International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

A Comprehensive Study of Mapping of Hypoxia And Acidosis Using Advanced MRI Technique

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Abstract:
Cancer is worldwide health problem which is faced by all over the world population. It is characterized by uncontrolled growth, division and metabolism of tissues and individual cells in order to feed the tumor and make it grow and develop resistance to treatment. Surgery, chemotherapy and radiation therapy are the conventional methods of treatment that have not proved effective treatment due to the major side effects at the site of tumor. To overcome this problem, the advanced MRI based techniques of perfusion, and metabolism imaging are used for assessing the tumor microenvironment which focuses on the partial pressure of the oxygen, which are critical for enhancing the Radiotherapy outcomes. The major cause of the Radiation resistance is caused by tumour hypoxia, and non-destructive imaging of tumour oxygenation and metabolic activity may improve treatment planning and monitoring. Since hypoxia is associated with poor treatment resistance against tumour . In this we can treat tumour hypoxia by non-destructive machine like MRI. There are various types of advanced MRI techniques which is useful for targeting the tumour site , these include DCE-MRI, BOLD-MRI, EPRI-MRI and Hyperpolarized 13C Metabolic MRI. These advanced MRI techniques examines how tumour behave during radiation therapy especially the oxygen levels and metabolism inside the tumour can be affected. The tumour with low oxygen level are difficult to kill with radiation . so the researchers explore these techniques to see how much the oxygen is in different parts of a tumour , monitor the blood flow and metabolism inside the tumour and improves the outcomes of the radiotherapy by customizing the treatment plans. EPRI-MRI (Electron Paramagnetic Resonance Imaging) is a spectrometric MRI technique which is based on NMR (nuclear magnetic resonance). It directly maps the oxygen levels in tissue and uses a special spin probe which is injected into the body called as OX063. It creates 3D maps which shows where the tumour is low oxygenated (Hypoxic) and where the tumour is gets well oxygen supply. This paper highlights the high resolution of oxygen levels (partial pressure of oxygen) , P_H gradient and inorganic phosphate (P_i) in the tumor microenvironment . These are the efforts which are utilized to improve the outcomes of the radiotherapy and we will more focussing on increasing the oxygen level in the tumors which results in the killing of the tumor tissue.

KEYWORDS: DCE MRI, BOLD, TOLD, EPRI, HYPERPOLARIZED 13C MRI

INTRODUCTION:

Cancer remains one of the leading cause of deaths in all over the world which is characterized by the uncontrolled growth, division, and invasiveness that form the tumor. The major cause of the occurrence

of the tumor is lack of oxygen and maintenance of P_h gradient reaching at the tumor site which is called as hypoxia and Acidosis [1]. To understand and minimizing the insufficient oxygen areas of tumor that promotes the effectiveness of the radiation therapy has been a research area of studying the tumor microenvironment [2]. The TME is the environment around a tumour , including immune cells , blood vessels , and signalling molecules. It influences the tumour growth , immune evasion , and therapy resistance . cancer cells reprogram their metabolism to survive, grow and escape the immune system [3]. Tumour progression, metastasis, immunological evasion, and treatment resistance are all significantly influenced by the TME [4] . Its impact on tumour metabolism, which is greatly impacted by variables like hypoxia, acidity, and nutrition availability, is among its most significant[5]. Hypoxia can cause variable changes in both systemic and local acid-base balance leading to the occurrence of Acidosis[6]. Hypoxia not only plays a physiological role in regulating normal metabolic and regeneration processes, but also plays an important role in abnormal stresses associated with disease and tissue injury[7]. In early century as on 1904, Hahn and Schwarz found that the impact of surface radium plaques and low-energy X-rays was influenced by compression that restricted the flow of blood . Although the role of oxygen in these effects was not immediately understood, these findings rapidly influenced by radiation sources [8] . Later, Mottram's histological studies on tumours in hamsters led to the conclusion that tumour growth could result in regions of vascular insufficiency, giving rise to localized hypoxia [9]. Around the same period, Crabtree and Cramer provided proofs in the 1930s that molecular oxygen played a key role in determining how cells respond to radiation. Molecular oxygen (O_2) is a strong chemical radiosensitizer[10]. Its radio sensitizing effect become apparent not from any metabolic or physiological functions, but from its strong electron-affinity and direct involvement in the chemical reactions that lead to DNA damage after ionising radiation is absorbed. Hypoxia not only involved in increased tumour aggressiveness and a higher risk of metastasis, but also minimizing the effectiveness of standard therapies involves radiation treatment, which depends on the presence of molecular oxygen to generate DNA-damaging free radicals. In addition to hypoxia, the tumour microenvironment often exhibits PH gradient, a result of poor blood flow and breaks cellular metabolism[10]. This acidic condition further enhances cancer development and provide resistance against treatment. By understanding the comprehensive distribution of oxygen and pH within tumours is crucial for improving therapeutic results and enhancing personalized treatment strategies[11]. Standard imaging techniques offer little insight into the physiological state of tumours. However, advancements in magnetic resonance-based technologies have enabled non-destructive evaluation of the tumour microenvironment[12]. Techniques such as Hyperpolarised 13C Metabolic MRI, Blood Oxygen Level Dependent (BOLD) MRI, and Dynamic Contrast-Enhanced MRI (DCE-MRI) have shown results in monitoring metabolic activity and tissue perfusion. Among these, Electron Paramagnetic Resonance Imaging (EPRI) has shown more positive outcomes due to its ability to directly map the tissue oxygen levels (p O_2) using a specialised spin probe[13]. Spin probe is a paramagnetic molecule which is a free radical that is used to make a contrast agent for imaging. These probes are designed in such a way so that they can interact with the magnetic environment of the tissue that allows us to visualize and quantify the specific physiological parameters such as oxygen levels in tissues which are particularly used in studying tumour hypoxia[14]. This paper explores the application of Electron Paramagnetic Resonance Imaging (EPRI) in assessing tumour hypoxia and acidosis by offering a detailed analysis of oxygen levels, pH gradients, and the distribution of inorganic phosphate (Pi). By analysing these key physiological parameters, EPRI holds the potential to enhance treatment planning and monitoring particularly in radiotherapy, where oxygen availability plays a critical role in determining therapeutic effectiveness[15].

