Cukurova Medical Journal

Araştırma Makalesi / Research Article

LMP-1 and Nasopharyngeal Carcinoma (NPC)

LMP-1 ve Nazofaringial Karsinoma

Anggun Indah Budiningrum[1], Achmad Rofi'i[2], Suharjono Suharjono[1], Fatchiyah Fatchiyah[1]

[1]Department of Biology, Faculty of Mathematics and Natural Sciences, University of Brawijaya, Malang, East Java, INDONESIA

[2]Departments of Otorhinolaryngology, Head and Neck, Ulin General Hospital, Faculty of Medicine, University of Lambung Mangkurat, Banjarmasin, South Kalimantan, INDONESIA

Cukurova Medical Journal 2014; 39 (3):480-487.

ABSTRACT

Purpose: This study aims to determine the interaction of LMP-1 with H_2O_2 , nitrosamine, and nicotine to predict the external factor that affected LMP-1, which activates lytic phase on nasopharyngeal carcinoma (NPC).

Materials and Methods: The amino acid sequence of human LMP1 (GI: 343177335) and compound structure of H_2O_2 (ID:784), nicotine (ID: 89594), and nitrosamine (ID: 37183) was obtained from database The National center of Biotechnology Information. The 3-dimension modeling structure of LMP-1 predicted using SWISS-MODEL web server. Hex 6.12 was used for the purpose of docking of the lead with the target molecule. Ligand details interaction then detected by LigPlus+ v.1.4.4 software. Molecular graphics and analysis were performed with PyMOL.

Results: LMP-1 has interaction with H_2O_2 , nitrosamine, and nicotine. Total energy that use of LMP-1 to interaction with H_2O_2 (-67,79 kJ/mol) is lowest than interaction with nicotine (-149,43 kJ/mol) and nitrosamine (-82,93 kJ/mol). The kind of interaction between LMP-1 and H_2O_2 is hydrogen bond whereas interaction of LMP-1 between nicotine and nitrosamine are hydrophobic bond.

Conclusions:LMP-1 has strongest interaction with H_2O_2 than nicotine and nitrosamine. This is predicted that H_2O_2 is external factor that affect LMP-1 switching to lytic infection in NPC patients.

Keywords: H_2O_2 , LMP-1; nasopharyngeal carcinoma, nicotine, nitrosamine

ÖZET

Amaç: Bu çalışmanın amacı nazofaringial karsinomun litik fazı üzerine etkili olduğu düşünülen LMP-1, ile H_2O_2 , nitrozamin ve nikotinin gibi dış faktörler arasındaki etkileşimi belirlemektir.

Materyal ve Metod: insan LMP-1 (GI: 343177335) aminoasid sekansı ile nikotin (ID: 89594), nitrozamin (ID: 37183) ve H_2O_2 (ID: 37183) nin yapısal içeriği The National center of Biotechnology Information'a ait veri tabanına göre açıklanmıştır. LMP-1 in 3 boyutlu yapısı SWISS-MODEL web ağı kullanarak tasarlandı. Hex 6,12 hedef moleküle öncülük etmesi için kullanıldı. LigPlus+1.4.4 software ile ligand detayları tarandı. Moleküler grafikler ve analizler PyMOL ile yapıldı.

Bulgular: LMP-1 in H_2O_2 , nitrozamin ve nikotin ile etkileşimi vardır. LMP-1 in H_2O_2 etkileşimi için harcadığı enerji (-67,79 kj/mol), nikotin (-149,43 kj/mol) ve nitrozamin (-82,93 kj/mol) için kullanılandan daha düşüktür. LMP-1 ile H_2O_2 arasındaki etkileşimin türü Hidrojen bağı iken LMP-1 ile nitrozamin ve nikotin arasındaki etkileşim hidrofobik bağdır.

Sonuç: LMP-1 H_2O_2 ile nitrozamin ve nikotine göre daha güçlü bağ kurar. Dış etken olan H_2O_2 nin LMP-1 i etkileyerek NPC li hastalarda litik enfeksiyon fazına geçişe neden olduğu tahmin edilmektedir.

Anahtar Kelimeler: H_2O_2 , LMP-1, nazofaringial karsinom, nikotin, nitrozamin.

