# CHAPTER 4

# DISCUSSION

## Biomarker(s) and proteome profile of human plasma

2DE has been proved to be an extremely promising method of protein analysis in proteomics (for reviews see Görg et al., 2004; López, 2006). Together with mass spectrophotometry (MS), several proteins/peptides that are related to metabolic systems and diseases including depressive disorder have been identified (for reviews see Steel et al., 2005; Zeugmann et al., 2010; Schiess et al., 2009; Brewis and Brennan, 2010). Many evidences suggested that peripheral fluids such as plasma are rich source of proteins marker (Adkins et al., 2002; for reviews see Anderson and Anderson, 2002) which some of them display complex combinations of post-translational modification, particularly involving glycosylation, that can be discriminated by proteomics techniques in particular 2DE (Gravel et al., 1994, 1996; for review sees Anderson and Anderson, 2002) Some of these proteins reflect a systemic metabolic signature or the changes in plasma secondary indicate to a disease-specific process in the brain, thus the disease stages and disease development may be defined and/or predicted (for review sees Schneider and Prvulovic, 2013). In this study, the proteins in plasma those are high potential to be used as markers for early prediction of the responsiveness to antidepressant (fluoxetine) of MDD were explored by 2DE and these proteins were identified by mass spectrophotometry.

The analysis between the plasma from the normal and MDD groups in this study revealed that the expression of several proteins including α1-antitrypsin, fibrinogen, haptoglobin, transcritption factor, apoliporpotein AI (apo AI), apolipoprotein AII (apo AII), apolipoprotein E (apo E), HDL associated protein and IgG light chain was high in MDD compared with the normal. All of these proteins, except apolipoproteins, function or are related to acute phase and inflammatory responsive systems (for reviews see Janciauskiene et al., 2011; Davalos and Akassoglou, 2012; Quaye, 2008; Salim et al., 2012; Zhu and Parks, 2012), and the increase of these protein levels in MDD well agree with that previous reported (Joyce et al., 1992; Zeugmann et al., 2010; Erdem et al., 2011; Papakostas et al, 2011).

Recently, the examination of the relationship between apo E ε4 (ApoE ε4) and depression in adults and elders showed high association of the symptom with the ApoE ε4 allele (Arnold et al., 2012; Michels et al., 2012). Together with the finding of higher expression of apo E in plasma of MDD than in the normal, it conceived that this apolipoprotein can be a biomarker for MDD, and may be used to classify between MDD and the normal.

Several controversial results have been reported for the relationship between apolipoprotein in particular apo A and depression. Either detection the protein in the serum of depressed patients at lower level of than in control or no significant difference between these two people groups were reported (Severus et al., 2001; Kopf et al., 2004; Sarandol et al., 2006). The severity of depression was also shown correlated with the decrement in serum apo A level (Sadeghi et al., 2011). The result obtained in this study, i.e. the levels of both apo AI and apo AII were higher in MDD than in the normal, therefore, was inversed to the previous reports, and may need to be validated such as by Western blot. However, since the correlation between the high expression of apo A with some diseases such as coronary heart disease has been evidenced (Yang et al., 2012). In addition, the recent searching on markers in plasma for predicting the development of Alzheimer disease (AD) showed that apo AII was one of the significant signatures that can differentiate AD from normal groups (Llano et al., 2012). It suggested that apo A may be a predictor for depression and its increase would be a risk factor for coronary heart disease particular in MDD (Sadeghi et al., 2011).

The absence or very low expression of complement C3 (C3) in the normal groups which led to extremely high expression level of the protein in MDD was observed in this study. The elevation of C3 but not C4 was reported associated with hostility, anger and depression (Boyle et al., 2007). However, it also was reported that no significant differences between the mean levels of C3 could be detected between depressed patients and controls, whereas the levels of C4 and C-reactive protein were significantly raised in the group with a depressive disorder (Berk et al., 1997). The previous controversial findings and the result observed in this study are still needed to be proved. Several observations showed inflammatory mechanisms as one of the causes of MDD (for reviews see Dean, 2011; Muller et al., 2011), and several proteins

in the system have been identified as markers for MDD. In addition, since C3 is one of the mediator in the inflammatory cascade and play important role particular in the alternative pathway of complement activation which provides host defense system and immune regulatory mechanisms (for reviews see Huang et al., 2008; Dinarello, 2010). High elevation of the level of C3 in plasma of MDD, thus, could confirm the association of the inflammatory and immune systems with depression.

To explore for a possible biomarkers in plasma for prediction of the responsiveness to antidepressant, 2DE spots from the plasma of NR, FR and SR were matched and analyzed. It showed the expression of α1-antitrypsin, apo AI, C3, haptoglobin and IgG light chain detected in the plasma of NR was higher than those of FR and SR. This indicated the association of proteins in the inflammatory and immune systems not only with the symptom of depression but also with the response to antidepressant. It seems likely that there was an inverse relationship between these body protective systems and the antidepressant effects. The expressions of transcription factor and Rap 1A, in contrast, were low in NR compared to FR and SR. Serotonin (5-HT) 5-HT1A autoreceptors (5-HT1AautoR) is one of key elements in the regulation of central 5-HT function and the responsiveness to antidepressant drugs in particular SSRIs (for review sees Descarries and Riad, 2012). Negatively regulation of expression of the receptor was shown be a possible clinical approach to improve the treatment of depression with SSRIs (Bortolozzi et al., 2011; for review sees Albert, 2012). It is possible that the transcription factor detected and had high expression in NR observed in this study may play role and associate to the low response to antidepressant of NR. To reveal whether this protein suppressive regulates 5-HT1AautoR or not, further identification and validation of the protein are necessary.

Synaptic plasticity confers adaptability and strength in response to changes of environment through modifications of the connectivity between neuronal cells and circuits. Deficits in synaptic plasticity were shown correlate to stress and depression (for review sees Marsden, 2012). Neurons, glia and many signaling systems including cAMP-protein kinase A complex (cAMP-PKA) signaling (for review sees Waltereit and Weller, 2003) participate in the synaptic networking. Dysfunctions in PKA and its substrates such as Rap1, small guanosine triphosphate (GTP)-binding protein

belonging to the ras family, are associated with depressive disorders (Perez et al., 2000; Odagaki et al., 2001; Perez et al., 2001; for reviews see Perez et al., 2000; Perez and Tardito, 2001). It reported that abnormalities in some components of cAMP signaling in patients were likely related to treatment rather than symptom of the disease (Lowther and Katona, 1997; Dowlatshahi et al., 1998). In addition, the knock-out (KO) of the rap1a gene showed impairment of synaptic plasticity (Pan et al., 2008). In this study, the expression level of Rap 1A was lower in MDD than in the normal group, in addition, lower in NR than in FR and SR. These findings should support the association of the cAMP-PKA signaling with depression and its relationship with the responsiveness to the treatment with antidepressant. Moreover, it implied to a possible use Rap 1A as one of biomarkers for the prediction of the treatment with antidepressant in particular fluoxetine in advanced.