JOURNAL OF
EMERGING INVESTIGATORS

Article

Protein kinases in phagocytosis (phagocytotic kinome): A promising biomarker set in cancer therapeutics

Sadhika Arumilli[1], Hengrui Liu[2,3]

[1] The Future Kids Global School, Rajahmundry, Andhra Pradesh, India
[2] Cancer Research Institute, Jinhan University, Guangzhou, China
[3] Yinuo Biomedical, Tianjin, China

SUMMARY

Cancer is a complex disease characterized by genetic diversity, often involving dysregu-lation of critical cellular pathways. Protein kinases, particularly those involved in phagocytosis, play pivotal roles in cellular homeostasis and immune response. This study systematically exam-ines the genetic alterations and expression profiles of protein kinases associated with phagocy-tosis (phagocytotic kinome) across cancer types, using data from the Cancer Genome Atlas (TCGA) and other publicly available verified resources. We hypothesized that the protein kinase genes show alterations and expressions in various cancer tissues, unlike in normal tissues, and that this is significant. Our results indicate that MET and MERTK were the most mutated protein kinase genes, with missense mutations predominating across cancers. Copy Number Variations (CNVs) are structural alterations in the genome where sections of DNA are duplicated or deleted. Analysis of CNV profiles of the protein kinase genes associated with phagocytosis re-vealed that heterozygous amplifications and deletions were predominant types with significant positive correlation to survival in some uterine and kidney cancers, while methylation analysis shows cancer-specific regulatory patterns influencing gene expression. Differential expression analysis uncovered distinct cancer-type-specific expression profiles, with genes like MET and BTK exhibiting significant variation. Crosstalk pathway analysis further demonstrated the in-volvement of these kinases in key cancer-related processes, importantly, epithelial-mesenchymal transition (EMT) and apoptosis. Drug sensitivity analysis identified potential thera-peutic targets, with gene expression significantly correlating with cancer cell line responsiveness to specific compounds. These discoveries underscore the importance of the phagocytotic ki-nome in cancer biology and suggest potential therapeutic approaches to enhance immune re-sponses and improve treatment outcomes in the future.

INTRODUCTION

The global cancer burden is expected to be 28.4 million cases in 2040, a 47% rise from 2020 (1, 2). Traditionally, cancer research has focused on the tissue of origin, which can be limiting as it may fail to capture the underlying molecular and genetic diversity of tumors (3). The paradigm shift towards pan-cancer research has opened new avenues of understanding. At the genetic and molecular levels, uncovering shared oncogenic pathways across different cancers has led to a deeper understanding of cancer biology and therapeutic targets (4, 5). Previous biomarker studies for cancer have been highly successful in identifying potential markers for cancer diagnosis and prognosis (6, 7).

In addition to other more commonly studied cell death pathways, such as apoptosis and necrosis, cells can die by being phagocytosed by other cells - a form of cell death termed phagoptosis, cell cannibalism, programmed cell removal, or primary phagocytosis (8). Immunity against cancer is also partly mediated by macrophage phagocytosis of cancer cells, but cancer cells can also phagocytose host cells and other cancer cells to survive (9). Protein kinases belong to the phosphoryl-transferases superfamily of enzymes, which activate enzymes via phosphorylation. These protein kinases are involved in almost all cellular functions such as transcription, translation, cell division, and apoptosis (10). Protein kinase-mediated phagocytosis is increasingly recognized as a critical mechanism for maintaining cell homeostasis and innate immunity (10). Emerging studies have demonstrated that innate immune checkpoints, which interfere with the detection and clearance of malignant cells through phagocytosis and suppress innate immune sensing, also have a key role in tumour-mediated immune escape and might, therefore, be potential targets for cancer immunotherapy (11). Hence, dysregulation of protein kinases in phagocytic cells can lead to impaired immune response, which may contribute to the development and progression of cancer (11, 12). Targeting the CD47-SIRPα axis is such an emerging cancer therapy strategy (11). Many types of cancer cells overexpress the immunoglobulin CD47 on their cell surfaces. CD47 forms a signalling complex with signal-regulatory protein α (SIRPα), enabling the escape of these cancer cells from macrophage-mediated phagocytosis (11). The CD47–SIRPα axis is a dual-function checkpoint. It suppresses innate immunity by preventing macrophage phagocytosis. It indirectly suppresses adaptive immunity by limiting antigen presentation and T cell activation. A growing number of studies have since demonstrated that inhibiting the CD47-SIRPα signalling pathway promotes immune response and enhances the phagocytosis of tumour cells by macrophages (11-15).

The kinome of an organism is the total set of genes in the genome that encode all the protein kinases. A subset of such kinases is crucial for mediating phagocytosis (16). We

https://doi.org/10.59720/24-352

hypothesized that a non-random and statistically significant relationship exists between recurring mutation patterns and expressions in the phagocytotic kinome of cancer cells. The study employed multi-omic data analysis from TCGA (The Cancer Genome Atlas), GDSC (Genomics of Drug Sensitivity in Cancer), CTRP (Cancer Therapeutics Response Portal) and other databases to examine mutations, copy number variations, methylation and expression of phagocytotic kinases across cancers, using tools like Maftools, GISTIC2.0 (Genomic Identification of Significant Targets in Cancer), GSVA (Gene Set Variation Analysis) and Cox regression models for survival and drug sensitivity correlations. This research reveals that protein kinases involved in phagocytosis are not just cellular workhorses but potential game-changers in cancer therapy. The phagocytotic kinome holds promise as a biomarker set and therapeutic target in cancer, offering new avenues for precision medicine and immune-based treatments.

RESULTS

To identify commonly mutated genes across cancer types, we analyzed the TGCA dataset for single-nucleotide variants (SNVs). An SNV is a change in a single nucleotide in the genome (17). SNVs are the most common type of sequence change in the human genome and play an important role in disease susceptibility and an individual›s response to therapy (18). The analysis of SNVs in protein kinase genes associated with phagocytosis revealed that *MET* and *MERTK* were the most frequently mutated across different cancer types. *MERTK* exhibited the highest mutation frequency with 51 SNV samples in 468 SKCM (Skin Cutaneous Melanoma). Overall, UCEC (Uterine Corpus Endometrial Carcinoma) had a higher SNV mutation rate across the genes; however, SKCM and COAD (Colon Adenocarcinoma) also presented significant mutation frequencies in several genes **(Figure 1A)**. The SNV landscape showed that most of the mutations were missense mutations and the most common SNV classes were C>T and C>A **(Figure 1B)**. Additionally, we analyzed the association between SNVs of the genes and patient survival. While SNVs of *TYRO3, LYN,* and *PTK3* genes significantly exhibit positive correlations with survival in specific cancer types, most other gene SNV expressions did not exhibit significant associations with survival outcomes across most cancer types analyzed **(Figure 1C)**.

