Review

Effects of Electromagnetic Radiation on Autophagy and its Regulation*

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With the ever increasing application of electronic technology, our exposure to artificial electromagnetic energy is also rapidly increasing. Electromagnetic radiation (EMR) is the fourth largest source of pollution, after air, water, and noise[1]. All populations are now exposed to varying degrees of EMR, and this poses a serious public health threat. The human level of exposure to EMR will continue to increase as technology advances and becomes an integral part of our day to day lives. EMR pollution has attracted widespread concern. Ashford and Porter (1962) discovered the phenomenon of 'self-eating' in the rat liver cells for the first time, which de Duve (1963) christened as 'autophagy.' There is strong interest in the field of biomedicine for pursuing research on cell autophagy. Autophagy is widely involved in many physiological and pathological processes, which is important for regulating cell function and maintaining cell homeostasis. It has been recognized that autophagy functions in the cell damage caused by EMR.

EMR

Concept and Frequency of EMR EMR is defined as waves of oscillating electric and magnetic fields that move at right angles to each other and outward from both the electric (E) and magnetic (B) oscillating field vectors, and is essentially non-ionizing radiation, as it does not carry enough energy to ionize atoms or molecules. The 'Controlling limits for electromagnetic environment' (GB 8702-2014) jointly issued by China's Ministry of Environmental Protection and the State Administration of Quality Supervision, Inspection and Quarantine was implemented on January 1, 2015, and there are corresponding provisions on the scope of exemption on the limit values, evaluation methods and related facilities (equipment) of electric, magnetic and electromagnetic fields (1 Hz to 300 GHz)[2]. The World Health Organization (WHO) established the International Electromagnetic Fields Project in 1996 to assess the scientific evidence of possible health and environmental effects of electromagnetic fields (EMF) in a frequency ranging from 0 Hz to 300 GHz[3]. The EMR discussed in this review has a frequency ranging from 0 Hz to 300 GHz, to which everyone in the world is exposed daily and has a great impact on our everyday life.

EMR of different frequency bands have different physical characteristics with different applications in various fields. This range (0 Hz to 300 GHz) is divided into static (0 Hz) field, which comes primarily from natural and man-made sources such as video displays, clinical diagnostic equipments like MRI and others, that are used in medical diagnostics and treatment as well as magnetic levitation technology or maglev that is being widely used in many applications. Extremely low frequency (ELF > 0 Hz to 300 kHz) fields, which come mainly from the power transmission and electrical equipments and intermediate frequency (IF > 300 Hz to 10 MHz) fields, which includes longwaves, medium waves, and partial shortwaves that are mainly derived from the radio system[3]; Radiofrequency (RF 100 kHz to 300 GHz) fields, which are applied in various facets of daily life, such as telecommunications (eg., mobile telephones), radio and TV transmission, diagnosis and treatments of disease, and in industry for heating and sealing materials[4]. Additionally, RF fields with frequencies ranging from 10 MHz to 30 MHz are called shortwaves, that are mainly used in radio broadcast and over-the-horizon (OTH) radar, while fields with frequencies from 300 MHz to 300 GHz are called microwaves (MW), which are mostly applied in radar and communications technology. Extremely high frequencies (EHF) fields from 100 GHz to 300 GHz are used in weapons systems, security screening, and medicine. Very little is known about the terahertz (THz) band (0.1 THz to 3 THz).

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Biological Effects of EMR The biological effects of EMR change with frequency, intensity, modulation mode and duration of exposure and cell microenvironment. Generally, the possible mechanisms of interactions of EMR with biological systems are often discussed in bioelectromagnetics in terms of thermal versus non-thermal mechanisms. The reported effects of EMR on tissues and organs are generally attributable to the thermal effects, whereas the non-thermal effects of EMR are still been actively researched. The thermal mechanisms are thought to play an important role in acute, high dose exposure causing toxicities. Meanwhile, there is a whole series of biologically important modifications appearing under weak static or alternating EMF action that could be explained only from the view point of non-thermal mechanisms. The thermal and non-thermal characteristics of interactions between EMR and living systems have been discussed elsewhere[5-6].

