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Review Article

Low-frequency magnetic field therapy for glioblastoma: Current advances, mechanisms, challenges and future perspectives

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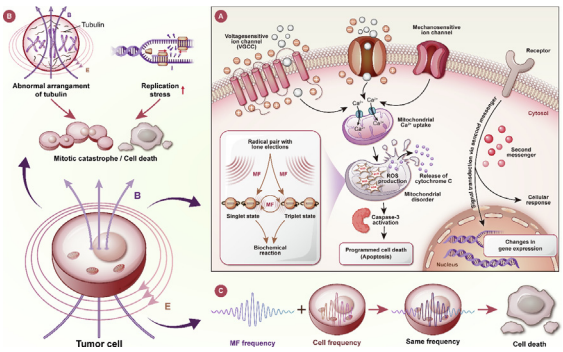
HIGHLIGHTS

- This review summarizes the types and parameters of magnetic fields that can inhibit the growth of gliomas.
- We summarise the landscape of LF-MFs clinical trials in GBM and consider how emerging preclinical data might inform future clinical applications for LF-MFs.
- We found that magnetic fields can mimic the effects of chemotherapy drugs, with efficacy equivalent to the drugs themselves.

GRAPHICAL ABSTRACT



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ABSTRACT

Background: Glioblastoma (GBM) is the most common malignant tumour of the central nervous system. Despite recent advances in multimodal GBM therapy incorporating surgery, radiotherapy, systemic therapy (chemotherapy, targeted therapy), and supportive care, the overall survival (OS) remains poor, and long-term survival is rare. Currently, the primary obstacles hindering the effectiveness of GBM treatment are still the blood-brain barrier and tumor heterogeneity. In light of its substantial advantages over conventional therapies, such as strong penetrative ability and minimal side effects, low-frequency magnetic fields (LF-MFs) therapy has gradually caught the attention of scientists.

Aim of Review: In this review, we shed the light on the current status of applying LF-MFs in the treatment of GBM. We specifically emphasize our current understanding of the mechanisms by which LF-MFs mediate anticancer effects and the challenges faced by LF-MFs in treating GBM cells. Furthermore, we discuss the prospective applications of magnetic field therapy in the future treatment of GBM.

Abbreviations: GBM, Glioblastoma; OS, Overall survival; TTF, Tumour-treating fields; LF-MFs, Low-frequency magnetic fields; PFS, progression-free survival time; BBB, Blood-brain barrier; MF, Magnetic Field; IARC, the International Agency for Research on Cancer; ELF-EMFs, extremely low-frequency electromagnetic fields; RF-EMFs, radiofrequency electromagnetic fields; ICNIRP, the International Commission on Non-Ionizing Radiation Protection; CNKI, China Knowledge Network; TMZ, Temozolomide; SQUID, the superconducting quantum interference device; *u*/RFE®, ultra-low radio frequency energy.

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Key scientific concepts of review: The review explores the current progress on the use of LF-MFs in the treatment of GBM with a special focus on the potential underlying mechanisms of LF-MFs in anticancer effects. Additionally, we also discussed the complex magnetic field features and biological characteristics related to magnetic bioeffects. Finally, we proposed a promising magnetic field treatment strategy for future applications in GBM therapy.

Contents

Introduction

Glioblastoma (GBM) is the most common malignant brain tumor, and surgical resection combined with radiotherapy and chemotherapy is the standard treatment for GBM. Nevertheless, the 5-year survival rate of GBM is less than 10%, with a mean survival time of less than 2 years [1]. Despite incremental advances in the therapeutic approach to GBM, including the use of targeted therapy and immunotherapy, there have been no breakthroughs. Currently, the treatment regimens approved by the United States Food and Drug Administration (FDA) for GBM include physical therapies such as tumor-treating fields (TTF) therapy, in addition to radiotherapy and chemotherapy. However, the efficacy remains limited [1]; thus, it is urgent to explore new methods for the treatment of GBM.

Two main reasons for the high failure rate of drug therapy for GBM are the blood–brain barrier limiting drug entry [2] and the high heterogeneity of tumors [3]. High heterogeneity makes monotherapy ineffective or causes short-term drug resistance [3], but patients treated with multiple drugs have to endure drug-related side effects [1]. Therefore, it is necessary to develop new therapies to address these disadvantages. Noninvasive approaches like light, electrical field, ultrasound, and magnetic fields(MFs) therapies are being explored as alternatives, with MFs therapy showing promise due to its strong penetration, multitarget effects, and minimal side effects [4]; thus, it can overcome the clinical challenges associated with the above drugs [5–8].

MFs are classified into static MFs and dynamic MFs based on intensity and direction. MFs are classified into weak MFs (<1 mT), medium-intensity MFs (1 mT – 1 T) and high-intensity MFs (>1 T) based on intensity [9]. According to the frequency, MFs can be divided into low-frequency MFs (<30 kHz), radiofrequency MFs (30–300 kHz), medium-frequency MFs (300 kHz-3 MHz) and higher-frequency MFs (>3 MHz) [10]. MFs therapy involves two main mechanisms: thermal effect (ionizing radiation) and non-thermal effect (non-ionizing radiation). Higher-frequency MFs, which include gamma rays, X-rays, and higher ultraviolet, directly cause DNA damage, while low- frequency MFs mainly affect biochemical reactions [10]. However, few risk factors are known for brain tumors, except for ionizing radiation [11]. In 2002 and 2011, the International Agency for Research on Cancer (IARC) classified extremely low-frequency electromagnetic fields (ELF-EMFs) and radiofrequency electromagnetic fields (RF-EMFs) as "possibly carcinogenic to humans" based on epidemiological studies [12,13]. However, in 2012, the International Commission on Non-Ionizing Radiation Protection (ICNIRP) found no evidence of long-term health effects from low-frequency electromagnetic field exposure [14]. Interestingly, several studies have shown that LF-EMFs have anticancer effects, with potential benefits in reducing the risk of certain tumors [15] and improving outcomes for cancer patients (Tables 1 and 2). Eventually, MFs may be developed as a strategy for cancer treatment (Fig. 1). This paper aims to conduct a systematic review and analysis of the findings from preclinical studies and clinical trials pertaining to the use of LF-MFs in the treatment of GBM. The focus is on elucidating the potential mechanisms, challenges, future application value of LF-MFs therapy for GBM.

Methods

Search strategy

A systematic search of the literature was conducted to identify published preclinical and clinical trials that reported studies related to LF-MFs and GBM as of May 1, 2023. The key words "MF or EMF (<30 kHz)", "ultra-low radio frequency energy", "glioma" and "GBM" were used to search the literature in the PubMed, Embase, Web of Science, China Knowledge Network (CNKI) and Wanfang databases, and the references of published trials and reviews were also searched for more qualified studies. Studies on the effects of LF-MFs on other organs and systems were excluded from the literature. The search identified 751 unique studies, and we included 41 studies (preclinical studies and clinical trials) that examined the effects of LF-MFs on glioma growth and patient outcomes after screening of title and abstract followed by screening of the full text (Fig. 2).

Data extraction

The following data were extracted from the included preclinical studies: MF type, cell line type, MF parameters, treatment time, effect on tumour, and references. The following parameters were extracted from the included clinical studies: MF type, number

Table 1
The effects of MFs therapy in Glioma.