The EPRI uses a special spin probe which is injected into the body and directly maps the oxygen levels in the tissue. It creates the 3D maps and shows where the tissue is hypoxic and well oxygenated. Electron Paramagnetic Resonance Imaging (EPRI) is a spectroscopic technique which is similar to the nuclear magnetic resonance[16]. It specifically detects the paramagnetic species such as free radicals (odd electron species) and transition metal complexes that possess unpaired electrons. Suitable agents for EPR imaging are assumed by simple spectra, safe dosages, and pharmacokinetics that ensure their presence throughout the imaging session[17]. In vivo, OX063 is a spin probe, a triaryl methyl radical (TAM) that serves as an effective paramagnetic probe for assessing tumour oxygen levels (p O_2), monitoring p O_2 fluctuations to differentiate between acute and chronic hypoxia, and evaluating treatment responses over time[18]. EPRI, using probes like OX063, is currently being explored as a non -invasive and quantitative method for mapping tissue oxygenation in living organisms[19].

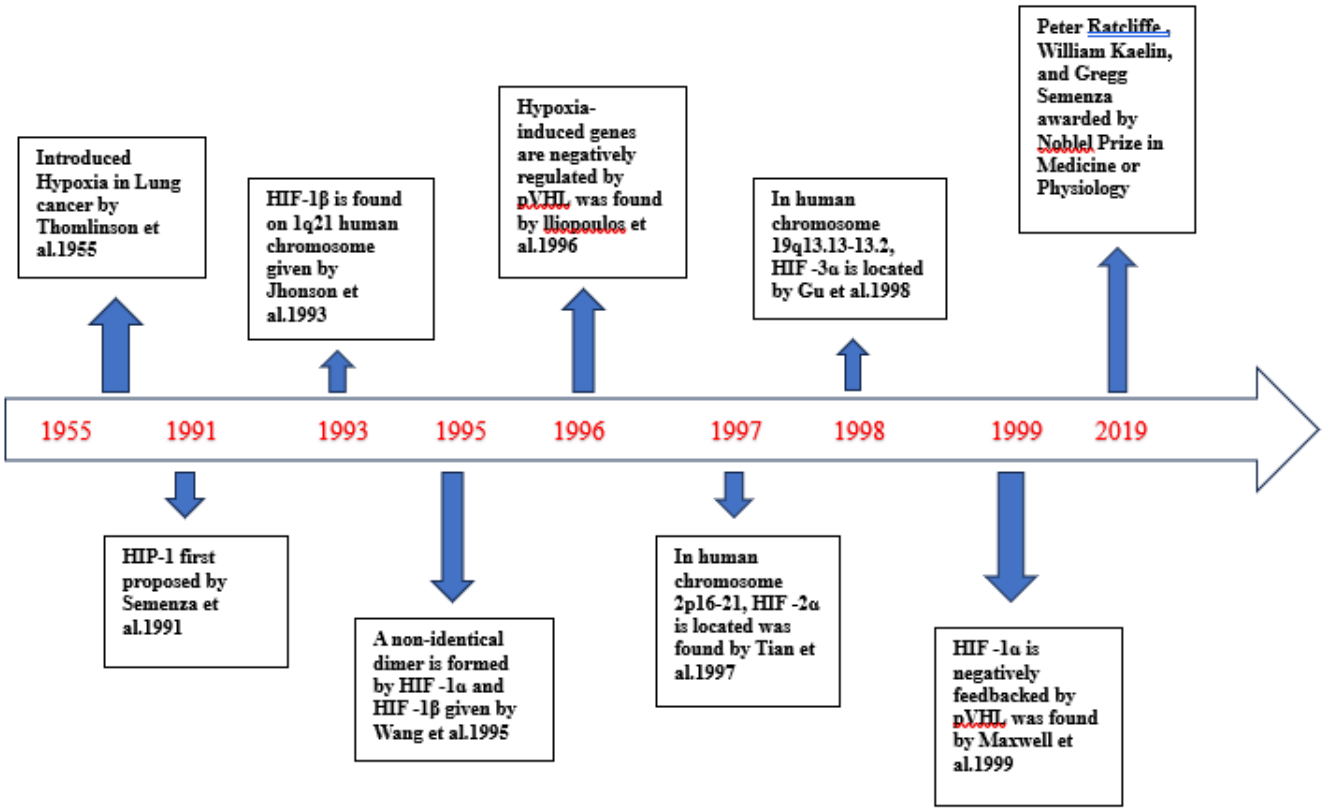
Tumour Microenvironment: Hypoxia and Acidosis