Multiple systems of the organisms are subjected to the impact of EMR, but the nervous, reproductive, cardiovascular, immune, hematopoietic, and endocrine systems are most commonly involved; thereby, they are extensively studied. Taking the central nervous system and stem cells as an example, we discuss the biological effects of EMR at the overall system and the cellular level, respectively. (1) The central nervous system. The brain is one of the target organs that are sensitive to EMR because the mitochondrial injury occurs here earlier and is more severe as compared to other tissues and organ systems in body. The mitochondrial injury disrupts the energy metabolism in brain and leads to brain dysfunction and structural brain damage because of depleted ATP stores. Epidemiological survey finds that EMR causes fatigue, headache, excitement, dreams, memory loss, and other symptoms of neurasthenia[7]. The studies conducted by us and other researchers have indicated that EMR is likely to cause brain dysfunction and synaptic plasticity injury, as mainly seen in cognitive impairment and structural damage[8-17]. Additionally, EMR has been reported to contribute to neurodegenerative diseases, such as Alzheimer's disease (AD) because of disruption of the signaling pathway[18-20]. In contrast, there is increasing evidence that EMR may help stimulate neuronal functions and protect against cognitive impairment in diseases, such as AD[21-23]. Although, the majority of data comes from animal studies that cannot be yet extrapolated to humans. (2) Stem cells reside in almost all tissues within the human body, and they exhibit various potentials. These cells are very important because they control homeostasis, regeneration, and healing. On one side, accumulating dose of EMR is thought to have devastating effects on stem cell proliferation[24], and on the other side, studies have demonstrated that EMR is able to regulate cellular processes related to decisions about the fate of stem cell via different ways, and properly adjusted values of EMR frequencies, times of stimulation as well as the microenvironmental niche may affect the impact of EMR on stem cell proliferation, differentiation, and migration to achieve the desired therapeutic outcomes[25-27].

The potential health hazards induced by EMR radiation are not negligible, and the public is not completely aware of potential dangers for human health deriving from EMR pollution and exposure to low radiation. Looking at the other side of the coin, EMR has found a wide range of potential clinical applications, as demonstrated by studies reporting the protective effects of EMR on AD and the ability of EMR to decide stem cell fate. Therefore, the underlying mechanisms that decide the biological effects of EMR are important and a controlled use of EMR may be useful for therapeutic purposes.

Autophagy

Concept and Classification of Autophagy Autophagy is an evolutionarily highly conserved, lysosome-dependent cellular recycling pathway existing in eukaryotic cells that transports cytoplasmic components, such as misfolded proteins and damaged organelles to lysosomes for degradation and eventually recycling of the degraded products. Autophagy plays a critical role in cell adaptation, clearance of intra-cellular organisms, antiaging, and tumor suppression. It is mediated through unique cell organelle called autophagosome. There are three main forms of autophagy: macroautophagy, microautophagy and chaperone-mediated autophagy (CMA), which differ based on the pathways of the substrates into the lysosomes[28]. During macroautophagy, cytoplasmic proteins or organelles are wrapped in double-membrane autophagosomes, which then fuse with lysosomes to form autolysosomes. In autolysosomes, the wrapped material is degraded by lysosomal hydrolases and the degradation products are recycled by cells[29]. Unlike macroautophagy, the cytoplasmic components are directly absorbed

through the invagination, protrusion, and septation of the lysosomal membrane in microautophagy and no intermediate vesicles are necessary. In contrast, CMA targets the cytoplasmic proteins with special sequence motifs recognized by heat shock cognate protein 70 (Hsc70), which is a cytosolic chaperone protein, and participates in many other cellular functions. Through the recognition of Hsc70 by lysosomal associated membrane protein 2A (LAMP-2A), which is a receptor on the lysosome surface, Hsc70-tagged proteins are captured by lysosomes, where the protein substrate is unfolded and translocated inside lysosomes for degradation[30]. Yeast and mammalian cells are commonly used as models in the autophagy research, and in many cases, the two are described together. In this paper, the autophagy we discussed focuses on mammalian macroautophagy, which is referred to as 'autophagy' herein.