MF type	Cell line or model	MF parameters	Treatment time	Potential effects	Reference
LF-MFs	CT2A	20 Hz, 100 μT	24 h, 48 h 72 h, 7 d	Increased cell viability Decreased cell activity	[98,111]
		30 Hz, 100 μT	24 h-7 d		
		50 Hz, 100 μT	24 h, 72 h 48 h, 7 d	Increased cell viability The effect was not statistically significant	
	C6 C6 SD model	40 Hz, 2.6 mT	3 h/d, 1–3 d 3 h/d for 7 d	Inhibited proliferation Prolonged OS	[106]
	U251	50 Hz (0.2, 0.4 mT)	24 h	Inhibited cell migration	[112]
	U251, A172	50 Hz, 2 mT	24 h	No effect on the proliferation or viability	[102,113]
	U87	50 Hz, 1 mT	1 h	Decreased sensitivity to irinotecan	[99]
		50 Hz, 7 mT	12–48 h	Inhibited proliferation alone; combined treatment decreased sensitivity to carboplatin	[81]
		50 Hz, 20 mT	24–72 h	Inhibited proliferation, migration and invasion	[101]
	U87MG xenograft model		8 h/d, 200 h in total	Tumour volume decreased compared with control	
	U87, T98G	100 Hz, 10 mT	144 h	Inhibited proliferation, induced oxidative stress, promoted cell differentiation and increased sensitivity to TMZ	[17,19,20]
	U87, LN229, LN18	50 Hz (0.1 mT, 0.5 mT, 1 mT)1 mT (50 Hz, 125 Hz, 200 Hz, 275 Hz)	24 h	Inhibited proliferation and induced apoptosis	[48]
	A172	60 Hz, 5 mT 10 Hz, 5 mT	5–30 h 96 h	Increased DNA damage induced by MMS or H_2O_2 Increased sensitivity to TMZ	[61] [18]
	SF767	60 Hz, 1.2 μT	3 h	Induced genomic and proteomic changes	[114]
LF-PEMFs	T98G	75 ± 2 Hz, 2.0 ± 0.2 mT	1 h	Epigenetic pro-apoptotic effects, increased sensitivity to TMZ	[77]
	A172, T98G	50 Hz, 7 mT	24–120 h	Decreased resistance to TMZ	[50]
	U87	50 Hz 10 mT, 100 Hz 10 mT, 10 Hz 5 mT, 50 Hz 5 mT	2–24 h	Promoted proliferation after 24-h 50 Hz, 10 mT exposure; inhibited proliferation and promoted apoptosis after 24-h 100 Hz, 10 mT and 10 Hz, 5 mT exposure	[16]
	U87MG, SH-SY5Y	75 Hz 2 mT	1−−24 h	Affected autophagy byregulating miR-3a	[115]
	U87MG	75 Hz 1.5 mTor 3 mT	24 h	Augmented the anti-tumor effects of A_3ARs	[62]
	U87MG	75 Hz 1 ± 0.2 mT	15 min	Protected GBM against oxidative stress	[65]
	U-373MG	50 Hz, 3 mT	24 h	Increased the intracellular Ca^{2+} concentration; no effect on cell proliferation or death	[40]
Static and ELF-MFs	C6	50 Hz, 30 μT	24 h	Elevated cytoplasmic superoxide dismutase levels without affecting cell viability	[116]
Sinusoidal MFs	132–1 N1	60 Hz, 30-120μT	3–72 h	Promoted proliferation of astrocytoma cells	[64]
OMFs	BT115, U87, BT175	50–350 Hz, 1–58 mT	2–4 h	Increased the ROS level and cell death	[117]
	GBM, DIPG	200–300 Hz, 80 mT		Disrupted mitochondrial electron transport, inhibited mitochondrial respiration, and promoted oxidative stress, loss of mitochondrial integrity, and apoptosis	[24]

LF-MFs, low-frequency magnetic fields; EMF-ELFs, extremely low-frequency electromagnetic fields; ELF-PEMFs, extremely low-frequency pulsed electromagnetic fields; EMFs, electromagnetic fields; OMFs, Oscillating magnetic fields.

and classification of patients, MF parameters, treatment time, effect on tumour, and references.

Possible mechanisms of LF-MFs on GBM

LF-MFs change the structure of GBM cells

Experimental studies have found that exposure of tumour cells, such as GBM cells, to LF-MFs not only causes changes in the overall structure of the tumour cells, such as cell process degeneration, cell elongation, and cell swelling [16–20], but also changes the subcellular structure of the tumour cells, for example, causing disorder of mitochondrial structure and enlargement of the endoplasmic reticulum and decreasing nuclear chromatin density [21–24]. These changes may be related to the rearrangement of the cytoskeleton and the changes in plasma membrane structure induced by MFs [25–27], which ultimately disturb the biological functions of tumour cells [28]. At present, it is suggested that the plasma membrane, mitochondria and microtubule spindles are the main targets of LF-MFs, which affect the proliferation, differentiation, migration and apoptosis of tumor cells [22,23,29,30].

LF-MFs induce Ca^{2+} influx into GBM cells

Abundant evidence has validated that effects of MFs on ion channels in the tumour cell membrane produce subsequent biological effects mainly through three mechanisms. First, ion channel proteins in cell membranes respond to changes in MFs [31]. Second, the phospholipid bilayer structure of the cell membrane is altered by MFs [32,33]. Third, the Lorentz force, that is the force of MFs on the moving charges, changes the permeability of the cell membrane to charged particles, thus affecting the depolarization of the membranes [34–36]. Among them, the calcium channel and the calcium signaling pathway may be the first step in the coupling of MFs and living organisms [19,37–41]. When calcium channel blockers are administered, the magnetobiological effect is significantly decreased [38,42]. The specific mechanism of LF-MF therapy

Table 2
The effects of MFs on tumour patients.

MF type	Tumour type	MF parameters	Treatment time	Potential effects on patients	Reference
LF-MFs	32 cases of postoperative recurrence of glioma with peritumoral oedema	30 ± 3 Hz, 0.22 ± 0.0 5 m T	48 min exposure daily for 10–14 days	Reduced the area of peritumoral oedema, with an effective rate of 78.13 %	[83]
Rotating LF-MFs	Rehabilitation and treatment of malignant tumours	380 mT/420 mT, 300–500 r/min	1–2 h exposure/d for 42 d	Complete remission or partial remission in tumour patients, with an effective rate of 93.8 %	[118]
	368 cases of malignant tumours	6.7 Hz (400 r/min), 0.4 T	2-h exposure daily / weekly for more than 42 days	Improved the quality of life of patients and prolonged the survival time of patients, with a total effective rate of 54.0–61.1 %	[119–121]
	13 cases of advanced NSCLC	7 Hz (420 r/min), 0.4 T	2-h exposure for 5 d per week, for 6–10 weeks	Relieved the general condition and prolonged the survival time	[122]
	Two cases of recurrent GBM	7.5 Hz (450 r/min), 0.4 T	2 h exposure/d for 42 d	Improved the quality of life of patients	[82]
SPMFs	A case of recurrent anaplastic astrocytoma	10–1000 Hz, < 30 mT	28-day SPMF therapy	Inhibited tumour growth and improved the subjective quality of life	[84]
OMFs	A case of recurrent GBM	> 1 mT	Intermittent OMF therapy for 5 weeks	Tumour volume shrinking by 31 % on day 31	[85]
AM-RFEMFs	One patient with breast cancer brain metastasis and 41 patients with advanced HCC	100 Hz-21 kHz	3 h/d	Prolonged survival with an antitumour effect	[38,109]
Ultra-Low Radio Frequency Energy	26 patients with recurrent glioblastoma	0–22 kHz, 2.5-4μT	Continuous treatment for more than one month	30 %–50 % of patients remained alive at 12 months.	[7,8]

LF-MFs, low-frequency magnetic fields; NSCLC, non-small cell lung cancer; SPMFs, sequentially programmed magnetic fields; OMFs, oscillating magnetic fields; AM-RFEMFs, amplitude-modulated radiofrequency electromagnetic fields; HCC, hepatocellular carcinoma; GBM, glioblastoma.