Copy Number Variations (CNVs) are segments of DNA that vary in copy number between individuals or between normal and diseased cells (19). CNVs are crucial in cancer genome studies because they often drive tumour development, progression, and treatment response (20). The analysis of the CNV profile of the protein kinase genes associated with phagocytosis revealed diverse CNV patterns with notable variations observed across different types of

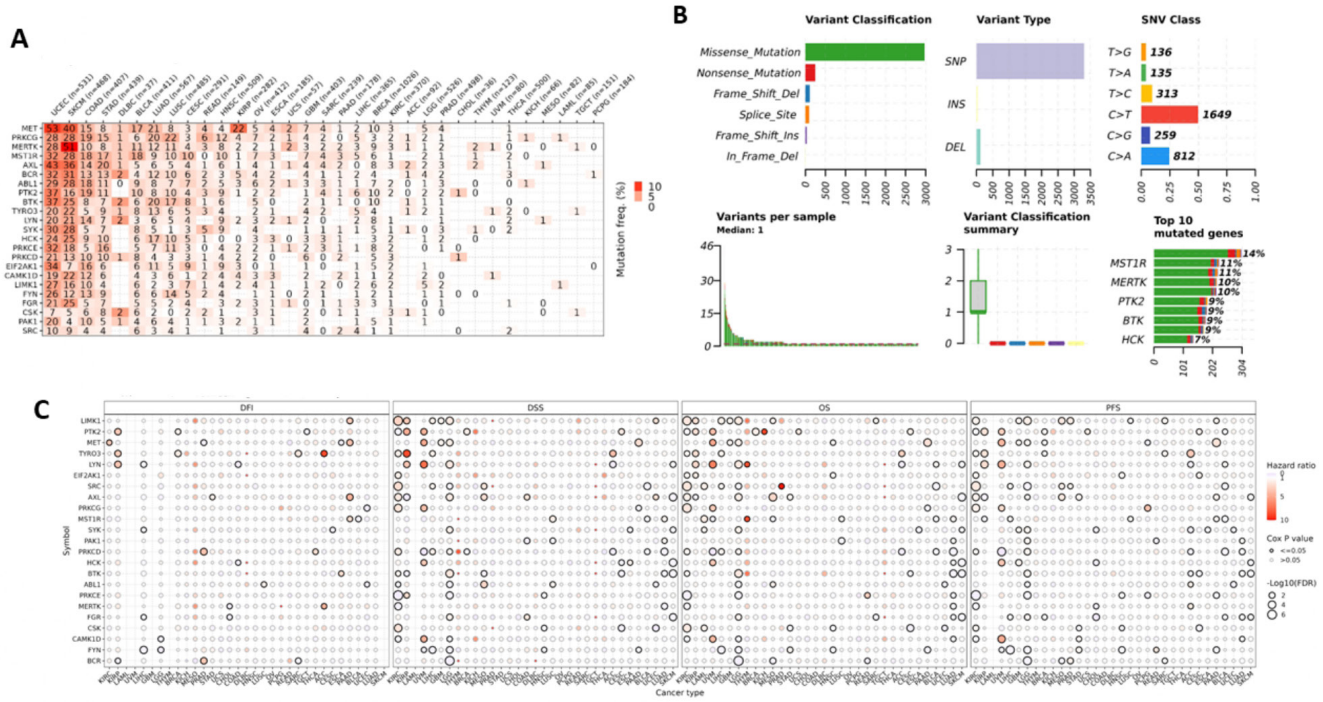

Figure 1:Protein kinase genome mutations in single-nucleotide variations (SNV) class and their relation to survival outcomes. A) Heatmap of mutation frequency. The color represents the frequency of mutations per cancer type; MERTK exhibited the highest mutation frequency, with 51 SNV samples in 468 SKCM (skin cutaneous melanoma). UCEC (Uterine Corpus Endometrial Carcinoma) had a higher SNV mutation rate across the genes. B) An overview of SNV classes in protein kinase genes showing the count of each type of harmful mutation and the number of different variant types (Single Nucleotide Polymorphism, Insertion, and Deletion). The SNV landscape shows that most of the mutations were missense mutations, and the most common SNV classes were C>T and C>A. C) Correlation between SNV and survival in normal and tumor samples. TYRO3, LYN and PTK3 show significant correlations with survival in specific cancer types, most gene expressions do not exhibit strong associations with survival outcomes across most cancer types analyzed.

https://doi.org/10.59720/24-352

cancer. The distribution of CNVs revealed that heterozygous amplifications and deletions were predominant among CNV types, and their prevalence across different cancer types was visualized to highlight tissue-specific patterns **(Figure 2A, 2B**). We evaluated how CNVs influence gene expression across various cancer types. The strongest correlations were observed in BRCA (Breast Invasive Carcinoma), OV (Ovarian Serous Cystadenocarcinoma), LUAD (Lung Adenocarcinoma), HNSC (Head and Neck Squamous Cell Carcinoma), and COAD (Colon Adenocarcinoma). While the strength of correlation varied, most cancers showed a generally positive relationship between CNV and gene expression **(Figure 2C)**. We found significant associations between CNV and survival across various cancer types, especially in UCEC, KIRP (Kidney renal papillary cell carcinoma), LGG (Brain Lower grade Glioma), and KIRC (Kidney renal clear cell carcinoma) **(Figure 2D)**. This indicates that CNV levels in these cancers may be linked to differences in patient survival. Our analysis suggests a possible association between heterozygous CNV of protein kinase genes involved in phagocytosis and cancer prognosis.

Studying DNA methylation differences between normal and cancer tissues helps uncover epigenetic alterations that drive tumor development and progression. The analysis of methylation differences between tumor and normal tissues revealed significant disparities. For instance, the genes *MERTK* and *LYN* show high methylation levels in *BRCA,* while *EIF2AK* has low methylation levels in the same cancer type. The methylation differences are not uniform across all cancer types, indicating that each cancer type has a unique methylation profile for these genes **(Figure 3A)**. The analysis of the correlation between methylation and mRNA expression profiles uncovered a consistent pattern across various cancer types, highlighting significant, largely negative associations between methylation levels and mRNA expression. However, there were certain genes like *PTK2* and *PRKCD* in UVM (Uveal Melanoma) which showed a strong positive association between their methylation levels and mRNA expression levels. **(Figure 3B)**. In the survival profile analysis, only a few cancers demonstrated either a consistent positive or negative relation between methylation levels and patient survival **(Figure 3C)**. The analysis suggests that hypermethylation may lead to the down-regulation of specific genes, impacting their expression and potentially influencing patient outcomes in various cancer types.