Biological Process of Autophagy Autophagy is a dynamic process that changes over time and is usually divided into the following four typical stages: (1) First stage involves induction, nucleation, and the formation of phagophores. After the process of autophagy is induced, proteins and lipids are recruited to form a bilayer membranous cup-like structure around the substrates to be degraded in the cytoplasm. This is called as pre-autophagosome structure (PAS) that then develops to form a phagophore, which is also known as an isolation membrane (IM). (2) Second stage involves complete elongation and sequestration of the expanding phagophore to form double-membraned organelle called as autophagosomes. (3) Third stage involves transfer of autophagosome and their fusion with the lysosomes compartment. Once autophagosome formation is complete, autophagosomes move along microtubules in a dynein motor-dependent manner and cluster close to the microtubule-organizing center near the nucleus, where they fuse with lysosomes. It has been proposed that autophagosomes first fuse with endosomes to form amphisomes, before fusion with lysosome. Subsequently, the inner vesicles are released into lysosomes, which results in the formation of monolayer autolysosomes. (4) Fourth stage involves degradation, recycling and reuse of the degraded material. After the formation of autolysosomes, the hydrolytic enzymes in the lysosomes are activated to degrade the contents of the vesicles and the amino acids, nucleotides and metabolites produced are reused by cells. In addition, the contents of the autophagic structure and lysosomes are also partially retrieved.

Mechanisms of Autophagy The genes involved in autophagy regulation are named as autophagy-related genes (*ATG*) and are a family of genes that are evolutionarily conserved. More than 30 *ATG* genes have been identified, and these genes are divided into different functional groups and play different roles in different stages of autophagy[31].

ULK1 complex plays a central role in inducing autophagy, by initiating the autophagosome formation. Mammalian ULK1 (unc-51-like activating kinase 1) complex contains the core proteins ULK1 or ULK2 (hereafter we only refer to ULK1), ATG (autophagy-related protein) 13, ATG101 and focal adhesion kinase interacting protein of 200 kD (FIP200), which is stable and required for the induction of autophagosome formation[32]. After activation of the ULK1 complex, it is transferred from the cytoplasm to the endoplasmic reticulum or other specific locations to form the autophagic prokaryotic nucleus, which then raises the downstream phosohatidylinositol-3-kinase catalytic subunit type 3 (PI3KC3) complex and microtubule-associated protein light chain 3 (MAP/LC3 or LC3) molecules to produce phagophores[33]. ULK1 activates the PI3KC3 complex and promotes autophagy, through phosphorylation of Ser14 in Beclin1[34]. Polyubiquitin-binding protein (p62) also known as sequestosome 1 (SQSTM1), is required both for the formation and the degradation of polyubiquitin-containing bodies by autophagy[35]. ULK1 enhances the binding affinity of p62 for ubiquitin through phosphorylating its corresponding sites[36]. Inhibition of ULK1/2 by knockout or drugs (such as MRT67307 etc.) blocks the occurrence of autophagy[37-39].

The PI3KC3 complex is necessary in the nucleation of the phagophore. The PI3KC3 complex contains the core proteins PI3KC3, p150, Beclin1, and ATG14. One of the key functions of the PI3K complex is the generation of phosphatidylinositol-3-phosphate (PI3P), which is a phosphoinositide that serves as a landmark on the membrane to recruit other factors, such as WD-repeat protein interacting with phosphoinositidesI1-4 (WIPI1-4) and double FYVE domain-containing protein 1 (DFCP1), which mark sites of autophagosome formation and lead to omegasome/cradle development[40-41]. The inhibition of PI3KC3 complex, by the common specific inhibitor of autophagic/lysosomal degradation

3-methyladenine (3-MA), wortmannin, and LY294002, etc., suppresses the autophagic activity.

After nucleation, the phagophore expands by membrane addition, which is accomplished by two ubiquitylation-like conjugation systems: the ATG12-ATG5-ATG16 and ATG8 (MAP1LC3, or briefly LC3 in mammals) conjugation systems[31,42-46], and the ATG8 conjugation system. The ATG12-ATG5-ATG16 conjugation system contains the core proteins ATG5, ATG12, ATG7, ATG10, ATG16, and autophagy related-like 1 (ATG16L1). The ATG8 conjugation system contains the core proteins LC3, ATG3, ATG4, and ATG7. The first of these systems covalently conjugates ATG12 to ATG5 by ATG10 (E2-like), whereas the second system conjugates LC3 to phosphatidylethanolamine (PE) by ATG3 (E2-like) after LC3 has been processed by the cysteine protease ATG4. Both the ATG8 and ATG12 proteins are activated by ATG7 (E1-like). The ATG12-ATG5 conjugate forms a complex with ATG16, which in turn promotes LC3-PE conjugation in an E3-like manner, although it is not essential for this process to occur.