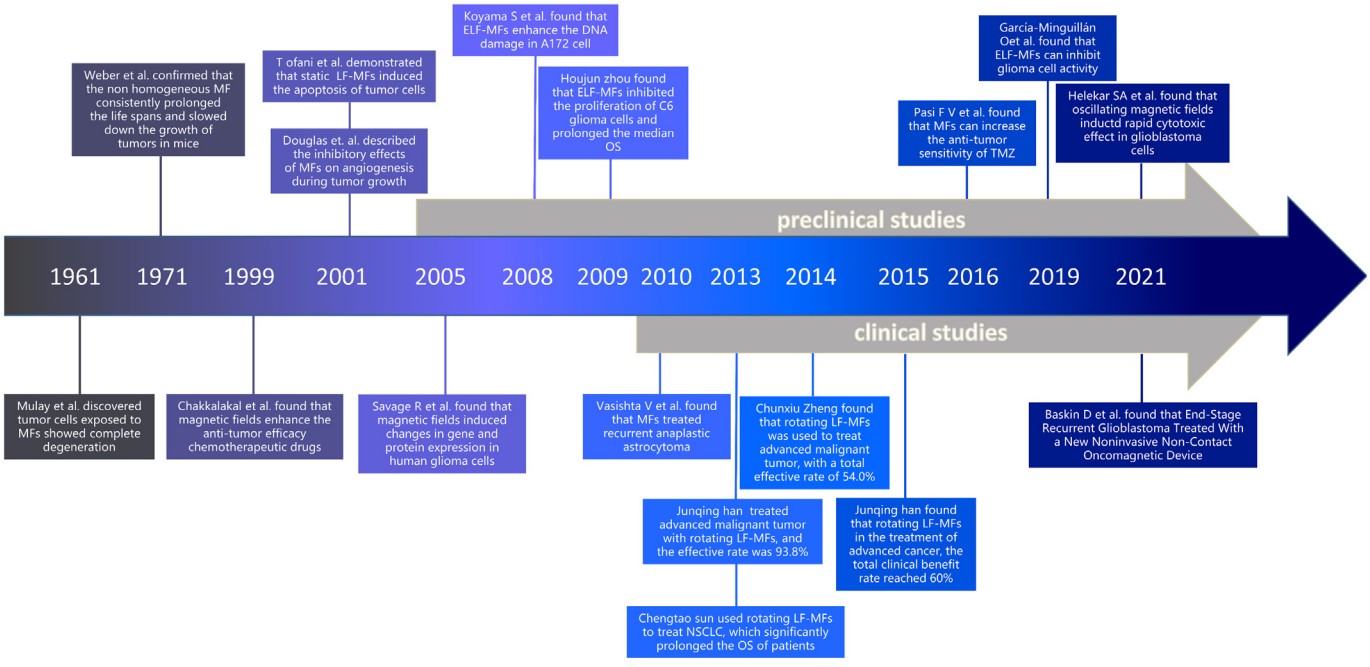

Fig. 1. Historical timeline of the emergence of MFs as novel therapy for tumour patients. In 1961 and 1971, two papers demonstrating the anticancer effects of MFs in vitro and in vivo were published. Following the promising preclinical data, a number of clinical trials investigating the safety and efficacy of MFs for the treatment of malignant tumours, including GBM, were completed (details described at each relevant date), and MF therapy is expected to be approved for the treatment of recurrent and newly diagnosed GBM in the future.

may be that it can exert corresponding biological effects by inducing extracellular Ca^{2+} influx into GBM cells [19,20,40], while changes of the Ca^{2+} signalling pathway have been widely proven to affect the proliferation, differentiation, apoptosis, angiogenesis and gene transcription of tumour cells [38,43] (Fig. 3). In addition, the increase of cytoplasmic Ca^{2+} concentration will lead to the increase of reactive oxygen species(ROS) and lipid peroxidation, while the increase of ROS will in turn stimulate the increase of intracellular Ca^{2+} concentration, the interaction between ROS and Ca^{2+} signaling pathway is bi-directional. ROS can regulate the Ca^{2+} signalling pathway, and the Ca^{2+} signalling pathway is very important for ROS production [44]. Therefore, the production of ROS and activation of the Ca^{2+} signalling pathway may be initial inducible effects induced by LF-MFs in living organisms [19,20].

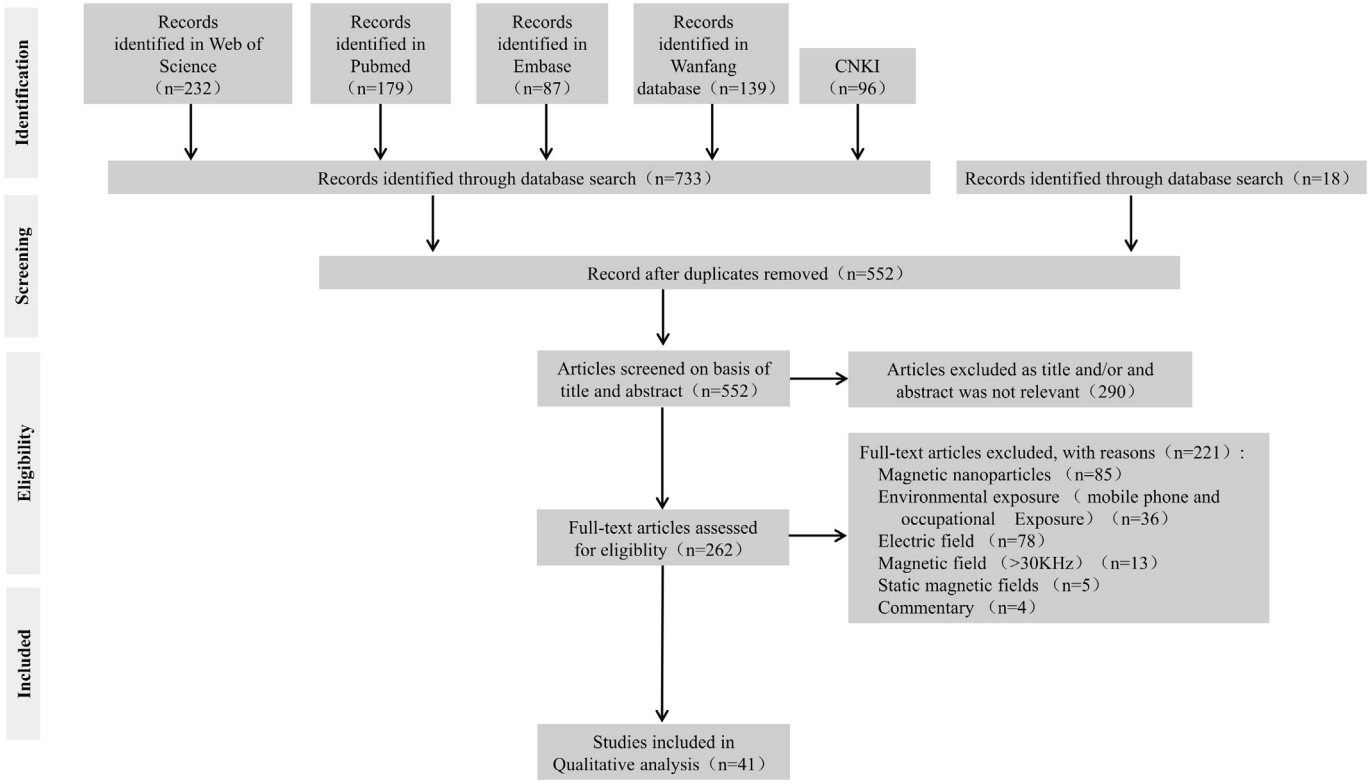

Fig. 2. Flow diagram of the study selection process.

