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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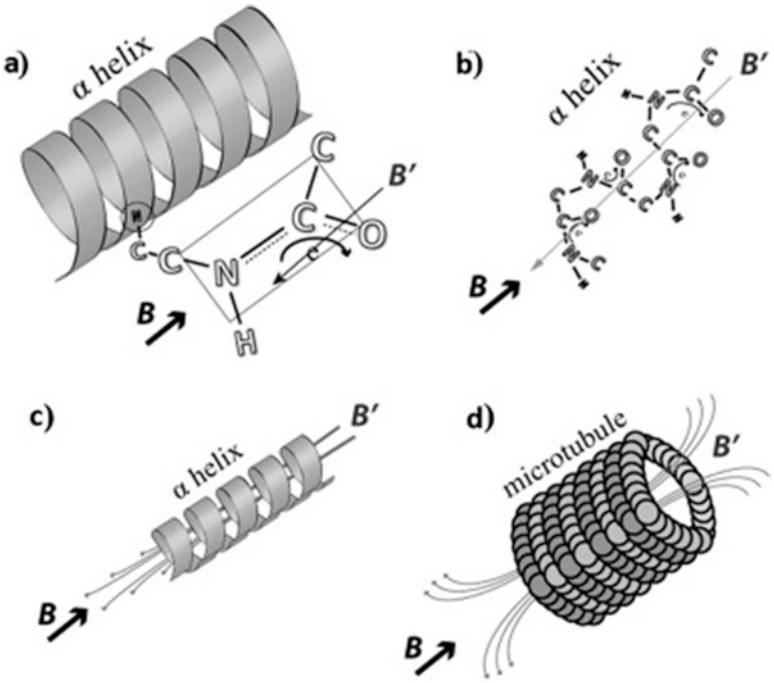

Fig. 4.1 Anisotropy diamagnetism in biological structures. Planar Peptide bonds present in α-helix give it large diamagnetic anisotropy **(a)**. **(b)** and **(c)** show the magnetic vector generated by the helical structures. In microtubules **(d)** the parallel alignment of the peptide bonds with the 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.

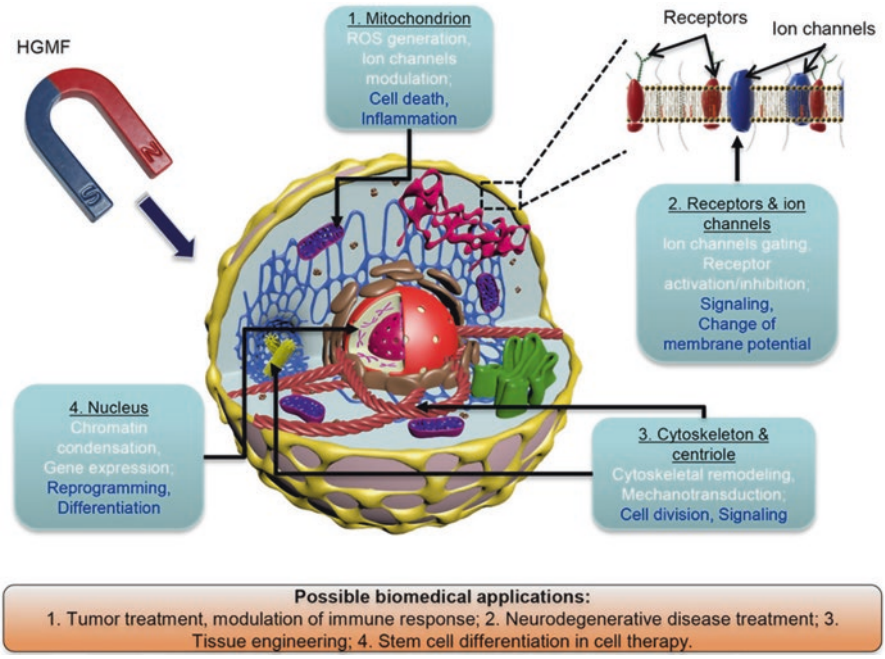

Fig. 4.2 A schematic illustration of the possible applications of high gradient magnetic fields (HGMFs) and intracellular effectors (Reprinted with permission from ref. (Zablotskii et al. 2016). Copyright © 2016, the Author(s). This work is licensed under a Creative Commons Attribution 4.0 International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