ATG9L1 contributes to vesicle retrieval. ATG9L1, which is a multi-spanning transmembrane protein, is localized to preautophagosomal structure (PAS), endosomes and the trans-Golgi network[47]. As a carrier of lipids, or as a platform responsible for raising other autophagy-related proteins to PAS, ATG9L1 is necessary for the formation of autophagic membranes and the recycling of some proteins[48].

Eeffects of EMR on Autophagy

Research on the effects of EMR on autophagy belongs to a relatively new field, and a consensus has yet not been reached. There have been conflicting reports on the role of EMR in inducing autophagy. Most studies are in support of EMR being positively involved in activating autophagy[49-56], while a few others suggest that there were no observed effects of EMR on autophagy[54,57-58]. Different laboratories have reached different conclusions, which may be explainable by the different parameters of EMR and different objectives of the experiments in the research methods. (Table 1).

Table 1. Effects of EMR on Autophagy

Year	Author	Parameters	Duration	Thermal Effect	Objects	Autophagy State
2016	Pasi F et al.[49]	2 mT, 75 Hz	Not clear	Not clear	Human glioblastoma cell line (T98G)	Activated
2016	Jiang DP et al.[50]	Repetition frequency 100 Hz, 50 kV/m, 100 to 100,000 pulses	Daily exposure for 8 months since 2-month-old	Not mentioned	SD rats, hippocampus	Activated
2014	Marchesi N et al.[51]	75 Hz, 5 mV, 1.3 ms, 2 mT	1 h	Not mentioned	SH-SY5Y	Activated
2014	Liu K et al.[52]	1800 MHz, 4 W/kg	24 h, 5 min on and 10 min off	Rise of approximately 0.08 °C	Mouse spermatocyte-derived GC-2 cells	Activated
2014	Koshkina NV et al.[53]	13.56 MHz, 600 to 900 W	2-5 min	Not mentioned	Panc-1 and AsPC-1	Activated
2014	Curley SA et al.[54,55]	13.56 MHz, 900 W	5 min	Elevation to approximately 46 °C	Panc-1, AsPC-1, MDA PATC-3	Activated
2012	Cao HL et al.[56]	12, 18, and 21 mV/cm^2	10 min	None	A549 cells	Activated
2015	Zuo WQ et al.[57]	1800 MHz, 4 W/kg	24 h, 5 min on and 10 min off	Rise by about 0.08 °C	Spiral ganglion neurons (SGN)	Unchanged
2015	Golbach LA et al.[58]	320, 730, 880, and 2600 Hz, 300 μT	4 h	Not mentioned	Human neutrophils	Unchanged
2014	Curley SA et al.[54]	13.56 MHz, 900 W	5 min	Elevation to approximately 46 °C	HPDE cells	Unchanged

The effects of EMR on autophagy are mainly achieved through its non-thermal effects. Curley SA et al.[54] exposed three human pancreatic cancer cell lines (Panc-1, MDA PATC-3, and AsPC-1) to the RF field at 13.56 MHz for 5 min or to conventional hyperthermia (HT) at 46 °C. RF treatment affected mitochondrial function in cancer cells more than HT treatment did, and unlike HT treatment, it was followed by an increase in autophagosomes in the cytoplasm of the cancer cells, and subsequent growth arrest of the cancer cells. The obtained data indicated that the effects of RF treatment were not limited to its hyperthermic property. Cao HL et al.[56] exposed human lung cancer A549 cells to the MW source for 10 min under ice bath conditions at doses of 12, 18, and 21 mV/cm^2, respectively. It was found that the non-thermal effects of MW could induce autophagy in human A549 cells, while no change in temperature occurred before and after radiation, as confirmed by a thermometer. Some researchers have been supportive of the idea that the non-thermal mechanisms contribute to the biological effects of EMR, especially wide band EMR. This conclusion is also supported by results of studies that show that the temperature was precisely controlled[54,59-60]. Therefore, we believe that the enhanced autophagy induced by EMR is likely achieved through its non-thermal characteristics, rather than the thermal characteristics.

The effects of EMR on autophagy are related to cell types. In the study of Curley SA et al., malignant and nonmalignant cells of pancreatic origin were both exposed to the RF field with same radiation intensity. RF treatment selectively inhibited the proliferation of tumor cells by inducing autophagy, while these effects were negligible in nonmalignant cells. The results indicate RF can provide a non-invasive treatment option to treat malignancies due to its tumor-specific cytotoxic effect through stimulation of autophagy in tumor cells. These effects exceed the hyperthermic properties of the RF field. All these outcomes require further investigation of the biological effects of RF treatment to stimulate the development of novel non-invasive approaches for cancer treatment using electromagnetic fields[54].

