Identification of transcriptomic hypoxia signature from tumour microenvironment by machine learning: a systematic review

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) (Liu et al., 2020). Traditional methods for identifying HSs, including in vivo–derived analyses using hypoxia markers(Marotta et al., 2011), meta-analysis of microarray-based gene signatures(Seigneuric et al., 2007), and gene function and co-expression patterns analysis (Buffa et al., 2010), provided foundational insights. However, since 2014 (Lalonde et al., 2014), machine learning has revolutionized this field. Its ability to efficiently analyse complex, large datasets has significantly enhanced the accuracy and depth of HS identification. This review synthesizes findings from a comprehensive analysis of literature focused on using machine learning methods to identify HSs in the tumour microenvironment, adhering to strict topic relevance criteria. This review reveals that LASSO and LASSO Cox regression are predominant in this area. Besides, while LASSO Cox regression is 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 (Toustrup et al., 2012), and an overall poorer clinical prognosis(Tawk et al., 2016). 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 (Hong et al., 2021). However, traditional HSs identification has faced significant limitations.

Machine learning, including techniques such as autoencoders (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 these advanced machine learning techniques, assessing their impact and potential in refining HS identification.

This review aims to systematically analyse and synthesize all the existing literature on the application of machine learning methods for identifying HSs in the tumour microenvironment. Given the nascent

nature of this research area, our scope will be broad and inclusive, covering studies across various tumour types and machine learning techniques.

To effectively collate and assess the findings from the selected papers, the narrative synthesis approach is employed. This method will allow qualitatively summarization and explanation of the diverse range of methodologies and outcomes observed in the studies. The narrative synthesis is particularly suitable given the expected heterogeneity in study designs, machine learning algorithms used, and types of tumours examined. By synthesizing this information narratively, we aim to draw comprehensive insights into the current state of machine learning applications in HS identification and highlight potential directions for future research.

Objectives

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?
2. How can AEs and other machine learning techniques be applied in identifying HSs?

Methodology

1. Identification of Studies
1. Searching Strategies

In this systematic review, we employed an "Iterative and Expert-Informed Search Strategy" to meticulously gather a comprehensive set of studies on machine learning applications in identifying HSs within tumour microenvironments. This approach involved dynamically refining search terms based on initial findings and ongoing insights.

Primarily PubMed is utilized, noted for its repository of peer-reviewed biomedical literature, ensuring access to high-quality scientific studies. Recognizing its limitations in encompassing newly published or grey literature, this review supplemented search with Google Scholar. While Google Scholar offers a broader range of literature, including grey and non-peer-reviewed articles, it may include sources with varying degrees of rigor.

Our search terms, carefully selected to cover critical aspects of our research theme, included (hypoxia), (signature), (transcriptomic), ((cancer) or (tumour)), ((micro-environment) or (TME) or (microenvironment)), and ((develop) or (autoencoders) or (AE) or (machine learning) or (deep learning)). Given the field's novelty, we imposed no date restrictions, with relevant studies dating back to 2014. This strategy yielded 90 papers from PubMed and 13 from Google Scholar, augmented by three expert-recommended papers, enriching the search's breadth and depth. For full transparency and reproducibility, the complete search methodology and iterations are detailed in Appendix A.

1.1. Inclusion/Exclusion Criteria and Screening

After the removal of duplicates, the screening of papers was conducted by the author. The initial stage involved evaluating titles and abstracts against the inclusion criteria outlined in Table 1. Papers were included in the review based on their alignment with the inclusion criteria, which encompassed topic relevance, utilization of specified technologies, and validation of results. This process resulted in the selection of 18 papers. Additionally, consultations with field experts led to the identification of three more pertinent publications, bringing the total count to 21 papers included in the review.

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 (Moons et al., 2014). This approach ensured that only terms relevant to the review's focus were included, with non-relevant terms being removed or adjusted appropriately. The extracted data was stored in a separate document for clarity and ease of analysis. 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 rigorously assessed by the author following the Critical Appraisal guidelines provided by the PROBAST tool (Wolff et al., 2019). This appraisal aimed to evaluate the risk of bias and the applicability of the studies included in the review.