Possible mechanisms underlying LF-MF effects on the regulation of the GBM cell cycle

The cell cycle is divided into the G1, S, G2 and M phases, which are closely linked with cell differentiation, growth and death. Abnormal expression of cyclins can accelerate the DNA replication of tumour cells [45]. Multiple studies have confirmed that MFs regulate G1/S and G2/M phase checkpoints and the cyclin-CDK-CKI signalling network of GBM cells by affecting the Rb-E2F and p53 signalling pathways, causing tumour cell death by damaging their DNA and inducing cell cycle arrest or decreasing GBM cell migration [16,46–50]. Studies have shown that the p53 signalling pathway induces cell apoptosis by arresting cell cycle progression via mediating cyclins [51], and different LF-MF parameters can activate the p53 signalling pathway in GBM cells [16–18]. Moreover, MFs are also capable of mediating the balance between cell cycle progression and apoptosis by activating the p38-MAPK signalling pathway [16,52,53] (Fig. 4).

Potential mechanisms of LF-MFs in regulating the apoptosis of GBM cells

Apoptosis is a process of programmed cell death that is vital in tumour treatment [54]. It is initiated by either the mitochondrial pathway (intrinsic pathway) or the death receptor pathway (extrinsic pathway). The former is mainly regulated by the Bcl-2 family. Through the change of mitochondrial permeability, various proapoptotic and antiapoptotic proteins are released to activate caspases and induce apoptosis [55]. Previous studies have suggested the role of LF-MFs in inducing tumor cell apoptosis via multiple ways [48,56–60] (Fig. 5). LF-MFs increase ROS levels in GBM cells [17–20,61], which further induces apoptosis via the mitochondrial pathway [55]. In addition, as a tumour suppressor gene, p53 is of great significance in cell apoptosis, and LF-MFs not only trigger the apoptosis of GBM cells by upregulating p53 and activating the mitochondrial pathway [17,18,62] but also sensitize GBM cells to temozolomide (TMZ) and inhibit migration by inducing p53-mediated MGMT inhibition [50,63]. In contrast, LF-MFs have also been reported to promote tumour cell proliferation and inhibit apoptosis [16,64], and protect GBM against oxidative stress [65]. The controversial findings regarding the role of LF-MFs in regulating cell apoptosis may be a result of the differences in the frequency, amplitude, exposure time, and cell and/or tissue types used in the experiments [66].

Potential mechanisms of LF-MFs in regulating the ferroptosis of GBM cells

Ferroptosis is an iron-dependent type of programmed cell death that is characterized by lipid peroxidation and the accumulation of ROS [67]. Studies have shown that the proliferation of GBM cells can be inhibited by various drugs via inducing ferroptosis, and the erastin, a ferroptosis activator, sensitizes GBM cells to TMZ [67,68]. Erastin can also induce cell death through the Ras-RAF-MEK-ERK pathway [69,70], while alternating MFs can regulate cell death by reducing ERK phosphorylation in glioma cells [71], it is indicated that MFs are able to determine the fate of GBM cells through regulating ferroptosis. A previous study revealed that differentially expressed proteins of tumor cells exposing to LF-MFs were mainly enriched in the p53 signaling pathway [48]. The p53 gene not only regulates the cell cycle, DNA repair, senescence, and apoptosis [49,51] but also affects ferroptosis in tumour cells by regulating SLC7A11 or iPLA2β [72,73]. It has been suggested that the p53 gene can be used not only as a drug target but also as a target of LF-MFs [17,18,49,70], and activation of the p53 signalling pathway is observed in GBM cells exposed to different frequencies or amplitudes of LF-MFs [17,18]. Therefore, LF-MFs may cause p53-induced ferroptosis to function as effective treatment for GBM (Fig. 6). Furthermore, LF-MFs can induce an increase in ROS levels in GBM cells [17–20,61], and the accumulation of ROS is a crucial indicator involved in ferroptosis [74], while NAC (ROS scavenger) can inhibit ferroptosis induced by H_2O_2 in GBM [75]. Additionally, previous research has revealed that MFs, composed

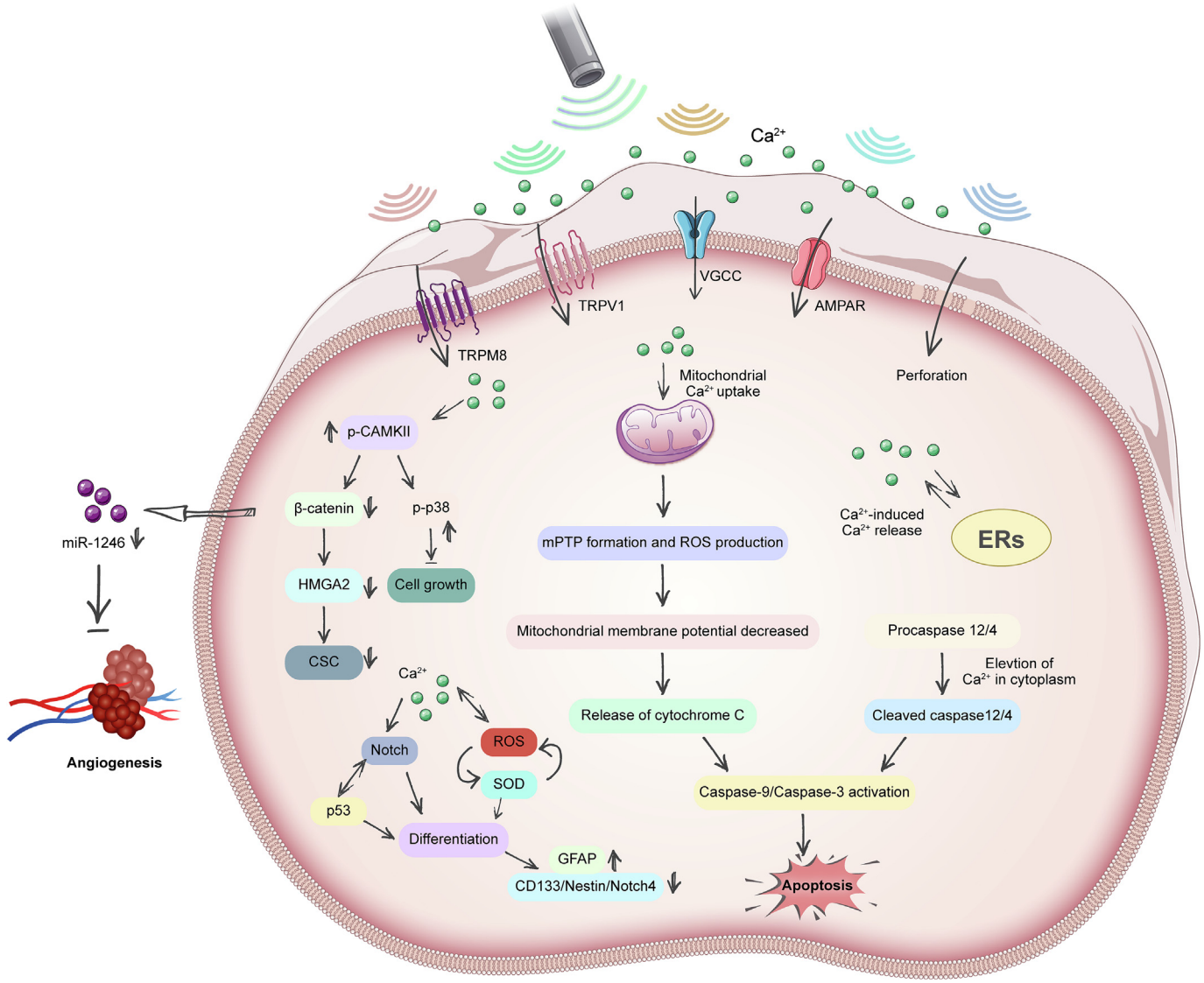

Fig. 3. Possible mechanisms of Ca2 + influx induced by LF-MFs. 1. LF-MFs may induce Ca2 + influx into the cell through ion channel proteins (e.g., TRPV1, VGCC, or TRPM8) in the cell membrane or through AMPAR or by promoting membrane permeability and membrane perforation, and the increase in the intracellular calcium concentration activates endoplasmic reticulum and/or mitochondrial apoptosis pathways to induce apoptosis and the CAMKII-p38 MAPK pathway and decreases HMGA2 expression through CAMKII-mediated β-catenin degradation to block the growth of cancer cells as well as CSCs; furthermore, the change suppresses angiogenesis in the tumour microenvironment by suppressing β-catenin-miR-1246 signalling. 2. Calcium can promote cell differentiation by activating p53 and Notch signalling, and overexpression of SOD induced by ROS increases cell differentiation.

of periodical SMF and ELF-MF modulations with time-averaged intensity, induce apoptosis and ferroptosis in other tumor cells through ROS-mediated DNA damage [76]. Therefore, we speculate that ROS accumulation plays a significant role in LF-MFs-induced ferroptosis in glioblastoma, but the specific mechanisms require further exploration.

