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Effect of Electromagnetic Fields on Growth of Human Cell Lines

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Abstract High Electromagnetic Field (EMF) with an intensity of 1 mT (Tesla) inhibited the growth of both human normal lung and immune T cell lines down to 20–30%, compared to that of an unexposed case. The human T-cells, Jurkat, were more severely affected by EMF than the human lung cells, which showed a relatively slow cell growth and substantial release of Ca^{+2} (3.5 times higher than the human T-cells). However, the growth of hepatoma carcinoma, Hep3B, was enhanced by twice that of an unexposed case. The EMF intensity and exposure time did not affect the growth of the cancer cells very much, while it significantly affected the growth of normal cells. Accordingly, it is possible that EMFs may play a role in the initiation of cancer. The EMFs disturbed the signal transduction and membrane systems, such that a five times higher amount of PKC-α was released from the cell membrane than in the control. Extended exposure to EMFs, for more than 48 hours, also led to a 90% necrotic death pattern from apoptotic cell death. Finally, EMF at an intensity of 1 mT with a 24-h exposure promoted the differentiation of HL-60 cells to monocytes/macrophages, possibly causing potential acute leukemia.

Key words: Electromagnetic fields, human cell lines, signal transduction, Ca^{+2} release, cell differentiation

Recent concern regarding the expanding use of electric devices, including cellular phones, has intensified since electromagnetic fields (EMFs) have been demonstrated as possible causes of cancer and other diseases [2 20]. The EMFs from many home and industrial appliances at 50 or 60 Hz have been measured to be within a range of 0.01–10 μT (tesla, 1 μT=0.01 Gauss) and even up to 100 μT, which is substantially higher than EMFs from naturally occurring fields of up to 7–10 μT. There have been several epidemiological and clinical studies on EMF-related cancers, particularly childhood leukemia [5, 12, 18, 25]. These studies have also included some cellular studies, describing EMF-induced effects on biological systems [3, 11]. However, there still lacks convincing evidence on EMF-related effects on the pathways regulating cell proliferation and cell growth. Relatively little attempts have been made to directly explain the effects [1, 27], even though several results have been made about the effects on the cell growth [5, 10, 15]. So far, the experimental findings have been almost uniformly unsuccessful, and have not covered all aspects of EMF-induced biological responses. Moreover, the conflicting results from these experiments complicate interpretation of the significance of EMFs on human health. As such, the effect of short- or long-term exposure to EMFs on the human pathology still remains controversial [23]. However, it is not so easy to conduct clinical trials by exposing EMFs directly to human beings. Therefore, it should be indirectly accomplished by carrying out cell culture experiments using human cells. From this standpoint, it is essential to conduct *in vitro* laboratory experiments that can provide understandable evidence about both physiological and biochemical mechanisms; for example, the changes of a key protein, such as protein kinase C (PKC-α) in signal transduction and the cell membrane (concentration of cytosol Ca^{+2}), plus cell proliferation and the cell-death pattern (apoptosis/necrosis), etc [4, 6, 9, 16, 17, 30]. Accordingly, this work investigated quantitative aspects of exposure to EMFs and changes at both the cellular and molecular levels.

Materials and Methods

Cell Culture and Exposure Apparatus

Normal human embryonic lung cells (HEL 299, ATCC, U.S.A.), human T-cells (Jurkat), and human hepatoma carcinoma cells (Hep3B, ATCC, U.S.A.) were all grown in

