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ADVANCES IN CANCER DIAGNOSIS: DIGITAL PATHOLOGY, BIOTECHNOLOGY, AND OMICS TECHNOLOGIES WITH AN EMPHASIS ON GLIOBLASTOMA

AVANÇOS NO DIAGNÓSTICO DO CÂNCER: PATOLOGIA DIGITAL, BIOTECNOLOGIA E TECNOLOGIAS ÔMICAS COM ÊNFASE EM GLIOBLASTOMA

AVANCES EN EL DIAGNÓSTICO DEL CÁNCER: PATOLOGÍA DIGITAL, BIOTECNOLOGÍA Y TECNOLOGÍAS ÓMICAS CON ÉNFASIS EN EL GLIOBLASTOMA

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ABSTRACT

Digital pathology emerges as a transformative innovation in the medical field, redefining traditional methods of analyzing and interpreting tissue samples. In this sense, based on the digitization of histological slides and the application of advanced image analysis technologies, this approach offers a new paradigm for diagnostic practice. This study was carried out as an integrative literature review. According to a predefined search strategy, six of the most common databases in the healthcare field were searched (i.e., MEDLINE Ovid (PubMed), Web of Science, CAPES, Biblioteca Virtual em Saúde (VHL), Google Scholar and Scopus). The following combination of keywords was used in the literature search: ("translational research" AND "digital pathology" AND "biotechnology") OR ("quantitative histomorphometry" AND "cancer") OR ("image analysis" AND "digital pathology " AND "cancer"). The insertion of artificial intelligence in digital pathology offers promising perspectives. Through sophisticated machine learning and image analysis algorithms, artificial intelligence can collaborate with pathologists to identify and analyze specific characteristics of samples, resulting in improved diagnostic efficiency and reduced time required for complex analyses. Thus, the integration of digital pathology with machine and deep learning emerges as a significant evolution in medical practice, providing tangible benefits in diagnostic effectiveness, accuracy and accessibility. In this context, the crucial importance of the synergy between technological advances and specialized medical expertise stands out, both in the development and improvement of algorithms and in the interpretation and application of results in clinical contexts.

KEYWORDS: *Cancer. Image analysis technologies. Machine learning. Whole Slide Imaging. Translational research.*

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RESUMO
A patologia digital surge como uma inovação transformadora na área médica, redefinindo os métodos tradicionais de análise e interpretação de amostras de tecido. Nesse sentido, baseada na digitalização de lâminas histológicas e na aplicação de tecnologias avançadas de análise de imagem, essa abordagem oferece um novo paradigma para a prática diagnóstica. Este estudo foi realizado como uma revisão integrativa da literatura. De acordo com uma estratégia de busca predefinida, foram pesquisadas seis das bases de dados mais comuns na área da saúde (MEDLINE Ovid (PubMed), Web of Science, CAPES, Biblioteca Virtual em Saúde (BVS), Google Scholar e Scopus). A seguinte combinação de palavras-chave foi utilizada na busca bibliográfica: ("pesquisa translacional" E "patologia digital" E "biotecnologia") OU ("histomorfometria quantitativa" E "câncer") OU ("análise de imagem" E "patologia digital" E "câncer"). A inserção da inteligência artificial na patologia digital oferece perspectivas promissoras. Por meio de sofisticados algoritmos de aprendizado de máquina e análise de imagens, a inteligência artificial pode colaborar com patologistas para identificar e analisar características específicas de amostras, resultando em maior eficiência diagnóstica e redução do tempo necessário para análises complexas. Assim, a integração da patologia digital com o aprendizado de máquina e o aprendizado profundo surge como uma evolução significativa na prática médica, proporcionando benefícios tangíveis em termos de eficácia diagnóstica, precisão e acessibilidade. Nesse contexto, destaca-se a importância crucial da sinergia entre os avanços tecnológicos e a expertise médica especializada, tanto no desenvolvimento e aprimoramento de algoritmos quanto na interpretação e aplicação dos resultados em contextos clínicos.

PALAVRAS-CHAVE: Câncer. Tecnologias de análise de imagem. Aprendizado de máquina. Imagem de lâmina inteira. Pesquisa translacional.

RESUMEN
La patología digital emerge como una innovación transformadora en el campo médico, redefiniendo los métodos tradicionales de análisis e interpretación de muestras de tejido. En este sentido, basada en la digitalización de preparaciones histológicas y la aplicación de tecnologías avanzadas de análisis de imágenes, este enfoque ofrece un nuevo paradigma para la práctica diagnóstica. Este estudio se llevó a cabo como una revisión integradora de la literatura. De acuerdo con una estrategia de búsqueda predefinida, se consultaron seis de las bases de datos más comunes en el campo de la salud (es decir, MEDLINE Ovid (PubMed), Web of Science, CAPES, Biblioteca Virtual em Saúde (VHL), Google Scholar y Scopus). Se utilizó la siguiente combinación de palabras clave en la búsqueda bibliográfica: ("investigación traslacional" Y "patología digital" Y "biotecnología") O ("histomorfometría cuantitativa" Y "cáncer") O ("análisis de imágenes" Y "patología digital" Y "cáncer"). La incorporación de la inteligencia artificial en la patología digital ofrece perspectivas prometedoras. Mediante sofisticados algoritmos de aprendizaje automático y análisis de imágenes, la inteligencia artificial puede colaborar con los patólogos para identificar y analizar características específicas de las muestras, lo que se traduce en una mayor eficiencia diagnóstica y una reducción del tiempo necesario para análisis complejos. De este modo, la integración de la patología digital con el aprendizaje automático y el aprendizaje profundo se presenta como una evolución significativa en la práctica médica, aportando beneficios tangibles en cuanto a eficacia, precisión y accesibilidad diagnósticas. En este contexto, destaca la importancia crucial de la sinergia entre los avances tecnológicos y la experiencia médica especializada, tanto en el desarrollo y la mejora de algoritmos como en la interpretación y aplicación de los resultados en contextos clínicos.

PALABRAS CLAVE*: Cáncer. Tecnologías de análisis de imágenes. Aprendizaje automático. Imagen de portaobjetos completo. Investigación traslacional*

1. INTRODUCTION

In pathology, the detailed examination of structural, biochemical and functional changes in cells constitutes the essence of this specialty, essential for the study and understanding of diseases. To this

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end, histological and cytological images of organs potentially affected by diseases, such as cancer, are analyzed. After the technical processing of these samples, pathologists are able to examine these structures in detail, contributing to more accurate diagnoses and guiding more effective clinical management (Bankhead, 2022).

