` packages. This allowed us to select the 31 probes overlapping the *MIR100HG* locus (chr11:122 030 075–122 239 846).

Clinical Metadata: Overall survival times, event indicators, pathologic stage, age, gender, and race were imported from the TCGA Pan-Cancer clinical supplementary table on Xena. We filtered to include only patients in PAAD, SKCM, LUAD, PRAD, and STAD, aligning clinical records to expression and methylation samples via standardized TCGA barcodes.

B. Data Preparation

Sample Harmonization and Grouping: TCGA barcodes were standardized by replacing hyphens with underscores to harmonize the expression, methylation, and clinical datasets. Samples lacking complete data across all three modalities or missing survival information were excluded. *MIR100HG* expression levels were dichotomized at the cohort median into "high" and "low" groups for case–control comparisons.

Normal Tissue Extraction: GTEx expression and phenotype files were joined on donor and tissue site identifiers. Only records corresponding to the five target tissues (pancreas, skin, lung, prostate, stomach) were retained. The resulting normal matrices were formatted analogously to the TCGA TPM data ($\log_2[\mathrm{TPM} + 0.001]$) and quantile-normalized within each tissue.

Gene Expression Processing: Within each cancer and matched normal cohort, genes in the lowest 10 % of variance were removed to reduce noise. Remaining genes were tested for differential expression between high and low *MIR100HG* groups using two-sample Student's *t*-tests with Benjamini–Hochberg correction. The top 100 upregulated and 100 downregulated genes (by absolute *t*-statistic) proceeded to downstream analyses.

DNA Methylation Handling: From the Illumina 450 K β-value files, 31 probes mapping to the *MIR100HG* locus were selected. Probes with $> 20\%$ missing entries were discarded; remaining missing β-values were imputed by probe-wise mean. Two-sided Student's *t*-tests identified probes differentially methylated between *MIR100HG* high and low groups ($p < 0.01$), and Pearson correlations with *MIR100HG* expression were computed for functional interpretation.

As summarized in Table I, the sample counts per tissue type following preprocessing were used in all subsequent analyses.

TABLE I
SAMPLE COUNTS PER TISSUE TYPE

Tissue Type	Cancer Samples	Normal Samples
Pancreas	178	167
Skin	102	812
Blood	513	288
Prostate	495	100
Stomach	414	174

V. RESULTS AND DISCUSSION

A. Gene Expression Analysis

To explore the role of *MIR100HG* in transcriptional regulation, we performed differential gene expression (DGE) analysis across five cancer types and their corresponding normal tissues. By stratifying samples into "high" and "low" *MIR100HG* expression groups, we identified key DEGs (differentially expressed genes) that may be directly or indirectly modulated by *MIR100HG*. This analysis helped uncover cross-cancer transcriptional trends and distinctions between cancerous and normal physiological contexts.

1) DEGs in Cancer Samples: Differential expression analysis was conducted separately for five cancer types—PAAD, SKCM, LUAD, PRAD, and STAD—by comparing high versus low *MIR100HG* expression samples. The top 100 upregulated and 100 downregulated genes were selected based on adjusted p-value < 0.01 and ranked using t-statistics.

In PAAD, strongly upregulated genes included *NEGR1*, a tumor suppressor implicated in neural adhesion and pancreatic tumor inhibition, and *CNN3*, associated with cytoskeletal dynamics and cancer progression [6]. On the other hand, downregulated genes such as *TMEM238* and *NR2F6* were suppressed in low *MIR100HG* samples; the latter regulates immune escape in T cells and has been implicated in immune checkpoint resistance [7].

In SKCM, notable upregulated genes included *LAMA4* and *FGF2*. *LAMA4* enhances melanoma cell invasion, and *FGF2* drives tumor angiogenesis and vascular remodeling [8]. Downregulated genes included *GSTP1*, a detoxifying enzyme frequently silenced in melanoma, with its suppression linked to poor prognosis and aggressiveness [9].

