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Expression of Gen Extracellular Matrix and Cell Adhesion Molecule of Brain Embrio 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 embrio at gestation days 10 (GD-10). The Electroforesis 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, Neurofilamen heavy, Neurofilament medium, Neurofilamen 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-difined temporal sequence of events which includes proliferation of ephitelial cells stem migration of neuronal precursors from ventricular zone to target area in neural tube. Neuron-glia cells interactions play a crucial role in several of these processes.

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In this context, cell adhesion molecules (CAMs) and the extracellular matrix (ECM) are involved in the control of neuronal migration and axon elongation [4]. The extracellular matrix (ECM) of the embryonic brain is composed of many types of molecules that have distinct patterns of spatial and temporal expression. Many of these components were originally discovered in non-neural tissues and include fibronectin (FN), tenascin (TNC), Neurofilament, Ncam, Vimentin.

Fibronectin is extracelllar matrix bilieved to be involeved in many cellular function such as cell adhession, wound healing and cell migration [5]. These protein is present in the central nervous system and is considered to play important roles in the development of embryonic neurons and regeneration of damaged adult nerves fibers.

Tenascin is extracellular matrix molecule too, synthesized and released by young astrocytes during embryonic and early postnatal development of the nervous system. Distinc spatial and temporal distributions of tenascin during developmental events suggest a role in the guidence neuronal.

The neural cell adhesion molecule (Ncam) is the one of most studied and well characterized cell adhesion molecules. It is implicated in various morphogenetic processes during development, such as proliferation, migration, differentiation, and synapse formation [3].

Vimentin is protein particulary useful as markers of glial differentiation [6]. Vimentin is intermediate filament component of astroglial cells. Function of vimentin in the cells is supposed to play role in neurogenesis because of its coexpression with neurofilament in neuroblast. More recently, evidence has been published on the role of vimentin in DNA replication and recombination, DNA repair and in gene expression.

Three sub-units of Neurofilament (NFs) are expressed at distinct stages of vertebrate development, triggered by neuron differentiation. Neurofilaments are intermediate filaments of neurons that are considered to add rigidity, tensile strength and possibly intracellular transport guidance to axons and dendrites. Exclusively expressed in neurons, NFs are members of the cytoskeleton proteins that act together to form and maintain cell shape and facilitate the transport of particles and organelles within the cytoplasm [7].

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.

TABLE I
SEQUEN OF PRIMER POSITION (F = FORWARD; R = REVERSE) AND % CONTENT OF G/C

Primer	Sequence	GC (%)
GAPDH f	CCA TCA CCA TCT TCC AGG AGC GA	56,5
GAPDH r	GGA TGA CCT TGC CCA CAG CCT TG	60,9
Fibronectin-f	AGG CAT AAG GTT CGG GAA GAG GT	52,2
Fibronectin -r	GCA GTT GTC ACA GCG CCA GCC	66,7
NCAM-f	GGT GCA GTT TGA TGA GCC AGA GG	56,5
NCAM-r	CGT CCT CTC CCA TCT GCC CTT C	63,6
Tenascin-f	CTA CAG CCT GGC AGA CCT GAG	61,9
Tenascin-r	CTT GTA GGT CCA CCC GGA GCT	61,9
Vimentin-f	CTG AGG CTG CCA ACC GGA ACA A	59,1
Vimentin-r	CCT CGC CTT CCA GCA GCT TCC	66,7
Nf h-f	AGG AGA TAA CTG AGT ACC GGC G	54,5
Nf h-r	CCA AAG CCA ATC CGA CAC TCT TC	52,2
Nf m-f	GTG GTT CAA ATG CCG CTA CGC C	59,1
Nf m-r	GAG GCC CGG TGA TGC TTC CTG	66,,7
Nf l-f	TGG CCT TGG ACA TCG AGA TTG CA	52,2
Nf l-r	GCT TCT CCT TCA GAG GGG GGC	66,7

When female mice achieved their sexual maturity (10-12 weeks old), they were mated with a male (1:1). A vagina plug detected the following morning was defined as day 0 of gestation day [8].The balck-6 mice pregnant were killed by cervical dislocation at GD-10. The pregnant mice were cut opened, next uterus was taken and put in falcon tube containing buffer solution. Uterus were opened, embrio was taken and brain were isolated, under stereo microscope. Brains sample were put in Nunc cryo tube, and kept in box which containing liquid nitrogen. Part of all the brain sample were put in the tube, which containing RNA-*later* (Sigma), for analysis DNA.