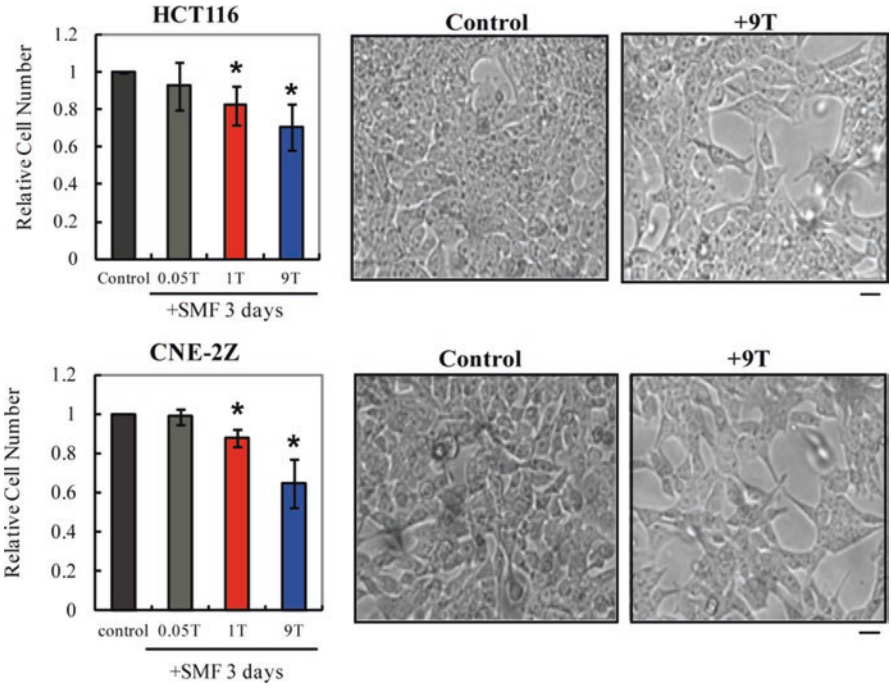

Fig. 4.3 Human nasopharyngeal cancer CNE-2Z and colon cancer HCT116 cell proliferation could be inhibited by SMFs in a magnetic field intensity-dependent manner. CNE-2Z and HCT116 cancer cell were exposed to SMFs of different intensities for 3 days. *Left panels* show the relative cell numbers. *p < 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).