The TME is the environment around a tumour , including immune cells , blood vessels , and signalling molecules. It influences the tumour growth , immune evasion , and therapy resistance . cancer cells reprogram their metabolism to survive, grow and escape the immune system. Tumour progression, metastasis, immunological evasion, and treatment resistance are all significantly influenced by the TME [4]. Hypoxia is the common feature of the tumour microenvironment which is closely linked with some processes includes the growth of new blood vessels that tumour needs to grow, metabolism, cell proliferation, and the immune response within the tumour tissue[20]. These interconnected factors can contribute to a poorer prognosis by promoting rapid tumour growth, enhancing aggressiveness, and increasing the potential for the spread of tumour. In solid tumours, the tumour microenvironment consists of a complex network of connective tissue in which various transformed cells are get enclosed [21].These include vascular endothelial cells (ECs), cancer-associated fibroblasts (CAFs), immune cells, and some components of the extracellular matrix (ECM), they all contribute to tumour development and progression. The local growth is promoted by the tumour cells and thereby also facilitates their spread by affecting the nearby healthy cells which are engaged in interactions, and also organizing a specialized tumour microenvironment [22]. This interaction between the tumour and host cells gives rise to a unorganized, organ-like structure that promotes the progression of the malignant cells [23].

HYPOXIA AND CELLULAR PROLIFERATION

A big problem in treating cancer with radiation therapy is something called as HYPOXIA. Hypoxia in general means that the part of the tumour tissue which does not get enough oxygen supply. Tumour with low oxygen are not able to kill or harder to kill with radiation. So the scientists explores the advanced MRI-based techniques of imaging so that they will know about how much oxygen is reaching in the different parts of a tumour and also get to know about the blood flow and metabolism by monitoring it [24]. Hypoxia is fundamentally linked to maximize the cellular proliferation by giving the sufficient amount of oxygen for ATP production [25]. Previous studies have showed that tumour cells situated near the blood vessels, where oxygen supply is enough which tend to multiply more rapidly than oxygenated cells, which are usually located 100–200 µm away from blood vessels and often near non- viable regions [26].

The Illustration of historical and researches in tumor hypoxia:



