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Comparative study of base nervous cells and base marrow nervous cells in sheep artificial nervous coarseness treatment

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Abstract. Our basic problem is to study fetus base cell of disproportionate tissue. Using grown up base cell of animal would solve the problem. Preliminary cells has been achieved by adult sheep brain is proportionate condition for transmission. Marrow stromal cell changes to specific cell to replace dead cells. They could alter to procreative nervous cells and be cultured along with nervous base cell by joint culture by the aid of beta-galactoside in artificial environment. Our study shows when transplanting marrow base cells and nervous under abdomen and brain shell of adult sheep and newborn lamb by experimental nervous damages, nervous damages has been removed fully. We have observed broad procreative potential in norogols of central nerve system during life and concluded adult base cells of the same sheep could solve problems related to cancer, genetic mutation, uncoordinated tissues in the two methods of fetus base cells and nervous base.

Keywords: Nerve base cells, marrow base cell, and otology. Beta-galactoside, sheep

1. INTRODUCTION

Recently, scientists has observed in marrow after birth of human, mouse, and rat, there is re-procreative cells in which we call them multipotent cells.(Reyes and et,al.2001schwarts and et,al.2002)these cells could change to nervous cell phenotype and activity.(Jiang and et,al.2003)we concluded using multipotent base cell could solve problems about cancer, mutation and disproportionate tissue in the two methods of using nerve base cells and marrow base cells of the sheep nervous coarseness.

2. MATERIAL AND METHOD

2.1. Separating, filtering and multipotent cell multiplication

In preliminary culture environment, adult multipotent cells have been separated fast after removing by sucking from marrow according to human extraction method. (Reyes and et, al.2001) although, we were not successful, success method of extracting multipotent cells has been done by below method. We have cut fetus sheep leg by the mean (10weekd pregnancy and after birth about 4days) and maintained in salty buffer solution of phosphate XI of MediaTak company and has added other materials as 5%albumine cow serum for 48hours at 4 degree centigrade. then we transferred marrow from leg by 15%albumine cow serum has been rinsed and added to salt solution of phosphate buffer. One-nucleus marrow cells has been separated by centrifuge apparatus (sigma-Aldrich; history pag-1077) and has been put in flask 150 by 10ccfrom 19ng/cc fibronektine (sigma-aldrich) 2×10, approximately on culture environment. By forming the first colonies in short time, its treatment has been put in another plate by trypsin and by the number of 100cells/ centimeter of space till forming other colonies and then any cell/ well has been put in a plate. (Plates by 8 nests) after multiplying colon, the nest has been selected. Cells have reached

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to the maximum number after 3-4days, it means, 4000cells in any centimeter before reaching again to 500 cells in any separate plate. Culture environment has been maintained in 5/5%carboinc gas, on 37 centigrade degree temperature by normal oxygen.

2.2. Neuroectoderm advantages

Multipotent cells are in plates by 3×3 cells in any centimeter in culture environment by base culture materials, cell distinguishing has been done by Q-RT-PCR. We have changed culture environment material every week by fresh one till days 28. General number of 34cells distinguishing multipotent cells from three donors and for PDs60-100 has been done.

2.3. Chemical study of immunology cells of tissue

In order to study cell altering, we have analyzed some parts substituting to forward and backward. Cell substitution to the sides and middle on tissue part includes transfusion limit, too. Five sheep by suppressed immunity section by 10miligram/kg weight by cyclosporine A (sigma) has been repeated fast before transplantation transfusion and every other day.

2.4. Forming different types of nervous cell

Similar distinguishing cells have been cultured by 10 miczomole Brd v for 2days after adding factor. Cells has been evaluated for immune-cyto-chemistry after 5, 3, 2 days electro physiologically and 7days after distinguishing.

2.5. Electron microscope study

Samples have been examined by liker E.M10A electron microscope by 1-16000x magnificent. Imaging has been done by digital CCD telescope. (Finger Nik instrument, Lima, New York) in order to analyze data, we have used of mean±s.e.m, student's test.

3. RESULT

3.1. Preliminary cells growth characteristic

Sheep brain tissue in temple has been separated from sheep having epilepsy. Tissue has been divided in small parts in hippocampus, under abdomen limit or gray material of cortex in temple. Separated sections has been multiplied in plastic layers in all the time of culture in feasible culture environment and has been maintained for standard protocol of nerve base cell culture.(Scheffler and etmal2005)after extracting, we have done immunocyto chemistry on elementary cells about 4days in artificial environment for recognizing phenotype signs of cell culture. It has shown discordant congestion in which almost are strosite. After multiplying in culture environment by culture materials, we have gotten multipotent cells and multiplying in which after 12days on artificial culture environment has been achieved. (Figure 1)

