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MACROPHAGE INFLAMMATORY PROTEIN 2-Alpha (MIP-2) IN HIV PATIENTS LEADING TO LUNG INJURY AFTER *Pneumocystis jiroveci* INFECTION

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ABSTRACT

Introduction: Exuberant inflammation during Pneumonia caused by *Pneumocystis jiroveci* strongly promotes pulmonary injury in HIV patients and suggested caused by Macrophage Inflammatory Protein 2-Alpha (MIP-2) as a strong chemoattractant.Aims: To understanding the potential roles of MIP-2 in the pulmonary injury in HIV patients.Methods: All complete coding sequences of human MIP-2 deposited inGenBank were downloadedand subjected for bioinformatics analysis.Results: Human MIP-2 lacks the highly conserved purine at position -3 but possess many features of the consensus sequence. The consensus polyadenylation signal was present in the hu-MIP-2a cDNA at position 1182-1187of the 3' untranslated region followed by a poly(A) beginning at nucleotide 1202. The cysteine alignment and the presence of non conserved amino acid were defining the properties of chemokines in case of what substances that could be attracted. The mechanism of lung injury perhaps does not caused by alteration of MIP-2 directly.

Key words: MIP-2, HIV, lung injury, *Pneumocystis jiroveci*

INTRODUCTION

Pneumocystis pneumonia (PCP) is the leading cause of morbidity and mortality in HIV-infected persons that is caused by *Pneumocystis jiroveci* (Morris *et al*., 2012). To eliminate *Pneumocystis jiroveci* infection, an effective host inflammatory response is required. One of the innate immunity regarding the host immune response to *Pneumocystis jiroveci*s by the activity of macrophage. In the lung, alveolar macrophages are the primary resident phagocytes that mediate the clearance of microorganisms. The uptake of *Pneumocystis jiroveci* by macrophages occurs through multiple receptor systems. That *Pneumocystisjiroveci*-receptor interaction makes alveolar macrophages produce a large variety of pro-inflammatory chemokines, cytokines and additional inflammatory mediators. These mediators generally participate in eradicating *Pneumocystis jiroveci* but also promote lung injury (Thomas and Limper, 2007).

Macrophage inflammatory protein 2 (MIP-2) is one of the chemokines that act as a potent chemoattractant for inflammatory cells, including neutrophils. Transcription of MIP-2 occurs through the action of macrophage mannose receptors interacting with major surface glycoprotein gpA/MSG on the surface of *Pneumocystis jiroveci*. The glucan cell wall components illicit an immunological response through a nuclear factor (NF)-kB mediated mechanism, in which p65/p50, heterodimeric form of NF-kB, translocate to the nucleus to increase transcription of tumour necrosis factor (TNF), interleukin (IL)-6, and MIP-2 (Thomas & Limper,

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2007).This mechanism may lead to the generation of effective immunity in a normal host, but contributes to immune-mediated lung injury in immunodeficient patient (Wang *et al*., 2005).

Exuberant inflammation during PCP strongly promotes pulmonary injury. Severe PCP is characterized by lung injury which induces diffuse alveolar damage and impairs gas exchange leading to respiratory failure(Thomas & Limper, 2007). Thechemotaxis property of MIP-2 is one of factors that could cause lung injury (Kelly & Shellito, 2010). The attracted neutrophils influx in the lung then associated with immune-mediated lung injury (Gigliotti & Wright, 2005). The mechanism and the exact role of MIP-2 to promote lung injury need a further explanation. The present study focusing on molecular analysis of MIP-2 that may defines its roles in immune response but also promotes lung injury.

MATERIALS AND METHODS

MIP-2 Gene Sequence and Analysis

All complete coding sequences of human MIP-2 (n= 18) deposited inGenBank were downloaded. The sequences then were analyzed by ClustalW for pairwise and multiple alignment.From the aligned sequence we reveal its consensus motif. The next step, we predicted all motifsand or variations that can be found in human MIP-2forboth nucleotide and amino acid sequences using MEME Suite and Geneious software.

RESULTS AND DISCUSSION

Sp1, Myf, and Ets motifs were found in nucleotide sequence of human MIP-2 (Table 1). Sp1 with 12 nucleotides in length that contains three Cys_2His_2-type zinc finger motifs plays important role in many cellular activities such as metabolism, cell growth, differentiation, angiogenesis and apoptosis (Raiber *et al.,* 2012), was found in negative strand of the sequence. Myfmotif related to myogenic regulatory factors (Wasserman & Fricket, 1998) was found both in negative and positive sequence. Ets, an 11 nucleotides-in-length DNA-binding domainrelated to proteins shown to be transcription activators (Wasylyk *et al.,* 1993), was found in positive sequence. Thosecis-elements on the sequence areresponsible for the regulation of the genesexpression which then translated into polypeptide and are folded into proteins (Maston *et al.,* 2006).

