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Review and Meta-analysis

Dissecting the Tumor Microenvironment (TME) to Decipher New Immunotherapy Targets by Using Artificial Intelligence

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Cancer remains one of the leading causes of death worldwide, second only to cardiovascular diseases. Standard cancer treatments, such as chemotherapy, radiation, and surgery, are effective for primary tumors but often fail to eliminate disseminated tumor cells responsible for metastasis. This limitation underscores the necessity for advanced therapeutic strategies, leading to the rise of immunotherapy, which leverages the immune system to combat cancer. Despite its promise, immunotherapy faces challenges, including variable patient responses and immune-related toxicities, complicating the prediction of treatment efficacy. Here, artificial intelligence (AI) emerges as a vital tool that can enhance the precision and effectiveness of immunotherapy by analyzing the intricate tumor microenvironment (TME). This paper explores the limitations of current immunotherapies and examines how AI can address these challenges. It discusses the TME's role in shaping immune responses, highlighting how understanding its complexities can improve predictive power and treatment outcomes. Furthermore, we address the limitations of AI in cancer research and propose future directions for its integration into clinical practice, with the potential to revolutionize personalized cancer therapy and improve overall patient care.

Introduction

Cancer continues to be one of the leading causes of mortality. According to recent statistics from the American Cancer Society, cancer remains the second most common cause of mortality in the USA, particularly among those under 85 years old. The COVID-19 pandemic exacerbated this crisis by delaying cancer diagnosis and treatment due to healthcare facility closures, economic uncertainties, and patients' fear of exposure to the virus. These delays have raised concerns about an increase in late-stage cancer diagnoses, potentially contributing to higher mortality rates at the community level [1]. The standard cancer treatments, such as surgery, chemotherapy, and radiotherapy are often effective at treating the primary tumor. However, their inability to eliminate dispersed tumor cells responsible for metastasis highlights the need for more advanced therapeutic strategies. This gap has led to the rise of immunotherapy, a promising treatment modality that harnesses the immune system to fight cancer [2]. While immunotherapy has shown significant success in some cancers, challenges such as variable patient responses, immune-related toxicities, and the complexity of predicting treatment efficacy remain. This is where artificial intelligence (AI) emerges as a critical tool, offering the potential to enhance the precision and effectiveness of immunotherapy. By leveraging AI to analyze the intricate tumor microenvironment (TME), researchers can gain insights into the dynamic interactions between cancer cells, immune cells, and other components. Understanding these interactions can reveal novel immunotherapy targets and improve treatment outcomes. AI can help predict responses, minimize toxicities, and guide

more personalized cancer therapies by unravelling the TME landscape. We will explore the limitations of current immunotherapies and examine how AI can address these challenges. We will discuss the role of the TME in shaping immune responses and how deciphering its complexities could enhance the predictive power and efficacy of cancer immunotherapy. Finally, we will explore the limitations of AI in cancer research and the future directions for its integration into clinical practice.

2. Cancer immunotherapy

Cancer immunotherapy represents a revolutionary approach that utilizes the body's immune system to combat cancer. Recently, this strategy has gained significant attention due to its promising results, with innovations ranging from immune checkpoint inhibitors to adoptive cell therapies [3, 4]. Today, cancer immunotherapy is employed across a various cancers, from hematological malignancies to solid tumors. This shift has been largely inspired by the remarkable successes of immune checkpoint inhibitors (ICIs) in melanoma patients and CAR-T cell therapies in blood cancers like leukemia and multiple myeloma [5, 6]. At the heart of this immunotherapeutic approach are immune checkpoints key inhibitory receptors that tumors exploit to evade T cell activity, a phenomenon known as immune escape. The most notable checkpoint inhibitors currently in use include programmed cell death 1 (PD-1), programmed death ligand 1 (PD-L1), and cytotoxic T lymphocyte-associated antigen 4 (CTLA-4). Immunotherapy drugs such as nivolumab (anti-PD-1), atezolizumab (anti-PD-L1), and ipilimumab (anti-CTLA-4) have shown considerable anti-tumor effects, heralding a new era in cancer treatment [5, 7]. While CAR-T cell therapy has demonstrated impressive outcomes in hematological cancers, it is now making strides in addressing the challenges posed by solid tumors. Advances in loco-regional delivery and the identification of new biomarkers are paving the way for targeted therapies that can tackle tumor heterogeneity and immune suppression—hallmarks of cancer [8-10]. However, despite these promising developments, the effectiveness of immunotherapy varies significantly among patients. High treatment costs and unpredictable responses remain major challenges. Some individuals experience substantial benefits, while others may show little to no response, sometimes accompanied by serious side effects and toxicities [11, 12]. Identifying patients who are most likely to benefit from these therapies is crucial for enhancing diagnostic accuracy and minimizing unnecessary toxicities. This could also alleviate the financial burden of treatment, making it more accessible, especially in developing countries. A few biomarkers, such as the expression of PD-L1, microsatellite instability (MSI), tumor mutational burden (TMB), and the number of tumor-infiltrating lymphocytes (TILs), have been identified to predict responses to immune checkpoint inhibitors [13, 14]. Yet, the predictive performance of these biomarkers whether used individually or in combination remains suboptimal. Some tumors exhibit resistance despite the presence of these biomarkers, while others may respond favorably without them [15].