The analysis of differential expression of protein kinase genes associated with phagocytosis across various cancer types revealed some notable expression differences

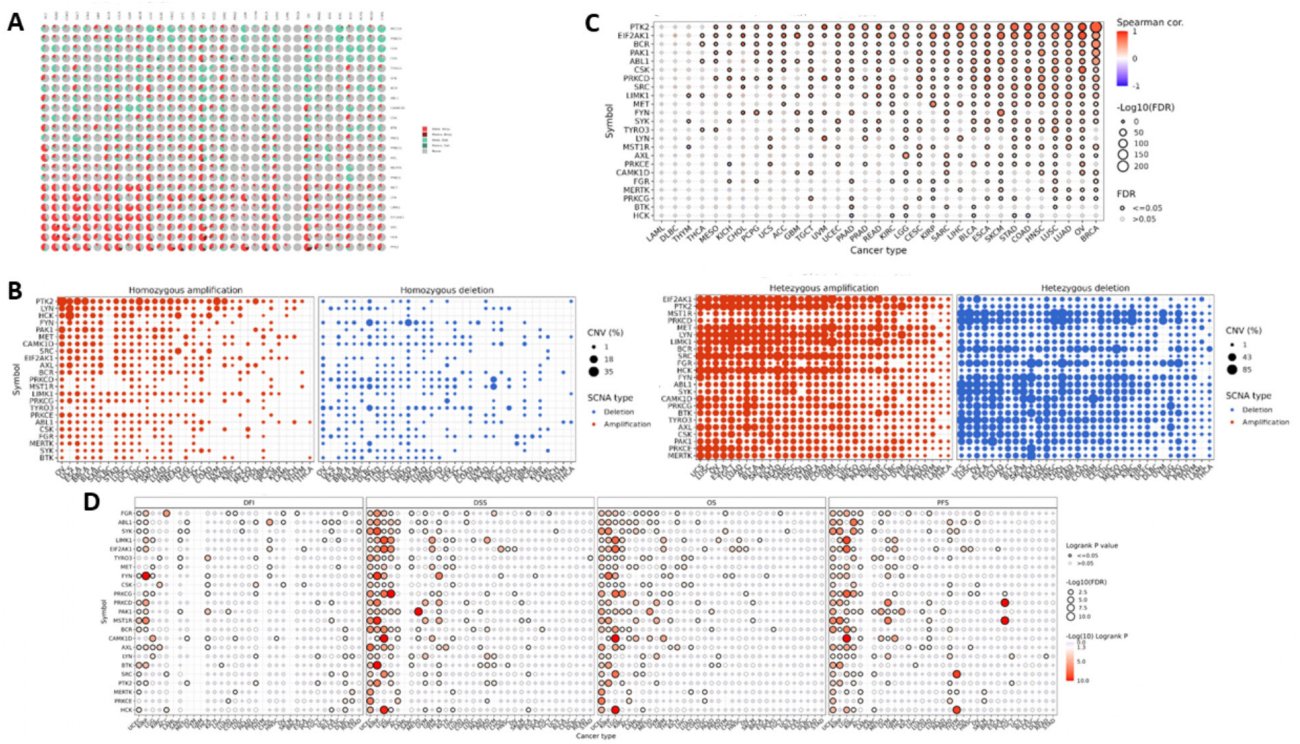

Figure 2: Protein kinase genome alterations in copy number variation (CNV) class and their correlation with mRNA expression and survival in cancers. A) Pie charts of CNV distribution across cancers showing that heterozygous amplifications and deletions were predominant among the various CNV types. B) Profile of heterozygous CNV and homozygous. The dark color of the graph shows the high percentage of heterozygous amplification and deletion of the CNVs for each gene in each cancer. C) Correlation between CNV and "mRNA" expression. The cancers with the strongest correlations were BRCA (Breast invasive carcinoma), OV (Ovarian serous cystadenocarcinoma), LUAD (Lung adenocarcinoma), HNSC (Head and Neck squamous cell carcinoma), and COAD (Colon adenocarcinoma); however, most cancers showed a positive correlation with expression. D) The survival difference between CNV groups. There is a significant association between CNV and survival across various cancer types, especially in UCEC (Uterine Corpus Endometrial Carcinoma), KIRP (Kidney renal papillary cell carcinoma), LGG (Brain Lower Grade Glioma) and KIRC (Kidney renal clear cell carcinoma).

https://doi.org/10.59720/24-352

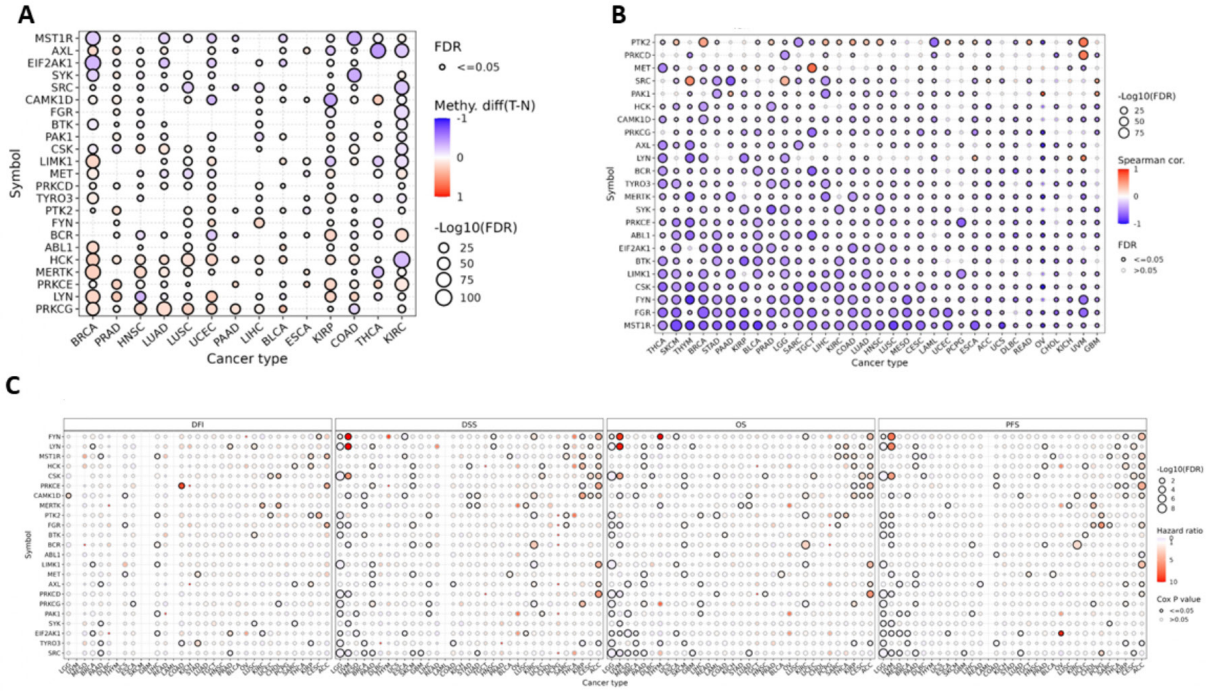

Figure 3: Methylation differences in cancer cells vs normal cells and the correlation between mRNA expression and survival. A) Methylation between normal and tumor samples, where methylation differences are not uniform across all cancer types, indicating that each cancer type has a unique methylation profile for these genes. B) The correlation between methylation and 'mRNA' expression uncovered a consistent pattern across various cancer types, highlighting significant associations between methylation levels and expression that were mostly negative. C) Difference in survival between high and low methylation of the genes. In the survival profile analysis, only a few cancers demonstrated a relation between methylation levels and patient survival.

