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Preface

With the development of modern technology, people are exposed to increased magnetic fields. Here we specifically focus on static magnetic field (SMF), which means the magnetic field strength does not change over time. SMF is different than the dynamic or time-varying magnetic field. For example, cellular phones or microwaves are pulsed magnetic fields with different frequencies, which belong to dynamic magnetic fields and will not be discussed in this book. Most commonly seen SMFs are the household magnets, the core component in magnetic resonance imaging (MRI) machines in hospitals, magnetic elevation trains, as well as the weak but widely existed earth magnetic fields. They are all SMFs, with different intensities. The magnetic field intensities people are exposed to vary from 0.05 mT (earth magnetic fields) to almost 10 T (high-field MRI in preclinical research).

To set up a safety standard for human exposure to SMFs, there are many related researches studying the effects of the magnetic fields at molecular, cellular, animal, as well as human body levels. Accordingly, WHO (World Health Organization) and ICNIRP (International Commission on Non-ionizing Radiation Protection) have published some guidelines for the SMF exposure of human bodies to ensure that people are not overexposed. At the same time, magnetic therapy, which was never in the mainstream medicine, has wide applications by many people as alternative or supplementary treatments. Most of them are currently used in pain relief, as well as some other nonurgent applications. However, the magnetic therapies in general are not substantiated by enough sound scientific proofs. Only with proper and detailed knowledge, people could try to maximize the proper usage of SMFs in our daily lives without hurting our bodies. We need to undertake serious and practical research into the magnetic effects on the biological systems so that we will have practical knowledge, both medically and scientifically.

It should be mentioned that we will not cover magnetic nanoparticle studies, which have a fast growing trend and have promising therapeutic applications for future medical treatments; we will focus on the externally applied magnetic fields on human and animal objects, but not the magnetic fields produced within living

organisms (biomagnetism). We try to cover most aspects of biological effects of SMFs on human cells but also want to apologize for any missed research findings that are not included in this book. Our goal is try to provide people with an overview of the current understanding of the biological effects of SMFs and hope to encourage more scientists to get involved in this field so that we can get a clearer view of this field in the near future.

There are three contributors to this book. All of them have done and are still currently working on the biological effects of magnetic fields. They are:

Dr. Xin Zhang, principal investigator at the Chinese Academy of Sciences, High Magnetic Field Laboratory, Hefei, China (Chaps. 1, 2, 4, and 6)

Dr. Kevin Yarema, associate professor of biomedical engineering at the Johns Hopkins University School of Medicine, USA (Chaps. 3 and 7)

Dr. An Xu, principal investigator at the Institute of Technical Biology and Agriculture Engineering, Hefei Institutes of Physical Science, Chinese Academy of Sciences, Hefei, China (Chap. 5)

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Contents

Abstract

With the development and growing popularity of modern appliances, including MRI in the hospitals, the potential impact of magnetic fields on human health is invoking increasing concerns. At the same time, static magnetic field (SMF) has been used in the clinical treatment of tumors and other diseases since decades ago. However, there are still some reservations and uncertainties about the exact effects of magnetic fields on human bodies, which are largely due to the differential biological effects reported in the literature. These experimental inconsistencies are mainly caused by variations such as different magnetic field types, intensities, treatment time, as well as biological samples examined. The purpose of this book is to review scientific evidences and summarize the emerging topic about the effects of SMF on biological samples ranging from single molecules, subcellular compartments, and cells to whole organisms, as well as the potential application of SMF in clinical treatment of cancer and other diseases. It will help clarify some dilemmas in this field and encourage further investigation in order to achieve a better understanding of the biological effects of SMF, aiming for a rational application of SMF in clinical diagnosis and therapy in the near future.

Part I
Introductory and Background Information

Chapter 1
Parameters of Magnetic Fields and Their Differential Biological Effects

Abstract This chapter summarizes different parameters of the magnetic fields, including magnetic field types, intensity, homogeneousness, field direction and exposure time. Various factors that contribute to the differential effects of magnetic fields on biological samples, which lead to the seemingly lack of consistencies in literature will be discussed.

Keywords Static magnetic fields (SMF) • Pulsed magnetic field (PMF) • Magnetic field intensity • Gradient magnetic fields • Differential effects of magnetic fields

1.1 Introduction

The biological effects of magnetic fields can be directly influenced by different parameters. Depending on whether the magnetic intensity changes over time, magnetic fields can be divided into static magnetic field (SMF) or dynamic/time-varying magnetic field, which can be further divided into different categories according to their frequency. Depending on the magnetic field intensity, there are weak, moderate, strong (high) and ultra-strong (ultra-high) magnetic fields. Depending on the magnetic field spatial distribution, there are homogeneous or inhomogeneous magnetic fields. Here we will discuss the major variations in magnetic field parameters and their differential effects on biological objects.

1.1.1 Static Magnetic Field vs. Dynamic Magnetic Field

When the magnetic field intensity does not change over time, it is called "static magnetic field". In contrast, if the magnetic field strength changes over time, it is called "dynamic magnetic field" or "time-varying magnetic field". Pulsed magnetic fields (PMFs) are the most commonly seen dynamic magnetic fields, such as the 50 Hz or 60 Hz power frequency alternating current (AC) magnetic fields and radio-frequency magnetic fields. Over the past few decades, there are emerging concerns about the growing exposure to these electromagnetic fields, which also encouraged

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Fig. 1.1 The international EMF (electromagnetic fields) project. The international EMF project is to assess health and environmental effects of human body exposure to static and time-varying electric and magnetic fields. It includes the most commonly seen electromagnetic exposure (Figure and information were from the WHO website http://www.who.int/entity/peh-emf/project/en/)

a huge amount of epidemiological and laboratory studies. Accordingly, WHO (World Health Organization) has initiated the International EMF (electromagnetic fields) project to assess health and environmental effects of exposure to static and time-varying electric and magnetic fields in the frequency range 0–300 GHz (Fig. 1.1).

From the current research, it is obvious that cells respond very differently to magnetic fields with different types and intensities. For example, a 50-Hz, 1 mT PMF could increase rat pituitary GH3 cell proliferation (Grassi et al. 2004) but a 0.5 T static magnetic field obviously inhibited GH3 cell proliferation (Rosen and Chastney 2009). In addition, multiple evidences showed that different types of magnetic fields of the same magnetic field intensity could produce totally different effects on the same sample examined. For example, a 0.4 mT 50 Hz and a 2 μT 1.8 GHz PMFs both increased epidermal growth factor receptor (EGFR) phosphorylation, which were reversed by incoherent ("noise") magnetic fields of the same intensities (Wang et al. 2010; Li et al. 2012). Although the mechanism of how the incoherent magnetic field reversed the effect of PMF is still unknown, it is clear that the magnetic type can directly affect the field effects.

Since PMFs have variable parameters, such as field intensity and frequency, it is relatively difficult to study the biological mechanisms of magnetic effects comprehensively and systematically. For example, it was shown that PMFs with different frequencies can have diverse effects on cell proliferation. In comparison to the

time-varying/dynamic magnetic fields, SMFs are more suitable to study the fundamental biological mechanisms because they have less changeable parameters. The most commonly exposed SMFs are the permanent magnets, such as the magnets on household refrigerators, toys and accessories, which are usually not very strong (below 1 T). In addition, the core component of the MRI (Magnetic Resonance Imaging) machine in the hospital is a strong magnet, which generates a SMF with field intensities usually range between 0.5–3 T in most hospitals nowadays.

SMFs usually generate much milder effects on human beings compared to time-varying magnetic fields and many of the effects are actually beneficial. Therefore we are much more interested in SMFs, and this book will only focus on discussing the biological effects of SMFs. For people who are interested in dynamic electromagnetic fields from power lines, microwave ovens and cell phones, there are many other resources, including some books, such as *Biological effects of magnetic and electromagnetic fields* by Shoogo Ueno (1996), *Biomagnetics: Principles and Applications of Biomagnetic Stimulation and Imaging* by Shoogo Ueno and Masaki Sekino (2015), *Electromagnetic Fields in Biology and Medicine* by Marko S. Markov (2015) as well as some other reviews (Simko and Mattsson 2004; Funk et al. 2009). In addition to the published books and ICNIRP (International commission on non-ionizing radiation protection) guidance in 2014, recent works in 2016 also show that there are no detrimental effects of radiofrequency PMFs on research animal models at the levels that people are exposed to (Gao et al. 2016; McNamee et al. 2016). Overall, as far as we know, there is still not enough evidence to show that the dynamic magnetic fields that many people are concerned have definite adverse impacts on human health. However, more careful and long-term investigations in both epidemiology and laboratory research are certainly needed to draw an unambiguous conclusion.

1.1.2 Different Magnetic Field Intensities: Weak, Moderate, High and Ultra-high Magnetic Field

According to their magnetic flux intensity, SMFs used in the biological effect studies could be classified as weak (<1 mT), moderate (1 mT to 1 T), high (1–20 T) and ultra-high (20 T and above).

$$1\ \text{T (Tesla)} = 10{,}000\ \text{G (Gauss)}$$
$$1\ \text{G} = 100\ \mu T$$

It should be mentioned that the classification of magnetic fields varies between different research areas. Therefore people should always clearly label the magnetic field intensity that they use. Despite the classification, with the development of modern technology, people nowadays have much increased exposure to various SMFs. Figure 1.2 shows some examples of different SMF intensities, including the

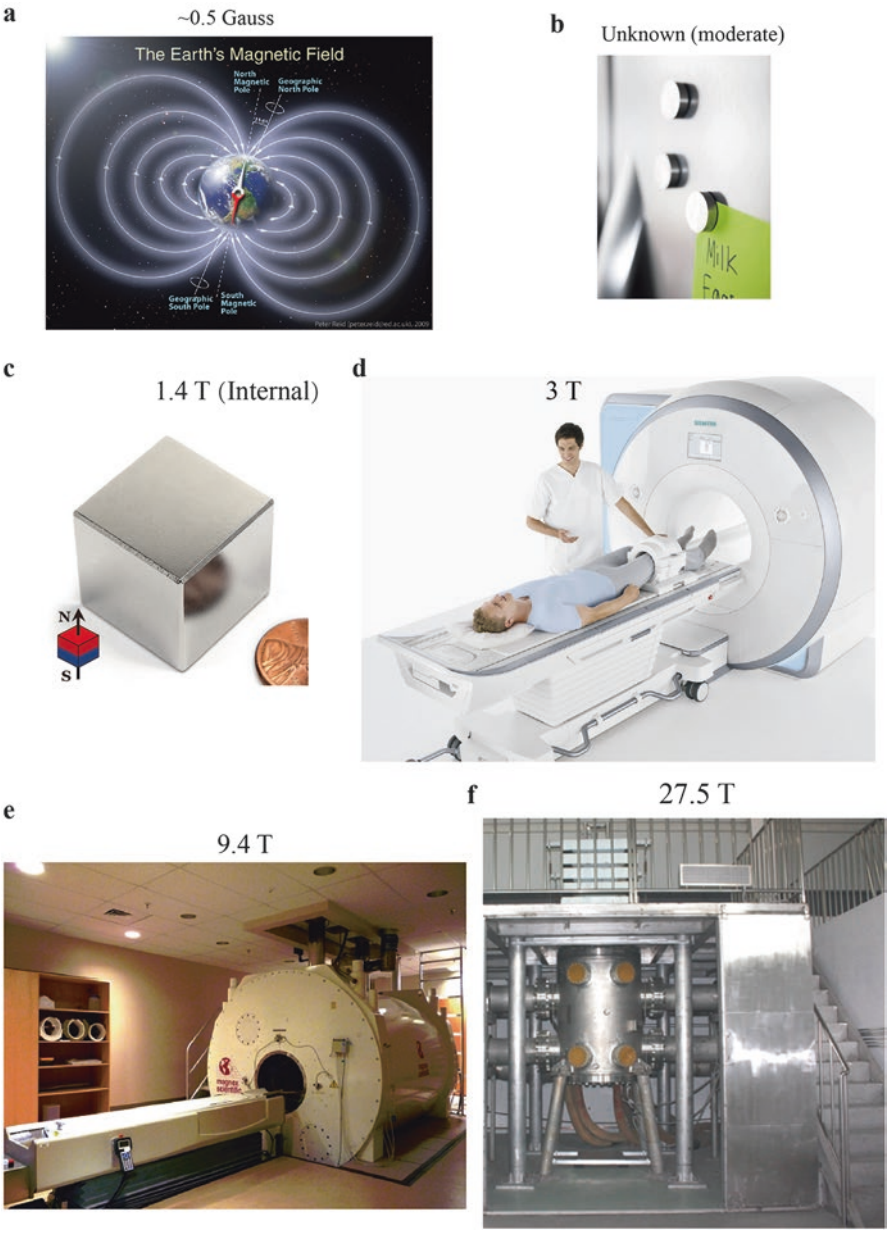

Fig. 1.2 Static magnetic fields of different intensities. (**a**) Earth magnetic field (~0.5 Gauss, 50 μT). The picture was from NASA website. **(b)** Small permanent magnets for household uses. The picture was from amazon.com, by MapMagnets. It shows a few small permanent magnets (22 × 6 mm) with unidentified magnetic field intensities. They are frequently used on whiteboards, refrigerators, and office cabinets. **(c)** A square shaped permanent magnet (Grade N50 with 14,200 internal Gauss=1.4 T). Its relative dimension can be compared to the penny by its side. The picture was also from amazon.com, by CMS Magnetics. **(d)** A 3 T MRI from SIEMENS. The picture was from SIEMENS website. **(e)** A 9.4 T MRI at University of Minnesota Medical School with 65 cm bore size that can be used on human head. **(f)** A water-cooled magnet in the Chinese High Magnetic Field Laboratory. It can provide up to 27.5 T ultra-high SMF

ubiquitous earth magnetic field of weak intensity, permanent magnets of various intensities (usually moderate intensity), MRI machines in hospitals and research institutes with high SMFs, as well as ultra-high magnets currently mainly used for research purposes (Fig. 1.2). It should be mentioned that the use of high and ultra-high magnetic fields is expanding quickly in recent years, which is no longer limited to the conventional investigation of condensed matter physics and material science, but expanded to diamagnetic materials, such as the majority components of our human bodies.

Because of the public sensitivity, the question of the possible effects of SMFs of 0.5–7 T, the range of the MRI machines in current hospitals as well as in preclinical researches (Fig. 1.2d), on human health is of paramount interest. The MRI process involves a combination of non-ionizing SMFs, gradient magnetic fields and pulsed radiofrequency fields. Currently the MRI scanners are considered to be safe and studies show that 7 T high field MRI is well tolerated by humans without excessive discomfort (Miyakoshi 2006; Simko 2007; Heilmaier et al. 2011), DNA damage (Fatahi et al. 2016) or other cellular abnormalities (Sakurai et al. 1999). At the same time, since stronger magnets could give better resolution and more detection possibilities, the researchers and engineers are currently investigating on building MRI machines with stronger magnetic fields. In fact, there are currently 9.4 T MRI machines (Fig. 1.2e) not only used on animal studies in research but also on healthy human volunteers at preclinical stage (Adair 2000; Miyakoshi 2005; Zhang et al. 2015). Moreover, 21.1 T MRI has already been developed and applied on samples such as mouse brain (Schweitzer et al. 2010; Schepkin et al. 2014; Nagel et al. 2016) (see Chap 2, Fig. 2.3).

Although FDA increases the limit of SMF field intensity with no significant risk to 8 T, whether longer time exposure to SMFs of this intensity is safe on human body is still not clear. In addition, whether higher fields above 8 T are safe on human is unclear either. There will be increasing safety concerns along with the development of ultra-high MRI machines. So far there are very limited studies that have investigated on high SMFs around 9 T on animal and human cells. In 2011, Zhao et al. studied human-hamster hybrid (*A*L) cells and found that 8.5 T SMF decreased cellular ATP level and increased ROS level (Zhao et al. 2011). Nakahara et al. found that 10 T SMF alone did not affect CHO (Chinese Hamster Ovary) cells for the cell cycle distribution or proliferation unless they were combined with X-ray treatment (Nakahara et al. 2002). Recently, we found that although 9 T SMF did not affect CHO cells, they could inhibit some human cancer cell growth, such as colon cancer HCT116 cells and nasopharyngeal cancer CNE-2Z cells (Zhang et al. 2016b). In addition, human glioblastoma A172 cells embedded in collagen gels, but not A172 cells alone, oriented perpendicular to the field direction of 10 T SMF (Hirose et al. 2003), which is largely due to the diamagnetic anisotropy of collagen fibers. Zhao et al. investigated the effects of 13 T SMF on immortalized hamster cells and human primary fibroblasts cells and found that both cell cycle and cell viability were not affected (Zhao et al. 2010). A high SMF of 14 T affected the morphology of smooth muscle cell assemblies, as well as cell colony shapes, which extended along the direction of the magnetic field (Iwasaka et al. 2003). Moreover, Rat2 rat

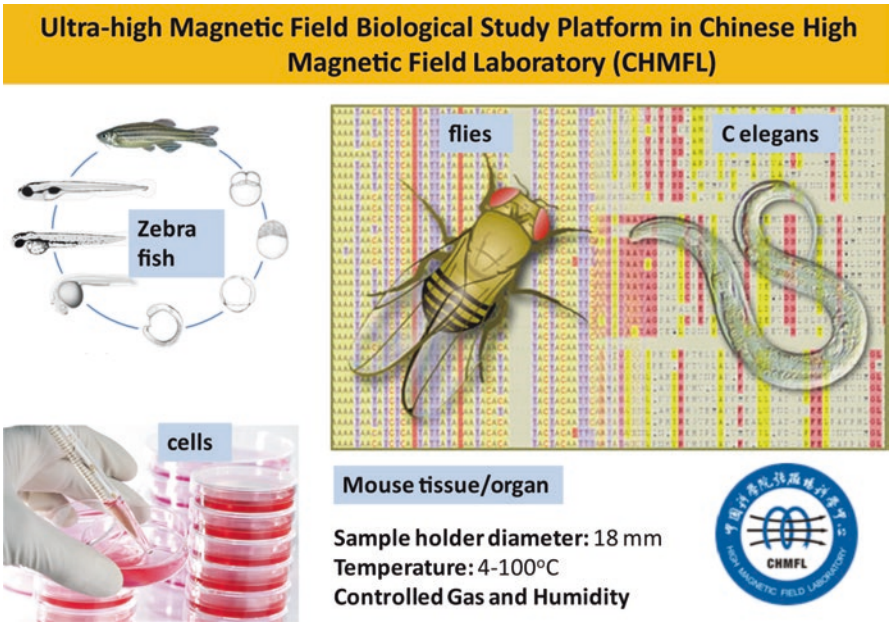

Fig. 1.3 Ultra-high magnetic field biological study platform in Chinese High Magnetic Field Laboratory (CHMFL). The biological study platform with 18 mm culture plates is suitable to study various cell cultures, including human and animal cells, eukaryotes and prokaryotes, as well as small animal models, such as fruit flies, *C elegans* and zebra fish

fibroblast cells, NIH-3T3 mouse cells, HeLa human cervical cancer cells and murine hippocampal cells were exposed to 7–17 T ultra-high SMFs, which affected Rat2, NIH-3T3 and HeLa cell attachment and neuron cell differentiation. Immunostaining analysis revealed that the actin cytoskeleton was affected by ultra-high SMFs (Valiron et al. 2005). A great deal of researches must be conducted to demonstrate the safety of ultra-high MRI before it can be fully applied on human bodies.

Due to technical limitations, the biological effects of strong field of ≥20 T on human cells have never been investigated until recently. Although the ultra-high field NMR (nuclear magnetic resonance) machines currently available can generate around 20 T SMFs, they have very narrow bore size that is impractical to accommodate cell culture plates. In addition, the animal and human cells need to be cultured with accurate temperature, humidity and gas control, which make the NMR machines unsuitable to do these experiments. For large bore SMF equipment, there are currently only very few magnets that can generate ≥20 T ultra-high SMFs, which are mostly used for material science and physical science studies. People need to construct special sample holders to make these magnets appropriate to study biological samples such as animal and human cells, as well as small animal models. We recently constructed a cell incubation system matching the large bore ultra-high magnet (Figs. 1.2f, 1.3). It can provide accurate temperature and gas control for cell cultures and some small

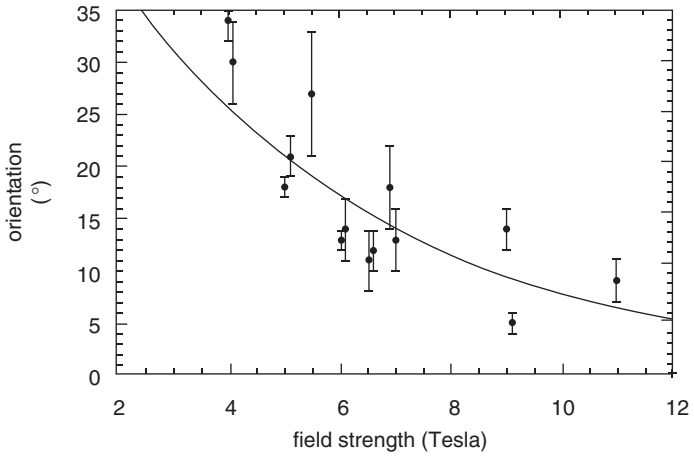

Fig. 1.4 Differential biological effects in different magnetic field intensities. Degree of orientation for microtubules assembled in the presence of SMFs as a function of magnetic field strength (The figure was reprint with permission from (Bras et al. (1998). Copyright © 1998 The Biophysical Society. Published by Elsevier Inc)

animal models. Recently we used a human nasopharyngeal carcinoma CNE-2Z cell line to study the effect of the 27 T ultra-strong SMF on its cell number, viability, cell cycle, and microtubule cytoskeleton. We found that the 27 T SMF did not have an immediate cytotoxic effect. However, it affected the spindle orientation and morphology (Zhang et al. 2017a).

There are many studies show that the magnetic field intensity is one of the key factors that cause the bio-effects differences. For example, Okano et al. found that moderate intensity gradient SMF of 0.7 T (Bmax) significantly reduced the nerve conduction velocity of frog nerve C fibers but gradient SMF of 0.21 T (Bmax) did not (Okano et al. 2012). Our recent findings showed that 0.4–9 T moderate and strong magnetic fields can affect EGFR orientation to inhibit its activity and cancer cell growth while weaker SMFs cannot (Zhang et al. 2016b). *In vitro* kinase assays using purified EGFR proteins showed that its kinase activity was inhibited by SMFs in an intensity-dependent manner (Zhang et al. 2016b). In addition, we recently found that 27 T ultra-strong SMF can affect spindle orientations in cells while moderate intensity SMFs cannot (Zhang et al. 2017a).

The magnetic field intensity and their effects on biological samples need to be examined case by case. Multiple studies show that some biological effects are directly correlated with the SMF intensity and the higher magnetic field intensities are frequently associated with stronger phenotypes (Bras et al. 1998; Takashima et al. 2004; Glade and Tabony 2005; Guevorkian and Valles 2006; Zhang et al. 2016b). For example, the microtubules can be aligned by SMFs and the alignment is increased in higher magnetic field intensity (Bras et al. 1998) (Fig. 1.4). Takashima et al. studied the DNA integrity of fruit fly in strong SMFs of 0.5–14 T and found that although an increase linearly dependent on the magnetic

flux density was observed between 0.5 T and 2 T, but it was saturated at exposure levels over 2 T and did not further increase at 5 or 14 T stronger SMFs (Takashima et al. 2004). However, higher magnetic field intensity may have different or even opposite biological effects compared to lower intensities. For example, Morris et al. showed that application of a 10 or 70 mT, but not a 400 mT, SMF for 15 or 30 min immediately following histamine-induced edema resulted in a significant reduction in edema formation (Morris and Skalak 2008). In addition, study in Shang's group demonstrated that 500 nT and 0.2 T SMFs promoted osteoclast differentiation, formation and resorption, while 16 T had an inhibitory effect (Zhang et al. 2016a).

1.1.3 Homogeneous vs. Inhomogeneous Magnetic Field

Depending on the spatial distribution of magnetic fields, SMFs can be classified as homogeneous SMF and inhomogeneous SMF, in which the field strength can be spatially constant (homogeneous) or different (inhomogeneous). Both homogeneous and inhomogeneous magnetic fields are present in many cases. For the electromagnets designed for SMFs, the center of the magnet provides a homogeneous magnetic field, as long as the samples are placed within a certain range. For example, Nakahara et al. showed the magnetic field intensity distribution as well as gradient distribution within a 10 T superconducting magnet (Nakahara et al. 2002). "0" indicates the center of the magnet, where the magnetic flux density is maximum and the field gradient is "0". However, if the samples are placed far away from the center, the magnetic field usually becomes inhomogeneous. For example, if the sample is placed around 20 cm from the center of their magnet, the magnetic field density becomes around 5 T and the field gradient is maximum. Figure 1.5 shows the magnetic field intensity distribution as well as gradient distribution within a 27 T water-cooled magnet (Fig. 1.5). At the center of the magnet, the magnetic field flux density is maximum and the field gradient is 0. In contrast, at around 7 cm away from the center, the field gradient is maximum while the magnetic field intensity decreases to < 20 T. Similarly, although the center of the MRI machine has a homogeneous magnetic field, MRI workers who stand step away from the MRI machines receive a gradient (inhomogeneous) magnetic field.

To help evaluate exposure to gradient magnetic fields (GMFs) of staff working with 1.5 and 3 T MRI machines, Iachininoto et al. used an exposure system reproducing measured signals of the 1.5 T and 3 T MRI (1.5 T-protocol and 3 T-protocol) and investigated their effects on hematopoietic stem cells. They exposed CD34+ cells obtained from six blood donors to 1.5 T-protocol and 3 T-protocol for 3 days and then cultured for 4 weeks. Results showed that *in vitro* GMF exposure did not affect cell proliferation but instead induced expansion of erythroid and monocytes progenitors soon after exposure and for the subsequent 3 weeks. However, CD34+ cells isolated from MRI workers behaved similarly to sham-exposed CD34+ cells, suggesting that other cells and/or microenvironment factors might prevent GMF

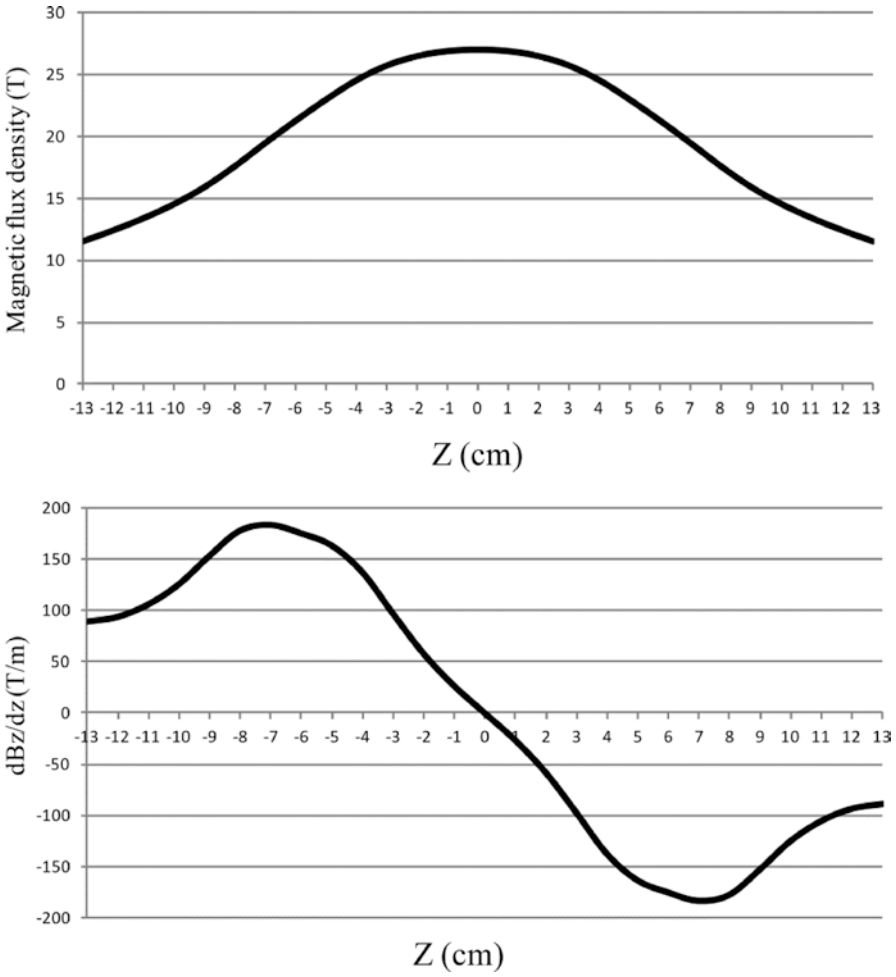

Fig. 1.5 Magnetic field intensity and gradient distribution within an ultra-high magnet that provides 27 T SMF at the center. This is based on the water-cooled magnet #4 in the Chinese Academy of Sciences, Hefei, China. *Upper panel* shows the magnetic flux density and the *lower panel* shows the magnetic field gradient. The *X axis* indicates distance from the center (Figure was provided by Lei Zhang)

effects on hematopoietic stem cells in human bodies (Iachininoto et al. 2016). So far there are no detrimental effects of MRI on regular MRI staff members have been reported.

The magnetic forces used in magnetic levitation belong to the inhomogeneous SMFs. The magnetic field intensity decreases along the upward direction away from the center so that the forces can point to the upward direction to balance gravity. The magnetic force acting on diamagnetic object is repulsive and if it is stronger than gravity, the object will be levitated. The famous "flying frog" used a 16 T supercon-

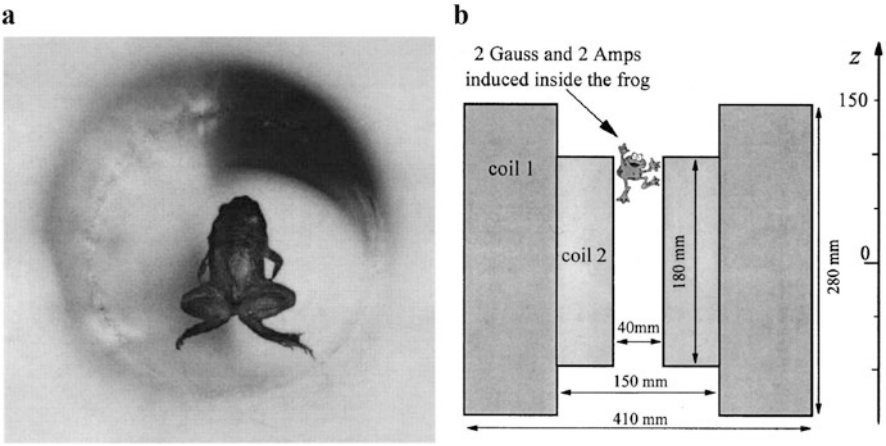

Fig. 1.6 The flying frog. (**a**) A small frog levitated in the stable zone within a 16 T magnet. (**b**) Illustration of the position of the frog within the magnet (The figures were adapted with permission from Simon and Geim (2000). Copyright © AIP Publishing LLC)

ducting magnet that provided a SMF with a gradient that is large enough to balance the gravity of the frog when it was placed at the upper part of the magnet, away from the center (Fig. 1.6). Apparently, magnetic levitation can only be achieved in static magnetic fields, but not in pulsed magnetic fields.

Besides the flying frog, there is another excellent example of using magnetic levitation to "fly" much smaller living objects, single cells. In 2015, Durmus et al. made a small magnetic levitation platform (Fig. 1.7a). This is based on the principle that each cell has a unique cellular magnetic signature, predominantly owing to the formation of intracellular paramagnetic reactive oxygen species. For example, cancer cells, white blood cells (WBC) and red blood cells (RBC) are all different from each other (Fig. 1.7b). Apparently this platform is much smaller than the one that is needed to fly a frog (Fig. 1.7c) and the magnetic field strength is also much weaker (Fig. 1.7d) because cells are much smaller and lighter than frogs. They actually used permanent magnets of moderate intensity (hundreds of militesla) in this platform (Fig. 1.7d). This relative simple set up actually can give ultrasensitive density measurements because each cell has a unique levitation profile (Fig. 1.7e) (Durmus et al. 2015). They proposed that this technique could be used in label-free identification and monitor of heterogeneous biological changes in various physiological conditions, including drug screening in personalized medicine.

In fact, multiple groups have utilized magnetic levitation technique to mimic the "weightless" condition and study its effects on cells. For example, the Shang group did a series of studies to investigate the effects of SMF with a vertical gradient using a large gradient strong magnet (Qian et al. 2009; Di et al. 2012; Qian et al. 2013). They compared the samples when they were placed at 0 gradient (1 g, indicate that the gravity is normal), or at above or down the magnet center, where the magnetic force is upward (0 g) or downward (2 g), respectively. The "0 g" position mimics the weightless condition and the "2 g" position has the double gravity forces

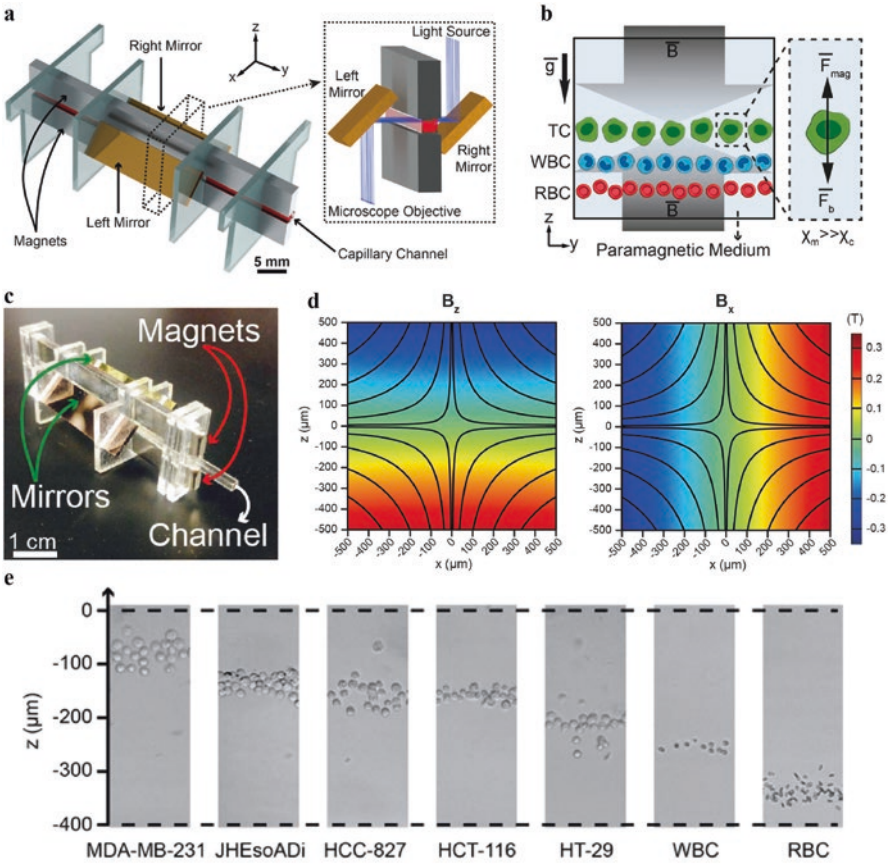

Fig. 1.7 Magnetic levitation of single cells using a densitometry platform, the MagDense cell density meter. (**a**) Illustration of the platform. (**b**) Final equilibrium height of cells in MagDense. Owing to the magnetic induction (B) and gravity (g), cells are levitated in the channel and are focused in an equilibrium plane where magnetic forces (Fmag) and buoyancy forces (Fb) equilibrate each other. Magnetic susceptibility of the medium (χm) is chosen to be bigger than the cells' magnetic susceptibility (χc). Different cell types with different densities, such as cancer cells (TC), WBC, and RBC, are separated from each other. (**c**) Photograph of densitometry platform. Capillary channel is introduced between two permanent neodymium magnets whose same poles are facing each other ("N" to "N" and "S" to "S"). Mirrors are used to image samples along the side of the channel. (**d**) FEM simulation results showing z and x component of magnetic induction (Bz, Bx) inside the channel. Total magnetic induction (Bz+Bx) is also presented as streamlines on the images. (E) Distribution of cancer and blood cells in the MagDense along the channel (HCC827, nonsmall cell lung adenocarcinoma cells; HCT116, colorectal carcinoma cells; HT29, colorectal adenocarcinoma cells; JHesoAD1, esophageal adenocarcinoma cells; MDA-MB-231, breast adenocarcinoma cells) (The figures were adapted from Durmus et al. (2015) (open access))

in the downward direction. Since "0 g" and "2 g" have identical magnetic field intensity of around 12.5 T and the magnetic field direction (B) is upward at both positions, their only difference is the direction of magnetic force. At "0 g" position, the magnetic force that is equivalent to the gravity in the opposite direction so that

"0 g" can be used to investigate the effect of weightless condition. At "2 g" the magnetic force is the same as the gravity so that it mimics the double weight condition. In the meantime, the "1 g" position provides homogenous SMF with no gradient so that it can be used to investigate the effect of magnetic field itself. Their results showed that the magnetic field and the reduced gravity worked together to affect integrin protein expression in osteoblast-like cells. Moreover, MTT assays also revealed that the 12–16 T SMFs could increase the cell number/viability of MG-63 and MC3T3-E1 cells since all three positions increased the MTT assay reading. However, they observed the difference between "1 g" of 16 T to "0 g" and "2 g" of 12 T, which is more likely due to the 4 T difference in magnetic field intensity.

There are some other studies indicate that the SMF homogeneousness have impacts on the biological effects. This is not surprising because the magnetic force acting on any particular object is proportional to the magnetic field intensity, field gradient, and the magnetic susceptibility of the object. Magnetic fields with low or no field gradients can be used to induce a magnetic torque, rather than a magnetic force, which acts on magnetic objects to move them along magnetic gradients. For example, Kiss et al. compared the homogeneous and inhomogeneous SMFs generated by permanent magnets and found that although both homogenous and inhomogenous SMFs of moderate intensity can significantly reduce pain in mice, the spatial SMF gradient might be responsible for the pain relief rather than the exposure to the SMF itself (Kiss et al. 2013). In addition, the SMFs with high gradient have been applied in red blood cell separation as well as malaria-infected red blood cell separation and diagnosis (Owen 1978; Paul et al. 1981; Nam et al. 2013), which will be further discussed in Chap. 4.

However, there is also some evidence shows that the magnetic intensity, rather than the gradient, is the key factor. For example, Denegre et al. found that the cleavage plane of frog eggs can be reoriented by SMF of 16.7 T and they did not observe differences when they placed the sample in the center (with homogeneous magnetic field) or away from the center (with inhomogeneous magnetic field) (Denegre et al. 1998). They thought that the magnetic field intensity, but not the gradient, generates effects on the samples. However, based on experimental and theoretical studies, we think their observation could because the cell division can be affected by both homogeneous and inhomogeneous SMFs as long as the magnetic field is strong enough. Whether the homogeneous and inhomogeneous field produce different phenotypes on other biological samples still needs more systematic investigations. At least one obvious difference is that the gradient field (inhomogeneous) with an upward direction could lift a frog, but a homogeneous field with no gradient could not.

1.1.4 Exposure Time

People are exposed to more and more electromagnetic radiation such as mobile phones and power lines, whose effects on human health are still debated. One of the constricting factors is that long-term exposure effects are still lacking. In contrast, the human exposure to most SMFs, other than earth magnetic fields, is only for a limited time. For example, the duration of the MRI examinations in hospitals is usually a few minutes to a couple of hours. Even for people who work with MRI, the exposure time is relative limited. So far there are no known detrimental effects of repetitive MRI exposure on human bodies, as long as they follow the MRI instructions.

It has been shown that exposure time is a key factor that contributes to the differential effects of magnetic fields on biological samples. Different exposure time will have variable effects to many aspects. For example, in 2003 Chionna et al. found that U937 cells exposed to 6 mT SMF showed cell surface microvilli shape change after 24 h exposure but they have distorted cell shape after longer exposure (Chionna et al. 2003). In 2005, Chionna et al. found that cytoskeleton was also modified in a time dependent manner in Hep G2 cells exposed to 6 mT SMF (Chionna et al. 2005). In 2008, Strieth et al. found that prolongation of the exposure time from 1 min to up to 3 h increased the 587 mT SMF-induced reduction effects on red blood cell velocity (vRBC) and functional vessel density (Strieth et al. 2008). In 2009, Rosen and Chastney exposed GH3 (rat pituitary tumor) cells to 0.5 T SMF for different time points and found that the effects on cell growth is time dependent. After 1-week 0.5 T SMF exposure, the cell growth of GH3 cells was reduced by 22% but returned to control level in a week after magnetic field retrieval. After 4-week 0.5 T SMF exposure, the cell growth of GH3 cells was reduced to 51% and returned back to control level after 4 weeks after magnetic field retrieval (Rosen and Chastney 2009). In 2011, Sullivan et al. found that ROS in fetal human lung fibroblast WI-38 cells was significantly increased by 18 h of moderate intensity SMF exposure but not 5 days of exposure (Sullivan et al. 2011) although the underlying mechanism is still unknown. Also in 2011, Tatarov et al. tested the effect of 100 mT SMF on mice bearing metastatic breast tumor EpH4-MEK-Bcl2 cells. They found that exposure of the mice to magnetic fields for 3 h or 6 h, but not 1 h, daily for as long as 4 weeks suppressed tumor growth (Tatarov et al. 2011). In 2014, Gellrich et al. found that although both SMF single exposure and repeated exposure increased the blood vessel leakiness and reduced functional tumor microvessels, the repeated SMF exposure had stronger effects (Gellrich et al. 2014). Recently, we tested the effect of 1 T SMF on human skin cancer A431 cells and also observed the time-dependent ROS changes (Fig. 1.8). All these studies show that the SMF exposure time is a key factor for their effects on biological systems and people should keep the exposure time in mind when they design their own experiments or analyze the literature.

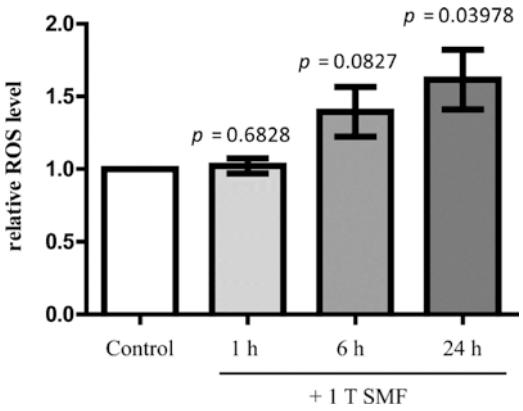

Fig. 1.8 1 T SMF increased ROS level in human skin cancer A431 cells in a time-dependent manner. 4–5 × 10^5 cells/ml of A431 cells were plated one night ahead and exposed to a 1 T SMF for different time points before the ROS levels were measured. The 1 T SMF was provided by placing the cell plate on the top center of a 5 cm × 5 cm × 5 cm neodymium permanent magnet, with the North pole up. The control group was placed with at least 30–40 cm away from the magnet with a measured magnetic field intensity background of 0.9 Gs, which was 10,000-fold lower than the 1 T experimental groups (Our lab unpublished data) (Figure was provided by Huizhen Wang)

1.1.5 Magnetic Poles and Different Field Directions

Although the scientific explanation is apparently missing, there are some reports saying that the different poles of a permanent magnet would have different effects on living organisms. Most of these points were brought up by people in the magnetic therapy field and the most famous claim was brought up by Dr. Albert Roy Davis and Walter C. Rawls. In 1974, Dr. Albert Roy Davis and Walter C. Rawls, Jr. wrote a very interesting book "*Magnetism and its effects on the living systems*". They claimed that the N pole and S pole of the magnet could have dramatically different effects on living systems. The original finding was actually from an "earthworm incident" in 1936, in which the earthworms had eaten through one side of the cardboard container near the S pole while the earthworms in the other container near N pole did not have obvious effects. The magnetic strength was around 3000 Gauss (0.3 T) in this "earthworm incident". Further analysis revealed that the earthworms near the South pole were "one- third larger, longer in length and larger in diameter and were extremely active". In this book, they also described many interesting findings about the differential effects of North vs. South magnetic pole on biological processes, such as the ripen speed of green tomatoes, radish seed germination, small animals, as well as cancers. Overall, they think the North pole is the "negative energy pole" which arrests life growth and/or development while and the South pole is the "positive energy pole" that increases life, growth and development. Although their claims have not been scientifically proven, there are many other non-scientific reports supporting the Davis and Rawls's claims. However, since no illustration or picture was provided in their book about these experiments, the relative

location of the earthworms or other samples they tested near the magnets is unclear. Moreover, there are many remaining questions. For example, whether the North or South pole magnets could generate the same effects when they were placed on the top vs. bottom, or at the side of the samples are completely unknown. Therefore, I think it is necessary for scientists to perform carefully designed and well controlled studies to test their claims. From my point of view, it is very likely that the magnetic field direction, but not the magnetic pole itself, could generate some differences on biological samples. More researches are needed to draw an explicit conclusion.

There are actually two studies have indicated that SMFs of different orientations could generate differential results in mice and cells. Milovanovich et al. found that 128 mT static magnetic fields affected various organs in mice (Milovanovich et al. 2016) (Fig. 1.9). They compared the SMFs with two opposite directions, the upward field direction (field direction was opposite to the gravity) and downward direction (field direction was the same to the gravity). In the mice serum, the HDL level was increased by both upward and downward SMFs. In addition, SMFs of both directions can decrease the amount of total white blood cell and lymphocytes in serum, granulocytes in spleen and inflammation in kidney (Fig. 1.9a) (Milovanovich et al. 2016). However, it is interesting that the upward SMF caused increased spleen cells but the downward SMF did not (Fig. 1.9b), while the downward SMF decreased the granulocytes number in serum but the upward SMF did not have as significant effect (Fig. 1.9a) (Milovanovich et al. 2016).

In addition, recently, a separate study by De Luka et al. also suggested that the moderate intensity magnetic fields with different orientations may have differential effects on copper level in mice brain (De Luka et al. 2016). They measured the zinc and copper levels in different organs in mice that were exposed to SMFs (98 mT max) of upward or downward directions. They found that SMF could change the zinc and copper levels differentially in different organs. More interesting, the SMF of downward direction seemed to have more obvious effects (De Luka et al. 2016). The difference was small but statistically significant.

At the same time, there was also evidence showing that the magnetic field direction or magnet pole does not make a difference. In 2011, Sullivan et al. examined fetal human lung fibroblast WI-38 cells for their response to magnetic fields that were generated by pairs of "N" vs. "S" magnetic pole facing each other but with different orientations (Fig. 1.10). In this way, the cells placing between the magnets were exposed to magnetic fields of different orientation, and they were also relative closer to either "N" pole or "S" pole. They examined cell attachment and cell growth curves and found that the different exposure methods both could decrease cell attachment and cell growth but there was no difference between them (Sullivan et al. 2011). I think the different observations in these studies are likely due to the differences in biological samples examined. It is interesting that not only both Milovanovich et al. (2016) and De Luka et al. (2016) observed differential responses of mice when they were exposed to magnetic fields of different directions, they also showed that different organs responded differently. Based on results from their studies, the magnetic field direction can make difference in some organs or on some types of cells while have no difference in other organs or cell types. Therefore it is

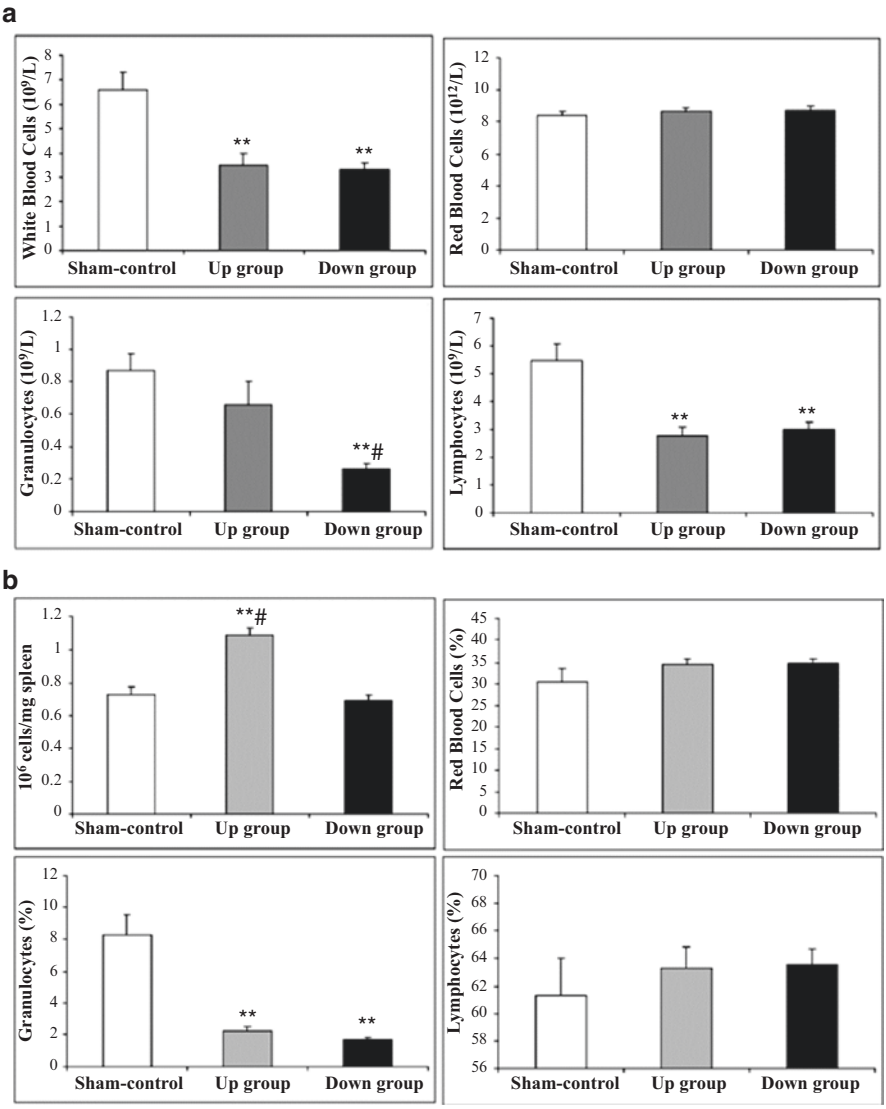

Fig. 1.9 Magnetic field direction influences the SMF effect on mice. "up group" means the group of mice that were exposed to SMF with the upward field direction. "down group" means the group of mice that were exposed to SMF with the downward field direction. Magnetic flux density is 128 mT. (**a**) Cell count of blood in mice exposed to SMFs of different direction, including total serum white blood cells, serum red blood cells, serum granulocytes and serum lymphocytes. **p<0.01 compared to control. # p<0.05 compared to up group. (**b**) Cell count in spleen of mice exposed to SMFs of different direction, including the total spleen cells, spleen red blood cells, spleen granulocytes as well as spleen lymphocytes. **p<0.01 compared to control. # p<0.01 compared to down group (The figures were adapted with permission from Milovanovich et al. (2016). Copyright © 2015, Springer-Verlag Berlin Heidelberg)

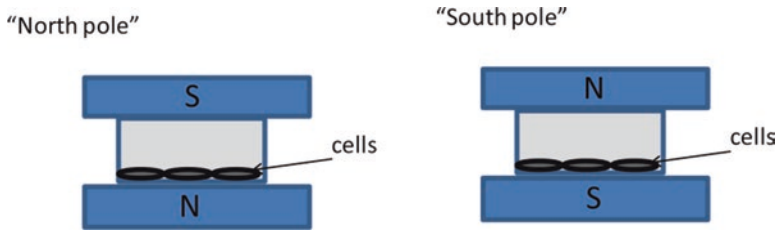

Fig. 1.10 Field direction did not make differences on human lung fibroblast WI-38 cell attachment or proliferation. In 2011, Sullivan et al. compared the different exposure methods on WI-38 cells attachment and proliferation but did not observe obvious difference (Figures are based on results from Sullivan et al. (2011))

not too surprising that Sullivan et al. examined only one cell type, fetal human lung fibroblast WI-38 cells, and did not observe any field direction-induced differences.

Overall the magnetic field direction or magnetic pole-induced bio-effects differences are not well supported yet. Scientific investigations are still lacking and mechanistic explanations are also missing. If the "N" vs. "S" magnetic pole induced dramatic differences in living systems proposed by Dr. Albert Roy Davis and Walter C. Rawls are real, it would help to explain some inconsistencies in current literature because most people did not pay attention to the magnetic poles in their studies, including us in our earlier experiments. However, based on our knowledge, the effects are likely not as simple and clear-cut as claimed by Dr. Albert Roy Davis and Walter C. Rawls. Our lab is currently investigating this issue systematically by comparing different magnetic poles and field directions on different types of cells for their effects on multiple aspects. Our initial data suggest that the effects seem to be cell type- and cellular activity-dependent (our unpublished data). Since most studies so far did not provide information about the magnetic pole information, we strongly recommend that people should pay attention to the magnets they use and keep a clear record about the magnetic field direction and/or the magnetic poles in their studies. This is actually crucial because the results could be totally different.

1.1.6 Factors Contributing to the Lack of Consistencies in Bioeffects Studies of Magnetic Fields

As mentioned above, despite the numerous scientific research and non-scientific case reports about the magnetic effects on living organisms, the magnetic field effects on biological systems are still looked upon with doubts and suspicions by many scientists outside of the field, as well as by the mainstream medical community. This is largely due to a lack of consensus on the biological effects in general that are backed up by solid scientific evidences and explanations. We have to admit that the countless scientific researches or non-scientific case reports are enriched with many seemingly contradictory results, which make many people confused and

hence become suspicious, including myself a few years ago. Then we carefully analyzed the evidence in the literature about the biological effects of magnetic fields to try to view them collectively in a scientific way. We found that most of these inconsistencies can be explained by the different parameters of either the magnetic fields or the biological samples people used in individual studies. For example, the magnetic field parameters mentioned above in this chapter all contribute to the differential effects, such as the types of magnetic fields, the field intensities and frequencies of magnetic fields, the homogeneity and directions of the fields, the magnetic poles and the exposure time. More importantly, we found that the biological samples people examined directly affect the magnetic effects. For example, we recently found that both cell types and cell densities have direct impact on the effects of 1 T SMF on cells (Zhang et al. 2017b). The cancer vs. non-cancer cells from the same tissue responded completely differently to the same magnetic field. Unexpectedly, the same cell line responded totally different when they were seeded at different cell density and we found that the EGFR-mTOR-Akt cell signaling pathway is likely involved in this regulation (Zhang et al. 2017b). In fact, even normal (non-cancer) cells from the same tissue have different responses to the magnetic field. The Shang group compared the effects of 500 nt, 0.2 T, 16 T on osteoblast MC3T3-E1 cells (Zhang et al. 2014b), as well as pre-osteoclast Raw264.7 cells (Zhang et al. 2016a) and found that the osteoblast and osteoclast cells responded totally opposite to these SMFs. Both hypo and moderate magnetic fields reduced osteoblast differentiation but promoted osteoclast differentiation, formation and resorption. In contrast, 16 T SMF increased osteoblast differentiation inhibited osteoclast differentiation. They also wrote a particular review to systematically summarize the effects of SMFs on bone that is worth to look into (Zhang et al. 2014a). More surprisingly, some people (including ourselves) found that even cell passage number could affect the experimental results, which will be further discussed in Chap. 4.

In 2009, Colbert et al. wrote a comprehensive review "Static Magnetic Field Therapy: A Critical Review of Treatment Parameters" (Colbert et al. 2009). Their purpose was to summarize SMF studies involving the application of permanent magnets in humans. In this review, they critically evaluated the reporting quality of ten essential SMF dosing and treatment parameters and proposed a set of criteria for reporting SMF treatment parameters in future clinical trials (Fig. 1.11). They reviewed 56 studies about magnetic therapy, in which 42 studies were done in patient populations and 14 studies were done in healthy volunteers. As we have discussed in earlier part of this Chapter, the magnetic field parameters greatly influence their effects on biological systems. However, by analyzing ten magnetic field related parameters in these studies, including the magnet materials, magnet dimensions, pole configuration, measure field strength, frequency of application, duration of application, site of application, magnet support device, target tissue, distance from magnet surface, and found that 61% of the studies failed to provide enough

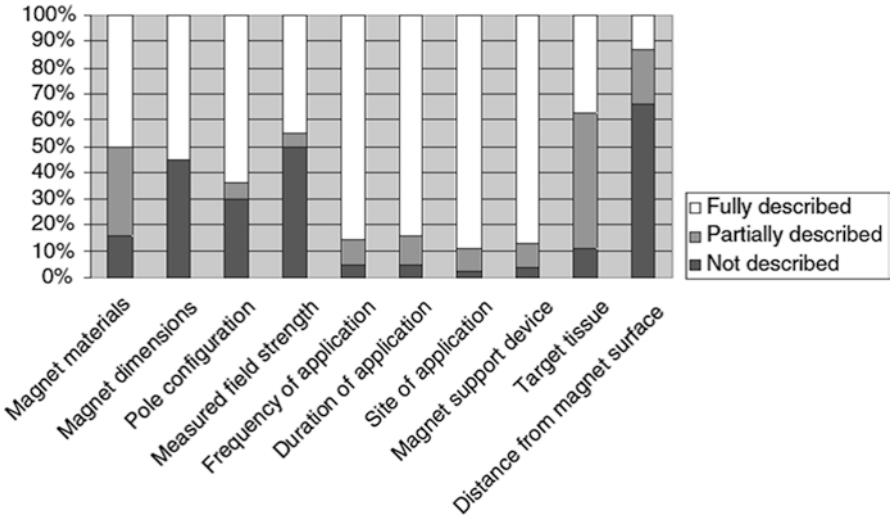

Fig. 1.11 Quality of reporting ten static magnetic field (SMF) dosage and treatment parameters was assessed in 56 human studies (The figure was from Colbert et al. (2009). Copyright © 2007 The Authors (open access))

experimental details about the SMF parameters to permit protocol replication by other investigators. Apparently, the lack of sufficiently detailed description of SMF parameters greatly prevented people from getting consensus conclusions from these studies. We strongly encourage people in the field of magnetic field studies to clearly label their parameters, such as the ten parameters listed in the Colbert paper, in their own research.

Last but not the least, there are also some other factors contributing to these differences, such as instrument and technical sensitivities, which have been greatly improved in the past few decades. Nowadays people have much advanced instruments and techniques, which should enable more findings that were not detectable before. The absence of magnetic field effects in some studies may simply due to the technical limitations and/or inadequate control of experimental conditions. We should take advantage of the modern technologies to answer related questions. For example, we recently used liquid-phase STM to get high resolution single molecular images of proteins (Wang et al. 2016) and combined with biochemistry, cell biology as well as molecular dynamics simulation to reveal that moderate and strong SMFs could change EGFR orientation to inhibit its activation and some cancer cell growth (Zhang et al. 2016b). At the same time, we should keep all relevant factors in mind, such as magnetic field type and intensity, cell type and density when we do our own research and analyze the relevant literature. This will help us reduce the diversity and contradictions in this field and also help us to correctly understand the mechanism of the biological effects caused by the magnetic field.

1.2 Conclusion

Since the human body itself is an electromagnetic object, it is not surprising that the magnetic fields could produce some effects on them. There are indeed many convincing experimental evidences as well as theoretical explanations about the effects of magnetic field on some biomolecules, such as the cytoskeleton microtubules, membrane, as well as some proteins (will be discussed in Chapter 3). In the meantime, most studies in the literature on the biological and health effects of magnetic fields had been inconclusive or contradictory, which was largely due to the various parameters used in individual studies, including the magnet fields themselves, samples examined, as well as the experimental set up. It seems that there is a large gap between atom/molecular level and cell/tissue/organism level that people need to fill in to correctly and scientifically understand the biological effects of magnetic field. For now, experimental and theoretical studies are both at a very preliminary stage. To help us get a more complete understanding of the biological effects of magnetic fields and their underlying mechanisms, more systematic, well controlled studies with fully described experimental details are strongly encouraged. Furthermore, increased collaborations between scientists in physics, biology and chemists are necessary to make substantial progresses in this emerging field.

Ethics The frog research studies in this chapter had their ethics approved. For Okano et al. 2012, it was stated that "the animal experiments were carried out with the approval of the Animal Ethics Committee of Chiba University (Chiba, Japan)".