Currently, most images studied are slide scans in RGB format - this means that each pixel is made up of three numbers, which together represent the red, green and blue components. In this way, a typical full-slide image can be considered as a Width x Height x 3 matrix; the former often exceed 100,000 pixels each - generating raw data comprising billions of numbers. Therefore, there is great effort in analyzing and identifying gigapixels, increasing the complexity of the process by considering biological, processing and scanning variations (Bankhead, 2022).

Digital pathology refers to the scanning of an entire slide of tissue with a slide scanner that captures partial images of the field of view, at high magnification - usually 20× or 40× - and stitches them together to construct a gigapixel image called a Whole Slide Image (WSI) or digital slides, which are viewed on computer monitors. As a result, the slides become easier to store, distribute and study, promoting easier sharing of cases, standardization of teaching and extraction of complex data in a reproducible way (Aeffner *et al.*, 2019; Klein *et al.*, 2021).

Given this scenario, advances in computer hardware and software speed up the digitization of glass slides, allowing the acquisition of images of entire slides more quickly and efficiently. Improvements in image analysis algorithms have made it possible to extract quantitative and objective features from large WSI files. Currently, digital pathology can provide specific analysis of different cellular components in tumor specimens, aiding in the classification of tumors, while image analysis algorithms can accurately define morphological and nuclear features, as well as determine tissue interfaces, allowing detailed analysis and comprehensiveness of the samples (Barsoum *et al.*, 2019).

Likewise, the use of an automated system for analyzing digitized images can potentially remove the subjective factor from professionals' analyses, turning qualitative factors (such as satellitosis, degree of differentiation, presence of specific cells) into quantitative ones, speeding up the performance of tasks repetitive procedures and increase diagnostic accuracy. In recent years, attempts have been made to use machine learning algorithms and artificial intelligence (AI) methods for this purpose (Klein *et al.*, 2021). In this context, it was possible to use convolutional neural networks with great success, an AI algorithm from the family of deep learning algorithms based on artificial neural networks designed for visual tasks. Since then, the convolutional neural network model has been successful in studies in different medical areas that work directly with images, making it important, therefore, to understand the bases behind these algorithms (Krizhevsky *et al.*, 2017).

Along the same lines, it is worth commenting on the main tasks that can be attributed to machine learning. Among them, the following stand out: classification, when dealing with discrete variables; regression, when dealing with continuous variables; grouping, which seeks to evaluate sampling

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distribution patterns; and dimensional reduction, which aims to represent data in smaller dimensions. To build an automatic image analysis system, you must initially have a set of images that are analyzed and classified by experts (as benign or malignant) and are then used as examples for learning the algorithm. Next, the characteristics of the images must be quantified. You can, for example, quantify colors, textures and positions of each pixel. Therefore, the characteristics are associated with the classification of the images and the algorithm is once again subjected to this data, so that it learns to automatically classify the images according to their characteristics (Klein *et al.*, 2021).

One of the main obstacles to digital pathology is the scarcity of robust and freely accessible histological image banks. In this sense, similar to humans, Convolutional Neural Networks (CNN) need to be trained to be able to differentiate healthy from malignant tissues. To overcome this limitation, different deep neural network training approaches were evaluated, such as training from scratch and transfer learning, demonstrating that deep learning provided better results compared to the manual method. Thus, transfer learning in the analysis of histopathological images, compared to training from scratch, was able to analyze WSIs in much less time (0.01 faster), without loss of accuracy, indicating that this strategy can be used to circumvent such obstacle (Abdolahi *et al.*, 2020).

Another challenge in digital pathology is the size of images, often exceeding 10 GB, which makes training computationally impractical. In this sense, approaches to deal with this include severe down sampling and division into patches for training and aggregation - however, both approaches result in loss of information, whether in fine details or in the arbitrariness of patch separation. Thus, the need to move beyond patch-based methods in digital pathology stands out (Caie *et al.*, 2020).

Given the above, the integration of artificial intelligence in pathology reveals a range of significant benefits, consolidating digital pathology as a unique promise for contemporary medicine. In this scenario, the digitalization of pathological data and the refinement of diagnostic reproducibility emerge as key elements, with the potential to revolutionize medical practice, enabling more effective detection and treatment of diseases, such as cancer. It is essential, therefore, to make a continuous investment in research in this area, combined with a collective collaboration of the academic community to overcome the challenges associated with the dissemination of images and the training of algorithms, to effectively take advantage of the fruits of this innovation.

2. METHODOLOGY

This study was carried out as an integrative literature review. The review was guided by the following research question: how can digital pathology, artificial intelligence, quantitative histomorphometry, and biotechnological approaches contribute to cancer diagnosis? According to a predefined search strategy, six of the most common databases in the healthcare field were searched (i.e., MEDLINE Ovid (PubMed), Web of Science, CAPES, Biblioteca Virtual em Saúde (VHL), Google

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Scholar and Scopus). The following combination of keywords was used in the literature search: ("translational research" AND "digital pathology" AND “biotechnology”) OR ("quantitative histomorphometry" AND “cancer”) OR ("image analysis" AND "digital pathology " AND “cancer”). Studies published in English, Portuguese, or Spanish were considered eligible. The inclusion criteria comprised studies addressing digital pathology, whole-slide imaging, artificial intelligence, machine learning, deep learning, quantitative histomorphometry, biotechnology, omics technologies, and cancer diagnosis. Studies unrelated to oncological diagnosis, duplicate records, articles without full-text availability, and publications not aligned with the guiding question were excluded. After duplicate removal, titles and abstracts were screened, followed by full-text reading of potentially eligible studies. In total, 344 articles were identified in the six databases, and 68 studies were included after screening. Data were extracted and analyzed narratively, then organized into thematic categories related to digital pathology, artificial intelligence-based image analysis, histomorphometry, biotechnological approaches, omics technologies, and translational challenges.

3. RESULTS AND DISCUSSION

Digital pathology (DP) has been defined as “a dynamic image-based environment that enables the acquisition, management, and interpretation of pathological information obtained from a glass slide” (Digital Pathology Association). By moving towards a digital workflow, using computer screens to analyze digitized histological slides, qualitative and quantitative analysis of the expression, patterns and phenotypes of biomarkers in complex tissues has been possible, resulting in significant progress in computerized tissue analysis.

