Investigating the Effect of the Moonlighting Protein GAPDH from *Lactobacillus gasseri* on the AhR Metabolic Pathway to Alleviate Atopic Dermatitis

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Abstract

Atopic dermatitis (AD) is a chronic inflammatory skin disease characterized by type 2 inflammation and barrier dysfunction. Current therapies rely on corticosteroids or immunosuppressants, but side effects limit long-term use. The aryl hydrocarbon receptor (AhR) is known to regulate inflammation and barrier integrity, and certain probiotics can act through AhR. Our recent studies identified LGp40, a moonlighting protein from *Lactobacillus gasseri*, which reprograms macrophages, suppresses Th2 responses, and ameliorates asthma and AD in mice. In this study, we investigated whether LGp40 could modulate AD via the AhR pathway. LGp40 upregulated barrier-related genes (*FLG*, *LOR*) and downregulated the inflammatory gene *CCL26*. However, LGp40 did not induce expression of the AhR canonical downstream gene *CYP1A1*, and LGp40 did not interact with AhR based on protein-protein interaction (PPI) analysis. Although LGp40 cannot act as an AhR agonist directly, RNA sequencing (RNA-seq) revealed regulation of AhR-associated genes and kynurenine pathway (KP) enzymes, suggesting indirect AhR activation through tryptophan metabolism. These findings highlight LGp40 as a promising candidate for restoring barrier function and alleviating inflammation in AD.