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Chapter 2
Static Magnetic Fields (SMFs) on Human Bodies

Abstract This chapter summarizes the effects of static magnetic fields (SMFs) on human bodies. Some commonly seen SMFs, such as the weak earth magnetic field that we are all exposed to, moderate to ultra-high field magnetic resonance imaging (MRI) in the hospitals and research institutes, as well as SMF-based magnetic therapies, which have a long history but still lack of solid explanation or sufficient experimentation from a scientific point of view. Magnetobiology and biomagnetism are also briefly discussed.

Keywords Static magnetic fields • Earth magnetic field • Magnetic resonance imaging (MRI) • Magnetic therapy

2.1 Introduction

From a simplified view, the human body is mainly composed of weak diamagnetic materials, including water, proteins and lipids. The term diamagnetic means that it repels with the externally applied magnetic field. In a magnetic field, the motions of electrons in diamagnetic molecules make small changes, which generate weak magnetic fields in the opposite direction to the externally applied magnetic field. Although the diamagnetic properties of most living organisms are very weak, since the repulsive force is proportional to the product of the field intensity and the field gradient, the forces can be amplified by strong magnetic field. The most famous case is the "flying frogs" about 20 years ago, which we mentioned in Chap. 1. People put small diamagnetic objects such as water drops, flowers, grasshoppers as well as small frogs in the 16 T strong SMF produced by a vertical electromagnet and levitated those small objects. Theoretically the human body could also be levitated. However, due to the size and weight of our human bodies, the levitation would need a much stronger magnet and has not been accomplished yet.

In recent years, people have increased exposure to different kinds of electric magnetic fields, most of which are dynamic magnetic fields, such 50–60 Hz power line electromagnetic fields as well as radiofrequency electromagnetic fields. Therefore these magnetic fields have attracted paramount interests in the past, especially around 1970–2000. In the late 1970s and early 1980s, there were multiple

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epidemiologic studies suggested a link between occupational electromagnetic exposure with an increased incidence of leukemia, as well as some other diseases, such as breast cancers. However, although these associations raised many public concerns, further investigations failed to establish a link between the magnetic exposure with these diseases. There are many reviews and books about this topic and we will not discuss about the details here. The focus of our book is SMFs, which have non-changing magnetic fields over time (0 Hz). For SMFs, the most common ones that people are exposed to include the weak but widely spread earth magnetic field (~0.5 Gauss, ~50 μT), MRI scanners in the hospitals (0.5–3 T), as well as permanent magnets of various magnetic intensities that some people may use as alternative medicine for some chronic medical conditions such as chronic pain relief, as well as small magnets that are frequently used in household items such as refrigerators, toys and accessories.

For SMFs, the most updated fact sheet and guidelines by WHO (World Health Organization) and ICNIRP (International commission on non-ionizing radiation protection) were in 2006. For a general view of the current agreement of the magnetic field exposure standards, people can always check the website of ICNIRP for the most updated guidance for electromagnetic exposure (http://www.icnirp.org/). ICNIRP is an independent organization, which provides people with scientific advice and guidance on the health and environmental effects of non-ionizing radiation (NIR) (http://www.icnirp.org/en/frequencies/index.html). NIR is electromagnetic radiation that does not have enough energy to ionize atoms or molecules. Other than SMFs, ICNIRP also cover multiple topics about non-ionizing electromagnetic radiation, such as the electromagnetic radiation from the sun, household electrical appliances, mobile phones, Wi-Fi, and microwave ovens. Although some people may not agree with some specific points, ICNIRP guidelines are still the most well accepted standards for public exposure to non-ionizing radiation. It should be mentioned that due to the public attention, rapid development of technology and huge amount of accompanied studies, the most updated fact sheet and guidelines for radiofrequency magnetic fields published by WHO and ICNIRP were in 2014. Meanwhile, the safety issues of SMFs caused much less worries compared to mobile phones. The current updated fact sheet and guidelines by WHO and ICNIRP about SMFs are in 2006, which is already 10 years from now. There are also some fine and comprehensive reviews that people can look into (Schenck 2000; Valentinuzzi 2004; Feychting 2005).

In the meantime, with the development of high field MRI machines in the hospitals, people have increased exposure to high magnetic fields, which unsurprisingly raised new concerns. In 2011, Yamaguchi-sekino et al. wrote an updated review about the biological effects of electromagnetic fields and updated safety guidelines for strong SMFs (Yamaguchi-Sekino et al. 2011) that people can find many useful information. At the same time, there are various researches started to unravel the potential beneficial effects of SMFs on human, which may provide some action mechanisms of the magnetic therapy that have a long but debating history. Therefore, the effects of static magnetic fields and their effects on human bodies certainly require more research to get a better understanding.

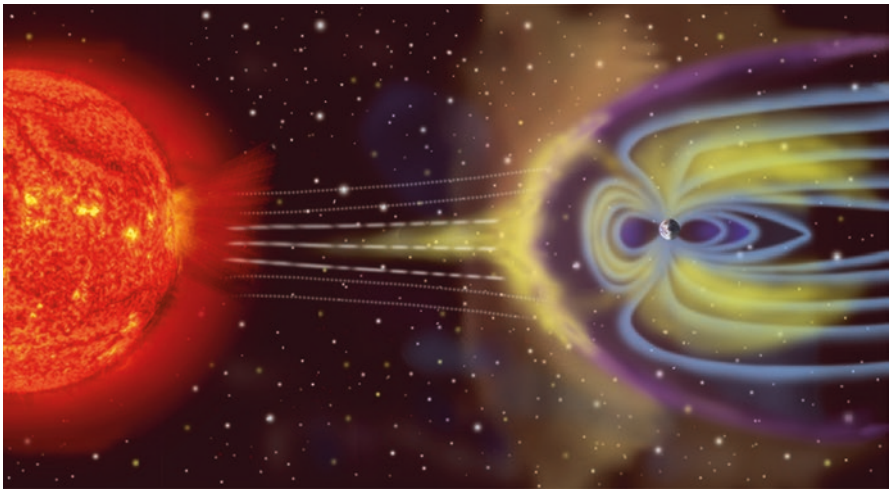

Fig. 2.1 Earth's magnetosphere (The picture was from the public domain created by NASA: https://commons.wikimedia.org/wiki/File:Magnetosphere_rendition.jpg)

2.2 Earth Magnetic Field

The most common SMF that all people are exposed to is the earth magnetic field, geomagnetic field (GMF), which is around 0.5 Gauss/50 μT (0.3–0.6 Gauss, depending on locations). It is actually quasi-static, which means it can fluctuate slightly. Geomagnetic field is much weaker compared to other types of SMF exposure but it is present virtually everywhere and is exceptionally important to the living organism on earth. It is proposed that planets without an intact global magnetic field are subject to atmospheric stripping by the solar wind. For example, people think that Mars does not have a global magnetic field so that the solar wind has contributed to the loss of water and the erosion of Mars' atmosphere. In contrast, the earth has its magnetic field (magnetosphere), which is proposed to protect our whole planet from solar wind stripping (Fig. 2.1).

It is well known that birds, bees, turtles and some other animals are shown to sense earth magnetic fields for direction during migration (Lohmann and Johnsen 2000; Wiltschko and Wiltschko 2005; Johnsen and Lohmann 2008). There are many studies about the earth magnetic fields and magnetoception of animals. It is believed that many birds have a compass in their eyes because their retinas have magnetic field sensors, which make them "see" the earth magnetic field in addition to their normal vision. The magnetic sensor was assigned to cryptochromes for many years until recently another protein was also found to participate in magnetic sensing (details will be discussed in Chap. 5). Both CRY (cryptochrome) and MagR seem to be important for the magnetoception in birds but more *in vivo* studies are necessary to draw a definite conclusion. In addition, it is interesting that recently Vidal-Gadea et al. found that the nematode *Caenorhabditis elegans* orients to the earth's

magnetic field during vertical burrowing and the migrations and magnetic orientation required the TAX-4 cyclic nucleotide-gated ion channel in the AFD sensory neuron pair (Rankin and Lin 2015; Vidal-Gadea et al. 2015).

More information about the SMF effects on microorganisms, plants and animals will be discussed in Chap. 5. Although the progress in this particular field is big in the past few years, more efforts are definitely needed to unravel the exact and detailed mechanisms to explain the animal behaviours related to earth magnetic fields. For example, people found some interesting but enigmatic phenomena that dogs like to align their bodies along the earth magnetic field when they excrete (defecation and urination) (Hart et al. 2013).

For humans, although we also have the proteins that are believed to be the receptors for the magnetic fields, such as CRY and MagR, there is no solid evidence to support the presence of magnetoception. Although for now, we think humans cannot detect, or at least cannot feel the earth magnetic field, the magnetic sensing is still one of the most significant unsolved problems in the biology field. Actually, researchers have knockout the cryptochrome in flies to make them insensitive to the magnetic field and found that the magnetic reception can be restored by the human cryptochrome (Foley et al. 2011). This means that human cryptochrome is functional as a magnetosensor, at least in flies. However, why humans do not sense magnetic fields as birds do? Roswitha Wiltschko, who was one of the scientists who first discovered the magnetic sense of birds, said, "*To sense the magnetic field, one does not only need a molecule like cryptochrome, but also an apparatus that picks up the changes in that molecule and mediates it to the brain. Drosophila obviously has this apparatus, but humans? I have my doubts.*" It is possible that we have other sensations that dominate the magnetoception, or just because we miss some key components along the magnetoception pathway. It is interesting that Thoss et al. indicate that the GMF could actually affect human visual system (Thoss and Bartsch 2003; Thoss and Bartsch 2007) although the mechanism is not completely understood. Apparently, this field still remains blurred and we are still far away from understanding the nature of it in both animals and potentially, in humans. More research is certainly needed to answer these fundamental questions.

It is interesting that there are some researches on humans show that GMF could produce some neurological and cardiovascular effects. Burch et al. indicate that the GMF can affect melatonin secretion (Burch et al. 2008), which is a possible mechanism for the neurological and cardiovascular effects of altered GMF. In addition, Lipnicki et al. show that there may even be some association between GMF activity with dream bizarreness (Lipnicki 2009). However, there are also some reports that reported negative results. For example, in 2002, Sastre et al. examined the effects of controlled changes in the GMF on fifty human volunteers for electroencephalogram (EEG) and did not find any obvious correlation (Sastre et al. 2002). Since different aspects were measured in these individual studies, they are not exactly comparable. It is obvious that more researches are needed to address this question.

On the other hand, there are also some evidences showing that in the absence of GMF, frequently referred to Hypomagnetic field (HMF, which is not high magnetic field in other cases), the gene expression, cell proliferation, migration and adhesion

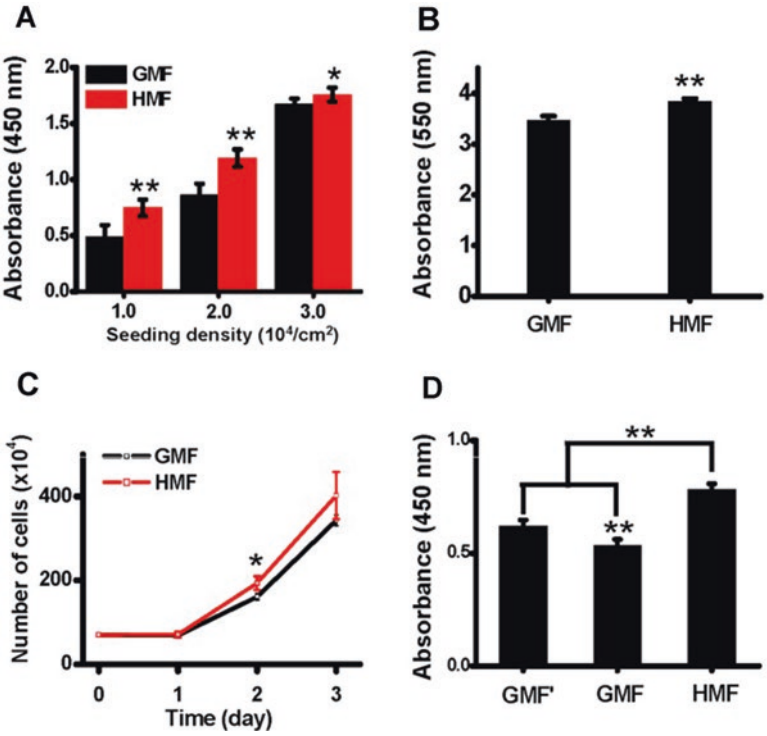

Fig. 2.2 The proliferation of SH-SY5Y neuroblastoma cells was accelerated in the Hypomagnetic field (HMF). (a) Cell proliferation assay by CCK-8 kit (n =6). **(b)** Cells were seeded at $2.0 \times 10^4/cm^2$ in 6-well plates and cell proliferation was measured by crystal violet staining after 48 h incubation in the GMF and HMF (n =6). **(c)** Cells were seeded at $2.0 \times 10^4/cm^2$ in 60 mm petri dishes and incubated for 48 h in the GMF and HMF. The numbers of SH-SY5Y cells were measured at day 1, day 2, and day 3 by hematocytometery (n =3). **(d)** Cells were seeded at 1.5×10^4 cells/cm^2 in 96-well plates. Cell proliferation was measured after 48 h incubation in the reference field (GMF′), in the GMF control shelf (GMF), and in the HMF (n =6). Error bar =s.d.; n =3; *p < 0.05; **p < 0.01 (Image was from Mo et al. 2013, an open access article)

of some human cancer cells could be affected (Martino and Castello 2011; Mo et al. 2013, 2014, 2016). For example, Mo et al. did multiple studies about the effects of HMF on human SH-SY5Y neuroblastoma cells. In 2013, they showed that continuous HMF exposure significantly increases the proliferation of human SH-SY5Y neuroblastoma cells (Fig. 2.2) by promoting cell cycle progression (Mo et al. 2013); In 2014, they compared the transcriptome profiles of SH-SY5Y cells exposed to either the HMF or the GMF and found multiple genes are differentially expressed, including MAPK1 and CRY2 (Mo et al. 2014). In 2016, they found that in HMF, SH-SY5Y cells have reduced F-actin cytoskeleton as well as reduced adhesion and migration (Mo et al. 2016). In addition, HMF was also found to reduce the ROS level in human pancreatic AsPC-1 cancer cell line and bovine pulmonary artery endothelial cells (PAEC) (Martino and Castello 2011), which is consistent with some studies reporting that SMFs could increase ROS in some cancer cells (will be

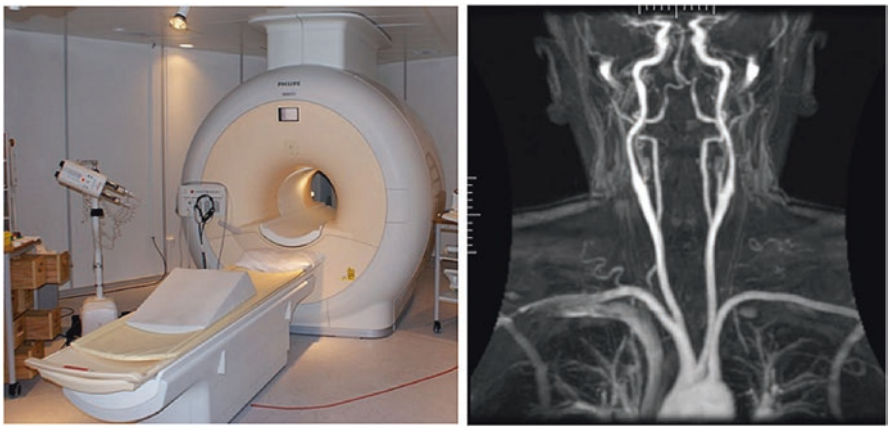

Fig. 2.3 Magnetic resonance imaging (MRI). *Left*: A MRI machine in the hospital (https://commons.wikimedia.org/wiki/File:MRI-Philips.JPG). *Right*: Magnetic resonance angiography (MRA), pictures of the arteries (https://commons.wikimedia.org/wiki/File:Mra1.jpg)

discussed in Chap. 4). In addition, they also did some studies in *Xenopus laevis* (African clawed frog) and found that HMF could cause a decrease in horizontal third cleavage furrows and abnormal morphogenesis in Xenopus embryos (Mo et al. 2012). Their results indicate that a brief (2 h) exposure to HMF is sufficient to interfere with the development of Xenopus embryos at cleavage stages. Although their study was done in frogs, the impact of HMF on mitotic spindle and cell division could also be potentially comparable in other organisms, including humans. This is especially critical for developing embryos.

In conclusion, based on the current available evidences, no matter whether or not humans can sense the earth magnetic fields for direction like some animals do, it is likely that our bodies are indeed affected, or more accurately, protected by the earth magnetic fields. However, more investigations are strongly needed to draw an unambiguous conclusion.

2.3 Magnetic Resonance Imaging

Besides the weak earth magnetic field (50 μT), nowadays people have more chances to get exposed to much stronger SMF, such as MRI scanners in the hospitals. MRI has a superior soft-tissue contrast compared to other radiological imaging methods, which makes it a powerful tool in many physiological and functional applications. The SMF of the MRI system is exceptionally strong compared to the earth magnetic field. Currently, most MRI scanners in hospitals for regular patients are 0.5–3 Tesla, which is around 10,000–60,000 times greater than the earth magnetic field. Figure 2.3 shows a MRI machine and a magnetic resonance angiography (MRA) picture achieved from MRI.

MRI is considered to be a safe technique as long as the operation follows the guidelines. So far, after several years of monitoring, there are no harmful effects reported on frequent MRI operators, patients or NMR (nuclear magnetic resonance) users. There are also some lab studies at cellular levels about the safety of MRI. For example, in 2003, Schiffer et al. used conditions that are relevant for patients during MRI for their effects on HL60 and EA2 cells. They examined different types of magnetic fields, including SMFs of 1.5 and 7.05 T, extremely low frequency magnetic gradient fields (ELFMGFs) with +/− 10 mT/m and 100 Hz, as well as +/− 100 mT/m and 100 Hz, pulsed high frequency MF in the radiofrequency (RF) range (63.6 MHz, 5.8 microT), and a combination of these different magnetic fields. They exposed the cells for up to 24 h and did not find cell cycle changes (Schiffer et al. 2003). Recently, Sammet wrote a review about the magnetic resonance safety (Sammet 2016). For example, people with pacemakers should not use MRI because the pacemakers may be reprogrammed or turned off by the magnetic field. People with some other implants, such as ferrous intra-cranial vascular clips, should also avoid MRI because the strong magnetic field of MRI may cause possible movement of the implants. Cell phones and credit cards may be damaged by the magnetic fields so that they should also be kept out of the MRI room. In addition, the patients should be moved slowly into the magnet bore to reduce the possibility of vertigo and nausea. It has been shown that no short term cardiac or cognitive effects are observed following significant exposure to 8 T (Kangarlu et al. 1999) and the 2009 ICNIRP guidance (ICNIRP 2009) concluded that there is no indication of serious health effects from acute exposure of stationary humans to SMFs of up to 8 T, except that people may have unpleasant feelings such as vertigo. Based on the available scientific data, the limit of exposure for general public was set to 400 mT. This is calculated by applying a reduction factor of 5 on 2 T, which has been proved to have no demonstrated robust effect on animals (Gaffey and Tenforde 1983; Tenforde 2005) or humans. The exposure of SMFs above 8 T requires approval of the research protocol by an Institutional Review Board as well as the informed consent of the subjects. It is well recognized that for the regular exposure to the MRI, there are some commonly experienced symptoms including nausea and headaches, which are all reversible.

Although the magnetic field intensities of the range of MRI machines in hospitals (0.5–3 T) are currently considered to be safe to human bodies, more investigations are still needed to achieve a more complete understanding. Large amount of data show that there is no increased risk for leukemia or other types of cancers by SMFs. In fact, increasing experimental evidences from biological labs indicate that the SMFs could inhibit cancer cell growth and have a potential in cancer treatment in the future, which will be discussed in later chapters in this book. In addition, the whole body exposure of mice to the 3 T homogeneous SMF of a clinical MR resulted in a statistically significant antinociceptive activity (Laszlo and Gyires 2009). However, besides the potential beneficial effects of MRI within the 0.5–3 T range, it should be mentioned that there are also some studies indicate that they may have some other effects on human. For example, 3 T SMF was shown to suppress human chondrocyte growth *in vitro* and affect recovery of damaged knee cartilage *in vivo* in the pig model (Hsieh et al. 2008).

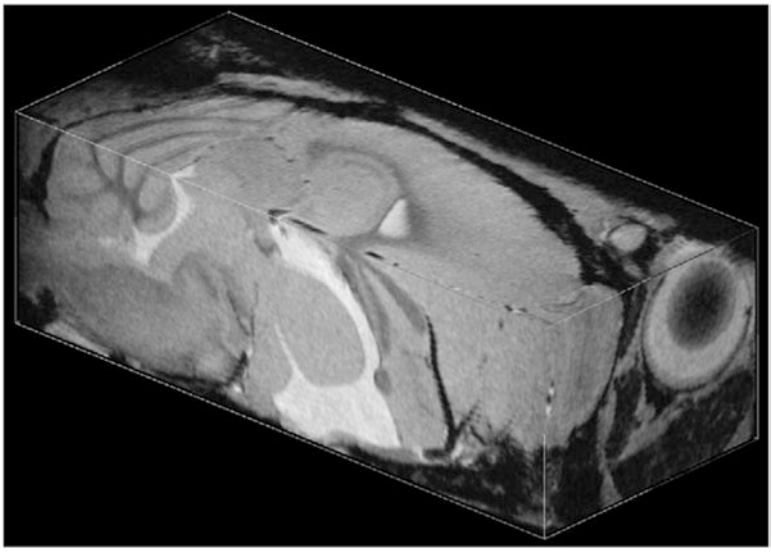

Fig. 2.4 A 21.1 T MRI used on mouse brain. MRI Gradient recalled (FLASH) proton *in vivo* MR image of a mouse head, in plane resolution for image is $50 \times 50\ \mu m^2$ and an apparent resolution in third direction of 50 μm (Image was adapted with permission from Schepkin et al. 2010. Copyright © 2010 Elsevier Inc.)

MRI machines with higher magnetic field strength are already developed. For now, there are 7–9.4 T MRI machines have been used on animal studies in research as well as on human bodies at preclinical stage (Kangarlu et al. 1999; Adair 2000; Miyakoshi 2005; Zhang et al. 2015). For the short-term exposures experienced by volunteers and patients, no readily demonstrated health risks were identified. In addition, since the publication of the 2009 ICNIRP guidance (ICNIRP 2009), there have been a large number of studies evaluating the physiological and neurobehavioral influence in human bodies exposed to SMFs of up to 9.4 T. Current findings show that for the SMFs used in MRI up to 9.4 T, there are no known detrimental biological effects on human bodies. In the meanwhile, people are currently investigating on building MRI machines with ultra-high magnetic fields. Increased magnetic fields can help providing enhanced sensitivity, higher resolution as well as decreased acquisition time. For example, high magnetic fields increased our capability to observe and investigate *in vivo* biological processes that are unavailable or obscure in low magnetic fields. In 2010, Schepkin et al. tested mouse and rat brains using a 21.1 T MRI, the highest field MRI to date, at the National High Magnetic Field Laboratory (NHMFL) in the United States. They were able to achieve imaging resolution of 50 μm (Fig. 2.4), which is much higher than the lower field MRIs. In addition, they also compared 21.1 T MRI to 9.4 MRI and found that the 21.1 T MRI can provide much more detailed features about the tissues and blood vessels in the rodent brain (Schepkin et al. 2010). This showed the promising future of developing similar MRI for human. However, it is still not very clear about the biological effects

of higher magnetic fields, especially the ultra-high magnetic field of 20 T and above. Since our knowledge of the biological effects of SMFs will guide us for future increase in magnetic field intensity for MRI to benefit medical diagnosis and treatment, more studies are definitely needed to investigate the biological effects of ultra-high magnetic fields, which are necessary for the future application of ultra-high field MRI machines on humans.

Therefore, although current MRI machines in the hospitals are considered to be safe, the long term consequences and their potential beneficial effects on human bodies are still incomplete identified. In addition, obvious advantages of ultra-high field MRI machines encourage people to create ultra-high field MRIs for technical benefits. This also calls for attention for necessary studies for the accompanied safety issues. More efforts are needed to help establish guidelines for occupational staff and patient exposures to high SMFs.

2.4 Magnetic Therapy Using SMFs

Looking back into history, magnetic therapy has been debated for thousands of years and there were multiple rounds of up and downs (Basford 2001). It is interesting that the lack of solid scientific explanation for the working mechanism of magnetic field on human bodies does not really prevent people from using magnets at their own wish. Although it is never a mainline medicine, there are still many people currently using magnetic therapy as an alternative and complementary treatment for some chronic diseases, such as arthritis, wound healing and analgesic therapy (pain relief). Every year, the magnetic therapy products have billions of dollars in sales worldwide. In fact this is mostly because many people using magnetic therapy do find themselves benefiting from them, such as some products designed for pain relief. For example, there are some magnetic therapy products on amazon.com. A few of these products have hundreds of positive comments claiming that they could alleviate the pain and discomfort, especially the magnet bracelet that has some relative stronger magnets embedded. By browsing the magnetic therapy products on the market, it is not surprising that the magnetic bracelets that received good reviews usually have their magnetic flux densities clearly labelled and most of them are within the range of hundreds to thousands of gausses (0.01–1 T).

Despite the fact that magnetic therapy has a long history, it is still not well accepted by the mainstream medicine. In some cases, it is even considered to be pseudoscience. The doubts people have are mainly due to the lack of consistency and scientific explanations (as discussed in Chap. 1). There are many efforts that have been devoted to trying to resolve this issue and some of them did provide positive results. For example, In 1997 Vallbona et al. conducted a well-controlled study on fifty post-polio patients and found that the 300–500 Gauss (0.03–0.05 T) SMFs (active magnetic device) significantly reduced the patient pain level from 9.6 to 4.4 ($p < 0.0001$) on a 10-point scale (Vallbona et al. 1997) (Table 2.1**, top**). It is interesting that the sham exposure system that maximally mimics the magnetic device

Table 2.1 Moderate intensity SMF reduced pain level in post-polio patients

Pretreatment and posttreatment pain scores			
	Active magnetic device (n = 29)	Inactive device (n = 21)	Significance
Pretreatment pain score (mean ± SD)	9.6 ± 0.7	9.5 ± 0.8	NS
Posttreatment pain score (mean ± SD)	4.4 ± 3.1	8.4 ± 1.8	P < 0.0001
Change in score (mean ± SD)	5.2 ± 3.2	1.1 ± 1.6	P < 0.0001
Proportion of subjects reporting pain improvement by magnetic activity of the treatment device			
	Active magnetic device (n = 29)	Inactive device (n = 21)	
Pain improved	N = 22 (76%)	N = 4 (19%)	
Pain not improved	N = 7 (24%)	N = 17 (81%)	

The *top* table shows that the pain score is efficiently reduced by active magnetic device. The *bottom* table shows that the % of patients that have effective pain relief is much higher in the active magnetic device group. Both tables were based on results from (Vallbona et al. 1997)

(inactive device) also had some placebo effects and reduce the patient pain level from 9.5 to 8.4. However, it is obvious that the pain level change in the SMF-treated group is fivefold more efficient than the placebo-device group (5.2 vs. 1.1, $p < 0.0001$). In addition, 76% of the patients in the active magnetic device group reported much reduced pain while the placebo-device group only have 19% patient (Vallbona et al. 1997) (Table 2.1**, bottom**). This study is well known and much better than most other magnetic therapy studies in a scientific point of view. It was done with proper controls, which provided people with convinced evidences that SMFs could indeed have beneficial effects on pain relief. More studies are needed to carry out in scientific way like this to test the claims made in the field of magnetic therapy.

Another two scientifically done studies in the field of magnetic therapy were by Alfano et al. and Juhasz et al. In 2001, Alfano et al. did a randomized, placebo-controlled, 6-month trial conducted from 1997 through 1998 on people with fibromyalgia (Alfano et al. 2001). In addition to sham controls, they compared a group of people that was exposed to sleep pads with magnets that provided low uniform static magnetic field of negative polarity (Functional Pad A) with a group exposed to sleep pads with magnets that varied both spatially and in polarity (Functional Pad B). In fact, they did find that the Functional Pad A had the most significant effects and both Functional Pad A and B groups showed improvements in functional status, pain intensity level, tender point count, and tender point intensity after 6 months of treatment, but they did not differ significantly from changes in the control groups (Alfano et al. 2001). Therefore although this study show that the magnetic sleep pads have the potential to work, the effects were not statistically significant. I think the major reason for the lack of efficiency in their study might be the magnetic field strength, which is too low (below 1 mT). Increasing the magnetic field strength to

hundred to thousand gauss might work. However, scientific studies are needed to be done to prove this. Moreover, in 2014, Juhász et al. did a randomized, self- and placebo-controlled, double-blind, pilot study included 16 patients diagnosed with erosive gastritis. They used inhomogeneous SMF-exposure intervention at the lower sternal region over the stomach with peak-to-peak magnetic induction of 3 mT and 30 mT m(−1) gradient at the target site. They did found clinically and statistically significant beneficial effect of the SMF- over sham-exposure on the erosive gastritis symptoms. The average effect of inhibition was 56% ($p = 0.001$). This indicates that inhomogeneous SMF could be a potential alternative or complementary method for erosive gastritis (Juhasz et al. 2014). It is interesting that their magnetic field intensity seems much lower than most other studies that have positive results.

Current evidences show that magnetic field strength is a key issue for potential magnetic therapy applications. Overall, it is believed that magnetic fields with too weak strength are not enough to produce enough energy. As mentioned above, the permanent magnets most people used for magnetic therapy have been proved to be effective ranging from hundreds to thousands of gauss. For example, in 2002, Brown et al. showed that 0.05 T SMF for 4 weeks could reduce chronic pelvic pain in patient (Brown et al. 2002). In 2011, Kovacs-Balint et al. did a research on 15 young healthy human volunteers and found that a inhomogeneous 0.33 T (Bmax) SMF exposure for 30 min could increase the thermal pain threshold (TPT) (Kovacs-Balint et al. 2011). However, it is possible, and very likely, that different symptoms have different requirement for the magnetic field intensity, as well as other magnetic field parameters.

Besides human studies, there are also some animal and cellular studies about the potential application of SMFs in multiple diseases. For example, in 2008 Gyires et al. showed that the inhomogeneous 2–754 mT SMF could significantly reduce the visceral pain (57%, $P < 0.005$) elicited by intraperitoneal injection of 0.6% acetic acid in mice (Gyires et al. 2008). In 2009 Laszlo et al. showed that 3 T MRI had significant beneficial effects on pain relief in mice (Laszlo and Gyires 2009). In 2012, Okano et al. found that gradient moderate intensity SMF of 0.7 T (Bmax) exposure for 4–6 hours could reduce the nerve conduction velocity of C fibers, which are responsible for pain transmission (Okano et al. 2012). In 2013 Kiss et al. did a study in mice show that moderate intensity of both inhomogeneous (3–477 mT) and homogeneous (145 mT) SMFs that are provided by permanent magnets can have a significant beneficial effects on pain relief (Kiss et al. 2013). In 2013, Vergallo et al. examined the effect of inhomogeneous SMF (0.476 T max) exposure on the production of different cytokines from human lymphocytes and macrophages (Vergallo et al. 2013). They found that the moderate intensity inhomogeneous SMF treatment for 6–24 h has a significant inhibitory effect on the release of pro-inflammatory cytokines IL-6, IL-8, and TNF-a from macrophages as compared to control. In addition, the SMF increased the production of anti-inflammatory cytokine IL-10 from lymphocytes. As brought up multiple times before, most of these magnetic field intensities that show positive results for pain relief and inflammation reduction are a few hundred to a few thousand Gauss. However, we do not exclude the possibility that lower magnetic field intensities will also have some effects on

some biological samples, or have some other different effects. There were also some mechanistic studies about the SMF-induced pain relief. Gyires et al. showed that the analgesic action induced by inhomogeneous 2–754 mT SMF could be inhibited by subcutaneous administration of naloxone, irreversible micro-opioid receptor antagonist beta-funaltrexamine and delta-opioid receptor antagonist naltrindole, but not the kappa-opioid receptor antagonist norbinaltorphimine, which suggests that the antinociceptive effect is likely to be mediated by micro and delta-opioid receptors (Gyires et al. 2008). More details and information are discussed in Chaps. 6 and 7 for the potential application of SMFs in cancer and other diseases.

In the meantime, not surprisingly, there are some experimental evidences showing that certain magnetic therapy products fail to produce positive effects, even for the magnets that have enough magnetic field intensities. For example, Richmond et al. compared a magnetic wrist strap with (1502–2365 gauss), a demagnetised (<20 gauss) wrist strap, an attenuated (250–350 gauss) magnetic wrist strap, and a copper bracelet. Their results show that wearing a magnetic wrist strap or a copper bracelet did not appear to have any meaningful therapeutic effect, beyond that of a placebo, for alleviating symptoms and combating disease activity in rheumatoid arthritis (Richmond et al. 2013). For now we are not sure about the reason for this lack of efficacy, however, as mentioned in Chap. 1, magnetic field parameters will greatly influence the effects of SMF on biological samples. In addition, there are multiple other factors that have led to the large variations in the clinical or research work about the SMFs, which we will discuss more in Chap. 4 in this book. For example, although lacking scientific mechanistic foundations so far, it is interesting that there are multiple claims about the differential effects of the two different magnetic poles on human bodies (Table 2.2). In fact, there are two recent papers observed differential effects of different magnetic field directions (De Luka et al. 2016; Milovanovich et al. 2016). Although more research is strongly needed to confirm their results, I think people should pay attention to the magnetic poles or directions when they investigate the biological effects of magnet fields in the laboratory, or simply want to try some magnetic therapy products.

The differential effects of the magnetic field direction and north/south poles need to be further confirmed by more scientific researches, and ultimately to provide clear scientific explanations. For now, I myself are not clear why two different poles can make any differences because there is no physical difference between the North and South pole of the magnet, at least from our current scientific knowledge. However, it is possible that some unknown mechanism indeed exists to explain these observations. Moreover, since it has already been shown that magnet could levitate single cells when the magnetic field is upward to balance the gravity (Durmus et al. 2015), it makes more sense to me if it is the magnetic field direction that made the differences that people claimed. More interestingly, Durmus et al. demonstrate that each cell type (i.e., cancer, blood, bacteria, and yeast) has a characteristic levitation profile, and they have identified unique differences in levitation and density blueprints between breast, esophageal, colorectal, and non-small cell

Table 2.2 The North and South magnetic poles are claimed to have different "healing effects" by some magnetic therapy manufactures

Claimed "healing effects" of different magnetic poles by many magnetic therapist	
North pole-"Negative"	South pole-"Positive"
Inhibits Relieves pain	Excites Increases pain
Reduces inflammation	Increases inflammation
Produces an alkaline effect	Produces an acid effect
Reduces symptoms	Intensifies symptoms
Fights infections	Promotes microorganisms
Supports healing	Inhibits healing
Reduces fluid retention	Increases fluid retention
Increases cellular oxygen	Decreases tissue oxygen
Encourages deep restorative sleep	Stimulates wakefulness
Produces a bright mental effect	Has an over productive effect
Reduces fatty deposits	Encourages fatty deposits
Establishes healing polarity	Polarity of an injury site
Stimulates meletonin production	Stimulates body function
Normalizes natural alkaline PH	

For now, it is not clear whether this is real. Different magnetic field direction could generate some differences. However, although from the scientific point of view there is no explanation for this, I do not exclude the possibility that their claim might be true. More scientific studies are encouraged to explore this question

lung cancer cell lines, as well as heterogeneity within these seemingly homogenous cell populations (Durmus et al. 2015). This indicates that various cell types in the human body might respond totally differently to the magnetic fields. More researches are needed to confirm this.

It is worth to mention that currently many researches related to magnetic therapy as well as the biological effect studies about magnetic fields are not well described or properly controlled. In 2008 and 2009, Colbert et al. wrote two important and comprehensive reviews (Colbert et al. 2008, 2009), which stated that *"Complete descriptions of the SMF dose that was applied to human participants are notably lacking in the majority of SMF therapy studies published to date. Without knowing the SMF dose that was delivered to the target tissue, we cannot draw meaningful inferences from clinical trial results. As research on SMF therapy progresses, engineers, physicists and clinicians need to continue to work together to optimize SMF dosage and treatment parameters for each clinical condition. Future publication of SMF studies should include an explicit assessment of the SMF dosage and treatment parameters outlined in this review, so as to be able to replicate previous studies, validly assess outcomes and make objective, scientific comparisons between studies."* The parameters they outlined include the magnet materials, magnet dimensions, pole configuration, measure field strength, frequency of application, duration of application, site of application, magnet support device, target tissue, distance from magnet surface,

Table 2.3 10 essential static magnetic field dosing parameters

	Static magnetic field dosing parameters
1	Target tissue(s)
2	Site of magnet application
3	Distance of Magnet surface from target tissue(s)
4	Magnetic field strength
5	Material composition of permanent magnet
6	Magnet dimensions: size, shape, and volume
7	Magnet polar configuration
8	Magnet support device
9	Frequency of magnet application
10	Duration of magnet application

Adapted from (Colbert et al. 2008). We recommend that people should all follow these standards when reporting their results

which all have great potential to directly affect the outcomes (Colbert et al. 2008, 2009) (Table 2.3). Many related researches need replication and we hope we can make great advancement after we have the proper knowledge of the magnetic field and biological systems, which will not only be helpful for WHO to assess any possible health consequences, but also improve the current status of magnetic therapy, which definitely needs much more rigorous experimentation. In fact, FDA has already approved the use of TTF (tumor treating fields), which delivers low-intensity, intermediate-frequency (100–300 kHz), alternating electric fields to treat newly diagnosed and recurrent glioblastoma, which works by disrupting cancer cell division, with no significant damage to normal non-dividing cells (Kirson et al. 2004; Pless and Weinberg 2011; Davies et al. 2013). Although TTF is a type of electromagnetic field therapy using low-intensity electrical fields, not SMFs, it may shed light on the SMF investigations for their potential clinical usage.

2.5 Magnetobiology and Biomagnetism

Generally speaking, magnetobiology is about the effects of magnetic fields on living organisms, which is the focus of this book. In contrast, biomagnetism refers to the magnetic fields that are generated by living organisms, which is not our main focus in this book, but will be briefly discussed here.

As mentioned in the beginning of this chapter, the human body is mainly composed of weak diamagnetic materials, such as water, proteins and lipids. However, our human bodies also generate currents that produce small magnetic fields (Cohen et al. 1980).

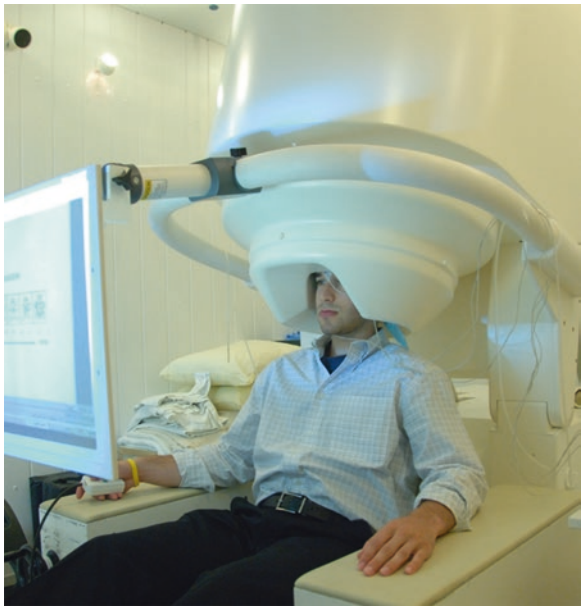

Fig. 2.5 MEG scanner with patient from National Institute of Mental Health (This image is on the public domain. Credit should be given to "National Institute of Mental Health, National Institutes of Health, Department of Health and Human Services". https://en.wikipedia.org/wiki/File:NIMH_MEG.jpg)

Neurons in our brain, nerve cells, and muscle fibers are all excitable cells that can generate currents when they are activated. Magnetic fields produced by the human body have been measured, which are actually very weak (10^{-10}–10^{-5} gauss). Most of the body's fluctuating magnetic fields, such as those from the heart or the brain have been extensively studied and developed. Electrocardiogram (ECG) measures the electrical activity of the heart and electroencephalogram (EEG) measures the electrical activity of the brain, both of which have been widely used in clinic.

It is well accepted that the human brain can be divided into multiple areas, and each of them are responsible for different aspects of behaviour. The accurate and efficient connectivity between these areas are critical for normal function of a healthy brain. Although a single neuron could only produce very weak current, it can be amplified when the neurons are clustered and aligned together and excited simultaneously. In this case, the neurons can produce magnetic fields that are strong enough to be detected using superconducting quantum interference devices (SQUIDs) (Zimmerman et al. 1970; Hamalainen et al. 1993). Weak alternating magnetic fields outside the human scalp, produced by alpha-rhythm currents, were demonstrated. The fields near the scalp are about 1×10^{-9} gauss (peak to peak) (Cohen 1968). Magnetoencephalography (MEG) (Fig. 2.5) is a non-invasive sophisticated technique that captures the magnetic fields generated by synchronized intraneuronal electrical activity, which yields rich information on the spatial, spectral and temporal signatures of human brain function. It is capable of imaging electrophysiological brain activity with good (~5 mm) spatial resolution and excellent (~1 ms) temporal

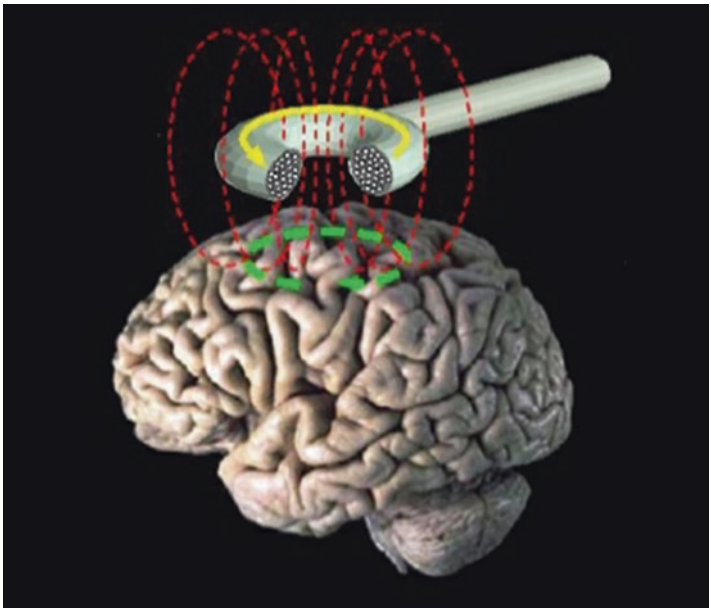

Fig. 2.6 A schematic diagram of transcranial magnetic stimulation (TMS) (This image is on the public domain and it contains materials that originally came from the National Institutes of Health. Picture website: https://en.wikipedia.org/wiki/Transcranial_magnetic_stimulation#/media/File:Transcranial_magnetic_stimulation.jpg)

resolution and provides significant value in elucidating the neural dynamics of the human connectome in health and disease (O'Neill et al. 2015). There are many very useful reviews and research articles for MEGs showing that neuroimaging methods like MEG represents an outstanding approach to better understand the mechanisms of both normal and abnormal brain functions (Brookes et al. 2011; He et al. 2011; Pizzella et al. 2014; Kida et al. 2015; O'Neill et al. 2015; Pang and Snead 2016; Stefan and Trinka 2016). Similarly, magnetocardiogram (MCG) measures the magnetic fields of the heart, which is a complementary or alternative tool for noninvasive detection of coronary artery disease (Kandori et al. 2010; Wu et al. 2013).

In addition, MEG appears to be more sensitive than EEG and can provide additional and different information compared to EEG (Cohen 1972). MEG is not only useful for functional neurosurgery but also for connectivity analyses. Since MEG could offer additional insights not possible by MRI when used to study complex network function, people are combining MEG (which has high temporal resolution) with functional MRI (fMRI), which has high spatial resolution, to provide more information on human brain function (Hall et al. 2014). In particular, MEG is most widely applied to the study of epilepsy, a brain disorder that causes people to have seizures (Kim et al. 2016; Pang and Snead 2016). In addition, simultaneous MEG/EEG recording and analysis could provide complimentary information and better detection sensitivity for tracing primary epileptic activity (Hunold et al. 2016;

Stefan and Trinka 2016). Moreover, for chronic neurological disorders such as epilepsy, functional connectivity detected through hemodynamic (fMRI) and electromagnetic techniques (EEG/MEG) help to identify the interactions between epileptic activity and physiological networks at different scales. fMRI and EEG/MEG functional connectivity help in localizing important drivers of epileptic activity and can also help in predicting postsurgical outcome (Pittau and Vulliemoz 2015). Beyond the diagnosis benefit of MEG, transcranial magnetic stimulation (TMS) (Fig. 2.6) is another electromagnetic method that uses a "coil" placed near the head to stimulate small regions of the brain and is used to diagnose or treat multiple diseases such as stroke and depression. In fact, TMS is currently covered by some health insurance in the United States to treat diseases like depression.

2.6 Conclusion

Since human body itself is an electromagnetic object, it is not surprising that the magnetic fields can produce some effects on us. However, the electrochemical processes within the human bodies are very complicated and still remain incompletely understood. Therefore the actual physical effects of magnetic fields on human bodies will still need continuous efforts to achieve a complete understanding. In the meantime, magnetic therapy may be an alternative or complementary method in the clinical use, especially in cases when conventional therapy options are unavailable. In addition, whether the magnetic therapy works does not depend on our understanding for its underlying biological mechanisms. As Dr. Basford said in his review (Basford 2001)*"An electric or magnetic therapy is first discovered by the populace, resisted by the medical establishment, and then discarded—only to arise again in the future in a slightly different form. Although sophistication has increased, this pattern is likely to continue into the future until clear treatment benefits and, one hopes, a convincing mechanism of action are established."* Currently, what we should do is to try our best to unravel the mysteries so that we can maximize the benefit we can get from these nature powers. In the meantime, we should alert people that there are numerous unreliable websites or products about magnetic therapy. We believe that with the increasing efforts to use legitimate and scientifically backed methods in the field of magnetic field research, we will gain more mechanistic insights to facilitate the clinical application of SMFs and make magnetic therapy scientifically respectable.

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Part II
Scientific Basis for Static Magnetic Field (SMF) Effects on Biological Systems, from Molecule, Cell to WHOLE Organism Level

Chapter 3
Molecular Mechanisms for Electromagnetic Field Biosensing

Abstract Almost all types of life that have been investigated appropriately have shown some indication of biological response to magnetic fields. An alluring application of the information that already has been, and continues to be, collected describing how biological systems sense magnetic fields and transduce this information into physiological response is to treat human disease. Towards that goal, this chapter summarizes what has been learned about electromagnetic biosensing in a diverse set of organisms across several phyla and discusses how the underlying mechanisms apply (and in some cases, don't apply) to humans.

Keywords Static magnetic fields (SMFs) • Biological magnetoreceptors • Magnetic field biosensing • Magnetite • Crytochromes • Chemical magnetosensing

3.1 Introduction

This chapter explores the biological basis for therapeutic effects of electomagentic fields (EMFs), with a focus on static magnetic fields (SMFs; basic definitions of magnetism are briefly reviewed in Sect. 3.2 of this chapter) in people. At present there is no clear and widely accepted mechanism by which SMFs benefits human health; indeed, there is considerable skepticism in the mainstream media (as well as in certain parts of the scientific literature) that SMFs have any effect at all. For example, a purported lack of beneficial effects sometimes is deduced from studies that EMFs (in general) have negligible detrimental effects. To illustrate, the bulk of the evidence indicates that living under (or near) high voltage electrical power lines does not increase the risk of cancer (Ahlbom et al. 2001; Anonymous 2002; Schüz 2011). To some, this lack of harm implies that EMF exposure (of any kind) likely has no beneficial effect either.

On the other hand, it is well established that a wide range of living organisms – ranging from bacteria, mollusks, crustaceans to fish, amphibians, reptiles, birds and mammals (Wiltschko and Wiltschko 2012) – use the earth's relatively weak magnetic field (i.e., geomagnetism) for orientation, navigation and direction finding as well as for additional purposes briefly covered in Sect. 3.3 of this chapter (with more detailed descriptions given in Chaps. 2 and 4 of this book). As will become

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evidence from this information, certain mechanisms that some species use for magnetoreception are highly specialized and do not apply directly to humans. In other cases, however, the underlying molecular basis of magnetic sensing and the broad mechanisms involved across many phyla provide at least a conceptual basis for how human cells, tissues, and organs can respond to SMFs. If nothing else, precedent from non-mammalian systems provides a starting point for investigation of magnetic field sensing in humans; one example lies in ongoing efforts to establish the presence and activity of magnetite – which was first described in prokaryotes half a century ago – in people (magnetite is discussed in Sect. 3.4 – along with other known magnetosensing mechanisms found in nature – and throughout this chapter as an exemplar of a "well known" magnetic biosensor).

Although much has been learned about how magnetoreception occurs, many aspects of magnetic field biosensing remain poorly understood and in some cases basic mechanisms may remain undiscovered. This is particularly likely to be the case for humans, where the very topic of whether people have any ability to sense magnetic fields, much less respond to them, remains controversial (as covered in more detail in Chap. 7). Here, in Sect. 3.5 an overview of sensing mechanisms found elsewhere in nature may apply to humans along with speculation of "novel" ways that human cells, tissues, and organs can sense and respond to magnetic fields.

3.2 Magnetism, Basic Definitions

This section briefly introduces basic concepts and definitions of magnetism related to biological systems; a more detailed description of magnetic phenomena is provided in Chap. 1 of this book (or introductory physics textbooks or fairly reliable internet sources such as Wikipedia). The information presented here is intended primarily to provide a sufficient basis for understanding the subsequent sections of this chapter without the need to refer to outside material.

3.2.1 Ferromagnetism, Paramagnetism, and Diamagnetism

Ferromagnetism is "everyday" magnetism; for example, permanent magnets (such as ubiquitous refrigerator magnets) or removable car bumper stickers are ferromagnetic. A ferromagnetic substance becomes magnetized when exposed to a magnetic field and retains this feature "permanently" after removal from the field. As a caveat, magnetism is not permanent in the strict sense of the word because field strength often wanes over time, and can be affected (i.e., field direction can be reversed) by exposure to a subsequently-applied field; nevertheless, field strengths of ferromagnets can be remarkably stable over long periods of time. As a second nuance, although the term "ferro" implicitly suggests that ferromagnets contain iron, several other metals have ferromagnetic properties including most alloys of nickel and

cobalt as well as several rare earth metals (neodymium is a well known example). A final feature, important for biological magnetosensing, is that these metals are not inherently magnetic but must have higher degrees of organization. For example, iron in solution or in prevalent biological contexts (e.g., when it is complexed with hemoglobin in erythrocytes) is not ferromagnetic. Instead the metal atoms (in the case of iron, usually as iron oxides) must be organized into distinct crystalline structures to be ferromagnetic; such structures occur abundantly in the form of lodestone (iron ore) in the mineral world and, in specialized situations in the biological realm, as magnetite.

Paramagnetic substances "become magnetic" while exposed to a magnetic field but this effect rapidly decays upon loss of the field. Examples of paramagnetic substances include free electrons found in metals and unpaired electrons found in many biological molecules. Indeed, in biology, many proteins are complexed with metals that have unpaired electrons, leading to the development of the commonly-used electron paramagnetic resonance (EPR) spectroscopy (Bertini et al. 2012). Diamagnetism is a property of all materials that describes the formation of an induced magnetic field in the direction opposite to an externally applied field; in other words the induced field attempts to repel the applied field (note that this is opposite to paramagnetism where the induced field is attracted to, and aligns with, the external field). Molecules found in biological systems ranging from water to bioorganic macromolecules are typically only very weakly diamagnetic and any resulting diagmagnetism can be overshadowed by external fields or by surrounding paramagnetic or ferromagnetic entities.

3.2.2 Field Types and Strengths

Life evolved in the presence of the Earth's magnetic field (i.e., "geomagnetism"); the geomagnetic field (GMF) fluctuates in direction and strength over time and space and currently has a magnitude at the Earth's surface that ranges from 25 to 65 microteslas (μT; or 0.25 to 0.65 gauss [1 T equals 10,000 guass]). This field is considered to be "weak" insofar as it cannot be detected by humans during their everyday activities in meaningful or noticeable ways without specialized instruments. To provide context for magnetic field strengths, the human brain emits a much weaker magnetic field (~0.1–1 picoT) while cardiac pacemakers produce fields about an order of magnitude stronger than GMFs (~500 μT); a refrigerator magnetic is yet another order of magnitude stronger (~5 mT); a device custom-built to treat cultured human cells (Fig. 3.1) provides another ~2 order jump in field strength (~0.25 T); another order of magnitude increase in strength (to ~1 to 3 T) represents typical stereo loudspeaker fields as well as MRI exposure; and finally, a 17 T field represents the strength needed to famously levitate a frog (*see* Chap. 1). For the purposes of this Chapter's discussion, magnetic fields in the range of geomagnetism are termed "weak." For higher strength fields, fields below 1 T are considered to be "moderate" strength and those above 1 T are considered to be "strong"

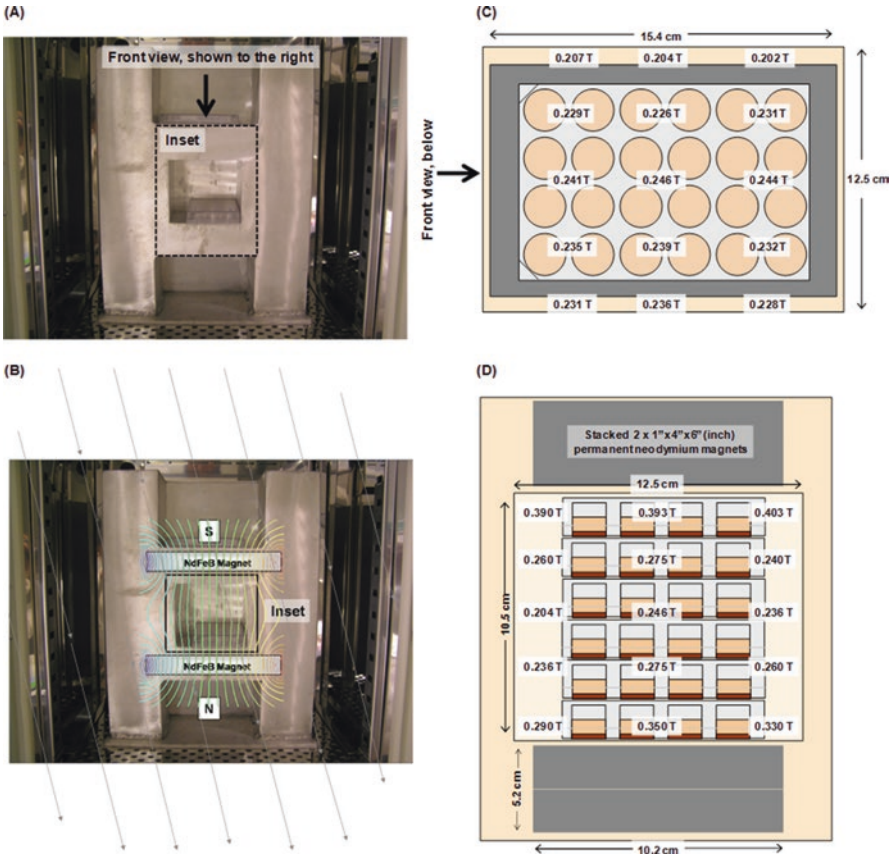

Fig. 3.1 Device used to treat cells with "therapeutic" strength SMFs. (**a**) The device is shown inside a standard-sized cell culture incubator with the position of permanent neodymium alloy magnets (inside a layer of aluminum covering) shown in *Panel* (**b**), which also indicates an approximate representation of the GMF (based on the positioning of the incubator in Baltimore, Maryland, USA) with the thin gray arrows and the applied SMFs (*colored lines*). (**c**) A top view of the "*inset*" (as indicated on *Panel* **a**) shows the dimensions and relative field strength at various positions on a standard 24-well cell culture plate located in one of the two centered (from top to bottom) slots indicated in the "*front view*" of the *inset* shown in *Panel* (**d**). *Panel* (**d**) also shows the position of the neodymium magnets and how field strength (measured at "cell level", indicated in *dark red*, *orange* indicates cell culture media) varies based on the vertical distance from the magnets. When used for biological studies, only the four centered (from top to bottom) positions were typically used (the bottom plate remained empty) resulting in cells typically being exposed to SMF strengths ranging from 0.23 to 0.28 T (Wang et al. 2009, 2010)

(most therapeutic magnetic treatments fall in the moderate strength category). Finally, although many electromagnetic fields (EMFs) involve a time varying component this chapter primarily focus on time invariant, i.e., "static" magnetic fields (which, as mentioned, are called "SMFs"); other EMF modalities used for human therapy are outlined briefly in Chap. 7). In the literature, it is sometimes assumed

that EMFs with frequencies of less than 100 Hz have biological effects similar or even identical to SMFs (Markov 2014), but unless otherwise noted, that convention is not followed in this discussion (i.e., unless otherwise stated, SMFs that are mentioned have no time-varying component).

3.3 Overview of Magnetoreception in Various Organisms

The ability to sense magnetic fields – or "magnetoreception" – has evolved across almost all phyla of mobile organisms starting with ancient magnetotactic bacteria that exploit the GMF to move up or down in the water column and nematodes that also use magnetic fields to move vertically (but on land). Other animals such as butterflies, birds, and even mammals use the Earth's magnetic field for navigation during long distance migrations. A sampling of such magnetosensing organisms is provided below, along with brief mechanistic insights. This information is not intended to be comprehensive (a more detailed description is provided in Chap. 5 of this book) but is instead meant to provide an overview of known and postulated molecular mechanisms found throughout nature as a prelude to a more detailed description of the three "well known" modes of magnetic sensing (magnetite, chemical, and inductive) in Sect. 3.4 of this chapter.

3.3.1 Bacteria

Magnetotactic bacteria were first described by Salvatore Bellini in a monograph published in 1963 (Bellini 1963) with a seminal peer-reviewed report by Robert Blakemore published 12 years later (Blakemore 1975) with a detailed review in the Annual Review of Microbiology published in 1982 (Blakemore 1982). These bacteria contain "permanent" magnets in the form of nano-sized (e.g., of an average size of ~420 Å) cuboidal to octahedral iron grains that allow them to orient themselves with the geomagnetic (0.25 to 0.65 gauss) or applied magnetic fields. Upon orientation, which is entirely passive in nature (e.g., even dead magnetotactic bacteria become aligned with an applied magnetic field), living bacteria actively swim along the field in a predominantly northward direction for bacteria harvested from the Northern Hemisphere and in a southward direction for bacteria from the Southern Hemisphere (Blakemore 1982). After half a century, investigation of magnetotactic bacteria remains robust with ever-increasing understanding of the integration of the iron grains into higher order structures such as "magnetosomes;" insights into the biosynthetic machinery for these structures; and insights into the dynamic control of these microorganism's iron-processing physiology in changing environments (Araujo et al. 2016). Although not directly related to human health or magnetic therapy in people, magnetotactic bacteria nicely illustrate how even very

"primitive" organisms have the ability to exploit magnetic sensing to enhance survival and gain an evolutionary advantage over competing species.

3.3.2 Invertebrates

As just described, even single celled organisms such as magnetotactic bacteria have a remarkable ability to exploit magnetoreception-based sensing for directional movement; we next turn to more complex creatures to illustrate how a diversity of life forms – using added biochemical strategies – have the ability to sense and respond to magnetic fields.

3.3.2.1 Nematodes

The soil-dwelling nematode worm *Caenorhabditis elegans* is extremely well studied in a laboratory setting and constitutes a facile model for investigation of "simple" multicellular organisms; for example all of this creature's neurons are mapped allowing for exquisitely sensitive investigation of brain function (at least to the extent that *C. elegans* have functioning brains) at the molecular and genetic levels. To complement and extend laboratory study of these nematodes, a recent paper by Vidal-Gadea and coauthors reported fascinating magnetoreception in "wild" *C. elegans* where populations isolated from different sites across the globe migrated at angles to an applied magnetic field that optimized vertical translation in their native soil, with northern- and southern-hemisphere worms displaying opposite migratory preferences (Vidal-Gadea et al. 2015). In these experiments, magnetotaxis was traced to genes expressed in the AFD pair of neurons that previously had been implicated in thermosensation (Mori 1999). The specific genes involved in magnetotaxis in these cells include two independent mutant alleles of ttx-1, important for AFD differentiation; the triple mutant lacking guanylyl cyclases, gcy-23, gcy-8, and gcy-18, which together are critical for AFD function; and two independent mutant alleles of each tax-4 and tax-2 genes that encode subunits of a cGMP-gated ion channel implicated in transduction of stimuli in sensory neurons (Vidal-Gadea et al. 2015).