The synergistic effect of LF-MF therapy and chemotherapy in GBM

A growing amount of evidence has validated that LF-MFs combined with chemotherapeutic drugs have a synergistic effect in the treatment of GBM; the combination promotes not only apoptosis by increasing the cytoplasmic Ca^{2+} concentration and regulating redox balance but also alleviates the aggravation of GBM by promoting cell differentiation and ultimately remarkably reduces the incidences of chemotherapy-induced adverse events and drug resistance [17–20,77]. Moreover, the combination inhibits the migration of GBM cells and enhances their sensitivity to chemotherapeutic drugs by regulating p53, cyclin D1 and MGMT [50].

Previous research reports that the interaction between the Raf/MEK/ERK and PI3K/AKT signalling pathways enhances the proliferation of tumour cells and their ability to avoid apoptosis [78]. The induction of protective autophagy in cells by inhibiting the Akt/mTOR signaling pathway may be one of the mechanisms by which GBM evades apoptosis and, simultaneously, could be a crucial mechanism in the development of drug resistance [79,80]. But it is noteworthy that MFs can inhibit drug-induced protective autophagy and enhance drug cytotoxicity by downregulating phosphorylated ERK [71] (Fig. 5). Therefore, as a type of adjuvant chemotherapy or radiotherapy, LF-MF therapy enhances sensitivity to chemotherapy drugs and reduces the required dose of antitumour drugs, thus reducing the incidence of adverse events. However, one study showed that exposure to LF-MFs alone inhibited the proliferation of U87 cells, while LF-MF therapy combined with carboplatin downregulated caspase-3 by regulating redox mechanism [81].

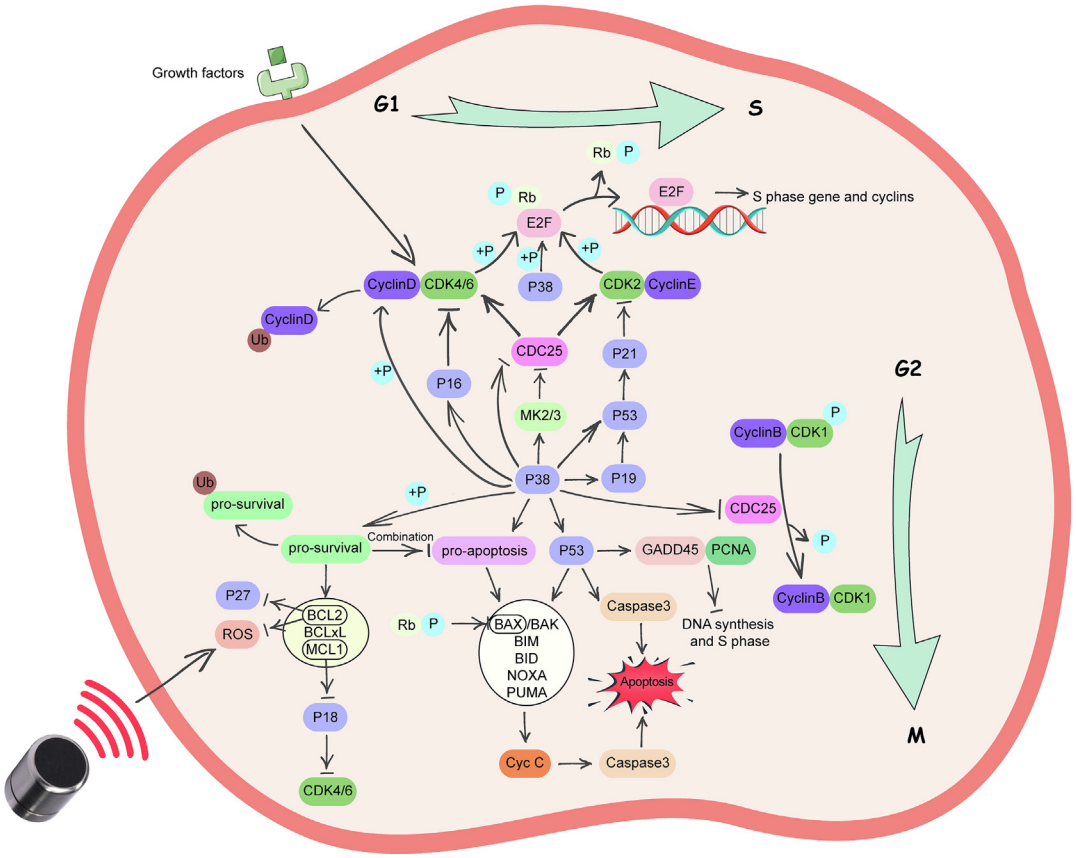

Fig. 4. Regulation of cell cycle checkpoints (G1/S and G2/M) and apoptosis by protein 38-mitogen activated protein kinases (P38-MAPKs) and LF-MFs. 1. LF-MFs activate the p38 pathway: MFs activate P38-MAPKs by inducing DNA damage and p38 activates MAPKAP-K2/3 (MK2/3). Both p38 and MK2/3 can regulate the G1/S and G2/M cell cycle transitions by phosphorylating and inhibiting the CDK-activating phosphatase, CDC25. 2. p38 regulates the G1/S cell cycle transition: Cyclin D binds and activates CDK4 and/or CDK6 in response to growth factor. In late G1, cyclin E binds and activates CDK2, and G1 CDKs hyperphosphorylate Rb, a major tumour suppressor and cell cycle inhibitor, which binds and inhibits E2F transcription factors on chromatin to induce E2F release and subsequent S phase gene activation. CDKs are controlled by CDK inhibitor proteins including the CIP/KIP family (p21, p27, p57) that inhibits CDK2 and the INK4 family (p15, p16, p18, p19) that inhibits CDK4 and CDK6, whereas p19 activates this pathway by activating p21 through p53. Both p19 and p53 are activated by p38. p38 also mediates G1 arrest via phosphorylation of cyclin D, resulting in its degradation, and p38 phosphorylates Rb at different sites, thereby enhancing E2F inhibition. 3. p38 regulates the G2/M cell cycle transition: During G2, phospho-CDK1 is inactive, and cyclin B/CDK1 activated dephosphorylation of CDK1 through CDC25, controlling the transition from G2 phase into mitosis. 4. p38 regulates the BCL2 family: In the context of DNA damage, active p38 upregulates the expression of BH3-only proteins (BIM, BID, PUMA, etc.), and the apoptotic effectors (BAK and BAX) from pores in the outer mitochondrial membrane resulting in cytochrome *c* release, caspase activation, and cell death. Active p38 phosphorylates several anti-apoptotic BCL2 proteins (BCL2, MCL1, and BCLxL), resulting in their ubiquitination and degradation, which leads to cell death. The BCL2 family also regulates the cell cycle; for example, BCL2 inhibits p27, and MCL1 inhibits CDK4/6 by inhibiting P18. However, phosphorylated Rb binds and inhibits BAX until Rb is dephosphorylated. 5. p38 regulates the cell cycle and apoptosis through p53: P53 activated by p38 inhibits CDK2 though activating p21 and increases the expression of apoptotic effectors (BAK and BAX), which activates a pathway upstream of caspase activation to induce apoptosis. p53 can also induce the synthesis of GADD45, which can bind PCNA and inhibit DNA synthesis, thus inhibiting cells from entering S phase.