To address these gaps, there is a pressing need for more accurate, reproducible, and cost-effective predictive biomarkers to inform clinical decisions. This is where artificial intelligence (AI) comes into play. By analyzing vast amounts of data from tumor microenvironments, AI has the potential to uncover new immunotherapy targets and improve patient outcomes in this evolving landscape of cancer treatment.

3. Artificial Intelligence

Artificial intelligence (AI) is a branch of computer science that enables machines to perform tasks typically reserved for humans, such as learning, thinking, and problem-solving [15, 16]. It includes subsets like machine learning (ML) and deep learning (DL), which have become widely used, including in cancer research. Researchers can utilize off-the-shelf AI products or develop custom software pipelines to enhance productivity, uncover hidden insights, and improve cancer

immunotherapy by better understanding the tumor microenvironment (TME) [11, 17]. Machine learning focuses on pattern recognition and is often called a learning machine due to its ability to learn from data. ML has been utilized in cancer research for quite some time and there are various tools available. A component or method of ML is artificial neural network or ANN which is kind of resembles brain neural networks and consists of components or units known as neurons organized into multiple layers. These layers include an input layer receiving the input data, an output layer producing the final output, and a few hidden layers involved in computation and abstraction [18, 19]. Deep learning is a kind of ML that utilizes multilayered ANNs, which makes it quite impressive. DL has shown major contribution or development in the field of image processing and the branch or method used is known as computer vision. These deep ANNs may use a component known as convolutions which reduces the raw pixel to relevant information [11]. Because of the expanded design of deep ANNs, higher degrees of computation and data representation are supported which enables deep neural networks to learn complicated patterns and abstract more information. Usually, large datasets are employed to train Deep neural networks. Today, in cancer research the terms AI and DL are used interchangeably quite a lot, the concept of AI overlaps with deep learning. During deep learning, the network learns to perform tasks from inputs. These inputs may be images such as histopathological or radiology images as in the case of oncology [20]. Additionally, Multimodal deep learning models integrate diverse data types, enhancing data interpretation by considering various sources and host factors [21]. By harnessing these advanced AI techniques, we can deepen our understanding of the tumor microenvironment (TME) and its implications for immunotherapy, particularly in response prediction and efficacy.