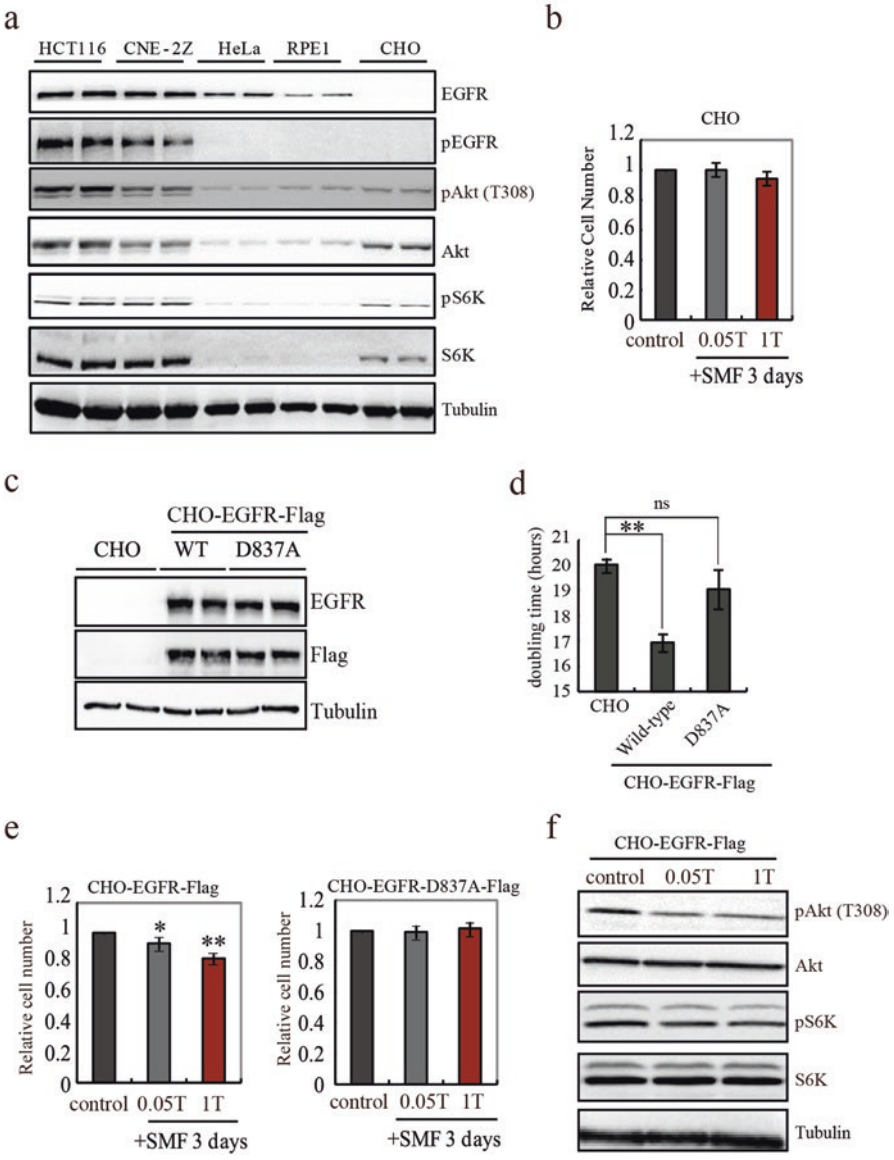

Fig. 4.4 EGFR expression and activity influence the SMF-induced cell proliferation inhibition. CHO cells and CHO cells stably expressing wild-type or D837A (kinase dead) EGFR were exposed to 0.05 T or 1 T SMFs for 3 days before their cell number were counted. (**a**) Representative Western blots are 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