Results

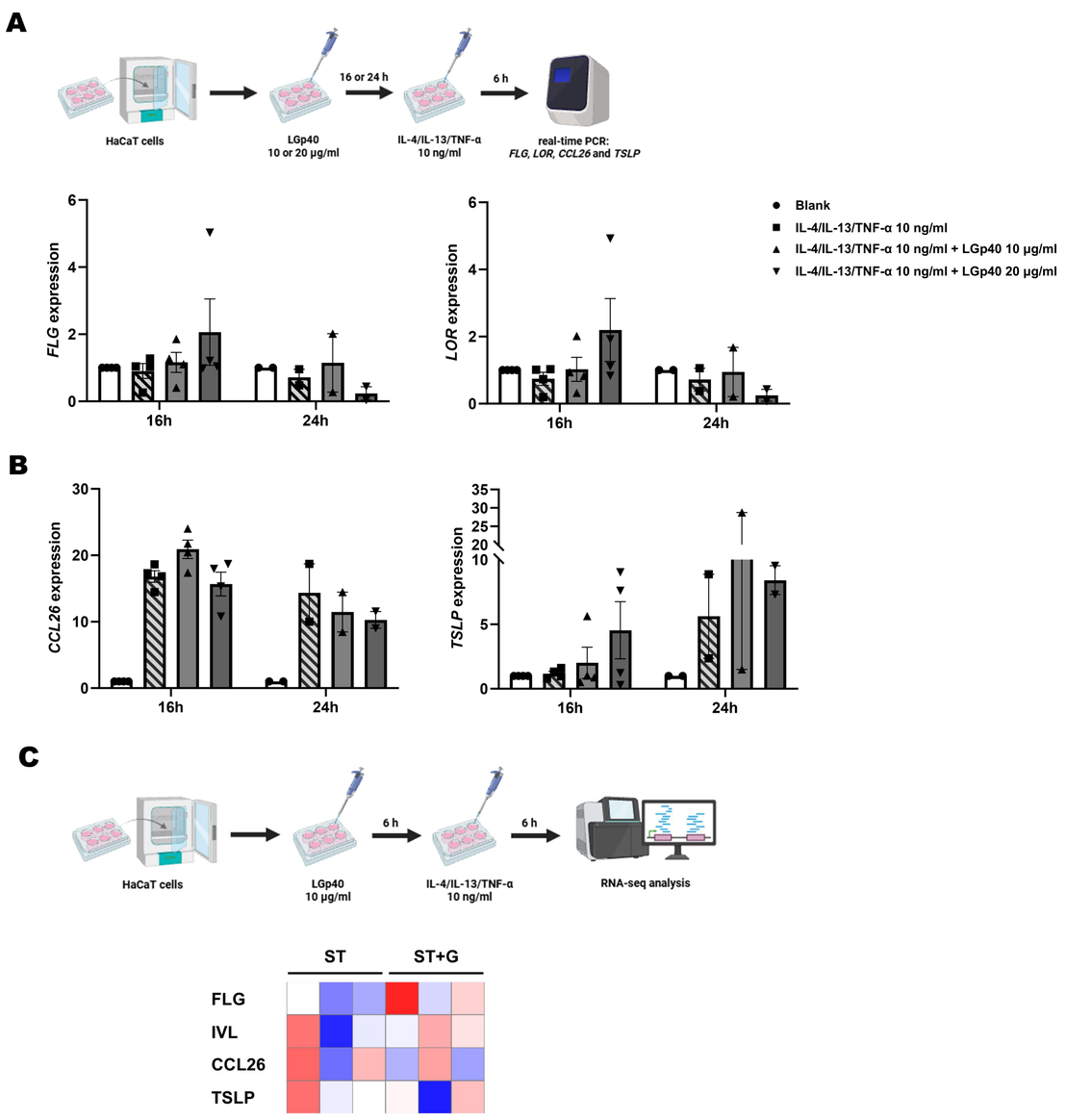

Figure 1. LGp40 promotes the expression of skin barrier gene and attenuates inflammatory cytokine genes in HaCaT cells.
HaCaT cells were treated with LGp40 (0, 10, or 20 μg/ml) for 16 or 24 h, followed by stimulation with IL-4, IL-13, and TNF-α (10 ng/ml) for 6 h. **(A)** LGp40 upregulated the expression of *FLG* and *LOR*. **(B)** LGp40 downregulated *CCL26* expression at 10 μg/ml after 16 h of pre-treatment, while *TSLP* expression remained unaffected (16 h: n = 4; 24 h: n = 2). **(C)** RNA-seq analysis showed that LGp40 modulated the expression of genes related to skin barrier function and inflammatory cytokines. (ST: stimulated with IL-4, IL-13, and TNF-α [10 ng/ml for 6 h]; ST+G: pretreated with LGp40 [10 μg/ml, 6 h], followed by cytokine stimulation).