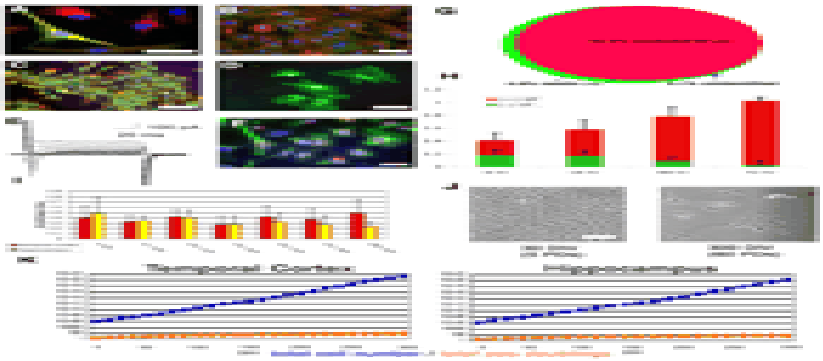

Figure 1. Preliminary cell growth characteristic

2. PLASTICITY SIGNS AND TRANSPLANTATION CAPABILITY OF ADULT SHEEP MULTIPOTENT CELLS (ASNP)

In order to measure adult sheep multipotent cell capability to continue life, composing and getting special phenotype in natural environment, we have transfused adult sheep multipotent cells in the right tissue of newborn sheep. Transplanted nerve cells have been used for recognizing transplanted cells by the aid of protein/ bio new color through composing and immunity phenotype on the next day. Immunity suppression system in lambs by cyclosporine-A decreased glia's reaction at last and increased discrepancy and living transplanted cells.(Figure 2) (2004 Svendsen) have supposed these cells for transmitting cells as a resource and gene content transmitting or changing gene route.

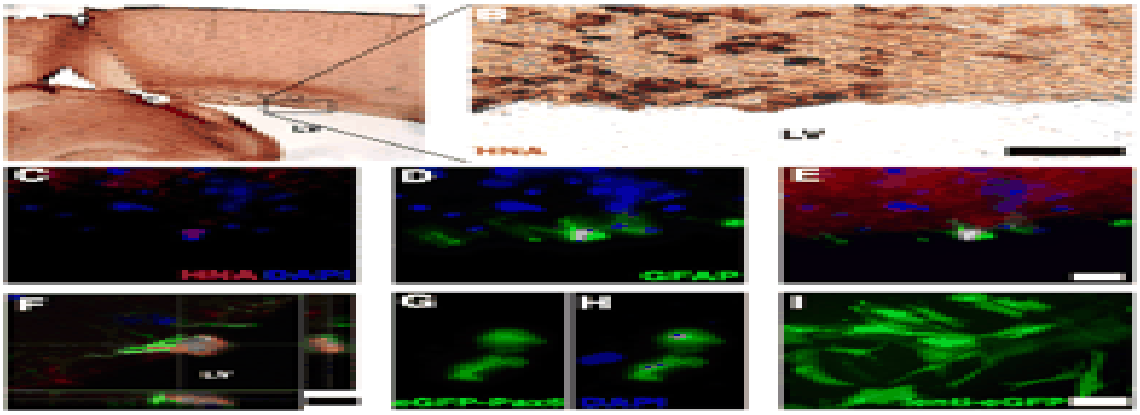

Figure 2. Plasticity signs and transplantation capability of adult sheep multipotent cells (ASNP)

3. ADULT SHEEP MULTIPOTENT CELL TRANSPLANTATION IN BRAIN CORTEX BY SUPPRESSED IMMUNITY SYSTEM

Adult sheep multipotent cell transplantation in brain cortex by suppressed immunity system does have broad nervous cell mood. Adult sheep multipotent cell has been recognized after 30 days of transplantation in transplantation environment. (Figure 3)

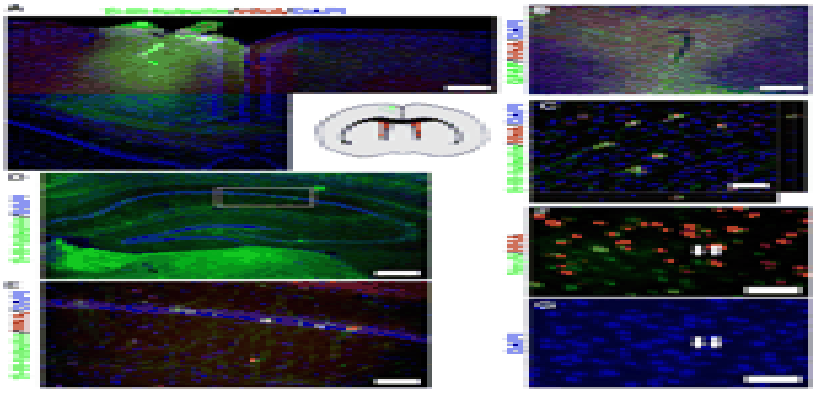

Figure 3. Adult sheep multipotent cell transplantation in brain cortex by suppressed immunity system.

3.1. Adult multipotent cell characteristic:

In order to form all adult multipotent colonies, the time of cell doubling is24hours for 30-40PDs, 36-48 when their culture reached to more than 40OD. According to the Figure 4 adult multipotent cells are round, triangular, somehow attached and less than 10micrometer diameter and dos have nucleus larger than cytoplasm. They could separate easily on multiplying cells. Based on analysis of 20cells in metaphase in any sample, less than Pd are diploids and there is no unnatural cytogenesis.