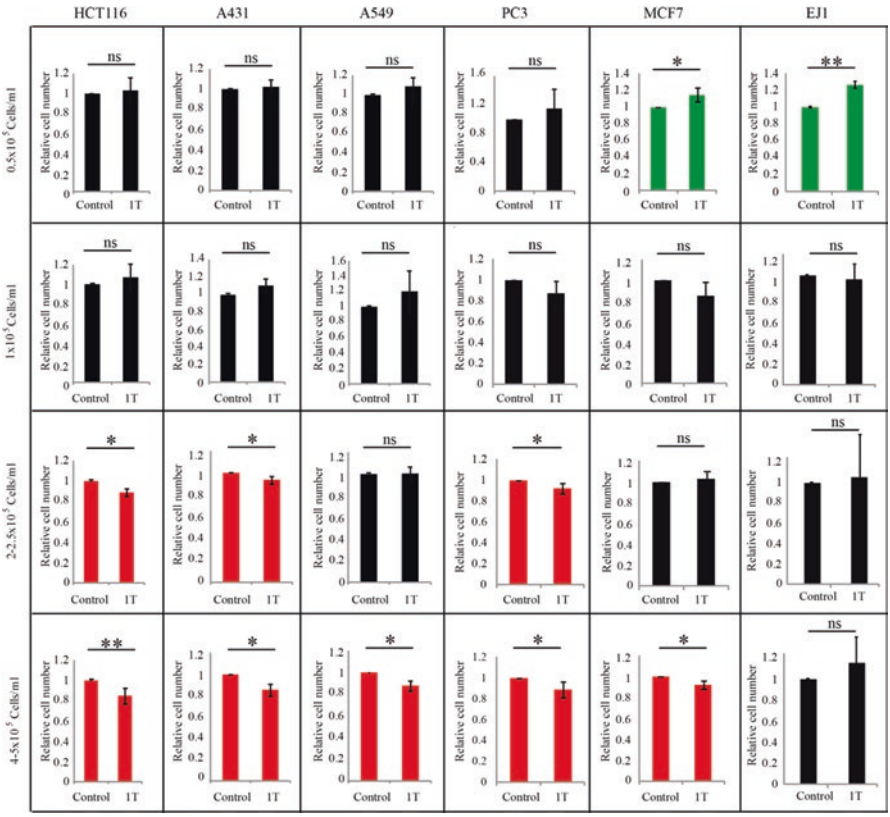

Fig. 4.5 1 T SMF affects multiple human cancer cell lines in a cell density-dependent manner. Different types of cells were plated at different densities one day ahead and treated with 1 T SMF for 2 days before they were counted. *ns* not significant; *, $p < 0.05$; **, $p < 0.01$. *Green* color indicates 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

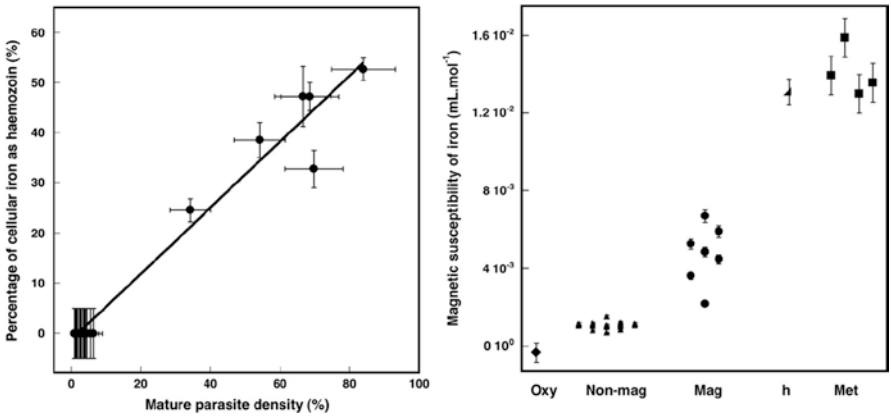

Fig. 4.6 Magnetic susceptibility of iron in malaria-infected red blood cells (RBCs). (*Left*) Percentage of cellular iron converted to haemozoin vs. mature parasite density. (*Right*) Scatter plot of the molar magnetic susceptibility of iron in standard samples of oxyhaemoglobin (Oxy — ◆), haematin (h — ◢), 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

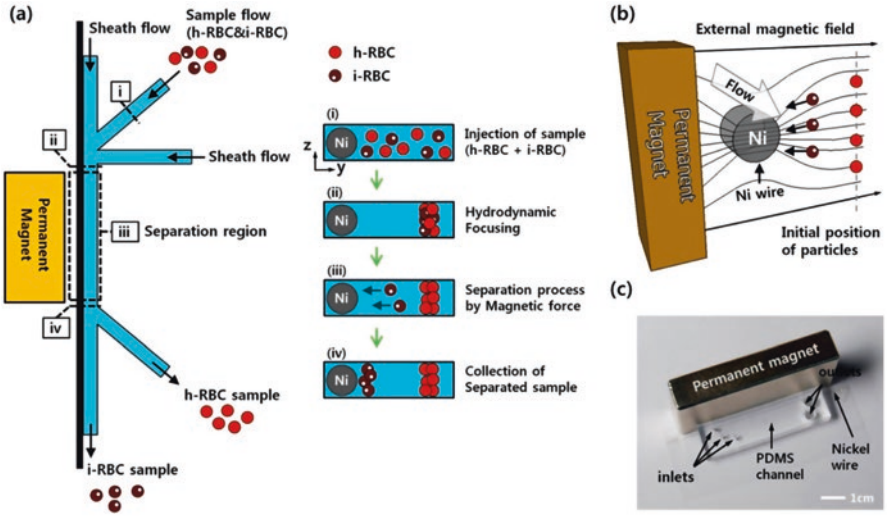

Fig. 4.7 Malaria-infected RBC separation using a high magnetic field gradient. (**a**) Schematic diagram of i-RBC (infected red blood cell) separation using the paramagnetic characteristics of hemozoin in i-RBCs. (**b**) Working principle of magnetophoretic separation with a ferromagnetic nickel wire 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