In human MIP-2 reference sequence, there is a feature to terminate transcription called polyadenylation signal (Proudfoot, 2011). The consensus polyadenylation signal (AATAAA) was present in the hu-MIP-2a cDNA at position 1182-1187 of the 3' untranslated region followed by a poly (A) beginning at nucleotide 1202. The AATAAA conserved sequence and poly (A) addition signal are required for transcription termination by RNA polymerase II. Deletion of this element would result to multiple rounds of transcription of the circular template because of a failure of transcription termination leading to the accumulation of RNA (Connelly & Manley, 1988).

Variants were found in aligned sequence of human MIP-2 after assembling the reference sequence to the aligned sequence resulted to contig. The contig then scanned to find any variation or polymorphism. We manually compared the amino acid sequence to its reference sequence to find any variation. We also tried to discover the same motif we found in reference sequence for the aligned sequence in order to analyze the variation. All polymor-

phism found did not change any amino acid residue (Table 2). Some substitutions in nucleotide which did change amino acid residue did not called as polymorphism because the number of these variations was detected below the minimum coverage that had been defined by the software. Most genetic variation is considered neutralbut single base changes in and around a genecan affect its expression or the function of its protein products (Ng & Henikoff, 2006).

Table 1. Motifs found in nucleotide and amino acid sequence of human MIP-2 with its biological

Motif	Motif Sequence	Position	Strand	Function
Nucleotide sequence				
Sp1	agcgccgccccca	19-31	-	Transcription factor and play role in metabolism, cell growth, differentiation, angiogenesis, apoptosis.
Myf	ctgctgctgctg	58-69 61-72 64-75 67-78 81-92	+ and - + and - + and - + and - +	Myogenic regulatory factor.
Ets	ggcaggaaggc	241-251	+	DNA-binding domain.
Amino acid sequence				
Signal peptide	MARATLSAAPSNPRL LRVALL	1 - 21		Post-targeting functions, mediate protein targeting into either cytosol orendoplasmic reticulum lumen.
GAG Binding Domain	APLATELRCQCLQTL QGIHLKNIQSVKVKS PGPHCAQTEVIATLK NGQKACLNPASPMV KKIIEKMLKNGKSN	35 - 107		Characterised of GAG binding protein.
ELR (Glu-Leu-Arg) Motif	ELR	40 - 42		Chemotactic to neutrophils.
GPH (Gly-Pro-His) Motif	GPH	67 - 69		Important in receptor binding.
RCxC Motif	RCQC	42 - 45		General requirement for binding to CXC chemokine receptors.

Table 2. Polymorphism data results from the analysis of the human MIP-2 assembled sequences with number of change that was found in assembled sequences (coverage).

Change	Start Position	End Position	Length	Coverage	Polymorphism Type
ATC -> GAG	475	477	3	17	Substitution
T -> C	469	469	1	17	SNP (transition)
A -> G	466	466	1	17	SNP (transition)
A -> G	454	454	1	17	SNP (transition)
A -> G	445	445	1	17	SNP (transition)
T -> G	439	439	1	17	SNP (transversion)
ATCG -> CAGC	427	430	4	17	Substitution
C -> G	418	418	1	17	SNP (transversion)
T -> C	415	415	1	17	SNP (transition)
T -> C	412	412	1	17	SNP (transition)
A -> G	409	409	1	17	SNP (transition)
G -> C	403	403	1	17	SNP (transversion)
T -> C	400	400	1	17	SNP (transition)
C -> G	394	394	1	17	SNP (transversion)
A -> C	391	391	1	17	SNP (transversion)
A -> C	385	385	1	17	SNP (transversion)
C -> G	382	382	1	17	SNP (transversion)
A -> G	379	379	1	17	SNP (transition)
A -> G	373	373	1	17	SNP (transition)
A -> C	358	358	1	17	SNP (transversion)
TC -> AG	350	351	2	17	Substitution
T -> C	337	337	1	17	SNP (transition)
A -> G	334	334	1	17	SNP (transition)
C -> G	322	322	1	17	SNP (transversion)
T -> C	316	316	1	17	SNP (transition)
A -> C	313	313	1	17	SNP (transversion)
T -> C	296	296	1	17	SNP (transition)
C -> G	286	286	1	17	SNP (transversion)
C -> A	284	284	1	17	SNP (transversion)
A -> G	280	280	1	17	SNP (transition)
T -> C	277	277	1	17	SNP (transition)
G -> C	265	265	1	17	SNP (transversion)
A -> C	262	262	1	16	SNP (transversion)
A -> C	259	259	1	17	SNP (transversion)
A -> C	256	256	1	17	SNP (transversion)
C -> G	253	253	1	17	SNP (transversion)
C -> A	251	251	1	17	SNP (transversion)
C -> A	248	248	1	17	SNP (transversion)
C -> G	232	232	1	17	SNP (transversion)
C -> G	226	226	1	17	SNP (transversion)
G -> C	217	217	1	17	SNP (transversion)
C -> A	209	209	1	17	SNP (transversion)
C -> G	205	205	1	17	SNP (transversion)
C -> A	200	200	1	17	SNP (transversion)
T -> C	196	196	1	17	SNP (transition)
CTC -> GAG	178	180	3	17	Substitution
G -> C	175	175	1	17	SNP (transversion)
C -> G	169	169	1	17	SNP (transversion)
C -> A	167	167	1	17	SNP (transversion)