3.3.2.2 Mollusks and Crustaceans

It has been know for three decades that the marine mollusk *Tritonia diomedea* has an ability for geomagnetic orientation (Lohmann and Willows 1987), that similar to nematodes, has been traced to specific neurons. In a series of studies beginning in the late 1980s, Lohmann and Willows first reported that perturbation of geomagnetic-strength magnetic fields change electrical activity in a single neuron (left pedal 5, LPd5) (Lohmann et al. 1991); subsequent experiments identified four such neurons

including LPd5 but also RPd5, LPd6 and RPd6 (Wang et al. 2004). These neurons fired an increased number of action potentials when the horizontal component of the ambient magnetic field was rotated. This response disappeared when all nerves emerging from the brain were cut, suggesting a peripheral locus for the geomagnetic transducer (Popescu and Willows 1999) leading to speculation that magnetic biosensors that affect brain function (in general) "could be in the big toe, or anywhere" (Hand 2016).

In addition to mollusks, crustaceans constitute another prominent category of sea creatures that respond to GMFs as exemplified by the spiny lobster (Lohmann and Ernst 2014). As Lohmann and Ersnt explain, spiny lobsters have a magnetic compass of the polarity type, similar to salmon and mole rats, that determines north using the horizontal component of the geomagnetic field (another type of magnetic compass found in nature is the inclination compass used by birds and sea turtles that defines "poleward" as the direction where the angle between the magnetic field vector and gravity vector is the smallest (Lohmann and Ernst 2014)). To date, similar to mollusks where the geomagnetic transducer is thought to exist outside of the brain *per se* and nematodes (where the actual molecular-level biosensor remains unknown), magnetoreceptors in crustaceans have yet to be identified definitively. One possibility is that magnetite nanoparticles ~50 nm in diameter similar to those found in magnetotactic bacteria act as the receptors. Evidence in support of this idea includes higher than background levels of magnetic material in shrimp and barnacles that respond to geomagnetism; significantly, these species experience disorientation upon demagnetization of these putative magnetite-based receptors (Buskirk and O'Brien 2013) and can also have their preferred orientation deflected by re-magnetization of the putative magnetite particles in a different direction (Lohmann and Ernst 2014).

Besides magnetite, a biosensing option in water-dwelling organisms is electromagnetic induction (as discussed in more detail in Sect. 3.4.3 below), which occurs when electrically conductive material moves through a magnetic field in any direction not parallel to the field (seawater is particularly conducive for transmission of electrical currents). As a result, positively and negatively charged particles move to the opposite sides of the object resulting in a voltage that depends on the velocity of the object relative to the magnetic field (Lohmann and Ernst 2014). Finally, as discussed in Sect. 3.4.2, chemical magnetoreceptors exist in many types of animals but have yet to be investigated in crustaceans (Lohmann and Ernst 2014).

3.3.2.3 Insects

The crustaceans just mentioned are arthropods, a phylum shared with insects; consequently it is not surprising that insects also provide numerous examples of magnetoreceptoin and magnetotaxis including ants (de Oliveira et al. 2010) and bees. Magnetoreception has been particularly well-studied in bees – no doubt because of their agricultural importance as pollinators; this role critically depends on their direction-finding ability and their innate compass, which allows them to find and

"remember" the location of food sources over distances up to five kilometers away. An early study showed that bees have magnetic remanance consistent with the presence of magnetite (Gould et al. 1978); subsequent electron paramagnetic resonance (EPR) imaging of honeybees showed magnetite was primarily located in the insects' abdomens (El-Jaick et al. 2001).

Despite decades of study, the precise mechanism of magnetoreception in bees remains controversial. For example, recent study of bumblebees showed that iron-based granules exhibiting magnetic character were not only located in their bodies (i.e., abdomens) as found in honeybees but were also abundant at peripheral sites on their wings and heads, thereby providing new models for how magnetite-based direction finding could work in these insects (Jandacka et al. 2015). Emerging evidence suggests that honeybees may have a dual sensing system that includes photochemical reactions (Válková and Vácha 2012). A recent study downplayed this complementary mechanism, based on experiments that showed honeybee magnetoreception worked in the total dark, where the requisite initiating photochemical reactions could not take place (Liang et al. 2016) (the radical pairs mechanism discussed in detail in Sect. 3.4.2, below explains the requirement for light for "chemical" magnetoreception). Support for dual-sensor magnetoreception in insects is provided by vertebrates – in particular several species of birds – that appear to rely on both magnetite and chemical magnetoreception for direction finding and migration over very long distances. Another possibility is that bees have both types of sensors that work independently with chemical magnetosensing acting as a "back up" mechanism (Dovey et al. 2013).

3.3.3 Vertebrates

3.3.3.1 Overview

The discussion up to now covered several "ancient" organisms that often have unique biological abilities that do not carry over to more advanced phyla such as vertebrates (for example, although magnetite appears in many types of more advanced animals, the specialized arrangement of the iron crystals into magnetosomes observed in bacteria has not been found above the prokaryotic level). It is clear, however, that magnetoreception *is* found in many higher organisms including vertebrates and, at least in some cases, detection of the magnetic fields rely on non-magnetite based biosensors. For example, several types of fish – exemplified by sharks – have specialized electrical sensing organs that are thought to also provide magnetoreception through induction, as discussed in Sect. 3.4.3. Other species, such as salmon, use magnetic fields for navigation over the vast distances they travel both in the open ocean and for returning to the precise site of their birth to procreate, which requires the correct choice between multiple river junctions as they move upstream. In addition, many amphibians and reptiles have the ability to detect and magnetic fields (Chap. 5). While fascinating, these examples will not be described

further here, instead we will briefly cover birds (Sect. 3.3.3.2), where chemical magnetoreception (Sect. 3.4.2) has become well established and then to mammals (Sect. 3.3.3.3) that share many biological similarities with humans and thus provide a reasonable scientific foundation to explain how magnetic fields can influence biological responses and work therapeutically in people.

3.3.3.2 Birds

Magnetoreception has been described in many birds – and indeed may be ubiquitous across the avian world – including remarkable examples such as the Arctic Tern that literally navigates from one end of the globe to the other. Although traveling shorter distances, the homing pigeon exemplifies precision direction finding ability that uses magnetic cues; in particular, a pigeon's homing ability in part is derived from magnetite-based receptors in the beak. These magnetoreceptors, however, only record magnetic intensity and as such, are just one component of a bird's multifactorial navigation mapping ability (Wiltschko and Wiltschko 2013). Increasingly compelling evidence suggests that birds, reminiscent of bees where a dual sensing system for GMFs has been proposed, use both magnetite and photoreceptors. The light sensing ability of birds has been linked to cryptochrome proteins, whose underlying chemistry is described in more detail in Sect. 3.4.2 below. Briefly here, these proteins have long been known to participate in circadian rhythms when located *in the nuclei* of certain retinal cells. Recently Bolte and coworkers discovered forms of cryptochrome (Cry1a and Cry1b) *in the cytosol* of retinal cells in migratory birds (e.g., European robins and homing pigeons) that depend on both light and magnetic fields for direction finding (Bolte et al. 2016). The unique cytosolic localization of these crytoptochromes suggests that they are not involved in circadian rhythms; instead their non-nuclear localization implicates their involvement in photosensing-based magnetoreception.

3.3.3.3 Mammals

The elucidation of magnetorececeptionin mammals has lagged other types of organisms, such as bacteria and birds for which much is now known even though mysteries remain. Nevertheless a fairly recent of magnetic field sensing in mammals (published in 2014 [(Begall et al. 2014)]), shows that many intriguing – albeit often preliminary and so-far inconclusive – pieces of evidence are available that together make the case that human's closest evolutionary relatives *do* respond to magnetic fields in several ways. In particular, magnetoreception studies have suggested that mammals can utilize GMFs for homing and direction finding; the focus on this "obvious" endpoint can be explained by researchers who were seeking to reproduce magnetotactic abilities already widely documented across many phyla. Briefly, these studies showed that cetaceans can migrate thousands of kilometers based on magnetic cues; rodents displaced hundreds of meters (or more) from their

homes can return successfully based on what the best evidence suggests is magnetic homing; bats preferentially build nests aligned to magnetic fields and have similar roosting preferences; and finally cattle, sheep, and deer – and even dogs – preferentially (for reasons otherwise unclear) align their bodies along N-S magnetic axes (Begall et al. 2014).

Indications are emerging that mammals can exploit geomagnetism for reasons beyond direction finding and homing. For example, the success of red foxes in hunting mice is correlated with the alignment of the direction of jumping attacks with GMFs when the fox's vision is obscured by snow or high vegetation (Červený et al. 2011). Relevant to the ultimate objective of this chapter and book – which is the evaluation of magnetic field therapy in humans – mice (presumably when safe from being hunted from red foxes) experience changes in stress-induced analgesia (the inability to feel pain) dependent on SMF exposure (Betancur et al. 1994). Subsequent studies showed that shielding of the ambient magnetic fields (to produce hypomagnetic fields or "HMFs") reduces stress-induced analgesia in these rodents (Choleris et al. 2002; Prato et al. 2005). These and other similar studies have established that exposure (or lack of exposure) to weak magnetic fields can affect mammalian biology in biomedically-relevant ways. In particular, these studies provide a foundation for the idea that magnetic therapy is viable in mammals even at low field strengths, and as discussed below (and in Chap. 7 of this book), these effects can in theory be complemented, augmented, and amplified by using stronger field strengths available in clinical settings using specialized treatment devices.

3.4 Types of Biological Magnetoreceptors

The overview of magnetoreception provided above in Sect. 3.3 highlighted two major molecular-level mechanisms underlying magnetic field detection across several classes of diverse organisms. The first, and most prevalent, is the exploitation of magnetite across many types of life for direction finding and additional biological responses (magnetite is discussed further in Sect. 3.4.1). Evidence is also consolidating behind a second modality for magnetoreception – with the important caveat that a full understanding of how any animal perceives magnetic fields has not yet been attained (Lohmann and Ernst 2014) – is the radical pairs mechanism (RPM) used in chemical magnetoreception. Biological iterations of the RPM widely use crytochrome proteins that are putatively utlized by organisms ranging from bumblebees to birds as part of their magnetic compass and even to mice where pain sensing was found more than 20 years ago to be modulated by both light and magnetic field exposure (Betancur et al. 1994). Cryptochromes and other chemistry-based possibilities for magnetoreception are discussed further in Sect. 3.4.2. Finally, a third more specialized mode of magnetic field detection, which is electrical induction, is covered in Sect. 3.4.3.

3.4.1 Magnetite

3.4.1.1 Structure and Biosynthesis in Prokaryotes

Magnetite can be considered to be the original biological magnetoreceptor. The "original" designation is based both on evolutionary history with magnetite present in early-evolving lifeforms (e.g., bacteria and unicellular algae) (Lefèvre and Bazylinski 2013) and also because it was the first magnetic biosensor discovered and characterized by modern science (it has been linked to behavioral responses in living organisms for half a century or more (Bellini 1963; Blakemore 1975)). Magnetite is common in the abiotic mineral world, comprising a major source of iron ore; chemically, magnetite is cystalline iron oxide (Fe_3O_4) that is ferromagnetic when in crystal form and therefore becomes a permanent magnet itself after exposure to an applied magnetic field. In bacteria, individual magnetite particle sizes can range from 35 to 120 nm with a particle size distribution much narrower than possible using chemical synthetic methods (Kahani and Yagini 2014); the size range of prokaryote-made magnetite is consistent with single-domain crystals that can be as small as 20 nm or as large as 100 nm (Mirabello et al. 2016). In magnetotactic bacteria, individual magnetite crystals are arranged into "magnetosomes," which are aggregates (usually linear chains) of ~20 magnetite crystals aligned along the long axis of the cell. Each magnetite crystal is surrounded by a membrane and is connected to the cell wall through cytoskeletal filaments (Mirabello et al. 2016). Magnetosome biosynthesis in prokaryotes, which involves the formation of these unique mineralized organelles, is increasingly being unraveled and is now known to require many genes that initiate nucleation and participate in the growth of the crystals (Arakaki et al. 2008; Lower and Bazylinski 2013; Mirabello et al. 2016; Murat et al. 2010).

3.4.1.2 Distribution and Function in Higher Organisms Including Humans

Magnetite has been discovered and studied across many species; it has now been detected in crustaceans, insects, birds, salmon, sea turtles, and other animals (even mammals such as cattle) that can orient themselves with respect to the Earth's magnetic field. Although controversial, magnetite has been reported to exist in the human brain (Kirschvink et al. 1992) as well as in the heart, spleen and liver (Schultheiss-Grassi et al. 1999). Magnetite isolated from higher animals typically exists as single-domain crystals similar to those found as chains in magnetosomes in magnetotactic bacteria (Johnsen and Lohmann 2008). The origin and source of magnetite in higher organisms such as people remains unclear, however, because counterparts to biosynthetic genes in bacteria (Mms5, Mms6, Mms7(MamD), Mms13(MamC), MamF, ManG, and MmsF (Mirabello et al. 2016)) do not seem to

be present and spontaneous chemical crystallization of magnetite results in larger size distributions than found in nature (Kahani and Yagini 2014).

Mechanistically, there are several ways that magnetite crystals have been postulated to transduce geomagnetic field information to the nervous system (or other organ systems) by Lohman and Ernst (Lohmann and Ernst 2014). These mechanisms are guided by lessons learned from magnetosomes bacteria, where each magnetite crystal is surrounded by a membrane and this larger structure is connected to the cell wall through cytoskeletal filaments providing a biochemical mechanism for force transduction (Mirabello et al. 2016). In particular, when the crystalline magnetite nanoparticles attempt to rotate to align with the GMF or other external field, torque would be transmitted from the magnetosome via the cytoskeleton. In higher organisms, if a similar system were in place, force could be transduced through a similar mechanism to secondary receptors (such as stretch receptors, hair cells, or mechanoreceptors); another possibility is that the rotation of intracellular magnetite crystals might directly (or indirectly) open ion channels (Cadiou and McNaughton 2010).

Indirect evidence for physical connections between magnetite and the cytoskeleton comes from the aforementioned studies where shrimp and barnacles, that experience disorientation upon demagnetization of these putative magnetite-based receptors (Buskirk and O'Brien 2013), can have their preferred orientation deflected by re-magnetization of the putative magnetite particles in a different direction (Lohmann and Ernst 2014). If magnetite crystals could freely rotate, they would quickly adopt random orientations inconsistent with these effects, which require all (or at least some) of the magnetite in the organism's body remain aligned in a certain way. Accordingly, magnetite presumably must be tethered to larger biomacromolecules, such as the cytoskeleton (which plays a dual role in both immobilizing the magnetite crystals and transducing force when the nanoparticles attempt to rotate to maintain alignment with the GMF or other magnetic field). Figure 3.2 conceptually illustrates the tethering of magnetite to the cytoskeleton and force transduction to membrane components while Cadiou and McNaughton present a detailed description of how this type of force transduction hypothetically functions in eukaryotic cells (Cadiou and McNaughton 2010).

3.4.2 Chemical Magnetosensing

3.4.2.1 Background: The Chemical Basis of the Radical Pair Mechanism (RPM)

Chemical reactions that proceed through radical intermediates can be influenced by magnetic field effects (MFEs) that alter reaction rate, yield, or product distribution (Rodgers 2009); the "radical pair mechanism" (RPM) underlies these effects. An RPM-influenced reaction begins when a ground-state precursor species (e.g., "A and B") are excited to produce two singlet radicals i.e., a spin-correlated radical pair

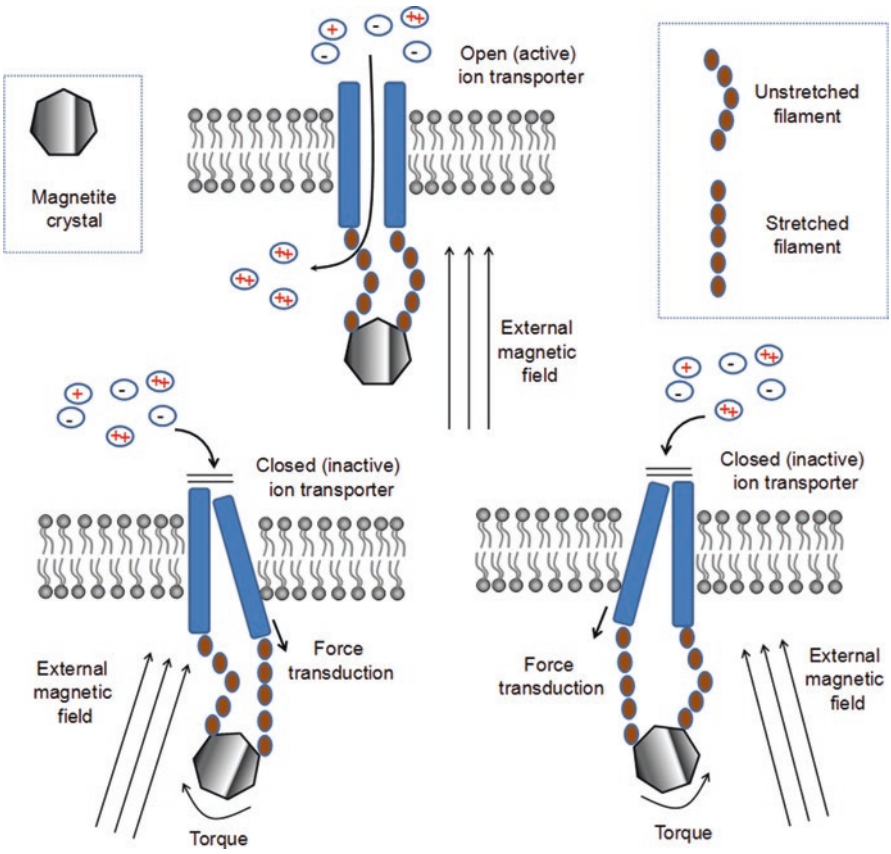

Fig. 3.2 Conceptualization of magnetite-based force transduction. (*Top*) An ion channel in the "open" conformation is shown along with connections to an intracellular magnetite particle via unstretched filaments; under these conditions the magnetic field of the magnetite is aligned with an externally-applied field. (*Bottom*) Upon misalignment of the magnetite and the external field, the magnetite turns in an attempt to re-align with the external field resulting in the generation of torque that can stretch the filaments and in the process, transduce force to membrane elements (in this depiction, the ion channel becomes distorted and subsequently experiences changes in activity)

(RP); the singlet RP electrons can undergo a spin-selective reaction to produce the singlet product (Fig. 3.3). However if coherent evolution of the spin state converts singlet RPs to triplet RPs on a similar (or faster) time scales as singlet product formation, the triplet product can be formed resulting in either different reaction kinetics or product composition for the chemical reaction.

The role of magnetism comes into play when S → T (singlet to triplet) conversion and the reverse T → S conversion of the spin-correlated RP are driven by magnetic interactions. Remarkably, even a weak applied magnetic field that has a much smaller effect on the reactants than factors such as thermal motion at physiological temperature can profoundly influence product formation in an RPM reaction. A

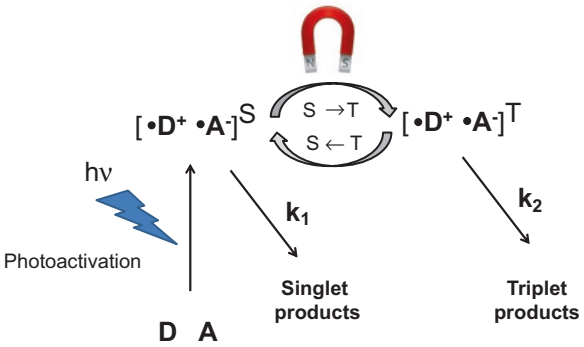

Fig. 3.3 Diagram outlining the radical pair mechanism (RPM). A spin-coupled radical pair is generated (by photoactivation in magnetoreception) resulting in a donor molecule (**D**) transferring an electron to an acceptor molecule (**A**). An external magnetic field affects interconversion between singlet (S) and triplet states (T) of the radical pair; typically the presence of an applied field increases the transient abundance of the triplet state resulting in more rapid production of triplet products (i.e., k_2 compared to k_1 increases upon application of an external force, or in the case of GMF sensing, appropriate alignment of the field with the reacting spin-coupled radical pair) (Adapted from Ritz et al. (2000), Rodgers (2009), and Wikipedia)

simplifying analogy provided by Rodgers to describe the influence of an external magnetic field is to consider a train approaching a railway switch (Rodgers 2009). The train is being propelled by a locomotive, which requires considerable energy, but the final destination (i.e., the composition of the reaction products) and time it takes to reach it (i.e., the reaction kinetics) completely depend on the expenditure of a small amount of energy (e.g., an amount that can be provided by a single person in a few seconds of effort) to change a junction switch in the track from one route to another destination. This relatively tiny force is equivalent to the role that magnetic fields – even weak GMFs – can play in determining the outcome of RPM reactions.

3.4.2.2 The RPM in Magnetic Field Biosensing

Based on the above explanation, RPM reactions provide a second biological transducer for weak SMFs (after magnetite) such as GMFs. The proposed mechanism requires the production of initiating free radical intermediates; in purely chemical systems appropriate radical-inducing catalysts could be introduced into the system for this purpose. In biological systems where such catalysts do not play a role, the production of the initiating radical pair (RP) is generally believed to require the absorption of a photon (i.e., from visible light). Accordingly, the receptors involved in such sensing need to be located on, or within a few hundreds of microns, of the surface of an organism where ambient light could penetrate. Most logically, these

receptors would be located in the eye, which is already optimized for photosensing; based on this reasoning, leading candidates for magnetoreception are cryptochrome proteins. Ritz and coauthors outlined how cryptochromes could function magnetoreception in the year 2000 (Ritz et al. 2000); this group described how, upon exposure to blue light, these proteins transfer an electron to flavin adenine dinucleotide (FAD) resulting in both the protein and the flavonoid having unpaired electrons – i.e., the "radical pair" required for an RPM reaction. It should be noted that the exact RPM reactants remain ambiguous; it is generally thought that in addition to the flavin, the other radical pair is one of three tryptophan residues on the cryptochrome protein although it has been speculated recently that the other reactant might be ascorbic acid rather than one of the suspected tryptophans (Lee et al. 2014). Nevertheless, an RPM reaction "activates" cryptochome proteins for chemical magnetosensing, often in conjunction with magnetite-based mechanisms as discussed above.

Cryptochrome reactions depend on continuous photoexcitation (Kattnig et al. 2016), explaining the requirement for both light and the presence of a magnetic field – i.e., the "dual sensing" mechanisms mentioned above for bees, birds, and mice. Because the spins of the two unpaired electrons are correlated with each other and subject to the surrounding magnetic field (e.g., the relative orientation with the geomagnetic or to an induced field) in an RPM reaction, the presence, absence, relative strength, and orientation of the magnetic field affects the length of time cryptochrome remains activated (Ritz et al. 2000). In turn, activation of cryptochrome affects the light-sensitivity of retinal neurons, with the overall result that a bird (or a bee) can see the color phase shift caused by the magnetic field (Ritz et al. 2000). In practice, the dependence of dual sensing mechanisms on continuous photoexcitation can be used empirically to deconvolute magnetosensing behavior; for example bees are known to have the biochemical machinery for RPM magnetoreception but their ability to utilize their magnetic compass in the total dark indicates that magnetic direction finding ability can function solely via a magnetite mechanism (Liang et al. 2016).

3.4.3 Electromagnetic Induction

3.4.3.1 Biological Precedent for Induction: The Ampullae of Lorenzini

Sharks, stingrays and certain cartilaginous fish have electroreceptive organs known as ampullae of Lorenzini that can detect changes to electric potential; these specialized structures allow these sea creatures detect direct (DC) electric currents in water and help sense the weak electric fields of prey and predators (Murray 1960). The ampullae of Lorenzini also allow sharks (and other animals with these physiological structures) to detect even very weak magnetic fields (Meyer et al. 2005). This ability

results from the phenomenon where the movement of electrically conductive material through a magnetic field in any direction other than parallel to the field lines results in the migration of positively and negatively charged particles migrate to opposite sides of the object (Roth 2012). As a result, a voltage is generated that depends on the velocity of the object's motion relative to the magnetic field. From a physics perspective, this phenomenon is known as the "Hall effect" that states that a magnetic field exerts force on a moving ionic current and as a result, a magnetic field perpendicular to the flow of an electric current will exert force to deflect and separate the charged ions. Presumably, specialized biological systems are then able to detect, and respond to, this mismatch in electrical charge potential as the host organism moves through misaligned SMFs.

3.4.3.2 The "Hall Effect" – Relevance Beyond Specialized Electroreceptive Organs?

The Hall effect has been – at least on the internet – used to explain the effects of magnetic fields in biological settings in some clearly misguided ways. For example, one claim is that electrons (being regarded as "charged particles") orbiting the nucleus of an atom (i.e., they are presumed to be moving in space) are propelled to higher velocities, thereby enhancing chemical reactivity. (In reality, applied magnetic fields only influence chemical reactivity through electron spin effects via the specialized RPM reactions described above in Sect. 3.4.2). Another common misconception is that the Hall effect can be used to explain changes to blood flow observed upon magnetic field exposure. While it is true that blood does contain copious amounts of charged (e.g., sodium and chloride ions) and paramagnetic (e.g., hemoglobin in certain oxidation states) entities, the physical forces generated by the Hall effect are dwarfed (they are generally orders of magnitude smaller) than kinetic energy associated with blood flow (which is generated through the mechanical action of the heart) not to mention thermal motion of biomolecules at body temperature. As a result, the idea that electromagnetic induction plays a role (outside of specialized ampullae of Lorenzini) in transducing magnetic field exposure into biological response is often met with disbelief.

3.5 Mechanisms for SMF Effects on Human Biology

Now that we have outlined biosensors found in the natural biological world for magnetoreception, we will revisit each of them in the context of human biology and provide a synopsis whether they plausibly play a role in magnetotherapy. As will be described in Sect. 3.5.1, the established magnetosensors do not provide a satisfying explanation for responses observed in humans, spurring speculation in Sect. 3.5.2 about "other" possibilities.

3.5.1 "Established" Biosensors/Magnetoreceptors

3.5.1.1 Magnetite

Over the past 30 years there have been periodic reports of magnetic iron (i.e., magnetite) in the human body with some of these studies being debunked because of possible contamination (Hand 2016); for context many of these studies came from the same era when aluminum "contamination" from cooking utensils and containers was (in retrospect implausibly) linked to plaques associated with Alzheimer's disease (Savory et al. 1996). Other reports of magnetite in humans, however, have remained plausible. One such study was published in the Proceedings of the National Academy (USA) that reported detailed parameters about magnetite-like iron assemblages in the human brain (Kirschvink et al. 1992). These crystal structures resembled magnetite from magnetotactic bacteria and fish and were present at minimum levels of five million single-domain crystals per gram for most types of brain tissues. Certain regions of the brain (e.g., pia- and dura-derived samples) had ~20-fold higher levels; further, the magnetite occurred in clumps of 50–100 crystals. The magnetite nanoparticles distributed (or, based on the numbers outlined in the next section, a better description might be "sparsely scattered") through neuronal and astroglial membranes have been proposed to play roles in perception, transduction and storage of information that arrives to the neocortex (Banaclocha et al. 2010).

To provide context for these findings, one gram of brain tissue has roughly one billion cells. Accordingly, if the magnetite clumps were intracellular, only about one in 500 to one in 20,000 cells – depending on the exact size of the clumps and which part of the brain was under analysis – could contain a magnetite clump. If the clumps were extracellular (which is not consistent with the proposed role of magnetitie-based force/signal transduction in eukaryotic cells, as outlined in Fig. 3.2 and by Cadiou and McNaughton (Cadiou and McNaughton 2010)), additional cells could be directly impacted by, or interact with, the magnetite. Either way based on the reported amount of magnetite, only a relatively fraction of brain cells could be involved in magnetoreception through a magnetite-based mechanism.

Another comparison is that honeybees have approximately 10^8 magnetite crystals in their bodies (Kirschvink and Gould 1981); based on a mass of ~100 mg a bee has ~10^9 (one billion) copies of magnetite per gram or about 200-fold more on a mass basis. While it is at least theoretically plausible that only a minor subset of neural cells might be involved in magnotosening in humans, the search for these cells constitutes a veritable "needle in the haystack" scenario and in the 25 years since this PNAS report was published (Kirschvink et al. 1992), magnetoreception *via* magnetite in the human brain remains unproven. Showing remarkable persistence, however, the lead author of the PNAS study, Joe Kirschvink, has continued to pursue the possibility of magnetic sensing in the human brain and was recently featured in a Science (the magazine) news article describing how he and his colleagues are embarking on the next generation of studies to pursue the elusive goal

of obtaining "definitive" proof for magnetotherapy or other magnetic field effects in humans (Hand 2016).

3.5.1.2 Chemical Magnetoreception via Cryptochromes

As just mentioned, efforts to confirm that magnetoreception exists in humans continue with exciting new initiatives being planned (Hand 2016). It should be noted that in addition to the decades old hypothesis that the human brain (and other tissue) contains magnetite, humans may have a dual sensing system similar to bees, birds, and mice based on cryptochromes. Two complementary lines of evidence support this idea. First, geomagnetic fields can influence the light sensitivity of the human visual system (Thoss et al. 2000; Thoss et al. 2002), evoking cryptochrome-based systems found in other species. Second, a biochemical foundation for this hypothesis is falling into place. In particular, humans express two cryptochromes (hCRY1 and hCRY2) in their eyes showing that – at least in theory – have the biochemical machinery for chemical magnetoreception (up to now, these proteins have primarily been linked to circadian rhythms). Foley and coworkers performed a critical experiment in support of this hypothesis by taking a transgenic cross-species approach to show that hCRY2, which is heavily expressed in the retina, can function as a magnetic field sensor in the magnetoreception system of *Drosophila* in a light-dependent manner (Foley et al. 2011). Although this result showed that hCRY2 has the molecular capability to function as a light-sensitive magnetosensor, it must be emphasized that even though hCRY proteins *can* function in magnetosensing roles as-of-yet there is no firm evidence that they actually perform this way in humans or even in other mammals such as dogs and apes that also exhibit certain GMF-sensing abilities and (perhaps entirely coincidentally) express cryptochromes in the retina (Nießner et al. 2016).

3.5.1.3 Induction: Revisiting the Effects of SMFs on Red Blood Cells

The idea that magnetic fields can influence blood flow and cardiovascular circulation is pervasive. As mentioned earlier, an often mentioned but fallacious scientific basis for this premise is that an applied magnetic field has inductive effects on iron-laden red blood cells (RBCs) that influence the overall circulation of the blood. On one hand, this idea is reasonable considering that RBCs typically constitute 40% or more of the volume of blood and if magnetic field-associated induction really was in play, the overall circulation of the blood easily could be affected but any inductive force (or, the "Hall effect") is too weak to measurably affect blood constituents. Secondly, there is considerable confusion and misinformation that iron in RBCs is "magnetic." Clearly it is not ferromagnetic because it is not organized in crystalline "magnetite" form; this iron, however, can be paramagnetic, which has long been recognized to have at least diagnostic value, but not without many nuances and caveats. For example, a paper from 1961 titled "Problems in the Measurement of

Blood Flow by Magnetic Induction" (Wyatt 1961) reported technical issues that bedeviled (at that time) already 20 year old efforts to exploit the electromagnetic fields generated by movement of paramagnetic iron found in RBCs to measure blood circulation.

In the intervening years, the study of iron in RBCs under the influence of magnetic fields has become increasingly sophisticated, for example a 2003 publication by Zborowski and coauthors (Zborowski et al. 2003) outlined the magnetophoretic mobility of different populations of deoxy and oxygenated erythrocytes (i.e., RBCs). This study showed that with the development of a new technology, cell tracking velocimetry, it was possible to measure the migration velocity of deoxygenated and metHb-containing erythrocytes exposed to a magnetic field of 1.4 T (i.e., a MRI-strength field). In this study erythrocytes with 100% deoxygenated hemoglobin had a magnetophoretic mobility of 3.86×10^{-6} mm^3 s/kg compared to 3.66×10^{-6} mm^3 s/kg for erythrocytes containing 100% metHb; in other words, both of these forms of hemoglobin displayed paramagnetic properties. By comparison, oxygenated erythrocytes had magnetophoretic mobilities ranging from -0.2×10^{-6} mm^3 s/kg to $+0.30 \times 10^{-6}$ mm^3 s/kg, indicating that these cells were primarily diamagnetic (Zborowski et al. 2003). The detection and analysis of these properties have matured since 2003, allowing dielectrophoretic and magnetophoretic methods to now be used for diagnosis of medical conditions such as malaria parasite-infected red blood cells (Kasetsirikul et al. 2016).

Although RBCs can now be studied and indeed, be used for diagnostic medical tests, by exploiting their magnetic properties it is less clear that externally applied magnetic fields have legitimate therapeutic effects on blood circulation, as is often claimed by vendors of "magnetic therapy" products (e.g., *see* Chap. 7). In particular, the impact of applied magnetic fields on unpaired electrons in contexts other than RPM reactions (as discussed above) is negligible under weak (i.e., geomagnetic strength) fields and even strong (i.e., 1.3 to 3 T MRI-strength fields) have negligible "chemical" effects. The lack of any such clear effect is evidenced in rather lax regulatory oversight by agencies including the United States FDA, which allows "wellness" magnetic field devices to be marketed to "treat" almost any type of ailment because safety is not an issue (Anonymous 2015). As a caveat, the word "treat" in the previous sentence is not completely accurate in a medical sense because the FDA specifically prohibits claims of therapeutic efficacy against any particular disease condition for magnetic field generating devices.

3.5.2 "Other" Human Biosensors

3.5.2.1 Human Cells Appear to Have Additional Magnetosensing Capacity

Evidence exists that humans *can* respond to magnetic fields; for example geomagnetic fields influence the geomagnetic field on the sensitivity of our eyes (Thoss and Bartsch 2007). This evidence remains controversial because (apart from unscientific

internet claims) a lack of clear evidence exists to explain how the three "canonical" modes of magnetoreception (magnetite, chemical magnetosensing, and induction) function in humans. In particular, even the first two modes required for visual geomagnetic perception in other species remain largely mysterious in humans. In part, progress is slow because many experiments performed with lower species (e.g., dissection of brains in living nematodes to uncover specific neurons involved in magnetorecption) cannot be performed in humans. In a way, ethical (commonsense, really) considerations that prevent such experimentation in humans have been a blessing in disguise, forcing researchers to use cell lines a surrogates for *in vivo* testing. These studies have led to the discovery of responses to magnetic fields at the cell level that do not involve any of the three "established" modes of magnetoreception. For example, immobilized cells maintained in the dark in incubators with unchanging SMFs are not expected to exhibit chemical magnetosensensing (because they're kept in the dark) or induction (because they do not move); similarly, there is no plausible mechanism for the presence of magnetite in these cells. To briefly illustrate this point, we next mention both an outside example based on HMF exposure (Sect. 3.5.2.1*)* and work from this author's lab using moderate strength SMFs (Sect. 3.5.2.2).

3.5.2.2 HMF Effects on Cell Behavior Are Mediated by the Cytoskeleton

Recently, Mo and coauthors found that HMFs repress expressions of genes associated with cell migration and cytoskeleton assembly in human neuroblastoma cells grown in cell culture conditions that were not plausibly subject to any of the magnetite, chemical magnetoreception, or induction mechanisms (Mo et al. 2016). Going beyond analysis of gene expression, they showed that HMF modulated "whole cell" behaviors in SH-SY5Y cells including control of cell morphology, adhesion and motility and traced these changes to the actin cytoskeleton. This study suggested that the elimination of geomagnetic field affects the assembly of the motility-related actin cytoskeleton, and implicates F-actin as a target of HMF exposure and positions it as a potential novel mediator of GMF sensation (Mo et al. 2016).

3.5.2.3 SMF Effects on Lipid Membranes and Downstream Signaling

The author's laboratory has published two studies that implicate biological membranes as the "biosensor" for magnetic fields in the apparent absence of canonical chemical (i.e., crytochrome-mediated) mechanisms. These studies were based on literature reports that SMFs alter the biophysical properties of lipids (Braganza et al. 1984) and by extension, higher order structures such as lipid bilayers (De Nicola et al. 2006; Nuccitelli et al. 2006; Rosen 2003b). Based on these studies, we postulated that biological membranes were the most reasonable "biosensor" for magnetoreception in cell culture investigations where magnetite was

absence and the cells involved had no obvious light-sensing ability. Further, based on the threshold of ~0.2 T reportedly required for SMFs to have an impact on biological membranes (Braganza et al. 1984), we undertook two studies where cells were treated with 0.23–0.28 T SMFs (the variation is due to different placement of tissue culture plates in the incubator device, *see* Fig. 3.1).

In one study, in part spurred by clinical efforts to use ~0.3 T SMFs to treat Parkinson's disease ((PD), as discussed in more detail in Chap. 7), we monitored the impact of similar magnetic fields on the adenosine A_{2A} receptor ($A_{2A}R$) in the PC12 rat adrenal pheochromocytoma cell line that displays metabolic features of PD. We found that SMF reproduced several responses elicited by ZM241385, a selective $A_{2A}R$ antagonist, including altered calcium flux, increased ATP levels, reduced cAMP levels, reduced nitric oxide production, reduced p44/42 MAPK phosphorylation, inhibited proliferation, and reduced iron uptake (Wang et al. 2010) (as shown in more detail in Chap. 4). Biological responses to ZM241385 result from direct binding to $A_{2A}R$. By contrast, SMF – not being a conventional small molecule pharmacological agent – must elicit cellular responses through a fundamentally different mode of action. A plausible mechanism, outlined in cartoon form in Fig. 3.4, is that ~0.25 SMF exposure directly alters the biophysical properties of lipid bilayers, which in turn rapidly modulates ion channel activity (Rosen 2003a) and thereby perturbs the intra-and extracellular levels of Ca^{2+} levels (Wang et al. 2010; Wang et al. 2009).

3.5.2.4 Lipid Membrane-Based Mechanisms Can (Speculatively) Account for Biphasic Kinetic Responses to Constant Magnetic Field Exposure

In a separate study, human embryoid body-derived (hEBD) LVEC cells were treated with the ~0.25 T fields for time periods of 15, 30, and 60 min, 2, 4 and 8 h, and finally up to 7 days (Wang et al. 2009). Software analysis of gene expression obtained by Affymetrix mRNA profiling of these cells showed that nine signaling networks responded to SMF; of these, detailed biochemical validation was performed for the network linked to the inflammatory cytokine interleukin 6 (IL-6). We found a biphasic response to SMF exposure (Fig. 3.5) where short-term (<4 h) activation of IL-6 mRNA expression occurred with coordinated up-regulation of toll-like receptor-4 (TLR4) and ST3GAL5, phosphorylation of p38, and calcium efflux. Interestingly, the initial multifaceted up-regulation of IL-6 mRNA was already being attenuated by 24 h but actual production of secreted IL-6 did not peak until day 2 after which it dropped to sub-steady levels by day 4.

A biochemical mechanism – outlined in Fig. 3.6 – for the biphasic kinetic response that puzzlingly occurs in the constant presence of a steady SMF, can be postulated based on the increased expression of NEU3 and the decreased expression ST3GAL5 at early time points. These enzymes work together to reduce levels of cell surface-displayed sialic acid including that found on ganglioside GM3. Specifically, NEU3 is a sialidase that removes the sialic acid from GM3, thereby

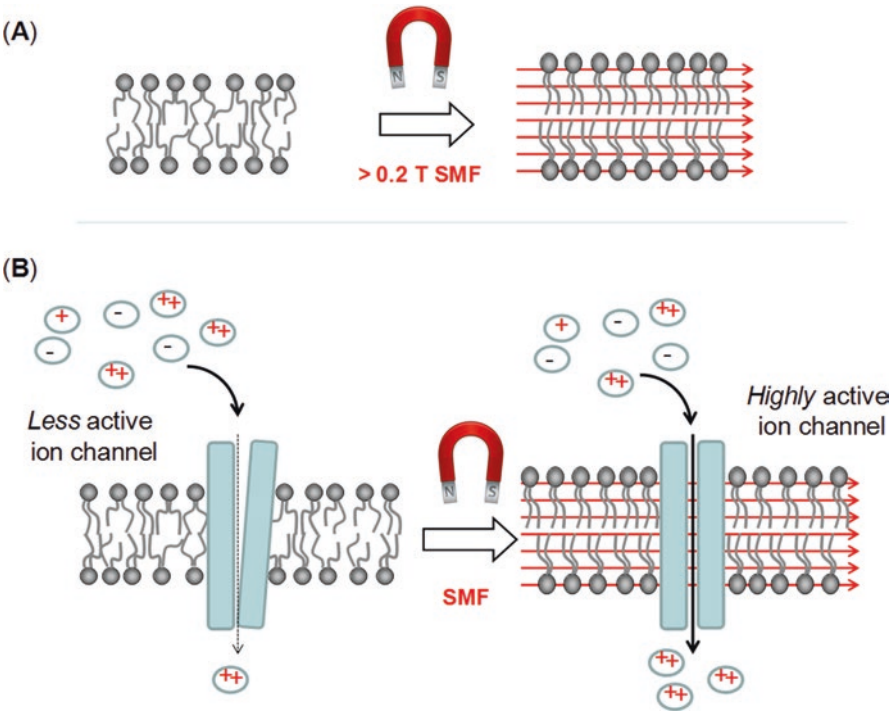

Fig. 3.4 Proposed mechanism for direct effects of SMF on biological membranes. (**a**) Based on literature reports of SMF effects on lipids (Braganza et al. 1984), we propose that field strengths of >0.2 T impose superdiagmagnetic organization on lipid bilayers. (**b**) Extending this concept to biological membranes (i.e., lipid bilayers with embedded proteins such as the cartoon of an ion channel as shown), we found that calcium ion flux rapidly responds to ~0.25 T fields (Wang et al. 2010; Wang et al. 2009). This response can be explained by allosteric regulation of ion channel activity by the relative membrane organization and biophysical properties in presence and absence of the external SMF. This response is conceptually similar to a variation of the magnetite-based mechanism shown in Fig. 3.2 where ion channel activity is not modulated by direct action on the channel (as shown in that figure) but instead results from magnetite's action on cis elements in a membrane that – upon perturbing membrane structure or organization – have an effect on proximally located ion channels (This mechanism is described in detail elsewhere (Cadiou and McNaughton 2010))

generating LacCer; at the same time loss of the biosynthetic enzyme ST3GAL5 prevent regeneration of GM3 (as well as other gangliosides such as GM1). The net effect of this functionally-coordinated response is diminution of cell surface levels of GM3, which we previously showed can affect cell surface signaling (Wang et al. 2006) and others have shown affect that gangliosides modulate calcium ion activity (Carlson et al. 1994). Accordingly, we speculate that SMF exposure immediately affects calcium ion channel activity through changes to the bulk biophysical properties of the surrounding membranes. This sets in motion a series of events that ultimately counteract the impact of SMF. In other words, the initial stimuli presented

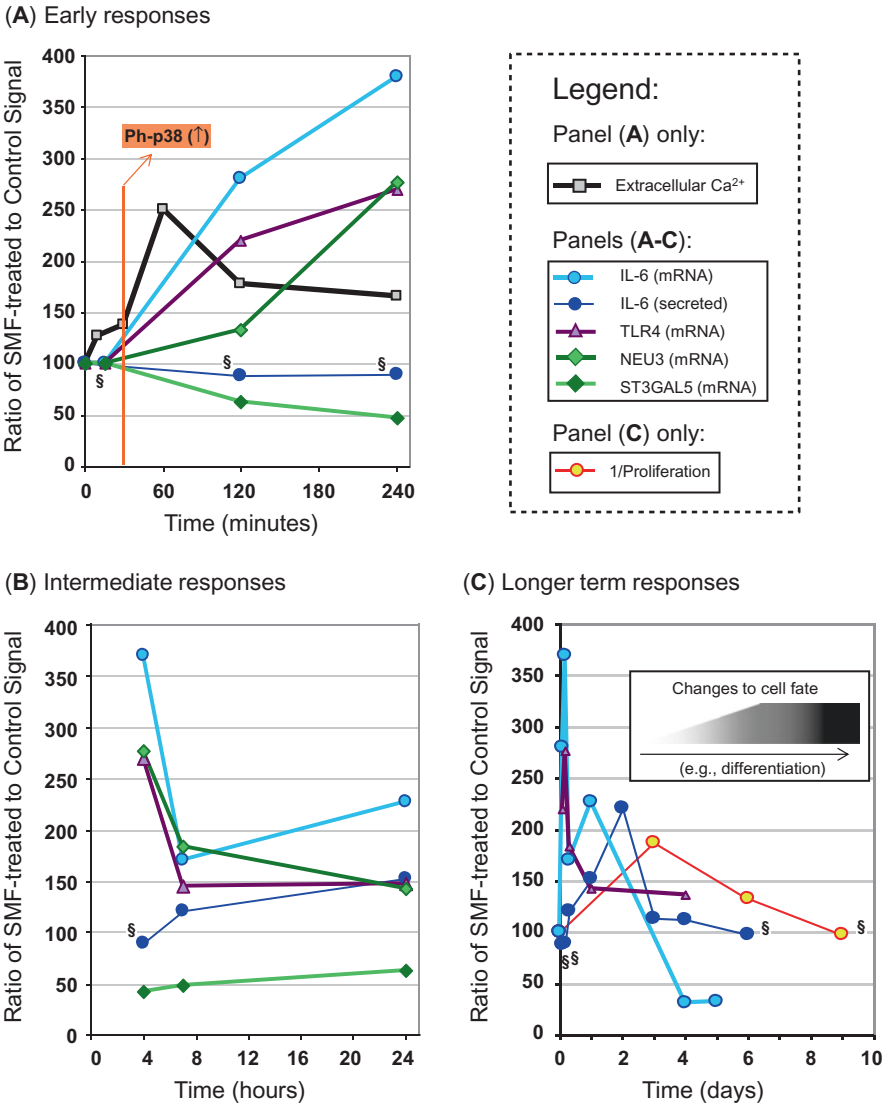

Fig. 3.5 Timeline of SMF-induced, IL-6 associated responses in hEBD LVEC cells. (**a**) early responses that occur within 4 h of the start of continuous SMF exposure include p38 phosphorylation by 30 min, calcium flux, and the other parameters denoted in panel (**b**), which shows intermediate responses that occur over the first day. Finally, (**c**) shows longer term responses over the first week or so of SMF exposure. Data is shown for $n \geq 3$ independent experiments and $p < 0.05$ for all data except for that indicated by "§" where $p > 0.05$ (these data were analyzed by SD but error bars are omitted from these graphs for clarity). All data shown – except for the proliferation data in *Panel* **c** that gives the reciprocal relationship for cell proliferation – compares SMF-exposed to control cells with a value of 100 as a baseline (This figure is adapted from Wang and coauthors (Wang et al. 2009))

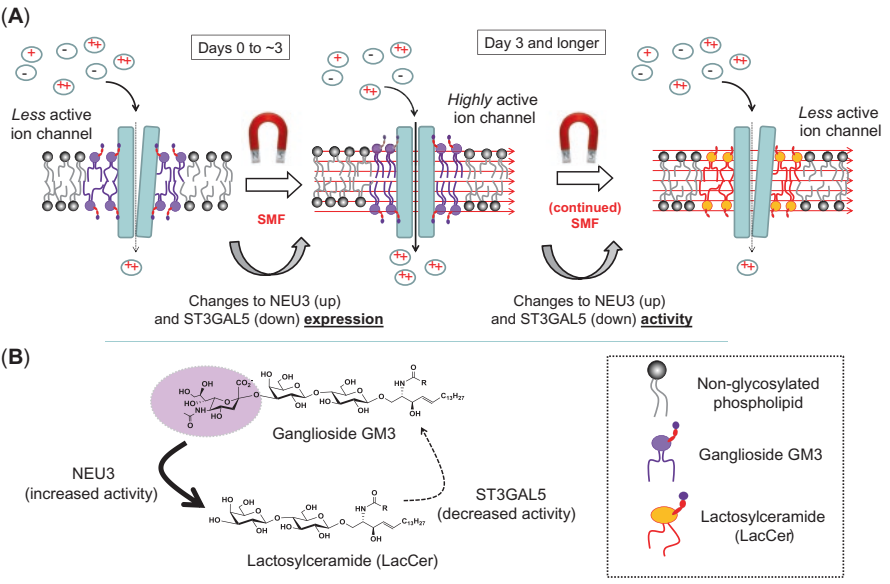

Fig. 3.6 Proposed mechanism for biphasic response to constant SMF exposure. (**a**) This proposal builds on the mechanism shown in Fig. 3.4 with the added provision that SMF exposure alone cannot fully convert low (or inactive) ion channels to a highly active form. Instead, the added contribution of ganglioside GM3 is required; for context GM3 is a prominent constituent of lipid rafts that surround membrane proteins and modulate their activity (this concept is explained in detail in Hakomori's "glycosynapse" publications (Hakomori 2002; Hakomori 2004a; Hakomori 2004b; Mitsuzuka et al. 2005; Toledo et al. 2004)). As a consequence, over the initial period of SMF treatment (e.g., Days 0 to 3), SMF exposure and GM3's impact combine to convert ion channels from a low to high activity state. During this time, however (as shown in Fig. 3.5), changes to the expression of NEU3 and ST3GAL5 lead to a later onset reduction in GM3 abundance (as shown in *Panel* **b**); in particular, GM3 becomes depleted after 2–3 days of SMF exposure when the newly made NEU3 becomes active. As proposed in the far right section of *Panel* **a**, SMF alone (i.e., in the absence of GM3) is not sufficient to maintain lipid conformation or biophysical properties to support "highly active" ion channel flux. (**b**) Biochemical details of NEU3 (a sialidase that removes sialic acid from gangliosided) and ST3GAL5 (the sialyltransferase that converts LacCer to GM3) are shown. The increase activity NEU3 converts GM3 to LacCer, which cannot be replenished at normal rates because of the concomitant decrease in ST3GAL3

by SMF exposure is counteracted by longer-term (also SMF-induced) loss of GM3 (i.e., GM3 ultimately proves to be a stronger mediator of the responses studied than SMF), which ultimately attenuates and in fact reverses IL-6 production over longer exposure periods.

3.5.2.5 Lipid Membranes as a Magnetic Field Biosensor – Revisiting Earlier Evidence

In addition to the speculative mechanism just presented, we briefly revisit magnetic sensing in nematodes (Sect. 3.3.2.1), where specific neurons have been identified to be responsive to the GMF. Early studies, consistent with findings in mollusks and

crustaceans Sect. 3.3.2.2), suggested that the actual biosensor was peripheral to the neurons found to respond to magnetic fields. A more recent study, however, provides convincing evidence that the neurons themselves have magnetic sensing ability of activated calcium flux and activation in the absence of synaptic input (Vidal-Gadea et al. 2015). This information is consistent with our cell-based findings where SMF exposure of human neural-like cells appeared to directly interact with membranes to trigger down-stream response. A counterpoint to this hypothesis, however, is that the nematode study monitored GMF-strength magnetic fields, which are much weaker than the ~0.2 T fields previously described as necessary for direct "magnetic field sensing" by changes to the biophysical properties of membranes; indeed, although not described in the recent (year 2015) study, nematodes have been reported to contain biogenetic magnetite (Cranfield et al. 2004). To conclude, membranes – in and of themselves – may provide added modes of magnetic field biosensing not detected up to now; like many aspects of "magnetobiology" confirmation of this possibility provides exciting future research opportunities.

3.6 Concluding Comments

This chapter revisits, albeit briefly, magnetic field biosensing abilities found across many diverse organisms and attempts to apply the information that has been compiled over the past half century or so to prospects for "magnetotherapy" in humans (this concept is extended in Chap. 7). As covered above, "Nature" has evolved two well-established modes of magnetoreception (magnetite and cryptochrome RPM mechanisms) and the more specialized inductive mechanism exemplified by certain fish that have "ampullae of Lorenzini" sensing organs. As summarized in Sect. 3.5, these mechanisms do not provide a fully compelling explanation for effects of magnetic field exposure in human cells, resulting in speculative models (in part based on this author's previous research) where moderate strength SMFs directly modulate the biophysical properties of biological membranes with profound consequences on downstream signaling pathways, gene expression, and ultimately cell fate.

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Chapter 4
Impact of Static Magnetic Fields (SMFs) on Cells

Abstract This chapter contains two parts. The first one is about parameters that influence the cellular effects of static magnetic fields (SMFs), including magnetic field intensity, cell types, cell densities as well as other cellular factors. The second part is about the various commonly seen cellular effects of SMFs, including cell orientation, proliferation, microtubule and cell division, actin, viability, attachment/adhesion, morphology, migration, membrane, cell cycle, chromosome and DNA, reactive oxygen species (ROS), adenosine triphosphate (ATP) as well as calcium. The focus of this chapter is on current evidence of SMFs on human cells and some animal cells, and especially on the potential factors that contributed to the different observations in individually reported studies.

Keywords Static magnetic field (SMF) • Cell type • Cell density • Red blood cell (RBC) • Orientation • Microtubule • Calcium

4.1 Introduction

Just like temperature and pressure, magnetic field is an important physical parameter that could have a general impact on multiple objects. The effect of magnetic field on object is mainly dependent on the magnetic susceptibility of the object, the magnetic field intensity and gradient. As discussed in Chap. 3, cells are filled with various cellular contents and biomolecules that could respond to the magnetic field, such as cell membrane, mitochondria, DNA and some proteins. For example, it has been shown that the peptide bonds united into organized structures, such as α-helix, which confers proteins diamagnetic anisotropy (Pauling 1979) (Fig. 4.1a–c). Organized polymers, such as microtubules that are composed of well organized tubulin (Fig. 4.1d), are also demonstrated to have strong diamagnetic anisotropy and could be aligned in the presence of magnetic fields (Vassilev et al. 1982; Bras et al. 1998, 2014). Both of them have been discussed in a recent review (Fig. 4.1) (Albuquerque et al. 2016). Obviously, the effects of magnetic fields on biological samples such as a human cell are not restricted to just a few components. In a recent work by Zablotskii et al., the theoretical calculation was provided to explain the effect of high gradient magnetic fields (HGMFs), which belong to SMFs because

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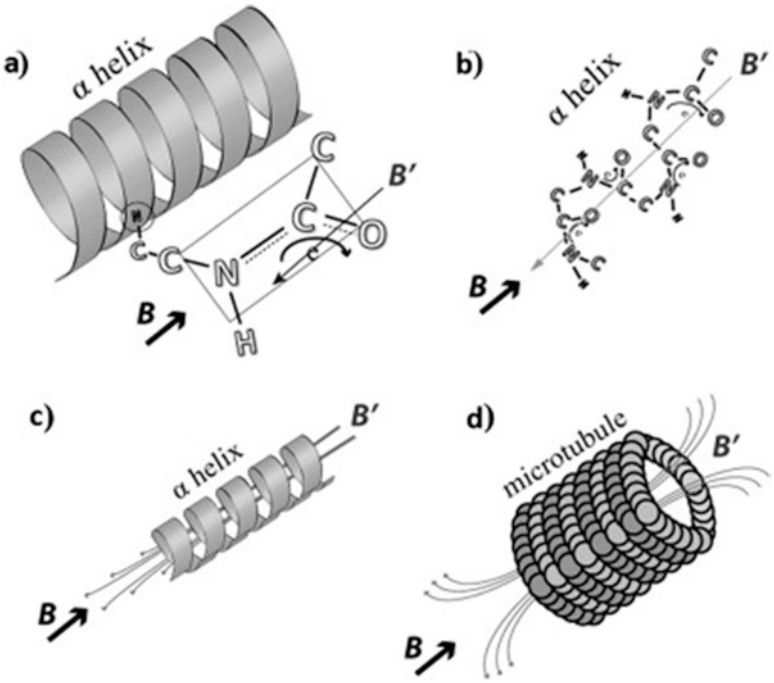

Fig. 4.1 Anisotropy diamagnetism in biological structures. Planar Peptide bonds present in α-helix give it large diamagnetic anisotropy **(a)**. **(b)** and **(c)** show the magnetic vector generated by the helical structures. In microtubules **(d)** the parallel alignment of the peptide bonds with the α-helix axis and their assembly internally to the circular structure increase the magnitude of the magnetic anisotropy as a summation of each secondary magnetic fields B' (Figure was reprinted with permission from ref. (Albuquerque et al. 2016). Copyright © 2016 Published by Elsevier Ltd)

the field intensity does not change over time but are inhomogeneous in space, on biological samples (Fig. 4.2) (Zablotskii et al. 2016). Since different cellular components have differential magnetic susceptibility, the exact cellular effects of a given SMF on a specific cell need to be examined specifically.

Multiple cellular components and molecules can be affected by SMFs, which was already discussed in Chap. 3. In fact, it has been found that even the dissolved oxygen in water could be modulated by high SMFs (Ueno and Harada 1982; Ueno et al. 1994, 1995). The effects of SMFs on cells have been reviewed and discussed previously (Adair 2000; Dini and Abbro 2005; Miyakoshi 2005; Miyakoshi 2006; Ueno 2012), which covered most related literature up to 2012. The recent review by Albuquerque et al. covered many progresses that people made in the past decades about the influence of SMFs on cells (Albuquerque et al. 2016). Here in this chapter, the focus is considerably different. I will try to provide an overview for the current evidence of SMFs on human cells and some animal cells, and especially will focus on the differential cellular effects reported in previous studies as well as their potential causes. Plants, bacteria and other organisms will not be discussed in this chapter, but in Chap. 5.

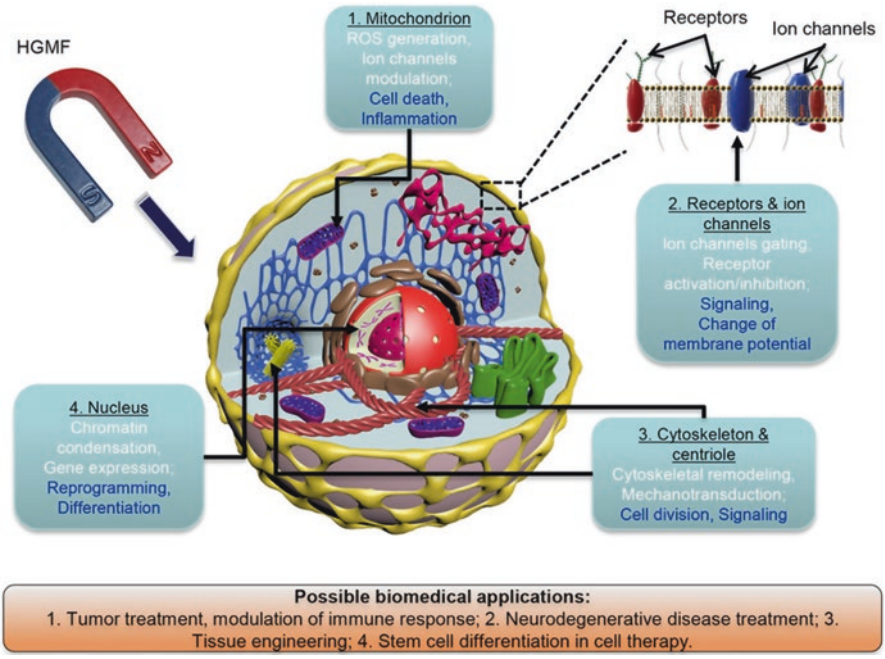

Fig. 4.2 A schematic illustration of the possible applications of high gradient magnetic fields (HGMFs) and intracellular effectors (Reprinted with permission from ref. (Zablotskii et al. 2016). Copyright © 2016, the Author(s). This work is licensed under a Creative Commons Attribution 4.0 International License)

4.2 Parameters That Influence the Cellular Effects of SMFs

In Chap. 1, we briefly mentioned that cellular effects of SMFs are dependent on multiple factors, which directly affect the experimental outcomes. Therefore it is not surprising that although there are numerous *in vitro* and *in vivo* experiments reporting the effects of magnetic field on biological systems, experimental coherence among them is still lacking. However, the seemingly inconsistent observations are mostly due to the different magnetic field parameters and experimental variables, such as magnetic field treatment time and magnetic field intensity, etc. It is obvious that different types of magnetic fields (static or dynamic, pulsed or noise), as well as magnetic fields with various intensities (weak, moderate or strong) or frequencies (extremely low frequency, low frequency or radiofrequency) can all lead to diverse and sometimes completely opposite results (Jia et al. 2007; Simko 2007; Sun et al. 2013; Zhang et al. 2015). In addition, there are also many cellular factors and experimental setup parameters that can have a direct influence on the experimental outcomes, which will be discussed in detail below.