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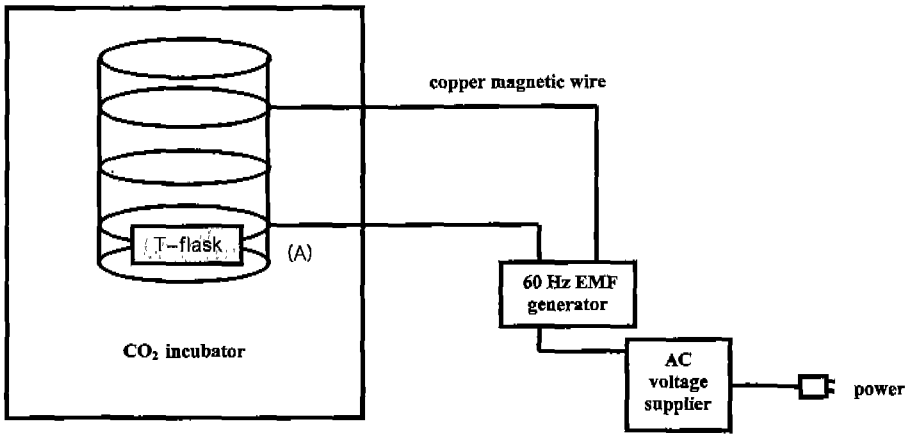

Fig. 1. Diagram of EMF-generating apparatus.
A 75-T-flask was located inside the main frame (A) which was then place in a 37°C CO_2 incubator for the growth of the human cells.

DMEM/F12 basal medium containing 365 mg/l of L-glutamine (GIBCO, U.S.A.) and 20% FBS (GIBCO, U.S.A.) in a 5% CO_2-supplying incubator at 37°C. Human promyelocyte, HL-60, was grown in an RMPI 1640 (GIBCO, U.S.A.) basal medium with 20% heat inactivated FBS (GIBCO, U.S.A.) and maintained in a 75 T-flask. All cultures were maintained to provide the same passage numbers and fresh medium so as to avoid any lack of nutrients before being put into an EMF generator.

A 75 T-flask with 5×10^4 viable cells/ml was placed inside an EMF generator consisting of 2,500 turns of copper magnetic wire in a near Helmholtz configuration, as shown in Fig. 1. The power was supplied by an AC voltage supplier (Kangdong Ind., Korea) with the current of 55~60 Hz frequency. The magnetic flux density was measured by a magnetometor (9200, F.W. Bell Inc., U.S.A.) on the top of a T-flask. The geomagnetic field without power to the coil was negligible. The EMF intensity was changed by controlling the output of the audio signal and sinusodial generator (DSG2, SETOL Co., U.S.A.), and was maintained at a maximum intensity of 1 mT. The EMF generator and cells were put into the 37°C CO_2 incubator with continuous exposure to EMFs for 12 to 48 h without changing the medium. All other environmental factors for each run were identical, except for the exposure time and intensity. The growth of the cells not exposed to EMFs was also observed as the control (referred to as the unexposed case).

Measurement of Cell Density and Biological Analysis

The cell density was measured every day using the trypan blue dye exclusion method [8]. One-hundred μl of the cell cultures were mixed with 4 μl of a dye solution which contained acridine orange and ethidium bromide (1:1 v/v). Then, the stained cells were counted through a fluorescent microscope (Olympus, Tokyo, Japan). The number of stained cells was divided by the number of total cells to estimate the percentage of apoptotic or necrotic cells. The ratio of apoptotic or necrotic cells was also calculated by dividing two percentages of the cells [8, 13]. The cytosol Ca^{+2} concentration was measured by first adding 2 g/l of Indo-1 penta acetoxymethyl solution into a T-flask containing 1×10^5 viable cells/ml. The cells were cultivated in a 37°C CO_2 incubator with continuous exposure to EMFs for 12 to 48 h. Next, the collected cells were suspended with 2 ml of a Na^- solution containing 135 mM NaCl, 5 mM KCl, 1 mM MgCl, 10 mM Glucose, and 20 mM HEPES. The fluorescent intensity, F, of the suspension was measured by fluorescent spectrophotometery (Perkin Elmer, U.S.A.) at 331/398 nm (excitation/emission). The Ca^{+2} ion concentration in the cytosol, $[Ca^{+2}]$, was calculated as follows:

$$[Ca^{+2}]=K_d(F-F_{min})/(F_{max}-F) \quad (1)$$

where K_d, the Ca^{+2} dissociation constant in the Indo-1 solution, was 250 nM, F_{max} is the fluorescent intensity of the suspension measured before exposure to EMFs, and F_{min} is the fluorescent intensity of the Indo-1 solution without Ca^{-2} calculated by the following equation (2).

