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The Effect Of Electromagnetic Fields On The Differentiation Of Hair Follicles Stem Cells Into Nerve Cells In Adult Male Rats

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Abstract: *Stem cells are found in different portions of the body, including the bone marrow, these cells are capable of differentiated into bone, ligament, muscle, nerve, pancreatic beta cells and etc. Thus, these cells can treat degenerative diseases such as brain injuries, spinal cord injuries, and other disease. Given the lack of research on various electromagnetic field associated with the hair follicle stem cell differentiation onto neuronal cells In this study, we evaluated the effects of electromagnetic fields on hair follicle stem cells to neuron.* ***Materials & Methods:*** *6 rats were selected .anesthetized and sterilized in their upper lip hair follicle cells under hood apart and separate them from the membrane. Then cultured in DMEM . After a week the cells were exposed to electromagnetic field characteristics(1-1.8 and 2.4 MT) with a period 7-10-14 and finally evaluated by immune histochemistry using antibodies specific for nestin were.* ***Results:*** *Electromagnetic field characteristics 1.8 in a period 10 day and 2.4 MT in a period 14 day cause cells express nestin. And other intensity and day cant cause express nestin.* ***Conclusion:*** *Electromagnetic field with different characteristic can cause differentiated hair follicle stem cells into neuron like cells.*

Keywords: hair follicle stem cells , Electromagnetic field , Nestin protein

Introduction: Stem cells are found in various parts of body, including the bone morrow these cell are capable of being differentiated into bone, ligament, muscle, nerve, pancreatic beta cells and etc. Thus these cells can be used for treating the degenerative diseases such as brain and spinal cord injuries and other diseases. Presence of NGF^2 factors in the field of stem cells differentiates the stem cells onto neural cells. Using the stem cells existed in skin and its accessories, has opened a view for researchers. Because they are a reservoir for the potential cells which they are capable of reproducing, differentiating and changing into the other cells such as neural cells (1). Today regarding the expanding the electronic systems, the electromagnetic fields are being increased in environment of life.

These fields are used for treating the neural disturbances. Also, they may cause some diseases such as bone marrow cancer, leukemia, breast cancer and cardiovascular disturbances.

These electromagnetic fields can also cause some changes in structure of cells, changes in ion conduction and changes in functional potential in the cell membrane. DNA duplication increases in the presence of electromagnetic field. Also studies show that electromagnetic field causes to reduce duplication and also causes to increase the differentiation of stem cells onto osteoblasts (2). A study shows that the electromagnetic field prevents the differentiation of stem cells onto lipid cells (3). Also

in other study, Effect of induction, of electromagnetic field in differentiating the stem cells onto has been proved (4).

Regarding the various effects of electromagnetic field and lack of any study in relation to the effect of electromagnetic field on differentiation of hair follicle stem cells onto neural cells, the researchers are going to study the effect of electromagnetic fields on differentiation of hair follicle stem cells onto neural cells.

The results of the studies which have been dons in different cell populations, tissues, organs and various microorganism encountering with electromagnetic field with the different severity, and frequency; including increasing the activity of duplication of the cells which are the receiver of light (5), increasing the speed of Restoration of tissue in rat(6), increasing the duplication of strocyte cells(7), improving the process of cell duplication and increasing the speed of bacteria duplication (9-8), differentiation of fetus stem cells of rat onto the cells of heart muscle, differentiation of the fetus tissue of rat into cartilage, differentiation of myeloid monocytes and high increasing the growth of spleen cells of rat have been proved(10). One of the most important populations of cells which the necessity of study of the effect of electromagnetic is felt on the stem cells which have the capability of high duplication and also have the capability of changing and differentiation onto the other cells including the blond, bone, muscular, cardio, neural and cartilage cells. Thus Induction of duplicating these cells can be a method to care the degenerative diseases, brain and spinal cord damages, like the presence of NGF in the culture of stem cells which differentiate the stem cell into the neural cells (11).