MECHANISM OF CELL PROLIFERATION

The vascular system is essential for transporting oxygen and substrates of energy to tissues [27]. In cancerous or pre-cancerous environments, the endothelial cells (ECs) lining the blood vessels are exposed to various harmful factors, such as drugs, carcinogens, infectious agents, and the distinct acidity of the tumour microenvironment (TME) [28]. These exposures can alter EC morphology such as causing swelling (oedema) and may also disrupt the normal function of the tumour vasculature [29]. Cancer cells grow and divide quickly, which demands a high level of energy. To support this fast-paced growth, they rely on an active metabolism and consume large amounts of energy to keep up with their needs. When the oxygen demand of cancer cells exceeds the available supply, oxygen-deficient areas develop and trigger metabolic adaptations [30]. This hypoxia mainly results from abnormal blood vessel formation and structure, driven by dysregulated angiogenesis. Although low oxygen conditions stimulate the release of erythropoietin (EPO) and angiogenic factors that promote the growth of endothelial cells, the resulting blood vessels are often disorganized and dysfunctional [31]. The rapid expansion of cancer often leads to hypoxia and inadequate blood vessel formation within the tumour region. As cancer cells grow farther from existing blood vessels and oxygen diffusion becomes less effective, the tumour microenvironment (TME) is subjected to sustained low oxygen levels [32]. This phenomenon has been observed in mouse models of breast cancer [33]. In fact, hypoxia is a common feature in solid tumours, typically occurring in tissues located more than 100–200 μm from a functional blood supply [28]. Research using a rat brain glioma model, further revealed that peritumoral blood vessels respond to hypoxia via alpha-smooth muscle actin, potentially exacerbating oxygen deficiency even in areas close to major vessels [34]. Ultimately, hypoxia stems from the presence of abnormal and poorly functioning vasculature [35] . Significant

alterations occur in the non-cancerous components and functions within the tumour microenvironment, including the activation and proliferation of stromal cells such as stellate cells and cancer-associated fibroblasts as well as an accumulation of stromal elements like fibrin [36] . These changes contribute to structural remodelling of the tumour, which may lead to vascular compression [37]. As a result, impaired blood flow and inadequate oxygen delivery can worsen tissue hypoxia and promote thrombosis [38]. Additionally, the physiological effects of certain substances, such as magnesium, can vary depending on the degree of hypoxia present [39].

Most solid tumours contain regions of hypoxia. Within these tumours, some areas exhibit transient hypoxia, others experience chronic hypoxia, and some contain a mix of both [40]. This results in the presence of three distinct cell populations. The first group consists of chronically hypoxic cells that, if left in place, eventually die off. These so-called "doomed" cells are considered the primary contributors to necrosis observed in the tumour core [28]. Interestingly, in cell survival experiments, these cells can survive independently once removed, but they do not influence the tumour's overall response [41]. The second group includes persistently hypoxic cells that can continue to survive as long as they remain in their hypoxic environment [28] . These cells adapt to low oxygen levels by altering gene expression, increasing resistance to drugs and radiation, and enhancing their proliferation rates [42]. The third type consists of transiently hypoxic cells, typically located near functional blood vessels, where the hypoxic state is short-lived [20]. Recent studies indicate that tumour cells actively adapt their signalling pathways in response to hypoxia [32]. This adaptation not only promotes malignant behaviours such as increased proliferation, migration, invasion, and the epithelial-mesenchymal transition (EMT) but also contributes to greater resistance to immunotherapy, chemotherapy, and radiotherapy [43].

ACIDOSIS IN TUMOUR MICROENVIRONMENT

The tumour microenvironment (TME) consists of a diverse array of cell types, including fibroblasts, adipocytes, endothelial cells (ECs), both innate and adaptive immune cells, extracellular matrix (ECM) components, signalling molecules, and antibodies [44] . These TME components, which are regulated and shaped by the tumour itself, interact in complex ways to create an environment that supports cancer cell adaptation, survival, and metastasis [45]. As the tumour grows and cancer cells proliferate, the TME undergoes both cellular and molecular changes [46].

Due to the increased glycolytic activity and the inadequate removal of products of metabolic acids the tumours often display an acidic extracellular pH (PHE), typically in combination with hypoxia [47]. This acidosis leads to several issuing effects, including:

- Altered drug absorption and reduced efficacy of pH-sensitive therapies [48].
- Enhanced invasiveness and metastatic potential [49].
- Immune suppression within the tumour microenvironment [50].

Cancer cells rely on anaerobic glycolysis to maintain intracellular energy levels in hypoxic conditions, driven by their high glucose demand [51]. This process leads to the accumulation of lactic acid and hydrogen ions (H+) within the tumour microenvironment (TME). The Warburg effect explains how, in aggressive tumours, oncogenes drive aerobic glycolysis even in the presence of sufficient oxygen, a phenomenon typically observed in hypoxic environments [52]. As glycolysis increases the production of lactic acid and hydrogen ions (H+), the intracellular pH (PHI) of cancer cells decreases [53] . Cellular processes such as adhesion, proliferation, metabolism, and apoptosis typically require a slightly alkaline pH range of 7.0 to 7.4.[54]. However, intracellular acidification can disrupt the cell cycle. A declining pH

also negatively impacts histone acetylation, which is crucial for allowing transcription factors access to chromatin [55].