This evolution is evident when image analysis and machine learning algorithms are employed to automatically identify regions of interest in tissues, segment individual cells or anatomical features, and classify these features based on biomarker expression levels and location (Aeffner *et al.*, 2019). Furthermore, these approaches have proven to be valuable in the field of immuno-oncology, allowing the decryption of the complex pathophysiology of cancer and the discovery of new biomarkers and therapeutic targets (Annaratone *et al.*, 2021; Baxi *et al.*, 2022).

Furthermore, they have made it possible to combine the analysis of digital histopathological images with artificial intelligence techniques, resulting in a more accurate and consistent assessment, overcoming the limitations of manual assessment. The integration of digital imaging into pathology expands the capabilities of the pathologist by enabling detailed identification and quantification of cellular and molecular features. This, in turn, could lead to the development of new diagnostic algorithms and prediction models. Despite regulatory challenges to be faced, computer-aided analysis and digital pathology are quickly becoming essential tools in clinical decision-making (Frascarelli *et al.*, 2023; Moscalu *et al.*, 2023). In view of this, the strategic application of traditional and modern pathological

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techniques, together with the collaboration of specialized pathologists, is essential for the success of translational research, ensuring the obtaining of robust and reproducible results, driving advances in medicine. This recognition highlights the crucial role of knowledge integration in the development of medicines and devices, as well as in understanding diseases and identifying environmental and health risks (Asiimwe *et al.*, 2021; Brayton *et al.*, 2018).

In this way, pathologists are encouraged to actively engage in reverse translational research, filling mechanistic gaps and addressing clinical questions. Although there are obstacles to overcome, such as ambiguous definitions, pathologists' mindsets, and scarcity of resources, collaboration and support can expand the research community and drive innovation. One of the ways to significantly advance is the use of computational, genetic/molecular and digital approaches (L. Zhang, 2022).

One of the techniques currently used is image digitization, which, despite being in its initial phase compared to Radiology, has considerable potential. Recent advances in techniques such as microcomputed tomography and high-resolution magnetic resonance imaging are expanding the capabilities of computer-assisted diagnosis (Ramamurthy *et al.*, 2015).

Regarding benefits in cancer diagnosis, the introduction of artificial intelligence (AI) into breast cancer pathology holds promise for improving diagnostic reproducibility, providing more accurate prognoses, and facilitating the practice of personalized medicine. As a result, advances in AI and the digitization of pathology data are fundamentally altering the approach to detecting and treating breast cancer. It is noteworthy that multiplexed imaging technologies offer a more comprehensive analysis of cellular subpopulations in the tumor microenvironment, boosting translational research and therapeutic development.

This occurs through a multiparametric assessment of immune cells and tumor-immune interactions, unlike traditional immunohistochemistry, which allows the detection of only one cellular marker at a time (Rojas *et al.*, 2022). However, there are technological challenges, such as algorithm training, as most current AI algorithms are developed based on small-scale data and images from a single implementation center. Therefore, to guarantee quality and rigorous standardization, it is necessary to develop large databases and data repositories. *Whole Slide Image* (WSI) annotated, validated, corrected and updated by a team of specialist pathologists (Hamilton *et al.*, 2014; Robertson *et al.*, 2018).

Regarding the benefits of WSI technology in renal pathology, it stands out especially the ability to correlate morphological data with molecular and genetic signatures. This integration represents a milestone in assessment and education, overcoming the limitations of conventional optical microscopy. Although not yet routinely used, digital pathology is already proving to be a powerful tool for translational research, clinical trials, and education in renal pathology, especially in genomic and proteomic contexts (Nast *et al.*, 2015; Salto-Tellez *et al.*, 2014).

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Studies like those of Kumar *et al.*, 2020 and Verghese *et al.*, 2023 highlight broad potential in present and future applications of WSI, although it still faces limitations and challenges. Its integration into clinical practice is faced with a series of operational, technical, regulatory, ethical and financial challenges, in addition to professional resistance to its adoption. Such obstacles include the demand for infrastructure for scanning and image management, lack of uniformity in data collection, patient privacy considerations, and substantial implementation costs. Despite these challenges, it becomes the potential of computational pathology to improve diagnosis, prognosis and identification of prognostic biomarkers is evident (Kumar *et al.*, 2020).

Furthermore, with the aim of trying to minimize errors caused by the manual process, which is a subjective, laborious and error-prone process, HistoQC was developed, an open-source quality control (QC) application that automates the identification of artifacts and regions unsuitable for computational analysis. HistoQC uses image metrics and supervised classifiers to detect technical artifacts and areas affected by batch effects, enabling accurate assessment of slide quality. Compared to manual QC, HistoQC has demonstrated high agreement and can be a valuable tool for improving the repeatability and robustness of PD workflows(Janowczyk *et al.*, 2019).

Finally, PD symbolizes the evolution of conventional pathology into the digital landscape, enabling the digitization of tissue samples for analysis and diagnosis. The integration of clinical, laboratory and radiological data with pathological images, through AI, is recognized as the future direction of diagnosis. Contrary to what many may think, PD does not aim to replace pathologists, but rather to enable them to make more accurate and consistent diagnoses (Barsoum *et al.*, 2019; Gupta *et al.*, 2023).

Continued advances in computing power, data storage, and network connectivity promise to pave the way for more innovative applications, setting the stage for the integration of these technologies into clinical practice soon. Therefore, the introduction of technology in pathology offers several advantages, such as remote access, space savings and preservation of slide quality. Despite the challenges, digital pathology is considered fundamental to the future of precision medicine, offering the potential for better understanding of the pathogenesis, progression and treatment response of diseases (Gupta *et al.*, 2023; Sadimin & Foran, 2012).

Quantitative histomorphometry and cancer

The increase in technologies allows the improvement of techniques such as digital microscopy which, in turn, accelerate the computerized processing of slides histological findings capable of be viewed in high definition (Acs and Hartman, 2020). In this trend, Madabhushi and Lee (Madabhushi and Lee, 2016) highlight the challenges and opportunities in using image analysis and machine learning in digital pathology (*machine learning* - ML - and *deep learning* - DL), an area that has benefited considerably from the advancement of artificial intelligence (AI). Further analysis delves into the

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prominent value of medical imaging informatics in personalized medicine, arguing that integrating imaging data with clinical and genetic information can lead to more accurate diagnoses and personalized treatments (Madabhushi and Lee, 2016).

