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Functional Analysis of the *Mycobacterium tuberculosis* Protein PE_PGRS

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Abstract

Tuberculosis (TB) remains an important infectious disease, causing ten million new cases and 1.4 million deaths a year worldwide. Elucidating the pathogenicity of *Mycobacterium tuberculosis*, the causative agent of TB will contribute to the development of new drugs, vaccines, and treatments. Proline-glutamic acid (PE)/proline-proline-glutamic acid (PPE) family accounts for approximately 10% of the coding region of the M. tuberculosis genome and its functions are largely unknown. PE proteins having polymorphic GC-rich repetitive sequences (PGRS) in the carboxyl-terminal are members of the PE_PGRS family. PE_PGRS62 and PE_PGRS30 are members of the PE_PGRS family and homologs of MAG24, the virulence factor of *M. marinum*. We are in the process of analyzing the functions of PE_PGRS62 and PE_PGRS30 and have results suggesting that PE_PGRS62 regulates autophagy, whereas PE_PGRS30 induces cell death.

Keywords

Mycobacterium tuberculosis
PE_PGRS
Autophagy
Cell death

1. Introduction

Tuberculosis (TB) remains an important infectious disease, affecting an estimated 1/3 of the world population, with approximately 10 million new cases and 1.4 million deaths annually [1]. In recent years, the emergence of drug-resistant *Mycobacterium tuberculosis* and the fact that TB is a major cause of death among HIV-infected people have become problems, and the development of new drugs and vaccines is urgently needed [1]. Identification and analysis of virulence factors of *Mycobacterium tuberculosis* is important for their development. In the *Mycobacterium tuberculosis* reference strain H37Rv genome, the proline-glutamic acid (PE) and proline-proline-glutamic acid (PPE) family members account for approximately 10% of the genomic coding region [2]. PE/PPE proteins are conserved in H37Rv, with 99 PE genes and 69 PPE genes, and are characterized by an N-terminal conserved PE/

PPE domain. The PE family includes PE_PGRS with polymorphic GC-rich repetitive sequences (PGRS) at the C-terminus and featureless PE genes. The PPE family includes PPE_MPTR with major polymorphic tandem repeats (MPTR), PPE_SVP with Gxx-SVPxxW motif, PPE_PPV with PxxPxxW motif, and featureless PPE genes. The C-termini of PE/PPE family proteins are highly variable, suggesting a role in pathogenicity, antigen diversity, or immune evasion [3].

2. Functions of PE_PGRS family proteins

Functions of PE_PGRS proteins have been reported to include enhanced adhesion and invasion of macrophages, modulation of resistance to antibiotics and other stresses, regulation of cytokine production by infected macrophages, regulation of apoptosis of infected cells, regulation of dendritic cell activation, and enhanced survival and proliferation in organs or within infected macrophages of infected mice. PE_PGRS with multiple functions have also been reported. In order to estimate the functions of PE_PGRS, the method of expressing PE_PGRS in *M. smegmatis*, a non-pathogenic, closely related bacterium of the same genus as *Mycobacterium* but without the *PE_PGRS* gene, is often used for various studies.

2.1. Enhanced adhesion and invasion of macrophages

M. smegmatis expressing PE_PGRS3 enhanced adhesion to mouse macrophages and human epithelial cells, and survival of the bacterium in mouse spleen via the arginine-rich C-terminal domain [4].

2.2. Regulation of resistance to stress

M. smegmatis expressing PE_PGRS41 increased cell wall permeability and enhanced sensitivity to several antibiotics [5].

2.3. Regulation of cytokine production by infected macrophages

Infection of macrophages with *M. smegmatis* expressing PE_PGRS16 increased the production of interleukin (IL)-12 [6]. When macrophages were infected with *M. smegmatis* expressing PE_PGRS18, the production of IL-6, IL-1β, and IL-10 was reduced and the production of IL-12p40 was increased [7]. When macrophages were infected with *M. smegmatis* expressing PE_PGRS33, PE_PGRS33 interacts with the Toll-like receptor (TLR)-2, resulting in increased production of tumor necrosis factor (TNF)-α [8]. PE_PGRS33 and PE_PGRS61 are glycine-rich, with a sequence motif GGXGXGXD/NXUX and Ca^{2+}-binding properties. When *M. smegmatis* expressing them was infected with the human monocyte-like cell line THP-1, it bound to TLR2 in a Ca^{2+}-dependent manner and enhanced the production of the anti-inflammatory cytokine IL-10 [9].