The HIF transcription factor family is pivotal in the management of intracellular pH (pHi) of a cell, especially during the more acidic phases. These transcription factors control the activity of the genes responsible for the efflux of lactate and hydrogen ions out of the cell [56]. Under HIF regulation, key proteins include glucose transporters GLUT-1 and GLUT-3 and various components of acid extrusion systems such as monocarboxylate transporters (MCTs), hydrogen/potassium ATPases (H^+/K^+ ATPases), vacuolar-type H^+-ATPases (V-ATPases), carbonic anhydrases (CAs), and sodium hydrogen exchangers (NHEs) [57]. They are turned on by hypoxia response elements (HREs) in the promoter regions of their genes [58]. All these systems function to ensure that there is a greater pHi during glycolysis by sequestering additional hydrogen ions which makes the extracellular space more acidic [59].

Although the lactate produced by cancer cells contributes to the acidosis of the TME, it additionally impairs immune response activities. Increased lactate concentrations are detected by immune cells and tumor cells, and it alters their functions and behaviors [60]. In the TME, the accumulation of lactate prevents immune cells from removing lactate because this process depends on a concentration gradient [61]. This lactate congestion in CD8+ cytotoxic T lymphocytes (CTLs) leads to chronic inhibition of interferon-gamma (IFN-γ) production and a blockade of NFAT activity, thus, rendered inactive cytotoxic function [62]. Furthermore, the lactate enhances expression of immune checkpoint molecules such as CTLA-4 and PD-L1 which further aids in the apoptosis of CTLs and NK cells [63].

Lactate tenders some impact on T cell polarization which results in the downregulation of anti-tumour T helper (Th1) cells and upregulation of Tregs [64]. Increased concentration of lactate leads to an increased reduction of nicotinamide adenine dinucleotide (NAD+) to NADH which reduces key precursors of glycolysis like serine and T cell proliferative substrates like glycerol-3-phosphate (G3P) and dihydroxyacetone phosphate (DHAP): gapdh, glucoso-6-phosphate-dehydrogenase, and PGK [65]. On top of that, lactate plays a fairly crucial role in the development and activation of DCs, processing and presentation of the antigens as well as the M2 type shift in TAMs [66].

COMPARISON OF EFFECT OF ACID IN TME:

TYPE OF CELL	EFFECT OF ACID ON TME	REFERENCES
MESENCHYMAL STEM CELLS		
FIBROBLASTS	The tumor microenvironment (TME) promotes cancer progression by generating signaling molecules. Increased amounts of CCL7 and tissue degrading enzymes called the matrix metalloproteinases (MMPs) result in increased distance of cell migration and invasion. A secreted molecule called CXCL1 is produced that allows tumors cells to differentiate. Molecules like CXCL12 and fibroblast growth factor (FGF) support tumor proliferation. Erythropoietin (EPO), FGF and vascular endothelial growth factor (VEGF) stimulate angiogenesis. Transforming growth factor-beta (TGF-β) secreted by tumor cells also dampens T cell san natural killer (NK) cell function. Tumour-associated macrophages (TAM) inside the TME also produce pro-inflammatory cytokines like interleukin-1β (IL-1β) and IL-6, IL-8. Cancer-associated fibroblast (CAFs) are also activated by signaling molecules, including IL-6 and TGF-β, IL-1, EGF, platelet-derived growth factor (PDGF) and FGF2.	[67],[68],[69],[70]

ENDOTHELIAL CELLS	VEGF and FGF causes angiogenesis and cell migration. Secretion of IL-6 and CSF-1 mediates immune modulation, while the recruitment of innate immune cells and Tregs build an immunosuppressive environment. The expression of PD-L1 causes downregulation of T cell activity. Tumor cells release polyunsaturated fats to enhance their own growth and manipulate apoptosis. ICAM-1 and VCAM-1 upregulation increases metastatic adhesion and ability to spread.	[71],[72]
PHAGOCYTIC AND ANTIGEN-PRESENTING CELLS		
Myeloid-derived suppressor cells (MDSC)	Myeloid-derived suppressor cells (MDSCs) are attracted to the tumor site where they secrete arginase and inducible nitric oxide synthase (iNOS) that suppress NK T cell and T cell activity and supports immune evasion.	[73]
Neutrophils	In the tumor microenvironment, neutrophils may take on the N2 phenotype that provides help to promote tumor development. Neutrophils secrete neutrophil extracellular traps (NETs), matrix metalloproteinases (MMP-8 and MMP-9), and neutrophil elastase (NE), all of which are important in extracellular matrix (ECM) remodeling. While neutrophils have the capacity to kill tumor cells, they secrete hydrogen peroxide, myeloperoxidase (MPO), and H^+ -ATPases, all of	[74],[75],[76]

