Identification of Transcriptomic Hypoxia Signature From Tumour Microenvironment by Machine Learning: A Systematic Review

Jucheng Hu

Abstract

This systematic review comprehensively analyses the application of machine learning in identifying transcriptomic Hypoxia Signatures (HSs) within the tumour microenvironment, a key factor in understanding tumour progression, tailoring treatment strategies, and potentially improving patient outcomes. Certain HSs have been validated to stratify patients into different risk groups and predict Overall Survival (OS). While traditional methods like in vivo analyses and meta-analysis of gene signatures laid the groundwork, machine learning, particularly since 2014, has dramatically advanced HS identification by efficiently handling complex, large datasets. This review reveals that LASSO and LASSO Cox regression are predominant in this area. Besides, while these two methods are well-established, this review highlights an untapped potential in the application of autoencoders (AEs). The insights gained from this review could pave the way for the HS identification for a broader range of tumour types and the development of novel identification methods.

Keywords: hypoxia signature, tumour, cancer, microenvironment, machine learning, prognosis, autoencoder

Introduction

A HS, a set of Differentially Expressed Genes (DEGs) under hypoxic conditions (low oxygen level) in the tumour microenvironment, plays a pivotal role in measuring tumour hypoxia. Hypoxia, prevalent in various tumours or cancers, is linked with increased tumour aggressiveness, reduced therapy response, especially to radiotherapy [1], and an overall poorer clinical prognosis [2]. Despite of the importance of hypoxia in tumours, its detection remains to be a challenge. Using identified HSs as biomarkers, the intratumoural hypoxia level can be inferred by measuring the expression level of certain HSs. Moreover, HS can also be use to predict more valuable information, including response to immunotherapy [3]. However, traditional HSs identification has faced significant limitations.

Machine learning, including techniques such as AE, presents a transformative approach in advancing the identification of HSs. AEs, specialized in unsupervised learning tasks like dimensionality reduction and feature learning, offer potential solutions for identifying gene signatures with increased precision. Compared to traditional methods, machine learning requires less empirical gene signature knowledge, automates the signature selection process, and efficiently handles larger, more complex gene datasets. This review is necessitated by the need to systematically examine machine learning techniques, assessing their impact and potential in refining HS identification.

The primary objective of this review is to explore and synthesize current methodologies and findings in the field of tumour microenvironment transcriptomics, particularly focusing on the development of HSs using AEs. The research questions guiding this review are:

1. How is machine learning being utilized to develop transcriptomic HSs in the tumour microenvironment? How effective are they?
2. How can AEs and other machine learning techniques be applied in identifying HSs?

Methodology

1. Identification of Studies

1.1 Searching Strategies

This systematic review used an "Iterative and Expert-Informed Search Strategy" to comprehensively collect studies on machine learning in identifying HSs within tumour microenvironments. This dynamic strategy involved refining search terms based on initial outcomes and insights.

Primarily, PubMed was chosen for its rich repository of peer-reviewed biomedical literature. To address its limitations, particularly in covering newly published or grey literature, Google Scholar was also used, recognizing its inclusion of a wider literature spectrum. The search terms, encompassing key aspects of our research theme, were hypoxia, signature, transcriptomic, tumour, micro-environment, and machine learning. Without date restrictions, we identified studies dating from 2014, resulting in 90 papers from PubMed, 13 from Google Scholar, and three expert-recommended papers. The complete search methodology is detailed in Appendix A.

1.2 Inclusion/Exclusion Criteria and Screening

Post-duplicate removal, papers were screened based on titles and abstracts using criteria outlined in Table 1, focusing on topic relevance, technology use, and results validation. This led to 18 papers, with expert consultations adding three more, totalling 21 included papers.

Inclusion Criteria	**Reason**
Focus on machine learning in signatures identification	Ensures that the paper contributes to the understanding of how machine learning is used in the identification of signatures, which is central to the review's aim.
Focus on HSs in tumour microenvironment	Directly aligns with the review's objective to understand how HSs are identified in the context of tumour microenvironments.
Development of signature conducted on real gene profiles	Ensures that the study's findings are based on empirical data, enhancing the practical applicability and relevance of the research.
Validation of signature development	Validation of the developed signatures is crucial to ascertain their reliability and utility in real-world applications.
Exclusion Criteria	**Reason**
Using Statistic Method only (e.g., only Cox regression or multivariate analysis)	Studies using statistic methods are not relevant to the specific focus on machine learning. This exclusion ensures the review concentrates on innovative, cutting-edge techniques.
Not focusing on HSs (e.g., immune signatures)	Studies focusing on other types of signatures are not relevant to the specific focus on HSs and thus fall outside the scope of the review.
Not focusing on gene signatures (e.g., HIF)	Studies not cantered on gene signatures do not contribute to the understanding of transcriptomic HSs, which is the core interest of the review.
Non-tumour studies (e.g., DSS-induced colitis)	The review is specifically interested in tumour-related hypoxia, so studies on other diseases are not within its scope.