2.4. Regulation of apoptosis in infected cells

PE_PGRS5 was present in late-stage granulomas of *Mycobacterium tuberculosis*-infected patients and induced ER stress-mediated apoptosis of host cells [10]. PE_PGRS33 targeted host cell mitochondria and caused apoptosis in macrophages or mouse splenocytes [11]. In addition, infection of macrophages with *M. smegmatis* expressing PE_PGRS33 increased the release of the necrosis marker lactate dehydrogenase [12].

2.5. Regulation of dendritic cell activation

PE_PGRS17 and PE_PGRS11 interacted with TLR2 to induce maturation and activation of human dendritic cells. Activated dendritic cells produced IL-6, IL-8, and IL-12, and induced proliferation of CD4+ T cells [13].

2.6. Enhanced survival and proliferation in organs or infected macrophages of infected mice

PE_PGRS47 gene disrupted *Mycobacterium tuberculosis*, which showed reduced proliferation and increased MHC class II-restricted antigen presentation in infected mice and within macrophages. PE_PGRS47

also inhibited phagosome maturation and autophagy in infected macrophages [14].

3. PE_PGRS62 functions

The transposon mutant strain L1D of *Mycobacterium tuberculosis* fish *M. marinum*, which cannot grow within the mouse monocyte-like cell line J774, contains a mutation in the gene *mag* 24-1 encoding the PE_PGRS protein, suggesting that its *M. tuberculosis* homologs PE_PGRS62 and PE_PGRS30 may have functions related to intramacrophage proliferation [15].

Regarding the expression of PE_PGRS62, the transcript level of PE_PGRS62 was increased when *Mycobacterium tuberculosis*-infected macrophages were incubated under hypoxic conditions [16]. In addition, PE_PGRS62 expression was confirmed in *Mycobacterium tuberculosis*-infected guinea-pig lungs [17]. Furthermore, PE_PGRS62 was localized on the surface of *Mycobacterium tuberculosis* [18]. Regarding the function of PE_PGRS62, a *PE_PGRS62* gene mutant strain of *M. bovis* Bacillus Calmette-Guerin (BCG), a weakened strain of bovine tuberculosis, was found to have a higher expression of PE_PGRS62 in macrophages compared to the wild strain [19]. Infection of macrophages with M. smegmatis expressing PE_PGRS62 resulted in reduced expression of IL-1 and IL-6 [20], impaired phagosome maturation [21,22], and decreased iNOS production [22]. The PGRS domain of PE_PGRS62 protected the PE domain from ubiquitin-proteasome-dependent degradation. This affected PE_PGRS62 recognition by CD8+ T cells, suggesting that it may evade immune recognition and bactericidal action of *Mycobacterium tuberculosis*-infected cells [23]. *M. marinum* expressing PE_PGRS62 showed increased sensitivity to surfactants [22].

A *PE_PGRS62* gene-deficient strain was generated using *Mycobacterium tuberculosis* standard strain Erdman and infected mice, which were attenuated compared to the parental strain, suggesting that PE_PGRS62 is a virulence factor of *M. tuberculosis* (**Figure 1**). To explore the function of PE_PGRS62, PE_PGRS62 was expressed in mammalian cells. PE_PGRS62 was not co-localized with lysosomes or LC3, while PE_PGRS62N, with its C-terminal region trimmed, was co-localized with them. Further analysis using Atg5 knockdown J774 cells suggested that PE_PGRS62 suppressed ATG5-dependent autophagy.

4. Functions of PE_PGRS30

PE_PGRS30 is a 1,011 amino acid-long protein containing a PGRS domain (506 amino acids long) followed by a unique 306 amino acid-long C-terminal domain. PE_PGRS30 was found to localize to the bacterial pole and the PGRS domain contributed to its localization [24]. Infection of macrophages with *M. smegmatis* expressing PE_PGRS30 resulted in reduced production of IL-12, TNF-α, and IL-6 [25]. Infection of mice with the PE_PGRS30 mutant strain of *Mycobacterium tuberculosis* resulted in less tissue damage in the lungs during the chronic phase compared to the wild-type infection group. Infection of macrophages with *M. smegmatis* expressing PE_PGRS30 resulted in increased cell death compared to the wild-type strain. These results suggest that PE_PGRS30 is a virulence factor for *Mycobacterium tuberculosis* [26]. Expression of PE_PGRS30 in murine monocyte-like cells RAW264.7 induced apoptosis, as shown in **Figure 2**. A search for the factors that interact with PE_PGRS30 revealed that prohibitin (PHB) 2 is involved in mitochondrial structural maintenance, and PE_PGRS30 may induce apoptosis by interacting with PHB2.