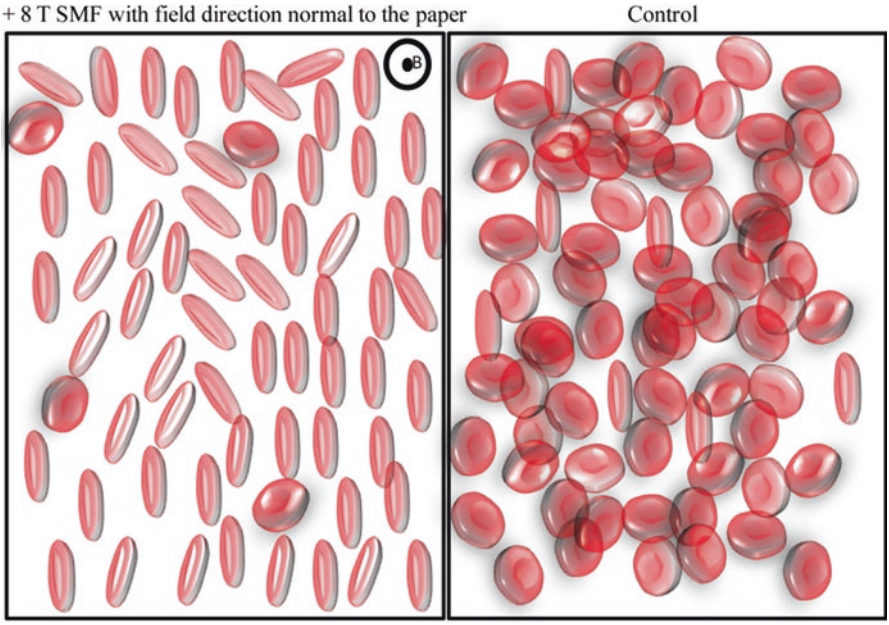

Fig. 4.8 Red blood cells were aligned by an 8 T SMF. Right: Red blood cells in control condition, with no SMF. *Left*: Red blood cells in an 8 T SMF. The field direction was normal to the paper (Illustration was drawn based on results from ref. (Higashi et al. 1993))

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

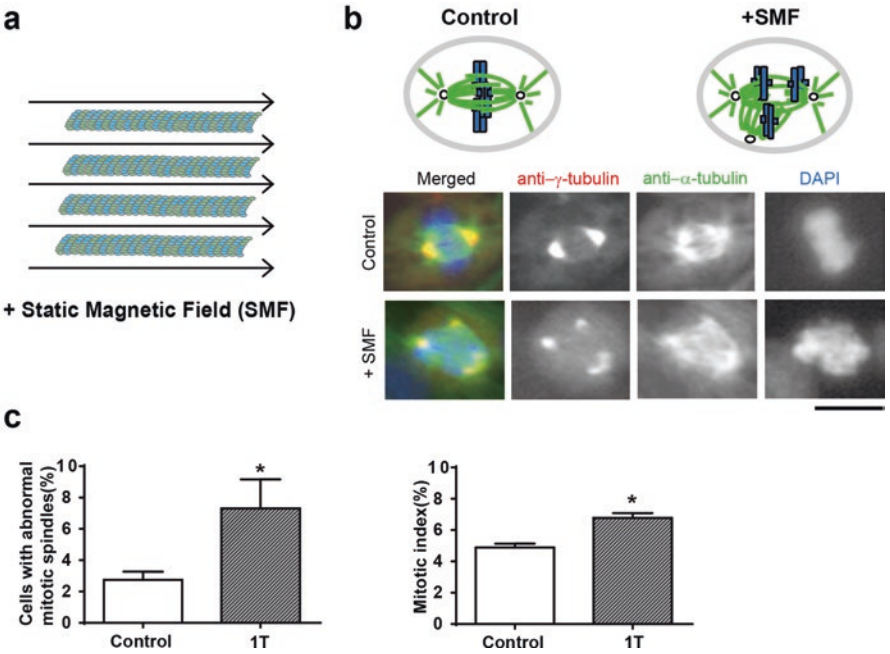

Fig. 4.9 1 T SMF affect mitotic spindles in HeLa cells. (**a**) Illustration of microtubules aligned under the SMF. (**b**) Cartoons and immunofluorescence images show normal bipolar spindles and abnormalmultipolar spindles. Representative images of cells with bipolar spindles and multi-polar spindles 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