4.2.1 *Magnetic Field Intensity-Dependent Cellular Effects of SMFs*

The magnetic fields with different intensities could generate differential cellular effects and multiple studies showed that magnetic fields with higher intensities could generate stronger phenotypes. For example, erythrocytes (red blood cells, RBCs) could be aligned by SMFs with their disk planes parallel to the magnetic field direction and the orientation degree was dependent on SMF intensity (Higashi et al. 1993). Specifically, 1 T SMF had only detectable alignment effect on erythrocytes while 4 T high SMF induced almost 100% alignment (Higashi et al. 1993). Moreover, Prina-Mello et al. reported that the p-JNK level was increased in rat cortical neuron cells after exposure to 2 T and 5 T SMFs but not the weaker SMFs of 0.1–1 T (Prina-Mello et al. 2006). In addition, our lab recently showed that the human nasopharyngeal cancer CNE-2Z cell and human colon cancer HCT116 cell proliferation could be inhibited by SMFs in a magnetic field intensity dependent manner (Zhang et al. 2016) (Fig. 4.3). Specifically, 1 T SMF exposure for 3 days reduced CNE-2Z and HCT116 cell number by ~15% and 9 T SMF for 3 days reduced their cell number by over 30%. In contrast, 0.05 T SMF did not have significant effects on these two cells (Zhang et al. 2016) (Fig. 4.3).

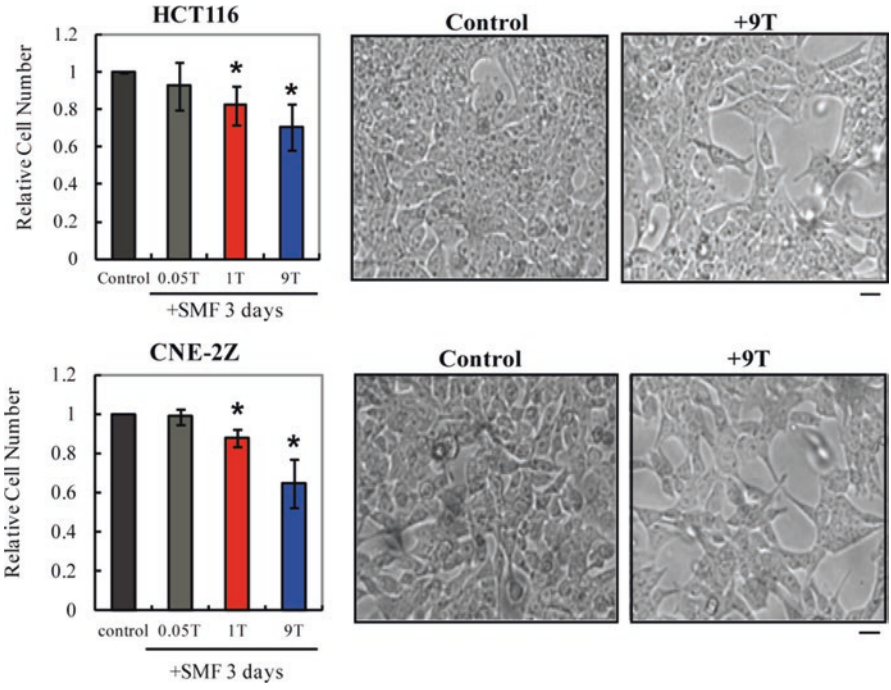

Fig. 4.3 Human nasopharyngeal cancer CNE-2Z and colon cancer HCT116 cell proliferation could be inhibited by SMFs in a magnetic field intensity-dependent manner. CNE-2Z and HCT116 cancer cell were exposed to SMFs of different intensities for 3 days. *Left panels* show the relative cell numbers. *p < 0.05. *Right panel* shows representative images in control and 9 T SMF treated cells. Scale bar, 20 μm (Reprinted from ref. (Zhang et al. 2016). Open access. Copyright © 2016 Impact Journals, LLC)

Although in many cases, higher magnetic field intensities can generate stronger effects than in lower field intensities, there are also evidences showing that different magnetic field intensities could cause completely different effects. For example, In 2006 Prina-Mello et al. showed that p-ERK was increased when rat cortical neuron cells were exposed to 0.75 T SMF but not 0.1 T, 0.5 T, 1 T, 2 T, or 5 T SMFs (Prina-Mello et al. 2006). In fact, 2 T SMF could reduce p-ERK level, which was opposite to the effect of 0.75 T (Prina-Mello et al. 2006). Ghibelli et al. also showed that although 6 mT SMF had an anti-apoptotic activity, 1 T SMF had a potentiating effect on small molecule induced apoptotic effects (Ghibelli et al. 2006). In 2014, the Shang group compared the effect of hypomagnetic field of 500 nt, moderate SMF of 0.2 T and high SMF of 16 T for their effects on mineral elements in MC3T3-E1 cells (Zhang et al. 2014b). They found that both hypo and moderate magnetic fields reduced osteoblast differentiation but the 16 T high magnetic field increased osteoblast differentiation. In addition, hypomagnetic field did not affect mineral elements levels but moderate magnetic field increased iron content and high magnetic field increased all mineral elements except copper (Zhang et al. 2014b). Therefore different magnetic field intensity could induce completely different effects at various biological systems. As Ghibelli et al. mentioned in their paper, the lack of a direct intensity-response curve may explain the existence of so many contradictory reports in the literature (Ghibelli et al. 2006). The exact effects of a given magnetic field on a specific cellular effect need to be examined case by case. More examples of SMF intensity induced cellular effects can be found in Chap. 1.

4.2.2 Cell Type-Dependent Cellular Effects of SMFs

Besides the various parameters of the magnetic fields, different cells in individual studies often have distinct genetic background, which makes them respond to the magnetic fields differentially. For example, as early as in 1992, Short et al. showed that a 4.7 T SMF could alter the ability of human malignant melanoma cells attachment onto the tissue culture plate, but had no effect on normal human fibroblasts (Short et al. 1992). In 1999 and 2003, Pacini et al. found that a 0.2 T SMF induced obvious morphology change in human neuronal FNC-B4 cell and human skin fibroblast cells but did not affect mouse leukemia or human breast carcinoma cells (Pacini et al. 1999a, 2003). In 2004, Ogiue-Ikeda and Ueno compared three different cell lines for their orientation changes under an 8 T SMF for 60 h exposure. They found that while the smooth muscle A7r5 cells and human glioma GI-1 cells could be aligned along the field direction of the 8 T SMF, the human kidney HFK293 cells were not aligned (Ogiue-Ikeda and Ueno 2004). In 2010, the high magnetic field of 16 T did not cause obvious changes in unicellular yeast (Anton-Leberre et al. 2010) but could induce frog egg division alteration (Denegre et al. 1998). In 2011, Sullivan et al. showed that moderate intensity (35–120 mT) SMF could affect attachment and growth of human fibroblast cells as well as growth of human melanoma cells, but not attachment or growth of adult adipose stem cells (Sullivan et al. 2011).

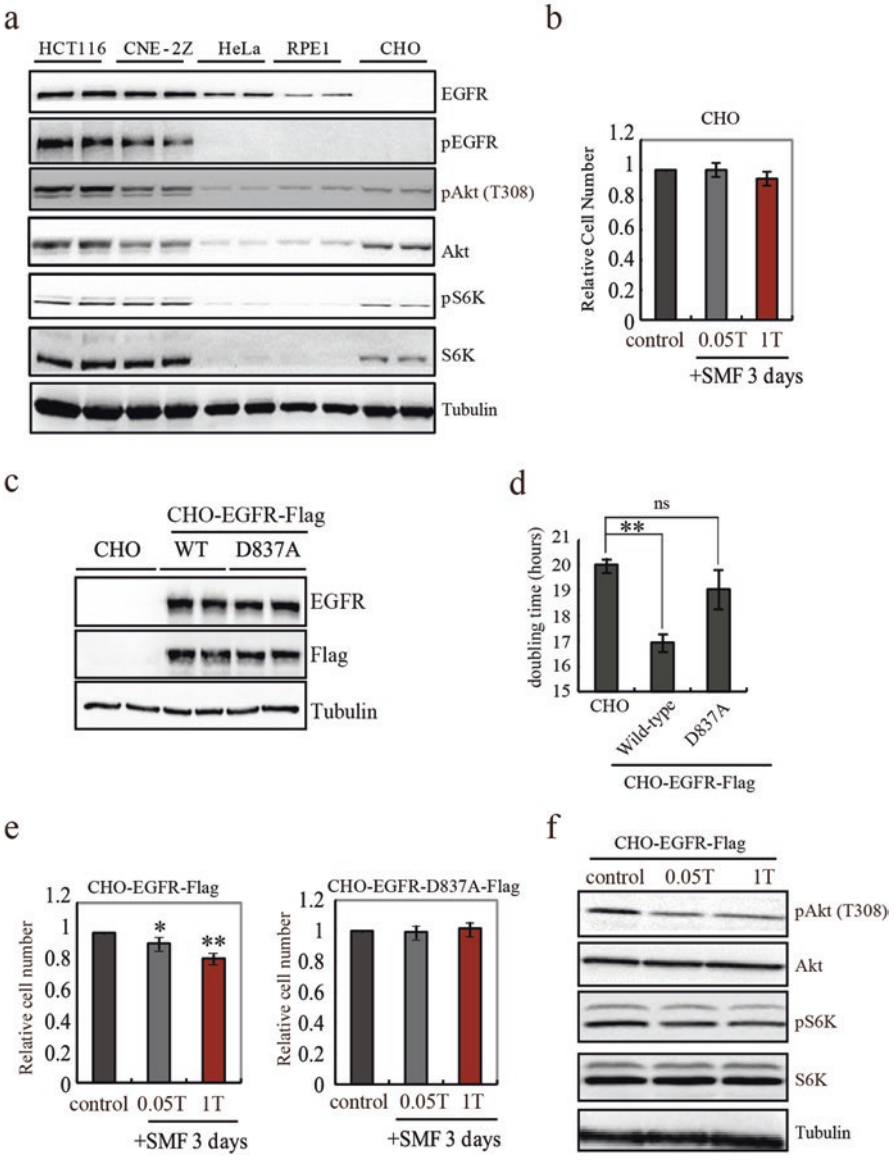

Fig. 4.4 EGFR expression and activity influence the SMF-induced cell proliferation inhibition. CHO cells and CHO cells stably expressing wild-type or D837A (kinase dead) EGFR were exposed to 0.05 T or 1 T SMFs for 3 days before their cell number were counted. (**a**) Representative Western blots are shown to compare the level of EGFR and pEGFR in five different cell lines. Samples were loaded in duplicate. (**b**) 0.05 T and 1 T SMFs do not affect CHO cells. Relative cell numbers of CHO cells after 3 days treatment in 0, 0.05, or 1 T SMFs are shown. (**c**) Representative Western blots comparing CHO cells and CHO cells stably expressing wild-type EGFR (CHO-EGFR-Flag) or kinase-dead EGFR (CHO-EGFR-D837AFlag). Anti-EGFR and anti-Flag antibodies show expression of EGFR-Flag, and anti-tubulin antibody shows loading control.

In 2013, Vergallo et al. show that inhomogeneous SMF (476 mT) exposure caused toxic effects on lymphocytes but not on macrophages (Vergallo et al. 2013). These studies all show that different cell types respond to SMFs differently.

The different cellular effects of SMFs on various cell types may because these cells were originated from different tissues. Since different tissues have totally distinct biological functions and genetic background, it is not surprising that they have different responses to SMF exposure. However, evidences show that even for cells from the same tissue, their response to the same SMF can be very different. For example, the Shang group has made series of progresses about the impact of SMFs on different types of bone cells. For example, they not only found that the differentiation and mineral elements can be differentially affected by low, moderate and high SMFs (Zhang et al. 2014b) but also found that different types of bone cells have obviously different cellular responses. The Shang group compared the effects of 500 nt, 0.2 T and 16 T SMFs on osteoblast MC3T3-E1 cells (Zhang et al. 2014b) and osteoclast differentiation from pre-osteoclast Raw264.7 cells (Zhang et al. 2017a). They found that both hypo and moderate SMFs reduced osteoblast differentiation but promoted osteoclast differentiation, formation and resorption. In contrast, 16 T high SMF increased osteoblast differentiation and inhibited osteoclast differentiation. Therefore the osteoblast and osteoclast cells responded totally opposite to these SMFs. Their studies revealed some parameters that could be used as a physical therapy for various bone disorders. They also summarized the effects of SMFs on bone in a very informative review (Zhang et al. 2014a).

It is interesting that many studies indicate that SMFs could have inhibitory effects on cancer cells but not non-cancer cells. For example, Aldinucci et al. found that 4.75 T SMF significantly inhibited Jurkat leukemia cell proliferation but did not affect normal lymphomonocytes (Aldinucci et al. 2003b). Rayman et al. show that growth of a few cancer cell lines can be inhibited by 7 T SMF (Raylman et al. 1996), but a few other studies showed that even 10–13 T strong SMFs did not induce obvious changes in non-cancer cells such as CHO (Chinese hamster ovary) cells or human fibroblast cells (Nakahara et al. 2002; Zhao et al. 2010). These results indicate that cell type is a very important factor that contributes to the differential responses of cells to SMFs. Recently we found that epidermal growth factor receptor (EGFR) and its downstream pathway play key roles in the SMF-induced cell proliferation inhibition. Our results showed that although CHO cells did not respond to moderate (1 T) or strong (9 T) SMFs, the transfected EGFR, but not the kinase-dead mutant of EGFR, could convert the SMF-insensitive CHO cells into SMF-sensitive cells and their cell growth could be inhibited by moderate and strong SMFs (Fig. 4.4). Detailed mechanisms will be discussed in Chap. 6, which focuses on the potential application of SMFs in cancer treatment.

Fig. 4.4 (continued) (**d**) Doubling time of CHO, CHO-EGFR-Flag and CHO-EGFR-D837A-Flag cells show that CHO-EGFR-Flag cells grow faster than CHO cells. (**e**) 0.05 T and 1 T SMFs reduce cell number in CHO-EGFR-Flag but not the kinase-dead mutant. Relative cell numbers of CHO-EGFR-Flag or CHO-EGFRD837A-Flag cells after 3 days treatment in 0, 0.05 or 1 T SMFs are shown. (**f**) Representative Western blots to examine the downstream components of EGFR in CHO-EGFR-Flag cells. $*p < 0.05$, $**p < 0.01$, *ns* not significant (Reprinted from ref. (Zhang et al. 2016). Open access. Copyright © 2016 Impact Journals, LLC)

Most individual studies so far have only investigated one or very few types of cells, which is not sufficient enough for people to comprehensively understand the effects of the magnetic fields on cells. Therefore, comparing different cell types side-by-side for their responses to the magnetic fields is strongly needed. In our recent work, we compared 15 different kinds of cells, including human cells and some rodent cells for their responses to 1 T SMF. Our results confirmed that SMFs could induce completely opposite effects in different cell types (Zhang et al. 2017b). However, since the biological systems are very complicated, the knowledge we have is still very limited. More studies are definitely needed for people to get a more complete understanding for the effects of SMFs on different types of cells.

4.2.3 Cell Density-Dependent Cellular Effects of SMFs

We recently found that the cell density also played very important roles in SMF-induced cellular effects. We originally found this by accident, when we were investigating the effects of 1 T SMF on human CNE-2Z nasopharyngeal cancer cell proliferation. We got diverse results when we plated the cells at different cell densities (Zhang et al. 2015). To verify this observation, we seeded CNE-2Z cells at 4 different cell densities and examined them side-by-side. We found that at lower cell density, 1 T SMF treatment for 2 days did not inhibit CNE-2Z cell proliferation and there was even a tendency of increased cell number after SMF treatment. However, when the cells were seeded at higher densities, it was interesting that 1 T SMF could consistently inhibit CNE-2Z cell proliferation (Zhang et al. 2017b). These results demonstrate that cell density can directly influence the effect of 1 T SMF on CNE-2Z cells.

We suspected that the cell density-induced variations must at least partly contribute to the lack of consistencies in the literature. Most researchers in the field of biological studies of magnetic fields, including us, did not really pay enough attention to the cell density, or at least did not realize that the cell density could cause dramatic variations in the experimental outcomes. However, it has been shown that cell density could directly cause variations in cell growth rate, protein expression, as well as alterations in some signaling pathways (Macieira 1967; Holley et al. 1977; Mcclain and Edelman 1980; Takahashi et al. 1996; Caceres-Cortes et al. 1999, 2001; Baba et al. 2001; Swat et al. 2009). Then we chose 6 other human cancer cell lines, including colon cancer HCT116, skin cancer A431, lung cancer A549, breast cancer MCF7, prostate cancer PC3 and bladder cancer EJ1 cells. We found that for most of these solid cancer cell lines, their cell number could be reduced by 1 T SMF when they were seeded at higher densities, but not at lower densities (Fig. 4.5). This indicates that cell density could generally influence the impact of SMFs on human cancer cell lines.

Then we further tested a few other non-cancer cell lines and found that cell density could directly influence the effects of SMFs on their proliferation as well. In addition, the pattern is different in different kinds of cells (Zhang et al. 2017b). Although the mechanism is still not completely understood, our data revealed that EGFR and its downstream pathways might contribute to the cell type- and cell

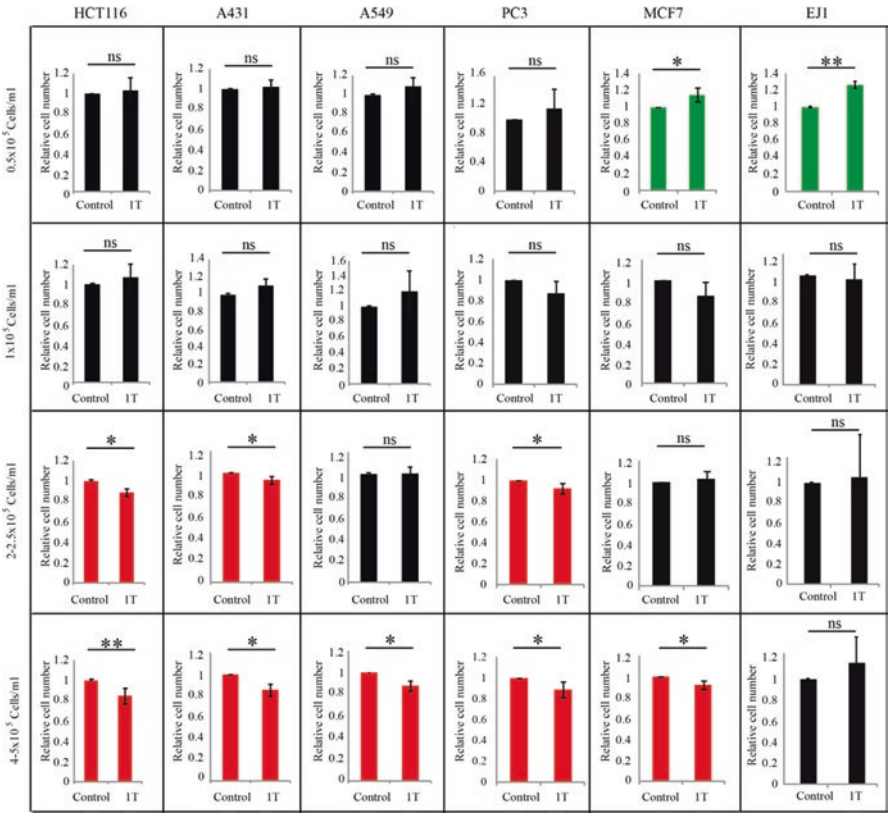

Fig. 4.5 1 T SMF affects multiple human cancer cell lines in a cell density-dependent manner. Different types of cells were plated at different densities one day ahead and treated with 1 T SMF for 2 days before they were counted. *ns* not significant; *, $p < 0.05$; **, $p < 0.01$. *Green* color indicates increase and *red* color indicates decrease (Reprinted from ref. (Zhang et al. 2017b). Open access. Copyright © 2017 Impact Journals, LLC)

density-induced variations (Zhang et al. 2017b). However, since cell density can have multiple effects on cells, such as calcium level (Carson et al. 1990) and signaling pathways, other factors are likely to be involved. For example, in 2004, Ogiue-Ikeda and Ueno found that A7r5 cells (smooth muscle cells, spindle shaped) and GI-1 cells (human glioma cells, spindle shaped) could orient in an 8 T SMF. However, it was interesting that the orientation did not occur when the cells were under the confluent condition at the start point of the magnetic field exposure, when the cell density was too high. They concluded that the magnetic field affected the cell division process, and only the proliferating cells at high density were oriented under the magnetic field (Ogiue-Ikeda and Ueno 2004). Apparently, further analysis is needed to unravel the complete mechanisms of cell density-dependent variations in SMF-induced cellular effects. However, before we have a clear understanding of the molecular mechanisms, people should always pay extra attention to the cell density in their own studies, as well as in literature reading.

4.2.4 Cell Status Influences the Cellular Effects of SMFs

Besides the cell type and density, cell status can also affect the cellular effects of SMFs. For example, in RBCs, the hemoglobin conditions can directly affect the magnetic properties of the whole cell. In normal RBCs, the hemoglobin is oxygenated and the cell is diamagnetic. In fact, they are slightly more diamagnetic than water because of the diamagnetic contribution of globin. However, when the cells were treated with isotonic sodium dithionite to make the haemoglobin in deoxygenated reduced state or treated with sodium nitrite to oxidize the haemoglobin (methemoglobin), the RBCs would become paramagnetic. Back in 1975, Melville et al. directly separated RBCs from whole blood using a 1.75 T SMF (Melville et al. 1975). In 1978 Owen used a 3.3 T SMF with high gradient to seperate RBCs (Owen 1978). The paramagnetic methemoglobin containing RBCs could be separated from diamagnetic untreated RBCs as well as diamagnetic leukocytes (white blood cells, WBCs) (Owen 1978). In fact, "magnetophoresis" has been applied in RBC, called RBC magnetophoresis, which uses an applied magnetic field to characterize and separate the cells based on the intrinsic and extrinsic magnetic properties of biological macromolecules in these cells (Zborowski et al. 2003; Moore et al. 2013). In 2013, Moore et al. designed an open gradient magnetic RBC sorter and tested on label-free cell mixtures (Moore et al. 2013). They showed that in the open gradient magnetic RBC sorter, the oxygenated RBCs were pushed away from the magnet and the deoxygenated RBCs were attracted to the magnet. Moreover, the effect for the oxygenated RBC's was very weak and comparable to that of other non-RBC cells in the blood, which do not contain hemoglobin and could be considered as non-magnetic. They proposed that the quantitative measurements of RBC mobility in cell suspension were the basis for engineering design, analysis and fabrication of a laboratory prototype magnetic RBC sorter built from commercially available, block permanent magnets to serve as a test bed for magnetic RBC separation experiments (Moore et al. 2013).

Another well studied example of cells with different magnetic property is malaria-infected RBCs. Researchers have utilized malaria byproduct, hemozoin, to study and seperate malaria-infected RBCs in a magnetic field gradient (Paul et al. 1981; Moore et al. 2006; Hackett et al. 2009; Kasetsirikul et al. 2016). During intra-erythrocytic maturation, malaria trophozoites could digest up to 80% of cellular hemoglobin, which accumulates toxic heme. To prevent haem iron from participating in cell-damaging reactions, the parasite polymerizes beta-hematin dimers to synthesize insoluble hemozoin crystals. In the process, the heme is converted to a high-spin ferriheme, whose magnetic properties were studied a long time ago (Pauling and Coryell 1936). In fact, in 2006, Moore et al. used magnetophoretic cell motion analysis to provide direct evidence for a graduated increase of live cell magnetic susceptibility with developing blood-stage parasites, which is compatible with hemozoin increase (Moore et al. 2006). In 2009, Hackett et al. experimentally determined the source of the cellular magnetic susceptibility during parasite growth (Fig. 4.6). They found that the parasites converted approximately 60% of host cell haemoglobin to haemozoin and this product was the primary source of the increase

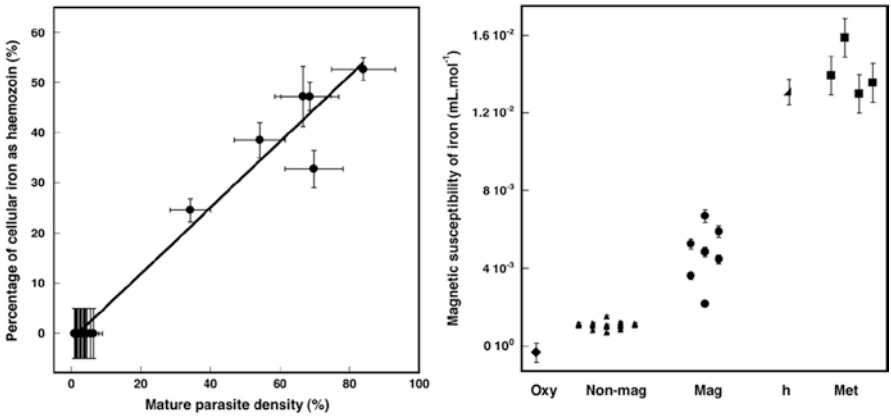

Fig. 4.6 Magnetic susceptibility of iron in malaria-infected red blood cells (RBCs). (*Left*) Percentage of cellular iron converted to haemozoin vs. mature parasite density. (*Right*) Scatter plot of the molar magnetic susceptibility of iron in standard samples of oxyhaemoglobin (Oxy — ◆), haematin (h — ◢), methaemoglobin (Met — ■), and for magnetic (Mag — ●) and non-magnetic (Non-mag— ▲) fractions of malaria-infected red cell cultures (Reprinted with permission from ref. (Hackett et al. 2009). Open access. Copyright © 2008 Elsevier B.V)

in cell magnetic susceptibility. While the magnetic susceptibility of uninfected cells was similar to water, the magnetically enriched parasitised cells have higher magnetic susceptibility (Hackett et al. 2009). Therefore the magnetic fields with gradient could be used in malaria diagnosis and malaria-infected RBC separation (Paul et al. 1981; Kasetsirikul et al. 2016).

Magnetic fractionation of erythrocytes infected with malaria has also been used in enrichment of infected cells from parasite cultures and separation of infected cells from uninfected cells in biological and epidemiological research, as well as clinical diagnosis. In 2010, Karl et al. used high gradient magnetic fractionation columns to quantitatively characterize the magnetic fractionation process. They found that the infected cells had approximately 350 times higher magnetic binding affinity to the column matrix compared to the uninfected cells (Karl et al. 2010). In addition, the distribution of captured parasite developmental stages shifted to mature stages as the number of infected cells in the initial samples and flow rate increased (Karl et al. 2010). Furthermore, in 2013, Nam et al. used permanent magnets and ferromagnetic wire to make a polydimethylsiloxane (PDMS) microfluidic channel integrated with a ferromagnetic wire fixed on a glass slide to separate infected RBCs in various developmental stages (Fig. 4.7). Late-stage infected RBCs were separated with a recovery rate of around 98.3%. Early-stage infected RBCs had been difficult to separate due to their low paramagnetic characteristics but can also be successfully separated with a recovery rate of 73%. Therefore it could provide a potential tool for malarial-related studies (Nam et al. 2013).

Besides the cell status mentioned above, the cell lifespan or cell age can also influence SMF-induced cellular effects. In 2011, Sullivan et al. found that various points during the lifespan of fetal human lung fibroblast WI-38 cells affected the

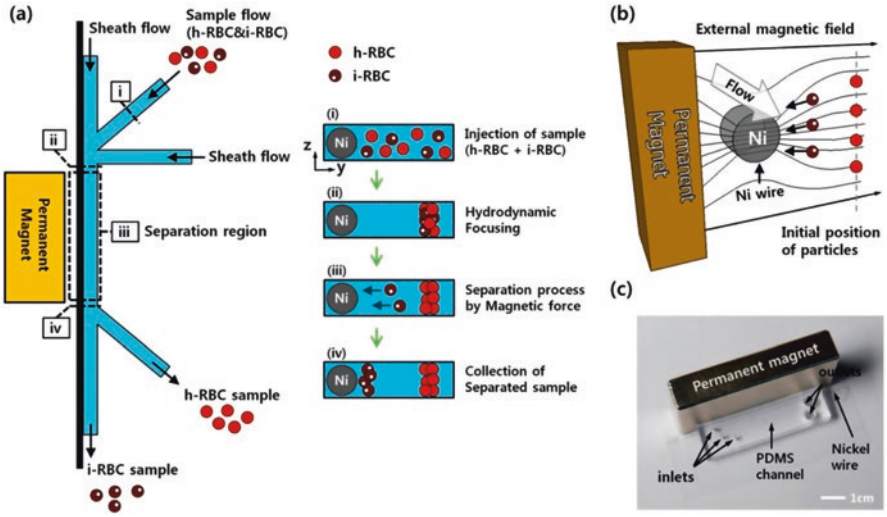

Fig. 4.7 Malaria-infected RBC separation using a high magnetic field gradient. (**a**) Schematic diagram of i-RBC (infected red blood cell) separation using the paramagnetic characteristics of hemozoin in i-RBCs. (**b**) Working principle of magnetophoretic separation with a ferromagnetic nickel wire in an external magnetic field. (**c**) Photograph of the permanent magnet for applying an external magnetic field in the microchannel and a microfluidic device consisting of the PDMS microchannel and a nickel wire (Reprinted with permission from ref. (Nam et al. 2013). Copyright © 2013, American Chemical Society)

cellular responses to moderate intensity SMF (Sullivan et al. 2011). SMF exposure decreased cell attachment by less than 10% in younger cultures (population doubling level 29) but can decrease cell attachment by more than 60% in older cultures (population doubling level 53). In 2004, Ogiue-Ikeda and Ueno found that the smooth muscle A7r5 cells could be aligned along an 8 T magnetic field direction only when the cells were actively proliferating at a higher density (Ogiue-Ikeda and Ueno 2004). In addition, In 2014 Surma et al. also found that fully differentiated myotubes at late stages of development were less sensitive to weak SMF and myotubes at the stage when electromechanical coupling was forming dramatically reduced the contraction frequency during the first minute's weak SMF exposure (Surma et al. 2014). These results demonstrate that even for the same cell type and same SMF exposure, the cellular effects could be influenced by their status, such as lifespan. The Underlying mechanisms are still unknown and need to be further investigated.

The above mentioned parameters, including magnetic field strength, cell types, cell density and cell status, are just a few examples that directly influence the cellular effects of SMFs. It is very likely that other aspects of cell status also contribute to the differential effects of SMF on cells. There are multiple other factors that complicate the situation, such as magnetic field exposure time, magnetic field direction, field gradient, etc. Interested readers can look into our Chap. 1 for more information. In the meantime, we recommend researcher in this field to provide as

detailed information as possible about their experimental setup as well as the biological samples, which will help us to understand better of the cellular effects of SMFs. Further investigations at both cellular and molecular levels are needed to get a comprehensive understanding.

4.3 Cellular Effects of SMFs

SMFs could induce multiple cellular effects depending on the magnetic field itself as well as the cells examined. Here I will mainly discuss some cellular effects that have been reported by multiple independent studies, such as SMF-induced changes in cell orientation, proliferation, microtubule and cell division, actin, viability, attachment/adhesion, morphology, migration, cell membrane, cell cycle, chromosome and DNA, intracellular reactive oxygen species (ROS) and calcium. Our focus here is mainly on human cells.

4.3.1 Cell Orientation

The orientation changes of biomolecules and cells are one of the most studied aspects of SMF bioeffects. When diamagnetic objects are exposed to strong SMFs, they align either parallel or perpendicular to the magnetic field direction due to the anisotropy of the magnetic susceptibility of the objects.

There are multiple examples for cells align themselves in parallel to the magnetic field direction. Among them, the best studied example was erythrocytes (red blood cells, RBCs). The first reported RBC orientation change induced by SMF was in 1965 by Murayama, who found that sickled RBCs were oriented perpendicular to a 0.35 T SMF (Murayama 1965). It is interesting that in 1993, a work carried out by Higashi et al. showed that normal RBCs were also aligned by an 8 T SMF but the orientation direction was different from what Muyama have observed (Higashi et al. 1993). Their results showed that normal RBCs oriented with their disk planes parallel to the field direction (Fig. 4.8). In 1995, they reported that the cell membrane components, including the transmembrane proteins and lipid bilayers were the major reasons for RBC alignment in 8 T SMF (Higashi et al. 1995). In addition, they found that the paramagnetism of membrane-bound hemoglobin contributes significantly to this orientation (Takeuchi et al. 1995; Higashi et al. 1996). These results clearly demonstrate that cells can be oriented by strong SMFs and the effects depend on the molecular components of the cell. Besides RBCs, more components in the blood stream have also been studied, such as platelets (Yamagishi et al. 1992; Higashi et al. 1997) and fibrinogen (Torbet et al. 1981; Yamagishi et al. 1990; Iwasaka et al. 1994).

Moreover, some other cells like osteoblast cells, smooth muscle cells and Schwann cells could also be aligned in parallel to the direction of the strong magnetic fields when they are exposed for a prolonged period. In 2000 and 2002, Kotani

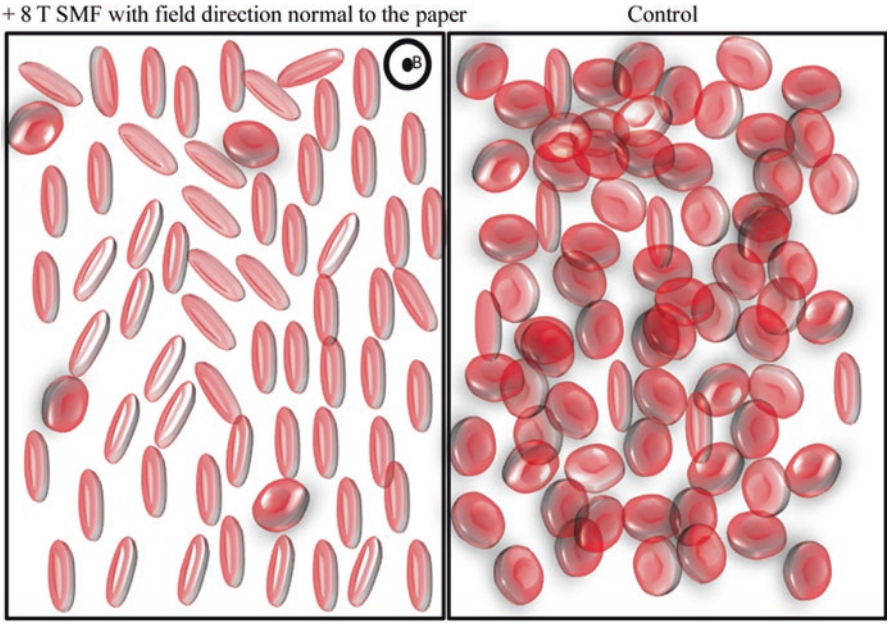

Fig. 4.8 Red blood cells were aligned by an 8 T SMF. Right: Red blood cells in control condition, with no SMF. *Left*: Red blood cells in an 8 T SMF. The field direction was normal to the paper (Illustration was drawn based on results from ref. (Higashi et al. 1993))

et al. found that osteoblast cells were oriented in parallel to the field direction by an 8 T SMF and the bone formation was significantly stimulated to grow along the direction of the magnetic field (Kotani et al. 2000, 2002). In 2001, Umeno et al. found that smooth muscle cell culture was aligned along the magnetic field direction after they were exposed to an 8 T SMF for 3 days (Umeno et al. 2001). In 2003, Iwasaka et al. found that the 14 T SMF aligned smooth muscle cell assemblies and the cell colonies were extended along the field direction (Iwasaka et al. 2003). In 2003, Eguchi et al. found that Schwann cells were also oriented in parallel to the 8 T SMF after 60 h exposure (Eguchi et al. 2003). They used linearly polarized light and observed changes in the intracellular macromolecule behaviour in 8 T and 14 T SMFs (Iwasaka and Ueno 2003a, b). In 2005, they also examined the actin cytoskeleton in Schwann cells and found that actin fibers were oriented in the direction of 8 T SMF (Eguchi and Ueno 2005). More interestingly, the Schwann cells did not orient in the 8 T SMF when an inhibitor of small GTPase (guanosine triphosphatase) Rho-associated kinase was added, which indicated that the SMF-induced Schwann cell orientation was dependent on Rho regulated actin fibers (Eguchi and Ueno 2005). In 2007, Coletti et al. found that 80 mT SMF induced myogenic cell line L6 cells to align in parallel bundles, an orientation conserved throughout differentiation. They proposed that SMF-enhanced parallel orientation of myotubes was relevant to tissue engineering of a highly organized tissue such as skeletal muscle (Coletti et al. 2007).

In the meantime, there are also multiple examples showing that cells could align in perpendicular to the direction of the magnetic fields, such as the bull sperm. The orientation of bull sperm was examined by a few studies, which actually showed stronger alignment effects than RBCs and platelets. The bull sperm cell has a fat head that mainly contains diamagnetic cell membrane and DNA. It also has a long tail with microtubules inside. In 2001, Emura et al. found that the orientation of bull sperm cells could be affected by SMFs in an intensity-dependent manner (Emura et al. 2001). They found that the bull sperm could reach 100% alignment perpendicular to the direction of the magnetic field at just below 1 T (Emura et al. 2001). In 2003, Emura et al. showed that the whole bull sperm and the sperm heads were orientated perpendicular to 1.7 T SMF while the paramecium cilia were aligned in parallel to 8 T SMF (Emura et al. 2003). It was interesting that the sperm tail is theoretically predicted to be in parallel with the field direction due to the diamagnetic anisotropy of microtubules, which will be discussed later. But why the whole sperm is aligned in perpendicular to the field direction is still unclear. It is possible that the sperm head has a stronger diamagnetic anisotropy, which dominates the whole sperm.

Another example of cell orientation in perpendicular to the direction of the magnetic field is neurite outgrowth. In 2008, Kim et al. showed that the application of 0.12 T SMF for 3–5 days could be used to modulate the orientation and direction of neurite formation in cultured human neuronal SH-SY5Y cells and PC12 cells (Kim et al. 2008). It is interesting that they found the neurites perpendicular to the SMF had long, thin and straight appearance while the neurites in parallel to the SMF direction had "thickeded or beaded" dystrophic appearance. More importantly, they not only found the neurites tended to orient perpendicular to the direction of SMF, the direction can also be changed after the SMF direction has changed (Kim et al. 2008).

From evidences mentioned above, we can conclude that SMF-induced cell orientation is cell type-dependent. Actually, as we have mentioned earlier, Ogiue-Ikeda and Ueno compared three different cell lines, including the smooth muscle A7r5 cells, human glioma GI-1 cells and human kidney HFK293 cells for their orientation changes under 8 T for 60 h exposure. They found that while the smooth muscle A7r5 cells and the human glioma GI-1 cells aligned along the field direction, the human kidney HEK293 cells were not aligned (Ogiue-Ikeda and Ueno 2004). They proposed that this was probably due to their different cell shapes because both A7r5 and GI-1 cells were spindle shaped while HEK293 cells were polygonal shaped. In addition, the orientation of adherent cells such as osteoblasts, smooth muscle cells and Schwann cells in strong SMFs usually took a few days while floating cells such as RBCs exhibited a diamagnetic torque rotation in only a few seconds under magnetic fields of the same intensity. This also implies that when our human bodies are exposed to externally applied magnetic fields, the free circulating blood cells would be affected more readily compared to other types of cells.

Table 4.1 summarizes some reported studies about the orientation of cells in SMFs (Table 4.1). It is apparent that other than cell types, the SMF-induced cell orientation change is largely dependent on the magnetic field intensity. The reported cell orientation changes were all achieved in SMFs of at least 80 mT, and actually most of them were done in strong magnets, such as in 8 T SMF. Therefore it is not surprising

Table 4.1 SMF-induced cell orientation in different studies

Cells examined	SMF strength	To the SMF direction	References
Myogenic cell line L6 cells	80 mT	Parallel	Coletti et al. (2007)
Paramecium cilia	8 T	Parallel	Emura et al. (2003)
Normal erythrocytes	8 T	Parallel	Higashi et al. (1993)
Osteoblast cells	8 T	Parallel	Kotani et al. (2000)
Smooth muscle cells	8 T	Parallel	Umeno et al. (2001)
Smooth muscle A7r5 cells and human glioma GI-1 cells	8 T	Parallel	Ogiue-Ikeda and Ueno (2004)
Schwann cells	8 T	Parallel	Eguchi et al. (2003)
Actin cytoskeleton in Schwann cells	8 T	Parallel	Eguchi and Ueno (2005)
Smooth muscle cell colonies	14 T	Parallel	Iwasaka et al. (2003)
Neurite growth of human neuronal SH-SY5Y cells and PC12 cells	0.12 T	Perpendicular	Kim et al. (2008)
Sickled erythrocytes	0.35 T	Perpendicular	Murayama (1965)
Bull sperm	~0.5-1.7 T	Perpendicular	Emura et al. (2001)
Whole bull sperm and bull sperm heads	1.7 T	Perpendicular	Emura et al. (2003)
Osteoblast cells mixed with collagen	8 T	Parallel	Kotani et al. (2000)
Schwann cells mixed with collagen	8 T	Parallel	Eguchi et al. (2003)
Human glioblastoma A172 cells embedded in collagen gels	10 T	Perpendicular	Hirose et al. (2003)
Cultured swine granulosa cells (GCs)	2 mT	No change	Gioia et al. (2013)
Schwann cells treated with an inhibitor of small GTPase Rho-associated kinase	8 T	No change	Eguchi and Ueno (2005)
Human kidney HFK293 cells	8 T	No change	Ogiue-Ikeda and Ueno (2004)
Human glioblastoma A172 cells	10 T	No change	Hirose et al. (2003)

Blue color indicates that SMF induces cells to align along the field direction. *Orange* color indicates that SMF induces cells to align perpendicular to the field direction. *Grey* color indicates that SMF does not affect cell orientation

when Gioia et al. investigated the effect of chronic exposure to a 2 mT SMF on *in vitro* cultured swine granulosa cells (GCs) and did not observe cell orientation changes (Gioia et al. 2013). In addition, the cell type is an important factor because most cells do not have strong structure characteristics like sperm cell, nor RBCs.

Besides the orientation change of cells themselves in magnetic fields, cells can also be oriented by moderate and strong SMFs when they are embedded in collagen, a macromolecule that has strong diamagnetic anisotropy (Torbet and Ronziere 1984).

In 1993, Guido and Tranquillo found that human foreskin fibroblasts embedded in collagen gel were oriented by 4.0 and 4.7 T SMFs (Guido and Tranquillo 1993). Human glioblastoma A172 cells embedded in collagen gels, but not A172 cells alone, oriented perpendicular to the field direction of 10 T SMF (Hirose et al. 2003). Therefore the orientation for cells embedded in collagen is largely due to the diamagnetic anisotropy of collagen fibers, which orient in perpendicular direction of SMF. Another example was provided in 2000 by Kotani et al., who found that osteoblast cells themselves were oriented in parallel to the field direction by an 8 T SMF, but the mixture of osteoblast cells and collagen oriented perpendicular to the magnetic fields (Kotani et al. 2000). This is interesting and promising because the stimulation of bone formation to an intended direction using a combination of strong SMF and potent osteogenic agents could possibly lead to a clinically viable treatment of bone fractures and defects. In addition, in 2003, Eguchi et al. found that Schwann cells themselves oriented in parallel to the 8 T SMF after 60 h exposure but when they were embedded in collagen, they were aligned in perpendicular to the field direction (Eguchi et al. 2003). These data all showed that the collagen has a strong alignment effect on cells embedded in SMFs.

The shapes of most mammalian somatic cells are close to symmetric and are also surrounded by and attached to their extracellular matrix and neighboring cells. Therefore they are less likely to have strong alignment effects in SMFs like sperm cells or RBCs in weak to moderate intensity SMFs. However, the SMF-induced orientation effects can potentially affect their cell division and subsequently affect the tissue development. In addition, it was very promising that Kotani et al. found that an 8 T SMF could cause osteoblasts to orient in parallel to the magnetic field and stimulate bone formation along the field direction. This implies that people may be able to apply SMFs in clinical treatment such as bone disorders. In fact, the orientation effects of RBCs might also provide some insights to help understanding the working mechanism of some magnetic therapy products. Continued efforts are encouraged to investigate more on blood cells, muscles, neurons, bones and sperms, as well as their potential medical applications in the future.

4.3.2 Cell Proliferation/Growth

Multiple evidences showed that SMFs could inhibit cell proliferation. For example, 1976, Malinin et al. exposed mouse fibroblast L-929 cells and human embryonic lung fibroblast WI-38 cells to 0.5 T SMF for 4–8 h after they were frozen in liquid nitrogen and found that the subsequent cell growth was significantly inhibited (Malinin et al. 1976). In 1999 Pacini et al. examined the effects of 0.2 T SMF in human breast cancer cells and found that 0.2 T not only reduced cell proliferation but also enhanced the vitamin D anti-proliferative effect (Pacini et al. 1999a). In 2003, Pacini et al. examined human skin fibroblasts for their effects in 0.2 T SMF generated by a magnetic resonance tomography and found that the cell proliferation was reduced (Pacini et al. 2003). In 2008, Hsieh et al. found that 3 T SMF inhibited

human chondrocytes growth *in vitro* and affected recovery of damaged knee cartilage *in vivo* in the pig model. They also mentioned that these results may be specific to the parameters used in this study and may not apply to other situations, field strengths, forms of cartilage injury, or animal species (Hsieh et al. 2008). In 2012, Li et al. found that the proliferation of human umbilical artery smooth muscle cells (hUASMCs) was significantly decreased after 5 mT SMF exposure for 48 h compared with the non-treated group (Li et al. 2012). In 2013, Mo et al. showed that magnetic shielding increased human SH-SY5Y neuroblastoma cell proliferation (Mo et al. 2013), which indicated that the geomagnetic field may have an inhibitory effect on SH-SY5Y neuroblastoma cell proliferation. In 2013, Gioia et al. investigated the effect of a 2 mT SMF on swine granulosa cells (GCs) and found that the doubling time was significantly reduced ($p < 0.05$) in exposed samples after 72 h of culture (Gioia et al. 2013). In 2016, Wang et al. exposed adipose-derived stem cells (ASCs) to 0.5 T SMF for 7 days and found that the cell proliferation was inhibited (Wang et al. 2016). Recently we found that 1 T and 9 T SMFs could inhibit the proliferation of human nasopharyngeal carcinoma CNE-2Z and colon cancer HCT116 cells (Zhang et al. 2015, 2016).

There are also some studies showing that SMFs could promote proliferation of some cell types, such as bone marrow cells, stem cells as well as endothelia cells. For example, Martino et al. found that 60 and 120 μT SMFs increased the cell proliferation of human umbilical vein endothelial cell (Martino et al. 2010). In 2013, Chuo et al. found that a 0.2 T SMF increased the proliferation of bone marrow stem cells (Chuo et al. 2013). In 2007, Stolfa et al. used MTT assay to study the effect of 0.6 T SMF on human chondrocytes and found that the MTT reading was increased by 0.6 T SMF (Stolfa et al. 2007), which was probably due to the increased cell proliferation and/or cell viability or metabolic activity. In 2016, Lew et al. showed that 0.4 T SMF enhanced dental pulp stem cell proliferation (Lew et al. 2016). Recently, Maredziak et al. found that 0.5 T SMF increased the proliferation rate of human adipose-derived mesenchymal stromal stem cells (hASCs) via activation of the phosphoinositide 3-kinase/Akt (PI3K/Akt) signaling pathway (Maredziak et al. 2017).

However, there are also some studies shown that cell proliferation was not affected by SMFs. For example, in 1992 Short et al. found that 4.7 T SMF treatment did not affect cell number of either human malignant melanoma cells or the normal human cells (Short et al. 1992). In 2005, Gao et al. found that even 14.1 T SMF (provided by an NMR spectrometer) exposure for 12 h did not affect cell growth of bacterial strain Shewanella oneidensis MR-1 (Gao et al. 2005). In 2007, Coletti et al. found that 80 mT SMF did not affect myotube cell proliferation (Coletti et al. 2007). In 2010, Hsu and Chang found that 0.29 T SMF did not affect the cell proliferation of dental pulp cells (Hsu and Chang 2010). In 2015, Reddig et al. found that exposure of unstimulated mononuclear blood cells to 7 T SMF alone or in combination with varying gradient magnetic fields and pulsed radiofrequency fields did not affect cell proliferation (Reddig et al. 2015). Recently, Iachininoto et al. investigated the effects of 1.5 T and 3 T gradient SMFs for their effects on hematopoietic stem cells and found that the cell proliferation was not affected (Iachininoto et al. 2016).

Therefore, not surprisingly, the effect of SMFs on cell proliferation is also cell type dependent. Table 4.2 summarizes some reported studies about the SMF-induced cell proliferation/growth changes (Table 4.2). For example, in 2003 Aldinucci et al. tested the effects of combining a 4.75 T SMF and a pulsed EMF of 0.7 mT generated by an NMR apparatus (NMRF). They found that the 4.75 SMF did not affect cell proliferation in both normal and PHA activated peripheral blood mononuclear cells (PBMC), but significantly reduced proliferation in Jurkat leukemia cells (Aldinucci et al. 2003b). We found that 1–9 T SMFs inhibited CNE-2Z and HCT116 cancer cells but not the Chinese hamster ovary (CHO) cells (Zhang et al. 2016). In addition, we found that the EGFR/Akt/mTOR signaling pathway, which was upregulated in many cancers, was involved in SMF-induced cancer cell proliferation inhibition (Zhang et al. 2015; Zhang et al. 2016). In addition, as we have mentioned before, SMF-induced effects on cell proliferation was not only cell type-dependent, but also dependent on magnetic field intensity as well as cell density. More investigations are needed to unravel additional mechanisms and specific effects of a given SMF on a specific cell type.

4.3.3 Microtubule and Cell Division

Purified microtubules have been known for a long time to be a target of SMFs as well as electric fields, which align along the magnetic field and electric field direction (Fig. 4.9a) due to diamagnetic anisotropy of tubulin dimers (Vassilev et al. 1982; Bras et al. 1998, 2014; Minoura and Muto 2006; Wang et al. 2008). It was also shown that tubulin assembly *in vitro* was disordered by a 10–100 nT hypogeomagnetic field (Wang et al. 2008). These studies demonstrated that microtubules could be affected by SMFs in *vitro*, but the effects of SMFs on microtubules in cells were less reported. In 2005 Valiron et al. showed that the microtubule and actin cytoskeleton could be affected by 7–17 T high SMFs in some cell types during interphase (Valiron et al. 2005). In 2013, Gioia observed actin and alpha-tubulin cytoskeleton modifications in swine granulosa cells after 3 days exposure to a 2 mT SMF (Gioia et al. 2013). However, this effect seems to be cell type- and/or exposure time-dependent because our group did not observe obvious microtubule abnormalities in CNE-2Z or RPE1 interphase cells when we exposed them to 1 T SMF for 3 days or 27 T ultra-strong SMF for 4 h (data not shown).

Microtubule is a key component for mitotic spindle, which is mainly composed of microtubules and chromosomes and are the fundamental machinery for cell division. However, information about the mitotic spindles in SMFs was not provided in above mentioned studies. In contrast, PMFs and electric fields have been shown to be able to affect mitotic spindle and cell division. For example, in 1999 Zhao et al. found that a small physiological electric field could orient cultured human corneal epithelial cells through affecting cell division (Zhao et al. 1999). In 2011, Schrader et al. observed spindle disturbances in human-hamster hybrid A(L) cells induced by the electrical component of the mobile communication frequency range signal

Table 4.2 SMF-induced cell proliferation/growth changes in different studies

Cells examined	SMF strength	Cell proliferation /growth	References
Swine granulosa cells (GCs)	2 mT	Inhibit	Gioia et al. (2013)
Human umbilical artery smooth muscle cells (hUASMCs)	5 mT	Inhibit	Li et al. (2012)
Human breast cancer cells	0.2 T	Inhibit	Pacini et al. (1999a)
Human skin fibroblasts	0.2 T	Inhibit	Pacini et al. (2003)
Adipose-derived Stem Cells (ASCs)	0.5 T	Inhibit	Wang et al. (2016)
Multiple cancer cell lines	1 T	Inhibit	Zhang et al. (2017b)
Human chondrocytes	3 T	Inhibit	Hsieh et al. (2008)
Jurkat cells	4.75 T	Inhibit	Aldinucci et al. (2003b)
Human nasopharyngeal carcinoma CNE-2Z and colon cancer HCT116 cells	1 and 9 T	Inhibit	Zhang et al. 2015, Zhang et al. (2016)
Human umbilical endothelial cells	60 and 120 μT	Promote	Martino et al. (2010)
Bone marrow stem cells	0.2 T	Promote	Chuo et al. (2013)
Dental pulp stem cell proliferation	0.4 T	Promote	Lew et al. (2016)
Human adipose-derived mesenchymal stromal stem cells (hASCs)	0.5 T	Promote	Maredziak et al. (2017)
Human Chondrocytes	0.6 T	Promote	Stolfa et al. (2007)
Human normal lung cells	1 T	Promote	Zhang et al. (2017b)
Myotube cell	80 mT	No change	Coletti et al. (2007)
Dental pulp cells	0.29 T	No change	Hsu and Chang (2010)
Hematopoietic stem cells	1.5 T and 3 T	No change	Iachininoto et al. (2016)
Human malignant melanoma cells and the normal human cells	4.7 T	No change	Short et al. (1992)
Normal and PHA activated peripheral blood mononuclear cells (PBMC)	4.75 T	No change	Aldinucci et al. (2003b)
Unstimulated mononuclear blood cells	7 T	No change	Reddig et al. (2015)
Chinese Hamster Ovary (CHO)	9 T	No change	Zhang et al. (2016)
Bacterial strain Shewanella oneidensis MR-1	14.1 T	No change	Gao et al. (2005)

Blue color indicates that SMFs inhibit cell proliferation/growth. *Orange* color indicates that SMFs promote cell proliferation/growth. *Grey* color indicates that SMF does not affect cell proliferation/growth

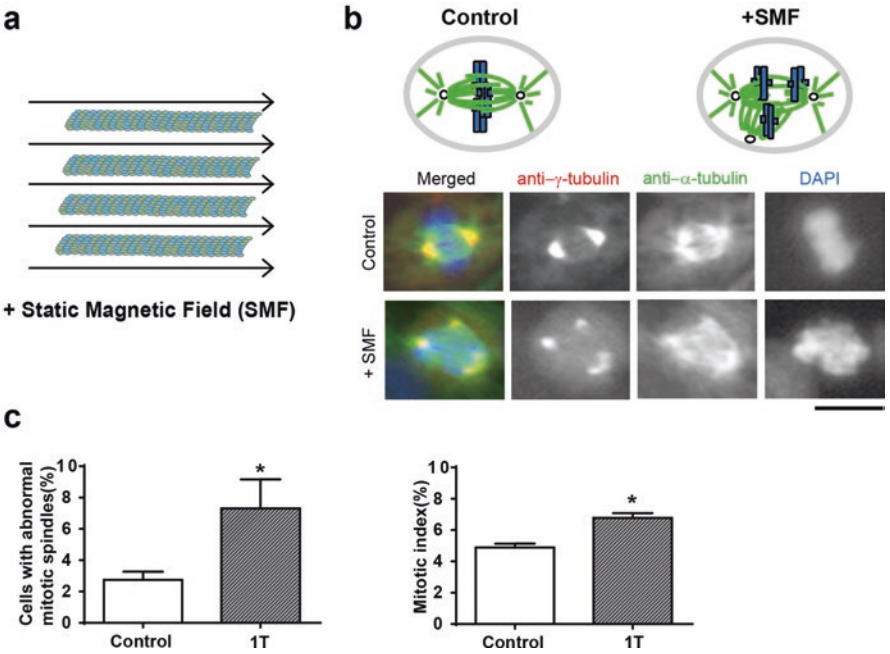

Fig. 4.9 1 T SMF affect mitotic spindles in HeLa cells. (**a**) Illustration of microtubules aligned under the SMF. (**b**) Cartoons and immunofluorescence images show normal bipolar spindles and abnormalmultipolar spindles. Representative images of cells with bipolar spindles and multi-polar spindles were shown. γ-Tubulin, microtubules and DNA were visualized by staining cells with γ-tubulin antibody (red), FITC-α-tubulin antibody (green) and DAPI (blue). (**c**) Quantification of abnormal mitotic spindles (*left*) and mitotic index (*right*) induced by 1 T SMF treatment for 7 days. Data represent the mean ± SD. *P < 0.05 (Figure was reprinted with permission from ref. (Luo et al. 2016). Copyright © 2015 Elsevier B.V)

(Schrader et al. 2011). However, for PMFs, people need to distinguish the effects caused by the magnetic fields per se or the thermal effect. In 2011, Ballardin et al. found that 2.45 GHz microwaves could disrupt spindle assembly (inducing multipolar spindles) in Chinese hamster V-79 cells, which was not due to the thermal effects (Ballardin et al. 2011). In contrast, in 2013, Samsonov and Popov found that exposure to 94 GHz radiation increased the rate of microtubule assembly and that effect was actually caused by the thermal effect (Samsonov and Popov 2013). The thermal effect in Samsonov and Popov's study is likely due to the high frequency compared to Ballardin et al.'s study. Moreover, there is a well known electromagnetic approach called tumor treating fields (TTF, TTFields) that uses low-intensity (1–3 V/cm) and intermediate-frequency (100–300 kHz) alternating electric fields to treat cancers such as glioblastoma. The mechanism has been proved to be mainly through disturbing mitotic spindle formation (Kirson et al. 2004; Pless and Weinberg 2011; Davies et al. 2013). TTFields destroy cells within the process of mitosis via apoptosis and have no effect on non-dividing cells (Pless and Weinberg 2011). In fact, the U.S. Food and Drug Administration has approved this technology for use in glioblastoma (Davis 2013), which was also discussed in Chap. 2.

We recently found that mitotic spindles could be affected by SMFs (Fig. 4.9b) (Luo et al. 2016). Our results show that 1 T SMF treatment for 7 days could increase the abnormal mitotic spindles (Fig. 4.9b, c) and mitotic index (% of cells in mitosis) in HeLa cells (Fig. 4.9c), which is likely due to the effect of SMF on microtubules. In addition, this phenotype is also time-dependent because when cells were treated for shorter time, the effects were not obvious. Although 1 T SMF did not affect the overall cell cycle distribution, it could delay the mitotic exit using synchronization experiment (Luo et al. 2016), which will be discussed in the cell cycle section later in this chapter.

Since purified microtubules can be aligned by SMFs, we predict that the spindle orientation could also be affected, which is a critical determining factor for cell division orientation. In fact, back in 1998, Denegre et al. found that 16.7 T large gradient high SMF could affect the division orientation of Xenopus eggs (Fig. 4.10) (Denegre et al. 1998). In 2006, Eguchi et al. showed that 8 T SMF could also change the cleavage plan formation in frog embryo division (Eguchi et al. 2006). It was proposed that SMFs may affect the orientation of astral microtubules and/or spindles, which was theoretically proven later by Valles (Valles 2002; Valles et al. 2002). In 2012, Mo et al. found that hypogeomagnetic field (HGMF; magnetic fields <200 nT) could cause a decrease in horizontal third cleavage furrows and abnormal morphogenesis in Xenopus embryos (Mo et al. 2012). In addition, they used immunofluorescence staining of tubulin to show the reorientation of the spindle of four-cell stage blastomeres. Their results indicated that a brief (2-h) exposure to HGMF was sufficient to interfere with the development of Xenopus embryos at cleavage stages. Also, the mitotic spindle could be an early sensor to the deprivation of the geomagnetic field, which provided a clue to the molecular mechanism underlying the morphological and other changes observed in the developing and/or developed embryos (Mo et al. 2012).