between cancer and non-cancer tissues. Using TCGA data, we examined the expression of protein kinase genes associated with phagocytosis in multiple cancer types **(Figure 4A).** In LUSC (Lung Squamous cell carcinoma), genes like *BTK, FGR* and *PRKCE* were significantly down-regulated. Conversely, in THCA, *MET* was up-regulated. Additionally, BRCA displayed a mix of up-regulated and down-regulated genes, with notable changes in *EIF2AK1* and *MET*. Overall, the expression profiles across cancer types indicate that, there are unique expression patterns that are specific to a few cancer types. Nonetheless, for most cancer types, there were minimal notable variations in the expression of the protein kinase genes controlling phagocytosis between cancerous and normal tissues. In the analysis of protein kinase gene expression subtype differences, the top cancers that showed consistently high expression were BRCA, KIRC, STAD (Stomach Adenocarcinoma), and LUAD, with FDR < 0.05 being significant **(Figure 4B)**. The survival analysis revealed that KIRC and LGG were the top cancer types associated with the phagocytotic kinome genes **(Figure 4C)**. *TYRO3* has a higher survival rate when overexpressed in KIRP and ACC (Adrenocortical Carcinoma). It also shows that overexpression of *LYN* in UVM is associated with a higher survival rate of patients. At this point, we can safely conclude that the expression of certain protein kinase genes predicts survival better than others across cancers. These indicate the statistical significance of each predictor, which has a diverse association with survival outcomes across a few cancer types.

Pathway activity scores for 7,876 samples were calculated using Reverse-phase protein array (RPPA) data from the TCPA (The Cancer Proteome Atlas) database. To investigate the potential cross-talk of protein kinase genes involved in phagocytosis with other cancer-related pathways, we analyzed the data from pathway activity scores for 7,876 samples from the TCPA database to determine the association of these genes with various biological processes. Among the 7,876 tumor samples analyzed, 44% of samples expressing *FYN*, 41% expressing *AXL* and *FGR*, and 38% expressing *HCK* showed EMT pathway activation, suggesting that these kinases may play a role in promoting cancer cell migration and invasion. **(Figure 5**) (21). Additionally, 41% of samples expressing *AXL* were linked to cell cycle inhibition, highlighting its potential role in controlling cell proliferation. In the context of apoptosis activation, genes such as *CSK*, *LYN, LIMK1*, and *FGR* showed associations, indicating their involvement in programmed cell death pathways. Furthermore, 38% of samples expressing *HCK* were related to hormone estrogen receptor activation (ER_A), and both *MET* and *LIMK1* showed associations with hormone androgen receptor inhibition (AR_I), suggesting a complex interplay between these protein kinases and hormone signaling pathways.

We also investigated the relationship between the expression of protein kinase genes in phagocytosis and the tumor immune microenvironment. We calculated a gene set signature (GSVA score) to evaluate the infiltration levels of different immune cells across various cancer types. The results indicated a significant correlation between the GSVA

https://doi.org/10.59720/24-352

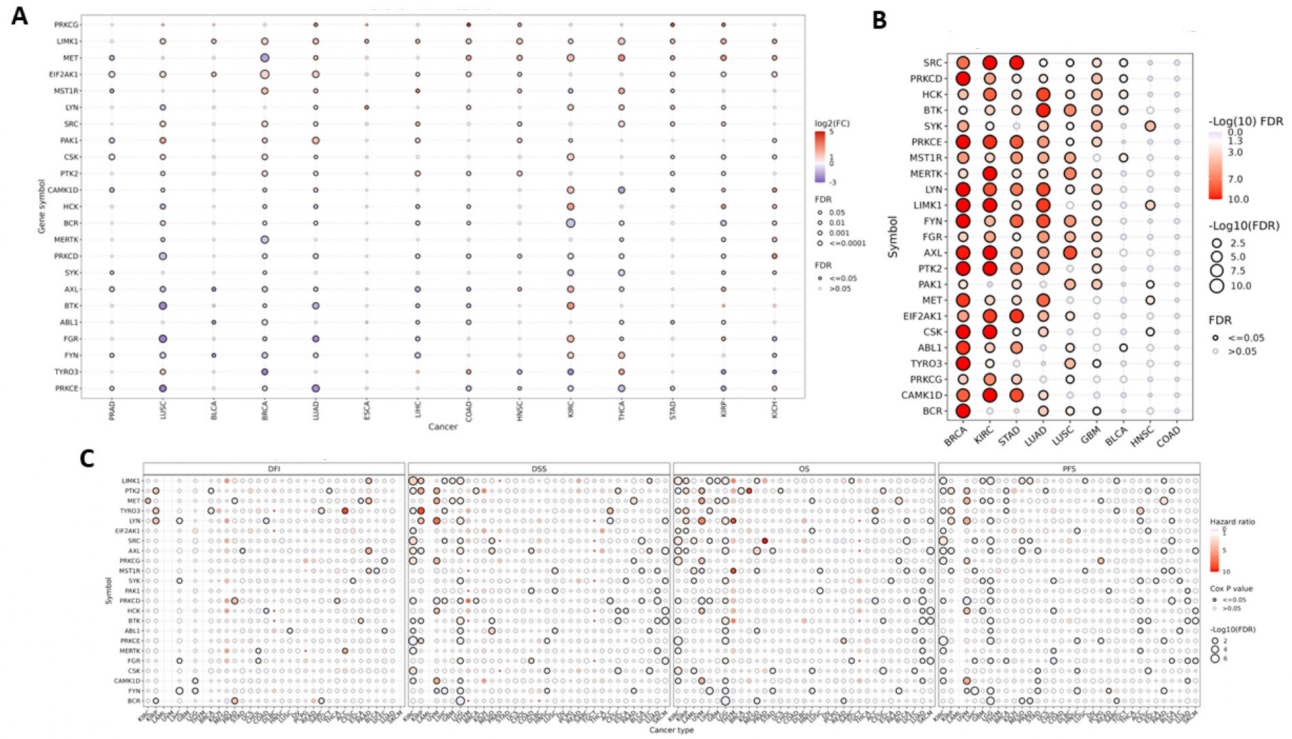

Figure 4: mRNA expression in protein kinase genes in normal and tumor samples. A) mRNA expression levels between normal and tumor samples. In LUSC (Lung squamous cell carcinoma), genes like BTK, FGR, and PRKCE were significantly down-regulated. Conversely, in THCA (Thyroid carcinoma), MET was up-regulated. Overall, the expression profiles across cancer types indicate that while there are some consistently up-regulated or down-regulated genes in certain cancers, there are unique expression patterns that are specific to each cancer type. B) Difference of expression levels between high and low subtypes. BRCA (Breast invasive carcinoma) and KIRC (Kidney renal clear cell carcinoma) emerged as the most significant cancer types. However, STAD (Stomach adenocarcinoma) and LUAD (Lung adenocarcinoma) also demonstrated notable significance. C) Difference of survival between high and low expression. The size of the dot represents the significance of the gene's impact on survival across different cancer types, while the color indicates the hazard ratio. DFI: disease-free interval; DSS: disease-specific survival; OS: overall survival; PFS: progression-free survival.

score and the infiltration levels of numerous immune cells. Particularly, the GSVA score showed a strong negative correlation with neutrophil cells and a positive correlation with macrophage cells (**Figure 6A**). Additionally, we examined the association between these protein kinase genes and drug sensitivity in cancer cell lines using data from the GDSC (Genomics of Drug Sensitivity in Cancer) and CTRP (Cancer Therapeutics Response Portal) databases. The results revealed that the protein kinase genes related to phagocytosis were significantly correlated with the sensitivity of cancer cells to multiple compounds (**Figure 6B, 6C**). These findings underscore the potential impact of protein kinase genes in phagocytosis on immune cell infiltration in cancers and suggest new avenues for targeted drug development.

