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Artificial intelligence: illuminating the depths of the tumor microenvironment

Ting Xie[1], Aoling Huang[1], Honglin Yan[1], Xianli Ju[1], Lingyan Xiang[1] and Jingping Yuan[1]*

Abstract

Artificial intelligence (AI) can acquire characteristics that are not yet known to humans through extensive learning, enabling to handle large amounts of pathology image data. Divided into machine learning and deep learning, AI has the advantage of handling large amounts of data and processing image analysis, consequently it also has a great potential in accurately assessing tumour microenvironment (TME) models. With the complex composition of the TME, in-depth study of TME contributes to new ideas for treatment, assessment of patient response to postoperative therapy and prognostic prediction. This leads to a review of the development of AI's application in TME assessment in this study, provides an overview of AI techniques applied to medicine, delves into the application of AI in analysing the quantitative and spatial location characteristics of various cells (tumour cells, immune and non-immune cells) in the TME, reveals the predictive prognostic value of TME and provides new ideas for tumour therapy, highlights the great potential for clinical applications. In addition, a discussion of its limitations and encouraging future directions for its practical clinical application is presented.

Keywords Tumor microenvironment, Artificial intelligence, Deep learning, Machine learning, Prognosis, Pathology images

Introduction

The extracellular matrix, a plethora of reactive chemicals, immune cells including lymphocytes, macrophages, and neutrophils, together with non-immune cells like fibroblasts and vascular endothelial cells, make up the highly complex components of the tumor microenvironment (TME). The TME exerts a crucial part in tumour occurrence, growth, prognosis and metastasis [1]. Characterisation of the tumour microenvironment as a predictor of a patient's prognosis, and also affects the sensitivity of the tumour to treatment, amongst other things, enabling doctors to assess a patient's disease progression and survival, and to develop a personalised treatment plan for the patient.

Current pathologists use histopathology-based microscopy to evaluate TME along with the quantification and localization of cells therein, which are prone to the risk of sample bias and subjectivity. Additionally, standard methodologies are unable to swiftly and intuitively extract the multi-dimensional features of the tumor microenvironment due to its multi-dimensional features, which encompass both quantitative and spatial characteristics of various parameters. Single-cell genomics, spatial transcriptomics, multiplex immunofluorescence and other analytical methods are applied to the study of TME [2]. Nevertheless, these approaches are costly, labor-intensive, time-consuming when dealing with large amounts of data that can only be obtained from a single data source (e.g., gene expression, cellular images), as well as insufficiently tapping into the large number of cellular interactions to completely capture the diversity and dynamics in the TME, hampering a thorough study of

*Correspondence:
Jingping Yuan
yuanjingping@whu.edu.cn
[1] Department of Pathology, Renmin Hospital of Wuhan University, 238 Jiefang-Road, Wuchang District, Wuhan 430060, People's Republic of China

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spatial aspects specific to the TME [3–5]. Therefore, it is necessary to apply more precise, convenient, and objective analytical methods to assess TME. Artificial intelligence (AI) has been used to digital pathology images for use in activities linked to cancer diagnosis, prognosis, and prediction. AI has the ability to integrate data from different sources, such as genomics, transcriptomics, imaging, etc., to provide more comprehensive information about the TME. When it comes to handling large data sets, AI also has a major advantage. By analyzing TME-related data quickly and efficiently, AI is able to discover hidden biological features, disease mechanisms or potential therapeutic targets in TME. Deep learning (DL) and machine learning (ML) are two aspects of AI. DL and ML techniques have become powerful tools to evaluate TME, for instance, it is used to study the interaction and number of immune cells and tumour-associated cells in TME for observing the impact on patient prognosis. AI learns the spatial location of each cell in the TME through supervised learning methods, so as to further analyses whether cells in various locations have varied relevance in the TME [6].

Drawing briefly on the application of deep learning and machine learning to pathological images based on hematoxylin and eosin (H&E) staining, this paper focuses on the study of the application of AI in analysing the quantity and spatial location of TME and its cellular components, mainly in terms of tumour cells, immune cells (TILs, TAMs, TANs), and non-immune cells (CAFs). It also highlights the considerable advantages that AI has in analysing TME, and the integration of AI with technologies such as spatial transcriptomics in the future will enable more precise access to cellular interactions and positional relationships, as well as reveal differences in the expression and spatial distribution of genes at the level of different regions within the tissues, different cell types, and even individual cells, which will help to explore the mechanisms of diseases, discover potential in TME therapeutic targets, etc.

Overview of artificial intelligence

AI is the computer system's simulation of human intelligence processes. By leveraging large-scale datasets, AI models learn intricate patterns and features, surpassing traditional methods in detecting subtle morphological changes indicative of various cancers. ML, a branch of AI that uses statistical techniques to optimize task-specific models [7]. Predictive models can be constructed by extracting information related to patient prognosis from tumour pathology images. DL, while one of the most advanced ML methods, applies neural networks to learn deep patterns in image data, which can enhance the analysis of images [8]. AI improves the digitisation

of pathology with the capability of effectively identifying tissue biological features on pathology slides. Numerous pathological image processing and classification activities, such as tumor classification, grading, prognosis prediction, and treatment, can also be accomplished with it. In addition to minimizing diagnostic errors brought on by pathologists' technical differences and conserving diagnostic time, AI makes it possible for pathology analysis to go from qualitative to quantitative analysis.

Machine learning

ML is the process of building predictive models by using labelled training set data, identifying and extracting features, applying the learned rules to new data and making predictions or decisions without the need for explicit programming. There are routine steps of data preparation, model selection, model training, model evaluation, parameter tuning and prediction in machine learning [9]. According to the training method, ML can be divided into three main categories, supervised learning, unsupervised learning [10], and reinforcement learning. Currently ML applied to pathology images is usually supervised learning, which requires professional pathologists to annotate the images before allowing the ML model to train the data for the further development of the prediction model. By integrating genomic, transcriptomic, proteomic, and metabolomic data, ML is able to reveal the complex interactions in the TME, which is now the latest application of ML in TME [11].

In machine learning methods such as Support Vector Machines (SVM) and Random Forests (RF), normally the features of most importance for tumor development and treatment response are extracted manually for modeling and classification, which reduces the data dimensions and improves the efficiency of the analysis [12]. However, pathological images often exhibit significant variations, requiring strong expertise for feature extraction, which can be incomplete and thus lead to lower classification accuracy.

Deep learning

Compared with machine learning, deep learning makes it possible to overcome the limitations of manual feature extraction and automatically extract complex non-linear features from data, which has been gradually and widely used in the classification of pathology images [13]. The application of DL algorithms to pathology images is expected to change the way malignant tumour pathology is diagnosed and stratified for treatment, and is another milestone event in the application of AI in medicine. DL frameworks build on the proposal that neural networks acquire representations and computations [14] similar to those of the biological brain by learning sample data

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to automatically determine the intrinsic regularities and levels of representation existed in the features from the input data.