In the context of this analysis, it is necessary to understand that the Quantitative histomorphometry technique is based on the analysis of images of tissues or tumor cells, to mathematically and biologically investigate morphological aspects relating to the shape, size and texture of the composition of cells and tissues (Banerji and Mitra, 2022).

This approach uses the fields of morphometry, histomorphometry and cytomorphometry, prominent in the field of digital pathology, by offering robust quantitative methods for tumor analysis. Nuclear morphometric studies are of fundamental importance for investigating tumor types and classifying them (Di Donato *et al.*, 2017).

When considering applications of morphometrics, (Nirschl *et al.*, 2017) highlights the use of deep learning in tissue segmentation in cardiac histopathological images, demonstrating how AI can identify complex tissue features that are challenging for manual analysis. This study complements the work of Penzias *et al.* (2018); Veltri *et al.* (2014), that investigate the morphological basis for characteristics radiomics in distinguishing prostate cancer grades and the application of histomorphometry in digital pathology, respectively.

The use of techniques powered by ML, DL and AI can facilitate the identification of patterns, previously invisible to the human eye, to begin to form a pillar of more accurate diagnoses and the discovery of relevant biological markers. Meanwhile, new histological and histomorphometric methods become accessible for the evaluation of implantable medical devices, illustrating how advanced image analysis techniques can improve the understanding of the mechanisms of integration and tissue response to the implant (Maglio *et al.*, 2020). Otherwise, studies of Monson *et al.* (2013); Tchanque-Fossuo *et al.* (2011) indicate that morphometry also provides cellular analyzes of the effects of radiation in murine models, contributing to the understanding of tissue responses.

In accordance with the studies cited, research such as that by Bean *et al.* (2018) point to the use of techniques from the field of digital pathology to examine subjective issues regarding the variability of adipocyte size in benign and malignant lipomatous tumors. The investigation of these images with the help of machines is facilitated, allowing more precise information for diagnoses to be determined. However, studies highlight the performance of DL AIs trained to classify types of cancer based on histomorphometric particularities of hematoxylin and eosin (H&E) slides, a staining technique for histological slides widely used in research (Acs and Hartman, 2020).

In line, according to Veltri *et al.* (2014) Waliszewski (2016) The use of digital histomorphometry also makes it possible to identify low and highly aggressive prostate carcinomas using quantitative criteria based on fractal dimensions, entropy and lacunarity. These advanced techniques enable more accurate and objective analysis of the distribution patterns of cancerous cell nuclei. In following the

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nuclear assessments, Whitney *et al.* (2018) expand the application of quantitative nuclear histomorphometry beyond prostate cancer, showing how it can predict Oncotype DX risk categories for early-stage ER+ breast cancer, offering a valuable tool in therapeutic decision-making. Likewise, preliminary findings on medulloblastomas indicate that nuclear histomorphometry can predict molecular subtypes and clinical outcomes, reinforcing the potential of this technique in personalizing oncological treatment (Whitney *et al.*, 2018).

Still in the light of histomorphometric research in the field of oncology, the investigation of A. Beltran-Bless *et al.*, (2021) opens the door to improving less invasive techniques for investigating patients with metastatic breast cancer. This technique was made possible by the combination of a less aggressive (Fralick *et al.*, 2012) bone biopsy step and subsequent analysis of the samples through investigation by histomorphometry of the extent of tumor invasion, as well as inference of bone renewal and formation parameters. From this perspective, Fralick *et al.* (2012) also tested the technique of using a 2 mm bone marrow trephine when searching for information regarding the stage of metastatic breast cancer in patients.

From the Ji *et al.* (2019) proposed research seeking to evaluate whether the computerized description by an image classifier based on morphometry (NGAHIC) could infer nuclear shape, texture and architecture consistent with reality and, therefore, predict the recurrence of gastric adenocarcinoma with negative intestinal nodule in investigated patients. In their examinations, the authors assess that computerized analysis can be an ally of precision medicine, especially in the oncology area.

It should be noted that bioimage analysis is a complex process that requires a lot of attention from specialist professionals. The diagnosis of pathologies through the help of ML tools supports a careful analysis of the information that the tissue sample must demonstrate. Therefore, the automation of this technique can prevent the loss of important information that is difficult to discern visually (Banerji and Mitra, 2022). Therefore, it is inferred that the improvement of ML can favor the identification of characteristics of digital slides, supporting routine diagnostic analyses by specialists (Acs and Hartman, 2020).

The study of nuclear morphometry and nucleomics in understanding prostate cancer progression emphasizes how variations in nuclear characteristics can be indicative of tumor aggressiveness (Roudier *et al.*, 2008; Veltri *et al.*, 2012). In this way, what is observed is a digital future of pathology, based on the digitization of histological slides and the application of AI, to improve efficiency and diagnostic accuracy (Pallua *et al.*, 2020).

Digital pathology, image analysis and cancer

The use of analysis of imaging to quantify histopathological features or known immunohistochemical results is a potential practice. In that regard, it is used to adjust the definition of color or fluorescence intensity thresholds, along with classifications based on the shape or texture of

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the object. Thus, this approach allows the quantification of characteristics such as lymphovascular density and protein expression, enabling a more personalized analysis through machine learning (Caie *et al.*, 2020).

Machine learning models can be trained according to four distinct paradigms. The first of these is called supervised learning, in which the machine maps an input based on an output, taking other input-output pairs as an example. To do this, labeled data and a collection of “training examples” are used. The second is unsupervised learning, which seeks to find patterns in unlabeled data without the need for human interference. The third is called semi-supervised learning, which evaluates both labeled and unlabeled data, seeking to be a complement to the last two models mentioned, especially in situations in which labeled data is of low quantity or quality. Finally, reinforcement learning is a model based on the reward and penalty system that seeks to train machines to perform actions in the most optimized way and is little used in computer image analysis (Sarker, 2021).