In the meantime, although it was shown that the microtubule and actin cytoskeleton in interphase cells could be affected by 7–17 T high SMFs in some cell types (Valiron et al. 2005), information about the mitotic spindle in high SMFs

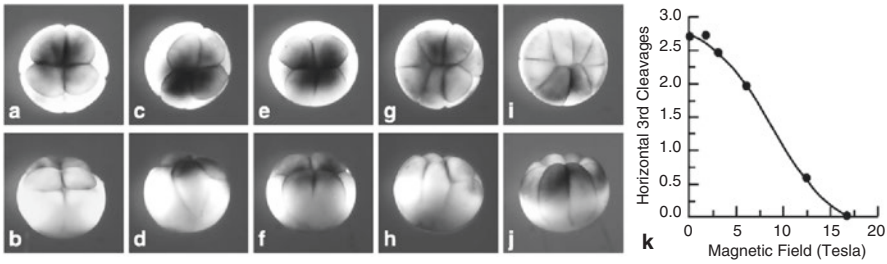

Fig. 4.10 Third cleavage in an AV-parallel SMF. Top (**a**, **c**, **e**, **g** and **i**) and side (**b**, **d**, **f**, **h** and **j**) views of eight –cell embryos from an AV-parallel field, showing the classes of third cleavage reorientation. For the side view, the embryo in the top view was rotated with the animal pole away from the viewer. The numbers of horizontal cleavages depicted are four (normal; **a** and **b**), three (**c** and **d**), two (**e** and **f**), one (**g** and **h**), and zero (**i** and **j**). (**k**) The average number of horizontal third cleavages per embryo as a function of field strength (Figure was reprinted with permission from from ref. (Denegre et al. 1998). Copyright © 1998, National Academy of Sciences, USA)

was not provided. Recently, using human nasopharyngeal cancer CNE-2Z cells and human retinal pigment epithelial RPE1 cells, we found that the spindle orientation could be altered by a 27 T ultra-high SMF. More interestingly, we found that the spindle orientation was determined by both microtubules and chromosomes (Zhang et al. 2017c).

4.3.4 Actin

Besides microtubules, the actin cytoskeleton has also been reported to be affected by SMFs in some cell types. For example, Mo et al. recently showed that in the absence of the geomagnetic field (GMF), the so-called hypomagnetic field (HMF) environment, the adhesion and migration of human neuroblastoma cells (SH-SY5Y cell line) were inhibited, which were accompanied with a reduction in cellular F-actin amount and disordered kinetics of actin assembly *in vitro* (Mo et al. 2016). These results indicated that elimination of the GMF affected assembly of the motility-related actin cytoskeleton, and suggested that F-actin was a target of HMF exposure and probably a mediator of GMF sensation (Mo et al. 2016).

Although whether actin could serve as a mediator of GMF sensation still needs to be further confirmed, there are multiple other studies have shown that actin could be affected in cells by SMFs. The most striking and convincing data was provided in 2005 by Eguchi and Ueno (Eguchi and Ueno 2005), which was briefly mentioned in the cell orientation section above. They examined the actin cytoskeleton in 8 T ultra-high SMF treated Schwann cells and found that actin fibers were oriented in the direction of the magnetic field. However, when the Schwann cells were treated with an inhibitor of small GTPase Rho-associated kinase, which disrupted actin fibers, the orientation phenotype induced by 8 T SMF no longer existed. This indicated that the SMF-induced Schwann cell orientation was dependent on Rho regulated actin fibers (Eguchi and Ueno 2005). Therefore their data directly showed that the Rho-regulated actin fibers were involved in SMF-induced cell orientation, at least in Schwann cell. Another example for SMF-induced actin alteration was in 2007 by Coletti et al., who used myogenic cell line L6 and found that 80 mT SMF promoted myogenic cell alignment and differentiation (Coletti et al. 2007), which was also introduced in the preious cell orientation section (Table 4.1). More specifically, they observed increased accumulation of actin and myosin as well as formation of large multinucleated myotubes, which was derived from increased cell fusion efficiency, but not cell proliferation (Coletti et al. 2007). In addition, a few other studies also showed SMF-induced actin alterations. For example, in 2009, Dini et al. found that 72 h of 6 mT SMF exposure caused human leukemia U937 cell F-actin modification (Dini et al. 2009). In 2013, Gioia found actin cytoskeleton modifications in swine granulosa cells after 3 days exposure to a 2 mT SMF (Gioia et al. 2013). Recently, Lew et al. found that 0.4 T SMF could increase the fluorescence intensity of the F-actin (Lew et al. 2016). Furthermore, Zhang et al. found that 16 T SMF disrupted actin formation in pre-osteoclast Raw264.7 cells but 500 nT and 0.2 T SMFs did not (Zhang et al. 2017a).

There are also some studies that reported the unchanged actin in SMF-treated cells. For example, in 2005, Bodega et al. examined primary cultures of astroglial cells for their responses to 1 mT sinusoidal, static, or combined magnetic field for various timepoints and did not observe any significant changes on actin (Bodega et al. 2005). In my opinion, the magnetic field strength in their study might be too low to induce actin alteration. Recently we examined multiple human cancer cells, such as human nasopharyngeal cancer CNE-2Z and colon cancer HCT116 cells, for their responses to 1 T SMF for 2–3 days and did not observe any significant changes on actin (data not shown). However, the cells we examined are different from above mentioned cell types that have actin alterations upon SMF exposure, such as neuroblastoma cells, Schwann cells and myogenic cell. These cells may have different actin regulation network than the cancer cell lines we examined. From the above mentioned studies, it is likely that actin cytoskeleton in cells respond to SMFs in a cell type- and magnetic field intensity-dependent way, which will need more systematic investigations.

4.3.5 Cell Viability

So far most studies showed that SMFs had minimum effects on cell viability. For example, In 1992, Short et al. found that 4.7 T SMF treatment did not affect cell viability in both human malignant melanoma cells and normal human fibroblast cells (Short et al. 1992). In 2003, Pacini et al. found that 0.2 T SMF could affect the cell morphology and proliferation but not the cell viability of human skin fibroblasts (Pacini et al. 2003). In 2009 Dini et al. reported that 72 h exposure of 6 mT SMF did not affect cell viability in human leukemia U937 cells (Dini et al. 2009). In 2013, Gioia investigated the effect of chronic exposure to a 2 mT SMF on *in vitro* cultured swine granulosa cells (GCs) and found that the SMF exposure did not affect the cell viability (Gioia et al. 2013). In 2016, Romeo et al. examined MRC-5 human foetal lung fibroblasts exposed to 370 mT SMF and found that the cell viability was not affected (Romeo et al. 2016). Recently we examined 1 T SMF induced effects on cell viability in 15 different cell lines, including human cancer cell lines CNE-2Z, A431 and A549, non-cancer cell line 293 T as well as CHO cells (Fig. 4.11). In fact, we checked four different cell densities and found that the cell viability was not obviously changed by 1 T SMF in any of these cell types (Fig. 4.11) (Zhang et al. 2017b). These studies, including more than 20 different cell types, showed that SMFs do not have obviously effect on cell viability.

However, there are a few studies indicate that SMFs could increase apoptosis in some cell types. In 2005, Chionna et al. reported that 6 mT SMF induced apoptosis in Hep G2 cells in a time-dependent manner. The apoptosis was almost negligible at the beginning of experiment but increased to about 20% after 24 h of continuous exposure (Chionna et al. 2005). In 2006, Tenuzzo et al. found that 6 mT SMF could promote apoptosis in thybridoma 3DO, human liver cancer Hep G2 cells and rat thyroid FRTL cells, but not human lymphocytes,

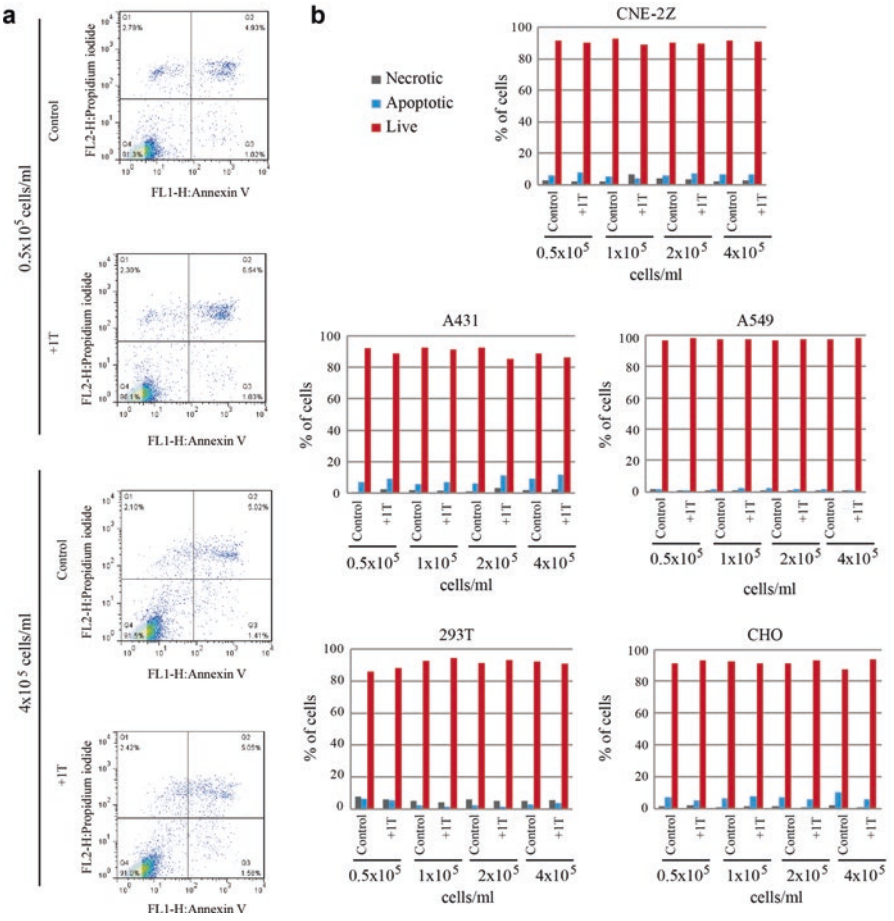

Fig. 4.11 1 T SMF exposure of two days does not promote cell death in multiple cells lines. Various cells were plated at different density one day ahead and treated with 1 T SMF for 48 h before they were analyzed for cell death using Annexin/PI stain and flow cytometry. Representative raw data (**a**) and quantification of live, apoptotic and necrotic cell numbers (**b**) are shown (Reprinted from ref. (Zhang et al. 2017b). Copyright © 2016 Impact Journals, LLC)

mice thymocytes, human histiocytic lymphoma or human cervical cancer HeLa cells (Tenuzzo et al. 2006). In 2008, Hsieh et al. found that 3 T SMF induced human chondrocytes apoptosis through p53, p21, p27 and Bax protein expression (Hsieh et al. 2008). In 2016, Wang et al. exposed adipose-derived Stem Cells (ASCs) to 0.5 T SMF for 7 days and found that the cell viability was inhibited (Wang et al. 2016).

It is interesting and puzzling that when SMFs are combined with some other treatments, they have been shown to have totally diverse effects. For example, in 2001, Tofani et al. found that when 3 mT SMF was combined with 3 mT 50 Hz PMF, the apoptosis of WiDr and MCF-7 cancer cells were increased while the

MRC-5 cells were not affected (Tofani et al. 2001). In 2006 Ghibelli et al. found that exposure to SMFs of NMR (1 T) could increase damage-induced apoptosis in tumor cells of haematopoietic origin, but not mononuclear white blood cells, showing that NMR may increase the differential cytotoxicity of antitumor drugs on tumor vs. normal cells (Ghibelli et al. 2006). These studies show that SMF could promote the apoptosis effects of PMF or antitumor drugs. However, there are also evidences showing that SMF could protect some cells from apoptosis. For example, in 1999 Fanelli et al. showed that 0.3–60 mT SMFs could reduce cell apoptosis induced by damaging agents such as etoposide (VP16) and puromycin (PMC) (Fanelli et al. 1999). It was also interesting that although Tenuzzo et al. found 6 mT SMF could promote apoptosis in thybridoma 3DO, human liver cancer Hep G2 cells and rat thyroid FRTL cells, when the SMF was combined with apoptotic inducing drugs, such as cycloheximide, puromycin, it had a protective effect because the majority of cells could be rescued from apoptosis, except for 3DO (Tenuzzo et al. 2006).

Therefore the effect of SMFs on cell apoptosis is magnetic field intensity, treatment time, and most importantly, cell type-dependent. In most reported cases, the cell viability was not affected by SMFs. However, there were also a few reports indicating that some cells could be affected. In addition, SMFs could have combinational or antagonistic effects when they are combined with other treatments, such as PMFs or different cell damaging agents. Further investigations are strongly needed to unravel the underlying mechanisms.

4.3.6 Cell Attachment/Adhesion

There are several studies showing that the cell attachment could be affected by SMFs. For example, in 2011 Sullivan et al. exposed the cells directly to SMFs right after seeding with an exposure time of 18 h and found that WI-38 (human fetal lung fibroblast cells) attachment was significantly reduced by 35–120 mT SMFs (Sullivan et al. 2011). In 2012, Li et al. exposed human umbilical artery smooth muscle cells (hUASMCs) to 5 mT SMF for 48 h and found that the cell adhesion was obviously decreased (Li et al. 2012). In 2014, Wang et al. found that moderate intensity SMFs of 0.26–0.33 T could reduce human breast cancer MCF-7 cell attachment (Wang et al. 2014).

Although these results indicate that cell attachment/adhesion may be affected by SMFs, the consensus result is still lacking. In most cases, SMFs seem to reduce the cell attachment/adhesion, there are also opposite evidences. For example, Mo et al. found that shielding of the geomagnetic field also inhibited cell adhesion and migration accompanied with a reduction in cellular F-actin amount in human neuroblastoma SH-SY5Y cells (Mo et al. 2016). This indicates that in the absence of SMF, the cell attachment could also be reduced. Moreover, in our own experience, the cell attachment/adhesion of most cells was not affected by moderate intensity SMFs.

Not surprisingly, the SMF-induced changes in cell attachment also seemed to be cell type-dependent. In 1992, Short et al. tested both human malignant melanoma

cells and the normal human cells and found that the malignant melanoma cells had reduced attachment to the tissue culture surface while the normal fibroblasts were not affected by the 4.7 T SMF (Short et al. 1992). More recently, Wang et al. found that although human breast cancer MCF-7 cell attachment was reduced by moderate intensity SMFs of 0.26–0.33 T, the HeLa cell attachment was not affected (Wang et al. 2014). In addition to the different cell types, the experimental procedure, such as the timing of SMF exposure before or after the cells have been attached to the cell culture plates, is also likely to be a key factor that influences the experimental outcomes. Moreover, we found that the supporting substrate, such as the cell culture plate and the coverslip, can also influence the experimental results about cell attachment/adhesion. Therefore more researches are certainly needed to examine the exact effects of SMFs on cell attachment/adhesion, as well as their consequences *in vivo*.

4.3.7 Cell Morphology

Multiple studies have shown that the cell shape can be altered by SMFs. In 2003, Pacini et al. found that the morphology of human skin fibroblast cells were modified by 0.2 T SMF (Pacini et al. 2003). In the same year, Iwasaka et al. found that 14 T SMF affected the morphology of smooth muscle cell assemblies, and the shapes of the cell colonies extended along the direction of the magnetic flux (Iwasaka et al. 2003). Chinonna et al. also reported time-dependent cell shape and membrane microvilli changes in human histiocytic lymphoma U937 cells and human lymphocytes by a 6 mT SMF (Chionna et al. 2003). In 2005, Chionna et al. found that Hep G2 cells exposed to 6 mT SMF for 24 h were elongated with many irregular microvilli randomly distributed on the cell surface, as well as a less flat shape due to partial detachment from the culture dishes. In addition, cytoskeleton was also modified in a time dependent manner (Chionna et al. 2005). In 2009, Dini et al. found that 72 h of 6 mT SMF caused human leukemia U937 cell shape change and F-actin modification, appearance of membrane roughness and large blebs and impaired expression of specific macrophagic markers on the cell surface (Dini et al. 2009). It was also interesting that although the cell growth was inhibited, the average cell size of rat pituitary adenoma GH3 cells was increased by prolonged exposure to 0.5 T SMF (Rosen and Chastney 2009). In 2013, Gioia found cell length and thickness changes, as well as actin and alpha-tubulin cytoskeleton modifications in swine granulosa cells after 3 days exposure to a 2 mT SMF (Gioia et al. 2013). Recently, Mo et al. found that magnetic shielding made the human neuroblastoma SH-SY5Y cells smaller in size and more round in shape, which was likely due to the disordered kinetics of actin assembly (Mo et al. 2016).

Not surprisingly, there are also many studies that did not observe cell morphology changes after SMF exposure. For example, in 1992 Sato et alo found that there were no cell shape changes in HeLa cells after 1.5 T SMF exposure for 96 h (Sato et al. 1992). In 2003, Iwasaka et al. found that no distinct changes in cell morphology in smooth muscle cells including cell membrane components occurred during

the 3 h exposure to 8 T magnetic field (Iwasaka and Ueno 2003b). In 2005, Bodega et al. examined primary cultures of astroglial cells for their responses to 1 mT sinusoidal, static, or combined magnetic fields for various timepoints and did not observe any significant changes on actin (Bodega et al. 2005). Again, the cell type may play a very important role in the SMF-induced cell morphology changes. For example, In 1999, Pacini et al. found that a 0.2 T magnetic field induced obvious morphology change in human neuronal FNC-B4 cell but did not affect mouse leukemia or human breast carcinoma cells (Pacini et al. 1999b).

In addition, multiple other factors could also determine whether people can observe cell morphology changes after SMF exposure, such as magnetic field intensity and exposure time, as well as detection techniques and experimental setup. There are two studies that both used freezing and SMF but the experimental results are totally different. The first one was in 1976, Malinin et al. exposed mouse fibroblast L-929 cells and human embryonic lung fibroblast WI-38 cells to 0.5 T SMF for 4–8 h after they were frozen and found that the cell morphology was significantly changed after they were thawed and cultured for 1–5 weeks (Malinin et al. 1976). In contrast, in 2013, Lin et al. found that when 0.4 or 0.8 T SMFs were used during the slow cooling procedures of RBCs, the survival rates of frozen-thawed RBCs were increased and there was no morphological changes (Lin et al. 2013). The mechanisms of the SMF + freezing-induced cell growth and/or morphological changes between these two studies are still unknown, which could due to the SMF + freezing procedure differences, or cell type differences. More studies are needed to test more cells in both procedures to reveal the underlying mechanisms.

4.3.8 Cell Migration

There are a few studies showing that SMFs could affect cell migration. Back in 1990, Papatheofanis found that 0.1 T SMF could inhibit cell migration of human polymorphonuclear leukocytes (PMNs) (Papatheofanis 1990). In 2012, Li et al. found that 5 mT SMF treatment for 48 h inhibited human umbilical artery smooth muscle cells (hUASMCs) migration (Li et al. 2012). In 2016, Mo et al. found that in the absence of the geomagnetic field (GMF), the so-called hypomagnetic field (HMF), cell migration was inhibited accompanied with a reduction in cellular F-actin amount (Mo et al. 2016). Besides SMFs, recently Kim et al. showed that TTF also inhibited U87 and U373 glioblastoma cell migration (Kim et al. 2016).

There are also many studies using gradient SMFs to separate different cell populations based on their different migration ability, which is called magnetophoresis. Based on the measured magnetic moments of hemoglobin and the relatively high hemoglobin concentration of human RBCs, the differential migration of RBCs was possible if exposed to a high gradient SMF. For example, in 2003, Zborowski et al. used a mean magnetic field of 1.40 T and a mean gradient of 0.131 T/mm to separate deoxygenated and methemoglobin (metHb)-containing RBCs (Zborowski et al. 2003). The existence of unpaired electrons in the four

heme groups of deoxy and metHb gives them paramagnetic properties, which is very different from the diamagnetic property of oxyhemoglobin. Zborowski et al. showed that the magnetophoretic mobility for erythrocytes with 100% deoxygenated hemoglobin and for erythrocytes containing 100% metHb were similar, while oxygenated erythrocytes were diamagnetic (Zborowski et al. 2003). Magnetophoresis could provide a way to characterize and separate cells based on magnetic properties of biological macromolecules in cells (Zborowski et al. 2003). In fact, this technique has been used in both malaria detection and infected erythrocyte separation. Although many other techniques are also available, magnetophoretic is very promising because their high specificity for malaria parasite-infected RBCs (Kasetsirikul et al. 2016).

There are also some studies using gradient SMFs to "guide" cell migration. For example, in 2013, Zablotskii et al. showed that SMF gradient could assist cell migration to those areas with the strongest magnetic field gradient, thereby allowing the buildup of tunable interconnected stem cell networks, which is an elegant route for tissue engineering and regenerative medicine (Zablotskii et al. 2013).

4.3.9 Cell Membrane

Multiple studies have shown that the cell membrane permeability can be increased by SMFs. For example, in 2011, Liu et al. used AFM (Atomic Force Microscope) to reveal that a 9 mT SMF could increase the number and size of the holes on the cell membrane of K562 cells, which may increase the membrane permeability and the flow of the anticancer drugs (Liu et al. 2011). In 2012, Bajpai et al. found that 0.1 T SMF could suppress both gram positive (*S. epidermidis*) and gram negative bacteria (*E. coli*) growth, which was likely due to SMF-induced cell membrane damages (Bajpai et al. 2012). There are also multiple studies indicated that SMFs could increase the membrane rigidity in cells. For example, in 2013, Lin et al. found that a 0.8 T SMF decreased membrane fluidity and enhanced erythrocyte membrane stability to resist dehydration damage caused by slow cooling procedures (Lin et al. 2013). They found that the SMF coupled with the slow cooling procedure increased the survival rates of frozen-thawed erythrocytes without obvious cellular damage. Therefore they proposed that the SMFs increased the biophysical stability of the cell membrane, which reduced dehydration damage to the erythrocyte membrane during the slow cooling procedure (Lin et al. 2013). In 2015, Hsieh et al. showed that dental pulp cells (DPCs) treated with a 0.4 T SMF had a higher tolerance to ipopolysaccharide (LPS)-induced inflammatory response when compared to untreated controls. They suggested that 0.4 T SMF attenuates LPS-induced inflammatory response to DPCs by changing cell membrane stability/rigidity (Hsieh et al. 2015). Recently, Lew et al. used 0.4 T SMF to treat dental pulp stem cells (DPSCs) and suggested that the cell membranes of the DPSCs were affected to influence intracellular calcium (Lew et al. 2016).

The effects of SMFs on cell membrane are also cell type-dependent. In 2006, Nuccitelli et al. showed that 6 mT SMF exposure for 5 min affected cell membrane

potential differently in various cell types. Specifically, the 6 mT SMF caused depolarization in Jurkat cells but hyperpolarization in U937 cells (Nuccitelli et al. 2006). In addition, high resolution imaging techniques like AFM or Electron Microscopy are also important to reveal the SMF-induced cell membrane changes, which have been used in multiple studies to reveal the membrane changes or membrane associated protein changes caused by SMFs (Jia et al. 2007; Liu et al. 2011; Wang et al. 2014). In contrast, low resolution imaging techniques are less likely to unravel the membrane changes. In 2010, Wang et al. used an illustration to show the potential mechanism of SMFs on cell membrane, some of the associated receptors and channel proteins, as well as the downstream effectors (Wang et al. 2010). They proposed that the cell membrane is one of the major targets of SMFs in cells, which is largely due to the diamagnetic anisotropy of phospholipid molecules in the lipid bilayer (Braganza et al. 1984). The phospholipid molecules would align or reorient in the SMFs, which consequently affect the bulk biophysical properties of the cell membrane. In addition, since membrane dynamics changes can affect the activity of membrane embedded proteins, SMFs may also affect some of the membrane associated proteins, such as mechanosensitive ion channels or other embedded proteins (Petrov and Martinac 2007; Wang et al. 2010).

4.3.10 Cell Cycle

There are a few studies indicating that SMFs may be able to affect cell cycle in some types of cells or at specific conditions. For example, in 2010 Chen et al. found that 8.8 mT SMF increased the G2/M phase and decreased G1 and S phases in K562 cells (Chen et al. 2010). In 2013 Mo et al. showed that magnetic shielding promoted cell cycle progression in the G1 phase of human neuroblastoma (SH-SY5Y) cells (Mo et al. 2013). Recently, we found that 1 T SMF could cause a mitotic arrest to reduce cell number in synchronized HeLa cells (Luo et al. 2016).

On the other hand, most other studies found that the cell cycle was not affected by SMFs. For example, in 2010 Hsu and Chang found that 0.29 T SMF did not affect the cell cycle of dental pulp cells (Hsu and Chang 2010). Also in 2010, Sarvestani et al. investigated the effects of a 15 mT SMF on cell cycle progression in rat bone marrow stem cells (BMSC) and did not find any cell cycle changes (Sarvestani et al. 2010). Recently we analyzed multiple cell types seeded at different cell densities for the effects of 1 T SMF (Zhang et al. 2017b). For all the cell lines we tested, 1 T SMF exposure for 2 days did not significantly affect the cell cycle (Fig. 4.12) (Zhang et al. 2017b). In addition, we exposed human colon cancer HCT116 cells and human nasopharyngeal cancer CNE-2Z cells to 9 T SMF for three days and did not find noticeable cell cycle changes (our unpublished data). Furthermore, we recently exposed CNE-2Z cells to an ultra-high 27 T SMF for 4 h and did not observe obvious cell cycle changes (Fig. 4.13).

However, the effect of SMFs on cell cycle is likely to be cell type-dependent, just like most other SMF-induced cellular effects. In 2010, Zhao et al. found that 13 T SMF had no obvious effect on the cell cycle distribution in both Chinese hamster ovary

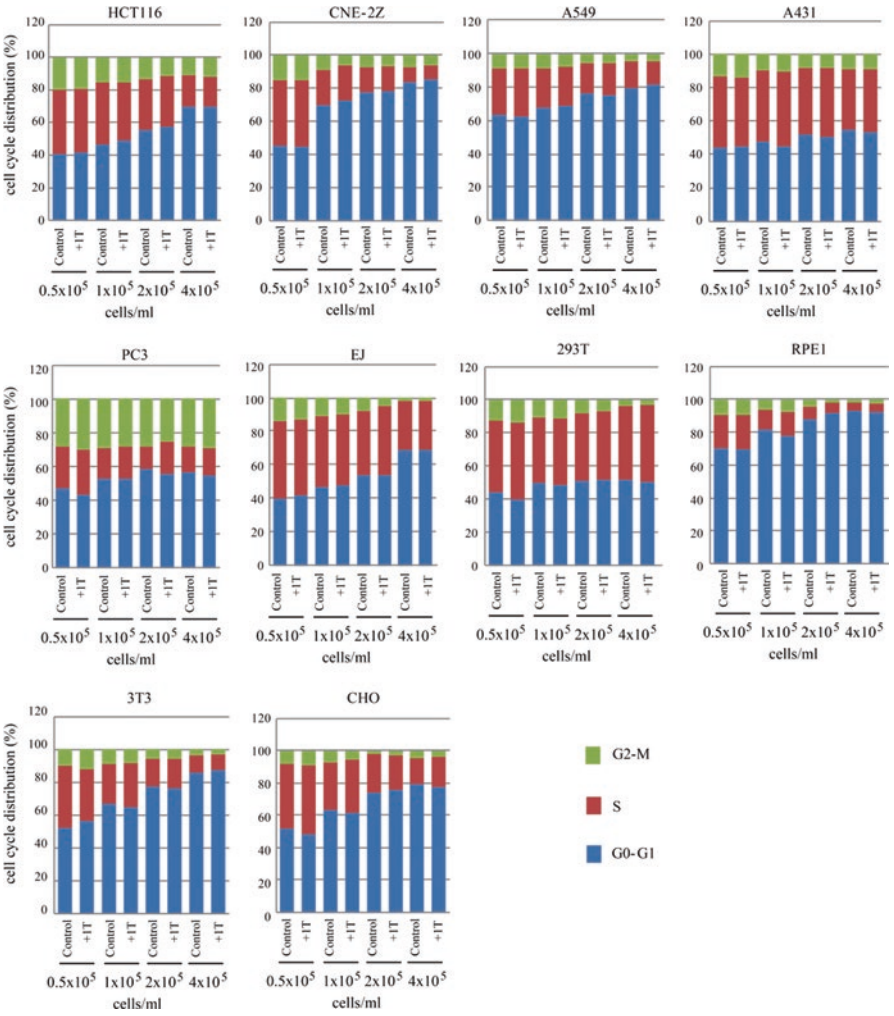

Fig. 4.12 1 T SMF has minimal effects on cell cycle. Various cells were plated at different densities one day ahead and treated with 1 T SMF for 2 days before they were analyzed for cell cycle by flow cytometry experiment. Experiments have been down for at least two times for each cell line and representative quantification results are shown (Reprinted from ref. (Zhang et al. 2017b). Copyright © 2016 Impact Journals, LLC)

(CHO) cells or DNA double-strand break repair deficient mutant XRS-5 cells, but decreased the G0/G1 phase and increased S phase cell percentage in human primary skin AG1522 cells (Zhao et al. 2010). This indicates that maybe SMFs have more effects on cell cycles in primary cells than immortalized cells. In addition, the specific cell cycle changes SMFs induced are different in reported studies (Chen et al. 2010; Zhao et al. 2010). More importantly, I think the methods people use make big differences. For example, flow cytometry (Figs. 4.12 and 4.13) could not reveal subtle changes in G2 or M phase because G2 and M are combined together. Therefore, further

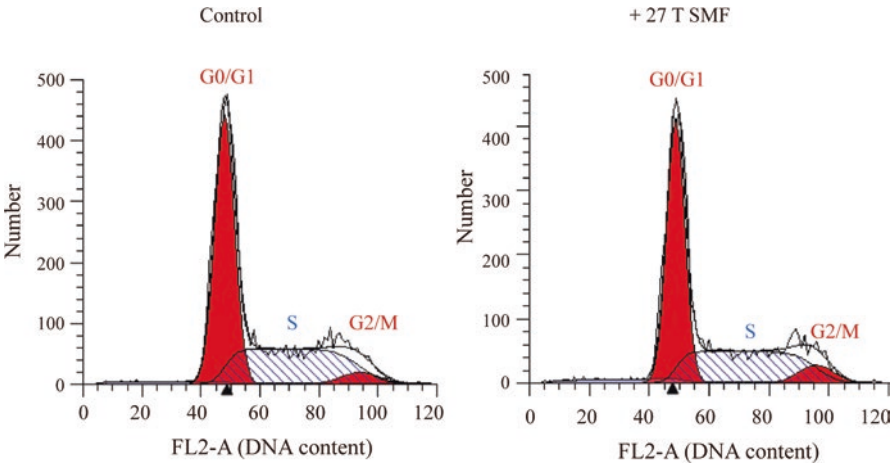

Fig. 4.13 27 T ultra-high SMF does not have obvious effect on cell cycle of human CNE-2Z cells in flow cytometry experiment. We recently exposed CNE-2Z cells to a 27 T ultra-strong SMF for 4 h and did not observe obvious cell cycle changes (Zhang et al. 2017c). The 27 T Ultra-strong SMF was provided by the water-cooled magnet #4 in Chinese Academy of Sciences, Hefei, China, with a biological sample investigation platform. It provides accurate temperature, gas and humidity for cell cultures (Figure was provided by Xinmiao Ji)

investigations with more methods are needed to examine more cell types and/or experimental conditions for the exact effect of SMFs on cell cycle.

4.3.11 Chromosome and DNA

Due to the public health concerns about the power lines, mobile phones and cancer, DNA integrity is frequently studied in pulsed magnetic fields (McCann et al. 1993; Cridland et al. 1996; Olsson et al. 2001; Zhou et al. 2002; Williams et al. 2006; Ruiz-Gomez et al. 2010). As early as 1984, Liboff et al. showed that DNA synthesis in cells could be increased by time varying magnetic fields (Liboff et al. 1984). Although so far there are still not enough evidences to confirm the harmful mutagenesis effects of these pulsed magnetic fields on human bodies, more researches are need to evaluate various exposure conditions.

In contrast, SMFs induced DNA damage and mutation is relatively less revealed. In 2004, Takashima et al. used somatic mutation and recombination test system in DNA repair-proficient and -deficient strains of *Drosophila melanogaster* to test strong SMFs for their possible effects on DNA damage and mutation in flies. They found that 2, 5, or 14 T fields exposure for 24 h caused a statistically significant enhancement in somatic recombination frequency in the postreplication repair-deficient flies, whereas the frequency remained unchanged in the nucleotide excision repair-deficient flies and in the DNA repair-proficient flies after exposure. In addition, they found that exposure to high magnetic fields induce somatic recombination in Drosophila and that the dose-response relationship is not linear (Takashima et al. 2004). Other than this work in flies,

most other studies revealed that SMFs do not cause DNA damage or mutation. For example, in 2015, Reddig et al. found that exposure of unstimulated human mononuclear blood cells to 7 T SMF alone or combined with varying gradient magnetic fields and pulsed radiofrequency fields did not induce DNA double-strand breaks (Reddig et al. 2015). In 2016 Romeo et al. examined human foetal lung fibroblasts MRC-5 exposed to 370 mT SMF and found that the DNA integrity was not affected (Romeo et al. 2016). Recently, Wang et al. exposed adipose-derived stem cells (ASCs) to 0.5 T SMF for seven days and did not observe DNA integrity changes (Wang et al. 2016). Therefore these studies did not reveal the direct DNA damage. Interestingly, in 2014 Teodori et al. found that the DNA damage in primary glioblastoma cells cause by X ray irradiation could be prevented by an 80 mT SMF exposure, which might because the SMF prevented the mitochondria membrane potential loss caused by X-ray irradiation (Teodori et al. 2014). So 80 mT SMF might have a protective role in X-ray induced DNA damage. However, it was also shown that combining 10 T SMF with X-ray-irradiation could promote the micronucleus formation, although the 10 T SMF itself does not have any effects on micronucleus formation (Nakahara et al. 2002).

It was reported that the DNA chain can be aligned by strong magnetic fields because its relative large diamagnetic anisotropy (Maret et al. 1975), which is mainly due to their stacked aromatic bases. In addition, it has been theoretically predicted that the highly compacted mitotic chromosome arms can generate electromagnetic fields along the chromosome arm direction (Zhao and Zhan 2012) and chromosomes should be able to be fully aligned by SMFs of around 1.4 T (Maret 1990). In addition, Andrews et al. showed that the isolated mitotic chromosomes can be aligned by an electric field (Andrews et al. 1980). We recently found that a 27 T ultra-high SMF could affect the mitotic spindle orientation in human cells, in which chromosomes played important roles (Zhang et al. 2017c).

The available evidences so far about SMF-induced DNA damage and mutation are still not sufficient to a solid conclusion. Most studies revealed that SMFs do not cause DNA damage or mutation in human cells. However, more investigations are encouraged to examine different cell types and magnetic field intensities to help us to achieve a more complete understanding on this issue.

4.3.12 Intracellular Reactive Oxygen Species (ROS)

Reactive oxygen species are highly active radicals, ions and molecules that have a single unpaired electron in their outer shell of electrons. ROS includes free oxygen radicals (O2•−, •OH, NO•, etc) and non-radical ROS (H2O2, N2O2, ROOH, HOCl etc). It is well known that low levels of ROS can act as intracellular signaling messengers that oxidize protein thiol groups, modify protein structure and functions while higher levels of ROS could nonspecifically attack proteins, lipids, and DNA to disrupt normal cellular processes (Liou and Storz 2010; Shi et al. 2014). There are also multiple studies showing that the elevated ROS levels in cancer cells compared to normal cells could contribute to the cancer progression (Gao et al. 2007). However, there are also some studies indicating that excessive oxidant stress slows

Table 4.3 SMF-induced ROS level changes in different studies

Cell line information	SMF intensity	SMF treatment time	ROS level	References
Human fibrosarcoma cancer cell line HT1080, pancreatic AsPC-1 cancer cell line, and bovine pulmonary artery endothelial cells (PAEC)	Shielding the geomagnetic field (decrease from 45–60 μT to 0.2–2 μT)	6–24 h	Decreased	Martino and Castello (2011)
Human SH-SY5Y neuronal-like cells	2.2 mT	24 h	Increased	Calabro et al. (2013)
Human histiocytic lymphoma U937 cells	6 mT	2 h	Increased	De Nicola et al. (2006)
Human-hamster hybrid A(L) cells, mitochondria-deficient rho(0) A(L) cells, and double-strand break (DSB) repair-deficient XRS-5 cells	8.5 T	3 h	Increased	Zhao et al. (2011)
WI-38 cells	230-250 mT	18 h	Increased	Sullivan et al. (2011)
MRC-5 human lung fibroblasts	370 mT	1 h/day for 4 days	No change	Romeo et al. (2016)
WI-38 cells	230–250 mT	5 days	No change	Sullivan et al. (2011)

Blue color indicates that SMF changes ROS level in cells. *Grey* color indicates that SMF does not affect ROS level in cells

cancer cell proliferation, threatens their survival and therapeutic interventions to further increase the oxidant stress level in newly formed tumor cells is likely to make them prone to death (Schumacker 2006, 2015; Trachootham et al. 2006).

There are multiple studies showing that SMFs could increase the cellular ROS (Table 4.3). For example, Calabro et al. showed that 2.2 mT SMF treatment for 24 h significantly decreased mitochondria membrane potential and increased ROS level in human SH-SY5Y neuronal-like cells (Calabro et al. 2013). De Nicola et al. found that 6 mT SMF increased the intracellular ROS of human histiocytic lymphoma U937 cells (De Nicola et al. 2006). In addition, Zhao et al. showed that ROS in the three cell lines, human-hamster hybrid A(L) cells, mitochondria-deficient rho(0) A(L) cells, and double-strand break (DSB) repair-deficient XRS-5 cells, were significantly increased by 3 h exposure of 8.5 T SMF (Zhao et al. 2011). In the meantime, Martino and Castello showed that shielding the geomagnetic field (decrease from 45–60 μT to 0.2–2 μT) could decrease the ROS production in human fibrosarcoma cancer cell line HT1080, pancreatic AsPC-1 cancer cell line, and bovine pulmonary artery endothelial cells (PAEC) (Martino and Castello 2011), which was consistent with the observations in other reports that SMFs could increase ROS level.

However, there was also a study showing that ROS was not affected by SMFs. Romeo et al. examined MRC-5 human lung fibroblasts exposed to 370 mT SMF and found that the intracellular ROS level was not affected (Romeo et al. 2016). These variations could be due to the cell type, magnetic field intensity, or even timepoint

differences. For example, Sullivan et al. showed that the oxidant production increased 37% in WI-38 cells exposed to SMF (230–250 mT) during the first 18 h after seeding, but no change was observed after a prolonged 5-day exposure (Sullivan et al. 2011), which indicates that the SMF-induced ROS elevation is time-dependent. Furthermore, ROS was known to be different in different cell types, as well as different cell densities (Limoli et al. 2004). We recently compared multiple cell lines and found that 1 T SMF increased ROS levels in some cell types but not the others. In some cell types, the ROS levels were even decreased by SMFs (our unpublished data). The molecular mechanism and the relation between ROS level changes and mitochondria alterations in SMF are still not clear. Further studies are necessary to explore the mechanisms in more details.

4.3.13 Adenosine Triphosphate (ATP)

Whether SMFs could affect the enzymatic ATP synthesis *in vitro* has been a big debate in the literature. In 2008, Buchachenko and Kuznetsov reported magnetic interactions on the rate of enzymatic synthesis of ATP *in vitro* (Buchachenko and Kuznetsov 2008). They found that the ATP synthesis can be significantly increased by 55 and 80 mT SMFs in the presence of $^{25}Mg^{2+}$. However, later studies by Crotty et al. failed to reproduce their results (Crotty et al. 2012) and the reason was still unclear (Hore 2012). Although the magnetic field intensities in these two studies were almost identical, the experimental details about the magnet setup were provided by Crotty et al., but not by Buchachenko and Kuznetsov. In addition, it is also possible that the difference was due to the fact that these two groups have used different sources of proteins. Buchachenko and Kuznetsov used a monomeric creatine kinase isozyme from snake venom, whereas Crotty et al. used dimeric creatine kinase. To my point of view, the above mentioned factors about both the magnetic fields and the protein itself could potentially produce seemingly inconsistent results. Therefore more investigations are encouraged to address this question.

Besides the *in vitro* studies, there are also some cellular works showing that the ATP level in cells could be affected by SMFs. However, the exact effects also seem to be case dependent. Back in 1995, Itegin et al. found that chronically applied SMF of 0.02 T had differential effects on various ATPase. The mean activities of Na(+)-K+ ATPase and Ca^{2+} ATPase were significantly increased by SMF but that of Mg^{2+} ATPase was non-significantly reduced (Itegin et al. 1995). It is possible that different cells have different ATPase network so that their responses to SMFs could be dissimilar. In 2010, Wang et al. tested moderate intensity SMF (~0.25 T) on PC12 cells (derived from a pheochromocytoma of the rat adrenal medulla) and found that the ATP level was moderately, but statistically significantly increased (Fig. 4.14). There was another study by Kurzeja et al. that also reported ATP level increase induced by SMF, although it was done in the presence of fluoride. In 2013, Kurzeja et al. found that moderate intensity SMFs (0.4, 0.6,

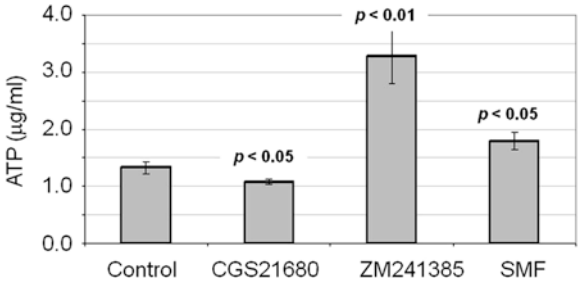

Fig. 4.14 Moderate intensity SMF increases cellular ATP level in PC12 cells. Cells were incubated with 1.0 μM CGS21680 (a selective adenosine A_{2A} receptor ($A_{2A}R$) agonis), ZM241385 (a potent, non-xanthine $A_{2A}R$ antagonist), or exposed to ~0.25T SMF for 6 h (Reprinted with permission from ref. (Wang et al. 2010). doi:10.1371/journal.pone.0013883.g004. Copyright ©2010 Wang et al. (open access))

and 0.7 T) could rescue fluoride-induced ATP decrease in fibroblasts. In addition, the effect was magnetic field intensity-dependent, in which 0.7 T SMF produced more significant effects than 0.4 and 0.6 T SMFs (Kurzeja et al. 2013).

There were also some studies showing that the cellular ATP level could be reduced by SMFs in a magnetic field intensity- and cell type-dependent manner. For example, in 2011, Zhao et al. used 8.5 T strong homogeneous SMF to test its effects in three cell lines, including human-hamster hybrid A(L) cells, mitochondria-deficient (rho(0) A(L)) cells, and double-strand break (DSB) repair-deficient (XRS-5) cells. They found that SMF-induced ATP content change was magnetic field intensity, time, as well as cell type-dependent (Zhao et al. 2011) (Table 4.4). Moreover, their results indicated that the 8.5 T SMF-induced cellular ATP decrease was partially mediated by mitochondria and the DNA DSB repair process because the ATP level in wild type A(L) cells could recover 12–24 h after SMF exposure but the mitochondria-deficient or double-strand break repair-deficient (XRS-5) cells could not (Table 4.4) (Zhao et al. 2011).

4.3.14 Calcium

Calcium plays important roles in a number of biological systems, especially in signal transduction cascades. The magnetic field-induced calcium changes in cells were mostly studied in PMFs (Walleczek and Budinger 1992; Barbier et al. 1996; Tonini et al. 2001; Zhou et al. 2002; Fassina et al. 2006; Yan et al. 2010) and were found to be dependent on cell status and field intensity (Walleczek and Budinger 1992) as well as other magnetic field parameters (Carson et al. 1990). There are multiple studies showing the calcium level was increased by 50–60 Hz magnetic fields (Barbier et al. 1996; Tonini et al. 2001; Fassina et al. 2006).

Similar to PMFs, there are also many studies showing that the calcium level was increased by SMFs. For example, in 1998, Flipo et al. examined the *in vitro*

Table 4.4 Summary of the SMF-induced ATP level changes in different cell lines in Zhao et al.'s study (2011)

Cell line information	SMF intensity and treatment time	Cellular ATP content
Human-hamster hybrid (A(L)) cells	1 T 3 h	No change
	1 T 5 h	No change
	4 T 3 h	No change
	4 T 5 h	No change
	8. 5 T 3 h	~20 % decrease
	8. 5 T 5 h	~20 % decrease
	8. 5 T 3 h, recover for 12 h	No change
	8. 5 T 3 h, recover for 24 h	No change
Mitochondria-deficient (rho(0) A(L)) cells	8. 5 T 3 h	~30 % decrease
	8. 5 T 3 h, recover for 12 h	~30 % decrease
	8. 5 T 3 h, recover for 24 h	~20 % decrease
Double-strand break (DSB) repair-deficient (XRS-5) cells	8. 5 T 3 h	~50 % decrease
	8. 5 T 3 h, recover for 12 h	~20 % decrease
	8. 5 T 3 h, recover for 24 h	~20 % decrease

Different color indicates different cell lines

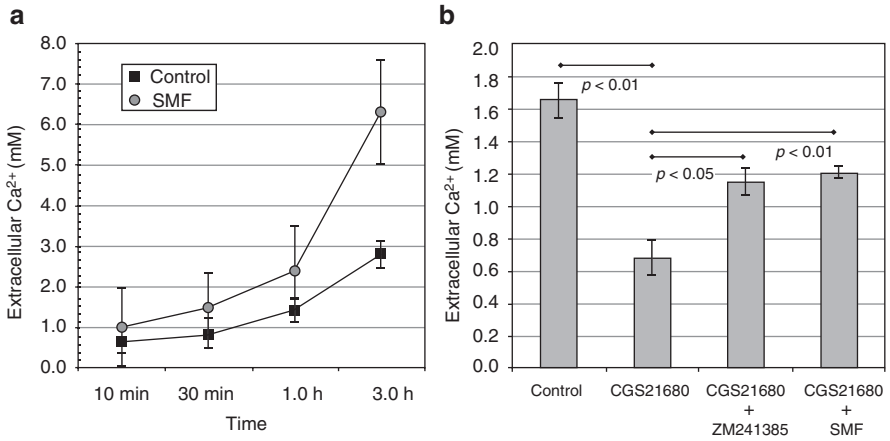

Fig. 4.15 Calcium levels in PC12 cells exposed to moderate intensity SMF, the $A_{2A}R$ agonist CGS21680 or antagonist ZM241385. SMF intensity was around 0.25T. (**a**) Extracellular Ca^{2+} level measured at different time points. $p < 0.05$, n =3. (**b**) Extracellular Ca^{2+} level measured at 3 h. n = 3 (Figure adapted from ref. (Wang et al. 2010). doi:10.1371/journal.pone.0013883.g001. Copyright ©2010 Wang et al. (open access))

effects of 0.025–0.15 T SMFs on the cellular immune parameters of the C57BI/6 murine macrophages, spleen lymphocytes, and thymic cells (Flipo et al. 1998). Exposure to the SMF for 24 h resulted in increased intracellular Ca^{2+} level in macrophages and increased Ca^{2+} influx in concanavalin A-stimulated lymphocytes (Flipo et al. 1998). In 2006, Tenuzzo et al. showed that 6 mT SMF could increase the calcium level in multiple cell lines (Tenuzzo et al. 2006). Prina-Mello et al. exposed rat cortical neurons to SMF of 0.75 T for 1 h and observed increased calcium level (Prina-Mello et al. 2006). In 2009 Dini et al. found that 6 mT SMF could cause significant increase in calcium level in human leukaemia U937 cells (Dini et al. 2009). In 2010, Wang et al. found that 0.23–0.28 T SMFs could increase extracellular calcium level in rat adrenal pheochromocytoma PC12 cells (Wang et al. 2010) (Fig. 4.15a). In addition, they found that SMFs could antagonize CGS21680-induced calcium reduction, which was similar to the effect of a selective A(2A)R antagonist ZM241385 (Wang et al. 2010) (Fig. 4.15b). In the same year, Hsu and Chang also found that 0.29 T SMF in combination with Dex/beta-GP significantly increased the extracellular calcium concentration at the early stage, followed by obvious calcium deposits later, which may contribute to the accelerated osteogenic differentiation and mineralization of Dental pulp cells DPCs (Hsu and Chang 2010). In 2014, Surma et al. found that weak SMFs increased the intracellular calcium and accelerated the development of skeletal muscle cells from newborn Wistar rats in primary culture (Surma et al. 2014). In the same year, Bernabo et al. showed that a 2 mT SMF could cause a reversible cell membrane depolarization wave (of about 1 min), which induced intracellular calcium increase and mitochondrial activity decrease in vital granulosa cells (Bernabo et al. 2014).

In the mean time, there are also some studies showing that the intracellular calcium was not affected by SMFs. For example, in 1986 Bellossi exposed neonatal isolated chick brains to uniform or nonuniform SMFs of 0.2–0.9 T and did not observe calcium efflux changes (Bellossi 1986). Papatheofanis et al. exposed mice to 1 T SMF for 30 min/day for 10 days and did not observe calcium alteration (Papatheofanis and Papatheofanis 1989). In 1990, Calson et al. found that 0.15 T SMF did not affect the cytosolic calcium level in HL-60 cells (Carson et al. 1990). In 1992, Yost and Liburdy combined extremely low frequency (ELF) time-varying magnetic fields with SMFs and examined their effects on calcium signaling in the lymphocyte (Yost and Liburdy 1992). Their results showed that a 1 h exposure of thymic lymphocytes to a 16 Hz, 42.1 μT magnetic field combined with a colinear SMF of 23.4 μT inhibited calcium influx in mitogen-activated cells but not resting lymphocytes. However, it was interesting that either the PMF or the SMF alone did not have such effects (Yost and Liburdy 1992). In 2008, Belton et al. found that application of 1, 10, or 100 mT SMF did not affect the calcium response to ATP in HL-60 cells (Belton et al. 2008). In 2009, Belton et al. and Rozanski et al. used a DEM to deplete GSH in HL-60 cells and then examined their responses to 0.1 T SMF and did not observe obvious calcium changes (Belton et al. 2009; Rozanski et al. 2009).

So far as we know, there are only a few studies that have reported the inhibition effect of SMFs on calcium. In 1992, Yost and Liburdy found that a combination of 16 Hz, 42.1 μT PMF with 23.4 μT SMF could decrease calcium level in thymic lymphocytes (Yost and Liburdy 1992). In 1996, Rosen et al. found that a 120 mT SMF caused a minor reduction in the peak calcium current amplitude and shift in the current-voltage relationship in cultured GH3 cells (Rosen 1996). In 2012, Li et al. found that 5 mT SMF could decrease cytosolic free calcium concentration in human vascular smooth muscle cells (VSMCs) (Li et al. 2012).

There are also many indirect evidences showing that calcium is involved in SMF-induced cellular effects. For example, in 1990 a study using human polymorphonuclear leukocytes (PMNs) showed that 0.1 T SMF could induce degranulation and cell migration inhibition, which could be prevented by pretreatment of calcium channel antagonists diltiazem, nifedipine, and verapamil in dose-dependent manner (Papatheofanis 1990). In 2005, Okano and Ohkuno found that neck exposure to 180 mT (B(max)) SMF alone for 5–8 weeks significantly suppressed or retarded the development of hypertension together with increased baroreflex sensitivity (BRS) in SMF group. Their results indicated that SMF may increase the L-type voltage-gated calcium channel blocker nicardipine-induced hypotension by more effectively antagonizing the Ca(2+) influx through the calcium channels compared with the nicardipine injection (NIC) treatment alone (Okano and Ohkubo 2005). In 2006, Ghibelli et al. found that 1 T SMF could potentiate the cytotoxic effects of puromycin and VP16, which could be prevented by calcium chelating agents EGTA and BAPTA-AM as well as the calcium channel blocker nifedipine (Ghibelli et al. 2006). In 2008, Yeh et al. found that 8 mT SMF increased the efficacy of synaptic-transmission in crayfish tail-flip escape circuit in a calcium-dependent way (Yeh et al. 2008). Also in 2008, Morris et al. used pharmacological agents for L-type calcium channel to show that SMF-induced anti-edema effect may work through the L-type calcium channels in vascular smooth muscle cells (Morris and Skalak 2008).

The differential effects of SMF-induced calcium changes are likely due to multiple reasons, such as cell types, magnetic field intensities as well as incubation time. There are multiple studies indicating that different cell types have differential calcium changes when exposed to SMFs. In 1999, Fanelli et al. found that the calcium level in different cell types responded to 6 mT SMF differently, which seemed to be correlated to the SMF-induced anti-apoptotic effect (Fanelli et al. 1999). They further found that both the protective and potentiating effects of 6 mT and 1 T SMFs in drug-treated cells were mediated by the Ca^{2+} influx from the extracellular medium, which only happened in some cell types (Fanelli et al. 1999; Ghibelli et al. 2006). In 2003, Aldinucci et al. tested the effects of combining a 4.75 T SMF and a pulsed EMF of 0.7 mT generated by an NMR apparatus for 1 h. They found that in Jurkat leukemia cells the calcium level was reduced significantly after exposure (Aldinucci et al. 2003b) but in normal or in PHA challenged lymphocytes the calcium level was increased (Aldinucci et al. 2003a). In addition, the SMF-induced calcium changes are also magnetic field intensity dependent. In 2006, Ghibelli et al. proposed that both the anti-apoptotic effect of a 6 mT SMF and the potentiating effect of a 1 T SMF were mediated by calcium influx (Ghibelli et al. 2006). In 2014, Zhang et al. examined multiple mineral elements for MC3T3-E1 cells during osteoblast mineralization when they were exposed to 500 nT, control geomagnetic field (C-GMF), 0.2 T, and 16 T SMFs. They found that the calcium level was decreased by 500 nT and 0.2 T SMFs but increased by the 16 T SMF (Zhang et al. 2014b). This magnetic field intensity-induced difference may have contributed to some of the inconsistencies in the literature, in addition to the cell type-induced variations. Moreover, the SMF-induced calcium changes are also likely to be time-dependent. In 2005, Chionna et al. found that Hep G2 cells exposed to 6 mT SMF had increased calcium level in a time-dependent manner and it reached the highest level at 4 h (Chionna et al. 2005). Table 4.5 summarizes the calcium changes induced by SMFs in the literature (Table 4.5).

Since calcium plays crucial roles in cellular processes such as cell proliferation as well as apoptosis, it is not surprising that different intensity SMFs could cause differential effects on calcium levels in various cell types, which lead to totally diverse cellular effects. In addition, there are also several studies that reported some signal transduction pathway changes, which are probably due to, or at least partially due to, the SMF-induced calcium modulation. For example, In 2012, Li et al. found that 5 mT SMF could influence the proliferation, migration, and adhesion of human umbilical artery smooth muscle cells (hUASMCs) by inhibiting the clustering of integrin beta1, decreasing cytosolic free calcium concentration, and inactivating FAK (Li et al. 2012). We previously found that 1 T SMF could inhibit human CNE-2Z cancer cell proliferation, which was related to the EGFR-Akt-mTOR pathways (Zhang et al. 2015; Zhang et al. 2016). As mentioned earlier in this chapter, we found that EGFR and its downstream pathways likely contribute to the cell type- and cell density-induced variations in SMF-induced cell proliferation changes (Zhang et al. 2017b). In fact, the kinase activity of EGFR protein itself could be

Table 4.5 SMF-induced calcium changes in different studies

Sample information	SMF intensity	Calcium level	References
Vital granulosa cells	2 mT	Increase	Bernabo et al. (2014)
Multiple cell lines	6 mT	Increase	Tenuzzo et al. (2006)
Human leukaemia U937 cells	6 mT	Increase	Dini et al. (2009)
Skeletal muscle cells from newborn Wistar rats in primary culture	60–400 μT	Increase	Surma et al. (2014)
Macrophages	0.025–0.15 T	Increase	Flipo et al. (1998)
Rat adrenal pheochromocytoma PC12 cells	0.23–0.28 T	Increase	Wang et al. (2010)
Dental pulp cells DPCs	0.29 T in combination with Dex/beta-GP	Increase	Hsu and Chang (2010)
Rat cortical neurons	0.75 T	Increase	Prina-Mello et al. (2006)
Thymic lymphocytes	23.4 μT	No change	Yost and Liburdy (1992)
HL-60 cells	0.1 T	No change	Belton et al. (2009), Rozanski et al. (2009)
HL-60 cells	0.15 T	No change	Carson et al. (1990)
Neonatal isolated chick brains	0.2-0.9 T	No change	Bellossi (1986)
Mice	1 T	No change	Papatheofanis and Papatheofanis (1989)
Thymic lymphocytes	16 Hz, 42.1 μT PMF + 23.4 μT SMF	Decrease	Yost and Liburdy (1992)
Human umbilical artery smooth muscle cells (hUASMCs)	5 mT	Decrease	Li et al. (2012)
GH3 cells	120 mT	Decrease	Rosen (1996)

Blue color indicates that SMF increases calcium level in cells. *Grey* color indicates that there is no effect. *Orange* color indicates that SMF decreases calcium level in cells

directly inhibited by SMFs (Zhang et al. 2016), which will be further discussed in Chap. 6. Recently, Lew et al. used 0.4 T SMF to treat dental pulp stem cells and found that the cell proliferation rate was increased. Their results indicated that 0.4 T SMF affected the cellular membranes of the DPSCs and activated intracellular calcium ions, which may activate p38 MAPK signaling to reorganize the cytoskeleton and increase cell proliferation of the DPSCs (Lew et al. 2016). Moreover, Maredziak et al. showed that 0.5 T SMF increased the proliferation rate of human adipose-derived mesenchymal stromal stem cells via activation of the phosphoinositide 3-kinase/Akt (PI3K/Akt) signaling pathway (Maredziak et al. 2017).

4.4 Conclusion

Since the human body is composed of various cells, which are filled with various components that can respond to the magnetic fields, most studies in the bioeffects of magnetic fields are carried out at cellular level. The parameters of the magnetic fields as well as the cells examined both have enormous impact on the experimental outcomes. So far most cellular effects of SMFs are largely dependent on magnetic field types, intensities, cell types, as well as other factors mentioned in this chapter. The cellular effects not only include the above mentioned aspects such as cell orientation, proliferation, calcium level changes, but also some other aspects that are relatively less studied and not included in this chapter, such as gene expression, mitochondria and immune system. It is obvious that further investigations are needed to get a more complete understanding of the cellular effects of SMFs. Overall, most cellular effects of SMFs are relative mild, except for the orientation changes in strong SMFs. In our own lab, to get unbiased and reproducible results throughout our studies, we always have at least two researchers to conduct the same sets of experiments independently and gathered their results together for data analysis. More importantly, people should know that the cellular effects of SMFs are influenced by various factors and parameters of magnetic field and the cells, as well as the way the experiments were done, such as incubation time and magnetic field direction. In addition, the absence of magnetic field effects in some experiments contrasted with the positive findings reported by other investigators. These discrepancies may be attributable to an inadequate detection capacity of instrument or techniques. Therefore, people should not only carefully record and analyze all experimental factors, but also try to take advantages of the advanced modern technologies to get a more comprehensive understanding of the cellular effects of SMFs.

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Chapter 5
Impact of Static Magnetic Field (SMF) on Microorganisms, Plants and Animals

5.1 Introduction

Static magnetic field (SMF) is a ubiquitous environmental factor for all living organisms during the evolutionary process. A variety of organisms including bacteria, algae, snails, planaria, honey bees, salmon, lobsters, salamanders, homing pigeons, robins, mice, and possibly humans have demonstrated the ability to sense the magnetic field (MF) for orientation in navigation, migration, homing, escaping and nest building (Gould 2010). Since the Industrial Revolution, human-made sources of SMFs have become an inevitable environmental factor for living organisms on the earth; in particular, the development of electromagnets and superconducting magnets made possible the exposure of organisms to intense magnetic fields. Acute and chronic exposures of organisms to SMFs, which are often ten or more times greater than geomagnetic fields, have been investigated for decades. Several hypothesis have been proposed to explain the interaction of SMF with biological systems, including magnetic induction, magnetite hypothesis and radical pair mechanism (Fedele et al. 2014). However, the exact mechanism(s) underlying the influence of SMF on living systems is still largely unknown, and until recently there was no unique theory about magnetic field-organism interaction. In this review, we limit our discussion to the evidence on the impact of exposure to SMFs with intensity ranging from a few mT to several Teslas (T) on microorganisms, plants, and animals and explore recent key results in the investigation of magnetoreception in these organisms.