INTRODUCTION

Nasopharyngeal carcinoma (NPC) is a rare malignancy of squamous cell carcinoma in most part of the world and it is one of the most confusing, commonly misdiagnosed and poorly understood disease[1,2]. NPC is an Epstein-Barr virus-associated malignant tumor and consistent elevation in EBV antibody titers is a well-established risk factor for the development of NPC[1,3]. While NPC tumors are known to express three EBV-encoded proteins, EBNA1, LMP-1, and LMP-2, they also express a large number of virus-encoded small RNAs (EBERs) and microRNAs (miRNAs). Among them, LMP-1 may be a central in the development of NPC[3,4]. LMP-1 is recognized as multifunctional genes which play in the development of NPC; early genetic alterations may predispose precancerous cells to EBV latent infection[3].

LMP-1 is an integral membrane protein, which acts like a constitutively active receptor, and confers a survival advantage to EBV infected cells. The mechanisms of LMP-1 sensitizing NPC cells to DNA damaging agents are far from being clear. LMP-1 can exacerbate cancer cells sensitivity to hydrogen peroxide[5]. The etiology of NPC is thought to be associated with a complex interaction of genetic, viral, environmental and dietary factors[1].

Previous study shows that the expression of LMP-1 detected in both NPC and healthy serum but BZLF-1 (35kDa) that predicted as lytic marker only expressed in NPC serum[6]. It's predicted that LMP-1 was affected by another factor that affect

activated of lytic phase. The transition of latent to lytic phase in Raji cell line was mediated by hydrogen peroxide (H_2O_2)[7]. Another study shows

that the high level of hydrogen peroxide (H_2O_2) detected in NPC patient compared to non NPC[8]. This is predicted that H_2O_2 interacted with LMP-1 to lytic phase.

Exogenous exposures associated with NPC previously included source of nitrosamine (dietary and other)[9]. Studies have shown an association between dietary consumption of salted fish and other preserved foods rich in nitrosamines and NPC risk[10]. Beside of nitrosamine consumption, NPC patients have positively smoking. Nicotine from smoking activity can stimulate downstream cellular pathways intimately related to the cancer process. Nicotine can enhance proliferation, decreasing apoptosis, and enhancing angiogenesis on oropharyngeal cancer[11]. Both nicotine and nitrosamine were predicted any interaction with LMP-1 as early oncoprotein to affect downstream pathways.

This study aims to investigate the interaction of LMP-1 with H_2O_2 , nicotine, and nitrosamine to predict the factor that affected LMP-1 to activated lytic phase on NPC.

MATERIALS and METHODS

The Intensity of LMP1, BZLF1, H_2O_2 expression (intensity/μm) on normal and NPC tissues were measured by immunofluorescence method and quantified with Olympus FluoView software (version 1.7a). To predict the interaction of LMP-1 with external factor, we were analyzed by in silico. The amino acid sequence of human LMP-1 (GI:343177335) and compound structure of H_2O_2 (ID:784), nicotine (ID: 89594), and nitrosamine (ID: 37183) was obtained from database The National center of Biotechnology Information, National Library of Medicine, National Institute of Health http://www.ncbi.nlm.nih.gov/. The 3-dimension modeling structure of LMP-1 predicted using SWISS-MODEL web server, www.swissmodel.expasy.org/[12]. Hex 6.12 was used for the purpose of docking of the lead with the target molecule. Docking of LMP-1 and compound target (H_2O_2 , nicotine, nitrosamine) was analyzed by using HEX software[13]. Ligand details

interaction then detected by LigPlus+ v.1.4.4 software[14]. Molecular graphics and analyses were performed with PyMOL[15].

RESULT

Our results show that in normal tissues the high level of LMP-1 will provide the high level of BZLF-1 and H_2O_2 (table1). The level of LMP-1 and H_2O_2 on NPC tissues were higher than normal tissues, meanwhile the BZLF-1 level was little bit higher than normal but not significant (P>0.05). To confirm interaction LMP-1 and H_2O_2 and/or other compounds, we analyze them by in silico.