Table 1. Inclusion and exclusion criteria

2. Coding and Analysing

2.1 Data Extraction and Critical Appraisal

The data extraction strategy employed in this systematic review was meticulously designed based on the CHARMS checklist [4]. This approach ensured that only terms relevant to the review's focus were included, with non-relevant terms being removed or adjusted appropriately. Key data elements extracted included TUMOUR type, data source, data size, model development, performance, evaluation results, and interpretative and discussion data.

The extracted data were then assessed following the Critical Appraisal guidelines provided by the PROBAST tool [5]. This appraisal aimed to evaluate the risk of bias and the applicability of the studies included in the review. The critical appraisal process reveals that approximately 40% of studies exhibited a high risk of bias and 30% for applicability, primarily in data and analysis assessment (details in Table 2).

Author, Year	Risk of Bias				Applicability			Overall	
	1. Participants	2. Predictors	3. Outcome	4. Analysis	1. Participants	2. Predictors	3. Outcome	Risk of Bias	Applicability
Jill M. Brooks, 2019	+	+	+	+	+	+	+	+	+
Edian F. Franco, 2013	+	+	+	+	+	+	+	+	+
Cheng-Peng Gui, 2021	-	+	+	?	+	+	+	-	+
Zhi Liu, 2021	+	+	?	?	+	+	+	?	+
Emilie Lalonde, 2014	+	+	+	+	+	+	+	+	+
Jia Li, 2022	+	+	+	-	+	+	-	-	-
Run Shi , 2021	+	+	+	+	-	+	+	+	-
Jun Shao, 2021	-	+	+	+	+	+	+	-	+
Baohui Zhang, 2020	-	+	+	-	+	+	+	-	+
Qiangnu Zhang, 2021	+	+	+	+	+	?	+	+	?
Fanhong Zeng, 2021	+	+	+	+	+	+	+	+	+
Brian Lane, 2022	-	-	+	?	+	+	-	-	-
Ke Wang, 2022	+	?	+	+	+	+	+	?	+
Xiong Tian, 2022	+	+	+	+	+	-	+	+	-
Dongjie Chen, 2021	+	+	+	+	+	+	-	+	-
Yanhong Shou, 2021	+	+	+	+	+	+	+	+	+
Xiangqian Zhang, 2023	-	+	+	+	+	+	+	-	+
Chenyu Nie, 2022	+	+	+	+	-	+	+	+	-
Jinman Zhong, 2021	-	+	+	+	+	+	+	-	+
Xia Yang, 2021	+	-	+	+	+	+	+	-	+

Table 2. Risk of bias and applicability assessment

2.2 Synthesis Strategy

The findings of this review are reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [6]. Given the heterogeneity in study design, tumour types, result measurement techniques, validation methods, and data sizes, a meta-analysis was deemed unsuitable. Instead, a narrative and comparative synthesis approach was utilized to integrally interpret the diverse studies focusing on the identification of HSs across various tumour types using a range of machine learning methodologies.

Findings and Discussion

Table 3 summarizes the characteristics and methods used for identifying Hypoxia Signatures (HSs) in the analysed studies. The findings categorize papers based on their employed methodologies. In cases where multiple methods were used, the function and effectiveness of each are discussed individually. Predominantly, LASSO and LASSO Cox Regression methods emerged as the most frequently used, being applied in 40% and 35% of the studies, respectively.

