Genomics & Informatics Vol. 2(1) 36-44, March 2004

In Silico Screening for Angiogenesis-Related Genes in Rat Astrocytes

Soo-Young Kim[1,2], Sae-Won Lee[1], Sung Yong You[1], Sun Young Rha[3] and Kyu-Won Kim[1]*

[1]Division of Pharmaceutical Bioscience, College of Pharmacy and Research Institute of Pharmaceutical Sciences, Seoul National University, Seoul 151-742, Korea
[2]Graduate Program in Bioinformatics, Seoul National University, Seoul 151-742, Korea
[3]Cancer Metastasis Research Center, Yonsei University College of Medicine, Seoul 120-752, Korea

Abstract

Astrocytes play supportive roles for neurons in the brain. Recently, they have been accepted to have various functions in the vascular system as well as in the nervous system. We investigated the differential gene expression in rat astrocytes according to the oxygen tension, which is a crucial factor for angiogenesis. A cDNA microarray was performed to find the genes whose expression was sensitive to oxygen tension. We found 26 genes in the astrocyte were found and classified into 4 groups. In order to show the genes' relevancy to angiogenesis, seven of the 26 genes were investigated to see whether they have capabilities of interaction with angiogenesis-related factors in AngioDB. Through this investigation, we found interactions of three proteins with angiogenesis-related factors. These genes were further investigated with a new focus on the vascular endothelial growth factor (VEGF) expression in an astrocyte based on our hypothesis that astrocytes can have effects on endothelial angiogenesis via the release of VEGF. Collectively, we identified several genes whose expressions were dependent on the oxygen concentration of the astrocyte. Furthermore, the relevancy of astrocytes to angiogenesis was investigated using preexisting information of AngioDB, and suggested a possible signaling pathway for VEGF expression in the aspects of brain endothelial angiogenesis by astrocytes.

Keywords: astrocytes, cDNA microarray, angiogenesis, AngioDB

* Corresponding author: E-mail qwonkim@plaza.snu.ac.kr, Tel +82-2-880-6988, Fax +82-2-872-1795
Accepted 11 March 2004

Introduction

Astrocytes, the most numerous glial cells, have been regarded as the supportive cells of the brain. They aid neurons in maintaining a good condition through nourishing, protecting, and modulating growth and excitability (Travis, 1994). They also function as scavengers that rapidly capture transmitters that are released during synaptic activity. It was also reported that some types of astrocytes can protect neurons from oxidative stress (Shih *et al.*, 2003).

Moreover, astrocytes are known to have various important roles that are related to the vascular system as well as being related to the supportive roles for neuron growth and maintenance. Therefore, astrocytes have come to be known as "the bridge" between the nervous and vascular systems (Parri *et al.*, 2003). In fact, astrocytes are in contact with both neuronal synapses and microvessels. For instance, signaling that involves the glutamate and calcium ions between the neuron and astrocyte is a significant factor in the control of brain microcirculation (Zonta *et al.*, 2003). Astrocytes are also involved in the process of blood-brain barrier (BBB) differentiation. BBB is a well-differentiated network of brain microvessels that provides homeostasis. It is known that BBB development can be classified into brain angiogenesis and BBB differentiation. Brain capillaries go through differentiation with the astrocyte, and these capillaries become more mature and are remodeled into a BBB that is impermeable (Janzer *et al.*, 1987, Lee *et al.*, 2003). Astrocytes can be involved in both the nervous system and the vascular system. Recently, many neurobiologists and vascular biologists have been interested in the coordinated interaction between the vascular and nervous systems (Park *et al.*, 2003). Herein, we held an anatomical and functional focus in exploring the characteristics of astrocytes that could be the linker between the two different systems.

Oxygen tension plays a crucial role in the vascular development (Maltepe *et al.*, 1998). Angiogenesis, which is the process of new vessel formation, is very tightly controlled by oxygen concentration levels. Therefore, we hypothesized that oxygen tension would be a key factor in gene expression of astrocytes that can be a part of the vascular system as well as the nervous system. Moreover, it is expected that some genes in the astrocyte,

which illustrated different expression patterns in various oxygen conditions, could have an effect on angiogenesis of brain microvessels. Therefore, a cDNA microarray was performed in order to screen genes whose expression level varied according to the oxygen conditions including normoxia (normal level of oxygen), hypoxia (low level of oxygen), and reoxygenation (recovery of oxygen level). Among the selected genes, Smad4, p62, and Bcl-2 associated death promoter (BAD) were investigated using some factors in AngioDB in order to examine their interaction with angiogenesis-related proteins. AngioDB is the database that consists of angiogenesis-related molecules and their signaling networks (Sohn *et al.*, 2002). The list of angiogenesis-related molecules in AngioDB was used, and it was focused on the VEGF signaling networks based on the fact that it is a key factor in the induction of angiogenesis.