Types of deep learning models
Convolutional neural network
Predominantly composed of convolutional, pooling, and fully connected layers, Convolutional Neural Networks (CNNs) are currently the most widely used deep learning algorithm for digital pathology image analysis [15]. CNNs are commonly used for pathology image analysis and visual feature extraction of tumor tissues to identify tumor regions and cell types. As can be seen in the Fig. 1, a CNN-based deep learning model extracts feature and performs learning and classification by performing convolutional operations and pooling operations on input data. Firstly, the WSIs are disassembled into small patches. Secondly, preprocess the data such as staining normalization and data enhancement. Lastly, construct an AI-based model. The processed data can be divided into training set and validation set, which are used for training optimization of CNN models. The trained models can be deployed to new data for testing to evaluate model performance. CNN has already been employed for

detection and segmentation tasks of pathological images with the ability to be used to identify and quantify cells on the one hand and classify them on the other hand. For instance, it is possible to sort out various cells in the TME such as neutrophils and lymphocytes at the cellular level [16], and also separate tumour from non-tumour regions, grade the malignancy of tumours, and so on.

Recurrent neural networks
The application of recurrent neural networks (RNNs) in medical image analysis is not very widespread than several other deep learning algorithms, instead it is often applied to text analysis or natural language processing [17]. RNNs are neural network model types specifically designed to cope with sequential data, which are also capable of capturing temporal information in medical images such as the response to treatment in tumor patients over time, or the temporal dynamics of processing gene expression data. Unlike CNNs, RNNs feature the ability to process image or numerical data and analyse tissue images obtained at distinct stages. As depicted in Fig. 2, inputs are based on HE-stained WSI, with the RNN model outputting classification results by combining channel attention and spatial attention, which further
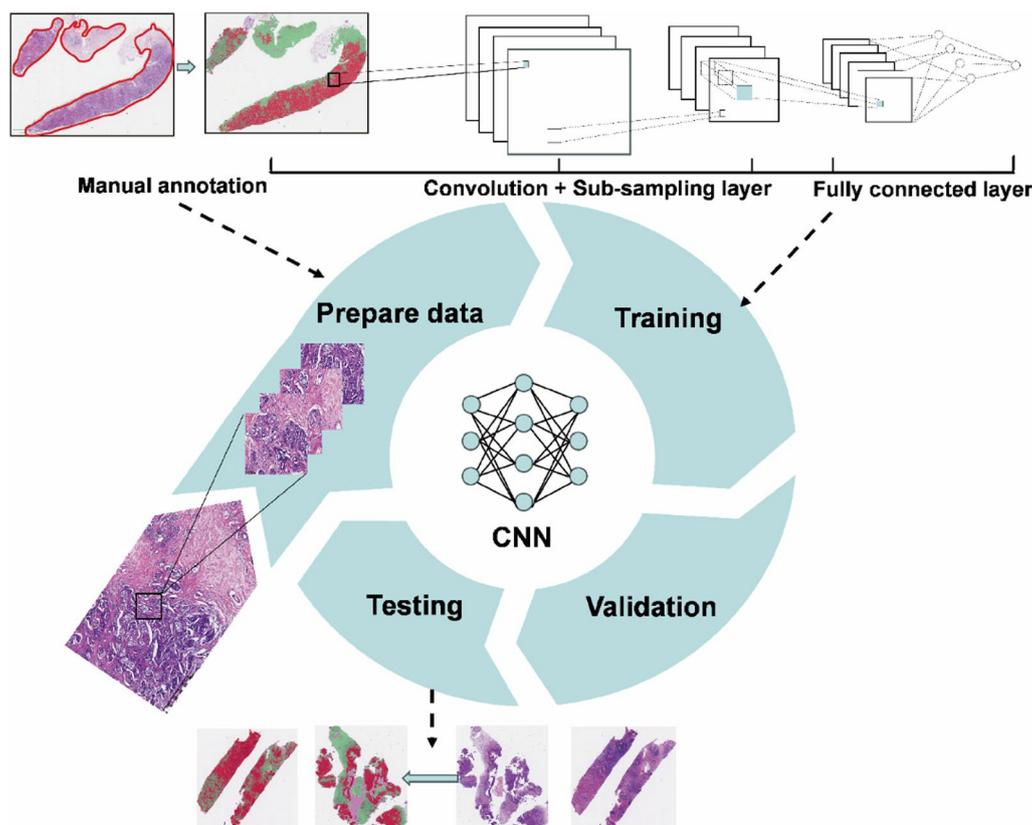

Fig. 1 Typical process of CNN-based approach for pathology image analysis

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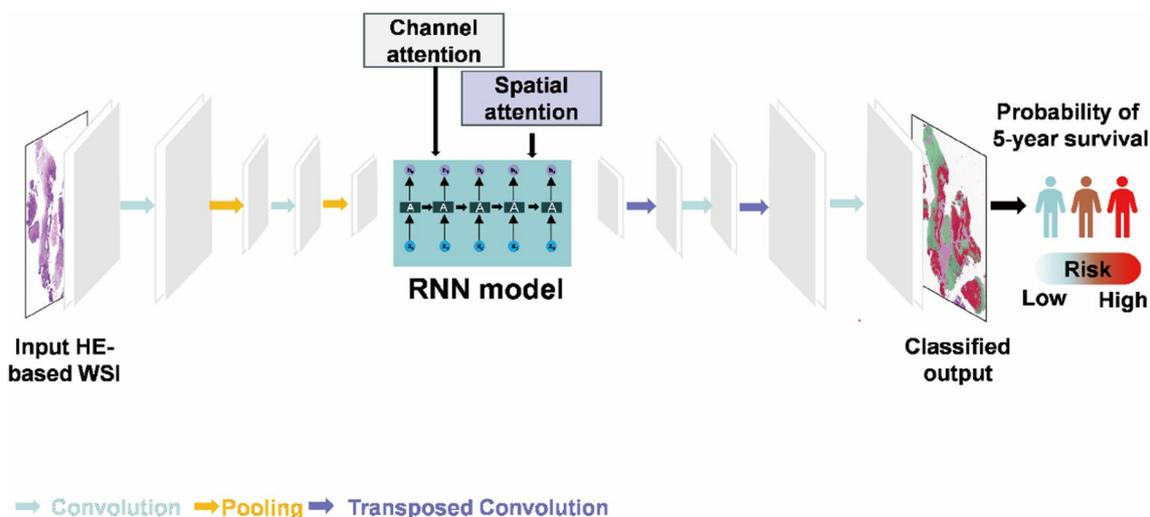⇒ Convolution ⇒ Pooling ⇒ Transposed Convolution

Fig. 2 The RNN model outputs classification results and predicts 5-year survival

predict 5-year survival [18]. There are numerous RNNs malformed networks, with the Long Short-Term Network (LSTM) being one of the most widely utilized networks. Targeted keywords that help pathologists write pathology reports can be obtained by combining LSTM with pathology picture recognition. A neural network model combining LSTM and CNN was deployed by Bychkov et al. [18] to predict the five-year survival rate of colorectal cancer patients using HE-stained pathology images. Research results indicated that the model's prediction accuracy was significantly better than other classifiers and higher than that of a visual risk score model.