$$F_{min}=(1/12)(F_{max}-F_{auto})+F_{auto} \quad (2)$$

F_{auto} was measured using the Indo-1 solution itself with the addition of just 2 mM $MnCl_2$ at 331/398 nm. The analysis of the PKC-α was performed using the Western blot method, where the EMF-exposed Hep3B cells were collected and suspended in a 1 ml buffer solution containing 2 mM EDTA, 4 mM EGTA, 10 μg/ml leupeptin, 25 μg/ml aprotinin, 100 μg/ml phenylmethylsulfonyl fluoride, 0.25 M sucrose, and 20 mM Tris-HCl, pH 7.5. The suspended cells were then broken by a sonicator in an ice-water bath. A pellet was collected by centrifugation at 14,000 ×g for 30 min at 4°C, and the collected pellet was resuspended in 0.5 ml of the above buffer solution with the addition of 1% SDS and 10% glycerol. The suspension was eletrotransferred by 50 cm^2 PDVF membrane electrophoresis at 400 mA for one

hour in a Towbin buffer. The human PKC IgG (Genzyme, U.S.A.) was blotted on a 3-mm paper and exposed to an X-ray film for 5–30 min [28].

The differentiation of the HL-60 cells was assayed using the nitro blue tetrazolium (NBT) dye reduction method. The EMF-exposed and -unexposed cells were washed with PBS and incubated for 45 min at 37°C with an equal volume of NBT solution (1 mg/ml). Therefore, the blue formazan deposit in the cells was determined by an inverted microscope [21]. Triplicate determinations of at least 100 cells were performed for each treatment condition.

RESULTS AND DISCUSSION

Figure 2 compares the effects of the EMFs on the growth of the normal cell lines, human lung cells (HEL 299), and human T-cell (Jurkat) at two different intensities for a 24-h exposure. As shown in Figs. 3, 5, and 6, the EMFs definitely influenced the normal human cell growth, since a high EMF intensity significantly reduced the cell growth compared to that in Fig. 2. At the highest EMF intensity of 1 mT, cell growth decreased to 20% compared to that of unexposed cell growth. It was also found that the higher EMF intensity reduced the cell growth more than the lower intensity, thereby comfirming that EMFs could inversely affect normal human cell growth. It is interesting that a sharper drop in the cell density was observed in the death phase at the high EMF intensity than in the exponential or

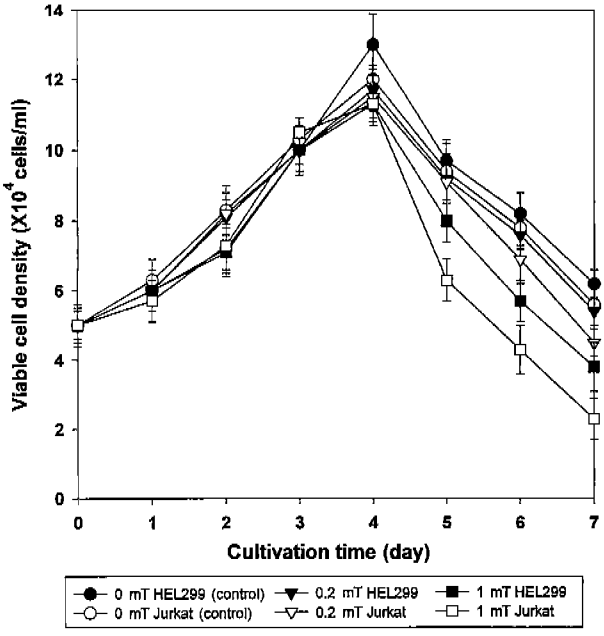

Fig. 2. Effect of electromagnetic field on growth of normal human lung cells, HEL299, and human T-cells, Jurkat, after 24-h exposure at two different electromagnetic intensities.