First Author	Publication Year	Methods	Tumour
Cheng-Peng Gui	2021	t-SNE and LASSO	ccRCC
Yifan Liu	2020	t-SNE and LASSO Cox Regression	Gastric Cancer

Zhi Liu	2021	LASSO	BLCA
Yanhong Shou	2021	LASSO	Melanoma
Jinman Zhong	2021	LASSO	AML
Xia Yang	2021	LASSO	Breast Cancer
Ke Wang	2022	LASSO	GBM
Chenyu Nie	2022	LASSO	Cervical Cancer
Xiong Tian	2022	LASSO	PAAD
Fanhong Zeng	2021	K-mean	HCC
Brian Lane	2022	K-mean	LUDA
Jun Shao	2021	K-mean and LASSO Cox Regression	LUAD
Baohui Zhang	2020	k-mean and LASSO Cox Regression	HCC
Jill M. Brooks	2019	UHC	HNC
Jia Li	2022	Random Forest	Breast Cancer
Qiangnu Zhang	2021	LASSO Cox Regression	HCC
Run Shi	2021	LASSO Cox Regression	LUDA
Dongjie Chen	2021	LASSO Cox Regression	PDAC
Xiangqian Zhang	2023	LASSO Cox regression	Gastric Cancer
Edian F. Franco	2013	Autoencoder	GBM

Table 3. Methods and Tumour Type of Selected Papers

1. Machine Learning Method Findings and Discussion

1.1 t-SNE

t-SNE is a nonparametric unsupervised algorithm for dimensionality reduction. Liu et al. [7] utilized t-SNE to classify 200 hypoxia hallmark gene sets into “hypoxia-high” and “hypoxia-low” groups for gastric cancer risk stratification. Similarly, Gui et al. [8] applied t-SNE in clear cell renal cell carcinoma (ccRCC), categorizing multiomics data into 'low-hypoxia', 'moderate-hypoxia', and 'high-hypoxia' to identify hypoxia-related DEGs.

1.2 LASSO

LASSO, a linear regression variant incorporating regularization for dimensionality reduction, has been widely applied in 28.6% studies of this review. Gui et al used LASSO on the multiomics dataset obtained from the Cancer Genome Atlas (TCGA) including the 'low-hypoxia & high-immunity' and 'high-hypoxia & low-immunity' DEGs to produce a refined set of variables including the signature that most relevant for prognosis of ccRCC. Differently, Shou et al. [9] applied LASSO on 200 hypoxia DEGs identified from the log-rank test for melanoma. Applying LASSO on DEGs obtained from univariate Cox regression, Tian et al. [10] implemented LASSO in pancreatic adenocarcinoma (PAAD) to select the most relevant signatures from hypoxia-stemness-related genes, Wang et al. [11] employed LASSO for hypoxia DEGs filtration in Glioblastoma Multiforme (GBM), similar to Liu et al. [12] in bladder cancer (BLCA), Shao et al. [13] in lung adenocarcinoma (LUDA), Nie et al. [14] in cervical cancer, Zhong et al. [15] in Acute Myeloid Leukaemia (AML) and Yang et al. [16] in triple-negative breast cancer.

1.3 K-mean

K-mean is an unsupervised clustering algorithm that partitioning a dataset into a set number of clusters. Lane et al. [17] employed K-mean on the TCGA-LUAD dataset to segregate genes into hypoxia and non-hypoxia in total two sets for DEG detection, with 35 hypoxia seed DEGs RNASeq data from four LUAD cell lines as primary clustering start point. Experimenting with a range of K values 2 to 9, Zeng et al. [18] identified LUAD HSs, using

K-mean to group genes data from TCGA based on expression similarities under hypoxic conditions, similar to Zhang et al. for hepatocellular carcinoma (HCC) [19] and Shao et al. [13] who used data from molecular signatures database for LUAD.

1.4 Unsupervised Hierarchical Clustering (UHC)

UHC is a clustering algorithm used to group data points based on their similarity without pre-labelled categories. Brooks et al. [20] focused on developing a prognostic classifier for Head and Neck Cancer (HNC), employing hierarchical clustering to subgroup a 54-gene hypoxia-immune signature derived from literature, using Spearman distance and the Ward criterion.

1.5 Random Survival Forest (RSF)

RSF is a an extension of the Random Forests algorithm that accounts for interactions and non-linear relationships between variables. Li et al. [21] applied RSF to identify the most relevant DEGs for breast cancer prognosis from univariate Cox regression, focusing on Hypoxia- and Lactate Metabolism-Related Signatures.

1.6 LASSO Cox Regression

LASSO Cox regression combines LASSO penalties with Cox proportional hazards models, suitable for predicting survival times in medical research. Shi et al. [22] identified 10 HSs for stage I lung adenocarcinoma, applying LASSO Cox regression to 199 candidate genes from the weighted gene co-expression network analysis (WGCNA). Zhang et al. [23] proposed a HS for HCC using LASSO Cox regression on TNM staging and hypoxia scores data, which includes HSs. Chen et al. [24] developed a Hypoxia- and Immune-Associated Signature for Pancreatic Ductal Adenocarcinoma, employing LASSO Cox regression post-Univariate Cox regression on DEGs. In a similar approach, after univariate cox regression, Zhang et al. [25] developed a hypoxia–glycolysis–lactylation-related gene signature using LASSO Cox regression and Liu et al. [7] developed a hypoxia-immune-related signature.

