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Short Communication Open Access

Unraveling the Progression of Ischemic Core Genome-Wide by Bioinformatics Analysis of Permanent Middle Cerebral Artery Occlusion (PMCAO) Mouse Model Brain Regions Genes

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Introduction

The neuropeptide, pituitary adenylate-cyclase activating polypeptide (PACAP) [1-3], is the major focus of research as a neuroprotective factor in the group of Prof. S. Shioda [4-16]. A major emphasis of the research is on the neuroprotective effects of PACAP38 on the brain, particularly in brain ischemia [17]. Therein, a permanent middle cerebral artery occlusion (hereafter referred to as PMCAO) mouse model has been established and used for unraveling of the genome-wide gene expression profiles by high-throughput omics approach, namely DNA microarray technology. DNA microarray analysis of the whole brain/ischemic hemisphere and specific brain regions of the ischemic core hereafter, the IC and penumbra hereafter, the P with or without PACAP38 treatment have been performed [11-16]. It is to be noted that the reason for utilizing intraluminal filament technique-based PMCAO model over the transient MCAO is to avoid reperfusion injury, in our research model (PMCAO). However, it should be emphasized that different research groups are using different stroke models such as the MCAO, resulting in greater insight into how PACAP treatment influences the brain ischemia. Overall, these genomic data on differential gene expression in the brain of mouse PMCAO model resulted in us i) obtaining the transcriptome profiles of ischemic brain hemispheres along with the diverse categories of gene families being modulated under the ischemic condition [11-13], ii) unraveling specific gene expressions and localization of molecular factors in the IC and P effected specifically by the PACAP38 treatment [14,15], and iii) providing the explanation and validation of the dye-swap, two-color DNA microarray approach [16]. The vast inventories of differential gene expressions generated from these numerous analyses have not only revealed the importance of both whole hemisphere and region-specific analyses in genome-wide identification of target molecular factors that might play a role in the neuroprotective function of PACAP38, but also provided a valuable resource for further study by the scientific community.

Short Communication

Our most recent data analyzing the specificity of the PACAP38 treatment in delineating the molecular expressions in the IC and the P at 6 h post-treatment (early) and 24 h (late) progression of the ischemia revealed that PACAP38 indeed has a positive influence in helping recover the ischemic insult [15]. However, we did not clarify the progression of ischemia itself, i.e. in the absence of any treatment (PACAP38). As also kindly suggested by an anonymous reviewer in our previous research paper [15] it would be important to investigate the progression of ischemia in order to effectively understand the neuroprotective effects of PACAP38, the overall goal of the research. Therefore, in this communication, we primarily present the DNA microarray analysis data of the IC and P regions at 6h in the ischemic brain compared to the healthy IC and P regions, as outlined in the experimental design (Figure 1). In other words, our research aimed to unravel differential gene expression profiles in the IC and P regions without PACAP38 treatment in order to know which genes are specifically functioning during progression of the ischemic core. To do so, we carried out a whole genome DNA microarray analysis (Agilent mouse whole genome 4×44K DNA chip; G4131F) of the healthy versus ischemic IC and P regions. Briefly, three mice each in PMCAO groups for IC and P regions in the ischemic brain over corresponding controls were used that exhibited neurological grades G1 and G2, for the subsequent downstream analysis. Ischemic core and P regions and corresponding healthy core and P regions were carefully removed with a sterile scalpel, and placed in sterile 2 ml Eppendorf tubes. Samples were

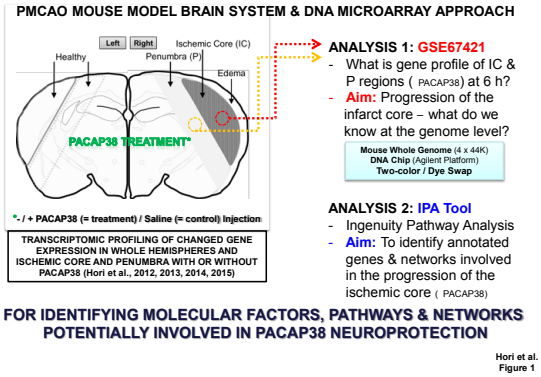

Figure 1: Strategy for analyses of the specific brain regions, ischemic core (IC) and the penumbra (P), in the permanent middle cerebral artery occlusion (PMCAO) mouse model and DNA microarray approach for identifying the molecular factors potentially involved in PACAP38-mediated neuroprotection. Two analyses aprpoaches are presented here for progression of infarct, first, gene profiling of the 6 h IC and P regions (minus PACAP38 treatment), and second, use of the Ingenuity Pathway Analysis (IPA) tool for identification of the annotated genes and newtorks therein.