Methods

Cell culture

A rat lung epithelial cell line (L2) as the control group in the microarray hybridization was cultured in RPMI 1640 with 10% FBS (Life Technologies), 100 U/ml penicillin streptomycin. Culture environment of L2 cell line was maintained at 37° C. An immortalized rat astrocyte cell line was cultured in DMEM supplemented with 10% FBS (Life Technologies), 20 mM Sodium bicarbonate, and 100 U/ml penicillin streptomycin. Hypoxic condition was maintained in a hypoxia chamber (Forma Scientific) that provided a low oxygen tension (5% CO_2 with 1% O_2, balanced with N_2) for hypoxic condition. After exposure to hypoxia, they were returned to the ambient atmosphere, supplied with fresh medium and continuously incubated for the condition of reoxygenation.

Table 1. Candidate genes from cDNA microarray analysis. (AC : accession number; N, normoxia; H, hypoxia; R1/2h, reoxygenation for 0.5 h; R9h, reoxygenation for 9h)

Name	AC	N	H	R 1/2h	R 9h
Increased at hypoxia					
PMF31	AA964295	1	3.52	1.73	1.54
EST	AA901267	1	3.5	1.25	1.77
Inhibitor of DNA binding 1	AA899322	1	3.29	2.31	1.86
EST	AA924991	1	3.18	2.18	0.91
Smad4	AA997371	1	2.98	1.29	1.22
EST	AA875316	1	2.94	2.34	1.36
EST	AA925307	1	2.91	1.53	1.34
ZIP	AA899656	1	2.02	1.31	0.82
Decreased at hypoxia and recovered at reoxygenation					
EST	AA875290	1	0.31	1.22	0.63
CL3BC	AI030464	1	0.3	0.71	0.92
EST	AA899667	1	0.33	0.57	1.03
Increased at early reoxygenation					
BAD	AA964163	1	0.89	8.41	1.45
EST	AI111936	1	1.5	8.33	4.35
EST	AA819560	1	1.21	4.55	2.07
ARL1	AI044423	1	1.06	3.44	1.34
EST	AA859013	1	0.96	3.27	1.66
eIF-2B α subunit	AA957084	1	1.01	3.24	1.33
EST	AA900088	1	1.13	2.89	1.64
PC3	AI146192	1	0.93	2.54	1.71
RGS8	AI144709	1	1.2	2.38	1.64
EST	AA899887	1	0.93	2.18	1.8
EST	AA817932	1	1.39	2.13	1.71
EST	AA819203	1	1.55	2.11	2.19
EST	AA817878	1	1.25	2.07	1.66
Decreased at early reoxygenation					
EST	AA925092	1	1.02	0.28	1.18
Stat5b	AA955730	1	0.95	0.36	1

Oxygen tension conditioning procedures and RNA isolation

Fig. 1 shows experimental scheme. Each experiment was done in tripicates. The duration of hypoxia was for 16 h. The temperature in hypoxia was maintained at 37° C. In this case, RNA isolation time point was adapted to different periods of reoxygenation (0.5 h, 9 h). RNA was extracted by standard methods. Cells were lysed using TRIzol reagent (Life Technologies), and total RNA was isolated according to the manufacturer's instructions. Extracted RNA was subjected to further purification using and RNeasy mini kit (Quiagen). The quality and quantity of extracted total RNA samples were evaluated by loading a specific amount of each sample on a denaturating agarose gel.

Microarray hybridization

Microarray experiment was performed according to the technical manual of DNA dendrimer probe reagents (3DNA™ Array 50™ Expression Array Detection reagents, Genisphere). Each RNA and Cy3 or Cy5 capture sequence primers were incubated for 10 min at 80° C. At 42° C an equal volume of reaction mix was added, and the reaction was incubated 2 h. The reaction was terminated by bringing it to 0.074 N NaOH and 7.4 mM EDTA. Concentrated cDNA tagged with capture sequence was mixed with hybridization buffer(40% formamide, 4x SSC, 1% SDS). The mixture was hybridized to GeneTrack rat arrays (GenomicTree), which contained more than 5000 of genes and expressed sequence tags. After incubation at 45° C overnight, the arrays were washed and dried. Then, 3DNA Array 50 Cy3 and Cy5 reagents (Genisphere) was hybridized to the arrays and incubated at 50° C for 3 h. After hybridization, the arrays were washed and dried. The hybridized probe array was scanned by microarray scanner GenePix 4000B (Axon Instruments) with GenePix Pro 4.0 (Axon Instruments) as analysis software. GenePix Pro 4.0 performed global normalization every scanning. R(http:// www.r-project.org) was used in normalization of all arrays. We used J-Express to cluster and generate candidates whose expression would be changed by oxygen tension (http://www.ii.uib.no/~bjarted/jexpress/).

Microarray data analysis

Gene expression profiles from the four different oxygen conditions were analyzed using the self-organizing map (SOM) algorithm. We constructed a 16-partition SOM (data not shown). A 16-partition SOM represented distinguishable expression pattern between clusters. Next, we selected only 4 groups of the gene expression patterns that were thought to be biologically meaningful: increased at hypoxia; decreased at hypoxia and recovered at reoxygenation; increased at early reoxygenation (30 min reoxygenation after exposure to hypoxia); decreased at early reoxygenation. After selection of 4 groups, we found the genes among them whose expression was more sensitive to oxygen tension under the criteria: 1) ratio was $\geq$ 2.0-fold as increased, 2) $\leq$0.5-fold as decreased. As shown in Table 1, there are 26 clones whose expressions are dramatically changed by oxygen concentration.