Generative adversarial network

The basic structure of Generative adversarial networks (GAN) includes a generator and a discriminator, which continuously optimize the loss function through their adversarial interplay to generate pseudo data highly similar to real data. GANs enable to be used for generating virtual tumor image data, for training data-poor models, or for simulating tumor behavior under different microenvironmental conditions. Therefore, GANs are often used for color normalization in pathological images to reduce the impact of color on classification, just as illustrated in Fig. 3. Moreover, extensive research applies GANs to virtual staining of pathological images, showing potential clinical applications [19]. In order to perform virtual immunohistochemistry (IHC) stained on the same slide, Xu et al. [20] employed a GAN network to transform HE-stained digital pathology images into IHC-stained images. This approach eliminated the negative consequences of destructive IHC-based tissue testing while simultaneously improving experimental efficiency

due to the little amount of manual labelling data required. Although virtual staining through GAN provides cost reduction, safety, etc., the use of it for clinical use is currently immature and requires standardization of staining, improved robustness of staining results, etc. In addition to applying GAN in the field of pathology images, there have been studies using it for magnetic resonance (MR) image processing. For instance, an adversarial learning framework for multimodal MR image fusion was trained and validated on the glioma dataset by Liu and his team [21], and the results revealed that the method outperforms some of the latest techniques in medical image fusion.

Transformers

Originally proposed by Vaswani et al. [22] in 2017, the Transformer model has become a groundbreaking technique that utilizes a "self-attention" mechanism to capture the intrinsic relationships in the input data without relying on traditional RNN or CNN structures, it contains mainly decoders and encoders. Expected results it delivers are better than other models, and it parallelizes training, which is fast and solves the problem of long-distance dependence well, with the exception that it is based entirely on self-attention, with a certain amount of loss of information about location. As shown in Fig. 4, the transformer model accomplishes the task of tumor segmentation based on HE-stained pathology images by means of a decoder, an encoder, and a multilayer perceptron used for feature transformations, with classification of the benign and malignant tumor. Subsequently, TransUNet (Transformers and U-Net) was presented, which opened up the application of Transformer in the field of
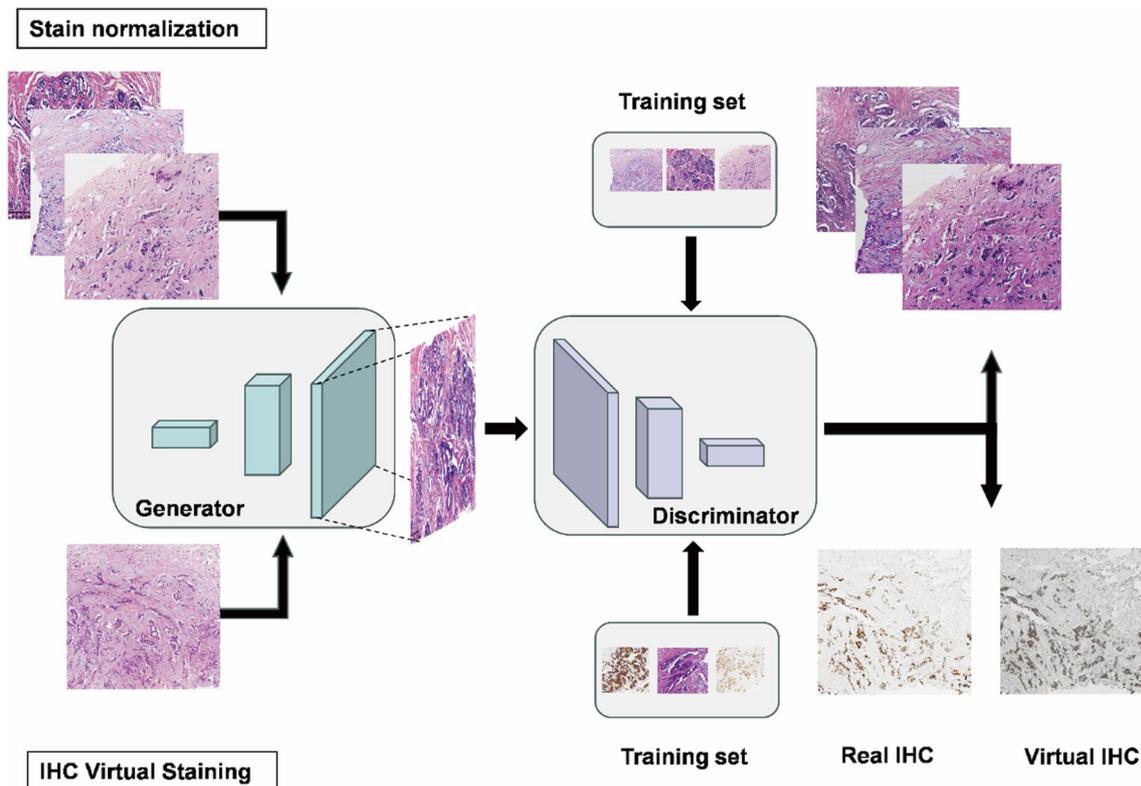
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Fig. 3 GAN model is capable of stain normalization and virtual IHC staining
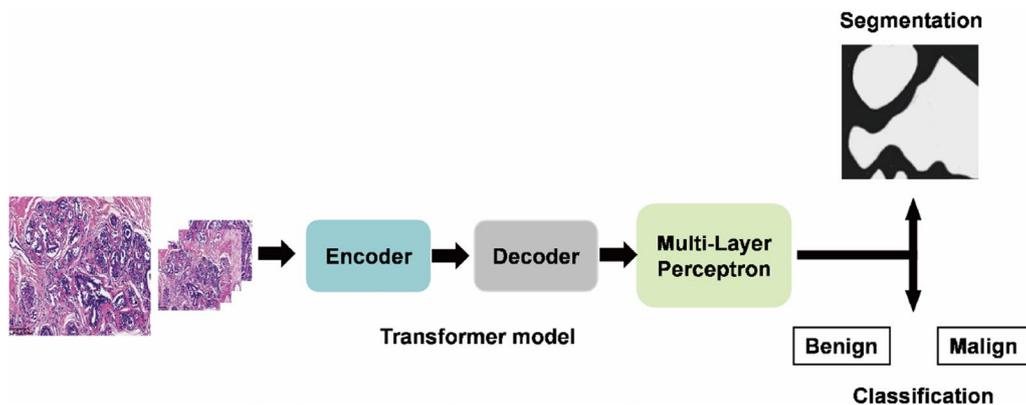
Fig. 4 Transformer model to segment pathology images and classify the benign and malignant of tumors

medical image segmentation. The fusion of Transformer and U-Net minimizes the amount of computation, and it provides a better advantage in large-scale datasets and captures important information effectively.