3.1 Unraveling the Tumor Microenvironment: Insights and Innovations Through AI

The tumor microenvironment (TME) refers to the complex cellular landscape in which tumors and cancer stem cells exist. It significantly influences the growth, behavior, and intercellular communication of cancer cells [22, 23]. Comprising a variety of elements, the TME includes immune cells such as neutrophils, macrophages, and lymphocytes, as well as non-immune cells like fibroblasts and vascular endothelial cells. This intricate environment is not just a mere aggregation of cancer cells; rather, it is a heterogeneous mix of resident and invading host cells, extracellular matrix components, and secreted factors. From the onset of tumor development, cancer cells and the constituents of the TME establish a dynamic and bidirectional relationship that fosters cancer cell survival, metastatic spread, and regional invasion. Immune cells play a crucial role in this interplay; they can both support and hinder tumor development, influencing carcinogenesis, tumor progression, metastasis, and recurrence [24, 25]. Traditional assessment methods, including western blotting, coimmunoprecipitation, and real-time quantitative polymerase chain reaction, have provided insights into the interactions between tumors and their microenvironments. However, newer high-throughput technologies, such as genomics, proteomics, and single-cell sequencing, have revealed the TME’s complexity, indicating that current approaches are insufficient [26-28]. Here, artificial intelligence (AI) can bridge the gap, utilizing deep learning to synthesize vast amounts of data from multiple sources and uncover novel insights. AI has the potential to transform TME analysis by managing extensive datasets and conducting sophisticated image analyses [29]. By evaluating quantitative and spatial characteristics of tumor and immune cells within the TME, AI can reveal the predictive prognostic value of the environment and provide new avenues for therapeutic intervention [30, 31]. Its deep learning algorithms are adept at extracting information from histopathological images, such as H & E-stained slides, allowing for accurate quantification of immune cells and tumor-associated structures [32]. Moreover, AI can predict biomarkers like TMB, MSI, and PD-L1 expression directly from imaging data, enhancing our understanding of tumor phenotypes and their evolution throughout treatment [30, 33, 34]. This capability is invaluable, as it enables real-time tracking of molecular changes that may influence therapeutic outcomes. Also, by integrating AI with advancements in spatial transcriptomics and other high-throughput technologies, researchers can uncover detailed insights into cellular interactions and positional relationships within the TME. Currently available research, however, has shown that the complex interactions with cancer cells can cause the precise activity of immune

cells to change and even reverse. Therefore, to comprehend this dynamic condition of TME, more research is required [27, 35, 36]. The issue with TME as it exists today is sample bias in pathology or expensive, unilateral information-based high-end approaches. Through cell quantification and localisation, pathologists use histological research to identify the TME. However, this approach is susceptible to sample bias, whereas methods like single-cell genomics and spatial transcriptomics are costly, time-consuming, and usually rely on information from a single source, like gene expression or images, and as such are unable to completely appreciate and express the depth, diversity and dynamism of TME [37-40]. Multimodal models are useful for combining multiple and diverse data entities and as such can combine both the spatial and non-spatial data together [41]. The emergence of such models, which converge diverse data types—such as genomic, clinical, and imaging data enhances our ability to identify relevant patterns and improve diagnostic accuracy than ever before [42, 43]. These sophisticated models can analyze complex relationships among various TME components, offering a comprehensive understanding of how these interactions affect patient prognosis and response to immunotherapy ultimately paving the way for enhanced therapeutic strategies.

3.2 How AI can help in Immunotherapy: response prediction and efficacy

AI is transforming the landscape of cancer immunotherapy by enhancing response prediction and efficacy. One of the key ways AI contributes is through its ability to identify new biomarkers and quantitatively assess existing ones, such as tumor mutational burden (TMB), microsatellite instability (MSI), and PD-1 expression [44]. By analyzing image data from oncology, including radiology and histopathology slides, AI can extract critical information that has often been overlooked in traditional medical settings. The core principle of image-based biomarkers lies in the recognition that routinely acquired images contain much more data than is currently utilized [11]. With the aid of deep learning algorithms, particularly those based on artificial neural networks, AI can abstract meaningful insights from these images. This capability is particularly relevant in oncology, where radiology images confirm malignancy and histopathology provides insights into tumor characteristics and staging. A significant advantage of AI is its ability to leverage the vast amount of imaging data available in cancer diagnosis and treatment. These images can serve as raw material for training AI models that predict immunotherapy responses and assess treatment efficacy. Deep learning systems can extract far more information from radiological and histological images than what is typically harnessed in healthcare settings.

Deep Radiomics: The emergence of deep radiomics a refined version of classical radiomics has revolutionized the extraction of features from medical images. While traditional radiomics software focused on a limited set of features such as shape, intensity, and texture, deep radiomics employs convolutional neural networks (CNNs) to access a broader spectrum of characteristics. This allows for the direct forecasting of target categories from radiology image data, enhancing flexibility and providing valuable insights relevant to immunotherapy. Additionally, AI can predict key biomarkers like TMB, MSI, and PD-L1 expression from radiology images, capturing how these markers change in response to treatment [32, 45]. Although the performance of these predictions may not yet be optimal, the ability to track molecular and phenotypic changes during treatment is invaluable for personalized cancer therapy [46, 47].