Analysis of interactions with factors in AngioDB.

The selected genes in the results from microarray screening were analyzed using the database, AngioDB (http://angiodb. snu.ac.kr). The analysis of interactions was based on the information in PubMed. We draw the network using Cytoscape (http://cytoscape.org), which is a bioinformatics software platform for visualizing molecular interaction networks.

Results

Selection of candidate genes and investigation of interactions with angiogenesis-related factors

Twenty-six genes from the cDNA microarray analysis were selected according to the criteria described in the Methods. In Table 1, the expression level was represented as the relative ratio to the level at normoxia. To study relevancy of the factors involved in angiogenesis, an investigation of interactions was performed using the data in AngioDB. AngioDB is a database of angiogenesis and angiogenesis-related molecules in human, which includes identification, functional classification, and bibliographic references. The known genes were the center of focus because we analyzed interactions using preexisting information in PubMed. Therefore, among the 26 genes, 15 ESTs were excluded at the beginning of the analysis. Then, some genes that were thought to be appropriate for this analysis were selected among the rest of the 11 known genes, which encode for PMF31, inhibitor of DNA binding 1 (Id-1), Smad4, PKC-ζ -interacting protein (ZIP), CL3BC, BAD, ADP-ribosylation factor-like 1 (ARL1), eIF-2B α subunit, NGF-inducible anti- proliferative protein PC3 (PC3), Regulator G protein signaling 8 (RGS8), and Stat5B. Among these, PMF31 and CL3BC were excluded because human homologs cannot be found. Additionally, inhibitor of DNA binding 1 and Stat5B were also excluded for our study because they were already well reported to be involved in angiogenic pathways (Benezra *et al.*, 2001; Dentelli *et*

al., 1999). Finally, 7 genes for the interaction analysis were selected: *Smad4* and *p62* in the group of genes that increased at hypoxia; *BAD, ARL1, eIF-2B α-subunit, PC3,* and *RGS8* in the group of genes that increased during early reoxygenation. In analyzing the interactions with angiogenesis-related factors, the results indicate that 4 out of 7 proteins, ARL1, eIF-2B α-subunit, PC3, and RGS8, had no interaction with factors stored in AngioDB. Fig. 2 shows the primary interactions of the remaining three proteins, Smad4, p62, and BAD, with angiogenesis-related factors. According to this interaction analysis, Smad4 interacts with 7 angiogenesis -related factors: Sp1, TGF-β, Jun, UBC9, CREB, Jab1, and haptoglobin; p62 interacts with p53, angiotensin, TrkA, Fos, PKC, epidermal growth factor receptor (EGFR), NF-κB, Ras, and Sp1; BAD interacts with eight other proteins: TGF-β, IL2, HBx, haptoglobin, PKC, NF-κB, BRCA 1, and nerve growth factor (NGF).

Extended investigation of interactions focusing on the VEGF signaling

Above, it was found that molecules are involved in angiogenesis and they interact with candidates that resulted from the cDNA microarray analysis by using preexisting information from AngioDB without additional biological experiments. It would be possible to further study these factors in order to discover the role in angiogenesis. However, such that trial would be still arduous; because it needs to screen a wide range of possibilities that could be considered as elucidating an obvious relationship between the gene expression in the astrocyte and brain angiogenesis. If astrocytes represent the effect of angiogenesis on endothelial cells, one of the possible paths for regulating angiogenesis can be the secretion of VEGF, an endothelial cell-specific mitogen that plays an important role in angiogenesis. Herein, the factors that can act on the expression of VEGF were extracted from the data in AngioDB, and then, the factors interacting with Smad4, p62, and BAD were investigated. The focus was on primary interactions of Smad4, p62, and BAD with the factors that influence VEGF expression. As shown in Fig. 3, we integrated interactions and constructed the networks. Smad4 can act on VEGF expression via Sp1 and TGF-β; p62 can do the same via angiotensin, Ras, PKC, and Sp1. The interaction of BAD was different from those of Smad4 and p62. On the interaction network, it was impossible to be linked via only one factor from BAD to VEGF. There were only two angiogenesis-related factors, NF-κB and IL2, receiving a signal from BAD on the interaction network. These proteins did not have a direct effect on VEGF on the network. Therefore, further investigation of primary interactions for NF-κB and IL2 was performed because it was expected that there would be some linkages to VEGF among interactions with other factors that were involved in angiogenesis. The network in Fig. 4 shows interactions concentrating on NF-κB, IL2, and the relationship with factors that directly interact with VEGF.

Inference of signaling pathways toward VEGF expression

Based on the networks, we inferred which signaling pathways Smad4, p62 and BAD were involved in (Table 2). First, Smads protein is involved in the TGF-β signaling pathway (Derynck *et al.*, 1998). Reportedly, TGF-β has the effect of angiogenesis-activation (Roberts *et al.*, 1986). Presumption for Smad4, however, is somehow complex. Smad4 plays a pivotal role affecting all TGF-β signaling and also has a tumor suppressor effect. Additionally, while TGF-β also has other attributes to inhibit epithelial cell growth, induce differentiation or apoptosis, and maintain genomic stability (Howe *et al.*, 1998), TGF-β has a neuroprotective effect by blocking apoptosis through phosphorylation of BAD (Zhu *et al.*, 2002).