AI for multidimensional characterization of TME
The phenotype and function of cells in TME may highly depend on the precise spatial location of cells and their interactions with neighboring cells. Therefore, Accurate

cell segmentation and classification are necessary to analyze the multidimensional spatial characteristics of TME. Currently, the assessment of TME by histological methods is prone to sample errors due to the difficulties associated with obtaining high-quality tissue sections, as well as the spatial heterogeneity within the tumor and the dynamic evolution of TME. Utilizing machine learning algorithms to process large-scale tumor tissue section images and single-cell data, it is able to construct models

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of cell types and spatial distribution in the tumor microenvironment. In addition, extracting features in tumor tissue sections, such as morphological and spatial distribution features, AI is able to perform cell type classification and spatial distribution analysis of TME [11].

Application of a single model to characterize TME

As opposed to traditional AI models for segmenting tumour images, Zhu et al. [23] developed a CNN-based brain tumour segmentation model in their recent study, which consisted of three modules combining multimodal, spatial and boundary information to analyse the global spatiality of the image, which facilitated the accurate acquisition of the tumour's location and interrelationships with other tissues on magnetic resonance images. The model was validated by external datasets with superiority in both performance and computational efficiency. Nagy et al. [24] improved the MultiOmyx analysis process based on a DL model developed in the NeoGenomics lab. This DL model produced biomarker intensities, phenotype counts, phenotype densities, and cellular morphological information in addition to segmenting and classifying images. In addition to this, it was able to perform advanced spatial analyses to pinpoint the clustering patterns of different phenotypes, which contributed to the investigation of complex cellular interactions in TME.

Application of fusion models to characterize TME

With the integration of learning, deep learning fusion models can combine the strengths of multiple single models to significantly improve predictive performance, enhance model robustness and stability, and support complex data and tasks at the same time.

In order to comprehensively analyze the spatial features of tumors, Liu et al. [25] combined the stronger local information extraction capability of CNNs and the excellent global representation capability of Transformers to build a hybrid model named TransSea for the task of brain tumor segmentation in medical images. By training and testing the BraTS2020 and BraTS2021 datasets, TransSea obtained Dice scores of 86.32% and 90.84%, respectively, which is a clear advantage over other models.

Although spatial transcriptomics is capable of in-depth analysis of the relationship between tumors and TME, it is costly and has limitations in practical clinical use. Based on this, Gao et al. [26] developed a deep learning model based on CNN and GAN (IGI-DL model), which is capable of predicting the expression of spatial transcriptomics in patients based on H&E-stained histological images by learning pixel intensities and structural features, effectively reducing the technical cost of using spatial transcriptomics. IGI-DL was also able to characterize the spatial features of TME, determine the heterogeneity of TME, and demonstrate that TME plays an important role in cancer prognosis. Comparative analysis with other models (e.g., HisToGene, DeepSpaCE, etc.) showed that IGI-DL exhibits optimal performance in predicting 179 target genes, both in the test and validation sets. Although the model currently performed well in only three cancer types, its ability to characterize TME could provide an effective bridge for probing spatial gene expression.

AI quantification of cells within the TME

Recent advancements in DL and ML techniques, have revolutionized the field of pathology by enabling precise cell identification, detection, quantification, and localization as well as identifying subtle changes in gene expression, metabolite levels or protein structure associated with disease [27].

Deep learning techniques based on segmentation of pathology images at the cellular level

DL techniques applied to segmentation of cellular level pathology images are U-Net [28], DeepCell [29], CellProfiler [30] and so on. With little cellular annotation, U-net based on CNN is able to rely on data augmentation to improve the robustness and invariance of the model [31]. As one of the pioneers in the field of AI-driven cellular analysis, DeepCell began by identifying cell populations based on morphology alone, and later was able to identify intracellular heterogeneity based on subtle morphological differences, its continuous development has provided an excellent platform for biological experiments at cellular level as well as for medical research. Owing to its ability to accurately differentiate between various immune cell subtypes, various cancer cells, and stromal cells, it is capable of being used for cellular profiling, cell and gene therapy development, and stem cell research, among others. On the basis of machine learning, CellProfiler [32] is able to automate the analysis of individual cells, quickly and accurately measuring various characteristics of the cells, such as size, shape, brightness, and so on.

Furthermore, the potential of machine learning to evaluate the TME is highlighted by the application of supervised machine learning to digital images of HE-stained tissue microarrays by Väyrynen et al. [33], which classified and counted lymphocytes, plasma cells, neutrophils and eosinophils in intra-epithelial and mesenchymal zones of colorectal cancer tumours. It's not hard to conclude that artificial intelligence-based analysis of WSIs will accelerate pathologists' assessment of the complex TME and increase the objectivity and reproducibility of predictions.

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AI in tumor cells

The technologies currently used to study the complexity and heterogeneity of cells in TME are predominantly single-cell sequencing technologies, flow cytometry and others. In contrast, AI approaches, such as image analysis of pathology slides, can offer insights into spatial relationships between different cell types and their distribution within the TME. Moreover, AI algorithms trained on large datasets can potentially identify and characterize rare cell types or subtle phenotypic changes with greater accuracy and efficiency [34], as illustrated in Table 1.

For the identification and capture of tumour cells, the application of CNN is preferable. In order to further optimise the cell detection and classification function of the VGGNet model, Li et al. [35] designed a CNN model with flow cytometry-derived datasets, which was able to achieve precise capture of cancer cells in a few milliseconds with more than 95% accuracy. In addition, for tumour cell classification, deep learning is able to extract features and achieve high accuracy in the classification of unlabelled cells. To distinguish cancer cells derived from cholangiocarcinoma within an unlabeled microscopy image, Chawan et al. [36] developed a proof-of-concept deep learning model by morphological differences. However, distinguishing cells by morphology alone can mistakenly miss identifying broken cells, cells with abnormally large morphology, and non-cellular objects that resemble cells, and whose accuracy therefore needs to be reconsidered. In addition, in order to differentiate between benign and malignant urothelial cells, Masatomo Kaneko et al. [34] developed a CNN model including the EfficientNet B6 and Arcface architectures which successfully differentiated between all cellular subtypes of urothelial cells, achieving up to 90% accuracy.

AI in immune cells

Tumor-infiltrating lymphocytes

Tumour-infiltrating lymphocytes (TILs) have been shown to be tumour-killing and exert an essential effect in the identification of tumour antigens [37, 38]. TILs contain both positively regulating immune response immune cells, such as CD4 T cells, CD8 T cells, NK cells, Th1 cells, and Tfh cells, which are capable of suppressing tumors [39, 40]. Conversely, myeloid suppressor (MDSC), Treg cells, Th2 cells, etc. are able to promote tumor growth, as is shown in the Fig. 5A.

The degree of TILs' infiltration within the tumour is usually positively correlated with the efficacy of immune checkpoint inhibitors, with higher levels of infiltration being associated with better efficacy and prognosis [41]. The number, type, and region of TILs within tumour tissues are important in predicting solid tumour clinical prognosis [42]. TILs can be used as a predictor of higher

pCR rates with neoadjuvant chemotherapy, As shown in Table 2. One of the studies had analyzed 498 patients with HER2-positive breast cancer treated with neoadjuvant treatment [43]. The results noted that TILs contribute to the prediction and prognosis of these patients.