The effects of EMR on autophagy are also affected by cellular microenvironment. Spiral ganglion neurons (SGN), which were obtained from neonatal (1- to 3-day-old) Sprague Dawley® (SD) rats, were treated with lipopolysaccharide (LPS) and then exposed to radiofrequency EMR (RF-EMR) at a specific absorption rate (SAR) of 4 W/kg. It seems that the occurrence of autophagy caused by EMR will increase in an LPS-induced inflammation *in vitro* model, while it did not have any effect on normal SGN not treated with LPS[57]. The sensitivity of cell autophagy to EMR changes with the microenvironment of the cells possibly. LPS, exemplified in this study, is also a known endotoxin and is often released by gram-negative bacteria that infect the body and increases the sensitivity of EMR exposed cells to autophagy. Other factors that can alter the cellular microenvironment may also cause changes in the sensitivity of autophagy to EMR, but further studies are still needed.

The effects of EMR on autophagy occur in a dose-dependent manner. Liu K et al.[52] exposed mouse spermatocyte-derived GC-2 cells to 1800 MHz Global System for Mobile Communication (GSM) signals in GSM-Talk mode at SAR values of 1 W/kg, 2 W/kg or 4 W/kg for 24 h intermittently. The results indicated that the activity of autophagy increased in a dose-dependent manner with RF exposure and peaked at the SAR value of 4 W/kg. In the study of Cao HL et al., within the dose ranging from 12 to 21 mV/cm^2, the activation of autophagy in A549 cells was also significantly enhanced in the exposed group with the increase of the radiation dose[56]. The results point to that the effects of EMR on cell autophagy obey a dose-response relationship, and to be specific, autophagy is more active with increasing doses of EMR under certain conditions.

The effects of EMR on autophagy obey a time-response relationship. In the study by Liu K et al. to study the protective effect of autophagy on mouse spermatocyte exposed to EMR, the LC3-II flux increased within 48 h after exposure, which indicates the increased activity of autophagy in this time range[52]. The time-response manner in the rate of autophagy induced by EMR may be affected by the exposed objects and the radiation conditions. To find out how autophagy alters after EMR is conducive in explaining the physiological roles played by autophagy in the effects of EMR.

In summary, the effects of EMR on autophagy are mainly achieved through non-thermal mechanisms, and these effects are affected by the cell types, as well as the cellular microenvironment and they follow dose-dependent and time-response patterns. However, the research field is still in its early stage, and there is a need for further studies and discussion.

Biological Effects of Autophagy in EMR-induced Cell Damage

The biological effects of autophagy function in two ways. For decades, autophagy has been debated as an active cell death pathway. Autophagy was usually observed in dying and starving cells, and is also known as autopathic cell death (ACD) or type II programmed cell death that occurs without chromatin condensation and is accompanied by large-scale autophagic vacuolization of the cytoplasm. In addition, there were studies that uncontrolled autophagy was shown to lead to a caspase-dependent and independent cell death under some conditions. However, more recent data support that autophagy exerts a cryoprotective effect and promoted cell survival. One of the key roles played by autophagy is to eliminate protein aggregates and damaged organelles, which may promote energy homeostasis or reduce the generation of reactive oxygen species (ROS) in different cases[61-62].

When cells were damaged by EMR and the injury could be compensated, activated autophagy functioned to protect cells and promote their survival. As mentioned before, in the study of Liu K et al., GC-2 cells were subjected to GSM signals at SAR values of 4 W/kg for 24 h. To understand the role of ROS-mediated autophagy on cell survival after RF exposure, 3-MA was used to block autophagy and then apoptosis was determined. Compared to RF exposure alone, co-treatment with 3-MA increased the percentage of apoptotic cells. These data indicated that autophagy may play an important role in the adaptive response mechanism to protect against EMR and ensure cell survival[52].