Table 2. Inferred signaling pathway from Smad4, p62, and BAD to VEGF expression by candidate genes related to angiogenesis.

Expression	Candidate	Inference of involved signaling pathway	Predictive effects on VEGF
Increased at hypoxia	Smad4	Activation of TGF-β signaling by Smad4	↑ VEGF expression
		Cooperation with Sp1 to induce transcriptional activity	
	p62	Association with PKC-ζ to activate VEGF	↑ VEGF expression
		Activation of Sp1 signaling	
		Association with angiotensin II	
		Negative regulation of Ras	↓ VEGF expression
Increased at reoxygenation	BAD	IL2 NF-κ B	TGF-β , TNF-α , and so on ↑ VEGF expression
			p53 ,Ras, and so on ↓ VEGF expression

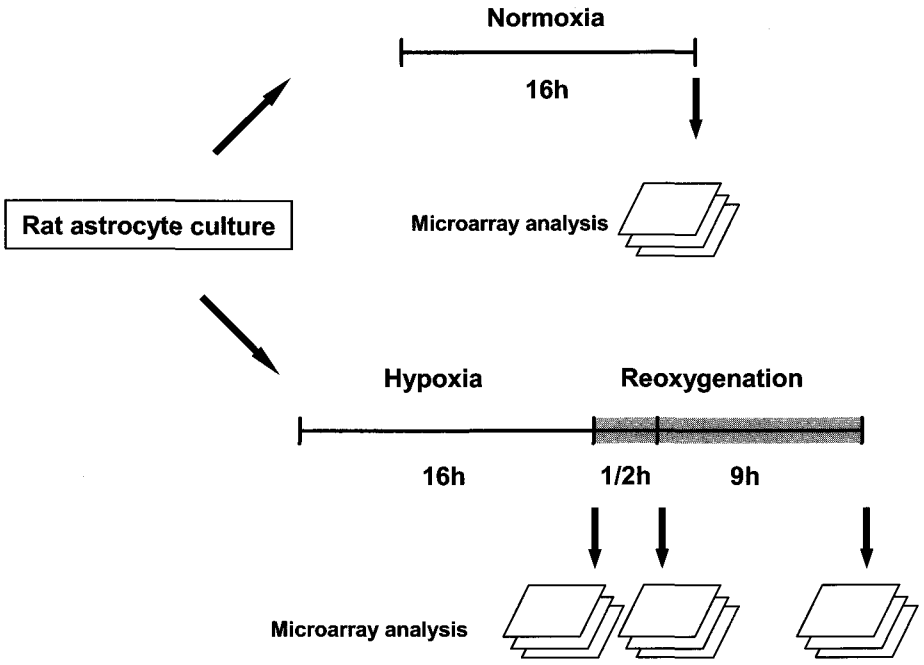

Fig. 1. Scheme of microarray analysis.
Oxygen tension was designed as hypoxia and reoxygenation compared to normoxia. Oxygen level state consisted of hypoxia for 16 hours and two reoxygenation conditions(0.5 and 9 hours). In the replicate study, pooled L2 RNA as control was labeled with Cy3, and RNA from the astrocyte cell line conditioned by oxygen tension was labeled with Cy5 for each slide.

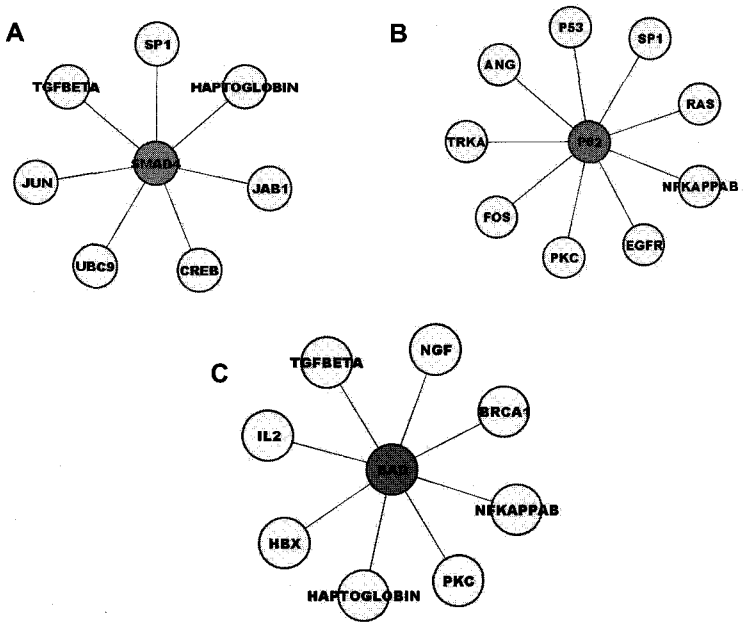

Fig. 2. Primary interactions with preexisting molecules in AngioDB.
Smad4 interacts with 7 angiogenesis-related factors: Sp1, TGF-β , Jun, UBC9, CREB, Jab1, and haptoglobin. p62 does with p53, angiotensin, TrkA, Fos, PKC, EGFR, NF-κ B, Ras, and Sp1. BAD whose mRNA expression was increased at early reoxygenation, interacts with 8 proteins: TGF-β , IL2, HBx, haptoglobin, PKC, NF-κ B, BRCA 1, and NGF.