Table 1. Summary of articles in the literature that demonstrated applications of AI algorithms in Digital Pathology

Reference	Applications for AI Algorithms in Digital Pathology
(Abels *et al.*, 2019)	Identification of tumor cells, counting mitoses, improving the accuracy of immunohistochemistry, applying standardized histological scoring criteria (e.g. Gleason Criteria), measuring areas, cells and objects in tissues.
(Aeffner *et al.*, 2019)	Use of algorithms to evaluate events or objects in sections of tissue, including those that may not be composed of individual cells.
(Caie *et al.*, 2020)	Use of learning algorithms, such as random forest, to automatically segment images, allowing the quantification of specific markers, such as Ki67, in specific areas, such as the tumor.
(Klein *et al.*, 2021)	Application of Convolutional Neural Networks (CNN) and other machine learning models to assist in the accurate diagnosis of patients.
(Abdolahi *et al.*, 2020)	Use of transfer learning in the analysis of histopathological images for faster and more accurate analyses, overcoming the scarcity of robust and freely accessible image banks.
(Md Zahangir Alom *et al.*, 2019)	Application of improved Deep Convolutional Neural Networks (DCNN) models for classification, segmentation and detection of pathological images, achieving promising results.
(Amerikanos and Maglogiannis, 2022)	Development of deep learning frameworks for detecting regions of interest (ROIs) in Whole Slide Images (WSIs), improving diagnostic accuracy and

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	efficiency.
(Bankhead, 2022)	Highlighting the importance of Free and Open Source Software in modern science, citing ImageJ, 3D Slicer, CellProfiler, QuPath and Cytomine as important tools for biomedical image analysis and digital pathology.
(Bankhead, 2022; Bankhead *et al.*, 2017; Humphries *et al.*, 2021)	Development of QuPath, an open-source platform for tissue-based image analysis, offering an easy-to-use interface and enabling automatic object detection in large images.

The main applications of cytological analysis, a cornerstone of digital pathology, include the identification of individual cells, their nuclei and other microscopic structures. Cell segmentation, fundamental in this process, can be challenging due to overlapping cells, which can lead to errors. Algorithms that use thresholding, segmentation *watershed* and active contour models are commonly employed to facilitate this analysis. Furthermore, features such as nuclear area and shape have independent prognostic value and can be quantified through image analysis. Other cellular features, such as texture and automated mitosis detection, can also be assessed to characterize tumors and predict clinical outcomes (Aeffner *et al.*, 2019).

Digital pathology is expanding to evaluate the tumor microenvironment in its entirety. With the advancement of WSI-based scanning methods, it is possible to map the spatial context of normal cells interacting with cancer cells, examining the complex intra-tumor network and its relationships with the immune and vascular systems. Although this article does not focus on this aspect, microenvironmental organization plays a crucial role in the evolution of cancer, influencing its resistance to treatment and development. Therefore, more studies using digital pathology in this context are essential for advances in oncological treatment in a translational manner (Heindl *et al.*, 2015).

Table 2. Summary of studies presented in the literature that demonstrated successful applications of AI algorithms in the analysis of histological cancer sections. Each study is tracked by the method used, the specific application of the algorithm, and the accuracy achieved when available

Application	Method	Precision	Author(s)
Classification of bone tumors	Deep Learning	Like experienced pathologists	(Tao *et al.*, 2021)
Prediction of metastasis in breast cancer	Deep Learning	-	(Xu *et al.*, 2021)
Accurate detection of invasive breast cancer	CNN mosaic classifier	76%	(Cruz-Roa *et al.*, 2018)
Indication or exclusion of	Rede Neural	89,8%	(S. Wang, Yang, *et*

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malignancy in lung biopsies	Convolucional (RNC)		*al.*, 2019)
Differentiation between stromal cells, tumor cells and lymphocytes	Rede Neural Convolucional (RNC)	-	(S. Wang, Wang, *et al.*, 2019)
Analysis of tumor angiogenesis	Automatic segmentation system	-	(Yi *et al.*, 2018)
Distinction between tumoral and normal epithelium in colorectal cancer	Slide gridding and resampling	79%	(Broad *et al.*, 2022)

In the field of tumor classification, an analytical approach has been devised that extracts features from segmented regions and uses a classifier to diagnose each representative tile. The method, used in cases of brain cancer, achieved an accuracy of 93.1% and demonstrated important stability and robustness, with an accuracy ranging from 95.5-100%. In this sense, they combined three components: (i) coarse profiling to sample tissue diversity, (ii) automatic identification of representative regions and (iii) allowing all representative regions to independently impact the final diagnosis, achieving good results in automatic differentiation. between brain subtypes: low-grade glioma and glioblastoma. Thus, the use of computer-aided diagnosis in digital pathology images can improve histopathological identification of glioma subtypes, reducing inter-reader variability and improving pathologists' workflow (Barker *et al.*, 2016).

The applications of digital pathology go beyond histopathological analysis. In this sense, the comparison of algorithms for identifying HER2 markers in breast cancer slides with the analysis of trained pathologists demonstrated that computer analysis was not inferior to human analysis [61]. By way of further examples, there are algorithms capable of quantifying lymphocytic invasion in testicular cancer and then associating it with the prediction of outcomes; improving the determination of the prognosis of colorectal cancer through the analysis of histological sections and analyze and quantify the presence of PD-L1 receptors (Bychkov *et al.*, 2018; Kapil *et al.*, 2018; Linder *et al.*, 2019; Mobadersany *et al.*, 2018; Ning *et al.*, 2020; Pell *et al.*, 2019; Schneider *et al.*, 2022).Therefore, this branch of medicine promises to improve the speed and accuracy of disease diagnosis, as it can detect such biomarkers, positively influencing the treatment strategy in different types of cancer (Abels *et al.*, 2019; Aeffner *et al.*, 2019).

Another possible application of the algorithms *deep learning* is the genomic analysis associated with the image. Although this task is complex due to the need for a greater number of pre processing steps and a greater number of data to ensure the association between microscopy and the genomic profile of the sample, it is an important tool for automated prediction of mortality (Schneider *et al.*, 2022).

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To exemplify, Ning *et al.* (2020) associated microscopy of clear cell renal carcinomas with the corresponding RNA sequencing of each sample, through a convolutional neural network, to predict cancer-specific survival.

The results were superior to the RNC trained only with the analysis of RNA from each sample but were considered comparable to the RNC trained to analyze only the microscopies. (Mobadersany *et al.* (2018) designed an RNC algorithm to analyze low-grade gliomas and glioblastomas by associating their images with previous data on the presence of isocitrate dehydrogenase mutation or codeletion of chromosomes 1p and 19q. This new approach promoted an increase in the agreement index, which evaluates how much the survival prediction coincided with what was expected for that stage of cancer, from 0.75 to 0.8 compared to an algorithm that analyzed the images alone. Finally, a pan-cancer model was developed to predict survival in 20 different types of malignancies. Using unsupervised learning, the model analyzed slides and their respective RNA sequencing, reaching a C-index of 0.78 - higher compared to the model that analyzed RNA sequencing alone, whose C-index was 0.60) (Ning *et al.*, 2020).