Clinical and recent research regarding the application of LF-MFs in the treatment of GBM

A large number of studies on the biological effects of LF-MFs on glioma cell lines have shown that LF-MFs can inhibit the proliferation of glioma cells (especially GBM cell lines) through a variety of molecular mechanisms and have synergistic or sensitizing effects when applied with tumour chemotherapeutic drugs (Table 1). These results provide support for the use of LF-MFs in the treatment of gliomas, and some researchers have come up with some surprising results (Table 2). For example, researchers have discovered that LF-MFs can improve the quality of life of patients with recurrent GBM, and alleviate the peritumoral edema in the surrounding areas of recurrent glioma [82,83]. Simultaneously, a patient with recurrent anaplastic astrocytoma underwent pulsed magnetic field. Over the course of 6–36 months of treatment, the tumor gradually reduced, and the clinical symptoms of the patient were alleviated [84]. Additionally, oscillating MF was applied to treat a patient with recurrent glioblastoma, resulting in a 31% reduction in tumor volume by the 31st day, with no apparent side effects [85]. The therapeutic efficacy of LF-MFs in GBM has been widely reported, although the underlying mechanisms remain largely unclear.

The latest research has found that the specific ultra-low radio frequency energy (*u*/RFE®) signal of a molecule (e.g., chemotherapy drug or siRNA) can be recorded by the superconducting quantum interference device (SQUID) [5–8]. The specific *u*/RFE® signal can be amplified and converted into MF energy, which can exert similar effects on GBM cells as anticancer drugs [6]. Based on this discovery, two randomized controlled clinical trials were conducted to treat 26 patients with recurrent GBM using two unique cognates (16 treated with A1A, a *u*/RFE® cognate that mimics the action of paclitaxel by inhibiting microtubule function [7,8]; and 10 treated with A2HU, a *u*/RFE® cognate that was derived from siRNA sequences known to inhibit the expression of CTLA-4 and PD-1 [8]). The fact that 30%–50% of patients in two clinical trials were alive 12 months after starting therapy is encouraging and confirmed that *u*/RFE® signal-based devices are efficacious in the treatment of GBM and have almost no treatment-related side effects. However, the mechanisms have not been deeply studied, so it is

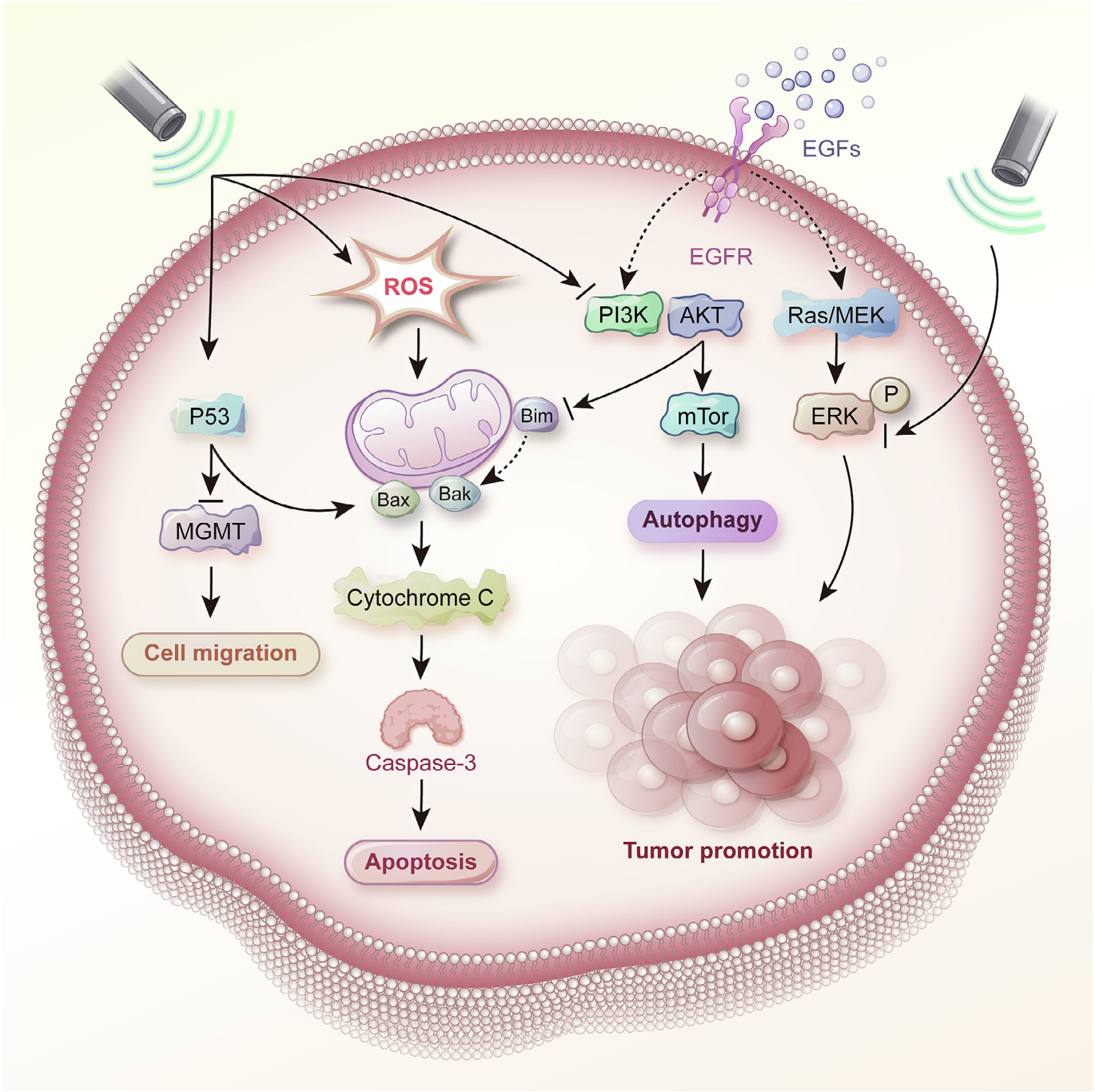

Fig. 5. Possible mechanisms of LF-MFs on apoptosis of GBM. LF-MFs may trigger apoptotic cell death by increasing the p53 level, decreasing ERK phosphorylation, or inhibiting PI3K/AKT signalling pathway; they may also function through the mitochondrial-dependent pathway.

not clear whether LF-MFs can simulate the effects of chemotherapeutic drugs.

Hypothesis on the mechanisms underlying the effects of LF-MFs on GBM cells

The mechanisms underlying the biological effect of MFs have not been fully elucidated due to the complexity of relevant parameters and the diversity of magnetic influences. At present, the following hypotheses have been proposed for explaining the non-thermal biological effects of MFs(Fig. 7). First, electromagnetic induction theory believes that an induced current and electric field are produced by MFs around living organisms. Cells are considered closed circuits in conductors due to the differences in the electromagnetic characteristics of tissues and organs caused by various charged particles. An additional voltage is produced on the cell membrane because of the intracellular induced electric field following the addition of external MFs [29]. The potential change further causes the opening or closing of voltage-sensitive ion channels such as voltage-gated Ca^{2+} channels (VGCCs) [86], thereafter influencing potential biological effects [38]. In addition, compared with non tumor cells, cancer cells have a larger volume and a larger magnetic flux passing through them, resulting in a larger induced