Therefore, the output of Smad4 and TGF-β signaling depends on various physiological phenomena and cell types. For our analysis, the focus was on the interactions that would be involved in VEGF expression when the oxygen condition was altered. Sp 1 was reported to activate VEGF transcription (Reisinger *et al.*, 2003), and Smad4 associates with and positively interacts with Sp1 (Pardali *et al.*, 2000). Therefore, Smad4 can mediate TGF-β signaling in VEGF expression, or Smad4 can be the upstream factor for Sp1-mediated VEGF induction. Taken together, Smad4, whose mRNA level was increasingly expressed at hypoxia, is possibly involved in the induction of VEGF via interactions with Sp1 and TGF-β. Furthermore, in the long run, it can lead to endothelial angiogenesis by astrocytes.

Second, like Smad4, ZIP was also increased at hypoxia in the results of the microarrays. ZIP was isolated from a rat brain cDNA library and was known to interact with the regulatory domain of PKC-ζ (Puls *et al.*, 1997). The human ZIP homolog that encodes a cytosoilc 62-kDa protein (p62) was identified and cloned (Park *et al.*, 1995). As the result of the interaction network, p62 can induce the VEGF expression via interactions with PKC, Sp1, angiotensin, and Ras. PKC-ζ is an intermediary molecule for Ras-mediated overexpression of VEGF (Pal *et al.*, 2001). Conversely, p62 acts as a negative regulator of Ras (Di Cristofano *et al.*, 2001). Therefore, p62 is likely to play a role as the decisive regulator of PKC-ζ signaling

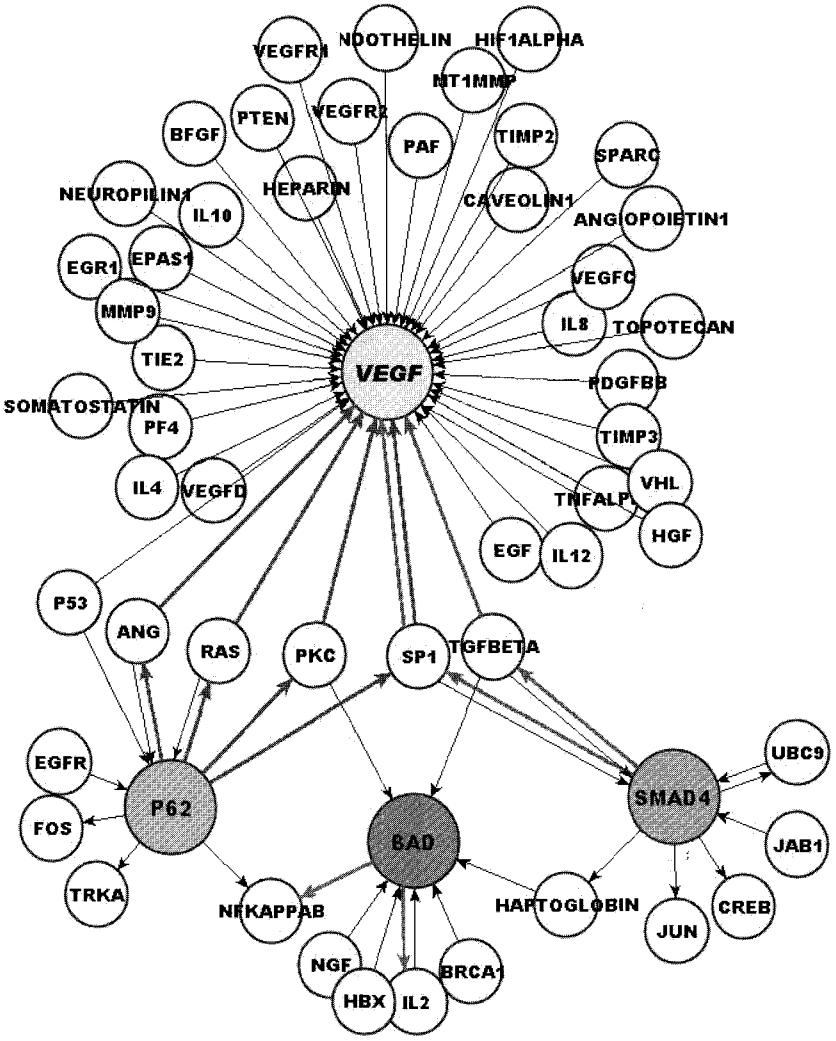

Fig. 3. The interaction network with factors affecting VEGF expression
Smad4 can act on VEGF expression via Sp1 and TGF-β ; p62 can do via angiotensin, Ras, PKC, and Sp1. However, angiogenesis-related factors receiving signal from BAD were just two, NF-κ B and IL-2 on the network. These proteins did not have a direct effect on VEGF on the interaction network.