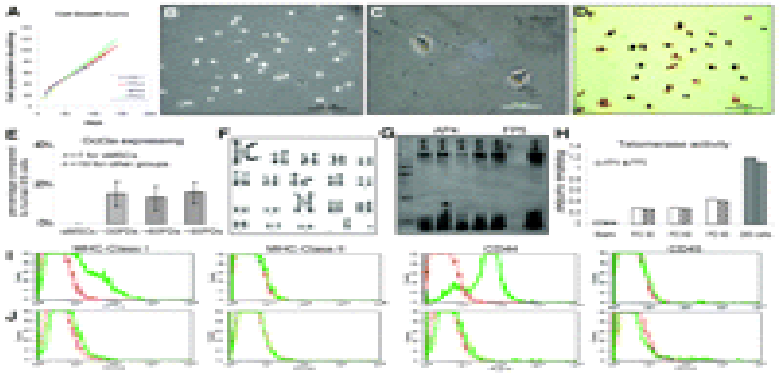

Figure 4. Adult multipotent cells characteristic.

3.2. Multipotent cells loss

When the number of multipotent cells reached to more than 8000cells after 3-6passes in any centimeter, we reach to CD44, MHRC phase's group of antigen on cell membrane and we lose oct3 by immunohistochemistry apparatus by Q-RT-PCR. We have noticed telomeres shortening in cells increases growth in high congestion. (Figure 5)

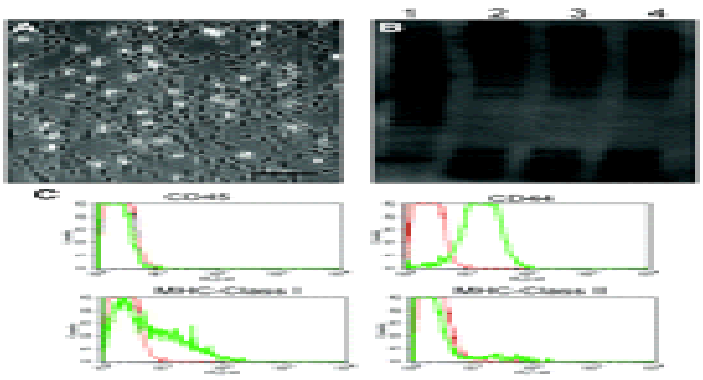

Figure 5. Losing multipotent cells.

3.3. Horse multipotent cell changes in artificial environment

These changes has been done in artificial environment to mesoderm, neuroectoderm, androderm by adding cytokine based on adult multipotent cells change studies.(Jiang and et,al. 2002 Reyes and et,al.2001 Schwartz and et,al.2002)study on fetus cell multipotent network and one lamb after birth and all changes has been determined so many times till doubling levels to 60-100 volume. In response to TGF, BMP4, multipotent cells have has first phenotype of B1 cartilage. Cells has shown Variable mass of kola gene MRNA. (Figure 6)

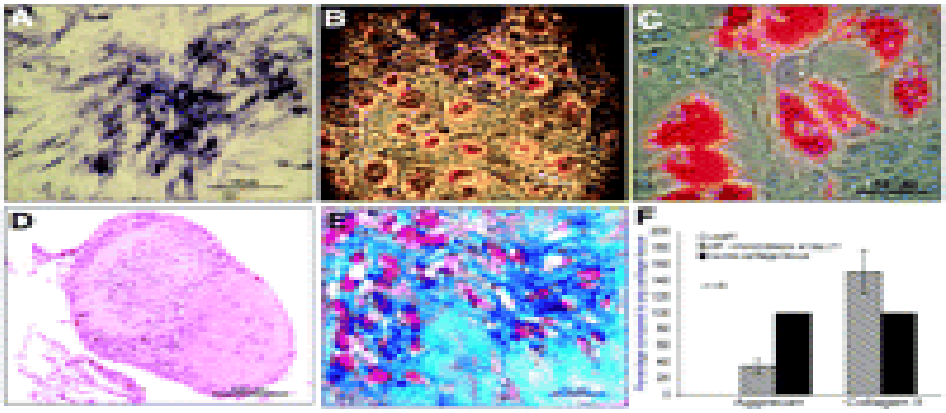

Figure 6. Horse multipotent cells changes in artificial environment.

4. DISCUSSION AND CONCLUSION

Marrow Struma cells does have different effect on signed materials and help damaged tissues and distinguishes them to special cells to substitute dead cells. Our study shows marrow base cell in experimental sheep by damaged nerve could eradicate damaged nerves. Nerve cells could treat brain damages. We have examined marrow base cells by common culture by the aid of β-galactoside along with elementary materials of nerve base cell has been recognized and could change to elementary nervous cells. We concluded, there is continuous growth of plasticity and multiplying potential in central nerve system glial during life. Using adult base otology cell could

remove cancer, mutation and disproportionate tissue in the two methods of using nerve base cells and marrow base cells in sheep.

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