The critical appraisal process revealed a significant level of bias within the studies: approximately 40% were found to have a high risk of bias, with 30% demonstrating significant concerns regarding

applicability. Notably, the most substantial risk of bias was concentrated in the domains of data and analysis assessment.

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

The findings of this review are reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Moher et al., 2009). Owing to considerable heterogeneity in study design, tumour types, result measurement techniques, validation methods, and data sizes, a meta-analysis was deemed unsuitable. Instead, a narrative synthesis approach was utilized to integrally interpret the diverse studies focusing on the identification of hypoxia signatures (HSs) across various tumour types using a range of machine learning methodologies.

Table 3 provides a comprehensive summary, detailing the characteristics and methods employed for HS identification in the included studies. These studies are systematically categorized based on the methodologies used, such as LASSO Cox regression, LASSO, Random Forest, K-means, Logistic Regression, Unsupervised Clustering, t-SNE, and Autoencoders. It is noted that some studies may fall

into multiple categories if they employed several methods. In such instances, the function and effectiveness of each method in relation to the study are discussed separately.

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
Qiangnu Zhang	2021	k-mean and LASSO Cox Regression	HCC
Jill M. Brooks	2019	Unsupervised Hierarchical Clustering	HNC
Jia Li	2022	Random Forest	Breast Cancer
Baohui Zhang	2020	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	/

Table 3. Methods and Tumour Type of Selected Papers

This methodical categorization has enabled the identification of distinct patterns and variations in the functionality and efficacy of these methods for HS identification across different tumour types. Such a structured approach provides a comprehensive understanding of how each technique contributes to the broader field of tumour microenvironment analysis and hypoxia signature identification.

need to synthesise this

3. Findings

For paper first mentioned, a brief description of the idea of the paper will be explained.

3.1 t-SNE

t-SNE is a nonparametric unsupervised algorithm for dimensionality reduction. Liu et al. (Liu et al., 2020) 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. (Gui et al., 2021) applied t-SNE in clear

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SHOULD BE ON ONE SECTION TOGETHER.

cell renal cell carcinoma (ccRCC), categorizing multiomics data into 'low-hypoxia', 'moderate-hypoxia', and 'high-hypoxia' to identify hypoxia-related DEGs.

3.2 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. (Shou et al., 2021) 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. (Tian et al., 2022) implemented LASSO in pancreatic adenocarcinoma (PAAD) to select the most relevant signatures from hypoxia-stemness-related genes, Wang et al. (Wang et al., 2022) employed LASSO for hypoxia DEGs filtration in Glioblastoma Multiforme (GBM), similar to Liu et al. (Liu et al., 2021) in bladder cancer (BLCA), Shao et al. (Shao et al., 2021) in lung adenocarcinoma (LUDA), Nie et al. (Nie et al., 2022) in cervical cancer, Zhong et al. (Zhong et al., 2021) in Acute Myeloid Leukaemia (AML) and Yang et al. (Yang et al., 2021) in triple-negative breast cancer.

3.3 K-mean

K-mean is an unsupervised clustering algorithm that partitioning a dataset into a set number of clusters. Lane et al. (Lane et al., 2022) employed K-mean on the TCGA-LUAD dataset to segregate genes into hypoxia and non-hypoxia in total two sets for DEG detection. Experimenting with a range of K values 2 to 9, Zeng et al. (Zeng et al., 2021) 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) (Zhang et al., 2020) and Shao et al. (Shao et al., 2021) who used data from molecular signatures database for LUAD.

3.4 Unsupervised Hierarchical Clustering

Unsupervised hierarchical clustering is a clustering algorithm used to group data points based on their similarity without pre-labelled categories. Brooks et al. (Brooks et al., 2019) 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.

3.5 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. (Li et al., 2022) applied RSF to identify the most relevant DEGs for breast cancer prognosis from univariate Cox regression, focusing on Hypoxia- and Lactate Metabolism-Related Signatures.