1.7 AE

AEs are unsupervised deep learning algorithms used for dimensionality reduction. Constructed from layers of simple neurons, where each layer's output feeds into the next, AEs typically exhibit a "butterfly" structure characterized by equal numbers of inputs and outputs and bottleneck hidden layers in the middle [26]. Franco et al. evaluated the performance of various AEs with different regularizations in detecting GBM subtypes. Their study utilized diverse data types, including mRNA, DNA methylation, and miRNA expression from colorectal adenocarcinoma datasets. The AEs processed features extracted from these datasets, with reconstructed data subsequently applied to unsupervised clustering, including K-means, to identify GBM subtypes. In a related study focusing on tumour subtype detection, Zhang et al. [23] employed UHC on pre-selected data for HCC.

1.8 Discussion

As a result of methods synthesis, this review notices a ambiguity in the application and description of LASSO and LASSO Cox Regression in the development of HSs. This ambiguity is exemplified in cases like Shou et al. [9], where LASSO Cox Regression is claimed in the abstract, but the method section reveals the use of LASSO. Additionally, the typical progression from LASSO to Multivariate Cox Regression, for instance as seen in Yang et al.'s work [16], further blurs the functional distinction between these two methodologies. Despite these inconsistencies, LASSO and LASSO Cox Regression have emerged as the predominant techniques in HS development, underlining their central role in this research domain.

The papers reviewed collectively suggest a generalized three-stage process for developing HSs in various tumour types:

1. Identification of Hypoxia DEGs: This initial step involves obtaining all hypoxia-related DEGs, which can be sourced through literature surveys, directly from databases, or identified using algorithms like K-mean or UHC.
2. Dimensionality Reduction on DEGs: Subsequently, dimensionality reduction techniques such as LASSO, LASSO Cox, or Random Forest are applied to these DEGs to pinpoint prognostic HSs.

3. Prognostic Model Development: The final step involves developing a prognostic model, which may take the form of a score or a more complex model incorporating additional features.

Moreover, our review has observed the emerging use of AEs in tasks similar to HS identification. Demonstrated capability in gene profile dimensionality reduction, AEs possess the unique ability to amalgamate the first two steps of HS identification. They accept raw gene data and reconstruct it to a simplified representation in a single step. The processed data thus can be effectively discerned using methods like K-mean clustering. Consequently, AEs show considerable promise for future applications in HS identification.

2. Machine Learning Method Performance Findings and Discussion

Due to the variance in tumour types, datasets used for HSs development, the result measurement and processes of HS identification, conducting comparative research on the performances of all different methods remains challenging. Note the overlapping focus on HCC and LUAD, the result findings and synthesis will focus on 6 studies developed HSs for these two tumours, respectively. The full signature list can be accessed in Appendix B.

2.1 HCC Results

As introduced in 3.1, three studies cantered on HCC.

Zeng et al. [18] utilize the K-mean algorithm during stage 1 the Identification of Hypoxia DEGs. This method led to the development of 4 genes including DCN, DDIT4, NDRG1, and PRKCA from the ICGC dataset, validated on ICGC databases. Their hypoxia-risk model demonstrated highest accuracy among all three HCC studies in predicting one-year and three-year OS. The AUC values for one-year, tow-year and three-year OS are 0.809, 0.771, and 0.791, respectively.

Zhang et al. [23] applied LASSO Cox Regression for the stage 2 the Dimensionality Reduction on DEGs. This approach identified 21 distinct genes, including ADM, BNIP3, BNIP3L, and CA9, from microarray data of hypoxia-treated cells, validated on the TCGA and GEO datasets. The AUC values for one, three, and five years were 0.71, 0.73, and 0.69, respectively, in TCGA-LIHC dataset and similar values in the GEO GSE14520 dataset.

Another Zhang et al. [19] employed both K-mean and LASSO Cox Regression in their respective stage 1 and 2. They identified 3 genes including PDSS1, SLC7A11, and CDCA8 from the TCGA-LIHC dataset and GEO GSE59729 and GSE41666 datasets. Validated on TCGA-LIHC test set, the AUCs for 0.5-, 1-, 3-, and 5-year OSwere 0.76, 0.78, 0.7, and 0.7, respectively.

2.2 LUDA Results

Another three studies focusing on LUAD.

