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# Low Intensity (0.2T) Static Magnetic Field for Dispersing Nucleus Ankyrin G in Human Cultured Glioblastoma Cells

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## Abstract

Biomagnetism is one of important biotechnology fields for manipulation of cell lines. However, its peri-cellular level regulation, upon stimulation is not fully developed. Glioblastoma U87 and U251 represent a malignant model in rapid growing cancer. We focused in cellular level dispersion of static magnetic fields (0.2T=2000 ± 600 Gauss), using its fast growing properties. As a result, cytoskeletal protein nuclear Ankyrin G was dispersed. Membrane barriers in TEM microscopy indicated the membranous apparatus change. Our findings bring an insight that static magnetic stimulation creates a specified cytoplasmic intracellular pattern.

**Keywords:** Static magnetic field; Glioblastoma; Ankyrin G

## Introduction

Glioblastoma multiforme, is a common malignant brain tumor, with a 5 year survival rate of less than 5% [1-3]. It is currently treated with radiotherapy and chemotherapy [4-6]. The current strategies are effective, however, the effect of current therapies is not sufficient [7,8] with high rate of recurrence [9-11]. Side effects include immune-depressant effects [12], secondary tumors [13]. Due to handicaps of widely applied established cures, alternative therapies are in clinical research such as specific maker target therapy [14-16] and supplemented magnetic therapy [17,18].

Static magnetic fields regulate the movements of molecules [19,20]. Previous literatures report effects in breast cancer [21] and showed changes of TUBGCP3 regulating material in the application of static magnetic field [22]. Ankyrin G is used for regulation of ion channels and formation of nuclear membrane apparatus along with spectrin [23].

Additional molecular evaluation was done on proteins associated in nuclear membrane formation and metastasis by assessing the localization of these molecules. TEM microscopy with applied 3D contrast image analysis was performed to confirm the molecular information.

## Materials and Methods

### Cell line

Human glioblastoma U87MG and U251MG cells (American Tissue Culture Collection) were cultured in DMEM, supplemented with 10% fetal bovine serum, 100 units/mL of penicillin and 100 μg/mL streptomycin, at 37°C in a humidified incubator containing 5% $CO_2$ and 95% air.

### Application of magnets

Static magnetic fields (1400-2600 Gauss, about 1/5 intensity of MRI, measured by GM08 Gaussmeter, Hirst Magnetic instruments Ltd., England) exerted by permanent magnets were applied to 24-well and 96-well by attaching magnets one the bottom of the well. The north (N) and south (S) poles were randomly arranged, or studied separately as there were controversial reports that elucidate to have differences or no effects of N and S poles [24,25]. The magnets were applied in bottom of the wells with a distance of 0.1-0.3 cm to the cells. The cells were cultured on a plastic shelf (75-T) 4.0 ± 0.2 cm above the metal shelf, so the metal shelf does not influence the magnetic field within the wells. Separate incubators were used for the control and treated wells so that the magnetic field would not affect the control. Incubation was done for 48 ± 4 h.

### Immunocytochemistry

The localization of ankyrin G was measured. Before immunostaining, the 24-well plates were applied with either N or S pole of static magnetic field and labeled. For immunostaining PBS-washed cells were fixed in 4% paraformaldehyde, permeabilized in 0.1% Triton X-100 in PBS, blocked in 1% bovine serum albumin in PBS and were immunostained with antibodies against Ankyrin G (Santa Cruz Biotechnology, 1:200 dilution, polyclonal). The additional statistical confirmations were made for the differently localized proteins according to application of static magnetic fields.

### Tunneling Electric Microscopy (TEM) imaging

TEM imaging was made for U87 and U251 before and after application of randomly arranged N and S pole magnets. U87 and U251 cells were fixed as 1 mm tissue blocks in 4% formaldehyde and 1% glutaraldehyde in 0.1 M PB (pH 7.4) for at least 2 h to overnight. The sample was then immersed in 8% (0.2 M) sucrose in 0.1 M PB 3 × 15 min. After all, the sample was post fixed in 1% osmium tetroxide in 0.1 M PB for an hour.

To assess 3D contrast of TEM microscopy, Parameterization-based Numerical Method for Isotropic and Anisotropic Diffusion Smoothing on Non-Flat Surfaces [25] was used. Using a parameterized representation of the surface, compute a solution to the diffusion equation in which metric tensors are used to account for the curvature of the surface and intrinsic distances.