In human MIP-2 protein sequence, a signal peptide sequence in N-terminal polypeptide chain was found (Table 1). The sequence comprised of a hydrophilic as those residues show a high hydropathy score. Because of its hydrophilic sequence, signal peptide direct the insertion of proteins into the membrane of the endoplasmic reticulum or post-targeting functions by mediating protein targeting into either cytosol or endoplasmic reticulum lumen (Kapp *et al.,* 2005). MIP-2 also needs hydrophilic sequence to pass cell membrane to the extracellular fluid or secretion as the consequence of the interaction between *Pneumocystis*

*jiroveci*and β-glucan receptor in alveolar epithelial cells induced NF-kB activation (Gigliotti & Wright, 2005).

As a member of CXC subfamilies that play roles in inflammatory, immune, and wound healing responses, MIP-2 needs to bind to other molecules, such as *glycosaminoglycan* (GAG) that serve as co-receptors of chemokines. GAG captures and presentschemokines on the surface of the homing tissue in order to establish a local concentration gradient directing the recruited cells (Cao*et al.,* 2000; Kungl, 2010). The GAG binding domain in MIP-2 amino acid sequence found at residue position 35 until 107.Chemokine induce cell migration and activation by binding to specific cell surface (Kungl, 2010). Binding to GAG is not yet enough to attract cells, MIP-2 also need to bind with its receptor for directing the attracted cells.

In MIP-2 sequence, there were three amino acids found that appear to be important in ligand/receptor interactions on neutrophils. Those three amino acids called ELR (glutamate-leucine-arginine) motif. ELR was found immediately before the first cysteine of the CXC motif. In all members of the CXC chemokine family that demonstrate biological activation of neutrophils, ELR is highly conserved and define the property to recruit different inflammatory cells between one chemokine to another. Chemokine that contain the ELR sequence in the N-terminus are being chemotactic to neutrophils and those that lack the ELR motif act on lymphocytes (Kungl, 2010). The neutrophils recruited specifically to alveolar regions of infection and not to uninfected alveoli (Wang *et al.,* 2005). The existence of ELR motif only defines MIP-2 chemotactic property to neutrophils. To really attract neutrophils, MIP-2 and other chemokine need to bind to its receptor and co-receptor.

Another conserved motif in amino acid sequence of MIP-2 found at 66-68 position called GPH (glysine-proline-histidine) motif. GPH motif is functionally important in receptor binding as MIP-2 interacts with chemokine receptors CXCR1 and CXCR2. For binding to CXC receptors, MIP-2 also has RCxC motif which is found in conserved domains of the sequence amino acid residue. Most receptors recognize more than one chemokine and vice versa (Kungl, 2010). MIP-2 interaction to receptors is important to express its chemotactic function in inflammatory, immune, or wound healing response.

In summary, all complete coding sequence of human MIP-2 had been analyzed to understand its role based on its sequence. Roles of MIP-2 in inflammatory, immune and wound healing responses strongly relates with its structure of amino acid and nucleotide sequences. Motifs that were found in MIP-2 sequence have biological function that defines MIP-2 properties. The mechanism of lung injury perhaps does not caused by alteration of MIP-2 directly but by the attraction of neutrophil via signaling pathway as the neutrophils were attracted to the site of infection because of the ELR motifs that was found in MIP-2 sequence.

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