Similar to Zeng et al. [18], Lane et al. [17] developed a 28-gene hypoxia signature from the TCGA-LUAD dataset, using K-mean in stage. The study focuses on qualitative prognostic assessment therefore using Hazard Ratio (HR), Confidence Interval (CI), and p-values of Kaplan-Meier analysis for performance measure. The signature's prognostic value for OS was confirmed in independent cohorts from TCGA-test and GEO datasets (GSE31210, GSE72094 and GSE50091) with result of HR 1.76, 95% CI 1.50–2.08 and $p < 0.0001$.

Following Zhang et al.'s work [23], Shi et al. [22] identified 10 genes using LASSO Cox Regression in stage 2. The HSs is developed from the GEO GSE72094 dataset and validated on datasets from U133A, U133 Plus 2.0 and TCGA with result HR = 6.738, 95% CI = 3.902-11.64 and p = 6.42e-09.

Shao et al. [13] developed a seven lncRNA gene from a combination of 13 microarray datasets from various platforms and one RNA-Seq dataset from TCGA. K-mean and Lasso cox regression is used in stage 1 and 2 respectively. This signature was validated on the TCGA validation set, achieving AUC values of 0.665, 0.693, and 0.652 for 1-, 3-, and 5-year overall survival, respectively. Though the main paper did not provide detailed HR, CI and p-value result, supplementary documents provide a separated Kaplan-Meier analysis result for all seven gens, with HR ranging from 0.61 to 1.65, CI from 0.42-0.88 to 1.39-1.95 and p-value from less than 0.001 to 0.277.

2.3 Discussion

The synthesis of results reveals that there is no uniformly superior method across all tumour types. For instance, Zeng et al.'s application of K-mean algorithm showed the most promising results in HCC studies, while Shi et al.'s use of LASSO Cox regression was most effective in LUAD. This variability underscores the necessity of selecting methods based on tumour type and dataset characteristics, rather than a uniform approach. It is evident that each tumour responds differently to various analytic techniques, emphasizing the need for a tailored methodological approach.

Besides different methods employed within the same tumour type led to the development of distinct HSs. This finding suggests that the choice of methodology significantly influences the nature of the identified signatures. Moreover, it appears that having a larger number of genes in a signature does not necessarily equate to better performance, indicating that the quality and relevance of the genes selected are more critical than quantity.

The synthesis also highlights challenges in HS research, notably in performance measurement standardization. The diverse predictive performance metrics used across studies (AUC, HR, CI) highlight the multifaceted nature of assessing HS efficacy. These metrics offer insights into different aspects of the HSs' predictive power, with AUC focusing on accuracy and HR and CI providing risk and confidence assessments. A more consistent approach to performance measurement could facilitate direct comparisons of HS efficacy across studies.

Conclusion

This systematic review has identified a prevalent three-step process in the development of HSs across various studies: the identification of hypoxia differentially expressed genes (DEGs), dimensionality reduction on these DEGs, and the subsequent development of prognostic models. A wide range of methods have been successfully applied in these stages, including LASSO, LASSO Cox Regression, K-mean, RSF, t-SNE and UHC.

The potential of AEs to efficiently identify hypoxia related DEGs and refine DEG list in a single step stands out, enhancing efficiency and opening new avenues for extracting relevant biological signatures from complex datasets. This capability signifies the growing importance of sophisticated machine learning techniques in tumour biology, especially as the field continues to delve into the complexities of the tumour microenvironment.

The synthesis of our results reveals that no single method is uniformly superior across all tumour types. For instance, Zeng et al.'s use of the K-mean algorithm excelled in HCC studies, while Shi et al.'s application of LASSO Cox regression showed significant efficacy in LUDA. This variability highlights the necessity of tailoring methodological approaches based on specific tumour types and dataset characteristics.

Moreover, different methods within the same tumour type have led to the development of distinct HSs, underscoring the significant influence of the chosen methodology on the nature of the identified signatures. It becomes apparent that a larger number of genes in a signature does not necessarily guarantee better performance, emphasizing the importance of gene quality and relevance over quantity.

Additionally, the review identifies challenges in standardizing performance measurement. The diverse predictive performance metrics (AUC, HR, CI) used across studies illustrate the multifaceted nature of assessing HS efficacy. These metrics provide insights into various aspects of the HSs' predictive power. Adopting a more consistent approach to performance measurement could enable more straightforward comparisons of HS efficacy across different studies.