Computational Pathology: AI has also given rise to the field of computational pathology, which improves the analysis of histopathology [30, 48]. Traditionally, pathologists examined hematoxylin and eosin (H&E) slides to assess tumor characteristics. Although conventional histopathology allows us to look for various types of cells and get a basic understanding of their quantitative and spatial features but is invasive and can be subject to sample bias. AI can quantify biomarkers like PD-L1 expression from H&E slides and provide scoring that aids in identifying patients sensitive to immune checkpoint inhibitors (ICIs) [32, 49, 50]. Furthermore, computational pathology utilizes deep learning to count immune cells, such as tumor-infiltrating lymphocytes (TILs). By abstracting complex visual patterns and analyzing cell morphology, deep learning can determine tumor

sensitivity to immunotherapy, assessing features such as cell shape, phenotype, and spatial relationships from raw histopathology images [51, 52].

The integration of these advanced AI techniques not only quantifies immunotherapy-related biomarkers but also enhances the prediction of therapeutic responses through scores like the immunoscore and immunophenoscore [53-55]. By effectively forecasting the probability of successful immunotherapy outcomes, AI is facilitating the development of more targeted and personalized approaches to cancer treatment.

4. Limitations of AI and what needs to be done

There are a few limitations to the use of AI, among them first one is related to trustworthiness. It has to do with the human mind's capacity to comprehend its intricate algorithms. The traditional or earlier ML models could be explained, but the more recent Deep ANNs-based DL models are quite intricate and the presence of multilayered neural networks with a substantial number of hidden layers for computation makes their algorithm hard to be explained by the human mind and have thus attained the moniker of "Black box models". Another limitation is related to the training of AI models such as DL-based models. Other than requiring a large amount of data, the quality of data should also be high. If the data is unclear, artefactual, or noisy, then it would be difficult to get accurate results. To compensate for this even larger amount of data is needed to reach a decent result [56]. Another issue is related to generalization and is quite prominent in a field where data varies a lot from location to location, between different healthcare centres and nations. The data should represent a real-world scenario so as to provide a better generalized result. Another problem related is data bias. The data may be bias based on ethnicity, age, and gender. For this large amount of data is needed, so as to make the model bias-free and generalized. Further proper validation is needed to utilize these models in the real-world scenario on a routinely basis [48, 57]. A major conceptual limitation of the AI model is based on histopathology and is related to computational pathology. As we know the H and E slides of IHC samples are acquired at the initial stage of reatment [51]. Immunotherapy is typically administered following two or three therapeutic modalities. Therefore, there is a few-month lag between initial treatments or regimens such as surgery, radiotherapy, and chemotherapy to immunotherapeutic treatment. The issue stems from data training, which is often carried out on initial histopathology samples, even if the tumor niche may have changed by the time immunotherapy is administered. One possible course of action is to collect samples at a later stage as well and switch immunotherapy to earlier treatment modalities.

In conclusion, immunotherapy has demonstrated remarkable promise in treating cancer, particularly in patients who have undergone two or more lines of conventional therapies and are at risk of recurrence. Immune checkpoint inhibitors and adoptive cell therapies have the potential not only to address primary tumors but also to target dispersed or disseminated malignant cells, leading to complete remission. However, challenges remain, including limited applicability to certain patient populations and high costs. As the saying goes, "Every problem has the seed of its own solution hidden within it." The vast data generated from routine procedures such as radiology and histopathology, combined with the complexity of cancer, presents a significant opportunity for training artificial intelligence models. These models can predict treatment responses and efficacy, allowing for more tailored therapies that benefit only those patients most likely to respond. This approach could significantly reduce the burden on healthcare systems and lower treatment costs.

This interdisciplinary collaboration has deepened our understanding of the tumor microenvironment, an area that has remained elusive despite extensive research. With advancements in immunomics and technologies like next-generation sequencing (NGS), omics, single-cell sequencing, and spatial transcriptomics, we are finally able to gain insights into this complex environment. AI plays a crucial role in integrating diverse data sources through its multilayered deep neural networks, enabling the extraction of relevant patterns and information. By combining these AI capabilities with spatial transcriptomics, we stand to enhance our

understanding of the cancer ecosystem, paving the way for the discovery of new therapeutic targets and improving predictions of immunotherapy responses and overall treatment efficacy in clinical settings.

As medical, analytical, and research technologies continue to advance alongside data collection methods and computer science, it is likely that AI and its deep learning subset will fundamentally transform cancer diagnosis and treatment.

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