Materials & Methods:

First, 6 adolescent rats weighted 200-250 grams were selected and using the following method, the follicles were separated and were under test.

Providing the stem cells: Rats go under general anesthesia with ether. After anesthesia their face and head are washed through Iodine (1%) and peroxide hydrogen within 3 minutes. Then their face area is shaved and then it is disinfected through 70% alcohol.

Then upper lip tissue with moustache of rat is cut. After removing the upper lip tissue under the hood in a sterile environment, the samples are put in DMEM/F12 containing penicillin (100 unit/ml), streptomycin (100mg/ml) and Amphotericin B in 30 minutes.

To separate the bulge area, these actions have been done: the samples were washed in Bafer phosphate then the connective tissues around follicles were removed and were thrown away. The sample was separated into small parts and were incubated in Collagenase II/ Dispase II solution (1mg/ml sigma aldherich) with 37^{oC} for 30minutes.After 30 minutes, the hair follicles were brought out by a fine pince form the lip tissue. Then the Bulge area was separated through taking out the collagenase capsule around the follicle from the follicles, then they were washed through buffer phosphate to culture the cells of bulge area.

This process was performed: The separated bulge area was incubated in ethylene diaminetetra aceetic acid (EDTA) within 30 minutes then the separated calls were centrifuged within 10 minutes with the speed of 1200 rpm.

After separation, the cells were poured in the plates wich had been couted with collagenase previously. The separated cells were cultured in DMEM/712 containing 10 mg/ml, 0.5 mg/m

hydrocortison, 10% FBS 3.4 mML, 10^{-9} M cholera toxin(sigma), Epidermal Growth factor (EGF,ƩigmoƩ), glutamin/SMS/ml insulin and 0.13 S mM adenis.

Culturing the cells in the form of clonally: Seven days after the early culture, the small parts of bulge are brought out the plate. Then the separated cells from the bulge which have sticked to the plate covered with collagenase are taken out with tripsine (12). In the early cultures, the cells have been dispersed. (Fig .1A, B). Cell suspension containing 20-35 cells is thrown away in the dish of cell culture which has been coated with collagenase. Cells stick to the bottom of plate within 24hours. Three hours after to plate, the cells at the bottom of dish are marked by using CD marker; a circle with diameter of 5 mm is drawn. During the culture, half of the culture media is changed each two days.

From the cell colonies which are formed within 7 days of the culture, are taken some photographs with microinjection camera.

Electromagnetic field: It is considered around the flasks containing stem cells with the cooperation of medical physics department. Regarding the considered aims in the study, mentioned frequencies are considered within 7-10 days and 14. in three groups of stem cells. It is allowed the cells to be duplicated and differentiated. In three periods; after one week, after 10 days and after two weeks, the differentiating operations are studied by using specific antibodies. Then the data are evaluated by using spss software.

Immionocytochemistry: Cells are fixed with parafromaldeide (4%) within 60 minutes at the temperature of $4^{\circ c}$. Then they were washed with PBS for three times within 15minutes:

1. Cuts were incubated within 10 minutes in a dark environment with the solution of methanol (10%) and H_2O_2 in order to block the internal peroxidase.
2. They were washed with PBS within 10 minutes.
3. Samples were inserted in 10% Goat serum (invitrogen) 0.3% Triton x-100 (Fluka) solution.
5. The early antibodies were used in the following concentration for 24 hours at the temperature of $4^{\circ c}$: Mouse auti nestin monoclonal antibody l: 1000 (MAB353, chemicon)
6. Then the cells were washed three times for 15 minutes with PBS.
7. Incubation with Biotin was done within 30 minutes.
8. Incubation was done in Proxidose iodine within 30 minutes
9. The following secondary antibodies were used with the following within two hours at the temperature of room;

Sheep anti- mouse biotinilated conjugate Ig G 1:2000 (F 2266; sigma).