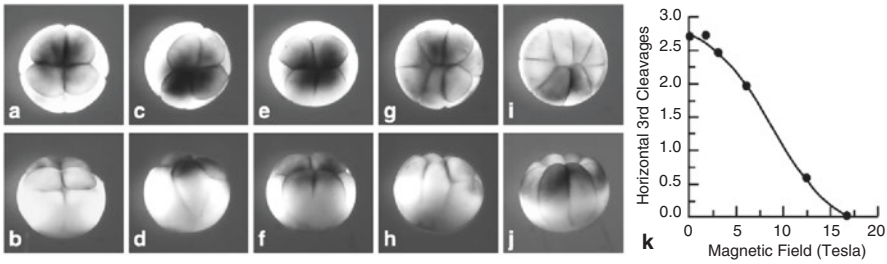

Fig. 4.10 Third cleavage in an AV-parallel SMF. Top (**a**, **c**, **e**, **g** and **i**) and side (**b**, **d**, **f**, **h** and **j**) views of eight –cell embryos from an AV-parallel field, showing the classes of third cleavage reorientation. For the side view, the embryo in the top view was rotated 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,

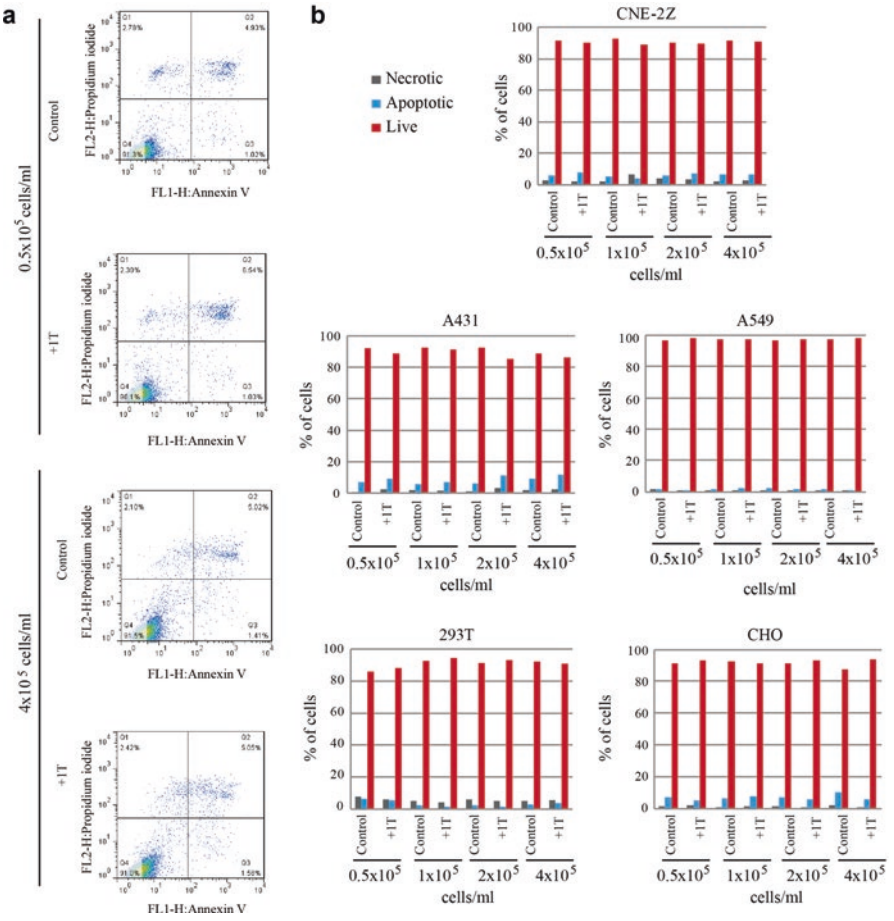

Fig. 4.11 1 T SMF exposure of two days does not promote cell death in multiple cells lines. Various cells were plated at different density one day ahead and treated with 1 T SMF for 48 h before they were analyzed for cell death using Annexin/PI stain and flow cytometry. Representative raw data (**a**) 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