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5.2 SMF on Microorganisms

5.2.1 SMF on Cellular Growth and Viability

The influence of magnetic fields with various flux densities on the growth rate and viability of microbes has been investigated in bacteria, yeast, and plant pathogenic fungi. Moore (1979) found that SMF of 30–60 mT were most inhibitory on the growth of several bacterial species. Bajpai et al. (2012) showed that SMF of 100 mT suppressed growth of both gram-positive (*S. epidermidis*) and gram-negative bacteria (*E. coli*), which was related to cellular membrane damage. Ji et al. (2009) showed that a 450 mT SMF inhibited growth and even killed *E. coli*, and the inhibitory effect increased with temperature. Morrow et al. (2007) investigated moderate SMF in the range of 50–500 mT on the growth of *Streptococcus pyogenes* and observed growth inhibition up to 300 mT, but an increase in growth rates when cells were exposed to 500 mT. Although an SMF of 300 mT had no influence on the growth of *E. coli* in nutrient rich Luria Bertani (LB) medium, it increased the density of bacterial cells during late growth in diluted LB (Potenza et al. 2004). EI May et al. (2009) also reported that SMF of 200 mT failed to alter cellular growth but induced a decrease of colony-forming units (CFU) between 3 and 6 h followed by an increase from 6 to 9 h. Kohno et al. (2000) compared the effect of SMF exposure of up to 100 mT on the culture of *Streptococcus* mutants (*S. aureus*) and *E. coli* grown in aerobic and anaerobic conditions. They found that bacterial growth was inhibited by SMFs in anaerobic conditions, but remained unaffected when applied in aerobic conditions, indicating that oxygen played an inhibitory role for magnetic fields.

In contrast to the inhibition effects of SMF of mT on the growth of bacteria, Nakamura et al. (1997) reported that the cellular growth of *Bacillus subtilis* MI113 and genetically transformed *B. subtilis* MI113 (pC112) significantly increased by exposure to homogeneous 7 T and inhomogeneous 5.2–6.1 T magnetic fields. Moreover, SMF of 5.2–6.1 T promoted survival rates of *E. coli* B cells in the stationary phase; CFU and the amount of S factor encoded by the *rpoS* gene were much higher than that under a geomagnetic field (Horiuchi et al. 2001).

Growth and sporulation of phytopathogenic fungi have been investigated under SMF. Nagy and Fischl (2004) showed that the applied magnetic field with flux densities ranging from 0.1 to 1 mT decreased the growth of phytopathogenic fungi colonies and the number of *Fusarium oxysporum* conidia, whereas the number of the developed conidia of *Alternaria alternata* and *Curvularia inaequalis* increased. Albertini et al. (2003) provided further evidence on the growth depression of fungi exposed to SMFs. However, Ruiz-Gómez et al. (2004) demonstrated that magnetic fields have no effect on fungal growth.

In yeast, Iwasaka et al. (2004) found that gradient magnetic fields of 14 T exhibited decelerated growth in a liquid-gas mixture system (Fig. 5.1). Santos et al. (2010) showed that SMF of 25 mT resulted in an increase in glutathione content and biomass in *Saccharomyces cerevisiae*. In contrast, Malko et al. (1994) reported that yeast cells subjected to a static MF of 1.5 T over the course of seven cell divisions

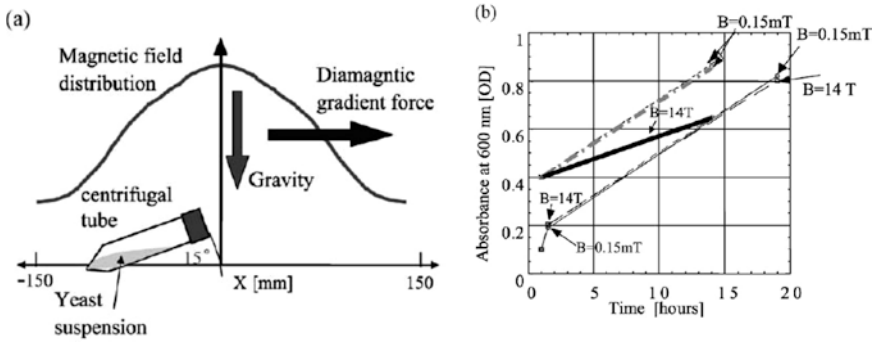

Fig. 5.1 Effects of magnetic fields of up to 14 T on the optical density of yeast suspension at wavelength 600 nm. The optical densities of the pair of sample tubes were measured after static incubation with and without exposure to magnetic fields. The upper panel shows the configuration of magnetic force acting on the diamagnetic solution and yeast, the direction of gravity, and the yeast culture tube. The bottom panel shows an example of the effects on the optical density of the yeast suspension (Reprinted from Iwasaka et al. (2004), Copyright 2016, with permission from Elsevier)

displayed growth rates similar to unexposed cells. Muniz et al. (2007) reported that the biomass (g/L) increment of *Saccharomyces cerevisiae* DAUFPE-1012 was 2.5 times greater in cultures exposed to a 200 mT SMF as compared with that in unexposed cultures.

5.2.2 SMF on Morphological and Biochemical Modifications

The morphological study of SMF-treated cells using a transmission electron microscope (TEM) revealed that the bacterial cell wall was ruptured by SMF exposure (Ji et al. 2009) (Fig. 5.2). SMFs of 200 mT significantly affected the phospholipid proportions in *S. typhimurium* wild type and *dam* mutant strain, with the most affected being the acidic phospholipids, cardiolipins (CL) (Mihoub et al. 2012). Egami et al. (2010) investigated the effect of SMFs on the budding of *Saccharomyces cerevisiae* and found that the size of budding yeast cells and the budding angle were affected by SMFs of 2.93 T. In a homogeneous magnetic field, the budding direction of daughter yeast cells was mainly oriented in the direction of magnetic field B; in contrast, in an inhomogeneous magnetic field, the daughter yeast cells tended to bud along the axis of capillary flow in regions where the magnetic gradient was high.

Microorganisms as models for analyzing fundamental metabolic responses to magnetic fields have great advantages, as they represent simple unicellular organisms. The inhibition of mycelia growth by SMF of 300 mT was accompanied by morphological and biochemical changes and Ca^{2+}-dependent signal transduction pathways were involved in conidia germination (Albertini et al. 2003). The patterns of metabolites released from *S. pyogenes* exposed to different magnetic flux

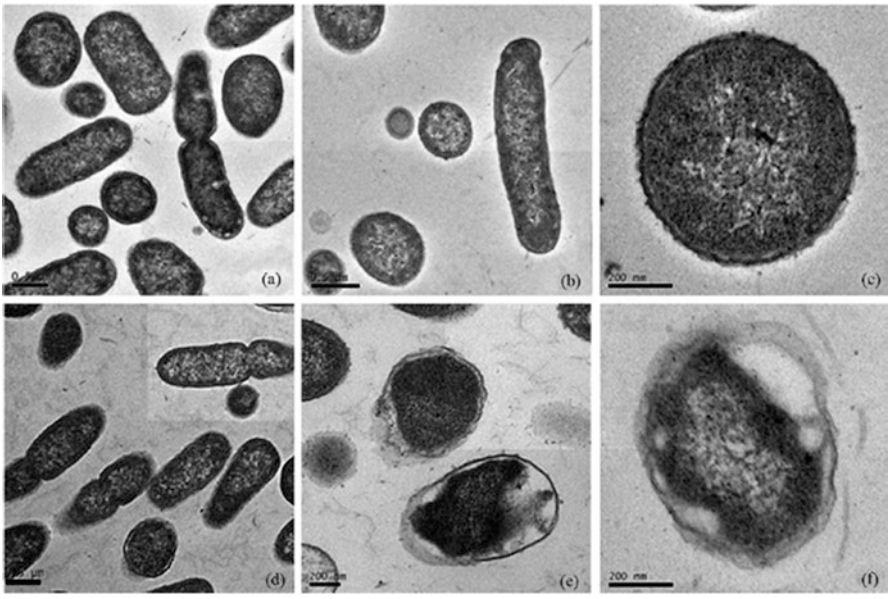

Fig. 5.2 TEM of SMFs-treated and untreated E. coli cells. (**a**)–(**c**) Images of untreated cells; (**d**)–(**f**) images of SMFs-treated cells. The cell walls of untreated samples were complete while the cell walls of SMFs-treated samples were obviously damaged (Reprinted from Ji et al. (2009), Copyright 2016, with permission from Elsevier)

densities ranging from 50 to 500 mT were significantly altered (Morrow et al. 2007). SMFs of 250–300 mT elicited the maximal release of the majority of metabolites. Hu and Qiu (2009) reported that an SMF of 10 T had significant effects on *E. coli* compared with *S. aureus*, which was reflected by the changes of spectral region of fourier-transform infrared (FTIR) spectroscopy combined with cluster analysis. The composition and conformation of the nucleic acid, protein, and fatty acid of *E. coli* were altered under the magnetic conditions. She et al. (2009) further found that 3.46–9.92% of the disorder coils in the secondary structures of protein in *E. coli* were turned into a-helices under SMF.

5.2.3 SMF on Genotoxicity

Mahdi et al. (1994) exposed various mutant strains of *E. coli* to a homogeneous static magnetic field of either 500 mT or 3 T. No evidence of increased DNA damage was detected in SMF-exposed *E. coli*, even with bacterial strains disabled for DNA repair. Ikehata et al. (1999) performed a bacterial mutation assay to determine the mutagenic potential of SMF. No mutagenic effects were detected in four uvrB strains of *Salmonella typhimurium* (TA98, TA100, TA1535 and TA1537) and *E. coli* WP2uvrA. Schreiber et al. (2001) also reported that exposures to an SMF of 7.2 T

did not show any alteration in the number of *His* $^{+}$ revertants in *Salmonella* mutagenicity test. Yoshie et al. (2012) reported that no statistically significant differences in the mutation frequency in thymine synthesis genes were observed between SMF-exposed cells and unexposed cells at any of the applied magnetic flux densities. SMFs up to 13 T caused neither mutagenicity nor co-mutagenicity in the SOD-deficient *E. coli* strain QC774 or in its parental strain GC4468, suggesting that exposure to strong SMFs does not affect the behavior of superoxides in these microorganisms.

The modification of chromatin conformation was reported in *E. coli* cells by Belyaev and Alipov (2001). Zhang et al. (2003) showed a dose-response relationship between the magnetic flux density (5 and 9 T SMF) and an increase in mutation frequency in the superoxide dismutase (SOD)-deficient *E. coli* strain QC774. Oxidative DNA damage plays an important role both in the aging process and environmental stress related diseases. However, exposure of cells to 300 mT significantly reduced the yield of 8-hydroxyguanine in extracted DNA compared to controls, suggesting some possible anti-oxidant protection to *S. pyogenes* at this field strength (Morrow et al. 2007). Carlioz and Touati (1986) showed an induction of the expression of a soxS::lacZ fusion gene following strong SMF exposures.

5.2.4 SMF on Gene and Protein Expression

Tsuchiya et al. (1999) reported that inhomogeneous magnetic field ranging from 5.2 to 6.1 T enhanced the transcription of the *rpoS* gene in *E. coli*. Three cDNAs were expressed only in *E. coli* exposed to 300 mT SMF, whereas one cDNA was expressed more in the controls (Potenza et al. 2004). EI May et al. (2009) found that the expression level of the 16S rRNA mRNA in *Salmonella Hadar* remained stable during exposure to 200 mT SMF, whereas mRNAs of *rpoA*, *katN*, and *dnaK* genes were over-expressed following 10 h of SMF exposure. Ikehata et al. (2003) reported that a slight decrease in the expression of genes related to respiration was observed in the budding yeast, *Saccharomyces cerevisiae*, exposed to 14 T SMF, whereas no changes were observed with field strengths <5 T. Although 14.1 T SMF caused little effects on cell growth of *S. onedensis* MR-1, apparent changes at transcriptional levels were detected in exposed cells, in which 21 genes were upregulated and other 44 genes were downregulated (Gao et al. 2005). In contrast, Potenza et al. (2012) reported that no differences were observed in gene expression in *Tuber borchii* mycelium after exposure to SMF, and only the activities of glucose 6-phosphate dehydrogenase and hexokinase were increased.

Snoussi and co-workers made a set of experiments on the out membrane protein expression in *S. Hadar* exposed to SMF of 200 mT (Snoussi et al. 2012, 2016). They found that a total of 11 proteins with more than a twofold change were differentially expressed in *S. Hadar* exposed to SMF. Among these changed proteins, 7 were up-regulated, while 4 were down-regulated. The proteomic analysis revealed that a total of 35 cytosolic proteins, which 25 were upregulated and 10 were downregulated,

were differentially expressed in SMF-exposed *S. Hadar*. Moreover, the overexpression of stress response proteins were determined in SMF-exposed *S. Hadar* as well. In other set of the experiments performed under the similar intensity of SMF, Mihoub et al. (2012) observed significantly changes in lipid proportions of membrane in exposed cells. SMF exposure caused an unusual accumulation of the acidic phospholipids cardiolipins, which the cyclic fatty acids and the total unsaturated fatty acids to total saturated fatty acids ratios were increased greatly.

5.2.5 Magnetosome Formation Sensing Magnetic Field

Microbial magnetosomes represent a special category of intracellular organelles that are synthesized by magnetotactic bacteria (MTB), which are a group of Gram-negative aquatic prokaryotes with a broad range of morphological types, including vibrioid, coccoid, rod and spirillum. MTB use the magnetosomes to sense and change their orientation in accordance with the magnetic field (Moisescu et al. 2014). Magnetosomes comprise magnetic iron-bearing inorganic crystals enveloped by an organic membrane (Staniland et al. 2007). Although the highly controlled process of magnetosome synthesis is not fully elucidated, magnetosome proteins are suggested to play an important role in biomineralization of magnetite crystals and formation of magnetosome chain (Komeili et al. 2004; Peigneux et al. 2016). Most of magnetosome proteins are encoded by *mam* and *mms* genes located in a conserved genomic region, known as magnetosome island (MAI), in *Magnetospirillum magneticum* (AMB-1) and *Magnetospirillum gryphiswaldense* MSR-1 (MSR-1) (Matsunaga et al. 2005; Ullrich et al. 2005; Fukuda et al. 2006). Among the various genes associated with magnetosome formation, the size and shape of magnetite particles are controlled by *mamCD* and *mms6*, while magnetosome biogenesis, magnetite biomineralization, magnetosome chain assembly, and iron transport are regulated by *mamAB* cluster (Nakamura et al. 1995; Grunberg et al. 2001; Amemiya et al. 2007; Komeili et al. 2004; Murat et al. 2010). Due to their superior crystalline and magnetic properties, magnetosomes have shown great prospect in biomineralization and medical applications, including drug delivery, magnetic resonance imaging, and array-based assaying (Yoshino and Matsunaga 2006; Matsunaga et al. 2007; Barber-Zucker et al. 2016).

The investigation on the influence of SMF on MTB and magnetosome formation is limited. Wang et al. (2008) found that exposure to SMF less than 500 nT restrained the growth of *M. magneticum* strain AMB-1 during the stationary phase, but increased the percentage of bacteria containing mature SD magnetosomes in their exponential growth phase. The average size of magnetic particles in SMF exposed cells was larger than 50 nm and a larger proportion of cells containing SD particles as compared to those grown in the geomagnetic field only. An SMF of 200 mT could impair cellular growth and raise Cmag values of the cultures. The number of magnetic particles per cell and the linearity of the magnetosome chain were affected by SMF exposure, which the expression of the *mamA*, *mms13*, and *magA* genes were up-regulated (Wang et al. 2009).

5.2.6 *Application of SMF on Antibiotic Resistance, Fermentation and Wastewater Treatment*

The application of SMF ranging from 0.5 ± 2 mT significantly enhanced the activity of the antibiotic gentamicin against *Pseudomonas aeruginosa* (Benson et al. 1994). Stansell et al. (2001) found that exposure of *E. coli* to SMF of 4.5 mT significantly increased its antibiotic resistance. Tagourti et al. (2010) showed that exposure to a 200 mT SMF increased the efficiency of gentamicin against *Salmonella Hadar* but did not affect the diameter of the inhibition zone of some other antibiotics actives on Enterobacteria: penicillin, oxacillin, cephalotin, neomycin, amikacin, tetracyclin, erythromycin, spiramycin, chloramphenicol, nalidixic acid and vancomycin. However, Grosman et al. (1992) reported that static magnetic fields of 0.5 ± 4.0 T had no significant influence on the growth of two strains of *E. coli* or *Staphylococcus aureus* after exposure time of 30 ± 120 min, nor were there any effects on sensitivity to several antibiotics.

The influence of SMF on the fermentation process has been investigated in biomass and enzyme activity. da Motta et al. (2001, 2004) showed that exposure to a 220 mT SMF significantly increased the biomass (g/L) of *S. cerevisiae* strain 2.5-fold and the concentration of ethanol by 3.4-fold as compared with SMF non-exposed cultures. Glucose consumption was higher in magnetized cultures, which correlated to the ethanol yield. Invertase is an enzyme (b-fructofuranosidase, EC 3.2.1.26) used to produce noncrystallizable sugar syrup from sucrose. Taskin et al. (2013) showed that the maximum invertase activity and biomass concentration were achieved with the spores exposed to 5 mT SMF.

Enhancement of biochemical processes by SMF has been applied in biological wastewater treatment. Jung et al. (1993) proved that SMF of 450 mT increased the efficiency of phenol biodegradation by 30% compared to the control sample. Krzemieniewski et al. (2003) reported that SMF of 400–600 mT stimulated the conditioning of wastewater sludge. Liu et al. (2008) found that a significant 30% increase in maximum nitrogen removal rate and an approximate 1/4 saving in cultivation time were achieved using SMF of 60 mT, indicating that the magnetic field was useful and reliable for fast start-up of the anammox process. Ji et al. (2010) showed that SMF up to 20 mT has a positive effect on bacterial growth in activated sludge and on wastewater biodegradation. Lebkowska et al. (2011) found that SMF of 7 mT had a positive effect on activated sludge biomass growth and dehydrogenase activity, which was similar to the observation in pnitroaniline removal with activated sludge. There was greater dehydrogenase and hydrolase activity in the activated sludge in the SMF. SMF of 7 and 21 mT increased the efficiency of the formation of polyhydroxybutyrate and the production of polyhydroxyvalerate, respectively, in sequencing batch reactors under working conditions. Niu et al. (2014) found that SMF intensity enhanced from 20 to 40 mT could promote microorganisms to produce more unsaturated fatty acids (UFAs) to stimulate the TTC dehydrogenase activity (TTC-DHA) in biological wastewater treatment. Křiklavová et al. (2014) reported that short term repeated exposure to an SMF of 370 mT

stimulated substrate (phenol) oxidation by around 34%, which, in turn, promoted Rhodococcus erythropolis growth by 28% while shortening the lag and exponential phases and increasing bacterial respiration activity by 10%. This was consistent with the observation that the degradation of phenolic waste liquors was enhanced by submersed microorganisms at a MF intensity of 22 mT. In algal-bacterial symbiotic system. Tu et al. (2015) reported that SMF stimulated both algal growth and oxygen production, suggesting that magnetic field could reduce the energy consumption required for aeration during the degradation of organic matter in municipal wastewater. Tomska and Wolny (2008) showed that a MF of 40 mT increases the removal of organic pollutants from wastewater, especially those containing nitrogen. Wang et al. (2012) found that 48 mT magnetic field could enhance the activities and growth of nitrite-oxidizing bacteria (NOB), suggesting that the magnetic field is helpful and reliable for accelerating the aerobic nitrifying granulation. In contrast, Mateescu et al. (2011) showed that SMF of 500 and 620 mT produced an atypical growth of the fungus that was characterized by fewer and swollen, bombastic colonies which did not spread on the entire surface of the culture medium. Filipic et al. (2012) reported that SMF (B = 17 mT) negatively influenced the growth of *E. coli* and *Pseudomonas putida* that are commonly found in wastewater treatment plants, but positively influenced enzymatic activity.

5.3 SMF on Plants

5.3.1 SMF on Germination

Magnetic seed treatment is one of the physical presowing seed treatments that have been reported to enhance the germination of crop plants. Martinez et al. (2000) showed that germinating barley seeds subjected to a magnetic field of 125 mT resulted in an increase in length and weight. Carbonell et al. (2009) reported that chronic exposure to a 150 mT magnetic field significantly increased the rate and percentage of germination in rice (*Oryza sativa* L.) seeds. Significant differences were obtained for seeds exposed to a 250 mT magnetic field for 20 min. Vashisth and Nagarajan (2008) showed that exposure of seeds of chickpea (*Cicer arietinum L.*) to SMF with a strength from 0 to 250 mT in steps of 50 mT significantly enhanced seed germination, speed of germination, seedling length and seedling dry weight. Furthermore, treatment of sunflower seeds in these magnetic fields increased the speed of germination, seedling length and seedling dry weight under laboratory germination tests, due to the higher enzyme activities of α-amylase, dehydrogenase and protease in magnetic field-treated sunflower seeds (Vashisth and Nagarajan 2010) (Fig. 5.3). Cakmak et al. (2010) found that SMF of 4 or 7 mT promoted the germination ratios in both bean and wheat seeds. The greatest germination and growth rates in both plants were from the test groups exposed to 7 mT MF. De Souza et al. (2010) reported that various combinations of SMF strength and

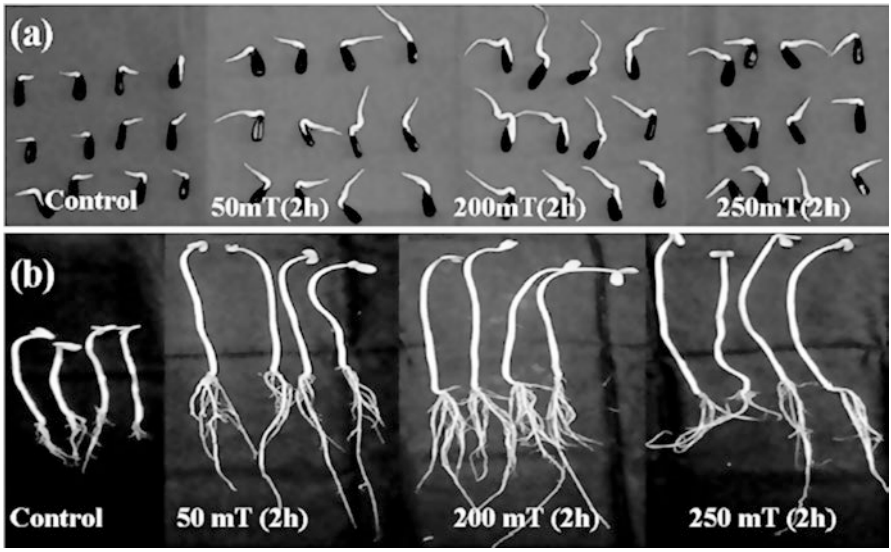

Fig. 5.3 Effect of pre-germination exposure of sunflower seeds on (**a**) speed of germination and (**b**) seedling vigor (Reprinted from Vashisth and Nagarajan (2010), Copyright 2016, with permission from Elsevier)

exposure time significantly improved tomato (*Solanum lycopersicum*) cv. Lignon seed performance. The combinations of 160 mT for 1 min and 200 mT for 1 min gave the best results. Naz et al. (2012) reported that pre-sowing magnetic treatments with an average magnetic intensity of 99 mT significantly increased the germination, growth and yield of okra (*Abelmoschus esculentus* cv. *Sapz pari*). Iqbal et al. (2012) reported that exposure of magnetic field strengths of 60 mT and 180 mT significantly enhanced the germination parameters of the seeds of the garden pea (*Pisum sativum L.* cv. *climax*), which the emergence index, final emergence index and vigor index was increased by 86.43%, 13.21% and 204.60%, respectively. Payez et al. (2013) reported that exposure to magnetic fields did not affect germination percent of the seeds, but increased the speed of germination and vigor index II, compared to the control group. Poinapen et al. (2013) investigated the magnetic flux density (R1 = 332.1 ± 37.8 mT; R2 = 108.7 ± 26.9 mT; and R3 = 50.6 ± 10.5 mT), together with exposure time, seed orientation (North and South polarity), and relative humidity (RH) (7.0, 25.5, and 75.5%) in tomato (*Solanum lycopersicum L.*) var. MST/32 seeds. They found that higher germination (∼11.0%) was observed in magnetically-exposed seeds than in non-exposed ones, suggesting a significant effect of non-uniform SMFs on seed performance with respect to RH, and more pronounced effects are observed during seed imbibition rather than during later developmental stages. Mahajan and Pandey (2014) found that the impact of SMF improved the germination of mung beans seeds even in off-season. Hozayn et al. (2015) reported that the magnetic field of 30 or 60 mT increased all germination and seedling growth characters in onion seeds (*c.v.Giza Red*) compared with control.

However, Flórez et al. (2004) reported that the mean germination time of rice (Oryzasativa) seeds exposed to SMF of 125 or 250 mT was significantly reduced compared to the controls.

5.3.2 SMF on Growth

Martinez et al. (2000) reported that MF of 125 mT stimulated the first stages of growth of barley seeds and increases in length and weight were observed. De Souza et al. (2006) showed that the mean fruit weight, the fruit yield per plant, the fruit yield per area, and the equatorial diameter of fruits increased significantly in tomatoes. Total dry matter was also significantly higher for plants from magnetically treated seeds than controls. Flórez et al. (2007) reported that continuously exposure to 125 or 250 mT MF produced corn plants that grew higher and heavier than the control, corresponding with an increase of total fresh weight. Carbonell et al. (2011) found that pea exposed to 125 or 250 mT SMF generated by magnets under laboratory conditions and continuous exposure were longer and heavier than the corresponding control. Yano et al. (2001) reported that the primary roots of radish (*Raphanus sativus L.*) seedlings responded tropically to the static magnetic field with the tropism appearing to be negative and the roots responded significantly to the south pole of the magnet. Subber et al. (2012) found that exposure to SMF of 50 mT significantly increased he root length, radicle length and protein percentage in *Zea mays*. Vashisth and Nagarajan (2008) reported that a dramatic increase in root length, root surface area and root volume was observed in chick pea exposed in batches to SMF of strength from 0 to 250 mT in steps of 50 mT. In the same conditions, sunflower seedlings showed higher seedling dry weight, root length, root surface area and root volume. Moreover, in germinating seeds, enzyme activities of α-amylase, dehydrogenase and protease were significantly higher in treated seeds than controls (Vashisth and Nagarajan 2010).

5.3.3 SMF on Gravitropism

Gravitropism is the most conspicuous response to the gravitational force in plants, which plays an essential role in maintaining the spatial orientation of seedlings and stable balance of massive plants. The ability of plants to sense gravity is largely attributed to starch-filled amyloplasts, which is a long-lived response throughout the entire life cycle. Kuznetsov and Hasenstein (1996) reported that high-gradient magnetic fields (HGMFs) induced intracellular magnetophoresis of amyloplasts. The shoots of *lazy-2* mutants of tomato plants (*Lycopersicon esculentum* Mill., cv. Ailsa Craig) exhibited negative gravitropism in the dark, but responded positively gravitropically in red light. The induced magnetophoretic curvature showed that *lazy-2* mutants perceived the displacement of amyloplasts in a similar manner to the wild

type and the high MF did not affect the graviresponse mechanism (Hasenstein and Kuznetsov 1999). Weise et al. (2000) reported that *Arabidopsis* stems positioned in a high gradient magnetic field (HGMF) on a rotating clinostat showed a lack of apical curvature after basal amyloplast displacement, indicating that gravity perception in the base was not transmitted to the apex. Hasenstein et al. (2013) examined the movement of starch grains of corn, wheat, and potato (*Solanum tuberosum*) in suspension during parabolic flights and found that magnetic gradients were able to move diamagnetic compounds under weightless or microgravity conditions and serve as directional stimulus during seed germination in low-gravity environments. Herranz et al. (2013) found that SMF itself produced a low number of proteomic alterations, but the combination of gravitational alteration and SMF exposure produced synergistic effects on the proteome of plants.

5.3.4 SMF on Photosynthesis

The increase in agricultural output are closely related to the efficiency of photosynthetic process. Shine et al. (2011) investigated the effects of SMF with magnetic field intensity ranging from 0 to 300 mT on the seeds of soybean (*Glycine max (L.) Merr. var.: JS-335*) and found that pre-sowing magnetic treatment could improve biomass accumulation and a higher fluorescence yield at J–I–P phase polyphasic chlorophyll a fluorescence (OJIP) transients was observed in exposed plants. Baghel et al. (2016) reported that the fluorescence yield at J–I–P phase of OJIP transients was increased in soybean seeds pretreated with 200 mT SMF under under salinity stress, which the growth, biomass accumulation, and carbon and nitrogen metabolism were enhanced in exposed plants as compare to controls. Anand et al. (2012) reported that SMF of 100 and 200 mT increased the photosynthesis, stomatal conductance and chlorophyll content in maize (*Zea mays* L.) var. Ganga Safed 2 seeds. Jan et al. (2015) compared the effects of reduced and enhanced geomagnetic field and SMF of 150 mT on the growth and efficiency of photosystem II in *Lemna minor* plants. They found that although geomagnetic field had no effect on the efficiency of photosystem II, 150 mT SMF had the potential to increase initial Chl a fluorescence and energy dissipation.

Yano et al. (2004) reported that CO_2 uptake rate, dry weight, and the cotyledon area of MF exposed radish seedlings was significantly lower than that of the control seedlings. However, Iimoto et al. (1996) showed that SMF of up to 4 mT had beneficial effects on the growth promotion and enhancement of CO_2 uptake of potato plantlets *in vitro*. In addition, Jovanic and Sarvan (2004) reported that SMF induced significant changes in bean leaf fluorescence spectra and temperature. The fluorescence intensity ratio (FIR) and change of leaf temperature βT increased with MF intensity.

5.3.5 SMF on Redox Status

The uncoupling of free radicals including reactive oxygen/nitrogen species (ROS/RNS) are involved in the underlying mechanism of SMF induced oxidative stress in plants. The activities of free radical scavenging enzymes, including catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR), glutathione trans- ferase (GT), peroxidase (POD), ascobtate peroxidase (APX), and polyphenoloxidase (POP), have been well documented to be altered by SMF exposure in various plants, including pea, radish (*Raphanus sativus*), *Leymus chinensis*, soybean, cucumber (*Cucumis stivus*), broad bean, corn, parsley (*Petroselinum crispum*), and wheat (Baby et al. 2011; Jouni et al. 2012). Cakmak et al. (2012) reported that SMF of 7 mT increased lipid peroxidation and H_2O_2 levels in shallot (*Allium ascalonicum*) leaves. Shine et al. (2012) showed that SMF of 150 and 200 mT enhanced production of ROS mediated by cell wall peroxidase, while the increase in the cytosolic peroxidase activity indicated that this antioxidant enzyme had a vital role in scavenging the increased H_2O_2 produced in seedlings from the magnetically treated soybean seeds. In mung bean seedlings treated with 600 mT SMF followed by cadmium stress, Chen et al. (2011) found that the concentration of malondialdehyde, H_2O_2, and $O^{-\cdot}$ were decreased in seedlings treated with 600 mT magnetic field followed by cadmium stress, while the NO concentration and NOS activity were increased compared to cadmium stress alone, indicating that MF compensates for the toxicological effects of cadmium exposure are related to NO signal.

5.3.6 Cryptochromes Sensing Magnetic Field

Cryptochromes (CRYs) are flavoproteins that direct a diverse array of developmental processes in response to blue light in plants (Yu et al. 2001). CRY has been suggested to be a potential magnetoreceptor for light-initiated electron transfer chemistry in CRY might be magnetically sensitive by virtue of the radical pair mechanism (Hore and Mouritsen 2016; Evans et al. 2013). Geomagnetic field (GMF) has been hypothesized to affect the redox balance of cryptochromes and the related signaling state (Vanderstraeten et al. 2015); however, the influence of strong SMF on the function of CRYs is still largely unexplored.

Three CRYs, CRY1, CRY2 and CRY3, are encoded in *Arabidopsis* genome (Lin and Todo 2005). CRY1 and CRY2 function as major blue-light receptors regulating blue light induced de-etiolation, photoperiodic flowering and circadian clock (Liu et al. 2016). Xu et al. (2014) found that SMF of 500 μT modified the function of CRYs. The blue light-dependent phosphorylations of CRY1 and CRY2 were enhanced in *Arabidopsis* seedlings grown in a 500 μT MF, whereas the near-null MF weakened the blue light-dependent phosphorylation of CRY2 but not CRY1. In the darkness, dephosphorylations of CRY1 and CRY2 were slowed down in 500 μT

MF, whereas dephosphorylations of CRY1 and CRY2 were accelerated in the near-null MF. According to the calculation of radical pair mechanisms in a relatively realistic model of the radical-pair system in *Arabidopsis* CRY1, Solov'yov et al. (2007) showed that 500 μT MF could increase the signaling activity of cryptochrome by up to 10%, suggesting that the function of CRYs was affected by magnetic fields. Ahmad et al. (2007) confirmed that 500 μT MF enhanced the blue light-dependent inhibition of hypocotyl growth of *Arabidopsis*. Hypocotyl growth of *Arabidopsis* mutants lacking cryptochromes was unaffected by the increase in magnetic intensity, while cryptochrome-dependent responses, such as blue-light-dependent anthocyanin accumulation and blue light dependent degradation of CRY2 protein, were enhanced at the higher magnetic intensity. However, with experimental conditions chosen to match Ahmad's study, Harris et al. (2009) found that in no case consistent, statistically significant MF responses were detected.

In addition to plants, the expression of CRYs has been detected in insects and vertebrate animals with distinct circadian clock functions (Möller et al. 2004; Nießner et al. 2013). In *Drosophila*, CRYs act as circadian photoreceptors according to the light and dark changes; in contrast, in vertebrates, CRYs act as transcriptional repressors in regulating the circadian feedback loop (Michael et al. 2017). Non-drosophilid insects such as butterfly have two types of CRYs: one act like *Drosophila*-CRY with light-sensing properties, whereas the other act like vertebrate-CRY with transcriptional repressive properties. Marley et al. (2014) reported that MF exposure coupled with blue light pulses had a substantial effect on seizure response in *Drosophila* larvae, which was dependent on CRYs. Giachello et al. (2016) provided new evidence that MF of 100 mT could increase neuronal action by stimulating the activity of CRY, indicating the link between magnetoreception and neuronal activity.

5.4 SMF on Animals

5.4.1 SMF on Caenorhabditis elegans

Caenorhabditis elegans (*C. elegans*) is a small free-living nematode. Due to the availability of the whole genome sequence, *C. elegans* has been widely used in studying fundamental issues of evolution, development, neurobiology, and genetics (Kaletta and Hengartner, 2006; Boyd et al. 2010). The unique advantages of *C. elegans* include the ease of maintenance, small size, short life cycle, genetic manipulability, stereotypical development, and high-throughput capability. Since 40–60% of its genes have human homologs, *C. elegans* based assays have been considered as an alternative to mammalian models in evaluating potential toxicity of physical and chemical mutagens and carcinogenes in humans (Dengg and van Meel 2004; Rajini et al. 2008; Sprando et al. 2009).

C. elegans has a simple nervous system with only 302 neurons in hermaphrodites. Vidal-Gadea et al. (2015) reported that AFD neurons, which sense environmental temperature and chemical stress, played a critical role in the orientation of *C. elegans* to geomagnetic field. Mutated stains, *tax-4* gene, which encodes an ion channel protein with similar function as a photoreceptor, was identified for magnetotaxis by using mutated strains. Rankin and Lin (2015) highlighted the significance of this study for it provided novel information on the navigation of living organisms sensing geomagnetic field.

The biological effects of SMFs on *C. elegans* have focused on aging and development, behavior, and global gene expression. Hung et al. (2010) reported that the development time and average lifespan were greatly reduced in wild-type nematodes exposed 200 mT SMF, which were related to the upregulation of *lim-7*, *clk-1*, *daf-2*, *unc-3* and *age-1*. Lee et al. (2012) screened 120 randomly selected genes in response to SMF exposure ranging from 0 to 200 mT and identified 26 differentially expressed genes that were related to apoptosis, oxidative stress, and cancer. The behavioral decline induced by SMF was suppressed in *ced-3*, *ced-4*, and *ced-9* mutants, indicating the involvement of key apoptotic pathway. Kimura et al. (2008) reported that SMF of 3 or 5 T significantly and transiently altered global gene expression in *C. elegans*, especially the upregulation of motor activity, cytoskeleton, actin binding, cell adhesion, Ca^{2+} binding, and cuticle related genes were found in SMF exposed nematodes. Wang et al. (2015) found that there was a time-dependent lifespan decrease and alteration of developmental pattern in *C. elegans* exposed to 8.5 T SMFs (Fig. 5.4). Furthermore, SMF exposure significantly increased germ cell apoptosis, which was mediated by apoptotic key signaling pathway and the generation of free radicals.

5.4.2 SMF on Insects

Magnetic fields have been shown to affect the orientation, oviposition development, fecundity, and behaviour for a wide variety of insects. The insect eggs have advantages in magnetic exposure as a large number of eggs can be placed into the magnet at the same time. The static magnetic field at 4.5 mT had no effect on egg lying, but increased mortality of eggs, larvae, and pupa, and diminished adult viability in Drosophila (Ramirez et al. 1983). Decreased hatching rate after exposure to a weak static magnetic field during early embryogenesis was also obtained in *D. melanogaster* and *Heliothis virescens (*tobacco bugworm) (Ho et al. 1992; Pan 1996). Apparent hatching delay from strong magnetic fields were observed in mosquito eggs in the center of 9.4 and 14.1 T magnets (Pan and Liu 2004). A significant increase of *Hylotrupes bajulus* viability and larval mass was reported after exposure to SMF of 98 mT (Rauš et al. 2009). A static magnetic field of 60 mT reduces the embryonic and post-embryonic development, and induces weaker viability in two different species, *Drosophila melanogaster* and *Drosophila hydei* (Savic et al.

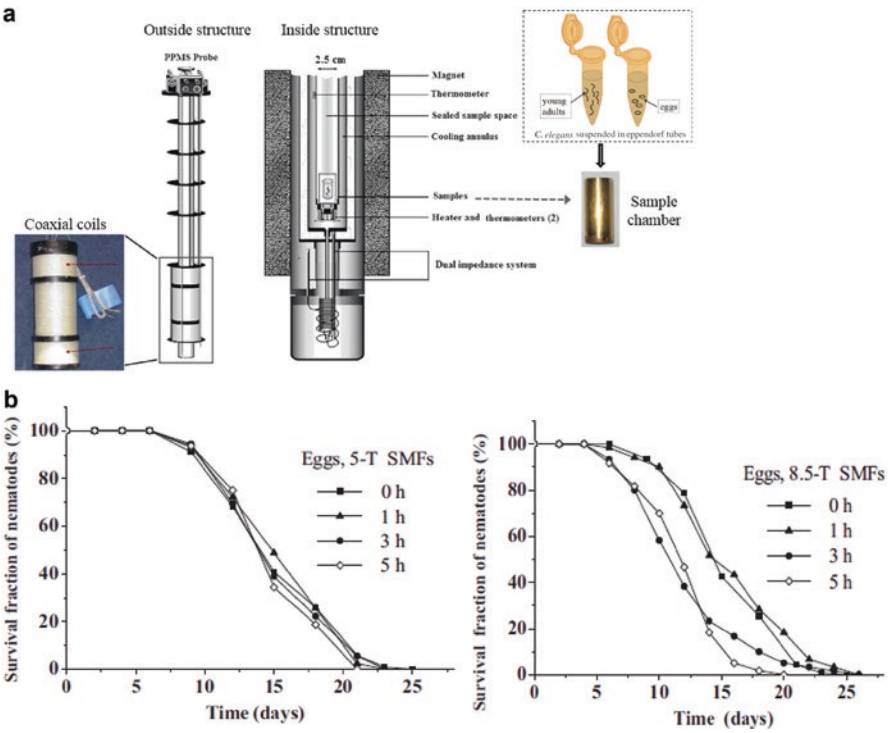

Fig. 5.4 (**a**) A Schematic diagram of the PPMS probe and SMF exposure system (Image courtesy of Quantum Design, San Diego, CA). (**b**) Effects of SMFs with different intensities and exposure times on lifespan of C. elegans. *Left*: Lifespan of C. elegans hatching from eggs exposed to 5 T SMF for 1, 3, 5 h. *Right*: Lifespan of C. elegans hatching from eggs exposed to 8.5 T SMF for 1, 3, 5 h. At least 60 worms were scored for each group

2011). Oak and beech populations of *Drosophila subobscura* had a longer development time and lower viability was observed in in N and S groups of 2.4 T SMF, which was mediated by oxidative stress (Todorovic et al. 2015).

In insects, the neuro-endocrine system is a main regulator of all aspects of life processes, such as development and behaviour, and the detection and activity of an external magnetic field may be transmitted by the neuroendocrine system. SMF of 375 mT caused a disturbance in the development and survival of pupae of the honeybee and *Tenebrio molitor*, the yellow mealworm (Prolic and Jovanovic 1986, Prolic and Nenadovic 1995). The morphometric parameters of the A1 and A2' neurosecretory neurons of the protocerebrum as well as the morphometric parameters of the *corpora allata* were changed by SMF of 320 mT (Peric-Mataruga et al. 2006, 2008). However, SMF of 50 mT had no effect on the pupa-adult development dynamic of two examine *Tenebrio* species, but they did modulated their motor behaviour (Todorovic et al. 2013).

The antennal lobe of *Drosophila* provides an ideal intact neural network model to investigate neural circuit function (Ng et al. 2002). Yang et al. (2011) found that SMF of 3.0 T modulated the rhythmic spontaneous activities of large LNs and correlated activity of ipsilateral pairs of large LN/LN in the *Drosophila* antennal lobe, indicating that *Drosophila* can be an ideal intact neural circuit model to evaluate the effects of magnetic field stimulations.

Mutagenic effects of a static magnetic field were investigated by increased mutation rate in a population of *Drosophila* exposed to a magnetic field 10–12 times greater than a geomagnetic one (Giorgi et al. 1992). Exposure to 2, 5, or 14 T fields caused a statistically significant enhancement in somatic recombination frequency in the post-replication repair-deficient flies, whereas the frequency of somatic recombination remained unchanged in the nucleotide excision repair-deficient flies and in DNA repair-proficient flies after exposure (Takashima et al. 2004).

5.4.3 SMF on Helix pomatia

Helix pomatia possess a simple nerve system and display simple behavioral repertoire. Single identified neurons have been documented as a good experimental model for the relatively large size, easy manipulation, consistent position on the surface of the ganglia, and consistent type of synaptic connections. Nikolic et al. (2008)reported that the magnetic field of 2.7 mT intensity caused changes in the amplitude and duration of the action potential of the Br neuron in in subesophageal ganglia of the garden snail *Helix pomatia*, whereas the 10 mT magnetic field changed the resting potential, amplitude spike, firing frequency, and duration of the action potential. Moreover, a significant increase of the activity of Na^+/K^+-ATPase and the expression of its α-subunit in the nervous system was observed in *Helix pomatia* exposed to 10 mT SMF (Nikolic et al. 2012, 2013). With single, 30-min long, and whole body exposure to 147 mT, Hernadi and Laszlo (2014) reported that SMF-exposure mediated the peripheral thermal nociceptive threshold by affecting the serotonerg as well as the opioiderg system.

5.4.4 SMF on Aquatic Animals

Sea urchins are the only invertebrates having development patterns similar to those of mammals. Moreover, the gametes of sea urchins can be obtained easily, the eggs and early embryos are transparent, and the early development of embryos is highly synchronous. SMF of 30 mT delayed the onset of mitosis in two species of sea urchins, *Lytechinus pictus* and *Strongylocentrotus purpuratus*. There was an eightfold increase in the incidence of exogasturlation in *L. pictus* embryos exposed to SMF, while magnetic fields had no effects on *S. purpuratus* embryos (Levin and

Ernst 1997). Exposure of fertilized eggs of *Echinometra mathaei* to 30, 40, and 50 mT magnetic fields delayed the onset of early cleavage division and significantly decreased the cleaved cells for exposed embryos. As the intensity of the magnetic field increased, earlier appearances of abnormalities were observed (Sakhnini and Dairi 2004).

The interaction among neurons in the escape circuit of crayfish has been well studied. Since the lateral giant (LG) neuron is easy to access for electrophysilogical study, Ye et al. (2004) found that exposure to SMF at 4.74–43.45 mT increased the amplitude of action potential (AP) in LG depending upon both the intensity of field and duration of field exposure, which was mediated by the increasing level of intracellular Ca^{2+} in the LG. The excitatory post synaptic potential (EPSP) produced via electrical and chemical synapses in the lateral giant neuron were enhanced after 30 min of SMF exposure (8.08 mT). Perfusion of field-exposed crayfish bath solution or preloading of Ca^{2+} chelator and intracellular Ca^{2+} release blocker failed to observe the SMF-induced enhancement on EPSP (Yeh et al. 2008).

As an increasingly important model species in genetic and neurobehavioral studies, zebrafish (*Danio rerio*) are an excellent organism for better understanding the biological mechanism of SMF. Using a fast, fully automated assay system relying on negative reinforcement, Shcherbakov et al. (2005) recorded statistically highly significant reactions to weak magnetic field changes in Mozambique tilapia, a fish migrating regularly between freshwater and the sea, and non-migratory zebrafish. Takebe et al. (2012) found that zebrafish responded to a magnetic field as weak as the geomagnetic field by bidirectional orientation with group-specific preferences regardless of close kinships. SMF with density from 4.7 to 11.7 T profoundly disturb the orientation and locomotion behaviors of adult zebrafish, and the independence of these effects from other sensory modalities suggests that they are mediated by the vestibular system (Ward et al. 2014) (Fig. 5.5). In addition, the static magnetic fields could be disrupting metabolism and immunity of the Caspian kutum fry during acute and subacute exposures (Loghmannia et al. 2015).

5.4.5 SMF on Xenopus laevis

Xenopus embryos are thought to be a useful tool for studying vertebrate development and gene expression for their embryogenesis is rapid and completed outside of the female. The hatching rate of embryos of the frog *Rana pipiens* subjected to the field of a 1 T permanent magnet was reduced (Neurath 1968). Ueno et al. (1984) investigated embryos of African clawed toads exposed to 1 T magnetic fields and found that the magnetic field exerted no harmful or modifying effects on gastrulation and neurulation; however, exposed embryos occasionally resulted tadpoles with reduced pigmentation, axial anomalies, or microcephaly. Compared to the first and the second cleavage, the third cleavage was the most susceptible to reorientation in a strong, static magnetic field. Exposure to SMF at 16.7 T altered the direction of

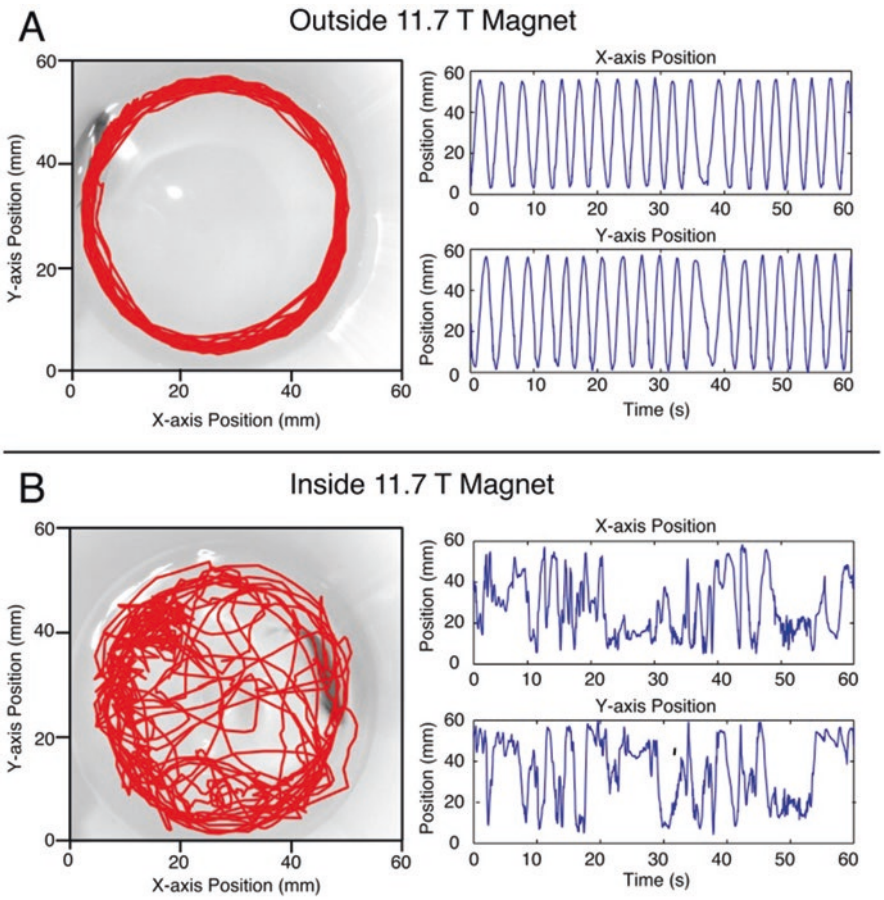

Fig. 5.5 Adult zebrafish behavior outside and inside of an 11.7 T vertical magnetic field. Tracing of adult zebrafish path in visible green light during 1 minute prior to magnetic field entry (**a**) and during 1 minute inside the magnet (**b**). X- and y-position coordinates are displayed as a function of time. Upon entry into the magnet, fish swimming becomes erratic, with frequent rolling, tight circling and increased swimming velocity (Reprinted from Ward et al. (2014), open access)

the third cleavage furrow from its normal horizontal type to the perpendicular type, which was confirmed by embryos exposed to 8 T (Denegre et al. 1998; Eguchi et al. 2006). These results indicated that SMF act directly on the microtubules of the mitotic apparatus to cause distortion of the third cleavage furrow (Fig. 5.6). Kawakami et al. (2006) found that SMF of 11–15 T significantly retarded normal development and induced microcephaly, two heads, abnormal cement glands and multiple malformations. Moreover, the gene expression of *Xotx2* (an important regulator of fore and midbrain morphogenesis) and *Xag1* (essential for cement gland

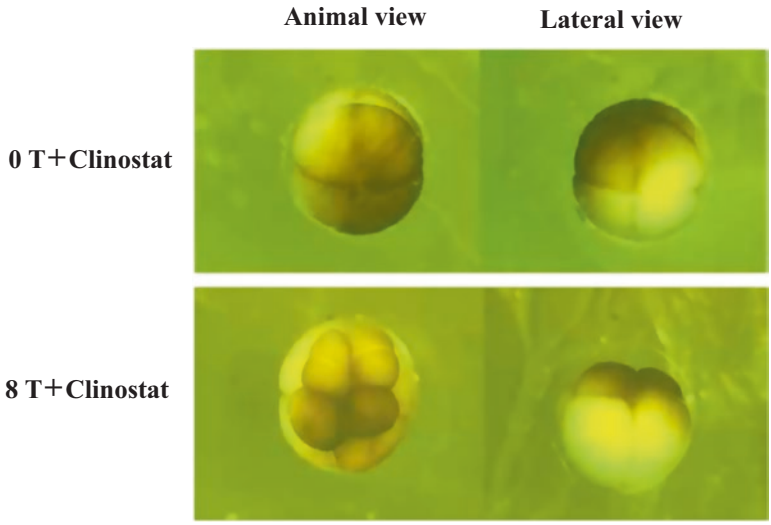

Fig. 5.6 An embryo rotated continuously using clinostat with and without the exposure to magnetic field of 8 T. *Upper row* shows the embryo rotated using clinostat under 0 T. *Lower row* shows the embryo rotated under 8 T. Note that the third cleavage furrows are all horizontal in the four blastomeres and rotated embryos without magnetic field tended to have larger animal blastomeres and rotated embryos with magnetic field tended to have smaller blastomeres compared to the control embryos (Reprinted from Eguchi et al. (2006), Copyright 2016, with permission from John Wiley and Sons)

formation) was greatly suppressed by strong SMF. Mietchen et al. (2005) investigated the morphology of fertilizable *Xenopus laevis* eggs with and without a jelly coat that were subjected to static magnetic fields of up to 9.4 T and found that no effect was observed when the jelly layers of the eggs were left intact, indicating the action of magnetic fields might involve cortical pigments or associated cytoskeletal structures normally held in place by the jelly layers.

The influence of SMF on nerve system have investigated in frog sciatic nerves. Edelman et al. (1979) placed the magnetic field around the axis of the nerve fibers and found that the amplitude of CAP in frog sciatic nerves was significantly increased by SMF of 385 or 600 mT. Although 8 T SMF had no effect on NCV of CAP, Eguchi et al. (2003) found that SMF altered the membrane excitation during the recovery process, which was mediated by the activation of ion channels. Satow et al. (1990, 2001) found that spatially homogeneous SMF of 0.65 T enhanced the excitability of bullfrog sartorius muscle during the recovery process. Okano et al. (2012) found that the nerve conduction velocity of C fibers were significantly reduced by 0.7 T SMF, instead of 0.21 T SMF.

5.4.6 SMF on Mice and Rats

5.4.6.1 SMF on Bone Growth, Healing and Loss

SMF has been utilized as a physical therapy option for bone health maintenance and treatment of bone disorders, since it can enhance bone fracture healing and formation by osteoblasts both *in vivo* and *in vitro* (Miyakoshi 2005; Saunders 2005; Trock 2000). With the implantation of magnetized rods into the middle diaphysis of rat femurs, Yan et al. (1998) found that the bone mineral density (BMD) and calcium content was significantly increased by SMF in the femurs adjacent to magnetized specimens as compared to unmagnetized specimen. With an ischemic rat femur model, Xu et al. (2001) found that bone weights were significantly increased in magnetized group as compared to unmagnetized one, which might be related to the improved blood circulation of the femur. Furthermore, SMF of 180 mT increased BMD of osteoporotic lumbar vertebrae in the ovariectomized rats (Xu et al. 2011). Kotani et al. (2002) showed that 8 T SMF stimulated ectopic bone formation, which was orientated parallel to the magnetic field, in mice implanted by bone morphogenetic protein (BMP) 2-containing pellets. Taniguchi et al. (2004) found that pain relief by SMF in mice rats with adjuvant arthritis adjuvant was attributed to the increased blood circulation, locomotor activity and BMD. In an ovariectomized (OVX) rat model, Taniguchi and Kanai (2007) that locomotor activity and BMD was increased by SMF. Yun et al. (2016) reported that SMF significantly enhanced the new bone formation in mouse calvarium implanted with magnetic scaffolds.

5.4.6.2 SMF on Cardiovascular System

Blood Pressure and Blood Flow

SMF in the mT range has been reported to modulate circulatory hemodynamics and/or arterial blood pressure (BP) and baroreflex sensitivity (BRS) (Okano and Ohkubo 2003, 2006; Morris and Skalak 2005). Okano et al. (2005) found that whole body exposure to SMF at 10 mT and 25 mT suppressed and delayed BP elevation in young, stoke resistant, spontaneously hypertensive rats (SHR), which was mediated by nitric oxide (NO) pathway and hormonal regulatory systems. SMF up to 180 mT enhanced nicardipine (NIC, an L-type voltage-gated Ca^{2+} channel blocker)-induced hypertension remarkably via more efficiently antagonizing the Ca^{2+} flux through the Ca^{2+} channels, which partially relate to the increase of NO metabolites. Continuous neck exposure to 12 mT SMF for at least 2 weeks either depressed or suppressed sympathetic agonists-induced hypertension, hemodynamics, and behavioral changes by modulating sympathetic nerve activity in Wistar rats (Okano and Ohkubo 2007).

It is well known that surface temperature and cutaneous blood flow closely parallel each other. Ichioka et al. (2003, 2000) reported that the whole body exposure of anesthetized rats to 8 T SMF was associated with reduced skin blood flow and temperature, which recovered after removal of the animal from the magnet. Both increases and decreases in skin and rectal temperatures were observed in mice exposed to SMF with intensities ranging from 0.4 to 8 T. In contrast to these observations, no evidence of a change in body temperature was found in rodents exposed to strong homogeneous or gradient magnetic fields (Tenforde 1986).

Cardiac Function

Blood flow in an applied magnetic field gives rise to induced voltages in the aorta and other major arteries of the central circulatory system, which can be observed as superimposed electrical signals in the electrocardiogram (ECG). The largest magnetically induced voltage occurs during pulsatile blood flow into the aorta, and results in an increased signal at the location of the T-wave in the ECG. Beischer and Knepton (1964) and Togawa et al. (1967) observed a marked increased T wave in the ECG records during exposure of squirrel monkeys to stationary fields of 2–7 T and rabbits exposed to 1 T SMF. A similar observation by Gaffey and Tenforde (1981) reported that a field strength dependent increase in the amplitude of the T-wave signal in the rat ECG was revealed during exposure to homogeneous stationary magnetic fields of 2 T, which might be due to a superimposed electrical potential generated by aortic blood flow in the presence of a stationary magnetic field. Morris and Skalak (2005, 2007)quantified the effect of localized SMF exposure on the diameter of microvessels in adult rat skeletal muscle *in vivo* and found that uniform 70 mT SMF altered arteriolar blood vessel diameter, while chronic SMF exposure altered the adaptive microvascular remodeling response to mechanical injury. The exposure of rats to SMF of 128 mT decreased the activities of glutathione peroxidase (GPx) and the superoxide dismutase (CuZn-SOD) in rat cardiac muscle (Amara et al. 2009).

Hematological Parameters

SMF is known to be lipolytic and glycogenolytic in rats. Sub-acute exposure to SMF of 128 mT stimulated the biosynthesis of plasma corticosterone and metallothionein activities in female rats (Chater et al. 2004). Moreover, 128 mT SMF increased blood glucose and decreased insulin release, leading to a diabetic-like state in pregnant rats (Chater et al. 2006a, b). Elferchichi et al. (2010) showed that an impaired glucose homeostasis and a deregulated lipid metabolism after SMF exposure in adult rats. Recent evidence suggested that supplementation with vitamin D corrected and restored glycemia and insulinemia in SMF-exposed rats (Lahbib et al. 2015a, b). Amara et al. (2006a, b) reported that SMF of 128 mT significantly decreased growth rates, but increased the plasmatic total protein levels,

hemoglobin, red blood cells, white blood cells, platelet number, and the activities of lactate dehydrogenase (LDH), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in male Wistar rats; in contrast, the glucose concentration was unaffected. A further study showed that selenium (Se) improved adverse oxidative stress in blood induced by SMF, whereas zinc supplementation could prevent toxic effects of SMF probably by its anti-oxidant proprieties (Ghodbane et al. 2011a, b). Atef et al. (1995) investigated changes of hemoglobin (Hb) characteristics in Swiss mice using hundreds of mT for 10 min and found that the rate of the Hb oxidative reaction declined at 350–400 mT. Elferchichi et al. (2016) noticed that SMF of 128 mT induced a pseudoanemia status with increased monocarboxylate transporters (MCT4) and glucose transporter 4 (Glut4). However, Djordjevich et al. (2012) found that differently oriented SMF of 16 mT did not alter hemoglobin and hematocrit, although the upward and downward fields caused statistically significant higher levels of serum transferrin. Milovanovich et al. (2016) showed that both upward- and downward-oriented SMF of 128 mT caused a reduction in the amount of total white blood cells (WBC).

5.4.6.3 SMF on Digestive System

An SMF of 128 mT increased total GSH levels and the activity of superoxide dismutase (SOD) and catalase (CAT), and hepatocyte apoptosis in rat liver through a caspase-independent pathway involving mitochondrial apoptosis-inducing factor (AIF), which was restored by selenium and vitamin E supplementations (Ghodbane et al. 2011a, b, 2015). Amara et al. (2007, 2009) reported that exposure of rats to SMF of 128 mT increased the 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodGuo) concentration in the kidney, while this biomarker of DNA oxidation remained unaffected in the liver and brain, which was in consistent with the observation of Chater et al. (2006a, b).