For the purpose of providing standardized and effective TIL quantification, automated image analysis approaches particularly AI-based methods are required, which offer standardized criteria for stringent validation by qualified pathologists and quality control by regulatory bodies [44–46]. Just as Table 1 illustrates, they also increase quantitative accuracy, reduce time, and make it easier to analyze more complicated spatial patterns. Joel et al. [47] developed a comprehensive approach and an interactive tool that incorporated expert feedback into a deep learning model based on extensive previous research, which could accurately generate TIL Maps from WSIs. This iterative feedback increased the overall accuracy of the TIL Maps. Both the necrosis segmentation CNN and the lymphocyte infiltration categorization CNN were applied. The first one distinguished between the little areas of the input image that had lymphocyte infiltration and those that did not. Initialized with an unsupervised convolutional autoencoder (CAE), it was a semi-supervised CNN. In order to reduce false positives in necrotic zones—where cell nuclei may resemble regions invaded by lymphocytes—the latter segmented the necrotic sections. The study revealed that the degree of TILs penetration may influence overall survival as well as the spatial aspects of the TME. Juha et al. [33] performed image analysis of HE-stained slides of the TME of CRC patients by a ML based approach to identify four types of immune cells in the TME: neutrophils, eosinophils, plasma cells, and other lymphocytes, which were subsequently classified. Results of the training showed that this automated approach to detect and classify immune cells using machine learning was highly consistent with pathologists and independently trained automated classifiers. The findings also revealed that high density of lymphocytes and eosinophils was known to be correlated with better survival.

Tumour-associated macrophages

As the most diverse immune cell in the TME: the tumour-associated macrophage (TAM), commonly linked to poor prognosis and drug resistance, is classified into two distinct subtypes according to morphological, phenotypic, and functional heterogeneity, namely the M1 and the M2 types [48, 49]. The two subtypes play diametrically opposed roles in the TME [50, 51]. As is depicted in the Fig. 5B, M1 TAMs with anticancer effects can release pro-inflammatory mediators such as IL-1, IL-12, IL-18, IL-23, and TNFα. M2 TAMs are triggered by IL4

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Table 1 Application of AI for assessment of cells in TME of different tumors

Cell type	Tumor type	Aim	Type of AI	Algorithm	Sample size			References
					Training	**Validation**	**Testing**	
Tumor cells	TNBC	To study cellular phenotypes at the tissue level	DL	PangNet, Fully Convolutionnal Net and DeconvNet	33 WSIs	–	–	[95]
	Various tumors	Segmentation of overlapping nuclei	CNN	VGG16	48 WSIs	11 WSIs	47 WSIs	[96]
	Neuroblastoma	Development of an automated nucleus segmentation method	DL	Morphological segmentation algorithm	–	20 WSIs	–	[97]
TILs	NSCLC	Develop an AI–powered spatial TIL analyzer	DL	–	3166 slides	2389 slides	–	[98]
	BC	Generate combined maps of cancer regions and TILs in routine diagnostic WSIs	CNN	VGG16, ResNet34 and Inception-v4	102 slides	7 slides	284 slides	[99]
	CRC	Explore the prognostic impact of spatial distribution of TILs	DL	Resent18, Resent34 and Shufflenet	9582 patches	1198 patches	1198 patches	[100]
	luminal BC	Assess the prognostic significance of TIL	CNN	The U-Net	1572 patients	318 patients	659 patients	[101]
	TNBC	Assess the clinical significance of AI-powered spatial TIL analysis in the prediction of pCR after NAC	DL	Lunit SCOPE IO, an AI-powered H&E WSI analyzer	954 patients	261 patients	–	[44]
	MIBC	Evaluate the prognostic value of TILs	ML	Artificial neural network classifier	133 patients	247 patients	–	[45]
TAMs	Lung cancer	Study cell interactions in the TME	DL	Mask-RCNN	12,000 cell nuclei	1227 cell nuclei	1086 cell nuclei	[59]
	-	Identification of macrophage subsets	ML	SVM, kNN, RF	50%	–	50%	[60]
	NSCLC	Identification the prognostic value of TAMs in the TME	ML	RF	477	–	204	[62]
TANs	CRC	Explore the prognostic value of TANs	ML	QuPath v0.1.2	80 patients	934 patients	570 patients	[33]
	Hematological diseases	Explore the morphological differences between neutrophils	CNN	Transfer learning algorithm	4892 neutrophils	1223 neutrophils	–	[72]

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Table 1 (continued)

| Cell type	Tumor type	Aim	Type of AI	Algorithm	Sample size			References
					Training	**Validation**	**Testing**	
CAFs	Hematological diseases	Autodetection of CAFs	DL	RetinaNet49, R-CNN	1000 patches	–	100 patches	[83]
	BC	Characterization of CAF subtypes	CNN	Resnet34	50,000 patches	–	–	[102]
	–	Development of immunosuppressive drugs	ML	SVM, RF, k-NN and ANN	1411 descriptors	–	942 descriptors	[86]
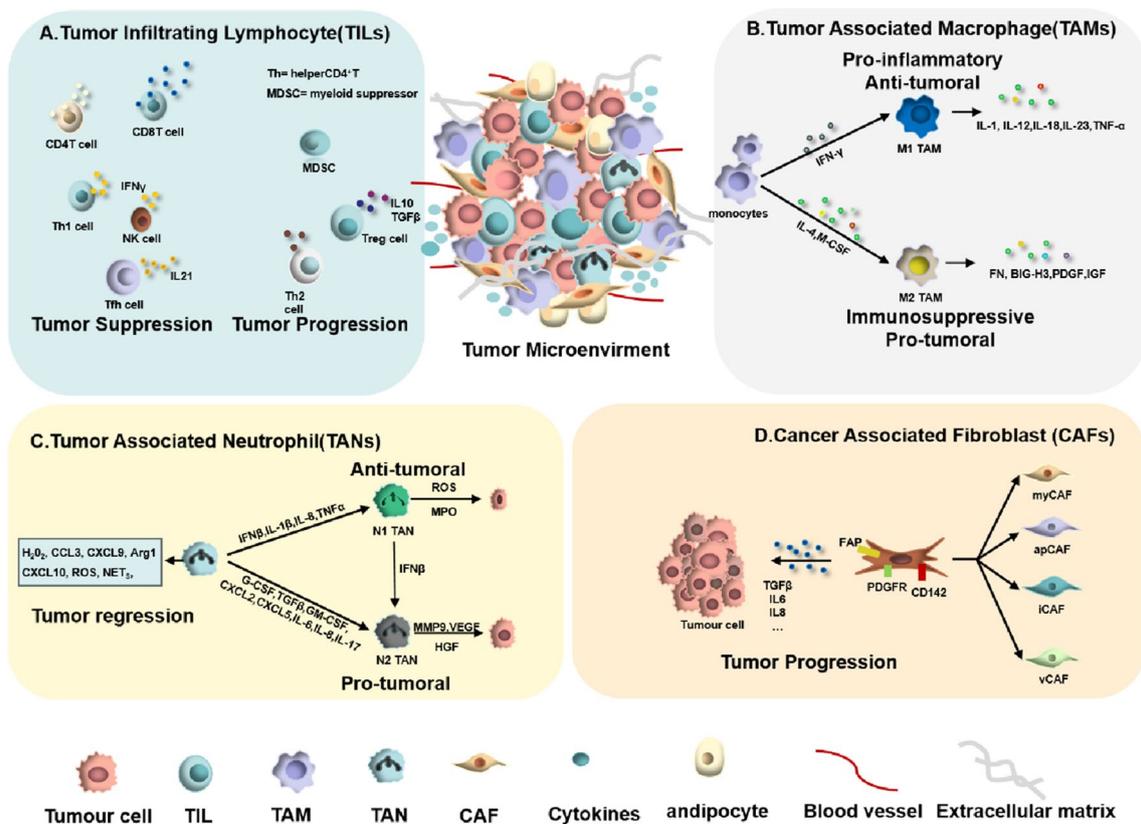
Fig. 5 The complex tumor microenvironment. **A** Tumour-infiltrating lymphocytes. **B** Tumour-associated macrophage. **C** Tumor-associated neutrophils. **D** Cancer-associated fibroblasts