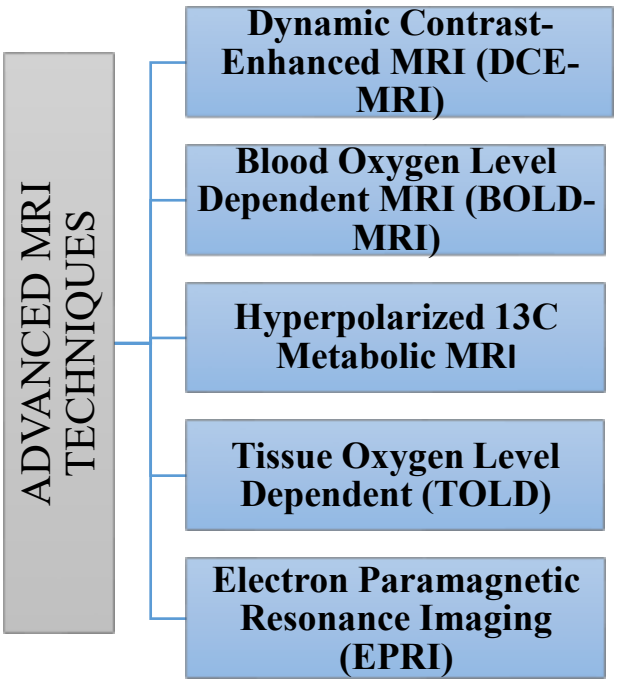
	which impair NK and T-cell function.	
Macrophages	Tumor-associated macrophages usually switch to an M2 phenotype, which encourages tumor growth. They secrete Th2-associated cytokines (like IL-10 and TGF-β) that inhibit T cell responses. They also generate reactive oxygen species (ROS), inducible nitric oxide synthetase (iNOS), and prostaglandins, all contributing to immunosuppression. In order to promote tumor cell viability and promote vascular growth, they secrete EGF, VEGF, CXCL8, and IL-6. They deposit MMPS to help with tissue invasion, while oncogenic extracellular vesicles (EV) production and reduced antigen presentation help the tumor hide from the immune system.	[77],[78],[79]
Dendritic cells	Macrophages can facilitate the release of anti-inflammatory cytokines, such as IL-10, and inhibit maturation/migration to lymph nodes with low HLA class II, resulting in inhibited antigen presentation and lower levels of co-stimulatory molecules (e.g., CD40, CD80, CD86). However, pro-inflammatory cytokines are also secreted by macrophages, such as IL-12. In addition, the expression of immune checkpoint molecules (PD-L1 and CTLA-4) increased, further decreasing immune responses.	[80],[81]
Natural Killer cells	Tumor-induced immune suppression leads to T-cell anergy and apoptosis, thus	[82],[83]

	reducing the cytotoxic activity of the T-cells. This includes decreased release of perforin and granzyme B, decreased expression and activation of NKG2D, and decreased production of IFN-gamma (IFN-γ), as well as decreased granule release and metabolic activity of T-cells. The T-cells are further inhibited by cytokines such as TGF-β and IL-10.	
T lymphocytes		
CD8+ (cytotoxic) and CD4+ (helper) T cells	Tumor-associated immune suppression causes T cell anergy and apoptosis. iNOS and arginase can diminish the availability of arginine to limit TCR activity that results in decreased expression of the CD3ζ chain and reduced cytokine production (eg IL-2, IFN-γ, TNF-α). In addition, the availability of perforin and granzyme is lower and often produces decreased cytotoxic activity. In addition, the activation of signaling molecules, such as: STAT5, ERK, AKT, JNK, c-Jun, and p38, as well as NFAT, occurs to a lesser extent, and T cell metabolism, chemotaxis, and proliferation are impaired. When TCR is engaged, indoleamine 2,3-dioxygenase (IDO) can also block the activation of T cells by converting tryptophan to kynurenine.	[84],[85],[86],[87]
Regulatory T cells (Tregs)	Within the tumor microenvironment, IDO, TGF-β, and CCL22 recruit increased anti-inflammatory Tregs to the environment, and lactic acid	[88],[89],[90]