Furthermore, radiohistomics (integration between radiology and histology) is an emerging field that combines radiological imaging with digital pathological imaging, genomics and clinical data for a more complete understanding and more effective treatments of cancer. Recent studies have shown correlations between magnetic resonance imaging parameters and distinct histological features, differentiating malignant tissue from normal. In addition, advanced radiology image analysis techniques can be applied to pathological scanning, allowing a more comprehensive and integrated analysis of the disease(Barsoum *et al.*, 2019).

Furthermore, genomic analysis can also be used to predict malignancy and identify cancer subtypes (Schneider *et al.*, 2022). He *et al.* (2020) developed a breast cancer analysis system that was trained to correlate morphological findings with genes most expressed in each aspect of the image. Without retraining based on new data, the algorithm was able to satisfactorily differentiate and predict breast cancer subtypes (invasive ductal carcinoma and invasive lobular carcinoma) when subjected to new clinical data. For prostate cancer, there is an algorithm capable of associating slides stained by H&E and the spatial expression of genes. The model demonstrates an accuracy of 0.91 in classifying malignant tumors versus benign tumors (the model that did not use imaging showed an accuracy of just 0.60) (Bae *et al.*, 2021).

To avoid errors that threaten the validity of studies, standardization of the pre-analytical and analytical phases is crucial to ensure the quality and consistency of data in digital pathology. Currently, criteria for ensuring quality control of histological preparations allow for a wide variation in artifacts between laboratories. These artifacts vary in severity and quality, affecting everything from the glass slides to the scanning process. Thus, heterogeneity in image presentation represents a challenge for computational analysis algorithms, which must be trained to deal with this variation. In this sense, strict

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quality controls and minimum processing criteria are necessary, based on current technologies and applications. For example, the standardization of color representation is essential to ensure robust and reproducible quantitative measurements, with computational tools such as *HistoQC*, combined with the automatic assessment of image quality, avoiding the systematization of errors. Finally, standardization of the post-analytical phase is also essential to improve precision and agreement between pathologists, facilitating the harmonization of data collection and the definition of pathological lesions through a controlled vocabulary. Therefore, such initiatives are essential to boost clinical research and enable the effective use of AI algorithms in digital pathology (Barisoni *et al.*, 2020).

Despite efforts to ensure safety and feasibility, large-scale implementation of this tool faces technical, logistical and financial challenges. In this vein, the field of pathology is undergoing a significant transformation with the introduction of digital pathology, as the integration of AI into diagnostics challenges human assessment as the gold standard. While this technology allows for real-time sharing of images and videos (telepathology) and the application of advanced computational analysis, such a transition faces challenges such as investments in infrastructure, regulations, occupational health and changes in the profession (Dawson, 2022; Jahn *et al.*, 2020).

Implementation of WSIs for routine pathological diagnosis is challenging and requires careful validation of equipment and software, as recommended by the guidelines of the *College of American Pathologists* (CAP). In this sense, the comparison of Glass slides with digital images and the guarantee of accurate calibration of monitors for color reproduction, which must be stored on secure servers, preferably using the DICOM standard to guarantee interoperability, stand out. Finally, it is worth pointing out that WSI scanners are authorized for use in the European Union and the United States, with different regulatory requirements. While in the EU, devices are approved under the In Vitro Diagnostic Medical Devices Directive, in the US they require specific approval from the FDA, with only two platforms being approved: Pathology Solution *Digital IntelliSite* from Philips and the DP Module from Sectra (Dawson, 2022; Jahn *et al.*, 2020).

Yet, it is necessary to consider that factors such as costs, shortage of skilled workers and regulatory considerations influence human replacement by automation. In pathology, technical advances and quality improvements are driving the introduction of AI into everyday life, making some tasks simpler, such as counting mitoses, to leave pathologists with more complex tasks. Therefore, even with automation, medical responsibility will continue to be human. Therefore, it is expected that the implementation of digital pathology, linked to AI tools, will make the work of pathologists more demanding, but also more dynamic (Jahn *et al.*, 2020).

New technologies for the study of glioblastomas

High-throughput technologies have revolutionized the study of gliomas. Tools such as Next Generation Sequencing (NGS), DNA Microarrays, and CRISPR-Cas9 gene editing allow for detailed

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analysis of the glioma genome by identifying key mutations, gene expression patterns, and protein changes that can influence prognosis and outcome. response to treatment. Furthermore, techniques such as flow cytometry and organoids have modeled cellular interaction within the tumor microenvironment (TME) and facilitated the development of more effective therapies (Wijethilake *et al.*, 2021). These cutting-edge technologies (Table 1) have not only improved understanding of gliomas but marked a breakthrough in the fight against this aggressive form of brain cancer.

Table 3. High-performance technologies and their advantages

High Performance Technology	Applications in the Study of Gliomas	Advantages	Reference
NGS	Genotyping, identification of mutations, gene expression.	Detailed analysis of the genome and transcriptome, identifying genetic changes specific to gliomas.	(Jovčevska, 2018)
Microarrays de DNA	Gene expression analysis, CGH (Comparative Genomic Hybridization).	Large-scale profiling of gene expression and genomic variations, identifying prognostic patterns.	(DeLay *et al.*, 2012)
Flow Cytometry	Analysis of cell populations, identification of tumor cells.	Detailed phenotypic characterization of cells in the tumor, including cancer stem cells.	(Vartholomatos *et al.*, 2021)

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CRISPR-Cas9	Gene editing for functional studies.	Targeted modification of genes to investigate their function in gliomas.	(Kang *et al.*, 2023)
High Throughput Immunohistochemistry	Analysis of protein expression in tissues.	Visualization and quantification of specific proteins in the tumor context.	(Orzan *et al.*, 2020)
Organoids	3D tumor modeling, cellular interaction studies and response to treatments.	Physiologically relevant system for studying tumors, simulating the tumor microenvironment.	(C. Zhang *et al.*, 2020)
Mass Spectrometry-Based Proteomics	Detailed analysis of the protein profile and signaling pathways in gliomas.	Allows a deep understanding of the protein composition and functional changes in tumor cells.	(Hutson *et al.*, 2024)
Single Cell RNA Sequencing (scRNA-seq)	Gene expression analysis at the single cell level.	Reveals cellular heterogeneity within gliomas, improving understanding of tumor dynamics	(Hernández Martínez *et al.*, 2022)

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	and resistance to therapy.