and M-CSF [52]. M2 TAMs exhibit high levels of expression for several factors involved in cell adhesion and proliferation, including insulin-like growth factor (IGF), platelet-derived growth factor (PDGF), betaig-h3 (BIG-H3), and fibronectin (FN) [53, 54]. TAMs are frequently linked to a poor clinical prognosis in cancer patients [48, 55, 56]. However, more recent research has shown that the prognostic significance of TAMs is debatable and that the positional distribution and function of TAMs affect a tumor's prognosis. As indicated in the Table 2, according to Li et al., a poor prognosis was closely linked to the accumulation of CD163 TAMs in lung cancer [55]. Interestingly, several clinical studies support the value of counting TAMs for prognostic and predictive outcomes. For example, an in-depth study performed by Ruffell et al. inticated that lymph node metastasis and inadequate pathological staging in patients with breast cancer were linked to macrophage infiltration (CD68+) [57]. Macrophages were not found in breast tissue in patients who did not receive chemotherapy; instead,

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Table 2 Prognostic value of different cells in the TME

Cell type	Tumour type	Assessment methods	Sample Size (Case)	Expression in cells	Study Result	References
TILs	BC	IHC	12439	CD8$^+$TILs and FOXP3$^+$	Infiltration of CD8$^+$ T cells led to a reduction in the risk of death	[103]
	HCC	IHC, quantitative PCR and flow cytometry	112	CD20$^+$ B cells and CD3$^+$ T cells	Density of tumour-infiltrating T and B cells correlated with higher survival	[104]
	HGSOCs	IHC	3196	CD8$^+$TILs	CD8$^+$TILs in HGSOCs were significantly associated with longer OS	[105]
	MIBC	H&E staining	203	–	High sTILs infiltration were associated with significantly higher OS, TSS and DFS	[106]
TAMs	MIBC	IHC, scRNA-seq and flow cytometry	520	CD8$^+$T cells and NK cells in IL-10 TAMs	IL-10 TAMs had a higher predictive value and were linked to a worse clinical prognosis	[107]
	BC	IHC	75	CD68$^+$ in TAMs	The presence of diffuse inflammation, particularly macrophages, was linked to increased tumor necrosis, tumor size, and tumor grade	[108]
	CRC	IHC and TMA	835	High CD206/CD68 ratio	The CD206/CD68 ratio is a useful biomarker for prognosis and prediction	[109]
TANs	HCC	IHC	832	CCL2 and CCL17	CD66b, CCL2, or CCL17 TANs were independent prognostic indicator	[110]
	ICC	Tissue microarray and IHC	123	IL-17, FOXP3, CD8, CD66b and CD34	IL-17 + cells, CD66b + TANs are powerful predictors of prognosis	[111]
	CRC	IHC	448	CD66b	Reduced neutrophil infiltration around the tumor front is a separate predictor of a worse prognosis	[112]
	PDAC	IF staining and IHC	119	MPO, CD11b and CD206	Higher TNM stage, increased likelihood of lymph node metastases, and worse tumor differentiation were all linked to lower N1/N2 ratios	[66]
CAFs	Rectal cancer	IHC	98	α-SMA and ki67	CAFs may favour tumour progression	[113]
	BC	IHC	132	Podoplanin, prolyl 4-hydroxylase, FAPα, S100A4, PDGFRα, PDGFRβ, and NG2	The prognostic value of CAF-associated proteins differed when the metastatic site of breast cancer differed in expression	[114]
	HCC	IF, western blot analysis and Real-time PCR	–	CCL2, CCL5, CCL7 and CXCL16	By activating the Hh and TGF-β pathways, CAF-derived CCL2, CCL5, CCL7, and CXCL16 increase HCC metastasis and were linked to a poor prognosis	[115]

they were more common in normal tissues that were not adjacent. In contrast, the tumor infiltrating macrophage levels were higher in patients undergoing neoadjuvant chemotherapy.

TAMs play a key role in TME and tumour biology, and few studies have applied AI to TAMs. On the one hand, it is because the development of AI and precision medicine needs to be further improved, and on the other hand, it

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is because TAMs are heterogeneous and their complexity makes the combination of AI and TAMs challenging [58]. Classification and medical picture segmentation are the main areas of application for neural networks and deep learning in TAMs. A recent study developed a deep learning-based computational model, Mask R-CNN, for segmenting cell nuclei from HE-stained pathological images of lung adenocarcinoma, which included segmentation and classification of macrophage nuclei [59]. Additionally, using machine learning techniques in the Orange Data Mining Toolbox, researchers were able to create a quick and easy imaging-based approach that could recognize various macrophage functional phenotypes based on cell size and morphology [60]. This machine learning approach, which solely examined macrophage morphology, demonstrated 90% average accuracy in identifying M1 and M2 phenotypes and differentiating them from naïve macrophages and monocytes. Random forest (RF) is an ML algorithm that ranks each variable's predictive potential and builds predictive models. It is a supervised learning technique based on feature stochastic vectors [61]. In 2022, in an attempt to investigate the prognostic significance of macrophages and their heterogeneous phenotypes in non-small cell lung cancer, Wu et al. [62] screened for prognostic markers using a machine learning algorithm with a RF model and constructed an immune-related risk score based on CD68 to predict disease-free survival.

To elucidate the regulatory role of macrophage infiltration in high-grade plasmacytoid ovarian cancer (HGSOC), Chang et al. developed a macrophage-associated predictive model utilizing the ML LASSO method and validated it in different HGSOC cohorts [63]. The results showed that high levels of M1 TAMs infiltration were related with favorable outcomes, but high levels of M2 TAMs infiltration were related with bad outcomes. Shen et al. [64] created a DL model to define the immune infiltration of the transcriptome with the goal of classifying brain tumours according to their distinct immune infiltration characteristics. To handle gene expression data, the model made use of an eighteen-layer ResNet feature encoder. The feature encoder was trained using close to 100,000 transcriptomes from various cancer types. The model identified two molecular subtypes, C1 and C2, of brain tumors, each with a distinct immunological infiltration profile and prognosis. It was determined that the considerable TAM infiltration of the C2 subtype was a key characteristic.