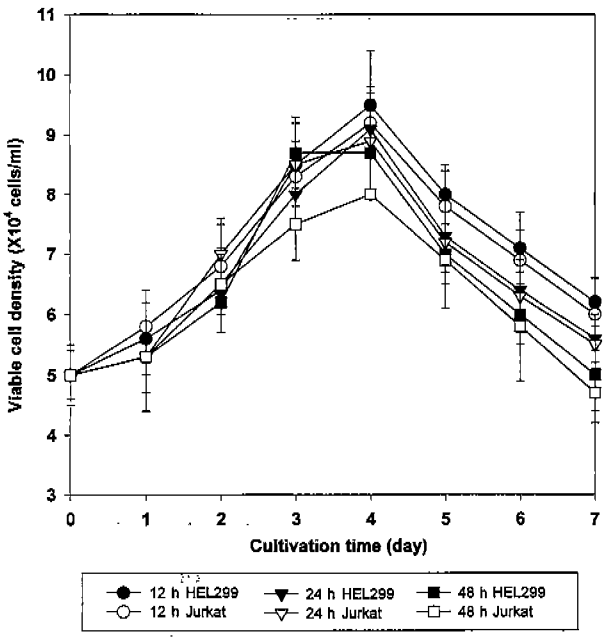

Fig. 3. Effect of exposure time to EMFs on growth of human normal lung cells, HEL299, and human T-cells, Jurkat, at an electromagnetic intensity of 1 mT.

stationary phase, compared to the low EMF intensity. Figure 3 shows the relationship between the EMF exposure time and cell growth at the highest EMF intensity, 1 mT, for two different cell lines. The growth of both cell lines was decreased as the exposure time increased, and, as shown in Fig. 2, the higher intensity affected the cell growth more severely than the lower intensity. The human T-cells seemed to be more influenced by the EMF exposure time than the normal lung cells, maintaining only 8×10^4 viable cells/ml as the lowest maximum cell density, while the normal lung cells maintained a relatively stable maximum cell density.

Figure 4 compares the growth patterns of human hepatocellular carcinoma cells under different EMF exposure conditions. The growth of the cancer cells dramatically increased up to two times higher than in the case of no exposure. However, the cell growth did not increase much as the exposure intensity and time (data not shown) increased. Not much difference in cell growth was found between the lowest (0.2 mT) and highest (1 mT) EMF intensities. This may indicate that EMFs can trigger the enhanced growth of cancer cells, however, they cannot affect the cell growth continuously. Both the intensity and the exposure time of the EMFs signficantly decreased the normal cell growth, as shown in Figs. 2 and 3, compared to the decrease of cancer cell growth. This is in support of the findings that a 60 Hz magnetic field enhanced the c-myc transcription level in human leukemia cells [8, 24]. It may suggest that EMFs play an important role in initiating cancer cells, yet not in proliferating them. Table 1 compares

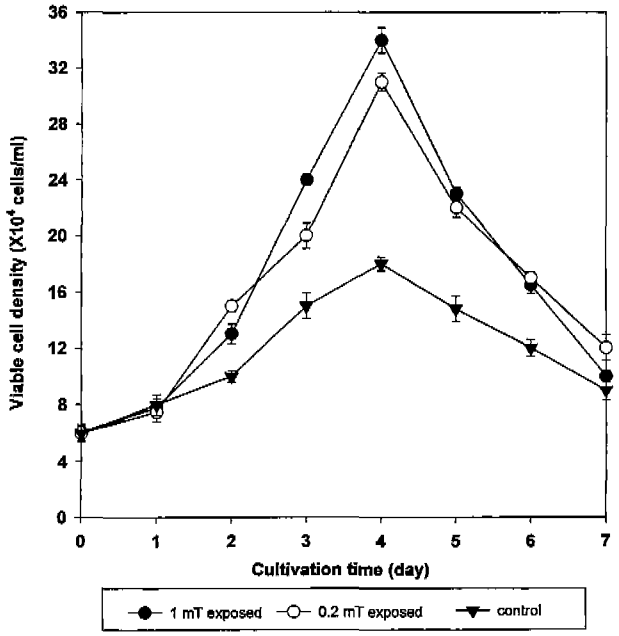

Fig. 4. Growth of human hepatocellular carcinoma, Hep3B, relative to various intensities of EMF after 24-h exposure.