When the EMR-induced damage was beyond the compensatory ability of cells, autophagy might be excessively activated and mediate cell death. Curley SA et al.[54] exposed cancer and nonmalignant cells of pancreatic origin to the RF with the average power of 900 W, for 5 min. Only RF treatment caused declines in cancer cell viability and proliferation alone with the elevation of autophagosomes in the cytoplasm of cancer cells, whereas the effects of RF treatment were negligible in nonmalignant cells. The results indicated that autophagy activation played a role in the decrease of the activity of pancreatic cancer cells induced by EMR. However, this study did not set up an autophagy intervention model and further explored the relationship between the activation of autophagy and the changes of tumor cell viability after radiation. Therefore, the numbers were not conclusive.

Mechanisms of EMR Affecting Autophagy

ROS ROS includes oxygen free radicals, such as superoxide anion radical ($O_2^{\cdot-}$), hydroxyl radical ($^{\cdot}OH$), and nonradical oxidants, such as hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$). Under physiological condition the balance between ROS production and its scavenging is strictly controlled and plays a part in cell homeostasis. EMR causes increased production of ROS, which in turn damages the mitochondrial respiratory chain resulting in electronic leakage (the main source of ROS) that ultimately leads to a vicious cycle[63-67]. Additionally, oxidative modification and increased ROS bring about dysfunction of cellular physiology, such as the activation or suppression of multiple signal paths, which is believed mostly to contribute to cell damage[68,69].

ROS contribute to the activation of autophagy after EMR. In the experiment of Liu and others, as described previously, pretreatment with antioxidant N-acetyl-cysteine (NAC) reduced the conversion of LC3-I to LC3-II and decreased the degradation of p62 in the RF-exposed group, which suggests that the enhanced autophagy by RF was achieved by increased ROS production[52]. There is strong consensus that increased ROS promotes the occurrence of autophagy, and Li Lulu et al.[70] have discussed the interactions between ROS and autophagy as well as the underlying mechanisms in detail. Briefly, the internal regulatory mechanisms of autophagy by ROS can be summarized as transcriptional and post-transcriptional regulation, which includes various molecular signal pathways such as ROS-FOXO3-LC3/BNIP3-autophagy, ROS-NRF2-P62-autophagy, ROS-HIF1-BNIP3/NIX-autophagy, and ROS-TIGAR-autophagy.

miRNAs microRNAs (miRNAs) are a class of endogenous, 22-24 nucleotide-long noncoding RNA molecules, that affect protein synthesis by impairing both the stability and translation of specific mRNAs, and they are involved in many processes in cells, including autophagy[71-72].

Down-regulated miR-30a activates autophagy after EMR through modulating the expression of *autophagy-promoting gene beclin1*. Among the previously identified microRNAs (miRNAs) in rat hippocampus that were sensitive to microwaves, we found that miR-30a was significantly down-regulated after exposure[73]. A study on the effects of a pulsed low-frequency EMF (LF-EMF, 75 Hz, 2 mT,

1.3 ms) exposure on miR-30a expression and downstream on *beclin1* mRNA and protein levels was conducted in human neuroblastoma cells, and the researchers found that LF-EMF induced a significant reduction of miR-30a with a concomitant increase in *beclin1* transcripts and its corresponding protein, which resulted in the activation of autophagy[51]. Studies have suggested that miR-30a negatively regulates the expression of *beclin1* and Atg12, which results in a decreased level of autophagy[74-80]. Thus, down-regulated miR-30a may be an important mechanism of autophagy activation induced by EMR.

Prospects Currently, the potential health hazards caused by EMR have attracted great attention from public as well as the scientific community around the world. Studies have mainly focused on mechanisms of EMR injuries at the cellular and molecular levels. Autophagy, is a topic of strong interest in the biomedical field, and it contributes to basal cellular and tissue homeostasis and is essential for physiological responses to stresses. The research on the effects of EMR on autophagy and the roles of autophagy in EMR-induced injury is still in its infancy, and there are many problems that have yet to be solved. Due to the complicated parameters of EMR, the results from different laboratories are not comparable, which creates obstacles for the formulation of any guidelines and development of this field. Furthermore, the effects of EMR on autophagy are not only related to the EMR itself but also affected by cell types and cell microenvironments. However, there has been little research in this area, and the conclusions made by this study should be carefully evaluated. The effects of EMR on autophagy, the relevant regulatory mechanisms and the roles of autophagy in the EMR-induced damage will be investigated in future research.

Author Contributions HAO Yan Hui participated in the design, collected and analyzed the data. ZHAO Li and PENG Rui Yun conceived the review and helped to draft the manuscript. All authors read and approved the final manuscript.

Conflict of Interest No conflict of interest to declare.

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