DISCUSSION

The results of this study provide important insights into the genetic and molecular alterations of protein kinase genes involved in phagocytosis across various cancer types. These findings underscore the relevance of these genes not only in cancer progression but also in their potential as therapeutic targets

The analysis of SNVs revealed that *MET* and *MERTK* were the most frequently mutated genes across multiple cancer types, with *MET* showing the highest mutation frequency in SKCM and an overall higher mutation rate in UCEC. The presence of predominantly missense mutations, particularly in *MET* and *MERTK,* suggests these genes may be critical in the oncogenic processes of various cancers. These findings are consistent with previous studies that highlight *MET*'s role in cancer progression, where its mutation and overexpression drive tumor growth and metastasis (22). Furthermore, while genes such as *TYRO3, LYN*, and *PTK3* exhibited significant correlations with survival in specific cancers, these associations were not universally observed across all cancer types, indicating a complex, context-dependent role of these kinases in cancer biology (23-25). Future studies should explore the mechanistic implications of these mutations to identify potential targets for therapeutic intervention.

CNV profiling revealed widespread heterozygous amplifications and deletions across cancer types, with significant survival associations observed particularly in UCEC, KIRP, and KIRC. The observed positive correlations between CNVs and gene expression in cancers like BRCA, OV, and LUAD suggest that CNVs play a critical role in modulating gene expression, which in turn may influence tumor behavior and patient outcomes. It is a well-established scientific fact that CNV profiling plays a critical role in cancer by identifying gene amplifications or deletions that influence tumour aggressiveness, treatment resistance, and patient

https://doi.org/10.59720/24-352

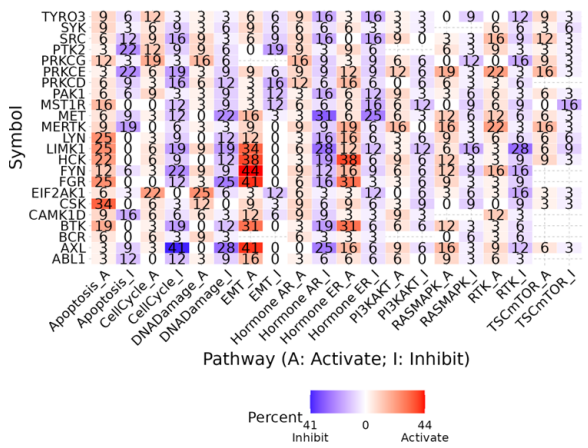

Figure 5: Correlation between expression and pathway activity of the genes. In the analysis of the total samples, 44% of FYN, 41% of AXL and FGR, and 38% of HCK were associated with the activation of the epithelial-mesenchymal transition (EMT) and 41% of AXL was linked to cell cycle inhibition, highlighting its potential role in controlling cell proliferation. In the context of apoptosis activation, genes such as CSK, LYN, LIMK1, and FGR showed associations. The percentages indicate the number of samples out of the total samples analysed, which showed a consistent effect (either activation / inhibition) on the specific cancer associated pathways tested.

prognosis (26). These genomic alterations help stratify patients for targeted therapies and guide clinical decisions, ultimately improving outcomes across cancers like breast, lung, glioblastoma, neuroblastoma, and colorectal cancer (9, 27). Hence, our findings align with previous reports that highlight CNVs as key drivers of cancer heterogeneity (28). The identification of significant CNV-survival associations further reinforces the clinical relevance of these alterations, suggesting that CNV profiling could serve as a valuable prognostic tool for personalized cancer treatment strategies.

DNA methylation is an epigenetic modification—a chemical change to DNA that does not alter the sequence but affects how genes are expressed. In cancer, the methylation process can silence protective genes and activate harmful ones (29). Our methylation analysis highlighted cancer-specific patterns, with genes such as *MERTK* and *LYN* showing hypermethylation in BRCA and an inverse relationship between methylation levels and mRNA expression in most cancers. Interestingly, while hypermethylation was generally associated with gene silencing, a few exceptions, such as *PTK2* in UVM, demonstrated a positive correlation between methylation and expression, suggesting alternative regulatory mechanisms at play (30, 31). These findings imply that methylation may serve as a regulatory mechanism for protein kinase gene expression in cancer, with potential implications for early diagnosis, formulating targeted epigenetic therapies, combating therapy resistance, synergizing immunotherapy, and methylation editing.

Differential expression analysis demonstrated significant cancer-specific variations in the expression of protein kinases associated with phagocytosis. While some genes, such as *MET,* were consistently upregulated in certain cancers like THCA, others, including *BTK* and *FGR*, were downregulated in LUSC. These results suggest that the phagocytotic kinome plays diverse roles in cancer biology, with specific genes either promoting or inhibiting tumor progression depending on the cancer type (32). The observed correlations between gene expression and survival, particularly for KIRC and LGG, offer new therapeutic avenues, like kinases being used as biomarkers and their levels for prognostication and targeting them with drugs might lead to better outcomes in some cancers at least.

The crosstalk between the phagocytotic kinome and key cancer-related pathways, such as EMT and apoptosis, further emphasizes the importance of these kinases in tumor progression. The involvement of genes like *FYN*, *AXL*, and *HCK* in EMT highlights their potential role in promoting cancer cell migration and invasion. In contrast, the association of genes such as *CSK* and *LYN* with apoptosis underscores their role in modulating cell death pathways. These findings suggest that targeting the crosstalk between the phagocytotic kinome and these critical pathways could disrupt key processes in cancer development and progression, presenting new opportunities for therapeutic intervention (33). There are many other cancer-related pathways, such as MAPK, RAD-related pathways, and IGFBP-related pathways, that should be studied in the future (34 - 36).

The observed correlations between protein kinase gene expression and drug sensitivity across multiple cancer cell lines indicate that these genes may influence cancer cell responsiveness to targeted therapies. In particular, the negative correlation between gene expression and sensitivity to certain compounds suggests that the overexpression of specific kinases may confer drug resistance. These findings offer valuable insights for the development of personalized cancer treatments, where targeting the phagocytotic kinome could enhance the efficacy of existing therapies or help overcome drug resistance (37). Targeted research is needed to validate how protein kinases influence cancer drug resistance, using molecular experiments, clinical trials, and computational models. Collaborative efforts across disciplines could lead to personalized treatments that overcome resistance by profiling kinase activity and modulating the tumour microenvironment.

