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Interactions between PBEF and oxidative stress proteins – A potential new mechanism underlying PBEF in the pathogenesis of acute lung injury

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Abstract Identification of pre-B-cell colony-enhancing factor (PBEF) interacting partners may reveal new molecular mechanisms of PBEF in the pathogenesis of acute lung injury (ALI). The interactions between PBEF and NADH dehydrogenase subunit 1(ND1), ferritin light chain and interferon induced transmembrane 3 (IFITM3) in human pulmonary vascular endothelial cells were identified and validated. ND1, ferritin and IFITM3 are involved in oxidative stress and inflammation. Overexpression of PBEF increased its interactions and intracellular oxidative stress, which can be attenuated by rotenone. The interaction modeling between PBEF and ND1 is consistent with the corresponding experimental finding. These interactions may underlie a novel role of PBEF in the pathogenesis of ALI.

Structured summary:
MINT-6538697: *PBEF* (uniprotkb:P43490) *physically interacts* (MI:0218) with *NADH1* (uniprotkb:P03886) by *two hybrid* (MI:0018)
MINT-6538811, MINT-6538868: *PBEF* (uniprotkb:P43490) *physically interacts* (MI:0218) with *interferon-induced transmembrane protein 3* (uniprotkb:Q01628) by *anti bait coimmunoprecipitation* (MI:0006)
MINT-6538787, MINT-6538841: *PBEF* (uniprotkb:P43490) *physically interacts* (MI:0218) with *NADH1* (uniprotkb:P03886) by *anti bait coimmunoprecipitation* (MI:0006)
MINT-6538755: *PBEF* (uniprotkb:P43490) *physically interacts* (MI:0218) with *γ-glutamyl-transferase* (uniprotkb:P19440) by *two hybrid* (MI:0018)
MINT-6538799, MINT-6538862:
PBEF (uniprotkb:P43490) *physically interacts* (MI:0218)with *Ferritin light chain* (uniprotkb:P02792) by *anti bait coimmunoprecipitation* (MI:0006)
MINT-6538769:
PBEF (uniprotkb:P43490) *physically interacts* (MI:0218) with *E2L6* (uniprotkb:O14933) by *two hybrid* (MI:0018)
MINT-6538741:
PBEF (uniprotkb:P43490) *physically interacts* (MI:0218) with *Adenosine A2aR* (uniprotkb:P29274) by *two hybrid* (MI:0018)
MINT-6538727:
PBEF (uniprotkb:P43490) *physically interacts* (MI:0218) with *interferon-induced transmembrane protein 3* (uniprotkb:Q01628) by *two hybrid* (MI:0018)
MINT-6538712:
PBEF (uniprotkb:P43490) *physically interacts* (MI:0218) with *Ferritin light chain* (uniprotkb:P02792) by *two hybrid* (MI:0018)

Keywords: PBEF; Interaction; Vascular permeability; Acute lung injury; Inflammation; Oxidative stress

1. Introduction

Acute lung injury (ALI) and its more severe form, acute respiratory distress syndrome (ARDS), are characterized by refractory hypoxemia associated with lung inflammation and increased pulmonary vascular permeability [1]. Although ARDS was first described by Ashbaugh and colleagues in the *Lancet* in 1967 [2] and considerable progress has been made, mortality of patients with ALI and ARDS remains 30–50%. This is because molecular mechanisms underlying the susceptibility and the severity of ARDS are still incompletely understood. Therefore, more studies are clearly needed to identify novel biochemical and genetic markers and elucidate their molecular involvements in ARDS.

In our previous study on animal models of ALI, we identified preB-cell colony enhancing factor (PBEF) as a significantly upregulated gene in ALI [3]. We also found a susceptible GC haplotype in the human PBEF gene promoter,

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Abbreviation: ALI, acute lung injury; ARDS, acute respiratory distress syndrome; A2aR, adenosine A2a receptor; ETC, electron transport chain; EC, endothelial cells; HPAEC, human primary pulmonary artery endothelial cells; HLMVEC, human primary lung microvascular endothelial cells; IFITM3, interferon induced transmembrane protein 3; ND1, NADH dehydrogenase subunit 1; PBEF, Pre-B-cell colony-enhancing factor; UCE2L6, γ-glutamyl transferase and ubiquitin conjugating enzyme E2L6; ROS, reactive oxygen species

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which conferred a 7.7-fold higher risk of ALI [3]. Our result was confirmed in a separate population by Bajwa et al. [4]. We further found that an inhibition of PBEF expression by PBEF siRNA significantly attenuated pulmonary vascular endothelial cell barrier dysfunction [5]. Taken together, these results strongly indicate PBEF as a potential novel candidate gene and biomarker in ALI.

This study aims to address the molecular mechanisms by which PBEF contributes to pathogenesis of ALI. Since the interactions between proteins are important for many biological functions and pathological processes, we applied BacterioMatch Two-Hybrid System (Stratagene, La Jolla, CA) [6] to identify potential human PBEF interacting proteins in the lung. With this system, several PBEF interacting partner proteins in the lung were identified and validated by coimmunoprecipitation experiments in pulmonary vascular endothelial cells. Several Interacting proteins to PBEF as well as effect of PBEF on intracellular oxidative stress were examined in the absence or presence of IL-1β-stimulation. The computer modeling was also employed to predict the interacting mode between PBEF and NADH dehydrogenase subunit 1 (ND1). These results may reveal a novel role of PBEF in the pathogenesis of ALI.