	preserves Treg metabolism. These agents, in combination to mediate Treg metabolism, produce TGF-β, IL-10, and IL-35 to immunosuppress the activated dendritic cells (DCs), B cells, T cells, and NK cells. Alongside this, tumor vasculature releases VEGF to promote angiogenesis.	
B cells B lymphocytes	B cell subsets are recruited to the tumor microenvironment (TME), where their effects vary depending on the cancer type.	[91]
Regulatory B cells (Bregs)	Tumor metabolites and IDO recruit Bregs to the tumor site, producing IL-10, IL-35, and TGF-β, inhibiting T cells/motility and inducing the expression of FoxP3 in Tregs. Bregs also express PD-1, PD-L1, and CTLA-4 to block T and NK cell, activity. In addition, adenosine created by Bregs prevents T cells from functioning, impairs Th17 responses and decreases IFN-γ from cytotoxic T lymphocytes (CTLs).	[92],[93],[94]

ADVANCED MRI TECHNIQUES FOR MAPPING OF TUMOUR HYPOXIA

Non-invasive imaging of tumour physiology is essential for analysing the tumour microenvironment and informing treatment approaches. Various advanced MRI techniques have been developed to evaluate in vivo perfusion, oxygenation, metabolism, and pH gradient [95] . Each method has its own advantages and drawbacks when it comes to visualizing the dynamic biological processes within tumours.



ADVANCED TECHNIQUE OF MRI	CONCEPT	RESULTS	DRAWBACKS
DCE-MRI (Dynamic Contrast Enhanced MRI)	It uses contrast agent into the blood for seeing how it flows through the tumor [96]. It gives hints about blood supply and oxygenation [97]. More Contrast ∝ Better blood flow and better oxygenation.	Shows more positive results in clinical applications as it is linked with better tumor oxygenation and shows effectiveness in radiation therapy [98].	It does not directly measures the partial pressure of oxygen (po_2) as it requires a contrast agent to measure perfusion [99].
BOLD-MRI (Blood Oxygen Level Dependent)	This method looks at how oxygen in the blood changes. It uses a special signal change in MRI related to Deoxyhemoglobin (oxygen poor blood) [100].	Does not show invasion, highly sensitive to oxygen levels [101].	Does not give accurate and precise results always. other things like blood flow and anemia can trouble with the readings [102].
TOLD-MRI (Tissue Oxygen Level Dependent)	It detects T1 changes caused by molecular oxygen based on the monitoring of the tissue oxygen levels [103].	Directly reflects tissue oxygenation and can be combined with BOLD imaging [104].	It does not Shows any experimental positive results and need to be further validate [105].
EPRI (Electron Paramagnetic	It is a spectroscopic MRI technique in which it directly maps oxygen levels in tissues.	It Provides real-time, 3D maps and quantify the pO_2 levels which is	Still experimented on small limited animals but does not

Resonance Imaging)	Uses a special spin probe injected into the body (OX063) [106]. It creates 3D maps showing where the tumor is hypoxic and where is oxygenated [107].	capable of differentiating between chronic and periodic hypoxia[108]. Still mostly used in animals [108].	experiment on human till [109].

Hyperpolarized 13C MRI	It is a metabolic MRI technique where they inject a molecule called 13C – labelled pyruvate [110]. It shows how tumour coverts pyruvate to lactate which is a sign of aggressive cancer metabolism [111].	Gives real time images of how the tumour is hungry which is super useful for seeing how it react to treatment [112]. It is safe and tested in humans in cased of Prostate cancer [112].	It is Limited to clinical studies due to the need for specialized DNP equipment [113].

RESULTS

1. **THE DCE-MRI** (Dynamic Contrast Enhanced MRI) Shows more positive results in clinical applications as it is linked with better tumor oxygenation and shows effectiveness in radiation therapy [98]. It does not directly measures the partial pressure of oxygen (po_2) as it requires a contrast agent to measure perfusion .
2. **THE BOLD-MRI** (Blood Oxygen Level Dependent) method looks at how oxygen in the blood changes. It uses a special signal change in MRI related to Deoxyhemoglobin and Does not show invasion, highly sensitive to oxygen levels [101]. It Does not give accurate and precise results always. other things like blood flow and anemia can trouble with the readings.
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4. **EPRI** (Electron Paramagnetic Resonance Imaging) is a spectroscopic MRI technique in which it directly maps oxygen levels in tissues. Uses a special spin probe injected into the body (OX063) [106]. It creates 3D maps showing where the tumor is hypoxic and where is oxygenated [107]. It Provides real-time, 3D maps and quantify the pO_2 levels which is capable of differentiating between chronic and periodic hypoxia[108]. Still experimented on small limited animals but does not experiment on human till [109].
5. Hyperpolarized 13C MRI is a metabolic MRI technique where they inject a molecule called 13C – labelled pyruvate [110]. It shows how tumour coverts pyruvate to lactate which is a sign of aggressive cancer metabolism [111]. Gives real time images of how the tumour is hungry which is super useful for seeing how it react to treatment [112]. It is safe and tested in humans in cased of Prostate cancer . It is Limited to clinical studies due to the need for specialized DNP equipment [113]