Future research should aim to refine these methods, compare their efficacies, and develop novel and streamlined approaches for HS identification. Such efforts are vital to deepen our understanding of tumour behaviour for more tumour types and inform targeted therapeutic strategies.

Bibliography

[1] K. Toustrup, B. S. Sørensen, J. Alsner, and J. Overgaard, "Hypoxia Gene Expression Signatures as Prognostic and Predictive Markers in Head and Neck Radiotherapy," *Seminars in Radiation Oncology,* vol. 22, no. 2, pp. 119-127, 2012/04/01/ 2012, doi: https://doi.org/10.1016/j.semradonc.2011.12.006.

[2] B. Tawk *et al.*, "Comparative analysis of transcriptomics based hypoxia signatures in head- and neck squamous cell carcinoma," *Radiotherapy and Oncology,* vol. 118, no. 2, pp. 350-358, 2016/02/01/ 2016, doi: https://doi.org/10.1016/j.radonc.2015.11.027.

[3] S. Hong *et al.*, "Hypoxic Characteristic Genes Predict Response to Immunotherapy for Urothelial Carcinoma," *Frontiers in Cell and Developmental Biology,* Original Research vol. 9, 2021. [Online]. Available: https://www.frontiersin.org/articles/10.3389/fcell.2021.762478.

[4] K. G. M. Moons *et al.*, "Critical Appraisal and Data Extraction for Systematic Reviews of Prediction Modelling Studies: The CHARMS Checklist," *PLOS Medicine,* vol. 11, no. 10, p. e1001744, 2014, doi: 10.1371/journal.pmed.1001744.

[5] R. F. Wolff *et al.*, "PROBAST: A Tool to Assess the Risk of Bias and Applicability of Prediction Model Studies," *Annals of Internal Medicine,* vol. 170, no. 1, pp. 51-58, 2019/01/01 2019, doi: 10.7326/M18-1376.

[6] D. Moher, A. Liberati, J. Tetzlaff, and D. G. Altman, "Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement," *Annals of Internal Medicine,* vol. 151, no. 4, pp. 264-269, 2009/08/18 2009, doi: 10.7326/0003-4819-151-4-200908180-00135.

[7] Y. Liu *et al.*, "Development and validation of a hypoxia-immune-based microenvironment gene signature for risk stratification in gastric cancer," *Journal of Translational Medicine,* vol. 18, no. 1, p. 201, 2020/05/14 2020, doi: 10.1186/s12967-020-02366-0.

[8] C.-P. Gui *et al.*, "A new thinking: extended application of genomic selection to screen multiomics data for development of novel hypoxia-immune biomarkers and target therapy of clear cell renal cell carcinoma," *Briefings in Bioinformatics,* vol. 22, no. 6, p. bbab173, 2021, doi: 10.1093/bib/bbab173.

[9] Y. Shou, L. Yang, Y. Yang, X. Zhu, F. Li, and J. Xu, "Determination of hypoxia signature to predict prognosis and the tumor immune microenvironment in melanoma," (in eng), *Mol Omics,* vol. 17, no. 2, pp. 307-316, Apr 1 2021, doi: 10.1039/d0mo00159g.

[10] X. Tian *et al.*, "Development and validation of a hypoxia-stemness-based prognostic signature in pancreatic adenocarcinoma," (in eng), *Front Pharmacol,* vol. 13, p. 939542, 2022, doi: 10.3389/fphar.2022.939542.

[11] K. Wang, Y. Lu, Z. Liu, M. Diao, and L. Yang, "Establishment and External Validation of a Hypoxia-Derived Gene Signature for Robustly Predicting Prognosis and Therapeutic Responses in Glioblastoma Multiforme," (in eng), *Biomed Res Int,* vol. 2022, p. 7858477, 2022, doi: 10.1155/2022/7858477.

[12] Z. Liu *et al.*, "A Robust Hypoxia Risk Score Predicts the Clinical Outcomes and Tumor Microenvironment Immune Characters in Bladder Cancer," (in English), *Frontiers in Immunology,* Original Research vol. 12, 2021-August-13 2021, doi: 10.3389/fimmu.2021.725223.

[13] J. Shao, B. Zhang, L. Kuai, and Q. Li, "Integrated analysis of hypoxia-associated lncRNA signature to predict prognosis and immune microenvironment of lung adenocarcinoma patients," *Bioengineered,* vol. 12, no. 1, pp. 6186-6200, 2021/01/01 2021, doi: 10.1080/21655979.2021.1973874.