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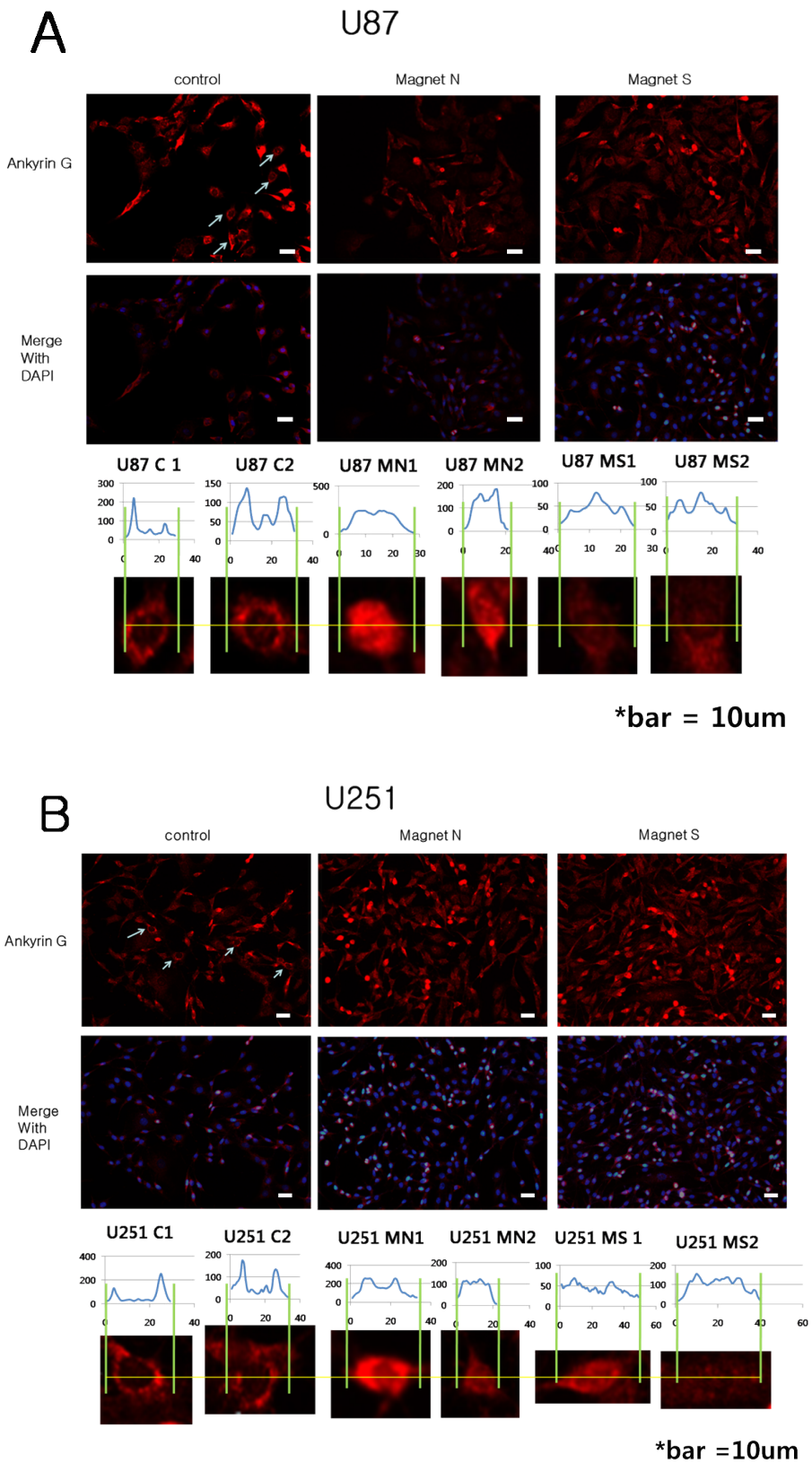


**Figure 1:** Magnet treatment and the immunocytochemistry of ankyrin-G (A:U87, B:U251). Controls seemed to have ankyrin-G configurations in circular structures around the nucleus, which supported the nuclear membrane (white arrows). However, we found these highly structured cells had a lower proportion of organized ankyrin after the application of N and S pole magnets, showing rather irregular protein distributions. The ankyrin-G staining was merged with the DAPI stain, to reveal the relative locus within the nucleus.