Next Generation Sequencing (NGS)

In the field of oncology, genetic sequencing manifests itself through three main strategies: complete genome sequencing, which maps all the DNA present in a sample; exome sequencing, which is dedicated to the segments of the genome responsible for protein coding; and the analysis focused on specific regions of the genes, prioritizing areas with a high incidence of mutations, known as "hotspots".

These approaches seek to discover therapeutic targets on a genomic and transcriptomic scale to help accurately classify tumors and project outcomes (Bewicke-Copley *et al.*, 2019).The complexity of tumors, characterized by the diversity of their genetic mutations and rearrangements, such as translocations and copy number changes, constitutes an obstacle. NGS emerges as a promising method, accompanied by specialized computational tools, allowing the distinction between hereditary and somatic mutations when analyzing genomes of tumors and normal tissues together (Gray *et al.*, 2015). However, processing and analyzing the extensive data generated by NGS poses a challenge due to its storage, transmission, and manipulation demands (Eck, 2018).

Zacher *et al.* (2017) carried out a retrospective analysis of 121 gliomas using a genetic panel that allowed the molecular classification of tumors into distinct biological groups. Findings include astrocytic gliomas with mutations in genes *IDH1 or IDH2*, often accompanied by mutations in genes *ATRX e TP53;* tumors oligodendrogliomatosos with mutation *IDH*, 1p/19q codeletion, mutation in the TERT promoter and frequent mutations in the gene *CIC;* e glioblastomas *IDH* wild type with frequent mutations in the promoter *TERT,* mutation in *PTEN* and/or amplification in *EGFR.* The findings suggest that gene panel sequencing offers a promising diagnostic technique that may facilitate integrated histological and molecular classification of gliomas.

Another valuable approach is small-scale sequencing (Small-seq), which has highlighted the heterogeneity of glioblastoma (Smith and Hutvagner, 2022). By analyzing diverse cells, including embryonic stem cells and different types of cancer, it was identified that tumor cells group into mixed clusters due to their cellular diversity and varied miRNA profiles (Smith & Hutvagner, 2022). This phenomenon contrasts with other cell types studied, which tend to group together more homogeneously. Furthermore, unique expression patterns and isomeric variants of microRNAs (isomiRs) have been discovered, providing new insights into the biology of glioblastoma (Smith and Hutvagner, 2022).

Further analysis of isomiRs revealed significant differences between cell types, highlighting a high similarity between embryonic stem cells and glioblastoma (Smith & Hutvagner, 2022).. These findings may open new directions for the development of targeted therapies and specific biomarkers for GBM (Smith and Hutvagner, 2022). Furthermore, the identification of long non-coding RNAs (lncRNAs), such as MALAT1, has been crucial to understanding tumor progression, especially its relationship with

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ERK/MAPK signaling and the pluripotency of GSCs (Hazra *et al.*, 2023). In-depth study of these lncRNAs may lead to the identification of new therapeutic targets and biomarkers (Hazra *et al.*, 2023).

The adoption of cutting-edge sequencing in glioblastoma diagnosis is expanding, with experimentation with genetic panels such as GlioSeq (Nikiforova *et al.*, 2016), which include frequently mutated genes, in different types of tumor samples, from fresh tissues to samples fixed in formalin and paraffin. This innovative approach is expanding understanding of new-age glioblastomas, promoting a more accurate clinical definition and considerable potential for elevating the quality of patient care (Jovčevska, 2018). In this context, GlioSeq technology was designed to address the challenge of accurately diagnosing and classifying gliomas, covering an extensive list of genetic alterations relevant to both adult and pediatric patients (Nikiforova *et al.*, 2016).

The panel is effective in detecting key genetic events, such as gene fusions (*BRAF com KIAA1549 or FAM131B*) and the mutation *BRAF* V600E, which are indicative of astrocitomas pilocytic. In children, the mutation in *BRAF,* especially when it occurs simultaneously with the deletion of *CDKN2A*, increases concern about the development of more aggressive tumors (Nahar Metu *et al.*, 2023). The mutations in *IDH*1/2 stand out for their association with grade II and III astrocytomas and oligodendrogliomas, in addition to grade IV glioblastomas. The panel also distinguishes between somatic mutations in chromatin remodeling pathways, such as *H3F3A, ATRX e TP53*, essential for the classification of pediatric gliomas. Specifically, mutation detection *H3F3A* using GlioSeq suggests a diagnosis of infiltrative glioma. However, caution should be exercised when interpreting results in pediatric populations, given the small sample size of pediatric cases studied (Nikiforova *et al.*, 2016).

NGS is increasingly used in the analysis of glioblastoma, particularly for its ability to detect multiple genetic mutations in a single biological tissue [91]. Its application in the analysis of cell-free circulating DNA in fluids such as cerebrospinal fluid and plasma, as well as in the investigation of the cellular content of extracellular vesicles, has shown promising potential as a source of biomarkers (Skouras *et al.*, 2023). NGS is characterized by its low sample requirement for analysis. This methodology is versatile and allows the identification of a wide range of genetic alterations, including Single Nucleotide Polymorphisms (SNPs), small insertions and deletions (indels), DNA amplifications, large areas of loss of heterozygosity (LOH) and changes in the number of copies (CNAs). Furthermore, this technology proved to be particularly effective in detecting all crucial pathogenic variants in gliomas and offered greater sensitivity and specificity than the Immunohistochemistry (IHC) method, according to Śledzińska *et al.* (2021).

Single Cell RNA Sequencing

The detailed study of individual cells through comprehensive techniques promises to transform our understanding of organisms, facilitating the identification of cell lineages and the mapping of heterogeneity within organs with a resolution never achieved. The *Single Cell RNA Sequencing* (scRNA-

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seq) has facilitated the exploration of experimental questions that require distinction at the single-cell level, such as the identification of cellular subpopulations that contribute to resistance to treatments, the characterization of novel or scarce cellular subtypes, the analysis of the composition of TME, or the study of tumor architecture and plasticity. For example, Couturier *et al.* (2020) explored the developmental hierarchy of glioblastoma cells compared to normal human brain development. Using scRNA-seq on 53,586 adult glioblastoma cells and 22,637 normal human fetal brain cells, the authors compare the lineage hierarchy of the developing human brain with the transcriptome of cancer cells. They discovered a conserved neural cancer hierarchy centered on glial progenitor-like cells, which contain the majority of cancer cells.