5. Conclusion

The functions of the PE/PPE family, which comprises approximately 10% of the *Mycobacterium tuberculosis* genome coding region, and the PE_PGRS proteins, the largest subfamily of the PE family, are gradually being elucidated. However, no similarities have been found between family proteins that show the same

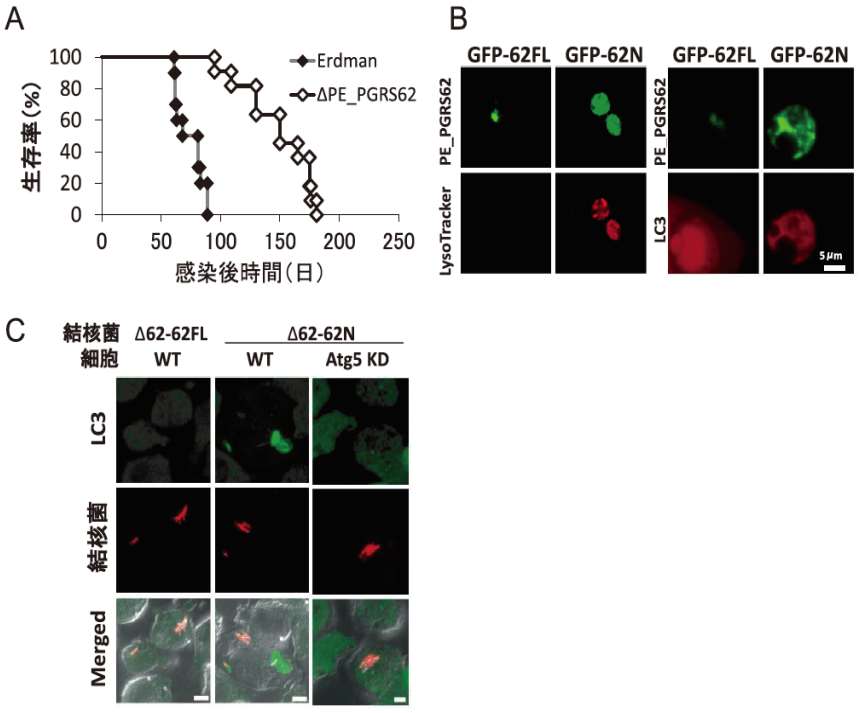

Figure 1. Functional analysis of PE_PGRS62. (A) *PE_PGRS62* gene disruption strain from *Mycobacterium tuberculosis* Erdman strain infected BALB/c mice via tail vein. The mean survival time was 73.9 ± 11.1 days in the wild-type group (◆: n = 10) and 148.7 ± 28.0 days in the *PE_PGRS62* gene disruption group (◇: n = 11); (B) GFP was fused to the N-terminus of PE_PGRS62 full-length (1–504aa: 62FL) and PE_PGRS62N lacking the C-terminal region (1–350aa: 62N) (GFP-62FL, GFP-62N), and expressed in mammalian cells, and co-localization with lysosomes and LC3 was observed. Lysosomes were stained with LysoTracker, and LC3 was detected with antibodies; (C) A complementary strain expressing 62FL (Δ62-62FL) and 62N (Δ62-62N) to the *PE_PGRS62* gene disruption strain of *M. tuberculosis* were infected with the mouse monocyte-like cell line J774 (WT) or *Atg5* gene knockdown cells of J774 (Atg5 KD), and co-localization with LC3 was observed. *Mycobacterium tuberculosis* expressed the fluorescent protein mCherry.