10. They were washed with PBS 3 times
11. Incubation was done in DAB.

All samples were studied through microscope (Olympus AX70)

Results :

Cell Duplication: Bulge smashed areas of hair follicles stickled to six – cell plates covered with collagenas. During one week after beginning the cell culture, cells were separated from the Bulge gradually and during the duplication, they migrate (fig A, B1). Comparison of photos (fig A, B, C, D2) shows that these cells have high growth rate. As you see in fig 2, the cell has much duplication in the culture media containing EGF cholera toxin.

Cells were observed in two sizes; small and big with circle shape.

The number of small cells was abundant with light color cytoplasm and low cytoplasmic granules but the number of big cells was low with dark color cytoplasm and abundant granules. The cells began to stick the bottom of flask from the second day. From the third day, the cell colonies were observed. But first the colonies were small but after one week; the number of colonies were increased and were with different sizes. Some of them were very big. The cells which had been sticked to the bottom of flask were seen into two kinds of phenotype. The first kind was similar to Fibroblast with elongated tails to the sides and a mass in center. The second one was the big cells with small Appendages and very big cell body, abundant cytoplasmic masses and a very active core in center. (Fig 3)

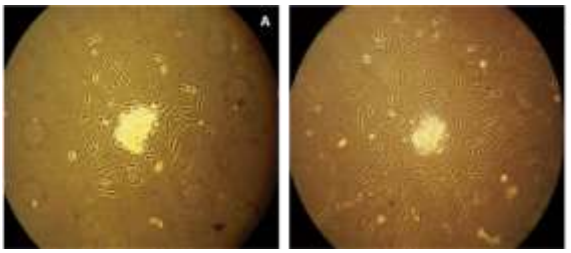


Fig 1-3: shows duplication and forming the cell colony which is identifiable

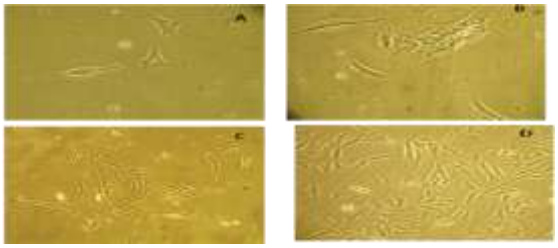


Fig 2-3: shows the cell duplication.

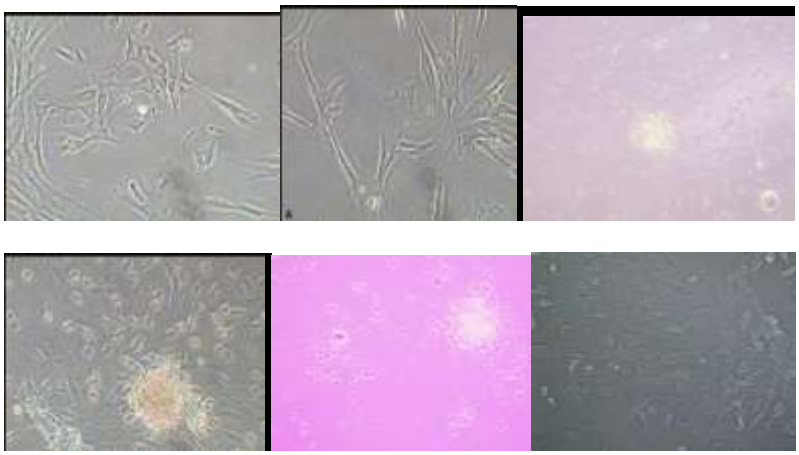
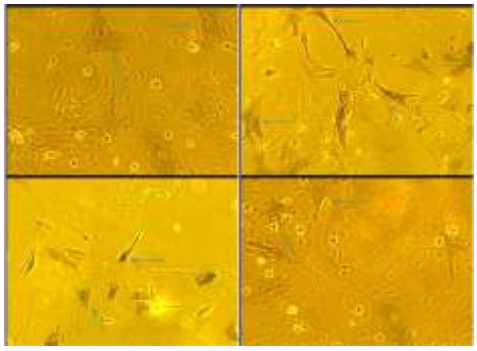


Fig 3-3: shows the cell colonies and various cells which have sticked to the bottom.