3.6 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. (Shi et al., 2021) 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. (Zhang et al., 2021) proposed a HS for hepatocellular carcinoma using LASSO Cox regression on TNM staging and hypoxia scores, which includes HSs. Chen et al. (Chen et al., 2021) 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. (Zhang et al., 2023) developed a hypoxia–glycolysis–lactylation-related gene signature using LASSO Cox regression and Liu et al. (Liu et al., 2020) developed a hypoxia-immune-related signature.

3.7 AE

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4. Discussion

   The general process of developing HSs can be concluded in three steps:

   1. Obtain all hypoxia DEGs, can be done by literature survey, directly from databases, by K-mean or unsupervised Hierarchical Clustering
   2. Apply dimensionality reduction techniques on DEGs to identify prognostic HSs, can be done by lasso, lasso cox or random forest
   3. Prognostic Model Development, can be a score, can be a model combine some other features

Due to the differences of the type of tumour, the measure of performance, process of HSs identification, it is hard to conduct researches on the efficiency of different method, although the quality of each study can be evaluated and have been done in the Critical Appraisal section.

Besides, in terms of autoencoders, literature shown its application on similarly tasks. Therefore potential can be used on HSs identification.

5. Conclusion

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Brooks, J. M., Menezes, A. N., Ibrahim, M., Archer, L., Lal, N., Bagnall, C. J., von Zeidler, S. V., Valentine, H. R., Spruce, R. J., Batis, N., Bryant, J. L., Hartley, M., Kaul, B., Ryan, G. B., Bao, R., Khattri, A., Lee, S. P., Ogbureke, K. U. E., Middleton, G., . . . Mehanna, H. (2019). Development and Validation of a Combined Hypoxia and Immune Prognostic Classifier for Head and Neck Cancer. *Clinical Cancer Research*, *25*(17), 5315-5328. https://doi.org/10.1158/1078-0432.CCR-18-3314

Buffa, F. M., Harris, A. L., West, C. M., & Miller, C. J. (2010). Large meta-analysis of multiple cancers reveals a common, compact and highly prognostic hypoxia metagene [Article]. *British Journal of Cancer*, *102*(2), 428-435. https://doi.org/10.1038/sj.bjc.6605450

Chen, D., Huang, H., Zang, L., Gao, W., Zhu, H., & Yu, X. (2021). Development and Verification of the Hypoxia- and Immune-Associated Prognostic Signature for Pancreatic Ductal Adenocarcinoma. *Front Immunol*, *12*, 728062. https://doi.org/10.3389/fimmu.2021.728062

Gui, C.-P., Wei, J.-H., Chen, Y.-H., Fu, L.-M., Tang, Y.-M., Cao, J.-Z., Chen, W., & Luo, J.-H. (2021). 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*, *22*(6), bbab173. https://doi.org/10.1093/bib/bbab173

Hong, S., Zhang, Y., Cao, M., Lin, A., Yang, Q., Zhang, J., Luo, P., & Guo, L. (2021). Hypoxic Characteristic Genes Predict Response to Immunotherapy for Urothelial Carcinoma [Original Research]. *Frontiers in Cell and Developmental Biology*, *9*. https://www.frontiersin.org/articles/10.3389/fcell.2021.762478

Lalonde, E., Ishkanian, A. S., Sykes, J., Fraser, M., Ross-Adams, H., Erho, N., Dunning, M. J., Halim, S., Lamb, A. D., Moon, N. C., Zafarana, G., Warren, A. Y., Meng, X., Thoms, J., Grzadkowski, M. R., Berlin, A., Have, C. L., Ramnarine, V. R., Yao, C. Q., . . . Bristow, R. G. (2014). Tumour genomic

and microenvironmental heterogeneity for integrated prediction of 5-year biochemical recurrence of prostate cancer: a retrospective cohort study. *The Lancet Oncology*, *15*(13), 1521-1532. https://doi.org/https://doi.org/10.1016/S1470-2045(14)71021-6