2. Materials and methods

2.1. Cell culture

Human primary pulmonary artery endothelial cells (HPAEC) and human primary lung microvascular endothelial cells (HLMVEC) were obtained from Cambrex Bio Science Inc. (Walkersville, MD). Routine cell culture and maintenance were performed as described before in our lab [3,5].

2.2. BacterioMatch Two-Hybrid System screenning

The application of BacterioMatch Two-Hybrid System (Stratagene) for screening PBEF interacting partners in lung tissues was carried out according to our established protocol [6]. The PBEF bait was prepared by subcloning the human PBEF cDNA into the pBait vector, pBT. A human PBEF coding cDNA prepared before in our lab [7], was PCR amplified and inserted in frame into EcoRI and BamHI sites of the pBT bait plasmid. Primers for the PCR amplification are: 5′-CTAGAATTCATGAATCCTGCGGCAGAAGCCG-3′and 5′-TATGGATCCATGTGCTGCTTCCAGTTCAATAT-3′, respectively. Underlined sequences are EcoRI and BamHI adapters, respectively. The target lung protein cDNA library in the target vector, pTRG, was obtained from the Stratagene.

2.3. Immunoprecipitation and complex analysis

Immunoprecipitation was performed as described before [8]. HPAEC and HLMVEC were grown to confluence and incubated in serum-free media with or without IL-1β (10 ng/ml) for 4 h. The cell lysate samples were immunoprecipitated with either rabbit anti-PBEF antibody (Bethyl Laboratory Inc.), mouse anti-ND1 antibody (Mitoscience), rabbit anti-Ferritin light chain antibody (ADI), mouse anti-IFITM3 antibody (Abnova), rabbit anti-A2aR antibody (Chemicon), mouse anti-γ-glutamyl-transferase antibody (Lab Vision) or mouse anti-Ubiquitin conjugating enzyme E2L 6 antibody (Abnova) followed by incubation with either goat anti-rabbit or goat anti-mouse conjugated sepharose beads (Sigma). Immunoprecipitated material and cellular lysates were run on SDS–PAGE on 4–15% polyacrylamide gels, transfer onto Immobilon™ membranes, and developed with specific primary and secondary antibodies. Visualization of immunoreactive bands was achieved using enhanced chemiluminescence (Amersham Biosciences).

2.4. Reactive oxygen species assay

HPAEC cells were transfected with the vehicle control (c), PBEF stealth siRNA (Si), scrambled RNA (Sc), PBEF overexpression (E) and vector control (V) for 48 h before subjected to the treatment without or with IL-1β (10 ng/ml) for 4 h. A549 cells, a lung Type II epithelial cell line, were similarly transfected for 48 h before treated with rotenone (Sigma, MO, USA) (10 μM) for 3 h. Reactive oxygen species assay in HPAEC cells was carried out according to the protocol of *World Precision Instruments* Superoxide/Reactive Oxygen Species Determination kit (Superluminal, Sarasota, FL, USA).

2.5. The binding mode modeling between PBEF and ND1

We further probed the interaction between PBEF and ND1 by modeling their binding mode. The structure of human PBEF was downloaded from the Protein Data Bank [9] [pdb code: 2GVG [10]]. Because no atomic structure is available for ND1, ND1 was modeled as follows: first, the sequence of human ND1 was extracted from the GenBank [Accession No.: NP536843, [11]]. Based on the ND1 sequence, similarity search was then performed against the sequences in the Protein Data Bank by using the BLAST program [12]. It was found that the C-terminal domain of the catalase-peroxidase KatG (pdb code: 1U2L) has a good sequence similarity with ND1 near the potential interacting residues (81–85). Next, the overall sequence alignment between 1U2L and ND1 was obtained through the FASTA program [13] provided on the PDB web site (see Fig. 2 A). Based on the sequence alignment, the protein structure of ND1 was created from the template structure 1U2L by using the homology modeling program MODELLER [14]. The constructed ND1 structure was then docked to the PBEF structure (2GVG) by using the protein–protein docking program ZDOCK 2.1 [15]. The best-scored orientation was predicted as the binding mode (Fig. 3).

3. Results

3.1. Several potential PBEF interacting partners in expressed lung cDNA library identified by the BacterioMatch Two-Hybrid System

Because most proteins work as complexes to regulate biological and/or pathological processes in cells and tissues, we set out to determine whether PBEF has any interacting partner in lung tissues using the BacterioMatch Two-Hybrid System. Six potential PBEF-interacting proteins in the lung including their protein accession numbers, involved key biological processes, interacting fragments and references are presented in Table 1. They are ND1, ferritin light chain, interferon induced transmembrane protein 3 (IFITM3), Adenosine A2a receptor (A2aR), γ-glutamyl transferase and ubiquitin conjugating enzyme E2L6 (UCE2L6).

3.2. Coimmunoprecipitation confirmation of PBEF interacting partners identified by the BacterioMatch Two-Hybrid System

We next employed a coimmunoprecipitation strategy to confirm whether those potential PBEF interacting partners identified above were true PBEF interacting partners. As presented in Fig. 1, all six genes (ND1, ferritin light chain, IFITM3, A2aR, γ-glutamyl transferase and UCE2L6) are expressed in both HPAEC and HLMVEC (Fig. 1A). However, the coimmunoprecipitation experiments revealed only ND1, ferritin light chain and IFITM3 out of the 6 PBEF interacting partners identified by the BacterioMatch Two-Hybrid System were confirmed in the precipitation complexed with PBEF (Fig. 1B). These interactions were highly enhanced in the IL1-β-treated cells. The reverse coimmunoprecipitation experiments corroborated that only ND1, ferritin light chain and IFITM3 interacted with PBEF (Fig. 1C).