5.4.6.4 SMF on Endocrine System

Static magnetic field (SMF) therapy, an inexpensive and accessible noninvasive method, has proven to be effective on various tissue repairs, such as fresh and nonunion fracture, skin wounds, ulcer, and nerve injury. Jing et al. (2010) found that 180 mT SMF exposure could significantly accelerate the diabetic wound (DW) closure process and enhance the wound tensile strength (TS); however, 180 mT local SMF exposure had no effect on insulin secretion or pancreatic cells of diabetic rats (Rosmalen et al. 2002). László et al. (2011) provided further evidence that daily SMF exposure repeated for several weeks was protective against the development of high blood glucose levels in diabetic mice. Lahbib et al. (2010, 2015a, b) showed that exposure to SMF of 128 mT induced an increase in plasma glucose level and a decrease in the plasma insulin concentration in rats, which could be corrected by vitamin D supplementation. Moreover, β cell insulin content, the expression of

glucose transporter GLUT2 and islet area were lower in SMF-exposed group compared to the control. Elferchichi et al. (2011) showed that the metabolic alterations following exposure to a SMF of moderate intensity could trigger the development of a pre-diabetic state. In addition, Abdelmelek et al. (2006) reported that SMF of 128 mT induced an increase in norepinephrine content in rat gastrocnemius muscle.

5.4.6.5 SMF on Lymphatic System

Bellossi (1986) showed that the lifetime was prolonged significantly by uniform SMF of 600 or 800 mT in female AKR mice, which develop spontaneous lymphoblastic leukaemia. Yang et al. (2009) observed that SMF of 200–400 mT prolonged the average lifetime of mice bearing L1210 leukemia cells and increased the spleen and thymus index in normal mice. Milovanovich et al. (2016) reported that SMF of 128 mT caused a reduction in the amount of lymphocytes in serum and a decrease of granulocytes in the spleen, kidney inflammation, a specific redistribution of proinflammatory cells in blood and various organs. De Luka et al. (2016) showed that SMF of 1 mT reduced the content of zinc in mouse spleen, while the copper amount remained unchanged.

5.4.6.6 SMF on Nervous System

The nervous system, including the brain, spiral cord, and neurons, is important target of magnetic field. SMF exposure had a strong modulatory effect on cell hydration in different tissues of rats including brain tissue. Deghoyan et al. (2014)showed that the initial state of tissue hydration could play a crucial role in animal age-dependent magnetic sensitivity, which could be an age-dependent dysfunction of Na^+/K^+ pump. Kristofiková et al. (2005) showed functional teratogenic risks of the alterations in the orientation of 140 mT SMF for postnatal brain development and functional specialization of both hippocampi in rats. Whole-body SMF exposure and local SMF exposure on the spine resulted in practically identical ear thicknesses and significant effects of the SMF may involve a lower spinal response to exposure (Gyires et al. 2008). Kiss et al. (2015) showed that local SMF exposure on the spine affected ear thickness, indicating that the place of local SMF action may be in the lower spinal region. Veliks et al. (2004) investigated the influence of 100 mT SMF on autonomic nervous system in rat brain by evaluating heart rate and rhythmicity and found that the effectiveness of SMF in large measure depended on both functional peculiarities and functional activities of brain autonomic centers. The activation of c-Jun N-terminal kinase (JNK) and extra cellular-regulated kinase (ERK) were significantly increased in primary cortical neuron exposed to magnetic field up to 5 T (Prina-Mello et al. 2006). The content of calcium and iron was raised sharply in rats spinal cord by exposure to 128 mT SMF, whereas magnesium and copper levels remained unchanged (Miryam et al. 2010). Sub-acute exposure to SMF altered the antioxidant response by decreasing the level of total selenium in rat brain

(Ghodbane et al. 2011a, b). Exposure to SMF of 1 mT increased the amount of zinc in mouse brain, while copper levels were decreased (De Luka et al. 2016).

Behavioral effects are essential response of nervous system function. Exposure to 128 mT SMF not only altered emotional behaviour of rats in the plus maze and long-term spatial memory, but also led to cognitive impairments or at least to substantial attention disorders in the Morris water maze (Ammari et al. 2008; Maaroufi et al. 2013). This showed that SMF exposure had no massive effect but affected long-term spatial memory. Weiss et al. (1992) confirmed that acute behavioral and neural effects on rats became apparent at 4 T in a simple T-maze study. A 30 min exposure of rats to a 9.4 T superconducting magnet induced tight circling locomotor activity, conditioned taste aversion (CTA), and the express of c-Fos in specific vestibular and visceral nuclei within the brainstem (Nolte et al. 1998; Snyder et al. 2000). Houpt et al. (2007, 2011, 2012) extended the studies on the relationship betwen rat behavior and an SMF of 7 or 14 T and found that depressed drinking, more circling and less rearing actions were observed in SMF exposed group, while CTA was acquired a short time later. The direction of circling was dependent on the orientation of the SMF to the rats (Fig. 5.7). The behavioral response of magnetic field exposure was abolished by chemical labyrinthectomy, suggesting that the vestibular apparatus of the intact inner ear is the locus of magnetic field interaction (Houpt et al. 2007; Cason et al. 2009). The generality of SMF induced behavioral effects was further demonstrated in mice exposed to SMFs with similar magnetic flux density (Tsuji et al. 1996; Lockwood et al. 2003). With rat model for Huntington disease, the static magnetic field north and south promoted a distinct behavioral profile and morphological preservation after 7 days of lesion with quinolinic acid associated with apomorphine (APO) (Giorgetto et al. 2015).

Magnetic therapy as a non-contact, non-invasive, and cheap physiotherapeutic method has been used for analgesic modulation. Gyires et al. (2008) reported that acute exposure of mice to 2–754 mT SMF resulted in an opioid-mediated analgesic action in the writhing test in the mouse. Exposure of mice to both inhomogeneous (3–477 mT) and homogeneous (145 mT) SMF generated an analgesic effect toward visceral pain elicited by chemically induced pain (Kiss et al. 2013). Antal and László (2009) found that inhomogeneous subchronic SMF could prohibit the increased sensitivity of mice to mechanical stimuli in neuralgia, which was in consistent with the pain suppression by SMF of clinical magnetic resonance order. However, Sekino et al. (2006) reported that SMF of 8 T upregulated the action potentials of nerve C fiber, which enhanced pain perception in rats as the C fiber functioned as a pain transmitter.

5.4.6.7 SMF on Reproduction and Development

The adverse effects of SMF on aspects of spermatogenesis, organogenesis, or even ontogenesis in humans have cause great concern in recent years. Narra et al. (1996) reported slight changes in spermatogenesis and embryogenesis in mice exposed to SMF of 1.5 T. Amara et al. (2006a, b) examined the exposure of rats to SMF for 128

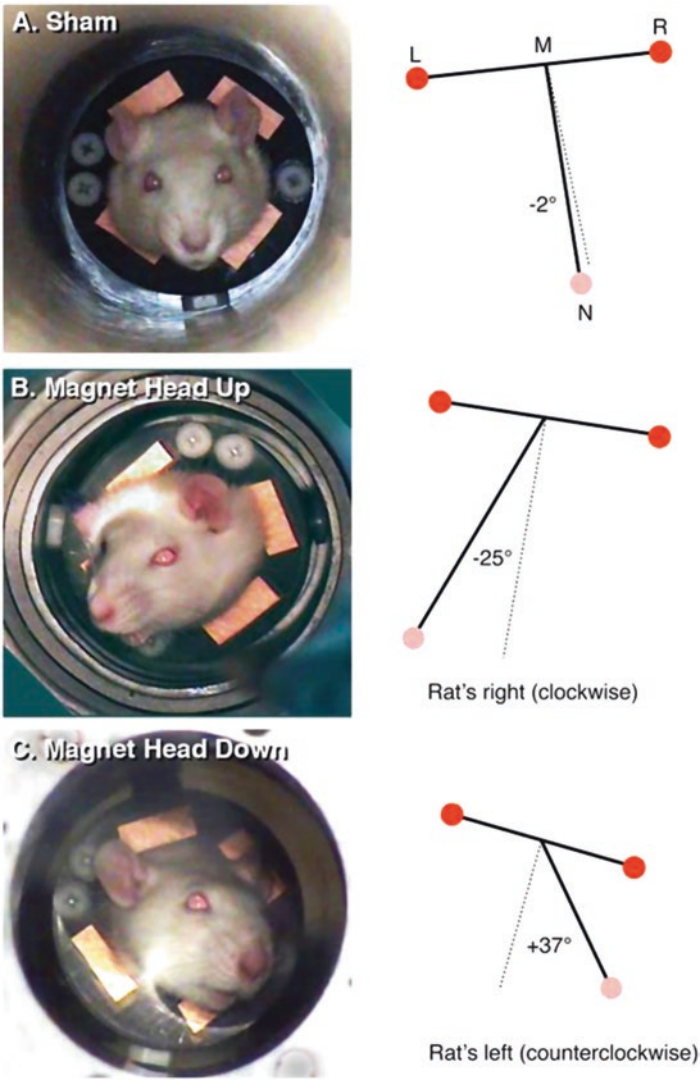

Fig. 5.7 Examples of rats during (**a**) sham exposure, (**b**) 14.1 T magnetic field with head up, and (**c**) 14.1 T magnetic field with head down. Panels on the *left* are frames from the video recording. Panels on the *right* demonstrate the quantification of head tilt calculated as the angle from the nose (N) to the midpoint (M) between the position of the left eye (L) and right eye (R). A deviation from the perpendicular towards the rat's right was assigned a negative angle (A), while a deviation towards the rat's left was assigned a positive angle (C) (Reprinted from Houpt et al. (2012), Copyright 2016, with permission from Elsevier)

mT for 30 days and found that there was no influences on spermatogenesis in rat testis, though the testosterone concentration reduced and oxidative stress increased significantly. High et al. (2000) reported that there were no adverse biologic effects in male and female adult rats or their progeny that could be attributed to a 10-week exposure to a 9.4 T SMF. Hoyer et al. (2012) chose offspring as subjectsobtained from pregnant dams exposed to 7 T SMF in utero and found that there were no any obvious effects on diverse behaviors like locomotion, exploration, or spatial learning. However, Tablado et al. (2000) reported that maturation of sperm production and motility, sperm morphology and mophometry, and postnatal testicular and epididymus development in mice was largely unaffected by either single, short-term exposure or continuous, long-term exposure at 500–700 mT. Ramadan et al. (2002) found that exposure of mice to magnetic field of a 20 mT caused a decrease in sperm count, motility and daily sperm production with marked testicular histopathological changes.

The development of an embryo is a highly sensitive process. An early study from Konermann and Monig (1986) showed no developmental effect from exposure to SMF of 1 T. Okazaki et al. (2001) reported that 4.7 T SMF had no significant effects on pregnant outbred mice and fetal development, which was confirmed by the finding in pregnant CD-1 mice exposed to 6.3 T SMF (Murakami et al. 1992). Fetal development and the delivery were normal in pregnant mice that were exposed to inhomogeneous SMF with 2.8–476.7 mT, but not treated with lipopolysaccharide (LPS) (László and Pórszász 2011). However, Mevissen et al. (1994) reported a significant decrease in the number of live fetuses per litter in rats exposed for the entire period of gestation to a 30 mT static field, indicating that such exposure might be embryotoxic. Saito et al. (2006) suggested that SMF had an obvious teratogenic influence on fetal development, according to data of various fetal malformations, even under 400 mT exposure for only 60 min a day during pregnancy. Recent evidence has shown that in utero-exposed male mice revealed no effect of magnetic field strength on weight of testes and epididymis or on sperm count, sperm morphology, or fertility; in pregnant mice that were daily exposed to SMF of 1.5 T and 7 T during fetal development in utero, no adverse effect was noted on duration of pregnancy, litter size, number of live births, or birth weight, and did not lead to teratogenic effects. However, a reduced placental weight of offspring of intrauterine exposed female mice was observed by a decrease in embryonic weight and developmental retardation could be observed postnatally with regard to weight gain and eye opening in mice exposed to static magnetic fields of up to 7 T (Zahedi et al. 2014; Zaun et al. 2014).

5.4.7 Magnetic Sensing Protein in Animals

Many animals have evolved to sense the direction of the geomagnetic field for orientation, navigation and migration over long distances. The blue light receptor cryptochromes (CRYs) that could form radical pairs after exposure to blue light was

suggested to be a magnetoreceptor based on the proposition that radical pairs were involved in the magnetoreception. CRYs are express not only in plant, but also in newts, fruit flies, birds and the eyes of mammals (Möller et al. 2004; Nießner et al. 2013). Gegear et al. (2008) reported that *cry* mutants of *Drosophila melanogaster* showed neither naive nor a magnetic field training response, while the wild-type flies showed significant naive and trained responses to the magnetic field. Expression of monarch butterfly (*Danaus plexippus*) cryptochrome gene in *Drosophila cry* mutants rescued the responses to the magnetic field (Gegear et al. 2010). Marley et al. (2014) reported that MF exposure coupled with CRY photoactivation during embryogenesis is sufficient to produce heightened seizure susceptibility in resultant *Drosophila* third instar (L3) larvae. Giachello et al. (2016) provided new evidence that exposure to MF of 100 mT was sufficient to potentiate the ability of light-activated CRY to increase neuronal action potential firing, indicating that the activity of CRY is sensitive to an external MF that is capable of modifying animal behavior. CRYs also function as circadian photoreceptors in the *Drosophila* brain, mediating the light resetting of the 24 h clock. In vertebrates, the CRYs act as the main negative regulators for the circadian feedback loop, due to the difference in light sensing (Yoshii et al. 2009; Fedele et al. 2014). Non-drosophilid insects encode CRY1 and CRY2, but CRY1 retain their light-sensing properties, whereas the CRY2s act as vertebrate-like negative regulators. Recently, Qin et al. (2016) identified a magnetoreceptor protein MagR, which co-localized with Cry in the pigeon retina. MagR as a novel biocompass-like model may help to clarify the underlying mechanism of magnetoreception in animal in response to magnetic field.

5.5 Conclusion and Perspectives

SMFs are constant fields that do not change in intensity or direction over time. The impact of SMF on a biological system largely depend on the target tissue(s), magnet characteristics, magnet support device, dosing regimen, and exposure manner and time. Although the effects of SMFs on living organisms have been investigated for decades, many inconsistencies and seemingly contradictory observations exist in the literature, which may due to the lack of appropriate systematic approaches to isolate the bioeffects of the treatment relative to other factors including geomagnetic field, the use of different exposure systems, different biological model systems, and the lack of uniformity in culture conditions and examination methods. In recent years, the magnetic flux density of SMFs used for medical and academic research purposes steadily increased; however, data on living organisms with exposure to strong SMFs have not been sufficient to evaluate these potential ecosystem risks and explore the function of magnetoreception.

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Part III
Opportunities for Static Magnetic Field (SMF)-Based Therapies

Chapter 6
Potential Applications of Static Magnetic Fields (SMFs) in Cancer Treatment

Abstract This chapter lists current evidence (from molecular level, cellular level, animal level to patient level) and some potential mechanisms for the effects of static magnetic field (SMF) on cancer inhibition. The prospective applications of SMF alone or in combination with chemotherapy drugs, pulsed magnetic field (PMF) as well as radiotherapy in cancer treatment are also discussed.

Keywords Static magnetic field (SMF) • Cancer cell • Epidermal growth factor receptor (EGFR) • Cell division • Alternative treatment • Pulsed magnetic field (PMF) • Combined therapy

6.1 Introduction

As briefly mentioned in Chap. 2, although magnetic therapy using SMF has been used by some people as alternative treatment on multiple chronic diseases for years, the scientific foundation is still lacking. As I have mentioned in Chap. 4, many studies have investigated the biological effects of magnetic fields on human cells, with results that depended on multiple factors including magnetic field frequency, intensity, exposure time and dynamics. More importantly, the difference in cell types made a significant impact. In particular, a large number of reported studies showed that the growth of multiple cancer cell types could be inhibited by SMFs, while most non-cancer cells were not, except for some specific cell types, such as embryonic or neuronal cells (Raylman et al. 1996; Tofani et al. 2001; Aldinucci et al. 2003b; Rosen and Chastney 2009; Ahmadianpour et al. 2013). These indicate that SMFs could differentially affect cancer vs. normal cells, which reveals their anticancer potentials. It is well known that cancer cells are different from normal cells in various aspects. For example, multiple types of cancers proliferate in response to signalling from oncoproteins such as EGFR (epidermal growth factor receptor). We recently found that SMF can affect EGFR orientation to reduce its activity as well as its related pathways to inhibit some cancer cell proliferation (Zhang et al. 2015; Zhang et al. 2016). Another difference is that most cancer cells are at a more active dividing state compared to normal cells. We recently also found that moderate and

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strong SMFs can interfere with microtubules as well as cell division, which are also the target of the Food and Drug Administration (FDA)-approved tumor treating fields (TTF) electromagnetic therapy. All of these cancer-related SMF studies will be discussed in this Chapter. The potential application of SMFs in other diseases will be discussed in Chap. 7.

6.2 SMF Effects on Cancer Cells

6.2.1 SMFs Could Inhibit Some Cancer Cell Growth While Have a Minimal Effect on Non-cancer Cells

As introduced in previous chapters, the exact cellular effects of SMFs on cells are largely dependent on cell types and so far there is no consensus effect of SMF on various kinds of cells. For example, Sullivan et al. examined the effect of 35–120 mT SMFs on four different types of cells and found that the effects varied greatly between them (Sullivan et al. 2011). However, among different cell types, the cell growth inhibition effects of SMF on cancer cells are much more consistent compared to other cell types. Multiple studies have shown that SMFs could inhibit cancer cell growth while had a minimal effect on non-cancer cells (Table 6.1). Although in each individual study, the cell types examined were very limited, we can see a clear trend that SMFs tend to inhibit cancer cells but not non-cancer cells. For example, in 1996, Rayman et al. showed that cell growth of a few cancer cell lines could be inhibited by 7 T SMF (Raylman et al. 1996). Later, a few studies used both cancer and non-cancer cells and found that they respond to the SMFs differentially. For example, in 2001, Tofani et al. found that the two cancer cell lines WiDr colon cancer and MCF-7 breast cancer cells were inhibited by 3 mT SMF plus 50 Hz PMF while the non-transformed cells MRC-5 was not affected (Tofani et al. 2001). In addition, they also used nude mice bearing WiDr colon cancer cells and exposed them to 70 min/day, 5 days a week for 4 consecutive weeks and found that the tumor growth could be reduced to up to 50% by 3–4 mT SMFs combined with 1.0–2.5 mT PMF (Tofani et al. 2001). In 2003, Aldinucci et al. found that 4.75 T SMF did not affect human peripheral blood mononuclear cells (PBMC) but inhibited Jurkat leukemia cell proliferation (Aldinucci et al. 2003b). In 2006, Ghibelli et al. showed that 1 T SMF could increase the chemotherapy-induced apoptosis in human tumor U937 monocytes but not mononuclear white blood cells (Ghibelli et al. 2006). In 2011, Tatarov et al. tested the effect of 100 mT SMF on mice bearing metastatic mouse breast tumor EpH4-MEK-Bcl2 cells. They found that exposure of the mice to magnetic fields for 3 h or 6 h, but not 1 h, daily for as long as 4 weeks suppressed tumor growth (Tatarov et al. 2011). Their study not only indicated that the moderate intensity SMF could inhibit mouse breast cancer growth, but also showed that the inhibition was directly correlated to the SMF exposure time (Tatarov et al. 2011). In 2015, Zafari et al. investigated the effects of SMF (5, 10, 20 and 30 mT) for 24–96 h on the viability of the human cervical cancer HeLa cells and fibroblast

Table 6.1 Multiple cellular studies indicated that cancer cells are more sensitive to SMFs

	Cell line information	SMF intensity	SMF treatment time	Effects of SMF on cells	References
Human cancer	WiDr colon cancer	3-30 mT	20 min exposure+3 hour latency time	Increased apoptosis	Tofani et al. (2001)
	WiDr colon cancer and MCF-7 breast cancer	3 mT SMF+3 mT 50 Hz PMF	20 min exposure+3 hour latency time	Increased apoptosis	Tofani et al. (2001)
	nude mice bearing WiDr cells	0-5 mT (SMF+50 Hz PMF)	70 min/day for 4 weeks	Tumor growth inhibition	Tofani et al. (2001)
	P53 mutant Jurkat cells	6 mT	24-72 hours	Induces apoptosis and cell cycle change in time dependent manner	Ahmadianpour et al. (2013)
	Jurkat cells	4.75 T	1 h	Reduce proliferation	Aldinucci et al. (2003b)
	HTB 63 (melanoma), HTB 77 IP3 (ovarian carcinoma), and CCL 86 and Raji cells (lymphoma)	7 T	64 h	Reduce cell number	Raylman et al. (1996)
	HCT116 colon cancer, CNE-2Z nasopharyngeal cancer	1-9 T	3 days	Reduce proliferation	Zhang et al. (2016)
Human non-cancer	human peripheral blood mononuclear cells (PBMC)	4.75 T	1 h	No effect	Aldinucci et al. (2003b)
	MRC-5 embryonal lung fibroblast	3 mT SMF+3 mT 50 Hz PMF	20 min exposure+3 h latency time	No effect	Tofani et al. (2001)
Rodent cancer	GH3 (rat pituitary tumor cell line)	0.5 T	4 weeks	Reduced tumor cell growth but increased cell size.	Rosen and Chastney (2009)
Rodent non-cancer	CHO (Chinese hamster ovary) cells	1-9 T	3 days	No effect	Zhang et al. (2016)
	CHO (Chinese hamster ovary) cells	10 T	4 days	No effect	Nakahara et al. (2002)
	CHO (Chinese hamster ovary) cells	13 T	3–5 h	No effect	Zhao et al. (2010)

Current literature indicates that multiple cancer cells can be inhibited by SMFs while non-cancer cells were not much affected. In each cell type category, the studies are arranged in a magnetic field intensity ascending order. In most studies, only SMFs were studied, except for Tofani et al. (shown in *brown*), in which PMF (pulsed magnetic field) was combined with SMF

cells. They found that the increase of SMF intensity and incubation time increased cell death percent and proliferation rate in HeLa cell more obviously compare to fibroblast cells (Zafari et al. 2015). Table 6.1 summaries some of the reported studies of SMF for the cell growth effect on cancer and non-cancer cells, which show that SMFs seem to inhibit cancer cell growth while have a minimal effect on non-cancer cells.

As mentioned above, most studies so far have only tested one or very few cell types in a given study, which prevented people from getting a comprehensive view of the cellular effects of SMF on different kinds of cells. Recently, our group did a systematic investigation to examine 15 different cell lines side by side, including 12 human (7 cancer cell lines and 5 non-cancer cell lines) and 3 rodent cell lines. We chose 1 T moderate intensity SMF because it was close to the magnetic exposure of patients to MRI (magnetic resonance imaging) in many hospitals, as well as some magnetic therapy field strength. To get unbiased and reproducible results, we tried to minimize experimental variations by doing the same sets of experiments for at least three times by two different researchers. They performed the experiments independently and their results were pooled together for statistic data analysis. After careful analysis, we found that the cell number of most solid cancer cells we tested could be inhibited by a 1 T moderate intensity SMF when the cells were seeded at higher cell densities (Table 6.2). In contrast, the cell numbers of the five human non-cancer cell lines were not reduced. Therefore, it is interesting that we found SMF not only affect cell proliferation in a cell type-dependent manner, the cell density also played indespensible roles (Table 6.2) (Zhang et al. 2017).

6.2.2 *SMFs Change EGFR Orientation and Inhibit Its Kinase Activity to Reduce Cancer Cell Proliferation*

SMFs have been shown to inhibit some cancer cell proliferation while have minimum effects on non-cancer cells in multiple studies, but the mechanism was unclear. Many types of cancer cells proliferate in response to signalling from Receptor Tyrosine Kinases (RTKs), and the effect of magnetic fields on EGFR phosphorylation has been investigated in several studies (Jia et al. 2007; Sun et al. 2008; Sun et al. 2013). It was shown that PMFs (both 0.4 mT 50 Hz low frequency and 2 μT 1.8 GHz radiofrequency) increased EGFR phosphorylation. However, it was very interesting that this effect could be reversed by incoherent ("noise") magnetic fields of the same magnetic field intensities (Sun et al. 2008; Sun et al. 2013). These results not only demonstrate that EGFR is a molecular target for magnetic fields, but also show that the different types of magnetic fields have differential effects on EGFR activities. However, whether and how EGFR could be affected by SMFs were unknown. Recently, we tested the effect of SMFs on EGFR and found that moderate and strong intensity SMFs could actually inhibit EGFR activity both *in vitro* and in cells in a magnetic field intensity-dependent way (Zhang et al. 2016) (Fig. 6.1a). We further explored the underlying mechanism using scanning tunnelling microscopy (STM) (Fig. 6.1b) and molecular dynamics simulation (Fig. 6.1c). We found that SMF could

Table 6.2 Systematic analysis of 15 different cell lines revealed that both cell type and cell density influenced the 1 T SMF induced effects on cells

	Cell line names	species	Cell line information	Effects of 1 T SMF on cell number	
				High density	Low density
Human solid cancer	CNE-2Z	Human	Nasopharyngeal cancer	**Reduction**	Increase
	HCT116	Human	Colon cancer	**Reduction**	No effect
	A431	Human	Skin cancer	**Reduction**	No effect
	A549	Human	Lung cancer	**Reduction**	No effect
	MCF7	Human	Breast cancer	**Reduction**	Increase
	PC3	Human	Prostate cancer	**Reduction**	No effect
	EJ1	Human	Bladder cancer	No effect	Increase
Human non-cancer	HSAEC2-KT	Human	Normal lung	Increase	Increase
	HSAEC30-KT	Human	Normal lung	Increase	No effect
	HBEC30-KT	Human	Normal lung	Increase	Increase
	RPE1	Human	Retinal pigment epithelial	No effect	No effect
	293T	Human	Embryonic kidney	No effect	No effect
Rodent	CHO	Hamster	Chinese hamster ovary	No effect	No effect
	CHO-EGFR	Hamster	Chinese hamster ovary, transfected with EGFR-Flag	**Reduction**	Increase
	NIH-3T3	Mouse	Mouse embryo fibroblast	**Reduction**	No effect

Table 6.2 (continued)

7 human solid cancer cell lines, 5 human non-cancer cell lines as well as 3 rodent cell lines were included. Cells were plated one day ahead for attachment to the culture plate before they were exposed to 1 T SMF for another 2 days. 4–5 × 10^5 cells were plated in the "high densities" group so that the cells were confluent at the end of experiments. 0.5 × 10^5 cells were plated in the "low densities" group so that the cells were around half confluent at the end of experiments. Experiments were repeated for 3–4 times by two independent researchers (Results were from Zhang et al. 2017. Copyright © 2016 Impact Journals, LLC)

affect the orientation of EGFR kinase domain, which interfered with the normal interaction between EGFR monomers to inhibit their activation. In addition, although the CHO (Chinese hamster ovary) cell number was not affected by 0.05 T, 1 T or 9 T SMFs, EGFR transfected CHO cells became responsive to SMFs and were effectively inhibited by 1 T and 9 T SMFs (Fig. 6.1d). Therefore, although we are aware that EGFR is not the only target of SMFs in cells, it is at least one of the key factors that contribute to SMF-induced cancer cell inhibition. In the meantime, the molecular mechanisms underlying these different magnetic field types-induced differential EGFR activity changes need to be further explored.

It is promising that our results showed that the cancer vs. non-cancer cells from the same tissue responded to the SMF totally differently. The growth of A549 lung cancer cells was inhibited by 1 T SMF when they were seeded at high density (Table 6.2). In contrast, the growth of normal lung cells was promoted by 1 T SMF at high cell seeding density (Table 6.2). We analyzed their EGFR-mTOR-Akt pathway and found that the A549 lung cancer and HSAEC2-KT non-cancer lung cells have dramatically different EGFR-mTOR-Akt pathway expression and activation (Fig. 6.2) (Zhang et al. 2017). The EGFR expression and phosphorylation levels are much higher in A549 lung cancer cells than in HSAEC2-KT normal lung cells. The mTOR and AKT expression and phosphorylation levels are also significantly higher in A549 lung cancer cells. These results, combined with the EGFR studies mentioned above (Zhang et al. 2016), demonstrate that EGFR-mTOR-Akt pathway is likely to be one of the key factors that contribute to the cell type differences in SMF-induced cell proliferation changes. In addition, it should be mentioned that the cell density also affected the A549 lung cancer cells and normal lung cells HSAEC2-KT in different pattern (Fig. 6.2). For example, the EGFR and 4EBP1 expression and phosphorylation level were increased in higher cell density compared to lower cell density in A549 lung cancer cells but not in HSAEC2-KT normal lung cells. These results indicate that EGFR-mTOR-Akt pathway may be a key factor that contributes to both cell type- and cell density-dependent SMF effects.

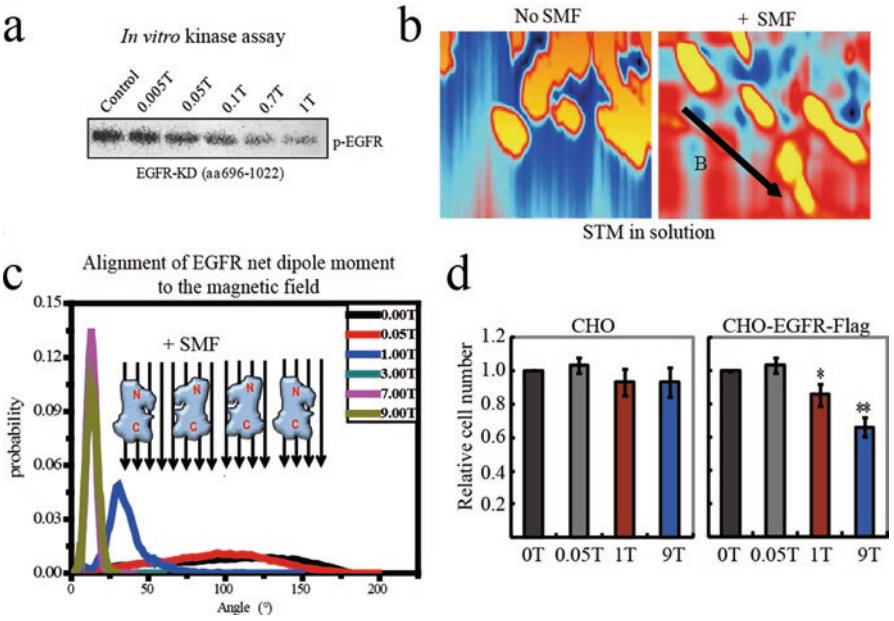

Fig. 6.1 SMFs inhibit EGFR activity by changing its orientation to inhibit cell proliferation. (**a**) *In vitro* kinase assays show that moderate intensity SMFs could inhibit EGFR kinase domain autophosphorylation. Western blot of phosphor-EGFR was shown. SMFs of 0.005–1 T were tested. Incubation time was 10 min. (**b**) Liquid-phase scanning tunneling microscopy (STM) shows that a 0.4 T SMF could change EGFR kinase domain orientation. (**c**) Computer-based calculation shows that the probability of the EGFR kinase domain net dipole moment aligns with SMF field direction in a magnetic field intensity-dependent manner. (**d**) The cell number of CHO cells was not affected by 0.05, 1, or 9 T SMF while the cell number of CHO cells overexpressing EGFR-Flag was significantly reduced by 1 T and 9 T SMFs. Incubation time was 3 days. $*p < 0.05$; $**p < 0.01$ (Figures were adapted from Zhang et al. 2016. Copyright © 2016 Impact Journals, LLC)

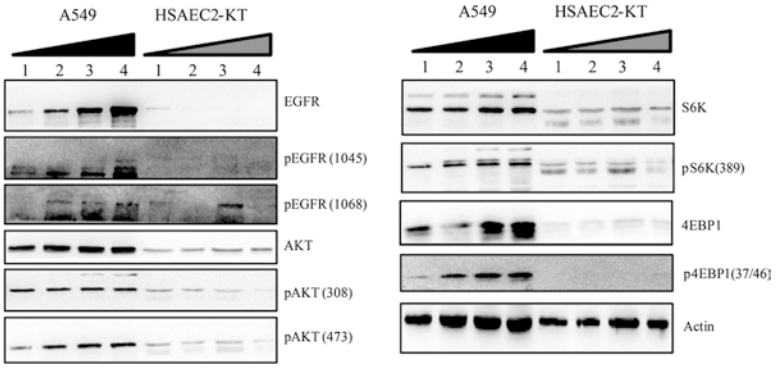

Fig. 6.2 Human lung cancer A549 and normal lung HSAEC2-KT cells have differential EGFR-Akt-mTOR pathway expression and phosphorylation. Human lung cancer A549 and normal lung cells HSAEC2-KT cells were plated at four different cell densities one day ahead before they were harvested for Western Blot (Reprinted with from Zhang et al. 2017. "*1*" indicates the lowest cell density. "*4*" indicates the highest cell density. Copyright © 2016 Impact Journals, LLC)

6.2.3 SMFs and Cell Division

Besides EGFR, there are other cellular components that play indespensible roles in SMF-induced cancer inhibition, such as cell division. Since cell division is a key step that leads to tumor growth, perturbations that disrupt or interfere with cell division could inhibit tumor growth. In fact, there are multiple chemodrugs that target cell division, such as Taxol. Moreover, cancer and non-cancer cells have been shown to respond differentially to cell cycle perturbations. For example, it has been reported that the human non-transformed cells and cancer cells have significant survival difference in response to the microtubule drugs treatment (Brito and Rieder 2009). Brito and Rieder found that both nocodazole and Taxol, two microtubule poisons, could kill much more HeLa and U2OS cancer cells than the non-cancer RPE1 cells. Specifically, 5 nM of Taxol, which is approximately the clinical concentration for chemotherapy, could kill 93% of HeLa cells and 46% of U2OS cells but only killed 1% of RPE1 cells (Brito and Rieder 2009). In addition, different types of cancer cells also have differential responses to microtubule drugs (Tang et al. 2013). Moreover, the depletion of plk1 (polo-like kinase), which is a vital regulator in multiple cellular processes, especially in cell cycle progression, caused significant cell proliferation and cell cycle abnormalities in human cervical cancer HeLa cells, but not the non-cancer RPE1 or MCF10A breast cells (Liu et al. 2006). Therefore, targeting microtubules or cell cycle could generate different effects on cancer vs. non-cancer cells or in different types of cancer cells.

The key structure that controls the whole cell division process is the mitotic spindle, which is mainly composed of microtubules. It is well known that microtubules can be affected by SMFs and recent evidences showed that cell division could also be affected by SMFs, which was discussed in Chap. 4. Although most results so far showed that SMFs did not change the overall cell cycle distribution of a given cell population, we found that prolonged exposure (7 days) to 1 T SMF could increase the abnormal spindle percentage and the mitotic index in HeLa cells, which was also discussed in Chap. 4. Moreover, we found that the duration of mitosis was increased by 1 T SMF (Fig. 6.3). Using cell synchronization experiment (Fig. 6.3a), we found that 1 T SMF could delay cells exiting from mitosis (Fig. 6.3). In the absence of 1 T SMF, most of the double thymidine synchronized cells exit from mitosis 12 h after thymidine release. However, there were a significantly increased number of HeLa cells staying in mitosis in the presence of 1 T SMF (Fig. 6.3c, d, e).

The mechanisms of the differential responses of cancer vs. non-cancer cells to SMFs still remain partially understood. However, SMF-induced microtubule interference is a broad impact on most dividing cells. Meanwhile, we should keep in mind that although EGFR and cell division are important, they are definitely not the only reasons that can explain the differences between SMF-induced differential effects among various cell types. Other factors are also likely involved. For example, Short et al. showed that 4.7 T SMF could alter the ability of human malignant melanoma cells attachment onto the tissue culture plate, but had no effect on normal human fibroblasts (Short et al. 1992), which indicated that the cell attachment was

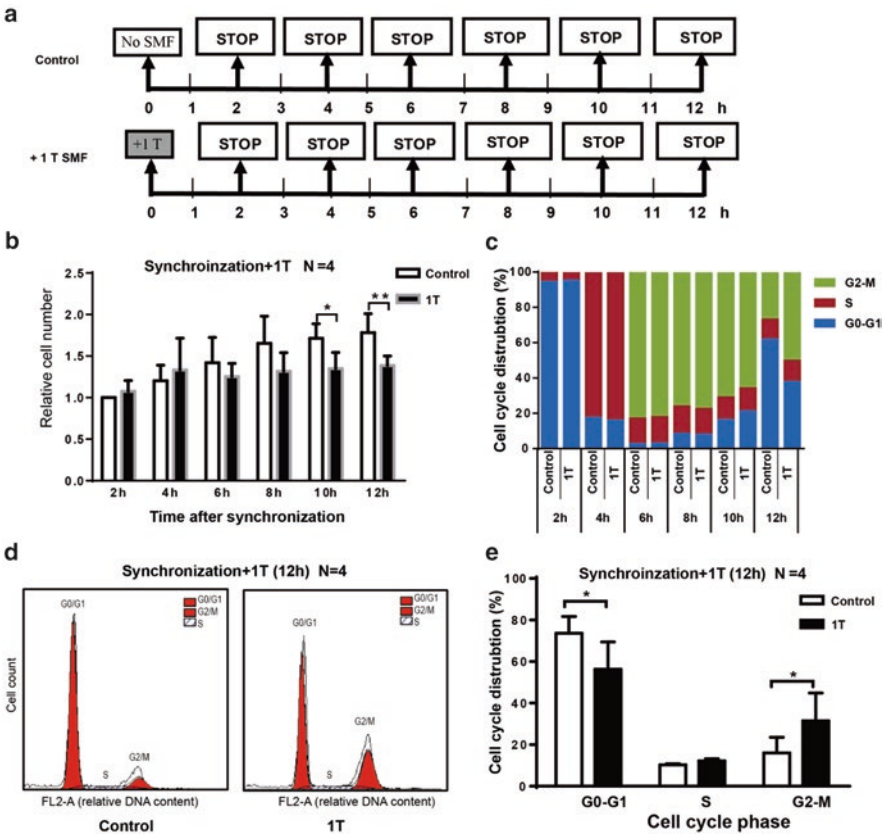

Fig. 6.3 1 T SMF delays mitotic exit and reduces HeLa cervical cancer cell number. **(a)** The schematic cartoon shows that double thymidine synchronized HeLa cells were released and treated with or without 1 T SMF for 2, 4, 6, 8, 10 and 12 h. "STOP" indicates the time point when cells were harvested for analysis. **(b)** Quantification of relative cell number at each time point. Cell numbers were normalized to the control (at 2 h point). **(c–e)** Flow cytometry analysis reveals that 1 T SMF delays the cells exit from mitosis. **(c)** The cell cycle distribution of HeLa cells as in (**a**). **(d)** Flow cytometry results show the increased G2/M phase after 12 h of exposure to SMFs. **(e)** Quantification of (**d**) from three independent experiments. The Student's t test was used for the comparisons between control and SMF-treated groups. Data represent the mean ± SD. $*p < 0.05$; $**p < 0.01$ (Figure was reprinted with permission from Luo et al. 2016. Copyright © 2015 Elsevier B.V)

differentially affected by SMF in cancer vs. non-cancer cells. Moreover, other aspects should also be carefully investigated, such as cell metabolism, mitochondria functions, ROS (reactive oxygen species) responses and ATP level, which could all be affected differentially in cancer vs. normal cells. Our group is currently working on these topics and we expect to have a much better understanding on this issue in the near future.

6.2.4 SMFs and Tumor Microcirculation

There are a few studies indicating that moderate intensity SMFs could inhibit angiogenesis and tumor microcirculation, which could inhibit cancer growth *in vivo*. For example, in 2008, Strieth et al. examined the effects of SMF (< 600 mT) on A-Mel-3 tumors growing in dorsal skinfold chamber preparations of Syrian Golden hamsters. They found that short-time exposure to SMF (about 150 mT) resulted in a significant reduction of red blood cell velocity (vRBC) and segmental blood flow in tumor microvessels (Strieth et al. 2008). At 587 mT, a reversible reduction of vRBC and a reduction of functional vessel density were observed. In addition, they found that prolongation of the exposure time from 1 min to up to 3 h had a more significant result. Moreover, SMFs not only reduced blood flow in tumor vessels but also activate and increase the adherence of platelets (Strieth et al. 2008). In 2009, Strelczyk et al. further evaluated the effects of prolonged exposure to SMFs on tumor angiogenesis and growth. They found that 586 mT SMF exposure for 3 h could inhibit both tumor angiogenesis and growth (Strelczyk et al. 2009). Detailed analysis revealed that the functional vessel density, vessel diameters and vRBC in tumors were all reduced by SMFs. In addition, they also observed increased edema after SMF exposure, which indicated that SMFs might increase tumor microvessel leakiness. In 2014, their group did some further analysis and found that the 587 mT SMF did increase the tumor microvessel permeability significantly in A-Mel-3-tumor-bearing hamsters (Gellrich et al. 2014) (Fig. 6.4). It was interesting but not surprising that the functional tumor microvessels, labeled by FITC-dextran, were much decreased after SMF exposure, especially after the repeated SMF exposure, which was likely due to the inhibited tumor angiogenesis. Nevertheless, it was obvious that both SMF single exposure and repeated exposure increased the blood vessel leakiness and the repeated SMF exposure had stronger effects. In addition, the increased microvessel permeability was likely the reason for the improved anti-tumor efficacy of SMFs in combination with paclitaxel (Fig. 6.5) (Gellrich et al. 2014).

An independent group also reported the effects of SMF on angiogenesis. In 2009, Wang et al. investigated the effects of the gradient SMF (0.2–0.4 T, 2.09 T/m, exposure time 1–11 days) on angiogenesis in the human umbilical veins endothelial cells (HUVECs) as well as two *in vivo* models, a chick chorioallantoic membrane (CAM) and a matrigel plug (Wang et al. 2009). Their results showed that the

HUVECs proliferation was significantly inhibited 24 h after exposure. In addition, the two *in vivo* models both showed decreased angiogenesis after 7 or 11 days of exposure (Wang et al. 2009). Although this study was not carried out in a tumor-related model, it showed the inhibition effect of moderate intensity SMFs on angiogenesis, which was consistent with the results reported by Strieth and co-workers (Strieth et al. 2008; Strelczyk et al. 2009). Taken together, these studies showed that moderate intensity SMFs of 0.1–0.6 T could reduce angiogenesis in some animal models, which implied their potential for tumor growth inhibition *in vivo*. Additional research is needed to ascertain this effect, such as the effects of other magnetic field intensities as well as more types of tumor models.

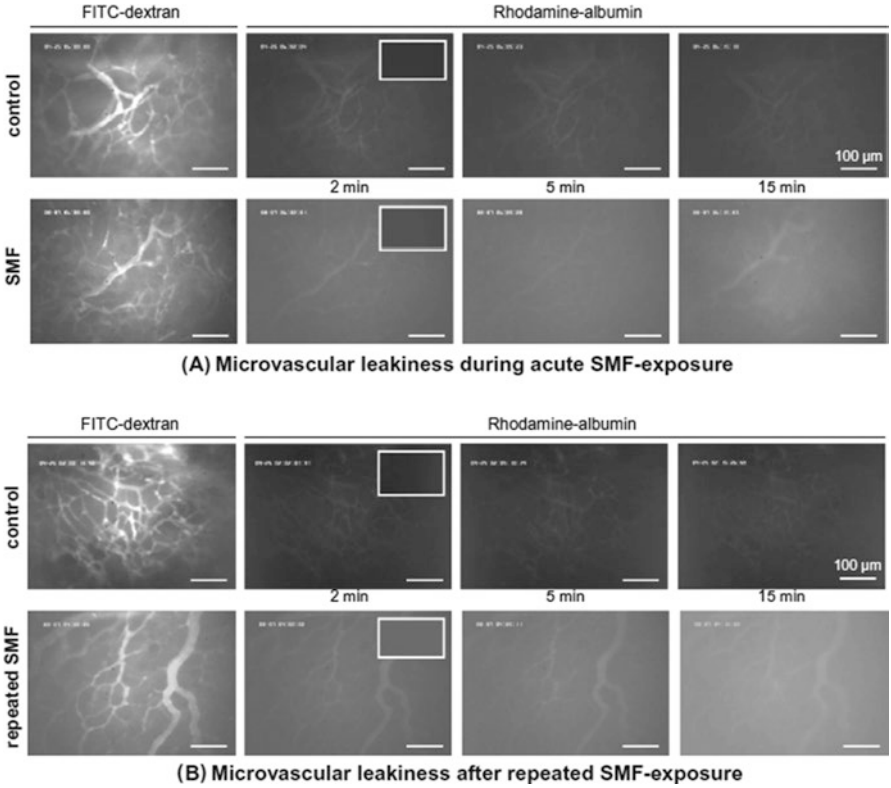

Fig. 6.4 A 587 mT SMF exposure induces intratumoral microvascular leakiness in A-Mel-3-tumor-bearing hamsters. On day 10 after tumor cell implantation representative ROIs (regions of interest) were chosen after FITC-dextran administration, highlighting functional tumor microvessels, before rhodamine-labeled albumin was given intravenously. In control groups, there was a continuous slight increase of fluorescent albumin in the extravascular compartment but the increase was stronger after SMF exposure. **(a)** *In vivo* fluorescence microscopy for analysis of microvascular leakiness during SMF-exposure. Animals were exposed to the sham control or the SMF of 587 mT during the whole *in vivo* assessment of microvascular permeability on day 10. **(b)** *In vivo* fluorescence microscopy of animals that have been repeatedly exposed to SMF of 587 mT for 3 h on day 5, 7, 9 after tumor implantation. The intratumoral microvascular leakiness was stronger in animals after repeated exposure to SMF even with regard to the obviously rather low functional vessel density (Reprinted with permission from Gellrich et al. 2014. Copyright © 2013 Elsevier Ireland Ltd)

6.3 SMFs in Combination with Other Treatments

6.3.1 SMFs in Combination with Chemodrugs

There are some researches implicated that moderate intensity SMFs could potentially work as an adjunctive treatment method for chemotherapy (Gray et al. 2000; Sabo et al. 2002; Ghibelli et al. 2006; Hao et al. 2011; Sun et al. 2012b; Ghodbane et al. 2013; Gellrich et al. 2014; Zhang et al. 2015, 2016; Luo et al. 2016). Multiple

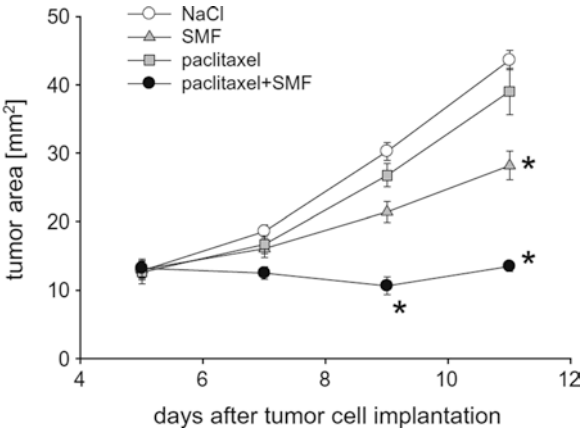

Fig. 6.5 Tumor growth inhibition by SMF in combination with chemodrug paclitaxel. In 2014, Gellrich et al. tested the effect of 587 mT SMF in the presence of absence of the chemodrug paclitaxel for their inhibition effects on A-Mel-3-tumor-bearing hamsters. After the third treatment, SMF alone could inhibit tumor growth. However, the combination therapy yielded a more pronounced tumor growth delay than any other group. *$p < 0.05$. The animals were randomly assigned to four groups (n = 6, each) 5 days after tumor inoculation. On day 5, 7 and 9 after tumor cell implantation, the "paclitaxel + SMF" group was treated by continuous intravenous infusion of paclitaxel over 90 min during exposure to 587 mT for 120 min. The "paclitaxel" group was treated with paclitaxel alone intravenously over 90 min without SMF-exposure. The "SMF" group was exposed to SMF of 587 mT over a period of 120 min without receiving paclitaxel. The "NaCl" group was injected with 0.9% NaCl intravenously over 90 min applying identical volumes as in the other experimental groups (Reprinted with permission from Gellrich et al. 2014. Copyright © 2013 Elsevier Ireland Ltd)

studies have investigated the combination effects of SMFs with chemodrugs and most of them achieved enhanced anti-tumor efficacy compared to SMF or chemodrugs alone. For example, in 2014, Gellrich et al. found that a 587 mT SMF could significantly increase the anti-tumor efficiency of paclitaxel chemotherapy in A-Mel-3-tumor-bearing hamsters (Fig. 6.5) because the 587 mT SMF inhibited tumor angiogenesis and increased tumor microvessel permeability significantly (Gellrich et al. 2014). Our group also found that 1 T moderate intensity SMF could increase the antitumor efficacy of mTOR inhibitors, EGFR inhibitors, Akt inhibitors, as well as Taxol and 5-Fu (Zhang et al. 2015, 2016, 2017; Luo et al. 2016). In addition, chemotherapy drug adriamycin had enhanced inhibition effect on the growth of leukemic cells K562 and transplanted mammary tumors in mice when it was combined with moderate intensity SMFs of 110 mT or 8.8 mT, respectively (Gray et al. 2000; Hao et al. 2011). For the leukemic cells K562, the combination of 8.8 mT SMF can also increase the working efficiency of the chemodrug paclitaxel (Sun et al. 2012b). For leukemic cell line HL-60, 1 T SMF also had combinational effects with a mixture of four chemodrugs (5-Fu, Cisplatin, doxorubicin and vincristine) (Sabo et al. 2002). In 2006, Ghibelli et al. showed that 1 T SMF increased apoptosis induced by anti-tumor drugs in human tumor U937 monocytes but not mononuclear white blood cells (Ghibelli et al. 2006). This indicated that the

combination of SMFs with chemodrugs might preferentially work on tumor cells but not normal cells although more studies are needed to confirm this point. It was proposed that the cell membrane permeability can be increased by SMFs to allow more drugs entering cells (Tofani et al. 2003; Liu et al. 2011; Gellrich et al. 2014). This is an appealing explanation because it can explain the combined effects of SMFs and chemodrugs. It is also explainable because SMFs were shown to have alignment effects on lipids (discussed in Chap. 3). However, it is puzzling that SMFs do not simply promote effects of all chemotherapy drugs. For example, Vergallo et al. showed that 31.7–232 mT SMFs did not promote the anticancer effect of Cisplatin in human neuroblastoma SH-SY5Y cells (Vergallo et al. 2014). In fact, our lab used four different human cancer cell lines, including cervical cancer HeLa, colon cancer HCT116, nasopharyngeal cancer CNE-2Z and breast cancer MCF7 cell lines and found that 1 T SMF can increase efficacy of 5-Fu and 5-Fu + Taxol but not Cisplatin in all four cell lines that we tested (Luo et al. 2016). This demonstrates that the combinational effects of SMFs with chemodrugs may be drug-specific and/or cell type-specific.

However, it should be mentioned that the current experimental results about combination of SMFs with Cisplatin are not completely consistent. Although we and Vergallo et al. found that SMFs did not increase the efficacy of Cisplatin and even had a tendency to antagonize the effects of Cisplatin, there are also some other evidence showing opposite results. For example, it was shown that SMFs could increase the antitumor effects of Cisplatin in mice bearing lewis lung carcinoma (Tofani et al. 2003) and leukemic cells K562 (Chen et al. 2010). We think this is probably due to the different magnetic intensities in independent studies (ranging from a few millitesla to a few hundred milliteslas) or cell type difference. Both of these factors could directly influence the magnetic effects as we have discussed earlier. More specifically, studies reported that SMFs of 1–10 mT could increase the antitumor efficacy of Cisplatin (Tofani et al. 2003; Chen et al. 2010) but in ours (Luo et al. 2016) and Vergallo et al.'s studies (Vergallo et al. 2014), we both used stronger magnetic fields (31.7–232 mT in Vergallo et al.'s study and 1 T in our study). Maybe lower magnetic field intensity could increase the Cisplatin efficacy while higher magnetic field intensity has the opposite functions. The exact effects and mechanisms of combining SMFs with Cisplatin in different cells need to be further investigated.

In fact, there are some studies indicated that both magnetic field intensity and cell type could influence the effect of SMF in combination with drugs. In 1999, Fanelli et al. found that SMFs with different intensities starting from 6 gauss could decrease the extent of cell death by apoptosis induced by several agents in different human cell systems via modulation of Ca^{2+} influx, and this effect was magnetic field intensity-dependent (Fanelli et al. 1999). This directly showed that the magnetic field intensity could influence the effect of SMFs with drugs. For cell type induced difference, in 2003, Aldinucci et al. tested a few different cell types for the effects of combining a 4.75 T SMF and a pulsed EMF of 0.7 mT generated by an NMR apparatus (NMRF) for 1 h. They found that in T cell leukemia Jurkat cells the calcium level was reduced significantly after exposure (Aldinucci et al. 2003b) but in normal or in PHA challenged lymphocytes the calcium level was increased

(Aldinucci et al. 2003a). Moreover, in 2006, Ghibelli et al. compared two different magnetic field intensities (1 T vs. 6 mT), four different cell lines (two cancer cell lines, human leukemic monocyte lymphoma U937 cells and T cell leukemia Jurkat cells as well as two types of normal cells, human monocytes and lymphocytes) (Ghibelli et al. 2006). It was not surprising that neither the 1 T nor the 6 mT SMF induced apoptosis in all four types of cells, which is consistent with what have discussed in Chap. 4. However, it is interesting that 1 T SMF increased puromycin (PMC)-induced apoptosis in U937 cells (Fig. 6.6), but not in other three cell types (Ghibelli et al. 2006). In addition, unlike 1 T SMF, the 6 mT SMF did not increase the PMC-induced apoptosis in any of the cells. In contrast, it reduced the PMC-induced apoptosis in U937 cells (Fig. 6.6) (Ghibelli et al. 2006). Moreover, Tenuzzo et al. used 6 mT SMF and apoptosis-inducing agents (cycloheximide, H2O2, puromycin, heat shock, etoposide) to compare their effects on human lymphocytes, mice thymocytes and cultures of 3DO, U937, HeLa, HepG2 and FRTL-5 cells. Their results showed that 6 mT SMF exposure interfered with apoptosis in a cell type- and exposure time-dependent manner (Tenuzzo et al. 2006). All above mentioned studies showed that both magnetic field intensity and cell type, and even exposure time, could influence the effect of SMF in combination with drugs.

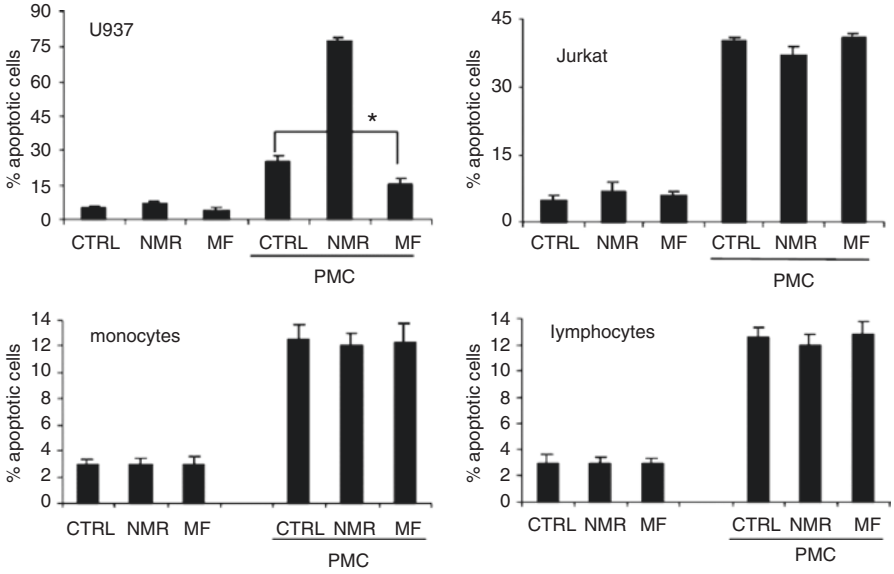

Fig. 6.6 The combinational effects of SMFs with chemicals are magnetic field intensity- and cell type-dependent. CTRL: control. NMR: 1 T SMF. MF: 6 mT SMF. PMC: puromycin 10 μg/ml. Four different human cells types (two cancer, U937 and Jurkat and two non-cancer, monocytes and lymphocytes) were exposed to SMFs and puromycin for 3–5 h before they were analyzed for apoptosis. 1 T SMF increased the efficacy of puromycin in U937 cells but not in other three cell types. 6 mT SMF inhibited the efficacy of puromycin in U937 cells but not in other three cell types (Reprinted with permission from Ghibelli et al. 2006. Copyright © Springer Science + Business Media, Inc. 2006)

In addition to the magnetic field intensity and cell type, the cell density and chemodrug concentration also affect the combinational effects of chemodrugs and SMFs. For example, we have reported that 1 T SMF could increase the efficacy of some chemodrugs (5-Fu, Taxol) in multiple human solid cancer cell lines, such as breast cancer MCF-7, colon cancer HCT116, nasophageal cancer CNE-2Z cells but only at some drug concentrations (Luo et al. 2016). In addition, we recently expanded our studies from solid tumor to leukemia cells. First of all, it was interesting that we found the cell growth of human leukemia K562 cells can be inhibited by a 0.5 T SMF at lower cell concentration but not at higher cell concentration (Fig. 6.7a), which was different from most of the solid cancer cell lines we tested (Table 6.2). Then we used the low cell concentration to test the combination effects of the 0.5 T SMF with Vincristine in K562 cells and found that SMF did increase the drug efficacy of Vincristine at 0.5, 1 as well as 2 nM but with slightly different effectiveness (Fig. 6.7b).

In conclusion, it is clear that although in most cases, SMFs could increase the efficacy of chemodrugs, there are also some studies showed that there were no synergetic or additive effects between SMFs and some chemodrugs (Table 6.3). These differential effects could be caused by cell type, field intensity as well as drug differences. Therefore the strategy of combining SMFs of different intensities with various chemodrugs in different cancer cells also needs to be further investigated. This is not only helpful to explore the potential application of combinational therapy of SMFs with chemodrugs, but also to alert people with specific chemotherapy, such as Cisplatin, for limited MRI or other types of SMF exposure in hospitals.