[14] C. Nie, H. Qin, and L. Zhang, "Identification and validation of a prognostic signature related to hypoxic tumor microenvironment in cervical cancer," *PLOS ONE,* vol. 17, no. 6, p. e0269462, 2022, doi: 10.1371/journal.pone.0269462.

[15] J. Zhong *et al.*, "Establishment of Prognosis Model in Acute Myeloid Leukemia Based on Hypoxia Microenvironment, and Exploration of Hypoxia-Related Mechanisms," (in English), *Frontiers in Genetics,* Original Research vol. 12, 2021-October-26 2021, doi: 10.3389/fgene.2021.727392.

[16] X. Yang *et al.*, "A combined hypoxia and immune gene signature for predicting survival and risk stratification in triple-negative breast cancer," (in eng), *Aging (Albany NY),* vol. 13, no. 15, pp. 19486-19509, Aug 2 2021, doi: 10.18632/aging.203360.

[17] B. Lane, M. T. Khan, A. Choudhury, A. Salem, and C. M. L. West, "Development and validation of a hypoxia-associated signature for lung adenocarcinoma," (in eng), *Sci Rep,* vol. 12, no. 1, p. 1290, Jan 25 2022, doi: 10.1038/s41598-022-05385-7.

[18] F. Zeng, Y. Zhang, X. Han, M. Zeng, Y. Gao, and J. Weng, "Employing hypoxia characterization to predict tumour immune microenvironment, treatment sensitivity and prognosis in hepatocellular carcinoma," *Computational and Structural Biotechnology Journal,* vol. 19, pp. 2775-2789, 2021/01/01/ 2021, doi: https://doi.org/10.1016/j.csbj.2021.03.033.

[19] B. Zhang, B. Tang, J. Gao, J. Li, L. Kong, and L. Qin, "A hypoxia-related signature for clinically predicting diagnosis, prognosis and immune microenvironment of hepatocellular carcinoma patients," (in eng), *J Transl Med,* vol. 18, no. 1, p. 342, Sep 4 2020, doi: 10.1186/s12967-020-02492-9.

[20] J. M. Brooks *et al.*, "Development and Validation of a Combined Hypoxia and Immune Prognostic Classifier for Head and Neck Cancer," *Clinical Cancer Research,* vol. 25, no. 17, pp. 5315-5328, 2019, doi: 10.1158/1078-0432.CCR-18-3314.

[21] J. Li *et al.*, "A novel hypoxia- and lactate metabolism-related signature to predict prognosis and immunotherapy responses for breast cancer by integrating machine learning and bioinformatic analyses," (in English), *Frontiers in Immunology,* Original Research vol. 13, 2022-October-07 2022, doi: 10.3389/fimmu.2022.998140.

[22] R. Shi *et al.*, "Identification and validation of hypoxia-derived gene signatures to predict clinical outcomes and therapeutic responses in stage I lung adenocarcinoma patients," *Theranostics,* Research Paper vol. 11, no. 10, pp. 5061-5076, 2021, doi: 10.7150/thno.56202.