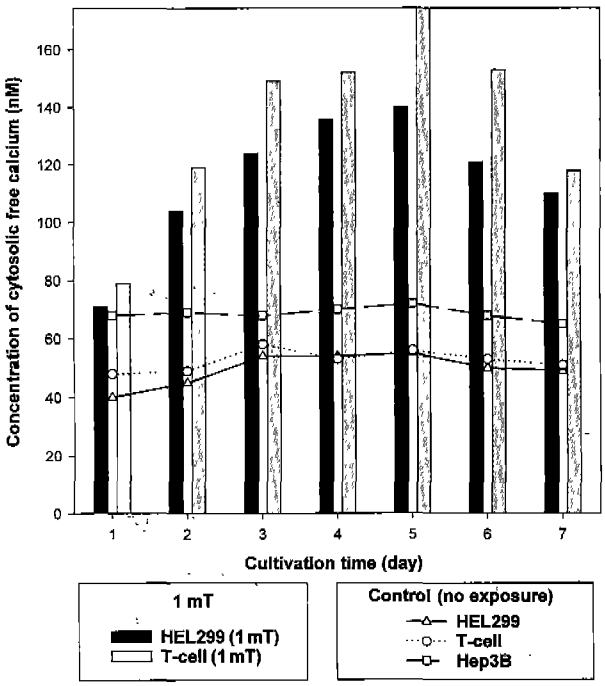

Fig. 5. Concentration of cytosolic free calcium from HEL299 and T-cell at 1 mT EMF intensity of 1 mT after 24-h exposure.

the growth characteristics of the normal and carcinoma cells with and without EMF exposure. The maximum cell density and maximum specific growth rate for both normal cell lines decreased as the EMF intensity increased, as shown in the previous figures. In contrast, the growth of the carcinoma cells dramatically increased at high EMF intensity, with the fastest growth rate at 0.309 (1/day). The decrease in the specific growth rate with EMF intensity was also very large for the human T cells, which maintained the lowest cell density.

Figure 5 shows the results on the cytosolic free calcium ions released from the cells. Several reports have proved that an increase of calcium ions in the cytosol causes membrane damage, resulting in malignant cells [6, 9]. It was found that there was not much change of released Ca^{+2} concentration at no exposure of the three cell lines, showing the range of 40 to 78 nM. The data presented in Fig. 5 also supports the hypothesis that EMFs strongly relate to the initiation of potentially cancerous changes in the normal cells, as shown in Figs. 2, 3, and 4. However, the release of cytosolic calcium ions from HEL 299 or T-cell lines after 24 h of exposure to an EMF intensity of 1 mT was greatly increased up to 3.5 times higher than that of control. The cytosolic calcium was the highest when the cell growth was the lowest, which indicated that EMFs affected the membrane of normal cells, resulting in a decrease of cell growth. The free calcium concentration in the human T cells was much higher than that in the HEL 299 cells, whose pattern was similar to the overall growth of both cells, exposed to EMFs. As such, it was clear that the EMFs had a more negative effect on the human T cells than on the other normal human cell, HEL 299, thereby hampering the signal transduction pathway.

Figure 6 illustrates the change in the death pattern of the normal lung cells at an intensity of 1 mT with 24 h of exposure. After exponential growth, 80–90% of the normal cells turned into necrotic cells. The number of apoptotic cells to the total number of viable cells remained relatively stable, and most of the cells became necrotic cells. The EMF-exposed cells could not maintain the apoptotic death phase, thereby resulting in a rapid drop in the viable cell numbers, as shown in Figs. 2 and 3. The necrotic cells never returned to apoptotic cells when the cells moved into

Table 1. Comparison of growth parameters of several cell lines exposed to EMFs for 12 h.

