Expression of Gen Extracellular Matrix and Cell Adhesion Molecule of Brain Embryo Mice at GD-10

By real time RT-PCR

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Abstract—research goal was to determine the expression levels cDNA of brain embryo at gestation days 10 (GD-10). The Electrophoresis DNA results showed that GAPDH, Fibronectin1, Ncam1, Tenascin, Vimentin, Neurofilament heavy, Neurofilament medium and Neurofilament low were 447 bp, 462 bp, 293 bp, 416 bp, 327 bp, 301 bp, 398 bp and 289 bp. Result of real-time RT-PCR on brain Embryo at gestation days 10 showed that the expression of copy gen Fibronectin 36 copies, Ncam 21,708 copies; Tenascin 24,505 copies; Vimentin 538,554 copies; Neurofilament heavy 2,419 copies; Neurofilament medium 92,928 copies; Neurofilament low 125,809 copies. Vimentin expressed gene copies is very high compared with other gene copies. This condition are caused by Vimentin, that contribute to proliferate of brain development. The vimentin role to cell proliferation of brain.

Keywords—GAPDH, Fibronectin, Ncam, Tenascin, vimentin, Neurofilament heavy, Neurofilament medium, Neurofilament low.

I. INTRODUCTION

ADHESIVE interaction between neurons and extracellular matrix play a key role in neuronal pattern formation. Molecules of the extracellular matrix have been implicated to play a pivotal role in tissue morphogenesis [1]. In addition to extracellular matrix and cell adhesion also plays a role in the process of brain morphogenesis. Cell adhesion systems should be regarded as molecular machineries that translate basic genetic information into complex three-dimensional patterns of cells in tissues [2]. Assembly of the central nervous system (CNS) architecture during development and maintenance of its circuitry throughout life are largely dependent on cell adhesion molecules (CAMs) capable of stabilizing and modulating cellular interactions. The neural cell adhesion molecule (NCAM) is well characterized cell adhesion molecules. It is implicated in various morphogenetic processes during development, such as proliferation, migration, differentiation, and synapse formation [3]. In the process of development of the nervous system or brain vertebrate evolves in a well-defined temporal sequence of events which includes proliferation of epithelial cells stem migration of neuronal precursor from ventricular zone to target area in neural tube. Neuron-glia cells interactions play a crucial role in several of these processes.

This study will determine the expression of gen that involved in brain development of mice embryos. Especially the gene for Extracellular Matrix and Cell Adhesion Molecule. primers used in this study were GAPDH, Fibronectin, Ncam, Tenascin, Vimentin, Neurofilament heavy, medium and low.
designed by Biotez Berlin-Buch GmbH, Berlin, Germany. Information on primers can be seen in Table 1.

II. MATERIAL AND METHODS

A. Experimental Animals and Sample Collection

Black-6 mice were used as experimental, from Charite Universitats Medizin Berlin, Jerman. Rearing the animals was done in a room at 23-27°C and 83% humidity. Food and water were given ad libitum.

B. Reverse Transcriptase and Real Time RT-PCR

The total RNA brain tissue was extracted with the RNeasy kit according to the manufacturer’s protocols. cDNA was synthesized from the total RNA using the Qiagen One Step RT-PCR Kit (Cat. No. 210210). PCR reactions using enzymes AidTM H Minus M-MuLV RT (Cat. No. 130 125 486) at a temperature of 95°C, 7 min, 45 cycles of PCR (20 sec, 95°C, 60°C, 20 sec, 72°C, 30 sec), 42°C, during 1 hour 15 minutes, 70°C elongation then followed with the temperature of 70°C, for 5 minutes. Quantitative analysis performed by Real-Time PCR. Analysis of Polymerase chain reaction (PCR) is done by adding each cDNA 9 µl of control brain and 1 µl of Primary-Mix into each different tube. In our experiments, Primary-Mix consists of eight primary types of GAPDH, Fibronectin1, vimentin, tenascin, Ncam1, NFh, Nfm, and the Nfl. Amplification products of DNA electrophoresis from brain mice black-6 were produced by using GAPDH, Vimentin, Fibronectin1, Ncam1, Tenascin, Neurofilament high, medium and low primers (Figure 1). Marker rainbow was used 1 kbp. Figure 1 was showed expression of cDNA FN 1 462 bp, Ncam 294 bp, Tnc 416 bp, Vim 327 bp, Nfh 301 bp, Nfn 398 bp dan Nfl 289 bp. All genes are expressed clearly, except in fibronectin, gene cDNA band is less clear.

III. RESULT

Product of DNA electrophoresis from brain mice black-6 were produced by using GAPDH, Vimentin, Fibronectin1, Ncam1, Tenascin, Neurofilament high, medium and low primers (Figure 1). Marker rainbow was used 1 kbp. Figure 1 was showed expression of cDNA FN 1 462 bp, Ncam 294 bp, Tnc 416 bp, Vim 327 bp, Nfh 301 bp, Nfn 398 bp dan Nfl 289 bp. All genes are expressed clearly, except in fibronectin, gene cDNA band is less clear.