Modeling protein of LMP-1 has important role to study interaction between LMP-1 and H_2O_2 , nitrosamine, and nicotine. Protein 3-dimensional structure of LMP-1 (Figure 1a) was generated using SWISS-MODEL server. From several predicted structure for LMP-1, the best model was picked based on Ramachandran plot analysis. The best model was picked based on highest percentages of residues in most favored regions and lowest percentages score in outlier region. The stereo chemical quality of predicted of LMP-1 structure was analyzed through residue by residue geometry and all geometry of protein structure using RAMPAGE server [16]. Ramachandran plots were drawn for this protein structure. In Ramachandran plots (Figure 1b), the most favored regions are indicated by dark blue patches, while areas show allowed regions. It was observed that LMP-1 has 88.0% number of residues in favored region, 8.9% number of residues in allowed region, 3.1% number of residues in outlier region.

LMP-1 has interaction with H_2O_2 , nitrosamine, and nicotine as shown in Fig 2. Total energy that use of LMP-1 to interaction with H_2O_2 (-67,79kJ/mol) is highest than interaction with nicotine (-149,43kJ/mol) and nitrosamine (-82,93kJ/mol). The kind of interaction between LMP-1 and H_2O_2 is hydrogen bond as shown with blue line whereas interaction of LMP-1 between nicotine and nitrosamine are hydrophobic bond.

Table1 Intensity of LMP1, BZLF1, H_2O_2 expression (intensity/µm) (P>0.05) on normal and NPC tissue by immunofluorescence and quantified with Olympus FluoView software (version 1.7a)

	LMP1	BZLF1	H_2O_2
Normal	998.56[a]	910.0313[a]	759.0233[a]
NPC	2036.78[b]	1024.474[a]	2600.137[b]