Cell Differentiation: In Bulge cells, Nestin is expressed with of immunocytochemistry showed that the Bulge cells which exist from the culture of hair rat, after ten days in the field of 108 millitesla, can

express the Nestin protein; in other words these cells are able to differentiate toward the neural cells. In the one millitesla intensity, in different days (7-10, 14 days), there was not any expression in the separated cells of follicles and there was not seen any differentiation toward the neuron. But the duplication cells in this group after 7 days in the field was suitable and the cells could quickly reach into a complete consolidation. In group of 2, 4 millitesla, was not also seen in cells within 7 and 10 days. But within 14 days in the field, the cells could express Nestin. Totally, only the intensities of 108 and 204 millitesla are able to express Nestin protein in the cells of the rat follicles within 10 and 14 days (Fig 3-4).



(Fig 3-4): some of the cells which have been colored by using the Nestin antibody, have been determined by flesh

Discussion:

Nestin is the marker of neural stem cells which in Syrian rat and human being is considered as the marker of the stem cells of hair follicle.

Expression of Nestin human's hair follicle and Syrian rat showing the existence of stem cell. In this study, we have studied Nest in expression in the cells of Bulge area of the rat's hair follicle which has been in the exposure of electromagnetic by using immunocytochemistry method. To study the stem cells of hair follicle, the rat's vibrissa follicle is available and it is big enough to manipulate and it is easy to have a biopsy.

The results of our study showed that the cells of Bulge of rat can be separated and they are cultured in the place containing the culture growth factors. Studies on the rat's vibrissa showed that in culture media of this are a 95% of colonels are formed (12). To analyze the formed colonels in our cultures media showed that the potential of duplication of Bulge cells are high because Bulge is near the connecting place of Arrestor pilli muscle and is located under the lipid mass (13). Also one of the results of this study was, to express Nestin in the cells which had been cultured for one week, but they did not express after differentiation. These results show that the cells of expressing Nestin not only have located in the Bulge area of the human's hair follicles and transgenic rat's follicles and transgenic rat's follicles but also they are in the Bulge area of the rat's hair follicle. Stem cells are located in the Bulge area.

However, there are some reports that these cells are located in the Bulge area of transgenic rats which express Nestin and CD34 (7). The result of this study showed that the Bulge cells of the rat's hair follicle can express Nestin after they were exposed under the electromagnetic fields. During the period of life, most of the organs in body, have the capability of producing the new cells after their

cells are destroyed by damages or physiological factors. Essentially, the capability of cell remedy depends on adolescent stem cells (1).

The adolescent stem cells are located in an area which is called niche: this area has been surrounded by the close differentiated cells (13). Skin is considered as the biggest organ in body and dies the important actions including; regulating the body temperatures keeping the balance of body fluid and preventing any damage to the body. Skin contains tissue supplements, epiderm, hair follicle and glands (14). Nestin is a kind of protein of visual filaments.

This protein is often expressed in some points of the neural cells which contribute in the growth of ax one.

In laboratory, they are in the form of Heterodymer or Hemotetradymer and they do not solely form visual filaments.

Preferably they from heterodymer structures with vimentin proteins and Internekcine (15). Nestin acts as the marker of duplicating cells and migration. During evolution, Nestin is expressed in must cells. But its expression is temporary and does not continue until the period of adolescence Nestin is expressed in the process of the early evaluation in the dividing cells PNS, CNS and meiosis tissues.

After differentiation, Nestin expression is reduced. During Neurogenesis and gliogenese, Nestin is alternated with GFAP. However Nestin is expressed in the pathologic conditions like forming glial scar and also it is expressed after any damage to CNS and during remedy of muscular tissues.

Studies have emphasis on the distribution and expression of Nestin in the dividing cells plays the complicated role in regulating the accumulation and separating the visual filaments for this protein.(16)

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