B. Reverse Transcriptase and Real Time RT-PCR

The total RNA brain tissue was extracted with the RNeasy kit according to the manufacture'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. Brain tissue of embrio, then was added by the component of reactions from SYBER Green kit Qiagen. Then reaction of Real Time RT-PCR showed the complete series of targetted cDNA, followed by Oligonucleotide primers. The Primers used in this study were synthesized into *Biotez Berlin-Buch GmbH, Berlin*, Germany.

C.DNA Electrophoresis

The separation of DNA in the agarose gel was performed according to base pair DNA, followed by separation marker 1kbp. Each of 1 µl sample brain GD-10, was pippet to well agarose gel. Before running at 80 V, agarose solution was added 5µl Ethidiumbromide . Gel agarose was transferred on to box foto UV, to see the bands of DNA.

III. Result

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

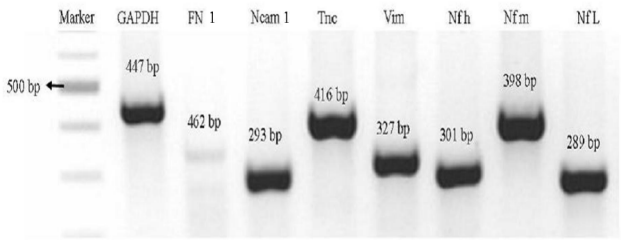

Fig. 1 Expression of cDNA brain embryo at gestation days 10 by DNA electroforesis. Marker, GAPDH, FN1;Fibronectin, Ncam 1,Tnc; Tenascin, Vim; Vimentin, Nf h; Neurofilament high, Nf m; Neurofilament medium, Nf L; Neurofilament low

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; Nefh 2,419 copies; Nefm 92,928 copies; Nefl 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 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.

TABLE II
DNA CYCLE CONDITONS OF BRAIN TISSUE EMBRYO AT GD-10 BY REAL TIME REVERSE TRANSCRIPTATION POLYMERASE CHAIN

primer	Ct	Take off	Amplification	Tm (°C)	Average of Tm	Calc. Conc (copies/ reaction)	Copies/1 Mio GAPDH
GAPDH	8.73	6.4	1.59	87.4		19,485,318	
FN1	32.36	29.7	1.87	76.3	85.4	705	36
Ncam1	17.58	15.4	1.76	87.4		422,996	21,708
Tnc	17.30	15.1	2.00	85.1		477,488	24,505
Vim	10.16	8.0	1.60	86.3		10,493,895	538,554
Nefh	22.65	19.8	1.69	87.1		47,143	2,419
Nefm	14.22	11.8	1.75	89.6		1,810,728	92,928
Nefl	13.52	11.1	1.72	86.9		2,451,429	125,809

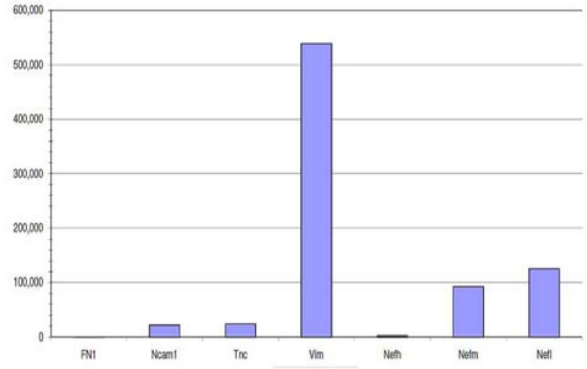

Fig. 2 Expression of cDNA FN ; Fibronectin, Ncam 1, Tnc; Tenascin, Vim; Vimentin, Nefh; Neurofilamen, Nefm; Neurofilamen, Nefl; Neurofilamen on embryonic day 10 by *Real Time* RT-PCR

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 rihgt 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 neuroephitelial 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 fobronectin necessary for late development of the nervous system, which occurs after birth. Similarly, the expression tenascin. 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 tenascin.

The real Time PCR on Embryo at GD-10 indicated that Expression cDNA Vimentin was higher than another cDNA. In brain tissue, vimentin is detecable as early as embriyonic 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 motitic figures. All regions exhibited vimentin-positive neuro-epithelial 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 cyto-skeleton 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 derivates of the neuroectoderm. The formation of

vimentin between days 7 and days 11 of mouse embryo-genesis.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 proliferate of cells. The study concluded that at GD10, Extracelular 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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