While this study provides a comprehensive overview of the genetic and molecular alterations of protein kinases involved in phagocytosis across cancers, several limitations must be acknowledged. The reliance on publicly available datasets, such as TCGA, may introduce biases related to sample composition and data quality. Additionally, while this study identified significant associations between genetic alterations and survival, further experimental validation is required to establish causal relationships. Future research should focus on the functional characterization of these protein kinase genes in cancer models to better understand their roles in tumor progression and immune modulation. In summary, this study aimed to present a comprehensive multi-omic analysis of protein kinase genes involved in phagocytosis—termed the phagocytotic kinome—across various cancer types. It reveals that genes like *MET* and *MERTK* are frequently mutated, with structural variations and methylation patterns significantly influencing gene expression and patient survival. Differential expression profiles and pathway crosstalk highlight their roles in tumor

https://doi.org/10.59720/24-352

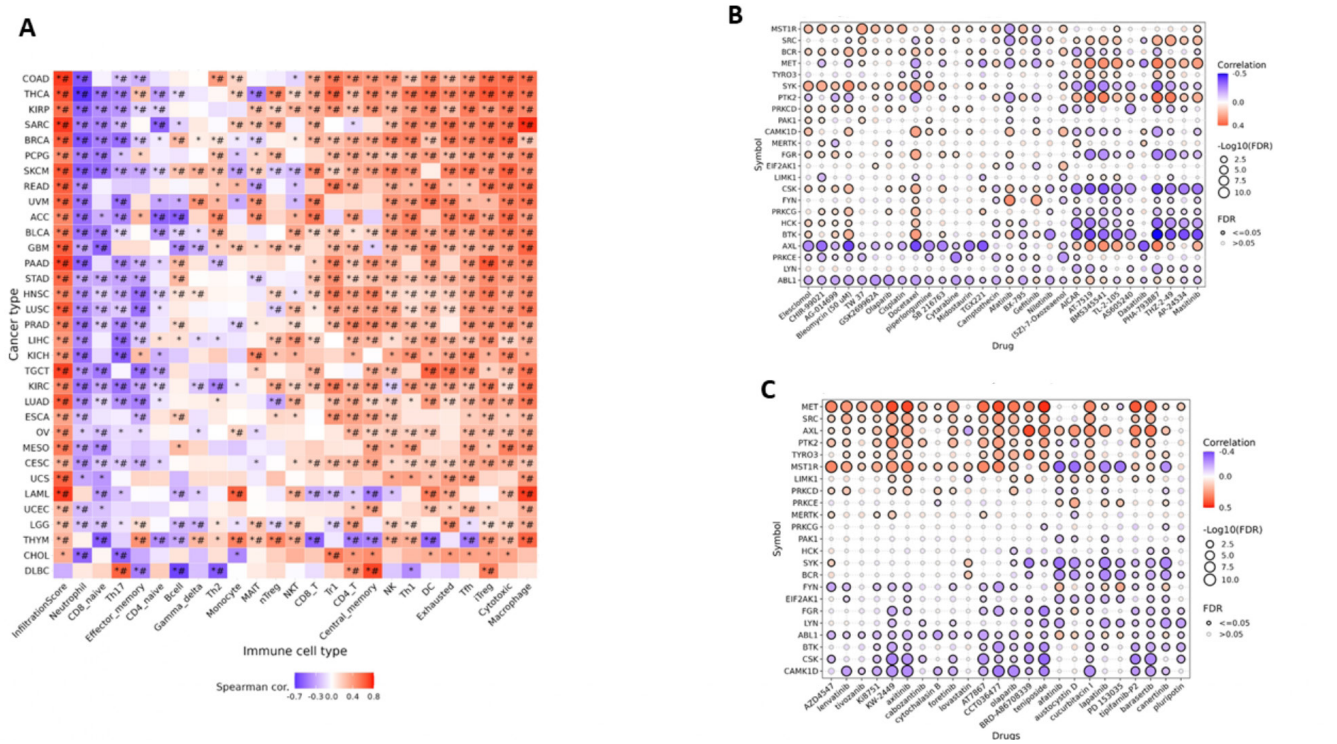

Figure 6: Relationship between Protein kinase genes with drug sensitivity in cancer cells. A) Association between GSVA (Gene Set Variation Analysis) score and activity of cancer-related pathways in selected cancers. *p-value ≤ 0.05; #: FDR ≤ 0.05. B) Expression of the genes and the sensitivity of cancer cell lines analyzed using GDSC (Genomics of Drug Sensitivity in Cancer) and CTRP (Cancer Therapeutics Response Portal) data, which showed the protein kinase genes related to phagocytosis were significantly correlated with the sensitivity of cancer cells to multiple compounds.

progression, immune cell modulation, and apoptosis. Importantly, these kinases show strong associations with immune cell infiltration and drug sensitivity, positioning them as promising biomarkers and therapeutic targets in precision oncology.

MATERIALS AND METHODS

The genome of protein kinases involved in phagocytosis, often referred to as the phagocytotic kinome, comprises a curated set of genes encoding kinases that regulate various stages of phagocytosis—from receptor signaling and cytoskeletal rearrangement to vesicle trafficking and immune modulation. This genome includes mainly *ABL1, AXL, BCR, BTK, CAMK1D, CSK, EIF2AK1, FGR, FYN, HCK, LIMK1, LYN, MERTK, MET, MST1R, PAK1, PRKCD, PRKCE, PRKCG, PTK2, SRC, SYK* and *TYRO3* (16).

Data Acquisition

Expression data, including clinical information, SNVs, CNVs, and methylation data, were obtained from TCGA and the NCI Genomic Data Commons (38, 39). Reverse phase protein array (RPPA) data were retrieved from The Cancer Proteome Atlas (TCPA) (40). Immunotherapy response and survival data were retrieved from the TIDE (Tumor Immune Dysfunction and Exclusion) database (41). Gene-drug sensitivity data were collected from the Genomic Drug Sensitivity in Cancer (GDSC) database and the Cancer Therapeutics Response Portal (CTRP) (42, 43). The data was pre-processed with normalisation to TPM (Transcripts per Million) using TPMCalculator version v0.0.1 (44).

Gene Alterations and Expression Analysis

SNV visualizations were generated with the Maftools v2.10.0 , while CNV data were processed with GISTIC2.0 v2.0.23 (45, 46). Correlation between CNV and mRNA expression, and between methylation levels and mRNA was assessed by Spearman correlation analysis. The statistical significance employed in this model is Cox's hazard ratio (P value and FDR). Before differential methylation analysis, correlation analysis was performed to filter the sites most negatively correlated with gene expression into this analysis. Differential analysis was performed using the Bioconductor package minfi v1.36.0 to compare methylation patterns between tumor and normal samples, and individual variations in gene expression were also assessed (47). Gene Set Variation Analysis (GSVA) was performed using the GSVA platform v1.46.0 to analyze mRNA expression data for the gene set using both the Wilcoxon test and ANOVA for statistical comparisons (48).

Survival Analysis

Disease-free interval, progression-free interval, overall survival, and disease-specific survival were evaluated at in survival analysis. Methylation data and clinical survival data were merged by sample barcode; the median methylation level was used to divide tumour samples into high and low methylation groups. A median value indicates a hazard ratio value of 1.0. If the hazard ratio was greater than or equal to 1.0, then it was binned as the higher methylation group; if the ratio was less than 1.0, then it was binned as the lower methylation group. Tumor samples were divided into high and

https://doi.org/10.59720/24-352

low groups based on median values or specific alterations, such as mutations and CNV classifications like amplification and deletion. Survival time and status were fitted using the R survival package, and the Cox Proportional-Hazards model was employed to calculate hazard ratios (HR) for each gene, indicating survival risk. Statistical significance of survival differences between groups was assessed using log-rank tests, and Kaplan-Meier survival analysis was conducted to further evaluate gene-specific survival impacts.