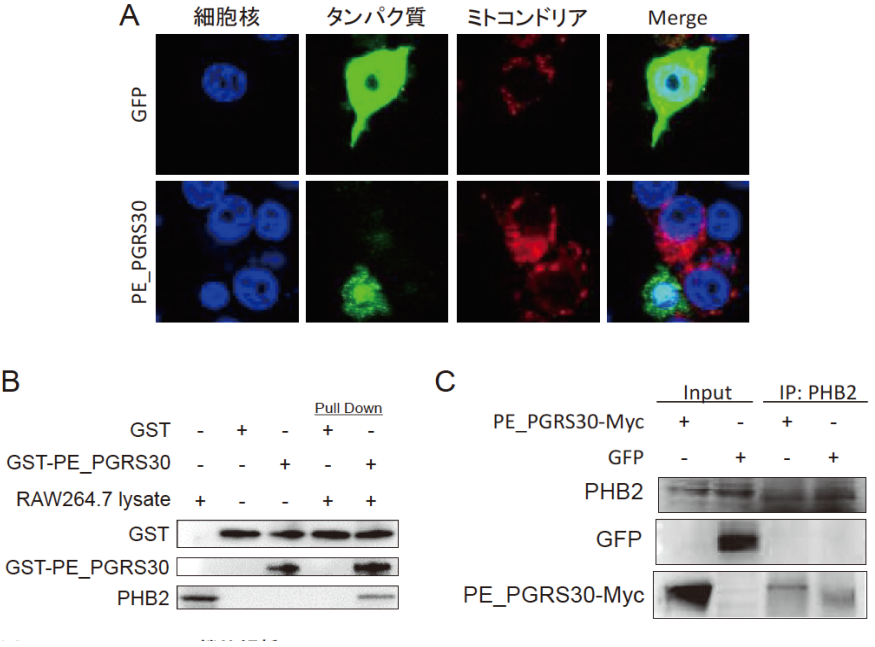

Figure 2. Functional analysis of PE_PGRS30. (A) GFP and PE_PGRS30-myc were expressed in the mouse monocyte-like cell line RAW264.7 and stained for cell nuclei (Hoechst 33342) and mitochondria (MitoTracker). PE_PGRS30 was detected with an anti-myc antibody. B: expressed in A pull-down assay was performed by mixing the cell lysate of RAW264.7 with GST-PE_PGRS30 expressed and purified in *E. coli* and GST-PE_PGRS30 fused to the N-terminus of PE_PGRS30. It was detected with anti-GST and anti-PHB2 antibodies. C: GFP and PE_PGRS30-myc were expressed in RAW264.7 and immunoprecipitated with anti-PHB2 antibody. GFP was detected with an anti-GFP antibody and PE_PGRS30-myc with anti-myc.

function in terms of the regions responsible for that function. In addition, the range of functions is broad, perhaps reflecting the polymorphism of the amino acid sequence in the PGRS region. Therefore, to date, no progress has been made in predicting the functions of PE_PGRS proteins, and it is necessary to elucidate the functions of individual PE_PGRS proteins. This applies not only to PE_PGRS but also to the rest of the PE/PPE family. In order to clarify the pathogenicity of *Mycobacterium tuberculosis*, it is necessary to elucidate the functions of these proteins one by one, to find commonalities and contribute to prediction.

Disclosure statement

The authors declare no conflict of interest.

References

[1] World Health Organization, 2019, Global Tuberculosis Report 2019, Geneva.

[2] Cole ST, Brosch R, Parkhill J, et al., 1998, Deciphering the Biology of *Mycobacterium tuberculosis* From the Complete Genome Sequence. Nature, 1998(393): 537–544.

[3] Yu X, Feng J, Huang L, et al., 2019, Molecular Basis Underlying Host Immunity Subversion by *Mycobacterium tuberculosis* PE/PPE Family Molecules. DNA Cell Biol, 2019(38): 1178– 1187.

[4] De Maio F, Battah B, Palmieri V, et al., 2018, PE_PGRS3 of *Mycobacterium tuberculosis* is Specifically Expressed at Low Phosphate Concentration, and its Arginine-Rich C-Terminal Domain Mediates Adhesion and Persistence in Host Tissues when Expressed in *Mycobacterium smegmatis*. Cell Microbiol, 2018(20): e12952.

[5] Deng W, Long Q, Zeng J, et al., 2017, *Mycobacterium tuberculosis* PE_PGRS41 Enhances the Intracellular Survival of *M. smegmatis* within Macrophages Via Blocking Innate Immunity and Inhibition of Host Defense. Sci Rep, 2017(7): 46716.

[6] Singh PP, Parra M, Cadieux N, et al., 2008, A Comparative Study of Host Response to Three *Mycobacterium tuberculosis* PE_PGRS Proteins. Microbiology, 2008(154): 3469–3479.

[7] Yang W, Deng W, Zeng J, et al., 2017, *Mycobacterium tuberculosis* PE_PGRS18 Enhances the Intracellular Survival of *M. smegmatis* via Altering Host Macrophage Cytokine Profiling and Attenuating the Cell Apoptosis. Apoptosis, 2017(22): 502–509.

[8] Zumbo A, Palucci I, Cascioferro A, et al., 2013, Functional Dissection of Protein Domains Involved in the Immunomodulatory Properties of PE_PGRS33 of *Mycobacterium tuberculosis*. Pathog Dis, 2013(69): 232–239.

[9] Yeruva VC, Kulkarni A, Khandelwal R, et al., 2016, The PE_PGRS Proteins of *Mycobacterium tuberculosis* are Ca^{2+} Binding Mediators of Host-Pathogen Interaction. Biochemistry, 2016(55): 4675–4687.