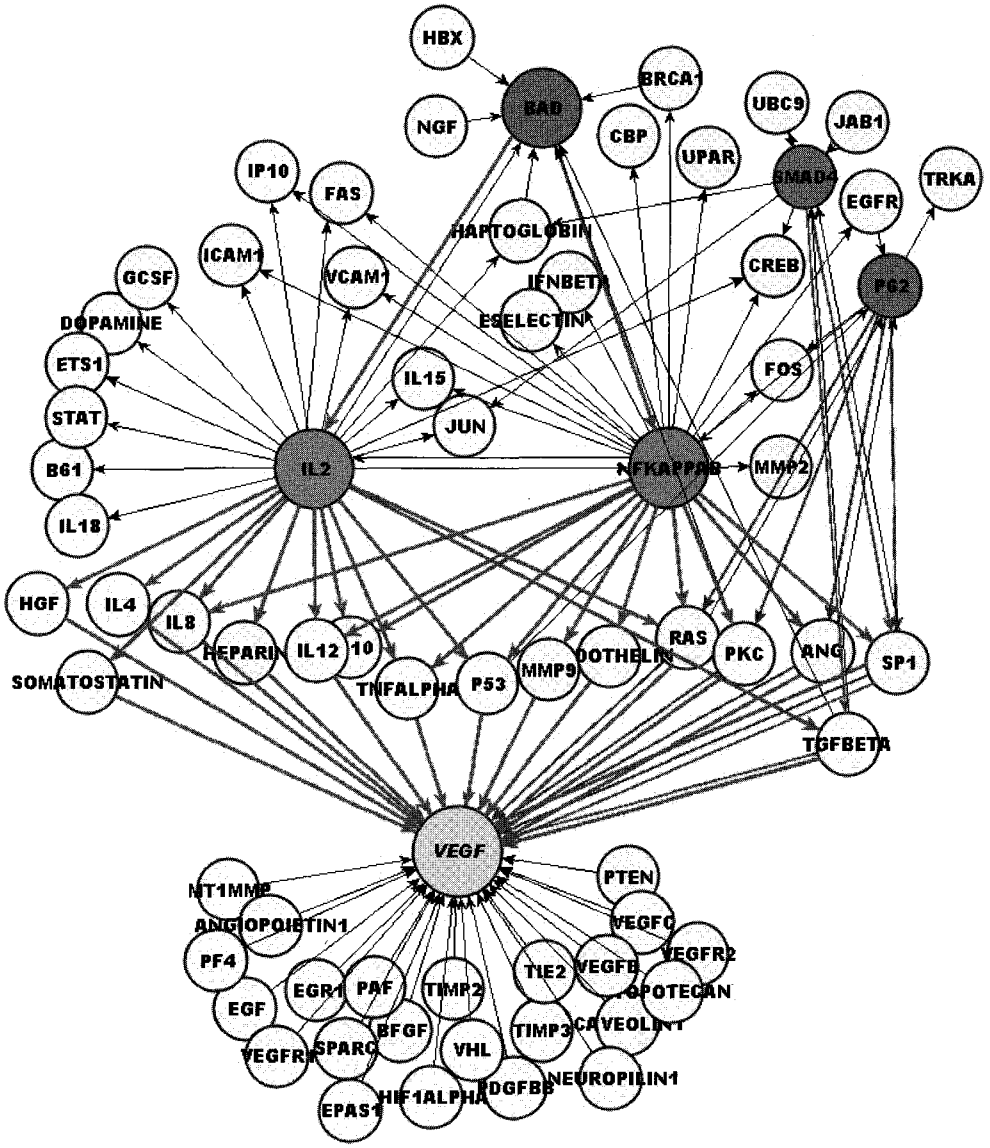

Fig. 4. Extended interaction network via NF kappa B and IL2
The network was an extended form of it in Fig. 3. This extended network shows interactions concentrating on NF-κ B, IL-2 and the relationship with factors directly interacting with VEGF.

for VEGF expression. In addition to signaling via PKC, Sp1, and Ras, p62 also interacts with angiotensin II, which has been reported to induce VEGF expression (Rizkalla *et al.*, 2003). Moreover, p62 is associated with angiotensin II and involved in angiotensin II-mediated signaling (Lu *et al.*, 1998). Therefore, p62 can be a regulator of either PKC-ζ-or angiotensin II- mediated signaling.

Finally, BAD is a distant member of the Bcl-2 family of proteins that was identified as a Bcl-2-interacting protein (Yang *et al.*, 1995). Bcl2 and Bcl-xL have anti-apoptotic effects, while other members of the Bcl-2 family, such as Bax and BAD are pro-apoptotic molecules. In terms of the interaction network, there was not a path via only one factor from BAD to VEGF. As shown in Fig. 4, there is a wider range of molecules involved in the pathway from BAD to VEGF through the extension to the secondary interaction of BAD via NF-κB and IL2. From the results of the microarray, both Smad4 and p62 were increased at hypoxia, while BAD was dramatically induced at the stage of early reoxygenation. Considering hypoxia is closely connected with angiogenesis, the fact that Smad4 and p62 were expressed notably at hypoxia

could be relevant to the linkage state with VEGF in just a 2-step interaction because less number of interactions might generate a faster effect on VEGF expression by upstream factors. BAD being increased during early reoxygenation is linked to VEGF not in a 2-step but 3-step interaction. It is possible for BAD to be linked to VEGF in a 3-step interaction due to NF-κB and IL2, which are major factors involving many interacting proteins. It means that BAD induce the VEGF expression via a number of pathways, but the specificity of BAD for VEGF expression might be reduced because once BAD interacts with NF-κB and IL2, which interact with a huge number of molecules in the various biological processes, the effect of BAD can possibly be dispersed.