EMF intensity (mT)	Maximum cell density $X\times10^5$ (viable cells/ml)			Specific growth rate μ (1/day)		
	HEL 299	Jurkat	Hep3B	HEL 299	Jurkat	Hep3B
0 (control)	1.32±0.01	1.21±0.09	1.82±0.03	0.242±0.001	0.236±0.012	0.229±0.011
0.2	1.24±0.04	1.15±0.07	3.04±0.02	0.231±0.004	0.211±0.009	0.294±0.007
1.0	1.10±0.15	1.02±0.05	3.43±0.08	0.229±0.004	0.208±0.014	0.309±0.010

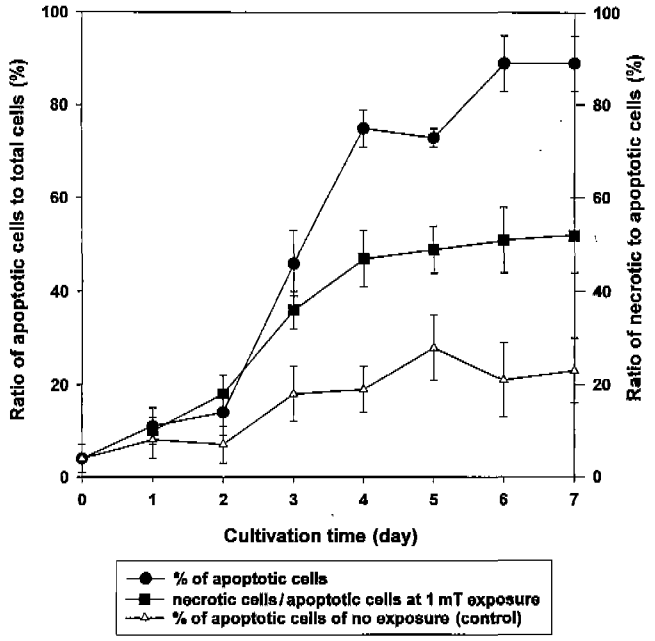

Fig. 6. Change in apoptotic and necrotic cell numbers for normal human lung cells, HEL299, at EMF intensity of 1 mT with 24-h exposure.

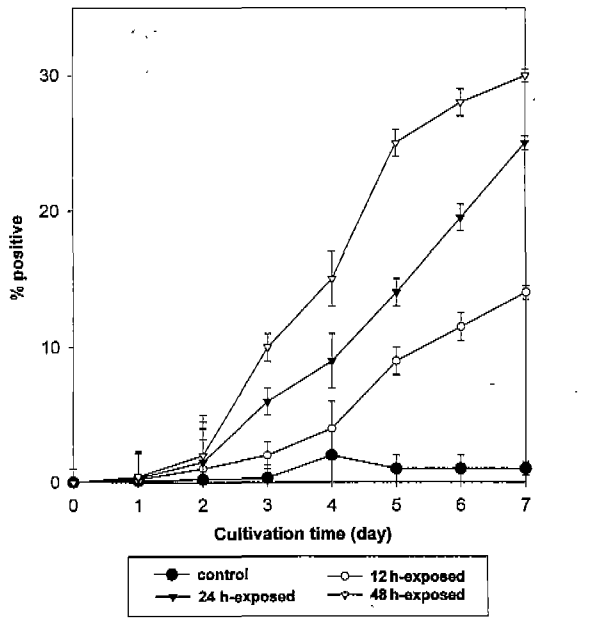

Fig. 8. Extent of differentiation of HL-60 cells after exposure to an EMF intensity of 1 mT for several exposure times.

the necrotic death phase. This death pattern seemed to be different from a normal cell death, compared to that of the control in Fig. 6, and a similar pattern was also reported elsewhere [22]. This is one of the reasons that the EMF-exposed cells sharply decreased at the latter periods of the cultivation. Figure 7 also shows the effect of EMF on the death pattern of the human carcinoma cells after several electromagetic intensities with 24-h exposure. An increase in the translocation of PKC-α is a sign of the potentiation of EMF-induced apoptosis in Hep3B cells, since most hepatoma cellular carcinoma cells express the multi-drug resistance-1 genes [14]. With a higher EMF intensity at over 24 h, higher amounts of PKC-α were translocated, meaning that EMF definitely initiated apoptotic to necrotic cell death.