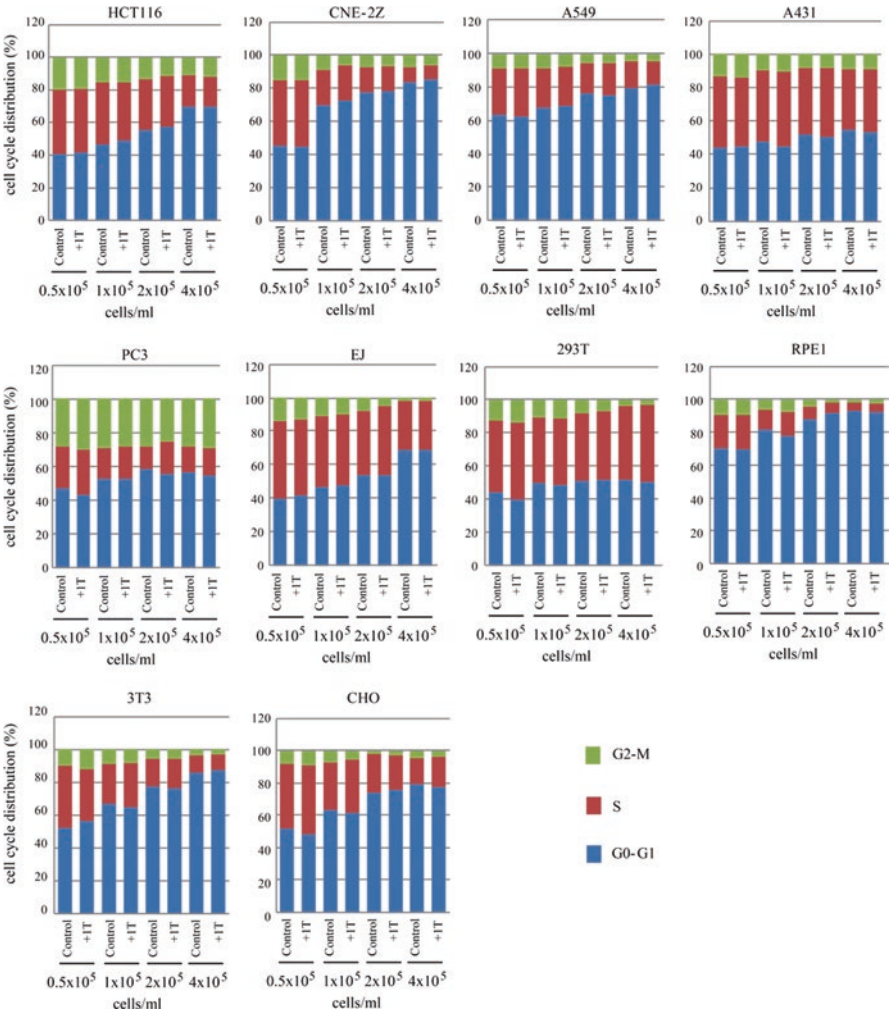

Fig. 4.12 1 T SMF has minimal effects on cell cycle. Various cells were plated at different densities one day ahead and treated with 1 T SMF for 2 days before they were analyzed for cell cycle by flow cytometry experiment. Experiments have been down for at least two times for each cell line and representative 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