Discussion

Recently, astrocytes have become the issue of interest because it has been reported to have various important effects on the vascular system and the nervous system. The major cell type involved in this process is the endothelial cell, which has surface receptors to which angiogenic factors bind. In the brain, astrocytes are associated with endothelial cells of the brain microvessels as well as to the neurons. Based on this concept that astrocytes can influence the vascular system, the investigation of differential gene expression in astrocytes according to oxygen tension is meaningful.

Through the investigation of interactions by using the data in AngioDB, the candidate genes, which resulted from the microarray experiment, were suggested to be involved in angiogenesis-related networks. To further study the effects on endothelial angiogenesis, we hypothesized that if astrocytes release VEGF depending on oxygen tension, VEGF can induce brain angiogenesis around them. Therefore, the effects of Smad4, p62, and BAD on endothelial angiogenesis were judged from their relationship with signaling pathways that are relevant to VEGF expression. Smad4 and p62, which were increased at hypoxia, can lead to VEGF via just one factor, such as SP1, Ras, and so on. On the other hand, BAD, which illustrated very high expression levels during early reoxygenation, can influence VEGF expression in a 3-step interaction. This shows that considering oxygen deficiency is a crucial factor for angiogenesis, and Smad4 and p62 were connected more closely with VEGF expression than BAD. This also signifies that Smad4 and p62 increasing at hypoxia can rapidly increase/regulate VEGF expression. In addition, through the inference of the signaling pathway for VEGF expression, it is possible to identify the underlying mechanism of some phenomena as well as the potential signaling pathways in angiogenesis. For example, both Smad4 and p62 can be linked to Sp1. VEGF expression by oxidative stress was known to be mediated by the Sp-1 and Sp-3 dependent mechanism (Schafer *et al.*, 2003). Of these, Smad4 and p62, which are sensitive to oxygen tension, can act as an intermediary factor for Sp-1 signaling that is related to oxygen concentration. For BAD, instead of being involved in the shorter signaling pathways toward VEGF expression, it might be involved in a number of interactions with factors having effects on VEGF expression via NF-κB and IL2. Then, it can have effects on various signaling for other processes as well as for VEGF expression. These inferences would be applied to other phenomena that occur in the process of reoxygenation aside from hypoxia, such as the maturation of the newly formed blood vessel and the formation of blood-brain barrier.

Collectively, in this study, the cDNA microarray was applied to the screening of differential gene expression patterns in the astrocyte according to oxygen tension. We suggests that further investigation of the gene expression data from microarray experiments can be extended by building interaction networks using previously characterized information, such as the data in AngioDB. As accepted, the cDNA microarray is one of the best methods for screening expression patterns of thousands of genes in a single experiment and finding candidate genes that are responsive to a specific condition among a number of genes. In the microarray analysis, the method by which the biological significance of these genes are analyzed and identified is the most important. We have illustrated new approaches for prediction through the use of the interaction network and the preexisting information that was previously characterized.

Acknowledgements

This work was supported by grants from the Korea Health 21 R&D Project (HMP-01-PJ1-PG1-01CH04-0005), the Ministry of Health and Welfare, Korea, Brain Research Center of the 21st Century Frontier Research Program (M103KV010005 03K2201 00520), and the National Research Laboratory Fund (2003-N-NL-01-C-015), the Ministry of Science and Technology, Korea (to K.-W. K).

References

Benezra R, Rafii S, and Lyden D (2001). The Id proteins and angiogenesis. *Oncogene* 20, 8334-8341.

Dentelli P, Del Sorbo L, Rosso A, Molinar A, Garbarino G, Camussi G, Pegoraro L, and Brizzi MF (1999). Human IL-3 stimulates endothelial cell motility and promotes in vivo new vessel formation.

J. Immunol. 163, 2151-2159.

Derynck R, Zhang Y, and FengXH (1998). Smads:transcriptional activators of TGF-beta responses. *Cell* 95, 737-40.

Di Cristofano A, Niki M, Zhao M, Karnell FG, Clarkson B, Pear WS, Van Aelst L, and Pandolfi PP (2001). p62(dok), a negative regulator of Ras and mitogen-activated protein kinase(MAPK) activity, opposes leukemogenesis by p210(bcr-abl). *J. Exp. Med.* 194, 275-284.

Howe JR, Roth S, Ringold JC, Summers RW, Jarvinen HJ, Sistonen P, Tomlinson IP, Houlston RS, Bevan S, Mitros FA, Stone EM, and Aaltonen LA (1998). Mutations in the SMAD4/DPC4 gene in juvenile polyposis. *Science* 280, 1086-1088.