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then quickly immersed in liquid nitrogen and stored in -80°C prior to further analysis. Animal care and experimental procedures were used as approved by the Institutional Animal Care and Use Committee of Showa University (School of Medicine), Tokyo, Japan, and the PMCAO model mice, (PACAP38 treatment), dissection of brain, sampling and storage, and total RNA extraction followed by DNA microarray analysis was performed using our established dye-swap approach as described previously [11-16]. The outputs of DNA microarray analysis are freely available to the public under the series number GSE 67421 [18] at the NCBI GEO public functional genomics data repository [19].

Additional bioinformatics analysis was carried out using the Ingenuity Pathway Analysis (IPA; Ingenuity® Systems, www.ingenuity.com; Content version: 23814503, Release Date: 2015-03-22, Qiagen) bioinformatics tool that provided us with the latest annotations of genes. The biological function and network analysis were also generated through the use of IPA. The data set from microarray (6 h, IC and P), which is the differentially expressed (≧/≦ 1.5/0.75-fold compared to saline control) genes, and their corresponding fold change values were uploaded as an Excel spread sheet into the IPA tool. To create gene networks, genes were overlaid onto a global molecular network developed from information contained in the ingenuity knowledge base. The functional analysis identified the biological functions that were most significant to the data set (p-value < 0.05) according to Right-tailed Fisher's exact test. Further, we also used the PACAP38 treatment data (GSE 62884) for the IC and P regions to generate a parallel list of annotated genes. In this PMCAO-PACAP38 model, an intracerebroventrically PACAP38 (1 pmol) injection over a control saline (0.9% sodium chloride, NaCl) treatment was used.

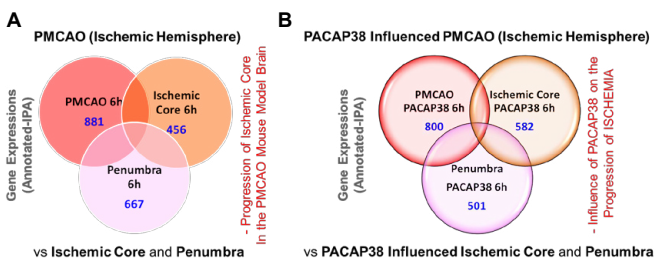

Figure 2: Venn diagram showing the numbers of differentially expressed annotated genes in the PMCAO ischemic hemisphere (881), as compared to the IC (456), and P (667) regions (A), and also in the PACAP38 influenced PMCAO ischemic hemisphere (800), as compared to the IC (582), and P (501) regions (B) at 6 h. Genes were annotated using the IPA bioinformatics tool. IC, ischemic core; P, penumbra.

Results presented in Figure 2A show the number of differentially expressed (up- and down-regulated) annotated genes in the IC (456) and P (667) compared to the whole brain (hemisphere, 881). Similarly, in Figure 2B, the number of differentially expressed annotated genes after PACAP38 treatment for the IC (582) and P (501) compared to the whole brain (hemisphere, 800) is presented. These genes are presented as a list of color-coded up-/down-regulated genes along with their Entrez gene names, Agilent probe number and fold-change, for clarity in Supplementary Tables 1 and 2 (Figure 2A) 3 and 4 (Figure