Lane, B., Khan, M. T., Choudhury, A., Salem, A., & West, C. M. L. (2022). Development and validation of a hypoxia-associated signature for lung adenocarcinoma. *Sci Rep*, *12*(1), 1290. https://doi.org/10.1038/s41598-022-05385-7

Li, J., Qiao, H., Wu, F., Sun, S., Feng, C., Li, C., Yan, W., Lv, W., Wu, H., Liu, M., Chen, X., Liu, X., Wang, W., Cai, Y., Zhang, Y., Zhou, Z., Zhang, Y., & Zhang, S. (2022). A novel hypoxia- and lactate metabolism-related signature to predict prognosis and immunotherapy responses for breast cancer by integrating machine learning and bioinformatic analyses [Original Research]. *Frontiers in Immunology*, *13*. https://doi.org/10.3389/fimmu.2022.998140

Liu, Y., Wu, J., Huang, W., Weng, S., Wang, B., Chen, Y., & Wang, H. (2020). Development and validation of a hypoxia-immune-based microenvironment gene signature for risk stratification in gastric cancer. *Journal of Translational Medicine*, *18*(1), 201. https://doi.org/10.1186/s12967-020-02366-0

Liu, Z., Tang, Q., Qi, T., Othmane, B., Yang, Z., Chen, J., Hu, J., & Zu, X. (2021). A Robust Hypoxia Risk Score Predicts the Clinical Outcomes and Tumor Microenvironment Immune Characters in Bladder Cancer [Original Research]. *Frontiers in Immunology*, *12*. https://doi.org/10.3389/fimmu.2021.725223

Marotta, D., Karar, J., Jenkins, W. T., Kumanova, M., Jenkins, K. W., Tobias, J. W., Baldwin, D., Hatzigeorgiou, A., Alexiou, P., Evans, S. M., Alarcon, R., Maity, A., Koch, C., & Koumenis, C. (2011). In vivo profiling of hypoxic gene expression in gliomas using the hypoxia marker EF5 and laser-capture microdissection [Article]. *Cancer Research*, *71*(3), 779-789. https://doi.org/10.1158/0008-5472.CAN-10-3061

Moher, D., Liberati, A., Tetzlaff, J., & Altman, D. G. (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. *Annals of Internal Medicine*, *151*(4), 264-269. https://doi.org/10.7326/0003-4819-151-4-200908180-00135

Moons, K. G. M., de Groot, J. A. H., Bouwmeester, W., Vergouwe, Y., Mallett, S., Altman, D. G., Reitsma, J. B., & Collins, G. S. (2014). Critical Appraisal and Data Extraction for Systematic Reviews of Prediction Modelling Studies: The CHARMS Checklist. *PLOS Medicine*, *11*(10), e1001744. https://doi.org/10.1371/journal.pmed.1001744

Nie, C., Qin, H., & Zhang, L. (2022). Identification and validation of a prognostic signature related to hypoxic tumor microenvironment in cervical cancer. *PLOS ONE*, *17*(6), e0269462. https://doi.org/10.1371/journal.pone.0269462

Seigneuric, R., Starmans, M. H. W., Fung, G., Krishnapuram, B., Nuyten, D. S. A., van Erk, A., Magagnin, M. G., Rouschop, K. M., Krishnan, S., Rao, R. B., Evelo, C. T. A., Begg, A. C., Wouters, B. G., & Lambin, P. (2007). Impact of supervised gene signatures of early hypoxia on patient survival [Article]. *Radiotherapy and Oncology*, *83*(3), 374-382. https://doi.org/10.1016/j.radonc.2007.05.002

Shao, J., Zhang, B., Kuai, L., & Li, Q. (2021). Integrated analysis of hypoxia-associated lncRNA signature to predict prognosis and immune microenvironment of lung adenocarcinoma patients. *Bioengineered*, *12*(1), 6186-6200. https://doi.org/10.1080/21655979.2021.1973874

