LAPORAN PENELITIAN HIBAH BERSAING
TAHUN 2009

Analisis Kelainan Fungsi Insulin Receptor Family pada
Penderita Diabetes Mellitus

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UNIVERSITAS BRAWIJAYA
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HALAMAN PENGESAHAN
LAPORAN PENELITIAN HIBAH BERSAING


2. Ketua Peneliti
   b. Jenis Kelamin : L/P
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   d. Jabatan Fungsional : Dosen Tetap Biologi Molekuler
   e. Jabatan Struktural :
   f. Bidang Keahlian : Biologi Molekuler
   g. Fakultas/Jurusan : MIPA / Biologi
   h. Perguruan Tinggi : Universitas Brawijaya, Malang
   i. Tim Peneliti

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<th>NAMA</th>
<th>BIDANG KEAHLIAN</th>
<th>FAKULTAS/ JURUSAN</th>
<th>PERGURUAN TINGGI</th>
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<td>Prof.dr. Djoko Wahono Soeatmadji, Sp.PD.K.E</td>
<td>Ilmu Penyakit Dalam</td>
<td>Fak. Kedokteran</td>
<td>Universitas Brawijaya</td>
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3. Pendanaan dan jangka waktu penelitian
   a. Jangka Waktu penelitian yang diusulkan : 3 (tiga) tahun
   b. Biaya Total yang diusulkan: Rp. 115.000.000,- (Seratus lima belas juta rupiah)
   c. Biaya yang disetujui tahun 2009 : Rp. 30.000.000,- (Tiga puluh juta rupiah)

Mengesahkan,
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RINGKASAN DAN SUMMARY

The pathogenesis of NIDDM has been studied in various ethnic groups, it appears that insulin resistance can precede the clinical onset of NIDDM. The human insulin receptor exists in two isoforms, hINSR-A and MNSR-B, which are generated by alternative splicing of a primary gene transcript and differ by a 12-amino acid insertion sequence in the a-subunit. The two receptor isoforms bind insulin with different affinities and are differentially expressed in human tissues. Mutations of human insulin and insulin receptor family can lead autosomal dominant syndrome on diabetes, fasting hyperinsulinemia, and insulin resistant. Activation of the insulin receptor on the plasma membrane of cells by binding of insulin is the initial event that triggers the insulin receptor-signaling cascade, leading to the multiple cellular responses induced by insulin (1, 2). The insulin receptor is a tetrameric membrane protein with an a2p2-subunit structure and is encoded by a single gene on chromosome 19 (2, 3). Processing of the primary a-p gene product yields the mature insulin receptor. Next to insulin receptors, most cells also express IGF-I receptors with similar structure and function (4).

Mutations of the human insulin receptor gene have been identified in patients with severe insulin resistance, and studies of these naturally occurring mutants may provide important insights into the relationship between structure and function of the receptor. Although 17 distinct insulin receptor mutations have been identified in 14 human subjects (1-5), there is as yet little known about the ability of natural mutants to alter the normal pattern of insulin-mediated signaling. In contrast, site-directed mutagenesis of residues within the intracellular P-subunit has led to new insights into the consequences of impaired tyrosine kinase activity for receptor signaling and the potential divergence of insulin-stimulated pathways (5).

Biochemical analyses of the various mutations seen in patients with insulin-resistant syndromes provide insight into the residues of the insulin receptor that are critical for correct functioning and processing of the receptor. Furthermore, by studying multiple patients with the same mutation, insight can be obtained into what extent the genetic background is an important modulator of phenotypic expression of insulin receptor gene mutations. Studies of the signaling properties of natural mutants are also important, not only because of unique insights into structure/function that may emerge, but also because it is possible that the diverse phenotypes associated with severe insulin resistance may in part be due to the ability of some mutations to differentially affect insulin-regulated cellular events.

The aim of our research is to characterize genomic and proteomic insulin receptor (hINSR) of Indonesian diabetes mellitus patients. The blood and serum were collected from normal and DM patient at some public clinics and Saiful Anwar Hospital, Malang. DNA and RNA were isolated from blood, and protein was isolated from serum. To find out the genomic and 3D-proteomic hINSR, DNA sequences were analyzed by in silico and the serum protein characterized by 2D-electroforesis analysis.

The result of research showed that the hINSR gene has two variant as same as GenelID 3643 at GeneBank, at chromosome 19p13.3-p13.2, and has 22 exons with mRNA 4200bp. The INSR and insulin has specific amino acid binding site as table 1 and Fig 1. The binding site amino acids between INSR and insulin is on L1 domain of INSR (aa 1108 until 1228). The mutation of amino acids of DM patients, we found it at exon 11, 14, 21and 22 of INSR and after we analyzed by in silico we got some mutation of amino acid sequence. The amino acids mutation provided the three dimention ligand structure of - and insulin (INS) that changed differently that normal ligand. This domain is suggested as the ATP-binding site of tyrosine kinase of INSR. According to Kodawaki research group (6) were identified two point mutations in the insulin receptor tyrosine kinase domain in subjects with the Type A syndrome of insulin resistance: Trp1200->r ° and Ala1134» Thr1134 (6). The other research report was one additional naturally ing amino acid substitution that is linked to an insulin resistance syndrome has been ified within the intracellular
P-subunit tyrosine kinase domain (3, 5). This mutation Gly\textsuperscript{1008} within the receptor ATP-binding site and results in complete loss of both tyrosins kinase activity and insulin-mediated biologic signaling; these effects closely resemble site-directed ATP-binding site (Lys\textsuperscript{1035}) mutations (3, 6).

To analyze the profile protein of diabates patient and control, we used tetrace!!-2D-gel electrophoresis; the gels were visualized and analyzed by using ChemiDoc-Imaging. We found protein profile is different between normal control and DM patients. The identification of protein are indicated that protein at ciecle 1 perhaps are IgA-a Chain (62.6/pH5.3), Hemopexin/p-1(3 glycoprotein (76.2/pH5.3), a-1-antitrypsin (69.3/pH 5.1) and/or a-1-antiplasmin (68/pH 5). We identified that at circle2 are Serum Albumins (±67/pH ±5.8), and in circle 3. C-reactive protein (23.7/pH5.1) and Apolipoprotein A-1 (22-23/pH 4.9-5.5). We need further analysis for inditify in details and figure out the specific protein.

We found specific protein of DM patient from 2D-protein profile and some type mutation of hINSR and can change the INSR 3D-protein structure and the structure of 3D ligand structure of INSR and insulin completely changed on DM patient. According to our result, we suggested that the INSR protein mutation of DM patient precede abnormally INSR function against tyrosine kinase and perhaps correlated with genetic syndrome of insulin resistance.
DAFTAR PUSTAKA


Kodawaki T, et al., 1990a. PNAS 87: 659-662


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