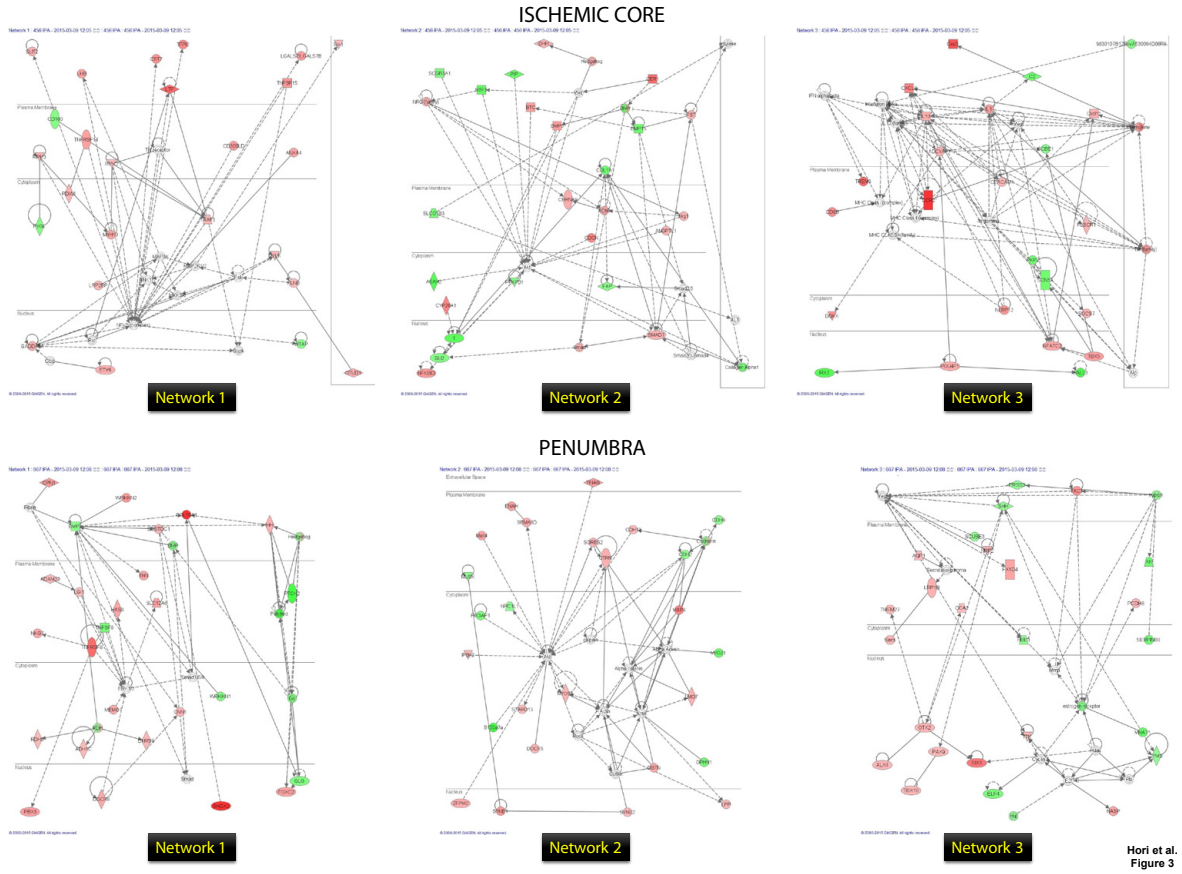

Figure 3: The top 3 networks for the IC (upper panel) and P (lower panel) regions by the IPA bioinformatics tool.

Ischemic Core (IC)		Penumbra (P)	
Fold Change up-regulated		**Fold Change up-regulated**	
Molecules	Exp. Value (+)	Molecules	Exp. Value (+)
Ngp	6.341	*CAMK2D*	8.03
CCR2	4.449	*SHOX2*	7.571
ANXA10	4.357	*COL10A1*	5.096
TMEM190	3.973	*SAMD3*	4.693
AVPR1A	3.858	*CCKAR*	4.29
FAM90A1	3.616	*OPN4*	3.736
Cxcl3	3.583	*KRT23*	3.522
LTF	3.217	*TNFRSF8*	3.35
TREM3	3.202	*SNCA*	3.132
NMU	3.157	*SIX3*	3.041
Fold Change down-regulated		**Fold Change down-regulated**	
Molecules	Exp. Value (-)	Molecules	Exp. Value (-)
TBX22	3.043	*TUB*	4.147
ADAMTS12	2.782	*TMEM199*	3.648
N4BP2	2.596	*SLC13A1*	3.116
FGF16	2.565	*CYP11A1*	2.78
RAB38	2.552	*ISL2*	2.647
MB	2.425	*SOX17*	2.613
ART4	2.368	*LY6D*	2.598
SAMD11	2.359	*OR2G6*	2.59
ITIH5	2.238	*S100a7a*	2.584
RAPGEF6	2.306	*PTCH2*	2.581

Table 1. The top molecules identified in the IPA analysis for the ischemic core (IC) progression. The penumbra (P) top molecules are also presented for comparison.

2B) (see Supplementary Information Tables). At a glance these data reveal differences in the type of genes expressed in the IC and P regions during progression of infarct, including that after PACAP38 treatment. Subsequently, these gene lists were used to generate the networks for the molecules/pathways being influenced in the IC and P regions (for without PACAP38 treatment), examples of which are shown in Figure 3. The top up- and down-regulated molecules or the IC and P regions are also presented in Table 1 and these genes might be linked to the progression of the ischemic core.

Here we have newly communicated the importance of using a specific analysis of the gene expression in IC and P brain regions compared to healthy control regions to know the gene profile status in the progression of IC using IPA bioinformatics tool providing the latest annotated gene lists and generated gene networks. This study will form the basis for a detailed bioinformatics analysis functionally identifying the genes (Figure 2A, Figure 3, and Table 1) involved specifically in the progression of ischemia, which is not well understood as yet. Furthermore, by revisiting the PACAP38 influenced annotated gene lists in light of the progression of ischemia we may be able to further understand the diversity and specific function of molecular factors, pathways and networks that may underlie the potential mechanism behind PACAP38 neuroprotective action in the ischemic brain. For this, functional genomics studies will be the only way forward to understand how and why PACAP38 is neuroprotective in the brain for any meaningful development of a stroke therapy using PACAP38 or its analogs.

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