Shi, R., Bao, X., Unger, K., Sun, J., Lu, S., Manapov, F., Wang, X., Belka, C., & Li, M. (2021). Identification and validation of hypoxia-derived gene signatures to predict clinical outcomes and therapeutic responses in stage I lung adenocarcinoma patients [Research Paper]. *Theranostics*, *11*(10), 5061-5076. https://doi.org/10.7150/thno.56202

Shou, Y., Yang, L., Yang, Y., Zhu, X., Li, F., & Xu, J. (2021). Determination of hypoxia signature to predict prognosis and the tumor immune microenvironment in melanoma. *Mol Omics*, *17*(2), 307-316. https://doi.org/10.1039/d0mo00159g

Tawk, B., Schwager, C., Deffaa, O., Dyckhoff, G., Warta, R., Linge, A., Krause, M., Weichert, W., Baumann, M., Herold-Mende, C., Debus, J., & Abdollahi, A. (2016). Comparative analysis of

transcriptomics based hypoxia signatures in head- and neck squamous cell carcinoma. *Radiotherapy and Oncology*, *118*(2), 350-358. https://doi.org/https://doi.org/10.1016/j.radonc.2015.11.027

Tian, X., Zheng, J., Mou, W., Lu, G., Chen, S., Du, J., Zheng, Y., Chen, S., Shen, B., Li, J., & Wang, N. (2022). Development and validation of a hypoxia-stemness-based prognostic signature in pancreatic adenocarcinoma. *Front Pharmacol*, *13*, 939542. https://doi.org/10.3389/fphar.2022.939542

Toustrup, K., Sørensen, B. S., Alsner, J., & Overgaard, J. (2012). Hypoxia Gene Expression Signatures as Prognostic and Predictive Markers in Head and Neck Radiotherapy. *Seminars in Radiation Oncology*, *22*(2), 119-127. https://doi.org/https://doi.org/10.1016/j.semradonc.2011.12.006

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

Wolff, R. F., Moons, K. G. M., Riley, R. D., Whiting, P. F., Westwood, M., Collins, G. S., Reitsma, J. B., Kleijnen, J., & Mallett, S. (2019). PROBAST: A Tool to Assess the Risk of Bias and Applicability of Prediction Model Studies. *Annals of Internal Medicine*, *170*(1), 51-58. https://doi.org/10.7326/M18-1376

Yang, X., Weng, X., Yang, Y., Zhang, M., Xiu, Y., Peng, W., Liao, X., Xu, M., Sun, Y., & Liu, X. (2021). A combined hypoxia and immune gene signature for predicting survival and risk stratification in triple-negative breast cancer. *Aging (Albany NY)*, *13*(15), 19486-19509. https://doi.org/10.18632/aging.203360

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

Zhang, B., Tang, B., Gao, J., Li, J., Kong, L., & Qin, L. (2020). A hypoxia-related signature for clinically predicting diagnosis, prognosis and immune microenvironment of hepatocellular carcinoma patients. *J Transl Med*, *18*(1), 342. https://doi.org/10.1186/s12967-020-02492-9

Zhang, Q., Qiao, L., Liao, J., Liu, Q., Liu, P., & Liu, L. (2021). A novel hypoxia gene signature indicates prognosis and immune microenvironments characters in patients with hepatocellular carcinoma. *J Cell Mol Med*, *25*(8), 3772-3784. https://doi.org/10.1111/jcmm.16249

Zhang, X., Li, Y., & Chen, Y. (2023). 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. *Biomedicines*, *11*(11). https://doi.org/10.3390/biomedicines11112948

Zhong, J., Wu, H., Bu, X., Li, W., Cai, S., Du, M., Gao, Y., & Ping, B. (2021). Establishment of Prognosis Model in Acute Myeloid Leukemia Based on Hypoxia Microenvironment, and Exploration of Hypoxia-Related Mechanisms [Original Research]. *Frontiers in Genetics*, *12*. https://doi.org/10.3389/fgene.2021.727392