For LUAD, genes such as *SGCD* and *DCN*, involved in extracellular matrix remodeling and tumor microenvironment modulation, were significantly overexpressed in high *MIR100HG* groups. Meanwhile, *MAP7-AS1* and *MRPS26*, likely involved in microtubule stabilization and mitochondrial translation, were strongly downregulated.

PRAD and STAD exhibited extreme gene expression contrasts, including overexpression of *CNRIP1* and *MGP* in high *MIR100HG* conditions. *CNRIP1*, also observed in normal prostate tissue, suggests *MIR100HG* relevance beyond malignancy. Downregulated genes such as *PPP1R14B* and *IFRD2*—both linked to cytoskeletal regulation and cell differentiation—showed notable fold changes [10].

- **Cross-Cancer Comparison:** Several DEGs overlapped across cancer types. For instance, *MAGI2-AS3* was significantly upregulated in both SKCM and PRAD, indicating possible pan-cancer regulation by *MIR100HG*. Genes involved in extracellular matrix remodeling (e.g., *PRELP*, *ANGPTL1*) were frequently upregulated in epithelial–origin cancers like PRAD and STAD. In contrast, downregulated genes such as *PLA2G4F* and *ALOX15B*, known for modulating inflammatory pathways, appeared repeatedly in adenocarcinomas (LUAD, PRAD), implying consistent suppression of anti-tumor immunity.

2) DEGs in Normal Tissues: In the corresponding normal tissues, stratified DGE analysis revealed more homogeneous patterns, though several tissue-specific distinctions were evident. In pancreas and blood, genes like *RAI1* and *DACT1* were significantly upregulated in high *MIR100HG* samples. Notably, *DACT1*, a Wnt signaling suppressor, was consistently upregulated across multiple normal tissues, hinting at a conserved role in physiological regulation [11]. In skin, genes such as *FBN1* and *FSTL1*, which contribute to structural integrity and fibrotic processes, showed strong upregulation with high *MIR100HG* levels. Downregulated genes included *TECR* and *GLTP*, involved in lipid metabolism and vesicular transport, respectively [12].

- **Cross-Normal Comparison:** Tissue-specific expression differences were prominent. However, genes like *DACT1* and *MAGI2-AS3* were recurrently upregulated across multiple normal tissues, implying that *MIR100HG* may influence shared signaling pathways in homeostatic regulation. Notably, no universal downregulated gene was identified across all tissues, highlighting that *MIR100HG*-associated repression is context-specific and tightly regulated in non-cancerous systems.

3) Cancer vs. Normal DEG Comparison: To directly quantify *MIR100HG*-associated transcriptional divergence between malignant and healthy contexts, we intersected each cancer's top 100 up- and down-regulated genes with its corresponding normal-tissue DEG lists. As shown in Fig. 2, overlap was generally low: PAAD shared only two up-regulated genes (*LRCH2*, *MOXD1*) and no down-regulated genes; SKCM shared four up-regulated genes (*IL6ST*, *ITGA1*, *LAMA4*, *ZEB1*) with no down; LUAD had six shared up (*DACT3*, *FGF7*, *LRRN4CL*, *MSC-AS1*, *PRRX1*, *TGFB3*) and two shared down (*LINC02894*, *TTC39A*); PRAD exhibited the highest concordance with 13 shared up (e.g., *MAGI2-AS3*, *CNRIP1*, *LAMA4*) and three shared down (*PYCR3*, *SPTBN2*, *SYNGR2*); and STAD shared 13 up-regulated genes (*ABCC9*, *BNC2*, *CARMN*, ...) with no down. No gene was consistently down-regulated across both states in more than one tissue, reinforcing the context-dependence of *MIR100HG*-mediated repression. Interestingly, pan-tissue homeostatic candidates like *MAGI2-AS3* in PRAD and *LAMA4* in SKCM suggest partial overlap of regulatory programs, while key cancer-specific DEGs such as *NEGR1* (PAAD) and *MGP* (STAD) remained uniquely cancer-associated.