Janzer RC and Raff MC (1987), Astrocytes induce blood-brain barrier properties in endothelial cells. *Nature* 325, 253-257.

Lee SW, Kim WJ, Choi YK, Song HS, Son MJ, Gelman IH, Kim YJ, and Kim KW (2003). SSeCKS regulates angiogenesis and tight junction formation in blood-brain barrier. *Nat. Med.* 9, 900-906.

Lu D, Yang H, and Raizada MK (1998). Involvement of p62 nucleoporin in angiotensin II-induced nuclear translocation of STAT3 in brain neurons. *J. Neurosci.* 18, 1329-1336.

Maltepe E and Simon MC(1998). Oxygen, genes, and devlopment: an analysis of the role of hypoxic gene regulation during murine vascular development. *J. Mol. Med.* 76, 391-401.

Pal S, Datta K, Khosravi-Far R, and Mukhopadhyay D (2001). Role of protein kinase Czeta in Ras-mediated transcriptional activation of vascular permeability factor/vascular endothelial growth factor expression. *J. Biol. Chem.* 276, 2395-2403.

Pardali K, Kurisaki A, Moren A, ten Dijke P, Kardassis D, and Moustakas A (2000). Role of Smad proteins and transcription factor Sp1 in p21(Waf1/Cip1) regulation by transforming growth factor-beta. *J. Biol. Chem.* 275, 29244-29256.

Park I, Chung J, Walsh CT, Yun Y, Strominger JL, and Shin J (1995). Phosphotyrosine-independent binding of a 62-kDa protein to the src homology 2(SH2) domain of p56lck and its regulation by phosphorylation of Ser-59 in the lck unique N-terminal region. *Proc. Natl. Acad. Sci. USA* 92, 12338-12342.

Park JA, Choi KS, Kim SY, and Kim KW (2003). Coordinated interaction of the vascular and nervous systems: from molecule-to cell-based approaches. *Biochem. Biophys. Res. Commun.* 311, 247-253.

Parri R and Crunelli V (2003). An astrocyte bridge from synapse to blood flow. *Nat. Neurosci.* 6. 5-6.

Puls A, Schmidt S, Grawe F, and Stabel S (1997). Interaction of protein kinase C zeta with ZIP, a novel protein kinase C-binding protein. *Proc. Natl. Acad. Sci. USA* 94, 6191-6196.

Reisinger K, Kaufmann R, and Gille J (2003). Increased Sp1 phosphorylation as a mechanism of hepatocyte growth factor (HGF/SF)-induced vascular endothelial growth factor(VEGF/VPF) transcription. *J. Cell. Sci.* 116, 225-238.

Rizkalla B, Forbes JM, Cooper ME, and Cao Z (2003). Increased renal vascular endothelial growth factor and angiopoietins by angiotensin II infusion is mediated by both AT1 and AT2 receptors. *J. Am. Soc. Nephrol.* 14, 3061-3071.

Roberts AB, Sporn MB, Assoian RK, Smith JM, Roche NS, Wakefield LM, Heine UI, Liotta LA, Falanga V, and Kehrl JH (1986). Transforming growth factor type : rapid induction of fibrosis and angiogenesis *in vivo* and stimulation of collagen formation *in vitro*. *Proc. Natl. Acad. Sci. USA* 83, 4167-4171.

Schafer G, Cramer T, Suske G, Kemmner W, Wiedenmann B, and Hocker M (2003). Oxidative stress regulates vascular endothelial growth factor-A gene transcription through Sp1- and Sp3-dependent activation of two proximal GC-rich promoter elements. *J. Biol. Chem.* 278, 8190-8198.

Shih AY, Johnson DA, Wong G, Kraft AD, Jiang L, Erb H, Johnson JA, and Murphy TH (2003). Coordinate regulation of glutathione biosynthesis and release by Nrf2-expressing glia potently protects neurons from oxidative stress. *J. Neurosci.* 23, 3394-406.

Sohn TK, Moon EJ, Lee SK, Cho HG, and Kim KW (2002). AngioDB: database of angiogenesis and angiogenesis-related molecules. *Nucleic. Acids. Res.* 30, 369-71.

Travis J (1994), Glia: the brain's other cells. *Science* 206, 970-922.

Yang E, Zha J, Jockel J, Boise LH, Thomson CB, and Korsemeyer SJ (1995), Bad, a heterodimeric partner for Bcl-xL and Bcl-2, displaces Bax and promotes cell death. *Cell* 80, 285-291.

Zhu Y, Yang GY, Ahlemeyer B, Pang L, Che XM, Culmsee C, Klumpp S, and Krieglstein J (2002). Transforming growth factor-beta 1 increases bad phosphorylation and protects neurons against damage. *J. Neurosci.* 22, 3898-3909.

Zonta M, Angulo MC, Gobbo S, Rosengarten B, Hossmann KA, Pozzan T, and Carmignoto G. (2003). Neuron-to-astrocyte signaling is central to the dynamic control of brain microcirculation. *Nat. Neurosci.* 6, 43-50.