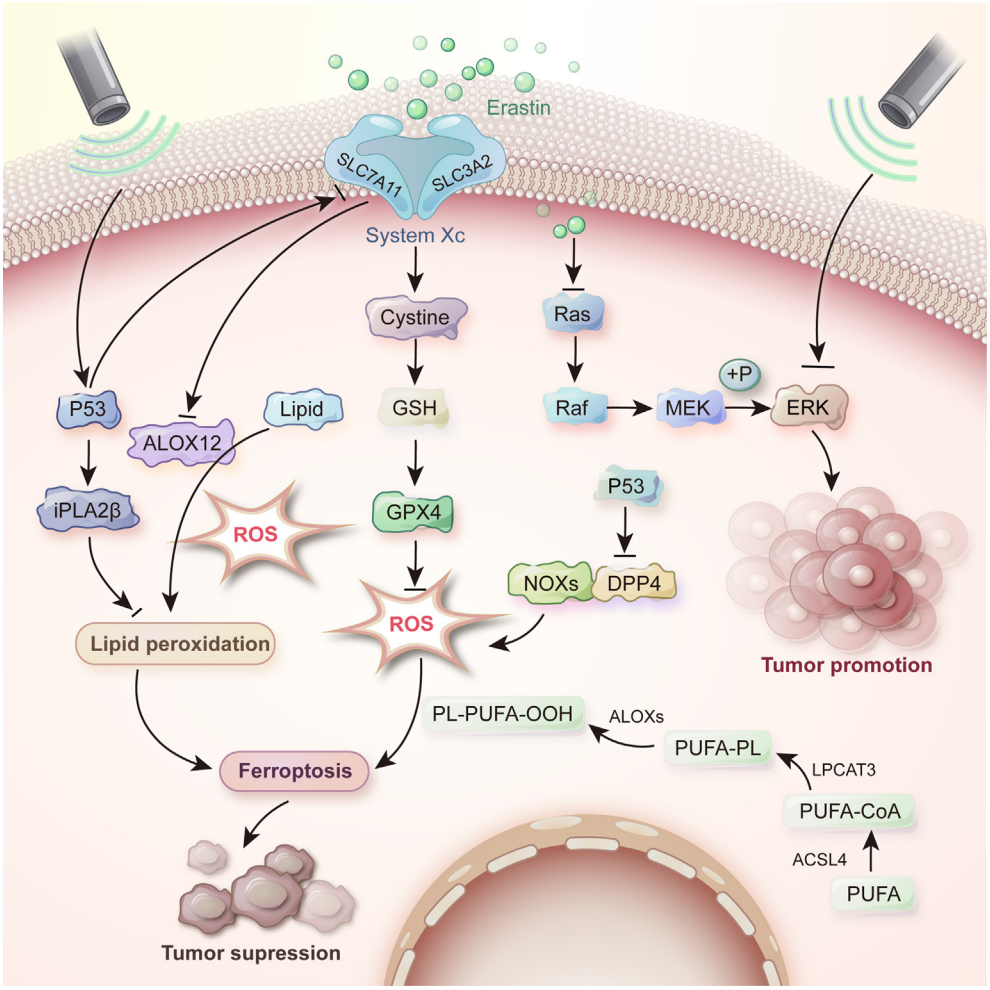

Fig. 6. Possible mechanisms by which LF-MFs affect GBM cell ferroptosis. LF-MFs can reduce the phosphorylation of ERK in GBM to regulate ferroptosis. The increased expression level of p53 induced by LF-MFs mediates ferroptosis of GBM cells through SLC7A11 transcription inhibition or direct binding of p53 with DPP4 to inhibit NOX.

current and induced electric field, and thus, there is a greater impact on cancer cells. Second, the Lorentz force is produced in the moving charged particles of living organisms by MFs. This force influences ion permeability by altering the cell membrane permeability to charged particles, thus regulating biological functions. For instance, the Lorentz force contributes to inducing cancer cell apoptosis by mediating the influx of Ca^{2+} [34–36]. Moreover, the Lorentz force induces conformational changes by altering the charge distribution of molecules or proteins, which will affect their biological activity [7]. For example, such changes can affect the charge distribution of tubulin, which modulates mitosis by enhancing the bond between the monomer and dimer, as well as tubulin polymerization [7]. Third, the magnetic susceptibility and magnetic anisotropy of biological samples determine the effectiveness of MF therapy. For example, the orientation of microtubules and DNA with magnetic anisotropy would change in the MF [87]. The diamagnetic anisotropy of cell membrane lipid molecules after an external MF changes the physical properties of the lipid bilayer, thereby regulating ion channels or receptors on the membrane, such as mechanosensitive ion channels [32,33] or transmembrane signaling of receptor [38,88]. Fourth, MFs exert biological effects by affecting the pairing mechanism of free radicals in tumour cells. Quantum theory posits that paired free radicals or ionic radicals can generate electron spins and magnetic moments, and magnetic interactions can induce changes in the electron spin state and magnetic moment to control biochemical reactions. In addition, as a spin nanoreactor, free radical pairs serve as common chemical keys for magnetism and very low-frequency signals by receiving MFs [89]. By fixing the magnetic moments and reducing electron spins, an additional MF can inhibit or catalyse biochemical reactions by interfering with the conversion of radical pairs from the singlet state to the triplet state [90]. Fifth, magnetobiology is achieved through narrow-band resonance. This theory holds that organisms are selective to MF signals. Tumour cells are believed to be selective to low-frequency alternating MF frequencies, and tumours have tumour-specific frequencies [91]. Such frequency signals can affect the rate of quantum mechanical state transition in biochemical reactions in biological systems. That is, a resonance effect is induced under the same frequency of reactions and magnetic field signals, thus presenting significant biological effects [92,93].

Challenges in using LF-MFs for management of GBM

The clinical use of LF-MFs for management of GBM is still in the early stages despite the plethora of preclinical studies in this field (Table 1). Firstly, MF-induced cell biological effects are the result of the interactions of MFs and cells and are closely related to the parameters of both. However, both biological systems and MFs are very complex, this complexity contributes to the intricate nature of the study of magnetic field biology. Secondly, Electrical characteristics vary greatly in different tissues and cells, resulting in contrary magnetic bioeffects. For instance, MFs can either promote the proliferation of GBM cells [16,64] or inhibit it [48,82]. Thirdly,