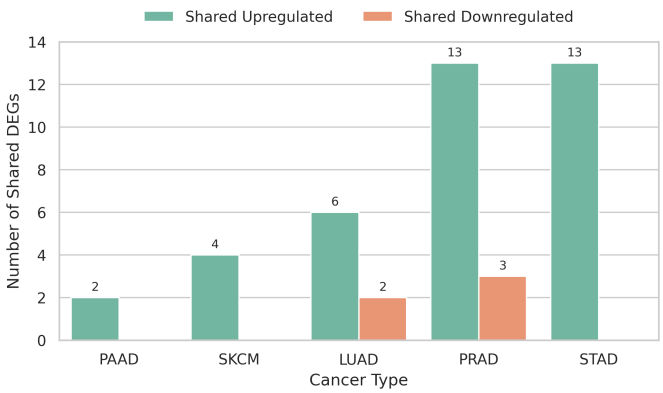

Fig. 2. Shared DEGs Between Cancer and Normal Samples

B. Transcription Factor Analysis

To better understand the regulatory mechanisms influenced by *MIR100HG* expression across different cancer types, we focused on upregulated transcription factors (TFs)—likely drivers of downstream gene expression in high *MIR100HG* states. TFs were identified via two-sample Student's *t*-tests between high and low *MIR100HG* expression samples, followed by Pearson correlation coefficients, r, with DEGs to infer *MIR100HG*-driven regulatory networks. The most influential TFs were extracted based on network centrality (hub scores), highlighting both common and cancer-specific regulatory axes.

1) TF Activity in Cancer: Across the five cancer types (PAAD, SKCM, LUAD, PRAD, and STAD), several transcription factors (TFs) consistently emerged as upregulated and central in regulatory networks. In PAAD, key hub TFs included *EP300*, *STAT3*, *FOXP2*, and *TCF12*. Among these, *EP300* is a well-known chromatin remodeler and transcriptional co-activator linked to tumor progression [5], while *STAT3* plays a canonical role in inflammation and metastasis. *FOXP2*, though previously studied in neuronal contexts, has been implicated in epithelial-to-mesenchymal transition (EMT) and prostate/gastric cancer invasiveness [3], [4]. SKCM featured *BACH1*, *REST*, and *CHD2* as top hubs, with *BACH1* known for roles in oxidative stress resistance and melanoma metastasis [16]. In LUAD, *NR3C1*, *FOSL2*, and *BACH1* dominated, with *NR3C1* acting as a glucocorticoid receptor with anti-inflammatory roles [17]. PRAD displayed upregulation of *FOXP2*, *GATA3*, and *MXI1*, involved in hormonal response regulation and prostate tumor development [18]. STAD was distinguished by *HDAC2*, *PBX3*, and *YY1*, where *HDAC2* is a potent histone deacetylase frequently linked to chromatin silencing and poor prognosis in gastric tumors [19]. Shared regulatory cores such as *TCF12*, *STAT3*, and *PBX3* were observed across multiple cancers, whereas others like *EP300* (PAAD), *HDAC2* (STAD), and *FOXP2* (PRAD, STAD) emerged as cancer-specific hubs.

2) TF Activity in Normal Tissues: In normal tissues, upregulated transcription factors formed broader, denser regulatory networks, with more even connectivity. Notably, *TCF12*, *REST*, *STAT3*, *NRF1*, and *PBX3* were consistently upregulated across all normal tissues. *REST*, a transcriptional repressor of neuronal genes, is essential for maintaining cellular identity and has been implicated in tumor suppression [20]. *NRF1*, associated with oxidative stress regulation and mitochondrial function, was another consistent hub across tissues. The high connectivity and balance in these TFs suggest homeostatic transcriptional regulation in contrast to the skewed networks observed in cancer.