Tumour-associated neutrophils

Analogously to TAMs, tumor-associated neutrophils (TANs) are fall into two distinct phenotypes: Anti-tumor phenotype N1 TANs and pro-tumor phenotype N2 TANs. As can be seen in the Fig. 5C, N1 TANs directly produce cytotoxic mediators such as reactive oxygen species (ROS) and myeloperoxidase (MPO). In the presence of interferon β (IFN-β), the N1 TANs are suppressed and then converted to the N2 TANs. N2 TANs promote tumor invasion and angiogenesis by producing cytokines such as matrix metalloproteinase 9 (MMP9), vascular endothelial growth factor (VEGF), and hepatocyte growth factor (HGF). In addition, neutrophils produce H_2O_2, CCL3, CXCL9, CXCL10, ROS, NETs and Arg1 to regress tumour.

As shown in the Table 2, the prognosis resulting from differences in the relative location of TAN versus tumour cells varies, as well as the results of several studies support a strong correlation between intratumoural neutrophils and poor prognosis and a weak correlation between peritumoural and mesenchymal neutrophils and poor prognosis [65]. Increased neutrophils have been linked to a worse prognosis in various malignancies, including glioma, metastatic melanoma, and gastrointestinal mesenchymal tumors, according to a number of studies conducted over the past few decades. Recently, an in-depth study performed by Chen et al. [66] explored the plasticity of N1 TANs and N2 TANs in TME of PDAC and the effect of their immune infiltration on the prognostic value of patients. A total of 119 patients undergoing radical resection were included in this study, and N1 TANs and N2 TANs were identified by immunofluorescence staining, and the plasticity of N1 and N2 was evaluated by the N1/N2 ratio. Multivariate factor analysis showed that a low N1/N2 ratio was associated with poorer tumour differentiation, milder lymph node metastasis and higher TNM stage. While this was not the case for N2 TANs, the group with a large number of N1 TANs had a significantly longer median OS and RFS than the group with a low number of N1 TANs.

It is shown in Table 1, using image processing technology and ML algorithms, the quantity of TANs in cancer tissue can be evaluated rapidly and precisely, providing a significant basis for tumour progression and prognosis assessment [67, 68]. Using computational simulation and machine learning technologies, the interaction process between TANs and tumour cells can be simulated to gain an in-depth understanding of their mechanism of action. Artificial intelligence-based immunosurveillance technology can monitor the dynamic changes of TANs in the TME in real time, providing real-time guidance for clinical treatment. In the area of drug development, combined with AI technology, TANs can be used as targets for drug screening and discovery of new tumour therapeutic drugs [69, 70].

Some researchers proposed a deep learning model for identifying myeloproliferative tumours based on

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neutrophil morphology construction, and chose the Pico-Det deep learning target detection method to determine whether a patient is a myeloproliferative tumour and myeloproliferative tumour subtype [71]. The outcomes demonstrated that PicoDet can identify the cells in the bone marrow smear more accurately and achieve four classifications of the dataset, whose average accuracy rates were all over 70%, achieving a good classification prediction effect. Bi et al. [72] subdivided 6115 neutrophils from the WSI of malignant hematological diseases, trained these neutrophils using a migration learning algorithm, built a convolutional neural network model based on the morphological phenotypes of the neutrophils to determine their disease classification, and evaluated the model using confusion matrices and subject arithmetic characteristic (ROC) curves. The results showed that neutrophils from various diseases could be categorized into distinct groups, and the accuracy of the DL model in judging neutrophils of different diseases reached 0.896. Differences in neutrophil nuclear morphology may underlie the heterogeneity. Therefore, some researchers have conducted a preliminary study of neutrophil phenotypic heterogeneity in different haematological diseases by deep learning [73]. Firstly, neutrophil images were manually segmented and then nuclei were segmented using the interactive semantic segmentation tool ilastik. Validation results showed that the model achieved an accuracy of 0.749. The study also analyzed the nuclear features of neutrophils through migration learning, a machine learning-based pixel classification technique.

Cancer-associated fibroblasts

Among non-immune cells, cancer-associated fibroblasts (CAF) are the most numerous of the TMEs, accounting for about 80%, with the involvement in tumour generation, growth and drug resistance, consequently they are considered to be pro-tumourigenic [74]. Through their interactions with other TME components, CAFs play a crucial role in shaping TMEs, indicating their potential utility as therapeutic targets and prognostic variables. CAFs are heterogeneous, in that CAFs also exert pleiotropic functions in TMEs. MyCAF, apCAF, iCAF, and vCAF are the four primary subtypes of CAF [75, 76]. By producing cytokines such as TGFβ, IL6 and IL8, CAF stimulates tumor growth (As is shown in the Fig. 5D).

A worse prognosis for patients has long been linked to the quantity, hardness, and other characteristics of the ECM [77]. When evaluating prognosis, it is important to measure the overall characteristics of the patient's CAFs and the prevalence of each subtype [78, 79]. Some CAF subtypes, such as iCAFs, show tumor suppressor function and are associated with improved treatment outcomes, in contrast to myCAFs and vCAFs

which often suggest a poor prognosis, whereas apCAFs appear to have no prognostic implications [80, 81]. Due to the heterogeneity of CAFs and their intra-tumour specificity, some researchers have investigated their value in the early diagnosis of carcinoma and prognosis prediction as is shown in the Table 2. Cai et al. employed the Estimate the Ratio of Immune and Cancer cells (EPIC) algorithm to calculate the proportion of CAFs in patients with locally advanced rectal cancer. The results indicated a significant difference in cancer-specific survival between the two subgroups, with patients with a high rate of CAF infiltration exhibiting worse clinical outcomes [82].

The morphology of CAFs exhibits spindle-shaped, mostly polygonal, and flattened stellate forms, providing morphological cues for AI recognition of CAFs. Therefore, AI can identify CAFs and automatically quantify their numbers or ratios. Shen and associates [83] developed an imaging system which had the ability to identify CAFs with the accuracy up to 93%. By integrating with faster R-CNN cell identification technique, in the first step, extensive manual labelling of the CAF was performed on slides, and in the second step, the model was trained in conjunction with fluorescent images. The approach could significantly advance cell-based biopsies that go on to diagnose cancer. Furthermore, to extract the morphological dynamics and motility properties of cells from unlabeled live cell imaging data from CAFs, several researchers have combined a range of unsupervised and supervised machine learning methods with a deep learning-based cell categorization strategy [84]. Wu et al. [85] observed that the fibroblast growth factor receptor (FGFR) signaling pathway was enriched in the immune-exclusion phenotype of triple-negative breast cancer (TNBC) samples from the TCGA dataset after using DL to analyze the TME.

