**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 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 effective are AEs in comparison to other machine learning techniques in identifying and characterizing HSs?

Methodology

1. Identification of Studies
2. 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. 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.

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| **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

1. Coding and Analysing
   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.

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **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 |
| Jill M. Brooks | 2019 | unsupervised clustering |
| Edian F. Franco | 2013 | autoencoder |
| Cheng-Peng Gui | 2021 | t-SNE and Lasso |
| Yifan Liu | 2020 | LASSO Cox regression |
| Zhi Liu | 2021 | LASSO Cox regression |
| Jia Li | 2022 | random forest |
| Run Shi | 2021 | LASSO Cox algorithm |
| Jun Shao | 2021 | LASSO Cox regression |
| Baohui Zhang | 2020 | LASSO |
| Qiangnu Zhang | 2021 | LASSO Cox regression |
| Fanhong Zeng | 2021 | K-mean |
| Brian Lane | 2022 | LASSO Cox regression |
| Ke Wang | 2022 | LASSO Cox regression |
| Emilie Lalonde | 2014 | logistic regression and LASSO regression |
| Dongjie Chen | 2021 | LASSO Cox regression |
| Yanhong Shou | 2021 | LASSO Cox regression |
| Xiangqian Zhang | 2023 | LASSO Cox regression |
| Chenyu Nie | 2022 | Unsupervised clustering |
| Jinman Zhong | 2021 | LASSO Cox regression |
| Xia Yang | 2021 | LASSO Cox regression |

Table 3. Methods Used in 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.

1. Findings

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

3.1 t-SNE

t-SNE is nonparametric unsupervised algorithm for dimensionality reduction.

Liu (Liu et al., 2020) introduced a hypoxia-immune-based signature for risk stratification in gastric cancer. In this study, t-SNE divides a hallmark gene sets of hypoxia including 200 genes into “hypoxia-high” and “hypoxia-low” groups.

Similarly, Gui et al.’s work (Gui et al., 2021) intends to develop hypoxia-immune signature as prognostic biomarkers for clear cell renal cell carcinoma (ccRCC) using multiomics data. In this study, t-SNE is used for dividing the data into groups such as 'low-hypoxia', 'moderate-hypoxia', and 'high-hypoxia' to identify hypoxia-related DEGs.

3.2 LASSO

LASSO is a type of linear regression that includes a regularization component for dimensionality reduction.

In Gui et al.’s work, LASSO is applied on the multiomics dataset 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 predicting the prognosis of ccRCC patients.

Tian (Tian et al., 2022) proposed a Hypoxia-Stemness-Based prognostic signature for pancreatic adenocarcinoma (PAAD). Here LASSO is used to identify eight the most relevant signature from all Hypoxia-Stemness-Related Genes.

Liu develops HSs for Bladder Cancer (Liu et al., 2021). In the study LASSO is applied on the DEGs stems from previous Cox Analysis to identify the optimal candidate hypoxia DEGs with the best discriminative capability.

Shao et al. develop HSs for lung adenocarcinoma (Shao et al., 2021). LASSO is applied after cox regression to shorten the DEGs, in this case precisely is lncRNAs.

Wang et al. (Wang et al., 2022) establish a HS for Glioblastoma Multiforme. In this case LASSO is introduced on the genes obtained by univariate cox regression for further simplification.

Shou (Shou et al., 2021) determine a HS for melanoma. LASSO is introduced to select genes from 200 hypoxia DEGs identified using the log-rank test.

3.3 K-mean

K-mean is an unsupervised clustering algorithm that partitioning a dataset into a set number of clusters.

Zheng et al.’s work (Zeng et al., 2021) identify HSs for lung adenocarcinoma (LUAD). Here K-mean is used to divide the dataset into two distinct groups based on the expression similarity of certain genes identified as being induced under hypoxic conditions in lung adenocarcinoma cell lines.

Zhang et al. develop HSs for hepatocellular carcinoma (Zhang et al., 2020). In his study K-mean is used for clustering data from the Cancer Genome Atlas (TCGA) to obtain hypoxia DEGs, a range of K (2-9) is tired.

Similar to Zhang, Shao et al. (Shao et al., 2021) utilized K-mean with a same strategy to 200 hypoxia-related genes collected from the molecular signatures database.

Lane et al. introduced a HS for lung adenocarcinoma (Lane et al., 2022). K-mean is used on the TCGA-LUAD dataset to cluster genes to hypoxia and non-hypoxia set for further DEGs detection.

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.’s work (Brooks et al., 2019) focus on developing a prognostic classifier based on gene expression related to hypoxia and immune responses for Head and Neck Cancer (HNC). In this study, hierarchical clustering algorithm clustering a 54-gene hypoxia-immune signature identified through literature review to subgroups based on Spearman distance and utilized the Ward criterion.

3.5 Random Survival Forest (RSF)

RSF is a an extension of the Random Forests algorithm that can account for interactions and non-linear relationships between variables.

Li et al.’s work (Li et al., 2022) intends to develop Hypoxia- and Lactate Metabolism-Related Signature for the prognosis of breast cancer. In this study RSF is applied to select most relevant DEGs from the Cox regression.

3.6 LASSO Cox Regression

LASSO Cox regression is a variant of the LASSO and adapts the penalty to reduce the model complexity to Cox proportional hazards models, which predicting survival times in medical research.

Liu employs LASSO Cox regression to select the most prognostic gene signature from all the identified hypoxia-immune-related prognostic DEGs within the discovery cohort (Liu et al., 2020).

LASSO Cox Regression is performed on the DEGs obtained from univariate Cox regression analysis.

Shi et al. identified (Shi et al., 2021) 10 HSs for stage I lung adenocarcinoma. LASSO Cox regression is used to identify the most robust prognostic genes among the 199 candidate genes obtained from the Weighted gene co-expression network analysis.

Zhang et al. (Zhang et al., 2021) proposed a HS for hepatocellular carcinoma. LASSO Cox regression is used on TNM staging and hypoxia score including HSs to simplify the model.

Chen et al. (Chen et al., 2021) develop Hypoxia- and Immune-Associated Signature for Pancreatic Ductal Adenocarcinoma. In this case, LASSO Cox regression is introduced after processing DEGs procced by Univariate Cox regression. Similarly, Zhang et al. develop a hypoxia–glycolysis–lactylation-related gene signature (Zhang et al., 2023), using LASSO Cox regression in the same way as Chen et al.

1. Discussion

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

1. Obtain all hypoxia DEGs.
2. Apply dimensionality reduction techniques on DEGs to identify prognostic HSs.
3. Prognostic Model Development

Different method can be used in all three steps with different purpose. Following will introduce the detailed application and result obtained.

1. Conclusion

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