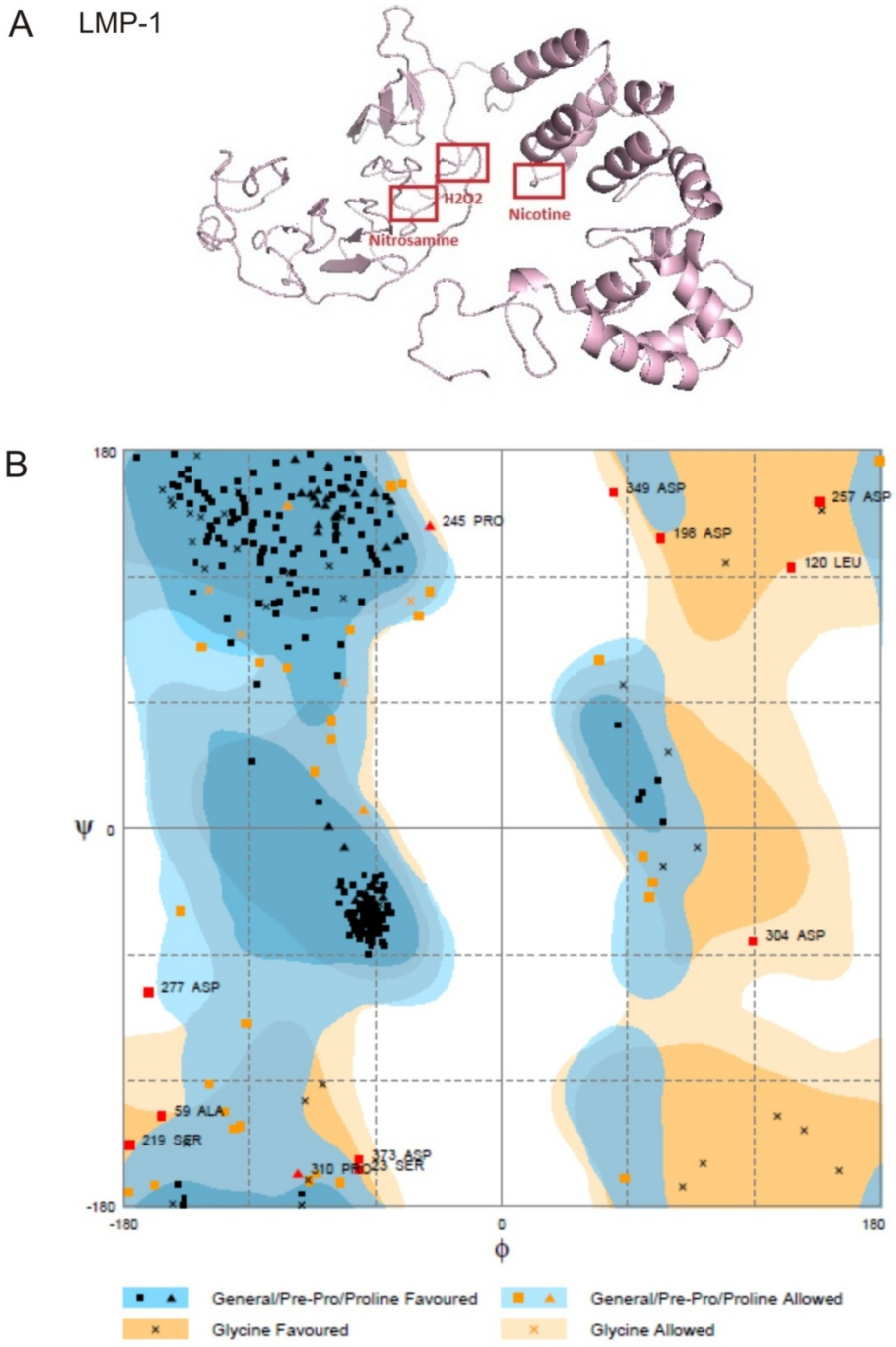


Figure 1. Budiningrum et al., 2014

Figure1.3D structure of LMP-1 protein (1a) and Ramachandran plots analysis for structure of LMP-1 protein (1b).

DISCUSSION

We found highest energy that used LMP-1 to interact with H_2O_2 . We are suggested that highest energy required to interaction between LMP-1 and H_2O_2 was little bit difficult. The interaction between LMP-1 and H_2O_2 is facilitated by hydrogen bond. The result of docking interaction analysis also showed that amino acid that directly involved in the interaction (Figure 2). In the interaction between LMP-1 and H_2O_2 , formed by 2 hydrogen bonds, those are H_2O_2 → Asp268 and H_2O_2 → Thr270, beside hydrogen bonds, this interaction formed by 4 hydrophobic bonds; those are H_2O_2 → Asp266, H_2O_2 → Pro267, H_2O_2 → Asp269, and H_2O_2 → Asp271.

In contrary, the lower energy provided the LMP-1 and nicotine stronger interaction than LMP-1 and H_2O_2 , and interestingly, this interaction is facilitated by hydrophobic bond. The interaction between LMP-1 and nicotine formed by 8 hydrophobic bonds; those are Nicotine (C10H14N2) with Ala157, Leu158, Leu160, Gln161, Asp268, Thr270, Asp271, Asp272. The interaction between LMP-1 and nitrosamine formed by 6 hydrophobic bonds; those are nitrosamine (H2N2O) with Glu254, Pro256, Gln265, Asp266, Pro267, Asp268.

The hydrogen bond is more stable than hydrophobic bond, although interaction between LMP-1 and H_2O_2 is difficult but the binding is more stable than nicotine and nitrosamine. LMP-1 is easy to interact with nicotine and nitrosamine but the binding is weak and not stable. Latent membrane protein 1 (LMP-1) is of special interest since it is generally considered to be the main EBV oncogene[16]. EBV, in conjunction with environmental and genetic factors, plays a role in the development of NPC[17]. According to the result of this interaction is consistent with previous study that H_2O_2 is highly detected in nasopharyngeal carcinoma patients than normal individual[8]. Hydrogen peroxide (H_2O_2) is non radical of reactive oxygen species that contributes to tumor initiation and progression solely by inducing genomic instability. ROS (reactive oxygen species) production or decrease in ROS-scavenger capacity disturbs redox homeostasis which leads to an overall increase of intracellular ROS level[18]. This is predicted that H_2O_2 more capable than nicotine and nitrosamine to caused LMP-1 to switch from latent to lytic that signed by BZLF-1 expression, because previous study shows that only the NPC patients have BZLF-1 at 32 kDa as regulatory lytic phase not in normal individual[6].

Nitrosamine might be importance in the development of NPC. This chemical carcinogen has a known tissue-specific oncogenic effect[19]. Smoking may also contribute as one of the environmental factors to NPC development[20]. Chronic exposure to various carcinogens may lead to genetic instability/damage of epithelial cells in the nasopharynx and may give rise to the development of multiple abnormal cell clones in the epithelium[21]. In this study, we found that both nicotine and nitrosamine have interaction with LMP-1 as first oncogenic of NPC, but this interaction is weak.