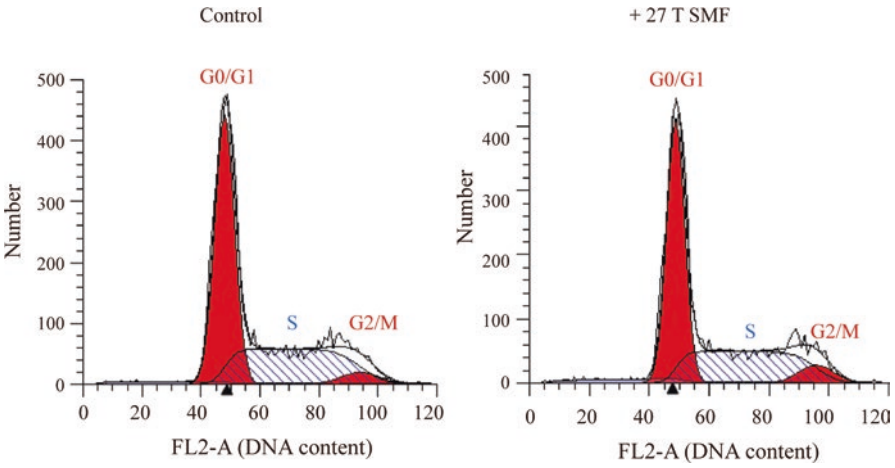

Fig. 4.13 27 T ultra-high SMF does not have obvious effect on cell cycle of human CNE-2Z cells in flow cytometry experiment. We recently exposed CNE-2Z cells to a 27 T ultra-strong SMF for 4 h and did not observe obvious cell cycle changes (Zhang et al. 2017c). The 27 T Ultra-strong SMF was provided 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,

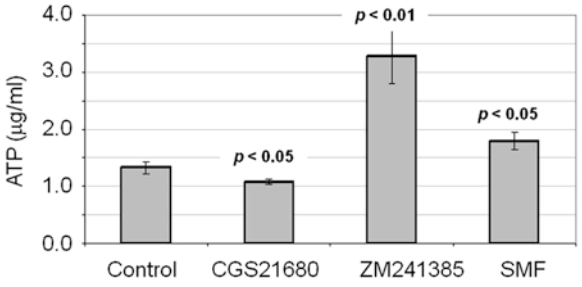

Fig. 4.14 Moderate intensity SMF increases cellular ATP level in PC12 cells. Cells were incubated with 1.0 μM CGS21680 (a selective adenosine A_{2A} receptor ($A_{2A}R$) agonis), ZM241385 (a potent, non-xanthine $A_{2A}R$ antagonist), or exposed to ~0.25T SMF for 6 h (Reprinted with permission from ref. (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

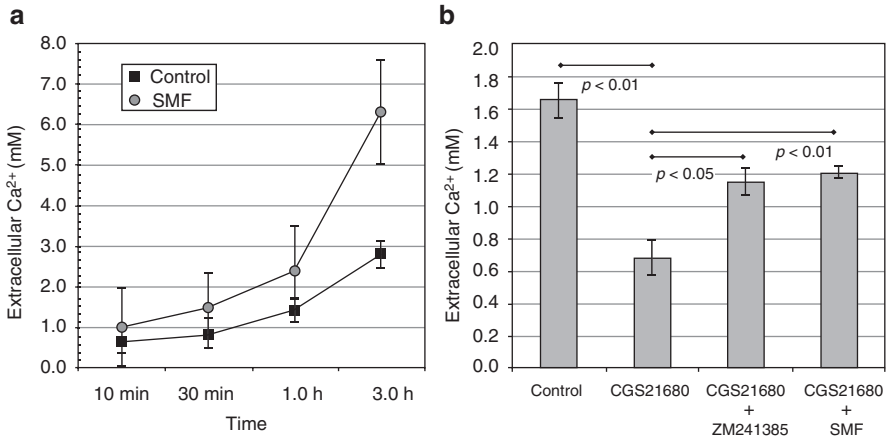

Fig. 4.15 Calcium levels in PC12 cells exposed to moderate intensity SMF, the $A_{2A}R$ agonist CGS21680 or antagonist ZM241385. SMF intensity was around 0.25T. (**a**) Extracellular Ca^{2+} level measured at different time points. $p < 0.05$, n =3. (**b**) Extracellular Ca^{2+} level measured at 3 h. 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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