To create more potent targeted medications for immunotherapy, Charan et al. [86] used a collection of 2356 compounds to create an artificial intelligence-based prediction model for FGFR1 inhibitors. Four machine learning algorithms were used in this study, including Support Vector Machines, Random Forest, K-Nearest Neighbors, and Artificial Neural Networks. With an accuracy of 89.8%, the Random Forest model was found to be the best-performing model. In addition to this, there were other relevant studies applying AI to the detection of CAF-related genes. Lv et al. [87] discovered a unique gene signature linked to CAF that may be used as a prognostic indicator and treatment response predictor. InvasionInverse convolutional algorithms, such as the xCell algorithm, which is based on the enrichment of gene signatures, the Estimated Proportion of Immune and Cancer Cells (EPIC) algorithm, and the Microenvironmental

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Cell Population Counter (MCP-counter) algorithm were used to calculate the abundance of CAFs in the study.

Other cellular components

Besides studying tumour cells and immune cells in TME, AI has a little application for other cells, such as adipocytes, blood vascular endothelial cells, pericytes, neurons and nerves etc. Adipocytes provide the energy needed by cancer cells for biosynthesis through a combination of adipokine secretion, lipolysis, and reprogramming of glucose metabolism. Similarly, neurons contribute to tumorigenesis. Compared to immune cells, these other cellular components of the TME seem to be in a position of underappreciation, and as such, they are not a hotspot for AI technology research.

Limitations and prospects of artificial intelligence

Traditional TME research generally depends on laboratory techniques and animal models, but is hampered by technological limitations and resource expenses. In recent years, the rapid growth of AI has brought new concepts and approaches for investigating the tumor microenvironment. Through technologies such as ML and DL, AI can extract useful information from large amounts of data, deepen our understanding of tumours and the TME, and thus guide more precise treatment strategies and prognostic assessments [88]. The development of AI technology has brought new opportunities and challenges to oncology research. Despite the significant progress made by AI in TME research, certain obstacles still need to be overcome.

Algorithms to keep up-to-date

The complexity of the TME makes it necessary for AI algorithms to be continuously optimized and updated to adapt to changing research needs.

Data quality and standardization

Absence of standardization of data, data imbalance and heterogeneity, as well as lack of training datasets can affect model training and prediction [89]. TME research involves many types of data, such as gene expression data, immune cell infiltration data, etc., and the quality and standardization of these data may be inconsistent, which needs to be judged by experienced experts to develop uniform standards [90].

Interpretability

The black-box nature of deep learning models may limit their application in tumor microenvironment research. While these models efficiently handle complex data, understanding their decision-making processes poses challenges for clinical practitioners and researchers [91].

Generalization ability

Models perform well on TME training data for certain cancers, while may have insufficient generalization ability on new data, i.e., results on the validation set are considerably divergent from the training set results [92]. This may result in the model not performing as well as expected in real-world environments, especially when the data distribution changes.

Sample size and diversity

Building effective AI models requires large and diverse datasets. However, obtaining diverse and ample samples for tumor microenvironment research, especially for rare cancer types or specific populations, may be challenging.

Ethical and privacy concerns

Large-scale data collection and usage raise ethical and privacy issues, including data security, patient consent, and data-sharing policies. These concerns may restrict data availability and hinder research progress.

Bright future prospects

With higher efficiency and cost-effectiveness, AI reduces the subjectivity and error rate of pathologists' diagnosis, and its clinical application will gradually spread, creating an automated and intelligent diagnostic environment for us.

The combination of AI with spatial transcription and single-cell sequencing has been carried out, as in the novel self-supervised deep learning framework called BIDCell [93], which combines single-cell transcriptome data and cellular morphology information, which not only provides good segmentation of cells, but also learns to spatially discriminate between gene expression and cellular morphology, providing a direction for multidimensional characterization of TME. Combining AI with other technologies can fully utilize the advantages of each technology, which is one of the ways to apply AI in the future. Future applications of AI alone could allow for spatial analysis of all aspects of TME, replacing other costly techniques.

By identifying potential immunotherapeutic targets within the TME, discovering more biomarkers for tumors, etc., AI has the ability to be further employed in drug development [94]. In addition, fusion of multiple AI algorithms can bring significant advantages in terms of improving prediction and decision-making accuracy, reducing the risk of a single model, increasing generalization ability and transparency, etc., which is one of the

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prominent strategies to promote the advancement of AI applications.

Conclusions

The application of AI in the TME has now moved from macro to micro. Macroscopically, AI has been gradually applied to e.g. tumour diagnosis, tumour metastasis identification, tumour grading and staging. On the microscopic level, AI has been used to analyse the TME in a more specific and detailed way by quantifying and locating immune cells such as TILs, TANs, TAMs, etc. in the TME, as well as non-immune cells such as CAFs, etc. Therefore, this paper mainly reviews the application of AI to these four types of cells, analyzes the multidimensional characteristics of TME, as well as details the similarities and differences between single AI models and fusion AI models in the study of TME. However, the TME is very complex and still has many components that have not been covered by the study, which means that we need to further develop AI models to explore the TME in depth. Through in-depth analysis of the complexity and dynamics of the TME, AI is able to reveal the mechanisms of tumour occurrence and development and provide powerful support for tumour treatment. Nonetheless, there are still certain obstacles to be addressed. Future applications of AI in tumor microenvironment research will be increasingly comprehensive and in-depth due to the ongoing advancement of technology.

Abbreviations

AI	Artificial intelligence
TME	Tumour microenvironment
H&E	Hematoxylin and eosin
TILs	Tumor infiltrating lymphocytes
TAMs	Tumor-associated macrophages
TANs	Tumor-associated neutrophils
CAFs	Cancer-associated fibroblasts
RNN	Recurrent neural network
LSTM	Long Short-Term Network
GAN	Generative adversarial networks
FGFR	Fibroblast growth factor receptor
MDSC	Myeloid suppressor
IGF	Insulin-like growth factor
PDGF	Platelet-derived growth factor
BIG-H3	Betaig-h3
FN	Fibronectin
ROS	Reactive oxygen species
MPO	Myeloperoxidase
IFN-β	Interferon β
MMP9	Matrix metalloproteinase 9
VEGF	Vascular endothelial growth factor
HGF	Hepatocyte growth factor
BC	Breast cancer
IHC	Immunohistochemistry
HCC	Hepatocellular carcinoma
HGSOCs	High-grade serous ovarian carcinomas
OS	Overall survival
IF	Immunofluorescence
TSS	Tumor-specific survival
DFS	Disease-free survival
CRC	Colorectal cancer
ICC	Intrahepatic cholangiocarcinoma
PDAC	Pancreatic ductal adenocarcinoma
TNBC	Triple-negative breast cancer
DL	Deep learning
CNN	Convolutional neural network
NSCLC	Non-small cell lung carcinoma
WSI	Whole slide image
ML	Machine learning
pCR	Pathologic complete response
NAC	Neoadjuvant chemotherapy
MIBC	Muscle-invasive bladder cancer
SVM	Support vector machine
kNN	K-nearest neighbour
RF	Random forest

Data availability
No data was used for the research described in the article.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors have declared that no competing interest exists.

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