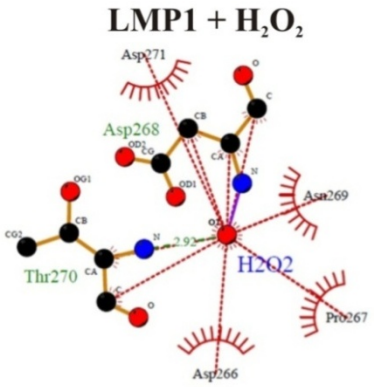
In summary, we found that LMP-1 has strongest interaction with H_2O_2 than nicotine and nitrosamine. This is predicted that H_2O_2 is external factor that affect LMP-1 switching to lytic infection in NPC patients.

ACKNOWLEDGMENT

This research is supported by Ulin General Hospital, Banjarmasin, and South Kalimantan for giving tissue slides. We also thank to Firli Rahmah Primula Dewi for in silico analysis assistant.

Conflict of interest statement

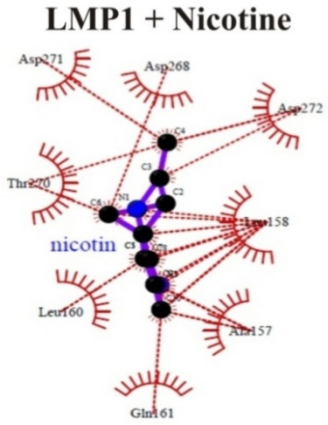
The authors declare that there are no conflicts of interest.



Compound ID: 784

Molecular Weight: 34.01468 [g/mol]
Molecular Formula: H_2O_2
XLogP3-AA: -0.9
H-Bond Donor: 2
H-Bond Acceptor: 2

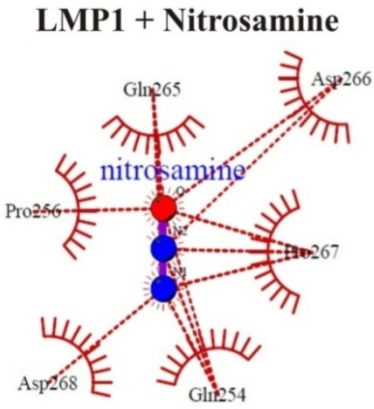
Etotal LMP1 + H_2O_2 = -67,79 kJ/mol



Compound ID: 89594

Molecular Weight: 162.23156 [g/mol]
Molecular Formula: $C_{10}H_{14}N_2$
XLogP3: 1.2
H-Bond Donor: 0
H-Bond Acceptor: 2

E total LMP1+nicotine = -149,43 kJ/mol



Compound ID: 37183

Molecular Weight: 46.02868 [g/mol]
Molecular Formula: H_2N_2O
XLogP3-AA: -0.6
H-Bond Donor: 1
H-Bond Acceptor: 3

E total LMP1+nitrosamine= -82,93 kJ/mol

Figure 2. Budiningrum et al., 2014

Figure2.Interaction of LMP-1 and H_2O_2, nicotine, and nitrosamine visualized by Hex 6.12 software.