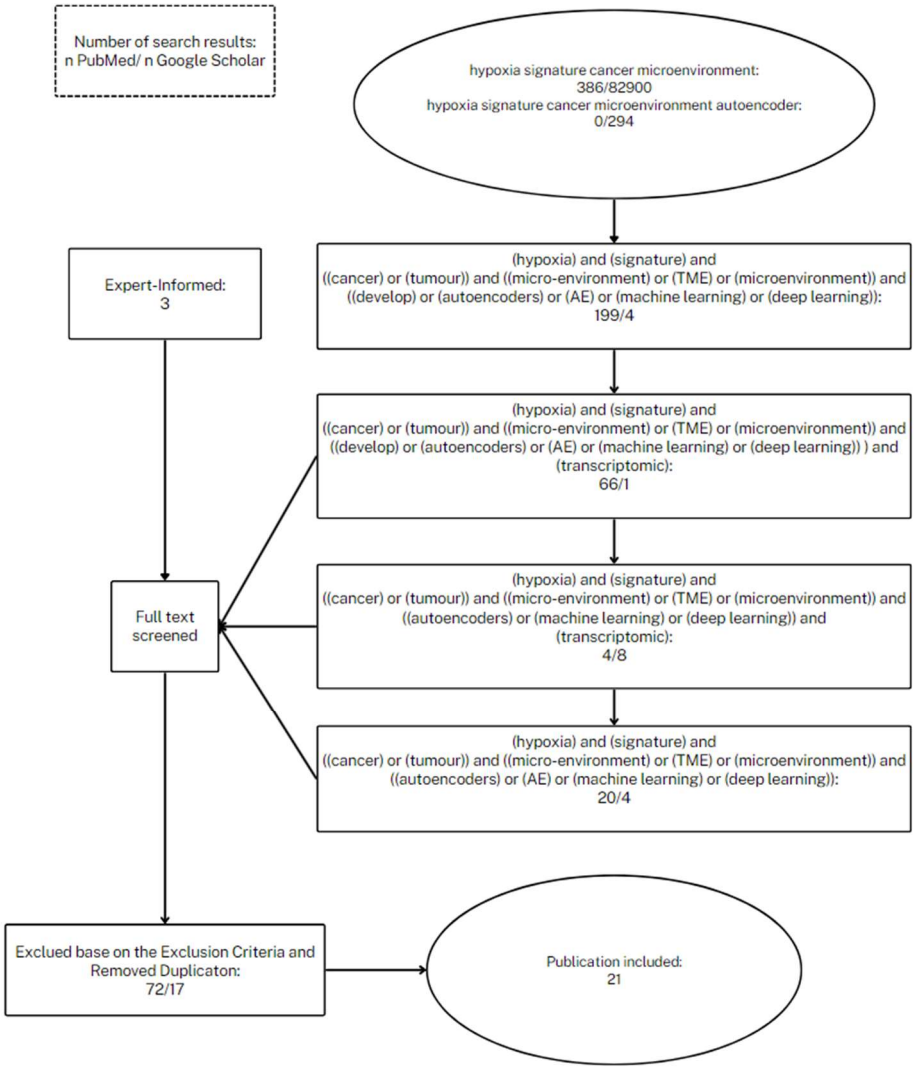
[23] Q. Zhang, L. Qiao, J. Liao, Q. Liu, P. Liu, and L. Liu, "A novel hypoxia gene signature indicates prognosis and immune microenvironments characters in patients with hepatocellular carcinoma," (in eng), *J Cell Mol Med,* vol. 25, no. 8, pp. 3772-3784, Apr 2021, doi: 10.1111/jcmm.16249.

[24] D. Chen, H. Huang, L. Zang, W. Gao, H. Zhu, and X. Yu, "Development and Verification of the Hypoxia- and Immune-Associated Prognostic Signature for Pancreatic Ductal Adenocarcinoma," (in eng), *Front Immunol,* vol. 12, p. 728062, 2021, doi: 10.3389/fimmu.2021.728062.

[25] X. Zhang, Y. Li, and Y. Chen, "Development of a Comprehensive Gene Signature Linking Hypoxia, Glycolysis, Lactylation, and Metabolomic Insights in Gastric Cancer through the Integration of Bulk and Single-Cell RNA-Seq Data," (in eng), *Biomedicines,* vol. 11, no. 11, Nov 1 2023, doi: 10.3390/biomedicines11112948.

[26] E. F. Franco *et al.*, "Performance Comparison of Deep Learning Autoencoders for Cancer Subtype Detection Using Multi-Omics Data," *Cancers*, vol. 13, no. 9*,* doi: 10.3390/cancers13092013.

Appendix A



Appendix B

Identifier	Signature
Zeng et al. 2021	DCN, DDIT4, NDRG1, PRKCA
Zhang et al. 2020	ADM, BNIP3, BNIP3L, CA9,EGLN3,GDF15,GYS1,HCAR3,HILPDA,HK2,INSIG2,JUN,KDM3A, PFKFB4,PLIN2,PTPRH,SLC2A3,SMAD3,SPAG4,TMEM45A,WSB1
Zhang et al. 2021	PDSS1、SLC7A11、CDCA8
Lane et al. 2022	CP,SLC2A3,SPAG4,WISP2,CYP26A1,YPEL1,RNF24,PGK1,NDRG1,BNIP3L,DLX4,PFKFB4, ERRFI1,LRP1,S1PR4,GAL3ST1,EGLN3,GUCY2D,BHLHE40,LDHA,RAB40B,EFNA3,PPFIA4, PNRC1,IGFBP3,IGFBP1,ADM,ANKZF1,CITED2,CASKIN1,DDIT4,PFKFB3,TMEM45A, SEMA4B,FUT11
Shi et al. 2021	CHST15, KLK6, TUBB3, TCEA3 SLC25A38, AQP7, GNMT, STX19, UQCR10,CXorf23
Shao et al. 2021	LINC00941, AC022784.1, AC079949.2, AC090001.1,LINC00707,AL161431.1, AC010980.2