[10] Grover S, Sharma T, Singh Y, et al., 2018, The PGRS Domain of *Mycobacterium tuberculosis* PE_PGRS Protein Rv0297 is Involved in Endoplasmic Reticulum Stress-Mediated Apoptosis through Toll-Like Receptor 4. mBio, 2018(9): e01017–18.

[11] Basu S, Pathak SK, Banerjee A, et al., 2007, Execution of Macrophage Apoptosis by PE_PGRS33 of *Mycobacterium tuberculosis* is Mediated by Toll-like Receptor 2-Dependent Release of Tumor Necrosis Factor-Alpha. J Biol Chem, 2007(282): 1039–1050.

[12] Dheenadhayalan V, Delogu G, Brennan MJ, 2006, Expression of the PE_PGRS 33 Protein in *Mycobacterium smegmatis* Triggers Necrosis in Macrophages and Enhanced Mycobacterial Survival. Microbes Infect, 2006(8): 262–272.

[13] Bansal K, Elluru SR, Narayana Y, et al., 2016, PE_PGRS Antigens of *Mycobacterium tuberculosis* Induce Maturation and Activation of Human Dendritic Cells. J Immunol, 2010(184): 3495–3504.

[14] Saini NK, Baena A, Ng TW, et al., 2016, Suppression of Autophagy and Antigen Presentation by *Mycobacterium tuberculosis* PE_PGRS47. Nat Microbiol, 2016(1): 16133.

[15] Ramakrishnan L, Federspiel NA, Falkow S, 2000, Granuloma-Specific Expression of *Mycobacterium* Virulence Proteins from the Glycine-Rich PE-PGRS Family. Science, 2000(288): 1436–1439.

[16] Vipond J, Vipond R, Allen-Vercoe E, et al., 2006, Selection of Novel TB Vaccine Candidates and Their Evaluation as DNA Vaccines Against Aerosol Challenge. Vaccine, 2006(24): 6340–6350.

[17] Kruh NA, Troudt J, Izzo A, et al., 2010, Portrait of a Pathogen: the *Mycobacterium tuberculosis* Proteome *in vivo*. PLoS One, 2010(5): e13938.

[18] Vani J, Shaila MS, Trinath J, et al., 2013, *Mycobacterium tuberculosis* Cell Wall-Associated Rv3812 Protein Induces Strong Dendritic Cell-Mediated Interferon γ Responses and Exhibits Vaccine Potential. J Infect Dis, 2013(208): 1034–1036.

[19] Stewart GR, Patel J, Robertson BD, et al., 2005, Mycobacterial Mutants with Defective Control of Phagosomal Acidification. PLoS Pathog, 2005(1): 269–278.

[20] Huang Y, Wang Y, Bai Y, et al., 2010, Expression of PE_PGRS 62 Protein in *Mycobacterium smegmatis* Decrease mRNA Expression of Proinflammatory Cytokines IL-1β, IL-6 in Macrophages. Mol Cell Biochem, 2010(340): 223–229.

[21] Huang Y, Zhou X, Bai Y, et al., 2012, Phagolysosome Maturation of Macrophages was Reduced by PE_PGRS 62 Protein Expressing in *Mycobacterium smegmatis* and Induced in IFN-γ Priming. Vet Microbiol, 2012(160): 117–125.

[22] Thi EP, Hong CJ, Sanghera G, et al., 2013, Identification of the *Mycobacterium tuberculosis* Protein PE_PGRS62 as a Novel Effector that Functions to Block Phagosome Maturation and Inhibit iNOS Expression. Cell Microbiol, 2013(15): 795–808.

[23] Koh KW, Lehming N, Seah GT, 2009, Degradation-Resistant Protein Domains Limit Host Cell Processing and Immune Detection of Mycobacteria. Mol Immunol, 2009(46): 1312–1318.

[24] De Maio F, Maulucci G, Minerva M, et al., 2014, Impact of Protein Domains on PE_PGRS30 Polar Localization in Mycobacteria. PLoS One, 2014(9): e112482.

[25] Chatrath S, Gupta VK, Dixit A, et al., 2016, PE_PGRS30 of *Mycobacterium tuberculosis* Mediates Suppression of Proinflammatory Immune Response in Macrophages Through its PGRS and PE Domains. Microbes Infect, 2016(18): 536–542.

[26] Iantomasi R, Sali M, Cascioferro A, et al., 2012, PE_PGRS30 is Required for the Full Virulence of *Mycobacterium tuberculosis*. Cell Microbiol, 2012(14): 356–367.