REFERENCES

1. Cho WC. Nasopharyngeal carcinoma: molecular biomarker discovery and progress. Molecular Cancer. 2007;6:1-9.
2. Lo KW, To KF, Huang DP. Focus on nasopharyngeal carcinoma. Cancer Cell. 2004;5: 423-8.
3. Yoshizaki T, Kondo S, Wakisaka N, Murono S, Endo K, Sugimoto H, Nakanishi S, Tsuji A, Ito M. Pathogenic role of Epstein-Barr virus latent membrane protein-1 in the development of nasopharyngeal carcinoma. Cancer Letters. 2013;337:1-7.
4. Young LS & Rickinson AB. Epstein-Barr virus: 40 years on. Nat Rev Cancer. 2004;4:757–68.
5. Du CW, Wen BG, Li DR, Lin YC, Zheng YW, Chen L, Chen JY, Lin W, Wu MY.Latent membrane protein-1 of Epstein-Barr virus increases sensitivity to arsenic trioxide-induced apoptosis in nasopharyngeal carcinoma cell. Exp Oncol. 2005;27:267-72.
6. Budiningrum AI, Rofi'i A, Suharjono S, Fatchiyah F. PARP-1 expression against Epstein-Barr virus LMP-1 and BZLF-1 in undifferentiated nasopharyngeal carcinoma. J Exp Integr Med. 2013;3:299-304.
7. Lassoued S, Gargouri B, El Feki Ael F, Attia H, van Pelt J. Transcription of the Epstein-Barr Virus lytic cycle activator BZLF-1 during oxidative stress induction. Biol Trace Elem Res. 2010;137:13-22.
8. Rofi'i A, Fatchiyah F, Rahayu P, Muhyi R, Sumitro SB. Reactive oxygen species, NF-κB, and p53 levels in tissue of undifferentiated nasopharyngeal carcinoma. Oxid Antioxid Med Sci. 2013;2:143-7.
9. Dood LE, Sengupta S, Chen I-H, den Bonn JA, Cheng Y-J, Westra W, Newton MA, Mittl BF, McShane L, Chen C-J, Ahlquist P, Hildesheim A. Genes involved in DNA repair and nitrosamine metabolism and those located on chromosome 14q32 are dysregulated in nasopharyngeal carcinoma. Cancer Epidemiol Biomarkers Prev. 2006;15:2216-25.
10. Yu MC & Yuan JM. Epidemiology of nasopharyngeal carcinoma. Semin Cancer Biol. 2002;12:421-9.
11. Shields PG. Long-term nicotine replacement therapy: cancer risk in context. Cancer Prev Res. 2011;4:1719-23.
12. Arnold K, Bordoli L, Kopp J, Schwede T. The SWISS-MODEL workspace: a web-based environment for protein structure homology modeling. Bioinformatics. 2006;22:195-201.
13. Macindoe GL, Mavridis V, Venkatraman MD, Devignes DW, Ritchie. HexServer: an FFT-based protein docking server powered by graphics processors. Nucleic Acids Res. 2010;38:445-9.
14. Cleave SS, Panda R, Suresh PK. In silico exploration of phenytoin binding site in two catalytic states of human P-glycoprotein models. Indian J. Biochem. Biophysic. 2013;50:7-13.
15. DeLano, W.L. The PyMOL Molecular Graphics System. DeLano Scientific, San Carlos, CA, USA. 2002. http://www.pymol.org
16. Lin JC, Cherng JM, Lin HJ, Tsang CW, Liu YX, Lee SP. Amino acid changes in functional domains of latent membrane protein 1 of Epstein-Barr virus in nasopharyngeal carcinoma of southern China and Taiwan: prevalence of an HLA-A2-resticted 'epitope-loss variant'. J Gen Virol. 2004;85:2023-34.
17. Tang YL, Lu JH, Cao L, Wu MH. Peng SP, Zhou HD, Huang C, Yang YX, Zhou YH, Chen Q, Li XL, Zhou M, Li GY. Genetic variations of EBV-LMP1 from nasopharyngeal carcinoma biopsies: potential loss of T cell epitopes. Brazilian Journal of Medical and Biological Research. 2008;41:110-6.
18. Liu J, Zhan X, Li M, Li G, Zhang P, Xiao Z, Shao M, Peng F, Hu R, Chen Z. Mitochondrial protemics of nasopharyngeal carcinoma metastasis. BMC Medical Genomics. 2012;5: 62-78.
19. Autrup H, Stoner GD. Metabolism of N-nitrosamines by cultured human and rat esophagus. Cancer Research. 1982;42:1307-11.
20. Yuan JM, Wang XL, Xiang YB, Gao YT, Ross RK, Yu MC. Non-dietary risk factor for nasopharyngeal carcinoma in Shanghai, China. Int J. Cancer. 2000;85:364-9.

21. Chan ASC, To KF, Lo KW, Mak KF, Pak W, Chiu B, Tse GMK, Ding M, Li X, Lee JCK, Huang DP. High frequency of chromosome 3p deletion in histologically normal nasopharyngeal epithelia from Southern Chinese. Cancer Res. 2000;60:5365-70.

Yazışma Adresi / Address for Correspondence:
Dr.Fatchiyah
Department of Biology
Faculty of Mathematic and Sciences, University of Brawijaya
Jl. Veteran, Malang, 65145, INDONESIA.
Fax: +62341575841.
E-mail: fatchiya@ub.ac.id ; fatchiya@gmail.com

Geliş tarihi/Received on: 06.01.2014
Kabul tarihi/Accepted on:14.02.2014