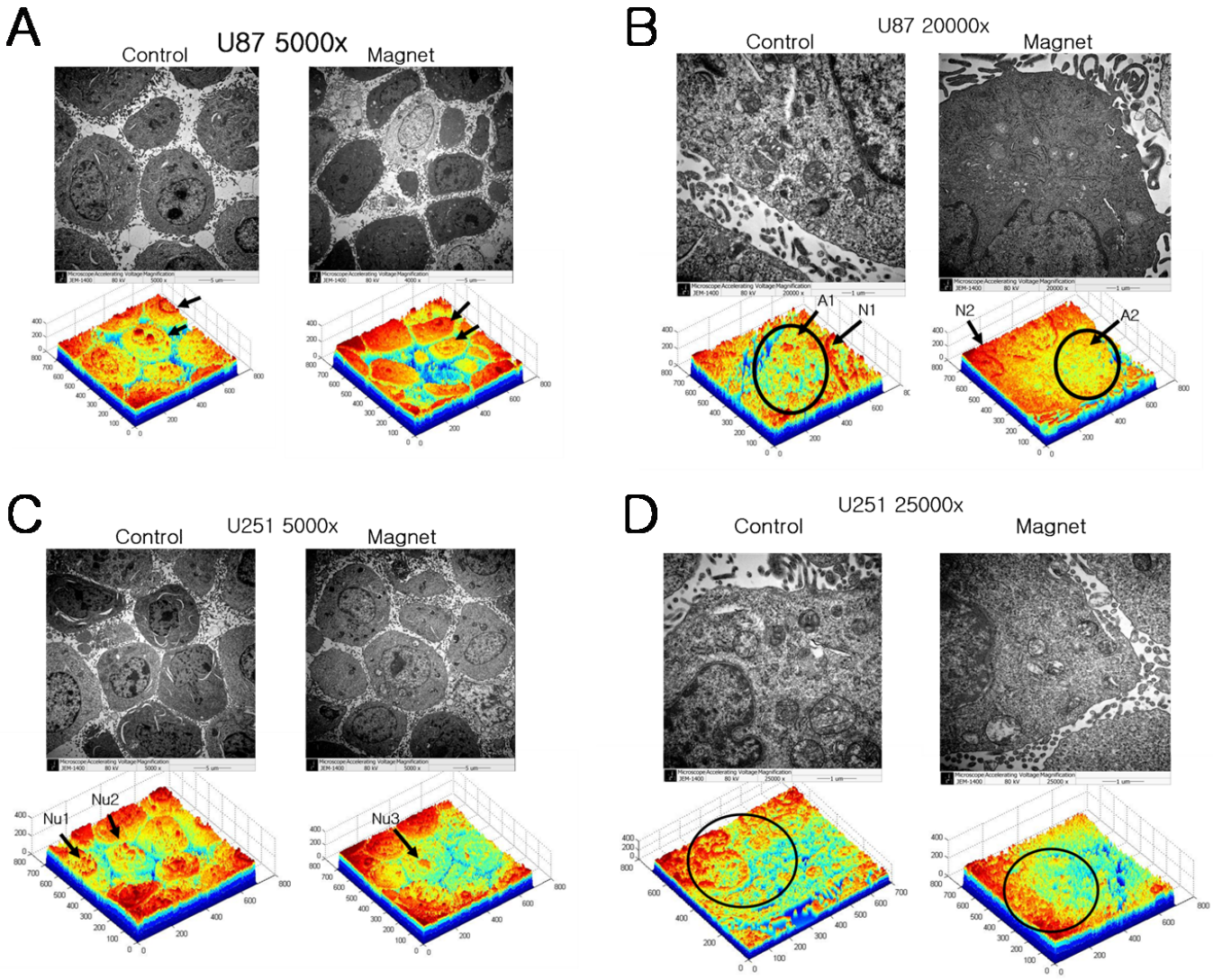


**Figure 2:** TEM microscopy and 3D contrast conversion of U87 and U251 cells. After magnet treatment (48 h, 2000 ± 600 Gauss), the nuclear membrane boundary shows less contrast as compared to the control group. The control's contrast is distinguishable, whereas the contrast is rather unclear in the magnet-treated group (A&C: 5000x and B,D: 20000x or 25000x). In addition, the subcellular organelles were highly organized and positioned in the controls (A1 arrow) but rather dispersed after exposure to magnetic fields (A2 arrow). In addition, nuclear boundaries became unclear upon treatment with magnets (compare N1 arrow and N2 arrow). In, U251 cells, with magnets applied (48 h, 2000 ± 600 Gauss), the nuclear membrane boundary shows less contrast than in the control group, which is consistent with the results for U87. Also, after application of the magnetic field, the nucleolus has a light image (A, Nucleoli Nu1 and Nu2 are dark shapes, whereas magnetic field-treated nucleolus Nu3 shows a light contrast).