Pathway Analysis

Pathway activity scores for 7,876 samples were calculated using reverse-phase protein array (RPPA) data from the TCPA database (40). The analysis covered ten cancer-associated pathways: hormone estrogen receptor (ER), hormone androgen receptor (AR), receptor tyrosine kinase (RTK), phosphatidylinositol-4,5-bisphosphate-3-kinase (PI3K)/protein kinase B (AKT), RAS/mitogen-activated protein kinase (MAPK), tuberous sclerosis complex (TSC)/mechanistic target of rapamycin (mTOR), epithelial-mesenchymal transition (EMT), cell cycle, and apoptosis pathways. Pathway scores were calculated by aggregating the relative concentrations of all positive regulatory proteins and subtracting those of negative regulators. To estimate Pathway Activity Scores (PAS), gene expression data were divided into high and low categories based on median values as in previous studies. PAS differences between these categories were assessed using Student's t-test, where the p-value was adjusted using the false discovery rate (FDR), given an FDR ≤ 0.05 was considered significant. A gene was found to exert an activating effect on a signaling pathway if PAS (Low expression of Gene A) < PAS (High expression of Gene A); otherwise, it was considered to exert an inhibitory effect. No specific IDs were given to the pathways apart from the names.

Immune Association Analysis

Immune cell infiltration levels within various cancers were analyzed using data from the TCGA database (38). The infiltrates of 24 immune cell types were evaluated using ImmuCellAI webtool (49). Gene set variation analysis (GSVA) scores of the genes were used to visualize the data. The relationship between immune cell infiltration and gene expression was quantified using Spearman correlation analysis, with correlation coefficients indicating the strength of associations. P-values were adjusted using FDR.

Drug Sensitivity Analysis

Drugs were screened based on their Centromere protein A (CENPA) correlation with gene expression and drug sensitivity, using a stringent significance cutoff ($p < 0.05$).
GDSC: The IC50 of 265 small molecules in 860 cell lines and the corresponding mRNA gene expression were collected from the Genomics of Drug Sensitivity in Cancer (GDSC) (42). The mRNA expression data and drug sensitivity data were merged. Pearson correlation analysis was performed to get the correlation between gene mRNA expression and drug IC50. P-value was adjusted by FDR.

CTRP: The IC50 of 481 small molecules in 1001 cell lines and the corresponding mRNA gene expression were collected from the Genomics of Therapeutics Response Portal (CTRP) (43). The mRNA expression data and drug sensitivity data were merged. Pearson correlation analysis was performed to get the correlation between gene mRNA expression and drug IC50. P-value was adjusted by FDR.

The analysis employed GSCA Lite (50) to calculate the area under the dose-response curve (AUC) values for drugs and the expression profiles of protein kinase genes involved in phagocytosis across various cancer cell lines. Drug sensitivity and gene expression data from the GDSC and CTRP databases were integrated for a comprehensive evaluation (42, 43). Spearman correlation analysis was used to assess the relationship between gene expression and drug sensitivity.

Statistical Analysis

Statistical analyses were conducted using R software v4.0.3 (51). The Spearman correlation test was used to assess correlation, and the Cox proportional hazards model was used to determine survival risk and HR. Kaplan-Meier curves and log-rank tests were used to evaluate prognostic values. T-tests or ANOVA were used for group comparisons, with a rank-sum test for two datasets unless specified otherwise. P-values have been FDR corrected.

Received: October 16, 2024
Accepted: March 03, 2025
Published: January 16, 2026

REFERENCES

1. Sung, Hyuna, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer Journal for Clinicians. 2021;71(3):209–249. https://doi.org/10.3322/caac.21660
2. Bray, Freddie, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer Journal for Clinicians. 2024;74(3):229–263. https://doi.org/10.3322/caac.21834
3. Eliyatkın, Nalan, et al. Molecular classification of breast carcinoma: from traditional to new-age approaches. Journal of Breast Health. 2015;11(2):59–66. https://doi.org/10.5152/tjbh.2015.1669
4. Chen, Fang, et al. Moving pan-cancer studies from basic research toward the clinic. Nature Cancer. 2021;2(9):879–890. https://doi.org/10.1038/s43018-021-00250-4
5. Bagaev, Artem, et al. Conserved pan-cancer microenvironment subtypes predict response to immunotherapy. Cancer Cell. 2021;39(6):845–865.e7. https://doi.org/10.1016/j.ccell.2021.04.014
6. Liu, Hao, et al. Voltage-gated sodium channels in cancers. Biomarker Research. 2024;12(1):70. https://doi.org/10.1186/s40364-024-00620-x
7. Liu, Hao, et al. A pan-cancer bioinformatic-based literature review of TRPM7 in cancers. Pharmacology and Therapeutics. 2022;238:108302. https://doi.org/10.1016/j.pharmthera.2022.108302
8. Brown, Geoffrey. Cell death by phagocytosis. Nature Reviews Immunology. 2024;24(2):91–102. https://doi.org/10.1038/s41577-023-00921-6
9. Chen, Shanshan, et al. Harnessing and enhancing macrophage phagocytosis for cancer therapy. Frontiers in Immunology. 2021;12:635173. https://doi.org/10.3389/