3) Cancer vs Normal TF Regulation: Comparing TF networks between cancer and normal samples revealed substantial regulatory reprogramming. While TFs like *TCF12*, *STAT3*, and *PBX3* remained conserved, their network centrality and correlation with DEGs intensified in cancer, pointing to increased regulatory dominance. For instance:

- In PAAD, cancer-specific hubs included *EP300* and *SIN3A*, absent in the normal tissue.
- In STAD, *HDAC2* emerged as a top cancer hub but was not prominent in normal stomach tissue.

These transitions suggest *MIR100HG* expression may drive context-dependent TF activation, potentially rewiring transcriptional programs to favor tumorigenesis.

To visualize the consistency and specificity of hub TFs across tissues, we present a heatmap (Fig. 3) of hub TFs, with color intensity indicating their centrality in correlation-based networks. Shared TFs like *STAT3*, *TCF12*, and *PBX3* show widespread presence, while *EP300*, *FOXP2*, and *HDAC2* display tissue-specific enrichment.

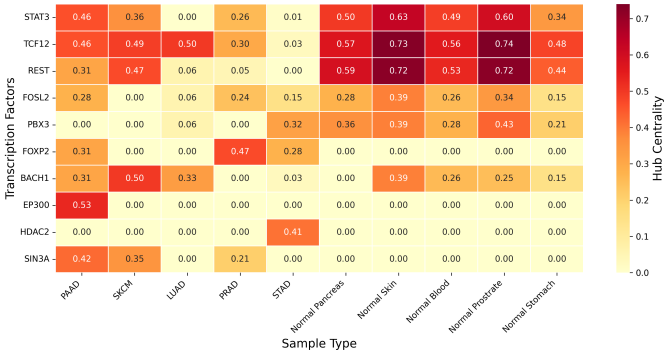

Fig. 3. Top Hub Transcription Factors Across Cancer and Normal Tissues

C. DNA Methylation

To investigate whether *MIR100HG* is epigenetically regulated across cancers, we examined methylation profiles of 31 probes located in its promoter, gene body, and upstream/downstream regulatory regions. These probes were analyzed across five cancer types: PAAD, SKCM, LUAD, PRAD, and STAD. Differential methylation testing and correlation with *MIR100HG* expression were used to evaluate the epigenetic control of this lncRNA in cancer progression.

1) Methylation in MIR100HG (Cancer): We performed a two-sided Student's *t*-test comparing methylation β-values in

high vs. low *MIR100HG* expression groups. All five cancers exhibited highly significant differences (p-values $< 1 \times 10^{-30}$), indicating consistent methylation alterations near the *MIR100HG* locus. These findings point toward a non-random, functionally relevant epigenetic modulation of *MIR100HG*. For instance, PRAD showed the strongest statistical signal ($p = 7.01 \times 10^{-117}$), followed by SKCM and LUAD. The differentially methylated probes are distributed across transcriptional regulatory regions, suggesting that *MIR100HG* expression may be influenced by both proximal promoter accessibility and long-range enhancer activity. These results are consistent with prior literature reporting that *MIR100HG* can be regulated by chromatin accessibility and methylation status, particularly in oncogenic contexts where it hosts the *miR*-100/*let*-7a/*miR*-125b cluster [1].

2) Correlation with Expression: To evaluate the functional relevance of differential methylation, we computed Pearson correlation coefficients between methylation β-values and *MIR100HG* expression levels across cancers. Probes were classified based on direction of correlation, strength, and recurrent appearance across tissues.

Key positively correlated probes:

- **cg14724899**: Strongly and consistently positively correlated in PAAD, LUAD, PRAD, and STAD. This probe lies upstream of the *MIR100HG* transcription start site, suggesting a putative enhancer or active chromatin region.
- **cg21854228**: Positively correlated in PAAD, PRAD, and STAD, located downstream, potentially marking enhancer-associated methylation.