### Migration assay

Cells were plated onto the 24-well plate to create a confluent monolayer and incubated the plate properly for approximately 6 h at 37°C, allowing cells to adhere and spread on the substrate completely. The required number of cells for a confluent monolayer depends on both the particular cell type and the size of dishes and need to be adjusted appropriately. The cell that penetrated the monolayer, with 8 μm in diameter, was counted. The cells were observed under a phase-contrast microscope [26].

### Statistical analysis

In immunocytochemistry, Pearson's Chi-Square test was assessed to confirm the relative localization of proteins; the ratio between total sum of nuclear area and total sum of protein area was calculated by depicting a mathematical boundary in control and magnet applied cells. After all, null hypothesis, which states that the ratio of (magnet treated group): (control)=1:1 and alternative hypothesis, which states that the ratio of (magnet-treated group):(control) ≠ 1:1 was set to be confirm the Chi-Square test. For making boundary contour lines, three dimensional contrast visual images was made with Gaussian Kernel Filtering method [25] and divided by sections according to its height portion range from 0 to 150.

## Results

### Protein localization and immunocytochemistry

Ankyrin G localization was unarranged in both N or S pole magnetic fields, whereas the boundary was clearer in control in both U87 and U251 cells (Figure 1). Cells with mitotic stage had high expression of