6.3.2 SMFs in Combination with Pulsed Magnetic Field (PMF)

There are multiple studies showing that SMFs combined with PMF could inhibit cancer cell growth (Tofani 2015) (Table 6.4). For example, Tofani et al. have made series progresses on the combination of SMF and 50 Hz PMF. In 2001, Tofani et al. showed that 3 mT SMF combined with 50 Hz PMF could induce more apoptosis in cells compared to SMF or the 50 Hz PMF alone (Tofani et al. 2001). In addition, it was interesting that apoptosis only occurred in the two transformed cell lines (WiDr human colon adenocarcinoma and MCF-7 human breast adenocarcinoma) but not the nontransformed cell line (MRC-5 embryonal lung fibroblast). They also tested them in nude mice xenografted with WiDr cells and exposed them for 70 min/day, 5 days/week, to ≤5 mT SMF in combination with PMF for 4 weeks and found that the tumor was significantly inhibited (up to 50%) (Tofani et al. 2001). In 2002, they further tested the effects of 5.5 mT SMF in combination with 50 Hz PMF and found that the survival time of nude mice with WiDr cells was increased by 31% when the mice were exposed to magnetic fields for 70 min/day for 4 weeks (Tofani et al. 2002). When the mice were exposed to the magnetic fields for 4 consecutive weeks, significant inhibition of tumor growth (40%) together with a decrement in tumor cell mitotic index and proliferative activity were observed. In addition, they also

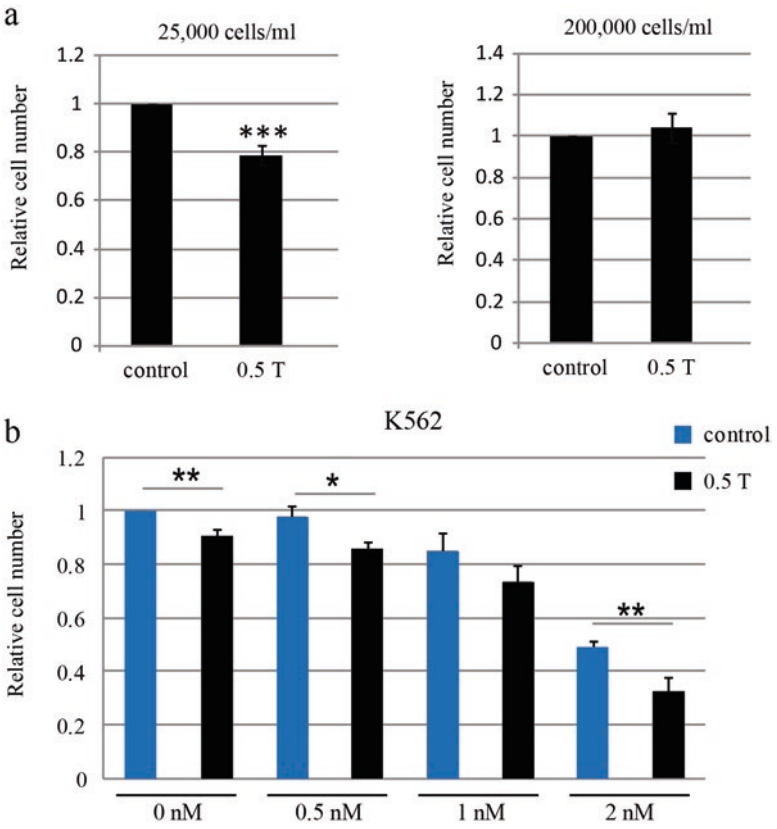

Fig. 6.7 0.5 T SMF increases the efficacy of Vincristine in K562 leukemia cells. (a) Two different cell concentrations of K562 cells respond to 0.5 T SMF differently. The cells were seeded at 25,000 or 200,000 cells/ml one day ahead, incubated in the absence or presence of a 0.5 T SMF for another 2 days before their cell numbers were quantified. **(b)** 0.5 T SMF increases the efficacy of Vincristine in K562 cells. 25,000 cells/ml of K562 cells were plated one night ahead and exposed to a 0.5 T SMF with different concentrations of Vincristine for 2 days before the cell numbers were measured. The 0.5 T SMF was provided by placing the cell plate on the top center of a 5 × 5 × 5 cm neodymium permanent magnet, with the North pole facing up. The control group was placed at at least 30–40 cm away from the magnet with a measured magnetic field intensity background of 0.9 Gs, which was around 5000 fold lower than the 0.5 T experimental group. Experiments were repeated for 3 times by two independent researchers. *$p < 0.05$; **$p < 0.01$; ***$p < 0.005$ (Our lab unpublished data) (Figure was provided by Xinmiao Ji and Meng Zha)

found a significant increase in apoptosis together with a reduction in immunoreactive p53 expression (Tofani et al. 2002). These works indicate that SMF + 50 Hz PMF of above 3 mT may have anti-cancer potentials. In contrast, lower magnetic field intensity, such as 1 mT SMF did not induce cell apoptosis as 3, 10 or 30 mT SMFs did (Tofani et al. 2001). Actually, their results could potentially explain why Bodega et al. did not observe any changes when they exposed cultured astroglial cells to a combined 1 mT SMF with sinusoidal 50 Hz PMF for 11 days (Bodega et al. 2005), which might due to the low magnetic field strength.

Table 6.3 A table to summarize current literature about combination of SMFs with different chemodrugs and cytotoxic drugs for their effects in different cells

Cell line/animal model information	Chemodrug	SMF intensity	Drug efficacy	References
Mice bearing lewis lung carcinoma	Cisplatin	3 mT	Increase	Tofani et al. (2003)
T hybridoma 3DO cells	Cycloheximide, puromycin	6 mT	Increase	Tenuzzo et al. (2006)
Leukemic cells K562	Cisplatin	8.8 mT	Increase	Chen et al. (2010)
Transplanted mammary tumors in mice	Adriamycin	8.8 mT	Increase	Hao et al. (2011)
Leukemic cells K562	Paclitaxel	8.8 mT	Increase	Sun et al. (2012b)
Leukemic cells K562	Adriamycin	110 mT	Increase	Gray et al. (2000)
Leukemic cells K562	Vincristine	500 mT	Increase	Our unpublished data. Figure 6.6
A-Mel-3-tumor-bearing hamsters	Paclitaxel	587 mT	Increase	Gellrich et al. (2014)
Human cancer cells, CNE-2Z and HCT116	mTOR inhibitors	1 T	Increase	Zhang et al. (2015)
Human cancer cells, CNE-2Z and HCT116	EGFR inhibitor Afatinib	1 T	Increase	Zhang et al. (2016)
Human cancer cells, CNE-2Z, MCF-7, HeLa and HCT116	Taxol and 5-Fu	1 T	Increase	Luo et al. (2016)
Leukemic cell line HL-60	mixture of 5-Fu, Cisplatin, Doxorubicin and Vincristine	1 T	Increase	Sabo et al. (2002)
Human tumor U937 monocytes	Puromycin, Etoposide, hydrogen peroxide	1 T	Increase	Ghibelli et al. (2006)
Human cancer CNE-2Z cells	AKT inhibitors (MK2206, BEZ-235)	1 T	Increase	Zhang et al. (2017)
Normal human monocytes, lymphocytes and tumor Jurkat cells	Puromycin	6 mT and 1 T	No effect	Ghibelli et al. (2006)
B16 melanotic melanoma	Cyclophosphamide	3 mT	No effect	Tofani et al. (2003)
Lymphocyte, thymocytes, U937, HepG2, HeLa, FRTL-5	Cycloheximide, Puromycin	6 mT	Reduced	Tenuzzo et al. (2006)
Human tumor U937 monocytes	Puromycin	6 mT	Reduced	Ghibelli et al. (2006)
Human neuroblastoma SH-SY5Y cells	Cisplatin	31.7–232 mT	Reduced	Vergallo et al. (2014)
Human cancer cells, CNE-2Z, MCF-7, HeLa and HCT116	Cisplatin	1 T	Reduced	Luo et al. (2016)

Blue color means that the SMFs increase the drug efficacy. *Grey color* means that there is no combinational effect. *Pink color* means that SMFs reduce the drug efficacy

Table 6.4 A table to summarize current literatures about combination of SMFs with PMFs for their effects in different cells

Cell line/animal model information	PMF intensity and frequency	SMF intensity	Anti-cancer effects	References
Cultured astroglial cells	50 Hz, 1 mT	1 mT	No effect	Bodega et al. (2005)
WiDr human colon adenocarcinoma	50 Hz, 3 mT	3 mT	Increase apoptosis	Tofani et al. (2001)
MCF-7 human breast adenocarcinoma	50 Hz, 3 mT	3 mT	Increase apoptosis	Tofani et al. (2001)
MRC-5 embryonal lung fibroblast	50 Hz, 3 mT	3 mT	No effect	Tofani et al. (2001)
nude mice with WiDr cells	50 Hz, 5 mT	5.5 mT	Increased survival time	Tofani et al. (2002)

Blue color means that there is an anti-cancer effect. *Grey color* means that there is no effect

To our knowledge, all reported studies used the combination of mili-Tesla SMFs (1–10 mT) with 50 Hz PMF of similar magnetic field intensity (Table 6.4). The combination effects of SMFs with higher magnetic field intensity and/or in combination with PMFs of other frequencies besides 50 Hz have not been reported. Whether the currently reported cancer inhibition effects of mili-Tesla SMFs with 50 Hz PMF can also be applied to other magnetic field parameters, such as different magnetic field intensity or frequency, is still unknown. In addition, since the three cell lines Tofani et al. tested showed different responses to the combinational treatment of SMF + PMF (increased apoptosis in two cancer cells lines WiDr and MCF-7 but not non-cancer cell line MRC-5), it is likely that the effects are also cell type-dependent. Whether other cancer cell types can also be inhibited by SMF + PMF still need more investigations.

6.3.3 SMFs in Combination with Radiotherapy

Radiation therapy (radiotherapy) is commonly used in cancer treatment. It uses high-energy radiation to kill cancer cells and reduce tumor size. Currently the most commonly used types of radiation are X-rays. In some cases, gamma rays and charged particles are also used for cancer treatment. In recent years, image-guided radiotherapy (IGRT) has greatly improved the precision and accuracy of radiotherapy, which takes advantage of modern imaging techniques such as ultrasound, X-ray and CT (computed tomography) scan. The information provided by these imaging techniques before and during radiotherapy treatment not only shows the size, shape and position of the tumor itself, the surrounding tissues and bones, but

Table 6.5 A table to summarize current literatures about combination of SMFs with different doses of X-ray radiation for their combined effects in different cell types

Cell line/animal model information	Irradiation	SMF intensity	Effects compared to radiation alone	References
Primary glioblastoma cells	5 Gy X-ray	80 mT	Reduced DNA damage	Teodori et al. (2014)
Chinese hamster ovary CHO-K1 cells	1 Gy X-ray	10 T	No effect	Nakahara et al. (2002)
Chinese hamster ovary CHO-K1 cells	2 Gy X-ray	10 T	No effect	Nakahara et al. (2002)
Rat bone marrow stem cells	0.5 Gy X-ray	15 mT	Increased G2/M cell cycle arrest	Sarvestani et al. (2010)
Chinese hamster ovary CHO-K1 cells	4 Gy X-ray	10 T	Increased micronucleus	Nakahara et al. (2002)

also allows instant correction for positioning deviations and thereby improves the precision of daily radiotherapy fractions. Although CT scan is mostly used in current IGRT, MRI-guided radiotherapy is attracting increasing attention. It is well known that MRI gives superior soft tissue contrast and more importantly, MRI could offer the advantage of providing IGRT without delivering an additional radiation dose to the patients compared to CT or X ray imaging. Currently there are multiple groups are building or starting to test MRI (0.3–0.5 T)-guided radiotherapy. Moreover, Elekta has announced its plans for the commercial release of Atlantic, a high-field MRI (1.5 T)-guided radiotherapy system, which may be launched in 2017–2018.

Along with the introduction of MRI-guided radiotherapy, the potential effects of SMFs on ionizing radiation have become increasingly important. However, the accompanied lab studies about the combinational effects of SMF and radiation is lacking. Although there are some evidences showing that the effects of ionizing radiation on cells could be strengthened by PMFs, such as 50 Hz magnetic fields (Francisco et al. 2013), the studies about SMFs in combination with radiotherapy are much less. So far there are only a few studies that have investigated the combinational effects of SMFs with ionizing radiation and most of these studies indicated that SMFs might be able to increase the effectiveness of radiotherapy (Table 6.5). For example, in 2002, Nakahara found that although 10 T SMF itself had no effect on CHO-K1 cell growth, cell cycle distribution, or micronucleus frequency, they could cause an increase in the micronucleus formation induced by 4 Gy X-rays (Nakahara et al. 2002). In 2010, Sarvestani et al. investigated the effects of a 15 mT SMF alone for 5 h or 0.5 Gy X-ray +15 mT SMF sequential exposures (first X ray and then SMF for 5 h) on cell cycle progression in rat bone marrow stem cells (BMSC). They did not find any cell cycle changes in SMF alone treated cells but

found that 15 mT SMF exposure could further increase the G2/M cell percentage induced by 0.5 Gy X-ray (Sarvestani et al. 2010). In 2014, Teodori et al. investigated the genotoxic effect of 80 mT SMF, both alone and in combination with X-ray irradiation, on primary glioblastoma cells. Their results showed that exposure of cells to 5 Gy of X-ray irradiation alone led to extensive DNA damage,which was significantly reduced by 80 mT SMF (Teodori et al. 2014). The DNA damage promotion effect of 10 T SMF in CHO-K1 cells (Nakahara et al. 2002) and the DNA damage reduction effect of 80 mT SMF in primary glioblastoma cells (Teodori et al. 2014) seem to be controversial. However, this difference could be due to the cell type or magnetic field intensity difference. In 2013, Politanski et al. investigated the combined effect of X-ray radiation and SMFs on reactive oxygen species (ROS) in lymphocytes from male albino Wistar rats. Their results indicated that 5 mT SMF increased the ROS increase changes induced by 3 Gy X-ray radiation while "0 mT" (50 μT magnetic field induction opposite to the geomagnetic field) always showed opposite effects compared to 5 mT SMF (Politanski et al. 2013). This indicated that different magnetic field intensity could directly influence its effect on radiation-induced effects. More researches are needed to get a complete understanding about different magnetic field intensities, especially around the range of MRI scanners, and their effects on radiation-induced effects on different cell types. Other types of radiation, such as gamma radiation, should also be investigated.

6.4 Patient Studies

It is interesting and promising that time-varying electromagnetic fields have been shown to be effective in multiple studies at patient level and was introduced as a novel cancer treatment modality. The most famous example was the tumor treating fields (TTF, or TTFields) therapy, which delivers low-intensity, intermediate-frequency (100–300 kHz), alternating electric fields that cause apoptosis or cell death by inducing mitotic catastrophe and can effectively inhibit the growth of a variety of human and rodent tumor cell lines, with no significant damage to normal non-dividing cells (Kirson et al. 2004; Pless and Weinberg 2011; Davies et al. 2013). In addition, Barbault et al. examined patients with various types of cancer using a noninvasive biofeedback method to identify "tumor-specific frequencies" (Barbault et al. 2009). They implied that cancer-related frequencies appeared to be tumor-specific and treatment with tumor-specific frequencies was feasible, well tolerated and may have biological efficacy in patients with advanced cancer (Barbault et al. 2009). Recently, Kim et al. used TTF to study the metastatic potential of U87 and U373 glioblastoma cell lines and found that TTF affected NF-κB, MAPK and PI3K/AKT signalling pathways as well as downregulated VEGF, HIF1α and matrix metalloproteinases 2 and 9, which indicated that TTF could be a promising novel anti-invasion and anti-angiogenesis therapeutic strategy for glioblastoma patients (Kim et al. 2016). More importantly, studies reported that treating recurrent glioblastoma patients with TTF improved overall survival (OS) and there was no

unexpected adverse effects (De Bonis et al. 2012; Rulseh et al. 2012). Due to these clinical outcomes, TTF was approved by the FDA as an alternative to the standard treatment for patients with recurrent and newly diagnosed glioblastoma.

In contrast, although a large number of *in vitro* and *in vivo* studies indicating the anticancer potentials of SMFs, there is only a very small amount of data concerning their application in clinical cancer treatment so far. In 2003, Salvatore et al. found that there was no increase in the severity of chemotherapy toxicity as measured by white blood cell count and platelet count in the participants exposed to SMF (Salvatore et al. 2003). In 2004, Ronchetto et al. examined eleven patients with "heavily pretreated" advanced cancer in a pilot study with different SMF exposure and found that the magnetic fields can be safely administrated according to their exposure schedules (Ronchetto et al. 2004). Although these studies indicated the safety of SMFs at patient level, the effectiveness of these SMFs on cancer inhibition is still lacking, which still needs to be proved. In fact, there are some clinical studies reported in some Chinese journals about the successful application of SMFs on some cancer treatment, which have been reviewed by Dr. Zhou, although also written in Chinese (Zhou 2000). In these studies, it seems that applying permanent magnets either alone or in combination with PMF or radiotherapy could have positive effects in cancer inhibition, and the effects are correlated with the magnetic field intensities. More specifically, it was shown that the SMF of 0.2 T and above had anti-cancer effects but SMFs blow 0.1 T did not. To my point of view, although these studies do not really meet the criteria of scientific investigations, they appear promising. However, more double blinded, well controlled clinical investigations are needed to confirm their claims.

In the meantime, it is interesting and promising that there are also some positive findings for magnetic devices that use permanent magnets, but spin them at low speed. They call them "extremely low-frequency magnetic fields" (Wang et al. 2011; Sun et al. 2012a; Nie et al. 2013a; Nie et al. 2013b). For example, in 2012, Sun et al. investigated the effects of 420 r/min, 0.4 T magnetic fields on the survival and palliation of general symptoms in 13 advanced non-small cell lung cancer (NSCLC) patients (Sun et al. 2012a). The patients were treated for 2 h/day, 5 days/week for 6–10 weeks. While the median survival of the advanced NSCLC patients receiving supportive care was 4 months, their "spinning magnetic device" could prolong the median survival to 6 months, which was 50% increase. Although 6 months median survival was still shorter than that of patients receiving chemotherapy (Cisplatin, 9.1 months; Carboplatin, 8.4 months), the magnetic field-treated patients had no severe toxicity or side-effects. More importantly, the 1-year survival rate was 31.7%, which was much higher than patients only receiving supportive care (15%), and comparable to patients receiving chemotherapy (Cisplatin, 37%; Carboplatin, 34%). In the meantime, the magnetic fields treated patients had improved physical conditions and alleviated symptoms in general (Sun et al. 2012a). In fact, the effect of this type of machine has also been proved to be effective on advanced cancer patients by another independent group in China (personal communications, unpublished work) as well as in cancer cell and mice models (Wang et al. 2011; Nie et al. 2013a; Nie et al. 2013b). Meanwhile, there are also other unofficial reports claiming that spinning magnets could be used

as alternative treatment for patients. Therefore, it is a promising field to explore but apparently these reported studies are still at very preliminary stage. In fact, an important criticism of these human case reports is the lack of control subjects. Therefore, more rigorous, well controlled and double-blinded clinical trials are strongly needed to prove the effectiveness of SMFs in cancer treatment. The magnetic field parameters, such as the field strength, fixed or spinning, exposure schedule and cancer types should all be tested.

6.5 Conclusion

Cancer is a heterogeneous disease and its complexity has hindered the development of effective and safe treatments. The studies listed in this chapter greatly helped us to understand some of the mechanisms that SMFs affect cancer cells and their potential applications in cancer treatment in the future. We only discussed about membrane receptor EGFR, cell division and microcirculation here, but it is likely that other aspects are also involved in SMF-induced cancer inhibition, such as ion channels, ROS, the immune system as well as metabolism. Moreover, current cellular studies and animal models of SMF effects on cancers are variable in reproducibility, and further systematic studies of different treatment parameters would be definitely beneficial. In the meantime, while some mechanisms of action have been proposed, their substantiation is needed. Although more research should be conducted to demonstrate its safety and efficacy, current experimental results indicate that SMF is relatively safe. Understanding and exploiting the potential application of SMFs would be an essential aspect of adjuvant therapies targeting conventional treatment-resistant tumors in the future.

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Chapter 7
Prospects, Pitfalls, and Opportunities for Human Static Magnetic Field (SMF) Therapy

Abstract This chapter provides an overview of the prospects of using electromagnetic fields (EMFs), with a specific focus on static magnetic fields (SMFs), for treatment of human disease. The information provided covers the underlying basis for widespread skepticism surrounding "magnetotherapy" – which in part is deserved based on overinflated claims by its practitioners over the past two centuries (or even longer). On the other hand, a compelling scientific foundation is in place to propel nascent efforts to use magnetotherapy from a questionable niche medical practice into the mainstream; a goal of this chapter is to provide a summary of this information using specific (but non-comprehensive) examples of human ailments that are expected (based on current information) to benefit from magnetic field treatment.

Keywords Magnetic field therapy • Magnetotherapy • Static magnetic fields (SMFs)

7.1 Introduction

Therapies that involve exposure to electromagnetic fields (EMFs) date back to the inception of practical methods to harness and exploit magnetism and electricity. Anecdotal folklore suggests that the subset of these therapies using time invariant (i.e., static) magnetic fields (SMFs) extend back two or even three thousand years (perhaps to 1000 BC (Mourino 1991)), when "lodestones" were thought to have the ability to draw disease out of a person's body (Palermo 2015; Zyss 2008). Jumping forward, by the early sixteenth century (AD) the Swiss physician Paracelsus was using magnets to treat epilepsy, diarrhea, and hemorrhage and in the mid eighteenth century Franz Mesmer, an Austrian doctor, had opened a healing salon in Paris to treat the untoward effects of the body's innate "animal magnetism" (Mourino 1991). With the advent of electricity as a power source, EMFs were added to the healing repertoire and were being used to assist bone healing as early as the mid nineteenth century with definitive literature reports verifying efficacy appearing in the 1970s (Bassett et al. 1974a, b).

Since World War II, magnetic field therapy (usually referred to as "magnetotherapy" in this chapter) has flourished across the globe – albeit unevenly with

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various levels of acceptance in different countries – with an estimated two million recipients each year (Markov 2009). Magnetotherapy has many attractive features including its relative low cost compared to many current treatment modalities, its (generally) non-invasive nature, and its established safety record (with obvious exceptions, such as individuals with medical device implants such as pacemakers or insulin pumps). On the other hand, magnetotherapy has a longstanding reputation for quackery. To give one example of the origins of this reputation, by the late nineteenth century, Thatcher's Chicago Magnetic Company (a mail order outfit) claimed that "magnetism properly applied will cure every curable disease no matter what the cause" (Macklis 1993).

Today, similar overblown rhetoric from some quarters continues to obscure valid scientific underpinnings of magnetotherapy. In part, magnetotherapy remains controversial because its opponents and proponents persist in making polarized blanket statements that either categorically rejects the possibility of beneficial health effects while other practitioners promise miracle cures for long lists of disparate ailment. The reality almost certainly lies between these extremes and the purpose of this chapter is to provide an overview of what is currently known about human magnetic field therapy, what is *not* known, and what *needs to be known* (and done) to move this field forward.

7.2 Overview of Electromagnetic Field (EMF) Treatment Modalities

Although rather arbitrary, EMF therapeutic modalities are generally categorized in five categories as outlined by Markov (some classification schemes give six categories) in an excellent synopsis of the influence of magnetic fields on human health (Markov 2014). These categories are briefly discussed below.

7.2.1 Low-Frequency Sine Waves

Low-frequency sine wave (LFS) electromagnetic fields are based on the predominant commercially-supplied electricity sources, which are 60 Hz in North American and generally 50 Hz in Europe and Asia (Markov 2014). One use of LFS is as an alternative to high frequency fields in deep brain stimulation for the treatment of epilepsy (Goodman 2005; Goodman et al. 2005). Another potential application is for the treatment of cancer (Blackman 2012); more broadly, efforts are underway to use diverse frequencies of EMFs to treat cancer (Zimmerman et al. 2012) including SMFs as covered in Chap. 6 of this book.

7.2.2 *Pulsed Electromagnetic Fields (PEMF)*

Pulsed electromagnetic fields (PEMF) are low frequency fields with specific wave shapes and amplitude (Markov 2014). PEMF treatment was introduced clinically in the 1970s by Bassett and colleagues, who used a specific biphasic low frequency signal for bone healing, in particular for the treatment of delayed fractures (Bassett et al. 1974a, b). Although reports continue to appear questioning the efficacy of PEMF therapy (Rose and Bryan-Frankson 2008), transcranial magnetic stimulation devices have been approved by the United States Food and Drug Administration (FDA) for patients not responsive to chemical anti-depressants (Anonymous 2011; Martiny et al. 2010). In addition, there are a profusion of PEMF devices that are sold and marketed as FDA-registered "wellness devices;" these products, however, are not permitted to claim efficacy for treating disease (Anonymous 2015).

7.2.3 *Pulsed Radiofrequency Fields (PRF)*

Pulsed radiofrequency field (PRF) therapy refers to the technique where radio frequency oscillations are generated at a defined rate of pulses per second with energies range from 1.0×10^4 Hz to 3.0×10^{11} Hz. Therapeutically, PRFs offer an alternative to continuous radiofrequency (CRF) therapy, which has been used since the 1970s, and offers the advantage of pain control without tissue destruction (Byrd and Mackey 2008). These therapies typically utilize frequencies between 300 and 750 kHz, are now delivered to precise locations in the body by catheter, and as mentioned are used in two primary modalities: in continuous mode these devices are designed to produce deep heat, while in pulsed (non thermal) mode, which uses short (e.g., 20 ms) high voltage bursts followed by a longer (e.g., 480 ms) silent phase to allow for heat dissipation, they are used for soft tissue stimulation (Markov 2014). Thermal PRF (i.e., CFR) therapy delivers high current focally to ablate the tissue of interest (e.g., a tumor or cardiac tissues that trigger arrhythmias) by heating to temperatures of 60–80 °C, resulting in focal tissue destruction (Byrd and Mackey 2008).

It remains controversial whether non-thermal PRF truly avoids biological effects due to heating; for example, although temperatures stay at or below 42 °C minimizing cell death or tissue destruction, heat shock response nonetheless could be triggered. Resolving this ambiguity will ultimately be necessary to fully define the biochemical mechanism of therapeutic responses associated with PRF therapy. Despite uncertainty over mechanism (and even efficacy), PRF is being used to treat a growing list of indications which are typically oriented towards amelioration of pain including axial pain, radicular pain, facial pain, inguinal pain and orchialgia, and miscellaneous pain syndromes (Byrd and Mackey 2008).

7.2.4 Transcranial Magnetic/Electric Stimulation (TMS)

Transcranial magnetic stimulation (TMS) involves applying very short magnetic pulses of up to 8 Tesla to selected portions of the brain (Markov 2014). During TMS, a magnetic field generator is placed in proximity to the head of the person receiving the treatment (Groppa et al. 2012). The coil produces electric currents in the region of the brain just under the coil through electromagnetic induction. TMS can be used to diagnose connections between the brain and a muscle to evaluate damage from several indications, including stroke, multiple sclerosis, amyotrophic lateral sclerosis, movement disorders, motor neuron disease and injuries (Groppa et al. 2012). Therapeutically, TMS has been evaluated for movement disorders, stroke, amyotrophic lateral sclerosis, multiple sclerosis, epilepsy, consciousness disorders, tinnitus, depression, anxiety disorders, obsessive-compulsive disorder, schizophrenia, craving/addiction, and conversion(Lefaucheur et al. 2014). In a recent review, Lefaucheur and coauthors concluded there is sufficient evidence to accept "definite efficacy" for the analgesic effect of high-frequency (HF) TMS of the primary motor cortex (M1) contralateral to the pain and the antidepressant effect of HF-TMS of the left dorsolateral prefrontal cortex (DLPFC). "Probable efficacy" is proposed for the antidepressant effect of low-frequency (LF) TMS of the right DLPFC, HF-TMS of the left DLPFC for the negative symptoms of schizophrenia, and LF-TMS of contralesional M1 in chronic motor stroke. Finally, TMS achieves "possible efficacy" in a number of indications including LF-TMS of the left temporoparietal cortex in tinnitus and auditory hallucinations (Lefaucheur et al. 2014).

7.2.5 Static/Permanent Magnetic Fields (SMF)

Time invariant – that is "static" – magnetic fields are a feature of various permanent magnets; alternatively they can be generated by passing direct current (DC) through a coil (Markov 2014). These fields – referred to "SMFs" (static magnetic fields) are the primary focus of this book with a more detailed description of the underlying physics provided in Chap. 1. In this chapter, SMFs will next be discussed based on their field strengths with Sect. 7.3.1 covering weak fields in the range of the Earth's magnetic field (< 0.65 gauss or ~65 μT), Sect. 7.3.2 will discuss the *absence* of these fields (which by default make a convincing case that humans *can* detect and (subconsciously) respond to weak magnetic fields; and finally Sect. 7.3.3 will provide an overview of the therapeutic use of more powerful moderate strength fields that range up to ~1 T (one Tesla or 10,000 gauss). Strong fields above one Tesla are rarely used in magnetotherapy *per se* but people are exposed to these field strengths during magnetic resonance imaging (MRI) generally without any discernible impact on health.

7.2.6 "Non-therapeutic" EMF Exposure Allays Safety Concerns

Over the past century (or so) humans have been increasingly subject to inadvertent exposure from "man-made" EMFs. For example, the rise of metal industries, welding processes, and certain electrified train systems in the late nineteenth centuries resulted in significant exposure for workers and even bystanders to SMFs; in 1921 Drinker and Thomson asked the question "Does the magnetic field constitute an industrial hazard?" and concluded that it didn't (Hartwig et al. 2009). Over the years as new "EMF"-based threats have emerged (Tucker and Schmitt 1978), such as living under high voltage power lines or the ubiquitous adoption of cell phones, which have raised fears of childhood and brain cancers, have been met with detailed scrutiny that have ruled out clear-cut evidence of harm. Ultimately meta-analysis of many such studies has cast doubt on the idea that EMF exposure causes any measurable detriment to human health in a way helpful for establishing the safety of magnetotherapy. On the other hand, the (general) lack of deleterious effect of EMFs has also been used to cast doubt on whether beneficial effects are possible based on the assumption that these fields likely have *no* meaningful impact on human health; a substantial portion of this chapter either directly or indirectly addresses this fallacy.

7.3 Biomedical Effects of SMF Therapies Categorized by Field Strength

7.3.1 "DIY" Treatments with Low to Moderate Strength SMFs Are Widespread but Unproven

The largest segment of extant "magnetic therapies" falls into the do-it-yourself (DIY) category where individuals use various types of permanent magnets that provide "always on" SMF exposure. This modality of magnetotherapy is used to treat a wide range of ailments with a quick internet search (conducted in January, 2017, but similar results have been obtained for at least 20 years) turning up magnetic bedding pads, magnets embedded in pillows, magnetic shoe insoles, magnetic back belts, magnetic leg and arm supports, magnetic bracelets, magnetic finger and toe rings, and – to wrap this up – multipurpose magnetic pads that can be customized to wear on virtually any part of the body. Note that no specific weblinks are provided here for several reasons. First, any particular commercial link is apt to be quickly out of date; second, this publication wishes to avoid the appearance of endorsing any particular product; and finally, to spur any interested reader to perform their own search for "magnetic therapy products" (or similar term). Such a search will almost certainly provide – above and beyond many sites selling these

products – numerous links running the gamut from "debunking" the entire idea of magnetotherapy and mocking consumers for falling for a billion dollar 'scam" (reportedly a conservative value for annual sales of these products, which was reported almost 20 years ago (Weintraub 1999)) to enthusiastic endorsements for efficacy against a broad gamut of human diseases; increasingly, products are coming available to treat one's pets as well!

Intuition alone makes a powerful case that many DIY magnetotherapy efforts are likely misguided and minimally effective. Even if the magnets used are "high quality" (e.g., constructed from latest neodymium-based alloys) as advertised, with field strengths reported in the range of tens to hundreds gauss (i.e., up two to three orders of magnitude stronger than the Earth's magnetic field), one key issue is that magnets themselves are NOT therapeutic. This point is discussed by Markov (2009) who describes how the term "magnetic therapy" is a misnomer. Instead, he emphasizes that the therapeutic effects of magnets emanate from the fields they generate and the subsequent interaction of these fields with the target tissue or organ in a person (note that the use of the "magnetotherapy" in this chapter implicitly denotes magnetic *field* therapy). In this regard, it is critical to note that field strength decreases exponentially with distance from the surface of a permanent magnet (for example, by ~2 orders of magnitude in only a few millimeters for magnets in the range of hundreds of gauss) and therefore field strength is negligible in deep tissue that would need to be penetrated to have an effect on many of the conditions purportedly treated with magnetotherapy.

One example of this pitfall is provided by a report where commercial magnetic wraps had no effect on blood circulation in horses (Steyn et al. 2000) or pain perception in people (Kuipers et al. 2007), which – because the field strengths used did not penetrate effectively into tissue to the depth where the target vessels or nerves were located – were not surprising results. More trivially, but still important, magnets placed in clothing or otherwise attached via wrappings that surround the body provide inconsistent magnetic field exposure to the intended target tissue if the clothing or wrapping is loose or not applied and worn consistently from day to day. An illustration of this point is that the field strength of a 500 gauss magnet can be as little as 1 gauss only one or two centimeters away from the magnet's surface. As a result, determination of dose – a key parameter in determining medical efficacy – is typically impossible to determine with any degree of accuracy in DIY magnetotherapy (Markov 2009).

7.3.2 Hypomagnetic Fields (HMF) – Evidence for Magnetotherapy by Default?

Interestingly, the impact of weak (to moderately strong) SMF on human health perhaps has been demonstrated most convincingly by default; that is, by observing the effects of the absence of geomagnetic strength magnetic fields. These studies have

exploited a century of efforts to develop materials designed to shield sensitive equipment from magnetic fields, such as submarine telegraph cables, electric power transformers, cathode ray tubes, and magnetic phonograph cartridges. To achieve the required shielding, "mu-metals" have been developed that have a representative composition of ~77% nickel, 16% iron, 5% copper and 2% chromium or molybdenum (Jiles 1998). In essence, a mu-metal is a high permeability alloy that does not block magnetic fields *per se*, but instead provides a path for the magnetic field lines to go around the area intended to be shielded. Details on magnetic field shielding are largely beyond the scope of this discussion but more information can be found online (e.g., in technical documents provided by vendors of magnetic shield products such as http://www.magnetic-shield.com/pdf/how_do_magnetic_shields_work.pdf). For this discussion, the key point is that products exist that can effectively shield objects from ambient magnetic fields that for practical purposes can isolate a research subject from a background (generally the Earth's) magnetic field. Geomagnetic field shielding produces what has come to be known as "hypomagnetic fields" (HMFs).

In the past few years a provocative set of experiments have emerged that HMF has numerous biological and biomedical effects across species up to and including in people. For example, long-term HMF exposure is associated with embryonic malformation in insects (Wan et al. 2014), amphibians (e.g., newts (Asashima et al. 1991) and frogs (Mo et al. 2012)), and rodents (e.g., mice (Fesenko et al. 2010)). Additional effects of HMF have been described in rodents including inhibition of stress-induced analgesia (Prato et al. 2005) and decreased noradrenaline release (Choleris et al. 2002; Zhang et al. 2007) and learning defects have been described in birds (Xu et al. 2003) and *Drosophila* (Zhang et al. 2004). Finally, the negative impact of HMF has been reported to extend to humans; these effects have often and most convincingly been deduced from space flight where the geomagnetic field is negligible in strength because it generally is not practical to confine a person to artificially-shielded HMF area. These studies have shown HMF effects in humans that include perturbed circadian rhythms (Bliss and Heppner 1976; Wever 1970) and weakened cognitive function (Binhi and Sarimov 2009).

The generally deleterious effects of HMF across several biological processes in many species, including the still-speculative but nevertheless plausible observations in people, have strengthened the case that weak magnetic fields *do* have legitimate biomedical relevance. For example, it appears that GMFs keep us healthy and contribute to normal physiology. Extrapolating from these observations, it has been hypothesized that because a lack of magnetic fields is harmful, field strengths stronger than the Earth's magnetic field might exacerbate and extend the beneficial impact of GMF exposure. A parallel drawn from pharmacology is that many natural "drugs" such as aspirin or the antioxidant resveratrol must be consumed at much higher levels to have a medical effect than a person can reasonably obtain from natural consumption (Scott et al. 2012). Similarly, arguments have been made – abutted with claims that humans evolved when the Earth's magnetic field was as much as an order of magnitude stronger than it is today (the earth's magnetic field is constantly waxing and waning, and even reverses polarity on a millions-of-years

time scale (Mori et al. 2013), an event associated with mass extinctions (Lipowski and Lipowska 2006)) – that to achieve maximum benefits from magnetotherapy, stronger magnetic fields should (or even "must") be used.

7.3.3 Stronger Magnetic Fields – Impacts on Human Health

7.3.3.1 Moderate Strength SMF Therapy

The benefits (or necessity) of using stronger than GMF-strength fields for human therapy have spurred efforts to use static magnetic fields much stronger than afforded naturally by today's geomagnetic fields. In some cases, these strategies involves "DIY" efforts with magnets in the tens to hundreds of milli-Tesla range, but, as discussed above, these efforts are likely ineffective for treatments that require deep penetration of tissue. As an alternative, medical devices, often from Europe, that create stronger electromagnetic fields have been marketed. The United States FDA generally permits these for "general wellness" (Anonymous 2015) while prohibiting claims for efficacy for treatment of any specific medical indication.

In some cases, proponents of magnetic therapy are pursuing more rigorous evidence of efficacy. One example is provided by continuing efforts of Joe Kirschvink and colleagues (as discussed in more detail in Chap. 3) to demonstrate that humans are affected by externally applied magnetic field in ways that are medically-relevant (Hand 2016). Another example of moving forward with therapeutic intervention is provided by the *Advanced Magnetic Research Institute* (AMRi) that has developed a "Magnetic Molecular Energizer™" (MME) device (Bonlie 2001) capable of producing SMFs of 0.3–0.5 T that completely penetrate the human body in an ~20 cm radius (Fig. 7.1). Based on the assumption that the "biosensor" for magnetic reception is located directly in the diseased or damaged tissue, a patient is position with field centered on the affected area. Double blind clinical trials seemingly showed efficacy against lower back pain (ClinicalTrials.gov Identifier: NCT00325377) and possibly against symptoms of diabetic neuropathy (ClinicalTrials.gov Identifier: NCT00134524). The results of these studies, however, were difficult to interpret because positive outcomes were not statistically different from placebo-treated patients, who also experienced marked improvement (Dean Bonlie, personal communication). These clinical studies illustrate two recurring themes in efforts to establish clinical efficacy for magnetotherapy; first, therapeutic effectiveness is most well established for pain perception (the subject of these tests) and second, the placebo effect is often overwhelming in magnetotherapy; both of these points are further elaborated in Sect. 7.5.3, below.

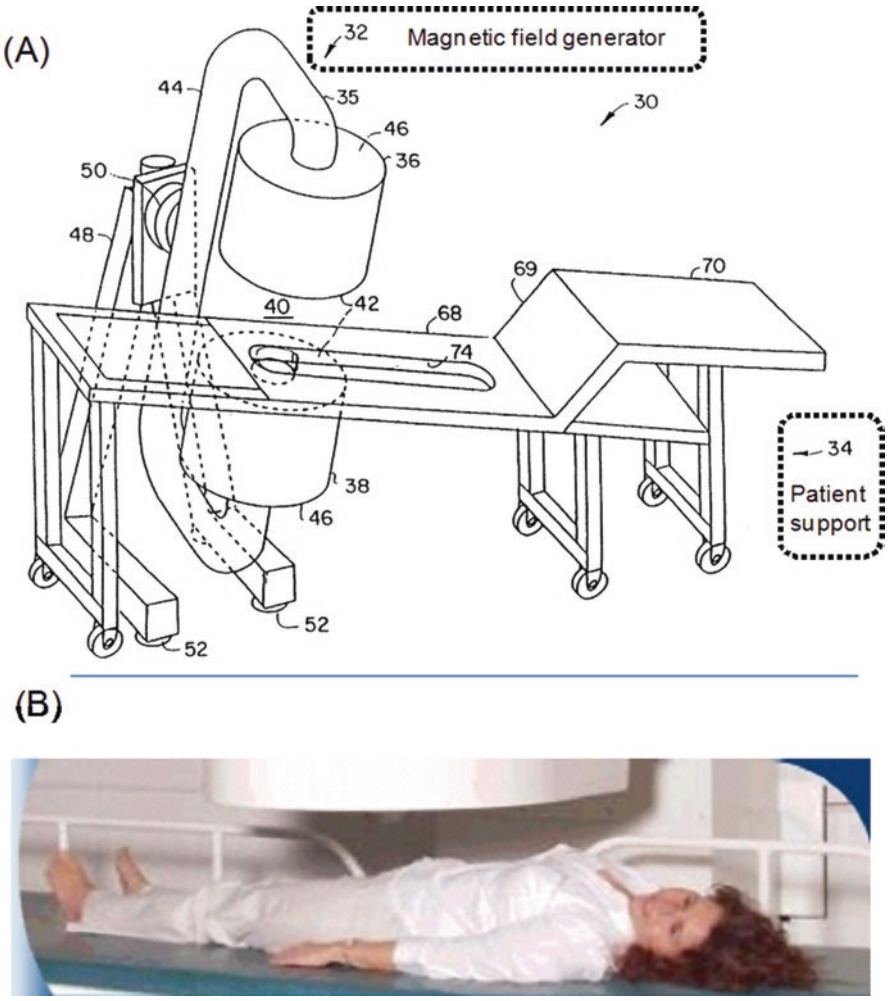

Fig. 7.1 The Molecular Magnetic Enegizer™ (MME) device and illustration of a patient during treatment. (**a**) The MME (as illustrated in U.S. Patent documents (Bonlie 2001)) consists of two major elements: a magnetic field generator (**32**) for producing a treating magnetic field and a patient support (**34**) for positioning a patient within the magnetic field. The magnetic field generator consists of a magnetic circuit (**35**) having an upper electromagnet (**36**) and a lower electromagnet (**38**) separated by a gap (**40**) on their adjacent pole faces (**42**) and connected by a C-shaped core (**44**) (or "C-core") on their opposing poles (**46**). In the embodiment shown C-core has a circular cross section with an 8 inch (20.3 cm) diameter. The electromagnets are wired in parallel with a power supply to create magnetic fields of the same sense. For example, the positive pole of the upper electromagnet **36** would face the negative pole of the lower electromagnet **38** (or vice versa). (**b**) A patient is shown positioned in the MME device in a supine position; it should be noted that the magnetic field generator apparatus can be rotated and otherwise adjusted via parts **48**, **50**, and **52** to accommodate patients who prefer to treated in other positions, for example, lying on their side

7.3.3.2 Higher Strength SMF Exposure

Strong fields above one Tesla are rarely used in magnetotherapy *per se* but people routinely are exposed to field strengths of 1.3 (and now up to 3) T during magnetic resonance imaging (MRI). As of 2016, over 150 million people have undergone MRI procedures with ~10 million undergoing examination each year (Anonymous 2016). Overall, it is accepted that MRI has little if any discernible impact on health either beneficial or deleterious (Schenck 2000). Based on this apparent lack of response, SMFs are generally regarded to be safe by regulatory agencies such as the United States Food and Drug Administration (FDA) (Anonymous 2015). Upon comprehensive review of the literature available described the *in vivo* and *ex vivo* effects of SMFs, Hartwig and coauthors confirmed that >1 T SMFs that accompany MRI are rarely harmful (Hartwig et al. 2009) with the possible exception of inconclusive reports where exposure led to acute neurobehavioral effects, such as eye–hand coordination speed and visual and auditory working memory problems (De Vocht et al. 2006) and a non statistically significant increase in spontaneous abortions in MRI workers (Evans et al. 1993). It should be noted that these reports dealt with MRI workers and, no doubt based on warnings raised by these speculative studies, safety standards have been tightened and follow-up and continuing problems have not been reported.

7.4 Prospects for Three Therapeutic Areas

Magnetotherapy has been applied to almost any imaginable human ailment. For example, MedicineNet (http://www.medicinenet.com/script/main/art.asp?articlekey=22961) summarizes conditions claimed to be diagnosed or treated using magnetic field therapy (largely through the "DIY" methods mentioned above) to include arthritis, cancer, circulatory disorders, diabetic neuropathy (nerve disease), fibromyalgia, HIV/AIDS, immune dysfunction, infection, inflammation, insomnia, multiple sclerosis, muscle pain, neuropathy, pain, rheumatoid arthritis, sciatica, stress and to increase energy and prolong life. The abovementioned AMRi Corporation, which utilizes stronger strength SMF therapy, is investigating the treatment of ailments that range from spinal cord injury, brain injury, stroke impairment, multiple sclerosis, muscular dystrophy, cerebral palsy, Parkinson's disease, Alzheimer's disease, congestive heart failure, to orthopedic conditions involving bone and joint repair. As described in Sect. 7.5 below, many find it implausible that a "one size fits all" treatment could be effective against so many indications and this doubt in part contributes to disbelief in therapeutic efficacy for magnetic field exposure. However, as discussed next, pain perception, blood flow and effects on the cardiovascular system, as well as the impact on cells found in the neurological system provide a compelling scientific basis for beneficial effects of SMFs that, if carefully and rigorously translated to the clinic, hold legitimate promise for human

therapy (cancer is another similar area that is covered in detail in Chap. 6 of this book).

7.4.1 Pain Perception

A substantial body of evidence has accumulated showing that exposure to EMFs affects pain sensitivity (nociception) and pain inhibition (analgesia); in particular acute exposure to various EMFs have been shown to inhibit analgesia in many studies (Del Seppia et al. 2007). In some studies, however, depending of the duration, intensity, frequency, and repeated nature of EMF exposure, increased analgesia has actually been observed (Del Seppia et al. 2007). While many of these studies – conducted in diverse organisms ranging from snails to mice to people – have involved time-varying fields, there is also substantial evidence that SMFs can affect pain perception. These findings have most convincingly come from HMF studies where mice apparently detect and respond to the absence of the ambient geomagnetic field. In a pioneering study, mice experienced a maximum analgesic response after 4–6 days of exposure (Prato et al. 2005). Follow up studies showed a more complex biphasic response, where geomagnetic shielding for 1 h per day for 10 consecutive days initially decreased the pain threshold over the first 2 days, followed by a sharp increase peaking by the fifth day, with a return to pre-exposure values within 8 days (Del Seppia et al. 2007). Interestingly, the kinetics of this response roughly mirror an *in vitro* cell-based assay response to moderate strength SMF (Wang et al. 2009) described in more detail below in Sect. 7.4.3.

7.4.2 Blood Flow/Vascularization

As discussed in more detail in Chap. 3, beneficial effects of magnetotherapy in humans often have been attributed to improved blood flow. Although many of the "internet" claims in this regard are nonsensical, for example the idea that a magnetic field attracts the iron in the blood is based on the misconception that hemoglobin is ferromagnetic. Instead, iron in oxygenated blood is diamagnetic which means there is a real, but almost negligible force, repelling the blood; on the other hand, deoxygenated blood is paramagnetic which means there will be a similarly almost negligible force attracting the blood (Zborowski et al. 2003). Either way, these effects are dwarfed by thermal motion and the ambient flow of the blood (as discussed in more detail in Chap. 3). Nevertheless, there is evidence – although inconclusive because of many conflicting or inclusive studies – that magnetic fields can legitimately modulate blood flow in humans (or other mammals). As an aside, some "negative" results can be accounted for by the trivial explanation that the magnetic fields used were not strong enough to penetrate deeply into the tissue where the target blood vessels were used. One example with horses was mentioned above (Steyn et al.

2000) and similarly, a study using 500 gauss (0.05 T) fields to measure blood flow in the forearms of healthy young men was equally ineffective (Martel et al. 2002); this is not surprising because field strength would be two to three orders of magnitude lower at the location of the targeted blood vessels embedded in tissue. A ~ 10-fold larger field (4042 gauss, or ~0.4 T), by contrast, *did* statistically affect blood flow in treated fingers (Mayrovitz and Groseclose 2005); interestingly this effect actually was a reduction in blood flow, which is against the direction generally thought to be therapeutically beneficial.

A set of studies in rabbits using similar strength fields (i.e., ~0.18–0.25 T) also showed legitimate effects of SMFs on blood flow (Gmitrov et al. 2002; Okano and Ohkubo 2001; Xu et al. 1998). These three studies demonstrated a biphasic response of blood flow where exposure enhanced vasodilation when the vessels were vasoconstricted and enhanced vasoconstriction in vessels that were vasodilated; in other words, the SMFs appeared to work to maintain circulatory homeostasis and "normalize" vascular function. A conceptually similar normalization effect was observed in mice where the impact of surgical intervention that would otherwise cause luminal diameter expansion in vascular networks was abrogated by continual exposure to SMFs over 4–7 days (Morris and Skalak 2007). Together, these studies suggest that while SMF exposure does have an interesting effect on blood flow, it likely is not mediated through magnetic or inductive effects on iron containing molecules (hemoglobin) or cells (RBCs) *per se*.

Instead therapeutic effects on blood flow are likely mediated by "non-canonical" mechanisms (i.e., *not* magnetite, chemomagnetic sensing, or inductive mechanisms, which are the three molecular mechanisms found throughout nature in many diverse organisms as discussed in detail in Chap. 3). Another interesting feature of these studies is that field strengths of greater than ~0.1 T (1000 gauss) were needed for efficacy; as mentioned the simple explanation is that weaker field strengths could not penetrate deeply enough into tissue to reach the intended site of action (i.e., the blood vessels themselves). Another explanation (again as discussed in more detail in Chap. 3), is that field strengths of ~0.2 T or higher can alter the biophysical properties of lipid assemblies (Braganza et al. 1984). As a result, the properties of lipid biolayers (i.e., biological membranes) are affected in ways that putatively explain many phenonema observed in magnetotherapy. For example changes in ion flux could reasonably be explained by allosteric changes to ion channels brought about changes to the biophysical properties of membranes rather than the less plausible explanation that SMF directly affects the movement of ions (i.e., through an inductive or "Hall effect," which has sometimes been postulated to explain the mechanism of magnetotherapy). Similarly, changes to signal pathway activity can be explained by the effects of magnetic field exposure on the biophysical properties of membranes, as discussed below for neural cells). Both of these topics are discussed in the next section in the context of studies performed in the author's laboratory.

7.4.3 In Vitro *Evidence for Treatment of Neurological Disease and Neural Regeneration*

In a study that was inspired by the need to find a scientific basis for coalescing evidence that magnetic field therapy may be a viable treatment option for neurological ailments through the use of moderate strength fields (i.e., 0.1–1 Tesla), we treated the PC12 rat adrenal pheochromocytoma cell line with ~0.25 T SMFs. PC12 cells display metabolic features of Parkinson's disease (PD) (Blum et al. 2000; Meng et al. 2007) such as possessing intracellular substrates for dopamine (DA) synthesis, metabolism, and transport and they also abundantly express adenosine A_{2A} receptors (e.g., $A_{2A}R$) implicated in PD (Kobayashi et al. 1998). In these studies we showed that SMF treatment reproduced several responses elicited by ZM241385, a selective $A_{2A}R$ antagonist; SMF exposure also counteracted several PD-relevant endpoints exacerbated by $A_{2A}R$ agonist CGS21680 in a manner similar to ZM241385 (Wang et al. 2010). These results raise the intriguing hypothesis that SMF can reproduce the effects of a promising class of non-dopaminergic PD drugs (i.e., ZM241385 and analogues) in a non-invasive manner and, more broadly, hold potential for ameliorating additional neurological disorders such as Alzheimer's and Huntington's diseases through modulation of $A_{2A}R$ (Takahashi et al. 2008).

In a second study from the author's laboratory, SMF-mediated responses associated with transient interleukin-6 (IL-6) signaling in human embryonic cells (the hEBD LVEC line (Shamblott et al. 2001)[as outlined in detail in Chap. 3]) translated into changes observable at the whole cell level (Wang et al. 2009). The response(s) observed in these cells began very rapidly after SMF exposure began, first observed within 15–30 min in increased transcription of mRNA for IL-6 with actual secretion of this pro-inflammatory cytokine increasing for the next 2–4 days.

Because IL-6 guides differentiation of neural stem cells primarily to astrocytes (Taga and Fukuda 2006) – which is generally a medically-unwanted outcome because hyperproliferation of this cell type leads to scar formation rather than regeneration – we investigated whether evidence of astrocytogenesis was seen in SMF-treated cells. Interestingly, responses consistent with astroctye differentiation (i.e., slowed proliferation and morphological changes) expected from IL-6 exposure were not seen; neither were biochemical markers of astrocyte differentiation (Fig. 7.2a). Instead, markers found in neurons (Fig. 7.2b) and oligodendrocytes were manifest (Fig. 7.2c, d), indicating that the other pathways modulated by SMFs (nine other signaling pathways besides IL-6 were affected by SMF exposure in this study (Wang et al. 2009)) tuned – and in fact reversed – the usual, and most-often unwanted, pro-inflammatory activity of IL-6. Ultimately, if oligodendrocyte formation can be promoted *in vivo* by SMF treatment without concomitant scar-forming astrocyte enhancement, this capability could lead to non-invasive therapies for conditions such as multiple sclerosis (MS) that are linked to oligodendrocyte pathologies.

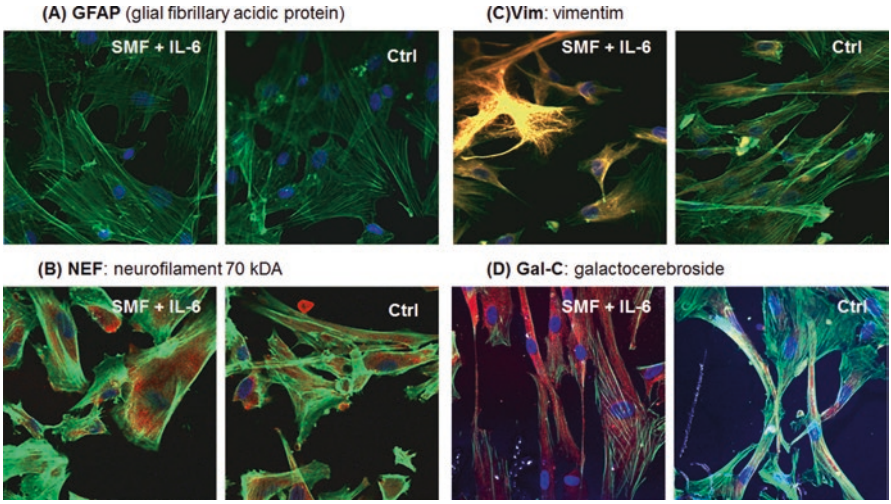

Fig. 7.2 SMF treatment reverses astrocyte differentiation in hEBD LVEC (human embryonic) cells (Adapted from Wang et al. 2009). In these experiments cells were treated with 4.0 ng/ml IL-6 and exposed to SMFs (control cells received neither stimuli) and the monolayers were co-stained with Oregon Green 488 phalloidin to visualize actin, the nuclear dye DAPI (*blue*), and one of the following markers (red). In Panel (**a**) the GFAP astrocyte marker was absent from both the control and treated cells (IL-6 treatment alone causes up-regulation, not shown). Panel (**b**) shows the neuron marker NEF and Panels (**c**) and (**d**) show expression of the pre-oligodendrocyte markers (**c**) Vim and (**d**) Gal-C, respectively upon combined IL-6 and SMF treatment (Images were obtained by confocal microscopy using identical exposure settings for each set of photographs)

7.5 Pitfalls with SMF Clinical Studies and Acceptance of Magnetotherapy

7.5.1 *Hyperbolic and Ambiguous Claims* vs. *Outright Rejection of Magnetotherapy*

It can be a daunting task to precisely match treatment parameters to various pathological indications even for long-standing medicines. For example, it has taken a century to understand how to fully exploit aspirin as a medicine; indeed, some aspects of this drug remain poorly understood. For example, at a pharmacological level, the need for esterase processing of aspirin is not fully elucidated (Lavis 2008). However much is known, including how higher doses delivered over short time intervals aspirin have anti-inflammatory and pain relief effects while lower does when administered consistently over time, it appear to reduce the risk of cardiovascular disease. On the other hand, no evidence exists that aspirin is effective against many other conditions, for example, pancreatic cancer or a neurological disorder such as Alzheimer's disease. Aspirin is again used here to illustrate pitfalls – and lessons to be learned – for magnetic field therapy. Just as aspirin, if tested against a

wrong medical indication – or at the wrong dose or duration – could be easily be proven to have no effect does not mean that it has no benefit for other ailments. Similarly, magnetic field therapy should not be considered to be debunked if a certain treatment modality shows no effect against a certain ailment; indeed, to the contrary, careful compiling of conditions that do *not* work could be extremely helpful in guiding treatments towards diseases and other ailments where the magnetotherapy *does* work.

Unfortunately, the efficacy of magnetic therapy has been clouded by ambiguity that results in large part from study design, as illustrated by a review of over 50 studies a decade ago (Colbert et al. 2007, 2009). In these studies, only two provided sufficiently-detailed experimental protocols to actually reproduce the work; although a more recent systematically-analyzed compilation of studies does not appear to be available, anecdotal perusal of the literature over the past decade suggests that the problem of incomplete reporting of experimental conditions persists up to today. As Markov forcefully editorializes, until parameters used in magnetic field therapy – starting at a very basic terminology level to overcome confusion over semantic differences between "magnetic therapy" and "magnetic field therapy" (i.e., magnets themselves have no therapeutic effect but the fields they produce do) – magnetotherapy is apt to remain marginalized and not fully accepted by the mainstream scientific and medical communities (Markov 2009). Indeed, Markov (and his colleagues) have been trying to educate about these issues for at least two decades, and in that vein, has proposed a set of parameters that must be considered and clearly defined; these endpoints are discussed next in Sect. 7.5.2.

7.5.2 Parameters Necessary to Be Controlled in Magnetotherapy

The variety of commercially-available EMF devices – often with poorly characterized and sometimes misrepresented field strength specifications – makes it difficult to compare the physical and engineering characteristics of any particular device used in any reported study, thus providing significant obstacles for analysis of clinical efficacy. Markov outlines a set of parameters that must be controlled, defined (and reported!) to be able to be able to evaluate magnetotherapy outcomes (Markov 2009); these are:

- **Type of field**
- **Intensity or induction**
- **Spatial Gradient (dB/dx)**
- **Localization**
- **Time of exposure**
- **Depth of penetration**
- *Temporary change (dB/dt)*
- *Frequency*

- *Pulse shape*
- *Component (electric or magnetic).*

- An attraction of SMF therapy is that the latter four parameters (indicated in *italics*) are not in play, thereby simplifying evaluation of this therapeutic modality, and in theory, increasing the reproducibility of the studies.

7.5.3 The Placebo Effect

As already alluded to above, pain response was the only medical outcome where magnetic fields unambiguously had a beneficial therapeutic effects based on the bulk of the literature reviewed by Del Seppia and coauthors a decade ago (Del Seppia et al. 2007). Many of the relevant studies were performed in animals, often rodents, where there presumably is no placebo effect but in humans placebo response cannot be discounted so easily. Indeed, difficulties in establishing benefits of magnetic therapy result in good part from designing experiments that account for the placebo effect. For example, a study from 1978 describes "the extreme cleverness with which perceptive individuals unintentionally used subtle auxiliary clues to develop impressive records of apparent magnetic field detection" (Tucker and Schmitt 1978). Of course, in many cases, not even "extreme cleverness" is for a test subject to figure out whether they are part of the placebo control arm of a study because real magnets have a propensity to attract loose magnetically-susceptible objects such as paper clips.

As discussed earlier, evidence suggests that deep-penetrating SMFs of at least 0.2 T are required to affect the biophysical properties of membranes (Braganza et al. 1984) implicated in therapeutic responses in humans at the cell level (Wang et al. 2009; 2010). The only plausible way to deliver these fields in a deeply-penetrating manner is to use electrical coils to generate the required moderate strength (e.g., 0.3–0.5 T) magnetic fields. One example of such an instrument is the MME device (Fig. 7.1) developed by AMRi (Bonlie 2001), which requires seven miles of copper coils situated above and below a patient (the entire apparatus is close two storeys in height). In theory, pitfalls that befall efforts to conduct controlled clinical trials using DIY-type wearable magnets (such as attracting, or not attracting) loose paperclips during everyday activities can be avoided by strictly monitoring the treatment environment. In reality, however, when in operation, electricity running through the device needed to generate the SMFs creates a perceptible humming noise, making it obvious whether or whether or not actual treatment is underway. As a result, control subjects in double blind clinical studies (ClinicalTrials.gov Identifier: NCT00325377 and NCT00134524) were subject to recorded MME device noise. Interestingly – and perhaps unsurprisingly – a large placebo effect was observed in these studies that plausibly can be explained by the belief of control subjects that they were undergoing legitimate SMF exposure.

The placebo effect – evidenced by sham-treated test subjects experiencing improvement to long-standing conditions (lower back pain and diabetic neuropathy) that were not responsive to conventional medical treatment at rates comparable to SMF-treated individuals – illustrates the growing realization that placebo treatment is not equivalent to "no treatment." Briefly, placebo effect depends on belief in the effectiveness of the treatment; in fact, the opposite "nocebo" effect has been proposed where a patient who disbelieves in a treatment may experience a worsening of symptoms (Kennedy 1961). Of note, "belief" is a rather ambiguous concept but in theory can be converted into physiological modulation through opoid neurotransmitters whose endogenous production is controlled by the brain.

The placebo effect can be powerful, with attempts to objectively measure its contribution to medical intervention overall ranging from 30 to 40% of overall observed effects of a medicine. The impact of the placebo effect varies amongst treatment modalities and disease conditions with one of the stronger responses reported for the effects of antitussive medicines in patients with acute upper respiratory tract infections. In these patients, 85% of the reduction in coughing was linked to the placebo effect and only 15% to the actual physiological effects of the pharmacological agents (Eccles 2002). It appears that the placebo effect might be equally pervasive and influential in response to SMF treatments and (in a lesson being learned from psychiatry (Horgan 2013)) the field should consider embracing – rather than being embarrassed – by this aspect of magnetotherapy.

7.6 Concluding Comments

This chapter describes various modes of EMF therapy, with the main focus on SMFs. Up to now, this therapeutic modality has both shown promise and has been downplayed, in part due to over-enthusiastic claims by its practitioners. Accordingly, strict guidelines have been proposed to maintain "quality control" when patients are being treated with magnetotherapy in efforts to rigorously establish efficacy against specific medical indications, several of which are mentioned and described in some detail (e.g., pain perception and management, blood flow and vascularization, and neurological regeneration in Sect. 7.4 of this chapter, as well as cancer in Chap. 6 of this book).

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