Multiplex qRT-PCR represented the standard strategy for investigating gene expression at the single-cell level but was confined to the analysis of only a few hundred genes, even employing high-parallel microfluidic systems (Perchet *et al.*, 2017). A critical limitation of qRT-PCR is its inherent bias towards the specific genes selected for study, therefore requiring a basis in pre-established hypotheses. On the other hand, microarrays enable transcriptome analysis on a broader scale but lose out to RNA-seq in terms of sensitivity and dynamic scope. Additionally, the amount of RNA required for hybridization methods is considerably greater, requiring micrograms of material, while RNA-seq requires only nanograms to prepare its libraries (Rai *et al.*, 2018). The requirement for many probes to cover the entire transcriptome, covering non-coding areas and detailing splice junctions, makes microarrays a costly alternative. Thus, following the trend observed in the study of cellular populations, RNA-seq is supplanting hybridization methods in the context of single-cell analysis (Wirka *et al.*, 2018). Table 4 contains a compilation of the major findings from 2023 and 2024 (Table 4) on glioblastomas using scRNA-seq techniques.

Table 4. Recent discoveries from 2023 - 2024

Authors	Method	Discovery
(Couturier *et al.*, 2020; S. Cui *et al.*, 2023; Garcia-Diaz *et al.*, 2023; He *et al.*, 2020; P. Liu *et al.*, 2024; Song *et al.*, 2023; Sun *et al.*, 2023; F.	scRNA-seq	5. Abundance of mural cells in meningiomas and abundance of dendritic cells in infratentorial tumors. 8. T cell-induced dormancy was found to prevail in the tumor bulk, while astrocyte-like differentiation occurred at the margin. 9. The gene *PLUAR* is associated with neutrophil activities and cell migration.

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Wang *et al.*, 2023; X. Wang *et al.*, 2023; Zhao *et al.*, 2023)		14. Genes associated with GB prognosis: *MDK, NBEAL1, HOXA2, HOXA7, MTRF1L, EREG, EN1, HOXA5, SLC37A3, LAP3, ERP29, AUTS2, HSPA5, SLC6A6 and ARMC10.* 16. Identified NRF2 essential for the senescence phenotype of GB 17. High expressions of type I collagen receptors in GB: I*TGAV, ITGB8, SDC1, SDC4 and CD44.* 18. Brain metastasis tumors, for which treatment with recurrent GB is more complex, requiring modification of these populations, can improve the effectiveness of immunotherapy. 21. Increased ECM gene expression in recurrent GB subpopulations than in primary GB 22. O6-methylguanine DNA methyltransferase in GB may contribute to drug resistance. 28. Bioactive molecules from exosomes can affect the signaling pathways of tumor cells and their gene expression.
(Al-Dalahmah *et al.*, 2023; Lin *et al.*, 2023)	snRNAseq	2. By comparing (A) normal brain tissue, (B) reactive/inflammatory tissue and (C) cellular/proliferative tumor: B - related to worse mortality and evaluating FASN as a promising target against GB. 11. The increase in INTS9 expression is related to the high proliferation of the tumor, related to the TP53 mutation.

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	single cell RNA	1. Gene expression in hypoxia in GB tumor-related cells: *IGFBP2, CP and LOX.* 6. The EGFR/AKT pathway promotes positive regulation of gene expression *ACSS3, ACSL3 and ELOVL2* in GB cells.
(Ahmed *et al.*, 2024; X. Cui *et al.*, 2023; Y. Liu *et al.*, 2023; Raviram *et al.*, 2023)	snATAC-seq; stRNA-seq	15. Mutations in the TP53 and ATRX genes are more sensitive to chemotherapy agents, such as temozolomide. 13. PpIX fluorescence resulting from 5-ALA treatment does not originate exclusively from neoplastic glioma cells. 3. The genes *ANXA1, RAB31, DSTN and SYPL1* positively regulate GB, and PRPC being a possible regulator.
(Boccacino *et al.*, 2024; Wu *et al.*, 2024)	bulk RNA sequencing data	4. Expression of glioma cells *FBXO2,* of cell-cell interaction in neurodevelopmental pathways 23. Podoplanin is related to the expression of CD68 and CD163 in glioma tissues, inducing macrophage polarization, contributes to the immunosuppression of GB.
(Yu *et al.*, 2023)	TCGA	26. High expression of the Activation Receptor expressed on myeloid cells 2, indicating shorter survival for patients

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		20. Some NPCTKO GB vascular cells were classified as malignant cells.
(Li *et al.*, 2024; P. Liu *et al.*, 2024; Verduin *et al.*, 2023; X. Wang *et al.*, 2023; Xing *et al.*, 2023)	More than 1 Technique	10. *TNFSF15* is upregulated in ARPC4 cells and *NDUFV1*, being an immunostimulant of T cells. 12. The transcription factor NFYB may be a new target for the treatment of GB. 19. Primary GB organoids can be used for screening to judge the best treatment approach, due to the level of heterogeneity. 24. Macrophages are much more concentrated in the tumor nucleus, and with this strong SPP1-CD44 interaction between macrophages and T cells in GB, it may be a point of tumor progression.

Legend: Single-cell RNA sequencing (scRNA-seq); Single-nucleus RNA sequencing (snRNAseq); RNA sequencing from the Cancer Genome Atlas (TCGA); Bulk RNA sequencing data; single-nucleus ATAC-seq (snATAC-Seq); spatial transcriptome sequencing (stRNA-seq

4. FINAL CONSIDERATIONS

Digital pathology emerges as a promising tool, enabling the visualization and understanding of structural, cellular and molecular information about cancer in a spatial and microenvironmental context. In this sense, this substantial advance significantly contributes to a better discernment of the clinical behavior of malignant tumors, so that the four-dimensional analysis of cancer, which considers not only temporal but also spatial aspects, becomes crucial in the era of precision medicine. The included studies were heterogeneous in terms of cancer type, methodological design, digital pathology tools, artificial intelligence models, biotechnological approaches, and clinical applicability, which limits direct comparison among findings. Although six databases were searched, the diversity of terms used in the literature on digital pathology, artificial intelligence, biotechnology, omics technologies, and cancer diagnosis may have restricted the retrieval of some relevant studies. In addition, most available evidence is still based on experimental, retrospective, or computational validation studies, with limited data on implementation in routine clinical practice. Therefore, the findings should be interpreted as a synthesis of current evidence and trends rather than as definitive clinical recommendations. Therefore, developing

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and improving artificial intelligence tools for PD means investing in adapting treatment plans in a functional way, aiming to maximize therapeutic efficacy and minimize adverse effects on patients.

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