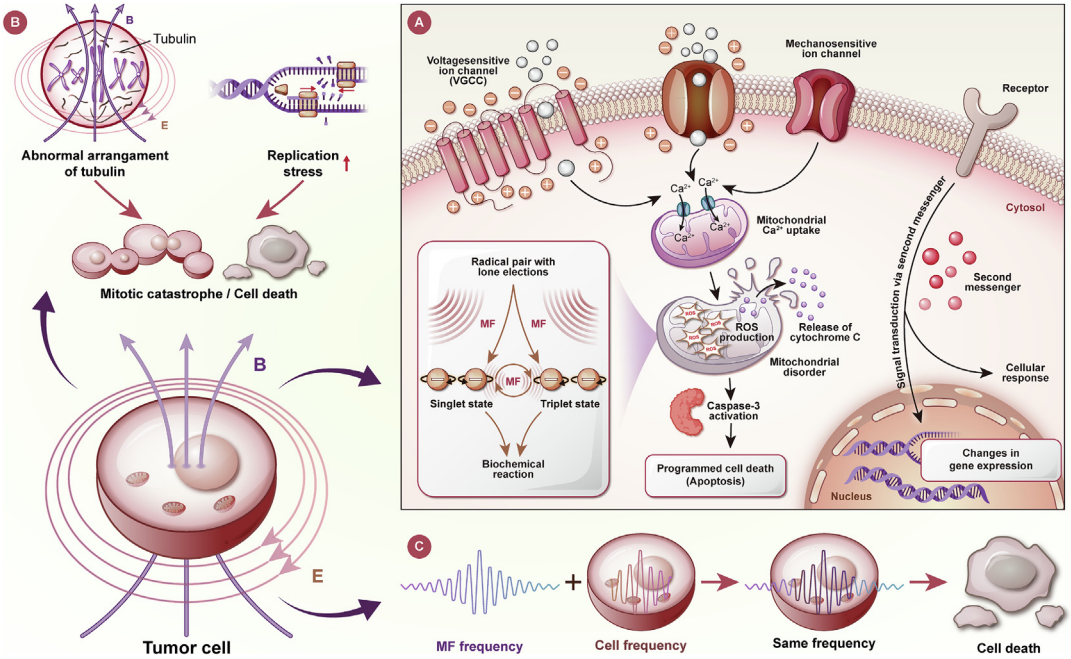

Fig. 7. Hypothesis on the mechanisms underlying the effects of LF-MFs on GBM cells. A. On the one hand, MFs can not only cause the opening of ion channels, such as voltage-gated Ca^{2+} channels (VGCCs) or mechanosensitive ion channels, but also generate Lorentz forces on moving charged particles, ultimately inducing ion influx, for example, MFs contributes to inducing cancer cell apoptosis by mediating the influx of Ca^{2+} through the mitochondrial pathway, and mitochondrial energy metabolism can produce paired free radicals or ion free radicals, MFs can inhibit or catalyse biochemical reactions by interfering with the conversion of radical pairs from the singlet state to the triplet state; On the other hand, MFs transmit the signals into cells by influencing the signal transduction mediated by cell receptors, thereby affecting biological effects. B. MFs can affect the arrangement of tubulin and DNA, thus interfere with mitosis and induce tumor cell death. C.The resonance effect occurs when the frequency of biochemical reaction mechanism is the same as that of magnetic field signal, and then shows significant biological effect.

tumor cells exhibit a obvious window effect on the response to LF-MFs. Reportedly, CT2A mouse glioma cells, originating from murine glioma (astrocytoma) and recapitulating several features of human high-grade glioma [94,95], are sensitive to the frequency of 33 Hz compared to other frequencies, exposure to which reduced cell activity by 40% [96–98]. Therefore, the frequency close to 30 Hz may produce athermo-biological effect, that is, a window effect [98]. However, at the same frequency, the intensity of MFs determines the cellular outcomes, but it is not simply a linear proportional relationship. It is known as the effect of intensity window [16,48,81,99–102]. Additionally, LF-MFs can enhance the sensitivity and anticancer capacity of tumor cells to traditional anticancer drugs [17,19,20,50,63,103–105]. However, they may also inhibit the anticancer efficacy of these drugs [50,81]. Therefore, in summary, it is extremely challenging to find the optimal magnetic field parameters(MF type, intensity, frequency, uniformity, direction and treatment time) for different tumor cells, which also makes their practical use more challenging.

More importantly, the same MF parameters can inhibit the proliferation of tumour cells, but there are contradictions and controversies about their curative effects in tumour-bearing rats [82,101,106]. The reasons for the above may be that 2D cell cultures cannot simulate the actual tumor microenvironment and cellular interactions [107]. As an alternative in vitro cell culture technique, 3D tumor spheroids can simulate various aspects of real glioblastoma, bridging the gap between in vitro and in vivo studies of anti-tumor effects [108]. Similarly, animal models used for in vivo assessment of anti-tumor effects, such as humanized animal models and animal models of species with immune systems more closely resembling humans, should be considered as alternative approaches for in vivo evaluation of magnetic field in GBM treatment.

Conclusions and future perspectives

Up to now, the primary reasons for the poor efficacy of GBM treatment are still the blood–brain barrier and tumor heterogeneity. These clinical challenges drove the research towards the development of more efficient therapeutic solutions for GBM. LF-MFs have gradually garnered researchers' attention and have the potential to become one of the most promising therapeutic approaches for treating GBM, owing to several intrinsic characteristic features such as strong penetration and few side effects. Critical analysis of important literature revealed that LF-MFs have been proven to inhibit the proliferation of tumour cells and induce apoptosis, while enhancing sensitivity to anticancer agents. At the same time, the clinical trials have also validated the excellent therapeutic efficacy of LF-MFs in prolonging OS and improving quality of life in GBM patients.

In cancer treatments involving different magnetic fields, besides the common LF-MFs, the specific magnetic fields detected by various techniques appear to have strong application potential due to the avoidance of spending a significant amount of time on blindly screening effective anti-tumor magnetic field parameters in the future (Table 2). Firstly, "Tumor-specific amplitude-modulated electromagnetic fields" refers to the tumor-specific frequencies present in tumors, which can be measured using non-invasive biofeedback techniques [38,91,109,110]. And Sambad Sharma et al. successfully extracted the tumor-specific amplitude-modulated electromagnetic fields from patients with breast cancer, significantly inhibiting tumor growth and preventing metastasis to the brain [38]. Secondly, "The specific u/RFE® signal" refers to the specific ultra-low radiofrequency magnetic field energy signals that can be detected by SQUID in the chemotherapy drugs or anti-cancer biological preparations, which can produce

therapeutic effects equivalent to those of anti-cancer drugs [7,8]. Thirdly, "Resonance generating fields™ (RGFIELDS™)" describes the utilization of this technology to generate ultra-low-intensity resonance frequencies from the oncogenic or mutated genes in tumors, which possess the capability to inhibit tumor proliferation [92].

Although the reports on the above-mentioned techniques in the treatment of human tumors have been limited, further exploration of these specific frequencies can lead to successful application of these techniques for GBM treatment. Therefore, in the future, if it becomes possible to detect the GBM-specific-frequencies or the magnetic field signals of drugs effective against GBM, the use of a specialized LF-MFs generation device holds the potential for implementing synchronized multi-targeted therapy for GBM. Additionally, with the increased use of whole genome sequencing, proteomics techniques and single-cell sequencing, a variety of oncogenes, proteins or pathogenic tumour cell subsets can be easily identified in tissues of tumour patients, so as to help to identify the optimal ultra-low intensity resonance frequencies needed for customized, personalized therapies that are precise. Furthermore, such strategies can be used to treat animals and patients immediately, which greatly shortens the time it takes for research to move from in vivo animal experiments to clinical trials.

However, this therapy is accompanied by some associated side effects of magnetic field treatment, such as headaches, seizures, amnesia, and aphasia [7,8]. Therefore, proactive preparation for preventing complications is crucial, such as early administration of oral antiepileptic drugs. Additionally, the limited number of cases included in clinical trials restricts the statistical assessment of the treatment's effectiveness and complications. Therefore, it is necessary to further expand the sample size through prospective controlled studies. Simultaneously, selecting appropriate glioblastoma multiforme (GBM) patients based on statistical results, considering factors like tumor size, spatial location, and peritumoral edema, is essential for reducing the probability of complications associated with MFs therapy. Finally, the effectiveness of LF-MFs therapy may be related to the duration of MFs treatment, and prolonging the treatment time may lead to better therapeutic outcomes. This aspect may require further research and exploration.

Although the studies discussed in this review provide a good understanding of the effects of magnetic fields on glioblastoma (GBM), further research is needed to confirm their efficacy in a clinical setting. Due to the strong dependence of treatment outcomes on magnetic field parameters and differences in experimental conditions, comparing results from different literature sources can be extremely challenging. Therefore, detailed and systematic research on the effects of LF-MFs on GBM is necessary in the future. Moreover, the in vitro tests using 2D cell cultures to assess the effectiveness of LF-MFs are limited. GBM patient-derived organoid (PDO) models will be a key direction for future research in investigating the efficacy of LF-MFs.

Compliance with ethics requirements

This article does not contain any studies with human or animal subjects.

CRediT author statement

Yinlong Liu: Conceptualization, Methodology, Resources, Writing–original draft, Writing – review & editing. **Qisheng Tang:** Methodology, Datacuration. **Quan Tao** Validation, Formal analysis, Datacuration. **Hui Dong:** Validation, Formal analysis, Datacuration. **Zhifeng Shi:** Validation, Formal analysis, Writing – review & editing. **Liangfu Zhou:** Validation, Methodology, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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