Key negatively correlated probes:

- **cg03261272**: Recurrent negative correlation in PAAD, PRAD, and STAD, located within the gene body, suggesting repression-associated methylation.
- **cg08087655**: Context-dependent behavior—positive in PAAD/STAD, but negative in SKCM—suggesting dynamic, cancer-specific regulatory influence.

These patterns reinforce that methylation is not uniformly repressive or activating, but instead may be contextually tuned across tissues depending on chromatin context and local transcription factor occupancy. To visualize the consistency of probe-level regulation, we generated a grouped bar chart (Fig. 4) of expression–methylation correlations for the top probes in all five cancers. Probes with recurrent strong correlations highlight regions under conserved epigenetic control, while divergent correlations reflect cancer-specific methylation dynamics.

D. Integrated Multi-Omics Feature Matrix

To evaluate the combined regulatory and transcriptional landscape influenced by *MIR100HG*, we constructed an integrated feature matrix composed of top hub transcription factors (TFs) and differentially expressed genes (DEGs). Only features that were shared across both cancer and corresponding normal samples were retained to ensure comparability and reduce dimensional bias. This matrix reflects both direct transcriptional

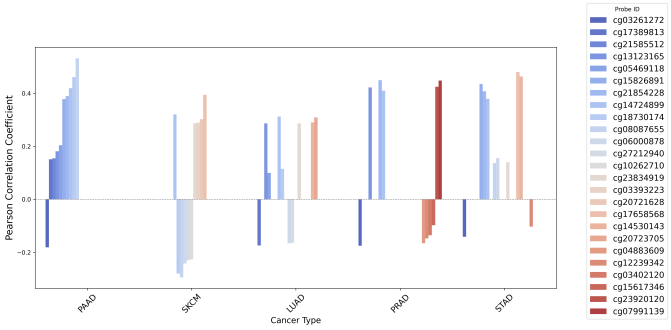

Fig. 4. Top Methylation Probes Correlated with MIR100HG Expression Across Cancers

outputs and upstream regulators influenced by *MIR100HG* expression.

1) Cancer vs. Normal Clustering: Principal Component Analysis (PCA) was performed on the integrated matrix for each tissue type to assess whether *MIR100HG*-associated multi-omics features can distinguish cancer from normal tissue states. Dimensionality reduction was applied after outlier filtering and feature scaling, and sample groupings were visualized along PC1 and PC2 axes. Across all five tissues—PAAD, SKCM, LUAD, PRAD, and STAD—PCA revealed clear and consistent separation between cancer and normal clusters (Fig. 5). The variance explained for PC1 ranged from 42.6% (LUAD) to 76.3% (PAAD), capturing a dominant biological signal likely reflective of *MIR100HG*-driven dysregulation. This pattern underscores the robust discriminatory power of *MIR100HG*-associated features when considered in an integrated multi-omics context. The cancer-specific reprogramming observed in PCA clustering aligns with previously reported roles of *MIR100HG* in oncogenic network modulation and immune regulation [22], [23].

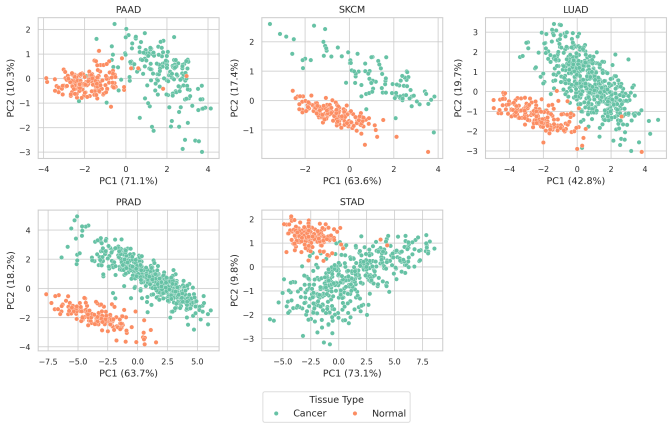

Fig. 5. PCA Grid of Integrated Feature Matrix for Cancer vs Normal Samples

2) Subtype Differentiation: In addition to binary cancer–normal separation, the integrated matrix revealed tissue-specific patterns of *MIR100HG* regulation, supporting its potential role in cancer subtype discrimination. For example:

- In SKCM, TFs such as *ZEB1* and *REST* co-occurred with DEGs involved in metastasis and EMT, revealing melanoma-specific regulation.
- In LUAD, distinct features like *DACT3* and *TGFB3* were retained, suggesting engagement with TGF-β signaling and stemness pathways.
- PRAD and STAD shared some features (e.g., *STAT3*, *PBX3*) but still maintained separable PCA profiles, indicating conserved yet divergently tuned regulatory circuits.

These distinctions support the idea that *MIR100HG*-associated networks not only mark cancer presence but also encapsulate subtype-level biological variation, which may aid future biomarker stratification or therapeutic targeting efforts [24], [25].

E. Clinical Outcome and Survival Analysis

To assess the clinical utility of *MIR100HG*-associated molecular signatures, we performed survival analysis across five cancer types using features derived from the integrated multi-omics matrix. These features encapsulated key regulators (hub TFs) and transcriptional targets (DEGs), hypothesized to reflect *MIR100HG*'s influence on tumor aggressiveness and patient prognosis.

1) Cox Proportional Hazards Modeling: We first applied Cox Proportional Hazards (CoxPH) modeling using the top 15 principal components (PCs) extracted from the integrated matrix for each cancer type. The model incorporated L_2 regularization (penalizer = 0.1) to ensure numerical stability in high-dimensional space. Among the five cancer types, PAAD and LUAD demonstrated statistically significant associations between select PCs and survival outcomes:

- **PAAD**: PC3 emerged as a strong prognostic marker ($p < 0.005$; HR = 1.16), indicating that this axis encapsulates a set of *MIR100HG*-driven alterations predictive of poorer survival. Additionally, PC4 showed a significant protective effect ($p = 0.01$; HR = 0.88).
- **LUAD**: Multiple components were predictive of outcome, including PC3 ($p < 0.005$; HR = 0.91), suggesting a protective role, alongside PC2 and PC4. These PCs are likely enriched for EMT, inflammation, and TGF-β signaling, all known *MIR100HG*-regulated mechanisms [20], [23].

In contrast, SKCM, PRAD, and STAD did not display significant associations across PCs (all $p > 0.05$), possibly due to lower event counts, weaker *MIR100HG* linkage, or higher survival heterogeneity in these contexts.

2) Kaplan–Meier Validation: To visually confirm the predictive relevance of PCs, we conducted Kaplan–Meier (KM) analysis by stratifying samples into "high" vs. "low" groups based on the median PC3 value—the top survival-associated component.

- In PAAD, the KM curve showed clear separation between the two groups, with the high-PC3 group displaying reduced overall survival (log-rank $p = 0.0116$).
- In LUAD, the stratification revealed an even more pronounced divergence in survival trends (log-rank $p = 0.0003$), reinforcing the prognostic value of this component.

These findings support the role of *MIR100HG*-associated PCs as compact representations of underlying molecular programs linked to disease progression.

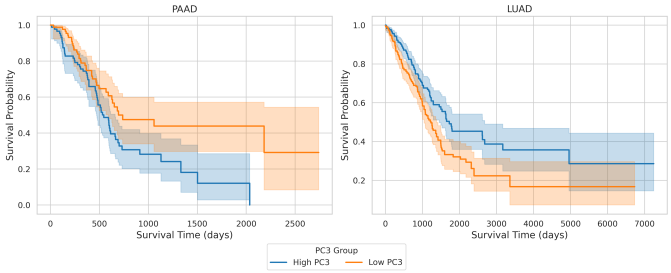

Fig. 6. Kaplan–Meier Grid Plot for PAAD and LUAD (PC3 High vs Low Groups)