DISCUSSION

1. The one of the main limitation of Electron Paramagnetic Resonance Imaging (EPRI) is that it has only been experimented to limited small animal models such as mice and rats. While the technique is effective in non-destructive mapping of tissue oxygen levels (PO_2). It does not closely connected to clinical trials due to limited lack of research in human, means that till now the trail of EPRI has not been experimented on humans. To Address this major issue will require advance researches in EPRI system design to compromise with humans, along with deep safety testing of spin probes like OX063. With these advancement in EPRI could play an important role in personalized cancer treatment by enabling real-time monitoring of tumour oxygenation.
2. Another limitation gap is the improper establishment of protocols for the combination of Electron Paramagnetic Resonance Imaging (EPRI) with other MRI techniques. Techniques like Hyperpolarised 13C-MRI, TOLD-MRI, and BOLD-MRI shows researches for metabolism, tissue oxygenation, and blood oxygen levels. However the integrated approaches remains undiscovered for Developing hybrid imaging systems that combines EPRI with functional and metabolic MRI which could provide a more complete understanding of tumour hypoxia, perfusion, and metabolism, ultimately leading to more personalized and accurate treatment strategies.
3. Although EPRI has shown potential in mapping tissue oxygen levels, its use in pH (acidosis) measurement is still in the early research phase. This limitation restricted to fully represent the metabolic environment of tumours, particularly in case of PH, which is important for the growth of tumour and responses against therapy. There is an urgent need to develop or enhance pH-sensitive spin probes capable of providing accurate, real-time imaging of tumour acidosis. Such advancements would enable a deeper understanding of the tumour microenvironment, which leads to target more therapies and improves treatment strategies.
4. Despite the promising potential of hypoxia imaging, the application of image-based therapy guidance for personalized treatment remains hypothetical. To enhance treatment strategies, clinical studies are necessary to evaluate the effectiveness of adaptive radiation therapy or chemotherapy guided by imaging biomarkers such as the lactate/pyruvate ratio and PO_2.
5. While the researches shows the role of lactate and acidity in immune suppression ,but there is an insufficient imaging studies that directly connects the immune modulation with MRI-based biomarkers. Future studies should combine imaging with immune profiling to fill this gap by identifying immunosuppressive regions and evaluating the combined effects of oxygenation strategies and immunotherapy.

FUTURE DIRECTIONS

AREA	FUTURE WORK
Clinical EPRI	To develop and test human-scale EPRI systems which ensures that the spin probes are safe for human use.
Probe Development	Develop dual-purpose spin probes which are capable of mapping both pH and PO_2 simultaneously.
Multi-Modal Imaging	Integrate EPRI with BOLD/TOLD and hyperpolarised 13C MRI in tumour mapping pipelines to achieve comprehensive imaging of oxygenation, perfusion, and metabolism.

Radiotherapy Personalization	It Utilizes real-time EPRI data during the treatment to effectively adjust radiation dose and fractionation which optimizes effectiveness of the therapy.
Therapy Response Monitoring	Validate the lactate/pyruvate ratio as a predictive biomarker across a broader range of cancer types such gliomas and prostate cancer.
Immune Environment Imaging	investigate imaging biomarkers, such as low pH and elevated lactate levels, that correlate with regions of immune suppression within tumours.
Artificial Intelligence	Manipulate AI to integrate genetic profiles, imaging biomarkers, and treatment response data for advanced predictive modelling and plan for personalized therapy.

CONCLUSION

The detailed study of hypoxia and acidosis within the tumor microenvironment is critical for improving the diagnosis of cancer and helps in giving better curing results. EPRI is the non -invasive technique which is useful for measurement of oxygen levels of tissue in the real time. For detailed visualization of oxygen distribution, we can use special spin probe such as OX063. EPRI is still experimented on small animals such as mice, rabbit. Development in clinical use demands improvement in the safety and reliability of spin probe, also development of dual function agents which monitors both PH and oxygen levels. The advance MRI techniques like BOLD, TOLD and DCE also gives understanding of tumor physiology and behavior. Further future studies should focus on the establishment of EPRI systems which is appropriate for the clinical use, enhancing the use of Artificial Intelligence to enable treatment planning.

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