Table II, shows the results of real time RT-PCR, with rotor gene machine. The number of copies produced per mio Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), were Fibronectin1 36 copies; Ncam 21,708 copies; Tenascin 24,505 copies; vimentin 538,554 copies; Nfh 125,809 copies; Nfl 19,485,318 copies; Nfl 92,928 copies; Nfl 2,419 copies; Nfn 92,928 copies; Nfl 125,809 copies. In this study the level of GAPDH mRNA expression in the brains of embryonic day-10 control is 19,485,318. GAPDH is commonly used as an endogenous control. GAPDH, an enzyme of glycolysis, this enzyme is also expressed in the events that are not related to the function of glycolysis. Therefore can be used as a control internal.
This data is more clearly visible with the figure 2. The copies gen of vimentin is very high compared with other genes. The copies gen of vimentin is 538,554 copies per mio GAPDH. This shows the expression of mRNA for vimentin protein is expected also very high. This shows that the expression of mRNA for vimentin protein is expected also very high. The opposite occurs on fibronectin, fibronectin gene expression is very low, these data support the results of DNA electrophoresis, in which DNA bands are very thin.

**IV. DISCUSSION**

Brain development involves a variety of events including epithelial cell proliferation and migration of precursor neurons into the right place in the neural tube [4]. Interaction of neurons and glial cells, play an important role in the migration process. In this case, extracellular matrix proteins that facilitate the process of migration and proliferation. The amplified Real Time RT-PCR from brain tissue of mice embryos of black-6, showed the changes of cDNA expression levels of extracellular Fibronectin1, Tenascin, Ncam1, Vimentin and neurofilament. Control typically used endogenous GAPDH. This enzyme is highly expressed in nearly every tissue in the body. GAPDH is usually found in the cytoplasm of healthy. Many researchers using GAPDH as endogenous control, because it shows high expression in all tissues. In other words, with increasing concentration of total cDNA in tissues is usually supported by high level expression of GAPDH cDNA in that tissue.

In this study, the level of GAPDH cDNA expression in embryonic brains of control is 19,485, 318. This means that the level of cDNA expression of the enzyme GAPDH, showed very high for the tissue brain. Therefore, this enzyme is used as an endogenous control in the analysis of quantitative real time RT-PCR [9]. Based on research [10], showed that the expression of fibronectin first appears in the neuroepithelial as small points of immunofluorescence among the earliest postmitotic that form preplate embryonic day 11 and 12. In this research, the expression the fibronectin was a little expressed. maybe this condition are caused by cell cell still proliferate. The cells that proliferate will express vimentin, and do not express fibronectin. Thus vimentin is expressed very high. Fibronectin plays a role in forming the migratory pathway for the growth cones of these axons [11]. The expression in the cortical proliferative zone is limited to the period of neurogenesis. Thus Fibronectin may be involved initially in supporting the cell division and fate determination that takes place in the neuroepithelium; later production by migrating neurons may play a role in the selection of radial glial pathways that lead to specific they form cortical layers within these region. so fibro as ECM, expressed very little, this is caused by fibronectin necessary for late development of the nervous system, which occurs after birth. Similarly, the expression tenasin. This is due at gestation days 10, the cells are still undergo mitosis, and do not differentiate to form a brain, although starting to look expressed tenasin.

The real Time PCR on Embryo at GD-10 indicated that Expression cDNA Vimentin was higher than another cDNA. In brain tissue, vimentin is detectable as early as embryonic day 11, the earliest stage and is located in radial fibers of neural tube, in ventricular cells [12]. The Vimentin is expressed in many cells of neuroectoderm in the fetal central nervous system [13]. The neuroepithelial cells in the neuroectoderm, which constitute the primordium of the CNS, are potentially capable of generating neuronal and glial cell lineages concomitantly. The appearance and morphological development of vimentin-positive neuroepithelial cells in human embryonic and fetal brain on 4–16 weeks [14]. In embryos aged 4-6 weeks, vimentin-reactivity was seen in all neuroepithelial cells, including those which exhibited mototic figures. All regions exhibited vimentin-positive neuroepithelial cells, the distribution and morphology of which gradually changed, resulting in lamination of the neural wall into two and subsequently three layers. Vimentin is a marker for cell lineage during early central nerve system development. It is suggested that all neuroepithelial cells differentiate to a stage where they express vimentin and that vimentin may have a role in cellular movements and migration. Vimentin is 57 kDa intermediate filament cytoskeleton protein widely expressed in immature cells, including those of the human fetal brain, that changes with maturation. Vimentin is synthesized early in mammalian embryogenesis, including derivatives of the neuroectoderm. The formation of
vimentin between days 7 and days 11 of mouse embryogenesis. Based on the data gained from this research is information of extracellular matrix and cell adhesion molecule expression development at gestation days 10. The fibronectin expression is very lower than the vimentin expression, because in the earliest stages of cortical development of brain, The data based on real time PCR that showed vimentin expression is higher than the other protein expression at embryo gestation days 10. This vimentin played a role in neurogenesis, specially in proliferation of cells. The study concluded that at GD10, Extracellular matrix and cell adhesion molecule expressed very low, and not required for the initial development of the brain, while vimentin expressed very high, because vimentin plays a role in proliferation and mitosis.

V. CONCLUSION

In the brains of embryonic day uk-10, expressed gen for vimentin protein was very clearly in comparison with other genes. the existence of genes associated with cellular functions during embryonic development

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REFERENCES