Figure 8 shows the induction of the differentiation of HL-60 cells exposed to an EMF intensity of 1 mT relative to the exposure time, since there has been a previous report that initiating the differentiation of HL-60 cells can induce

Fig. 7. Effect of EMF on PKC-α of human hepatocellular carcinoma, Hep3B, relative to various intensities of electromagnetic field with 24-h exposure.
A: control; B: 0.2 mT; C: 0.4 mT; D: 0.6 mT; E: 0.8 mT; F: 1.0 mT.

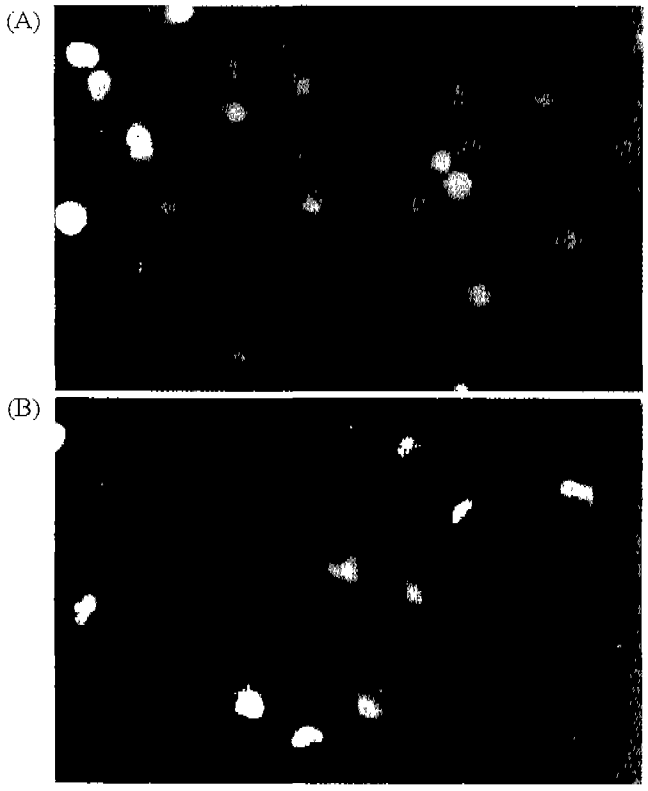

Fig. 9. Morphological changes of HL-60 cells before (A) and after (B) being exposed to an EMF intensity of 1 mT for 24 h.

complete remission in patients with acute promyelocytic leukemia [7]. It was found that the exposure time is very important in differentiating HL-60 cells, because relatively large portions of the cells (<55%) were differentiated, while only 10–20% of the cells was differentiated for 12–24-h exposure. A sharp increase of differentiated cell numbers was also observed for the case of long exposure time (48 h) at the latter period of culture. However, a very small number of HL-60 cells (>5–6%) were differentiated for the unexposed case. It means that HL-60 cells may not be spontaneously differentiated into monocytes/macrophages under normal conditions for short-period cultivation, and long exposure of EMF triggers the differentiation of the cells. Figure 9 shows the morphological change in the HL-60 cells based on a comparison of the shape of the cells before and after being exposed to 1 mT intensity. Most of the cells were sharp and round before being treated (A). Then, after 24 h of exposure, many cells differentiated to macrophages or monocytes. This also demonstrates that EMFs can induce the differentiation of HL-60 cells, as shown in Fig. 8.

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