- **Clinical Interpretation**: The predictive PCs in PAAD and LUAD encompass features such as *STAT3*, *TGFB3*, and *HGF*, all of which have been independently associated with poor prognosis in the literature [24], [25]. The ability of these latent dimensions to stratify survival outcomes highlights the potential of *MIR100HG*-driven molecular programs to serve as biomarker axes for clinical risk prediction. In contrast, the non-significant outcomes in SKCM, PRAD, and STAD suggest that either alternative regulatory axes dominate prognosis in these contexts, or that *MIR100HG*-linked signals may be more tumor subtype-specific in their influence.

VI. Further Work and Improvement

While this study provides a robust computational framework, several avenues remain for deeper exploration:

- **Experimental Validation**: Functional assays (e.g., *siRNA* knockdown, *CRISPR*) are necessary to confirm the direct regulatory roles of *MIR100HG* on the identified DEGs and TFs, particularly in contexts like PAAD and LUAD where clinical relevance is strongest.
- **Network Refinement with Time-Series Data**: Incorporating longitudinal expression or methylation data could enable dynamic modeling of *MIR100HG*'s regulatory impact over time or during treatment.
- **Single-cell Resolution**: Integrating single-cell RNA sequencing and methylation data could help identify specific regulatory patterns based on cell types, clarifying the role of *MIR100HG* in tumor microenvironment heterogeneity.
- **Subtype-Specific Modeling**: Cancer subtypes may harbor distinct *MIR100HG* networks. Subtype-stratified models would refine prognostic predictions and may help uncover subtype-selective therapeutic vulnerabilities.
- **Therapeutic Targeting Potential**: Given the link of *MIR100HG* to known oncogenic regulators (e.g.,

STAT3, *HGF*, *TGFB3*), investigating druggable interactions within the *MIR100HG*-centered network could guide the development of targeted therapies.

In general, this work serves as the foundation for both hypothesis generation and translational applications involving *MIR100HG*, strengthening its potential as a master regulator and biomarker in diverse cancer landscapes.

VII. Conclusion

This study presents an integrative multi-omics investigation into the regulatory influence of *MIR100HG* across five cancer types and their corresponding normal tissues. By combining differential gene expression, transcription factor profiling, and DNA methylation, we constructed a unified feature matrix reflective of *MIR100HG*-associated molecular programs.

Key findings from the expression analysis revealed context-specific upregulation and suppression of genes involved in immune evasion, extracellular matrix (ECM) remodeling, and cell differentiation, suggesting both shared and cancer-specific roles of *MIR100HG*. The transcription factor (TF) analysis underscored *STAT3*, *TCF12*, and *PBX3* as recurrent regulatory hubs, while *FOXP2* and *HDAC2* emerged as tissue-specific regulators in cancer contexts. DNA methylation analysis further supported the regulatory potential of *MIR100HG*, highlighting distinct epigenetic signatures that correlated with expression levels and possibly reflected active or repressive chromatin states.

Integrated multi-omics principal component analysis (PCA) confirmed the ability of these features to stratify cancer vs. normal conditions effectively, with strong clustering observed in all five cancer types. Furthermore, Cox proportional hazards (CoxPH) survival modeling and Kaplan–Meier analysis demonstrated that select principal components (notably PC3 in pancreatic adenocarcinoma (PAAD) and lung adenocarcinoma (LUAD)) are significantly associated with clinical outcomes, thereby positioning *MIR100HG*-associated features as potential prognostic biomarkers.

Altogether, our results position *MIR100HG* as a multi-modal regulator whose activity spans transcriptional, regulatory, and epigenetic levels, impacting both tumor biology and patient prognosis in a cancer-type-specific manner.

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GitHub URL: https://github.com/orgs/EMATM0050-2024/teams/groupm22