fimmu.2021.635173
10. Sarkar, Niladri, et al. Protein kinases: role of their dysregulation in carcinogenesis, identification, and inhibition. Drug Research. 2023;73(4):189–199. https://doi.org/10.1055/a-1989-1856
11. Feng, Min, et al. Phagocytosis checkpoints as new targets for cancer immunotherapy. Nature Reviews Cancer. 2019;19(10):568–586. https://doi.org/10.1038/s41568-019-0183-z
12. Li, Wei, et al. Targeting macrophages in haematological malignancies: recent advances and future directions. Journal of Hematology and Oncology. 2022;15:110. https://doi.org/10.1186/s13045-022-01328-x
13. Bhullar, Kamaljit, et al. Kinase-targeted cancer therapies: progress, challenges and future directions. Molecular Cancer. 2018;17:48. https://doi.org/10.1186/s12943-018-0804-2
14. Yang, Hui, et al. Engineering macrophages to phagocytose cancer cells by blocking the CD47/SIRPα axis. Cancer Medicine. 2019;8(9):4245–4253. https://doi.org/10.1002/cam4.2332
15. Zhang, Wei, et al. Advances in anti-tumor treatments targeting the CD47/SIRPα axis. Frontiers in Immunology. 2020;11:18. https://doi.org/10.3389/fimmu.2020.00018
16. Liberzon, Arthur, et al. Molecular signatures database (MSigDB) 3.0. Bioinformatics. 2011;27(12):1739–1740. https://doi.org/10.1093/bioinformatics/btr260
17. Shendure, Jay, et al. DNA sequencing at 40: past, present and future. Nature. 2017;550(7676):345–353. https://doi.org/10.1038/nature24286
18. Manolio, Teri, et al. Finding the missing heritability of complex diseases. Nature. 2009;461(7265):747–753. https://doi.org/10.1038/nature08494
19. Duan, Jing, et al. Common copy number variation detection from multiple sequenced samples. IEEE Transactions on Biomedical Engineering. 2014;61(3):928–937. https://doi.org/10.1109/TBME.2013.2292588
20. Zhang, Feng and James Lupski. Copy number variation in human health, disease, and evolution. Annual Review of Genomics and Human Genetics. 2009;10:451–481. https://doi.org/10.1146/annurev.genom.9.081307.164217
21. Hunter, Matthew, et al. Mechanical confinement governs phenotypic plasticity in melanoma. Nature. 2025;625(7987):112–118. https://doi.org/10.1038/s41586-025-09445-6
22. Toledo, Rodrigo. Genetics of pheochromocytomas and paragangliomas: recently implicated genes. Endocrinology and Metabolism Clinics of North America. 2017;46(2):459–489. https://doi.org/10.1016/j.ecl.2017.01.009
23. Kim, Jieun, et al. MerTK inhibition by RXDX-106 in MerTK-activated gastric cancer cell lines. Oncotarget. 2017;8(62):105727–105734. https://doi.org/10.18632/oncotarget.22394
24. Smart, Shannon, et al. The emerging role of TYRO3 as a therapeutic target in cancer. Cancers. 2018;10(12):474. https://doi.org/10.3390/cancers10120474
25. Jiang, Chao, et al. Immune characteristics of LYN in the tumor microenvironment of gliomas. Frontiers in Cell and Developmental Biology. 2022;9:760929. https://doi.org/10.3389/fcell.2021.760929
26. Carey-Smith, Sarah, et al. Clinical, biological and therapeutic impact of copy number alteration in cancer. International Journal of Molecular Sciences. 2024;25(13):6815. https://doi.org/10.3390/ijms25136815
27. Lozupone, Francesca and Stefano Fais. Cancer cell cannibalism: a primeval option to survive. Current Molecular Medicine. 2015;15(9):836–841. https://doi.org/10.2174/1566524015666151026100916
28. Sakuma, Shinichi, et al. Receptor protein tyrosine kinase DDR is up-regulated by p53 protein. FEBS Letters. 1996;398(2–3):165–169. https://doi.org/10.1016/S0014-5793(96)01234-3
29. Ahsan, Mujtaba, et al. Relative contribution of DNA methylation and genetic variants to protein biomarkers. PLOS Genetics. 2017;13(9):e1007005. https://doi.org/10.1371/journal.pgen.1007005
30. Yan, Kai, et al. Copy number variant landscape of multiple cancers and clinical applications. Annals of Medicine. 2023;55(2):2280708. https://doi.org/10.1080/07853890.2023.2280708
31. Tang, Xiaofeng, et al. Hypermethylation of death-associated protein kinase promoter attenuates TRAIL-induced apoptosis. Molecular Cancer Research. 2004;2(12):685–691. https://doi.org/10.1158/1541-7786.MCR-04-0123
32. Nikolic, Nikola, et al. Methylation of tumour suppressor genes in salivary gland tumours. Epigenetics. 2022;17(12):1661–1676. https://doi.org/10.1080/15592294.2022.2052426
33. Sahib, Zainab, et al. Crosstalk between autophagy and oncogenic signaling pathways. Biochimica et Biophysica Acta – Reviews on Cancer. 2021;1876(1):188565. https://doi.org/10.1016/j.bbcan.2021.188565
34. Chhatwal, Karan and Hao Liu. RAD50 as a biomarker for breast cancer diagnosis and prognosis. bioRxiv. 2024. https://doi.org/10.1101/2024.09.07.611821
35. Liu, Hao and Tao Tang. IGFBPs in glioma: diagnostic, prognostic and therapeutic value. American Journal of Translational Research. 2023;15(3):2140–2155. www.pubmed.ncbi.nlm.nih.gov/37056850/
36. Liu, Hao, et al. Genetic expression in cancer research: challenges and complexity. Gene Reports. 2024;36:102042. https://doi.org/10.1016/j.gene.2024.102042
37. Ou, Lin, et al. Helicobacter pylori infection facilitates cell migration in gastric cancer. Heliyon. 2024;10(17):e37046. https://doi.org/10.1016/j.heliyon.2024.e37046
38. Weinstein, John, et al. The Cancer Genome Atlas pan-cancer analysis project. Nature Genetics. 2013;45(10):1113–1120. https://doi.org/10.1038/ng.2764
39. Heath, Andrew, et al. The NCI genomic data commons. Nature Genetics. 2021;53:257–262. https://doi.org/10.1038/s41588-021-00791-5
40. Li, Jiayin, et al. TCPA: a resource for cancer functional proteomics data. Nature Methods. 2013;10(11):1046–1047. https://doi.org/10.1038/nmeth.2650
41. Jiang, Peng, et al. Signatures of T cell dysfunction and exclusion predict immunotherapy response. Nature Medicine. 2018;24(10):1550–1558. https://doi.org/10.1038/s41591-018-0136-1
42. Yang, Wei, et al. Genomics of drug sensitivity in cancer (GDSC). Nucleic Acids Research. 2013;41(D1):D955–D961. https://doi.org/10.1093/nar/gks1111
43. Seashore-Ludlow, Brooke, et al. Harnessing connectivity in a large-scale small-molecule sensitivity dataset. Cancer Discovery. 2015;5(11):1210–1223. https://doi.

https://doi.org/10.59720/24-352

org/10.1158/2159-8290.CD-15-0235
44. Vera Alvarez, Ruben, et al. TPM Calculator: one-step software to quantify mRNA abundance. Bioinformatics. 2019;35(11):1960–1962.https://doi.org/10.1093/bioinformatics/bty896
45. Mayakonda, Anand, et al. Maftools: efficient analysis of somatic variants in cancer. Genome Research. 2018;28(11):1747–1756.https://doi.org/10.1101/gr.239244.118
46. Mermel, Craig, et al. GISTIC2.0 facilitates sensitive localization of somatic copy-number alterations. Genome Biology. 2011;12:R41. https://doi.org/10.1186/gb-2011-12-4-r41
47. Aryee, Martin, et al. Minfi: a Bioconductor package for DNA methylation analysis. Bioinformatics. 2014;30(10):1363–1369. https://doi.org/10.1093/bioinformatics/btu049
48. Hänzelmann, Sonja, et al. GSVA: gene set variation analysis for RNA-seq data. BMC Bioinformatics. 2013;14:7. https://doi.org/10.1186/1471-2105-14-7
49. Miao, Yu-Ru, et al. ImmuCellAI: prediction of T-cell subset abundance. Advanced Science. 2020;7(13):2001280. https://doi.org/10.1002/advs.201902880
50. Liu, Chao-Jun, et al. GSCALite: a web server for gene set cancer analysis. Bioinformatics. 2018;34(21):3771–3772. https://doi.org/10.1093/bioinformatics/bty411
51. R Core Team. R: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing; 2020. https://doi.org/10.5281/zenodo.3954507