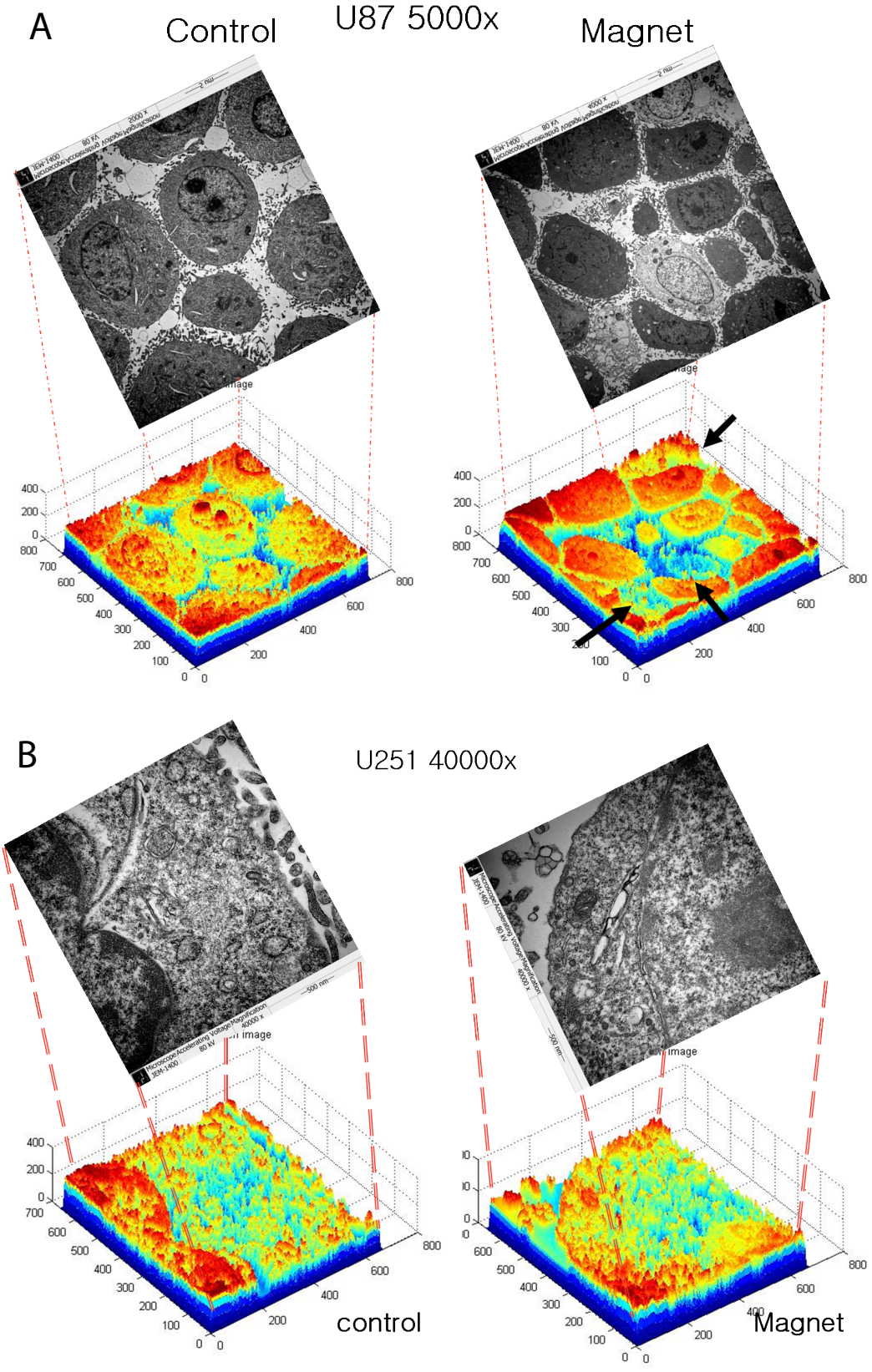


**Figure 3:** Cytopathic differences and envelope focus in magnetic treatment. **A:** We found the white-appearing cells in the groups of cells that received magnet treatment but not in the control group. **B:** For nuclear envelopes, they appear with a blue color or do not appear at all in 3D contrast conversion imaging.

Ankyrin G, but the reason was not clarified. Confocal microscopy was done to confirm the changes in immunocytochemistry of fluorescence microscopy.

### TEM microscopy by using static magnetic fields

TEM microscopy image was obtained and was analyzed by 3D contrast conversion. In U87 and U251 cells, with the application of magnets with random poles (48 hours, 2000 ± 600 Gauss), the nuclear membrane boundary shows less contrast compared to the control group. Also, the subcellular organelles were highly organized and positioned in control (A1 arrow, Figure 2A) but rather dispersed by the application of magnetic fields (A2 arrow, Figure 2B). Nuclear boundaries become unclear in the application of magnets (compare N1 arrow and N2 arrow, Figure 2B). Figure 3 shows TEM microscopy and 3D contrast conversion. The nucleolus also showed light image with static magnetic fields (Nucleolus Nu1 and Nu2 show dark shape whereas magnetic field applied nucleolus Nu3 shows light contrast).

The nuclear membrane structure was relatively unclear in the cells with static magnetic fields applied. The dielectric constant of water molecules are altered by static magnetic fields and phosphate and energy degradation process can also be interfered by alterations in dielectric constant of water molecules [27]. Therefore, the sole role of facilitating hydrolysis in phosphates by static magnetic fields in molecular level might have changed the envelope properties of the cell. Cellular process including signal transduction requires phosphate as energy source and signaling.

## Discussion and Conclusion

Our study focused in the subcellular structure orientation and the regulatory effects of static magnetic fields in U87 and U251. Especially, Ankyrin G, which were associated with the cytoskeletal and proliferation properties showed cytoplasmic delocalization. Nuclear membrane formations were an issue in TEM microscopy.

Ankyrin G formation had circular apparatus that arranges the nucleus, but showed relatively random distribution by application of magnets. The localization was concentrated in the nucleus in the control group, whereas it was localized in cytoplasm by applied N pole static magnets (Figure 2). Ankyrin G results are related with ion channeling targeting of cyclic nucleotide-gated (CNG) channels to the rod outer segment required their interaction with ankyrin G. Ankyrin G localized exclusively to rod outer segments, co-immunoprecipitated with the CNG channel, and bound to the C-terminal domain of the channel beta-1 subunit. Depletion of these proteins in neonatal mouse retinas markedly reduced CNG channel expression [23,27]. By this respect, reports that calcium channels alteration by a patch clamp study [28] and its role of facilitating hydrolysis of nucleotide phosphates in phosphates in the application of static magnetic fields might have association with the envelope properties of molecular levels in the cell.

For an enzyme to perform such mechanisms as phosphatase and hydrolysis activity of phosophodiester bonds, the activation energy barrier, which is dependent in dielectric constant of water molecules, is a major factor that regulates the speed of reaction [29,30]. However, the dielectric constant of water molecules is altered by static magnetic fields [27].

There are some reports that cytoskeletons and subcellular structures are altered by static magnetic fields [18,22]. It reveals that cytoskeleton and Gamma Complex Protein3 structure is altered and this correlates with our data that proliferation might be delayed by these reasons. Some studies report that calcium channels can be altered by static magnetic field in a patch clamp study [28]. Following with these studies, Notable structural changes were observed in TEM microscopy when static magnetic fields were applied.

Cell mobility is related on adhesion molecules in cellular membrane surface, and these complex adhesion molecules have a role in invasion [31], which is linked to multiple signaling pathways below the plasma membrane [32]. There is a link with nuclear membrane lightening via ankyrin G localization. As the nuclear membrane apparatus is dispersed or disorganized due to complex mechanism of magnetic field, nuclear proteins might be delocalized to cytoplasm by this manner. These results together indicate that the magnetic field may make physical changes in terms of energy and molecular status alterations, which links to specific chemical changes of certain proteins.

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