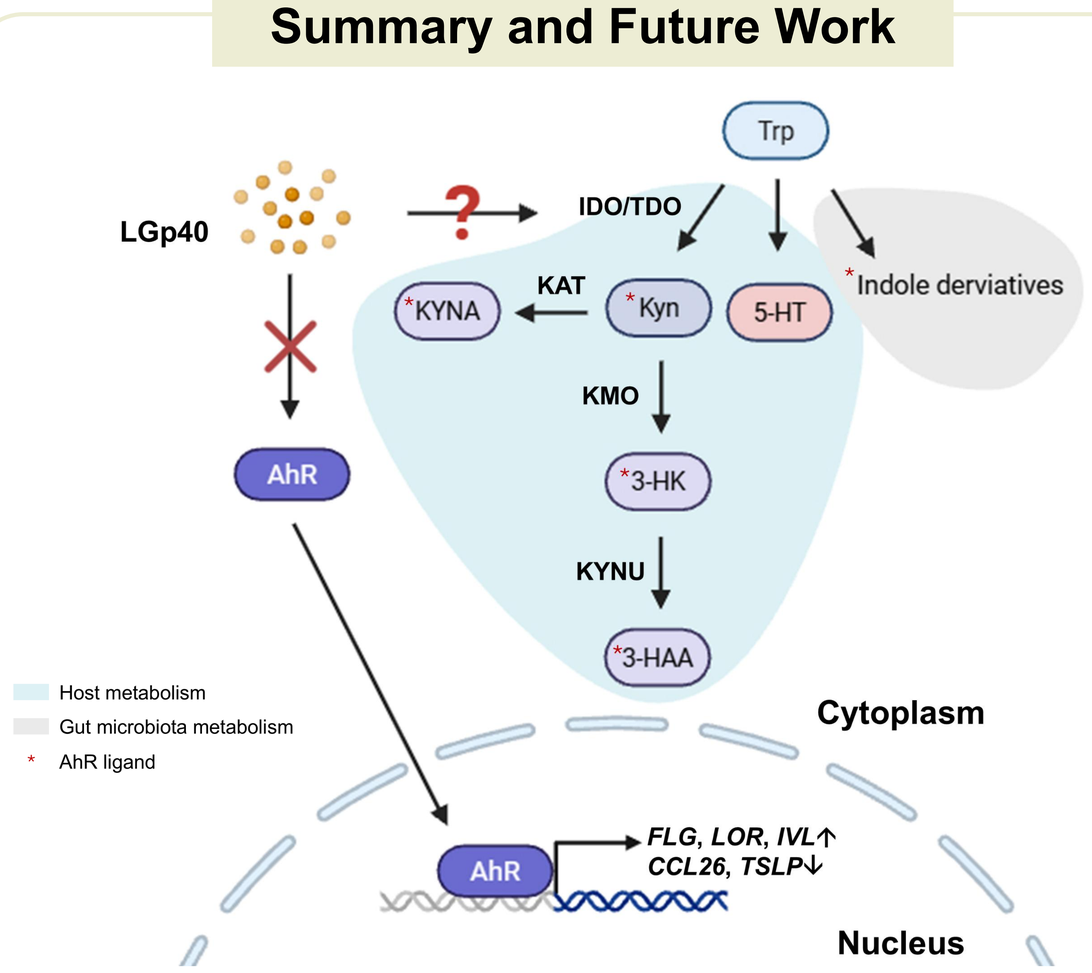

Figure 2. LGp40 does not directly activate AhR but modulates AhR-related pathways.
(A) HaCaT cells were treated with LGp40 (2, 5, or 10 μg/ml) or FICZ (1 μM) for 24 h. LGp40 did not upregulate *AHR* or *CYP1A1* expression, indicating that it is not an AhR agonist (n=4). **(B)** RNA-seq analysis showed that LGp40 modulated the expression of AhR pathway genes in HaCaT cells. The experimental design is identical to that described in Figure 1C. **(C)** Protein structure prediction and protein–protein interaction (PPI) analysis using AlphaFold revealed no direct interaction between LGp40 and AhR.

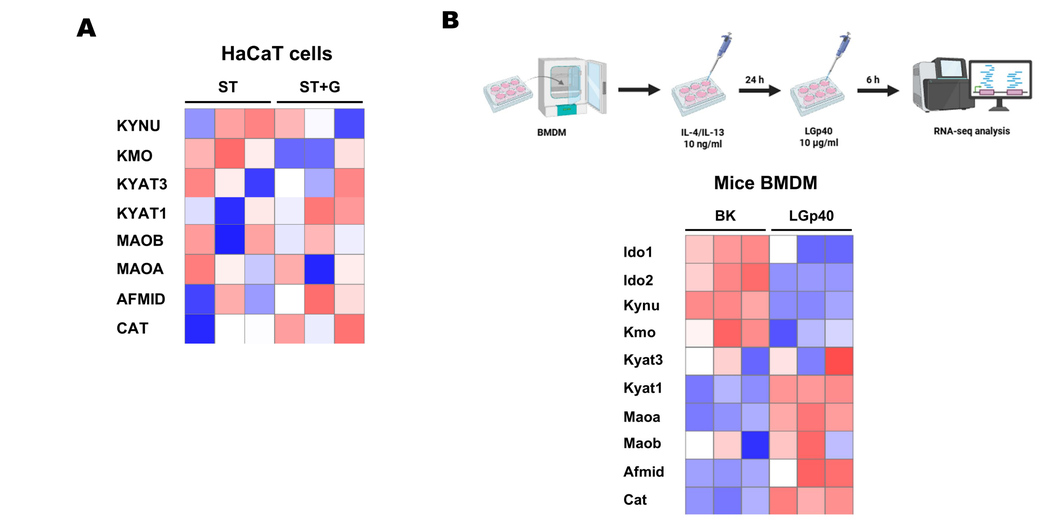

Figure 3. LGp40 has the potential to upregulate enzymes involved in the kynurenine pathway (KP).
(A) RNA-seq analysis showed that LGp40 increased the expression of KP-related enzymes in HaCaT cells. The experimental design is identical to that described in Figure 1C. **(B)** RNA-seq analysis also demonstrated that LGp40 modulated the expression of KP-related enzymes in mouse bone marrow-derived macrophages (BMDM) stimulated with IL-4 and IL-13. (BK, blank: stimulated with IL-4 and IL-13 [10 ng/ml, 24 h], followed by medium for 6 h; LGp40: stimulated with cytokines, followed by LGp40 [10 μg/ml, 6 h]).

Summary and Future Work

Future Work

1. To determine whether LGp40 induces KP-related enzymes in macrophages and promotes AhR ligand production.
2. To investigate the correlation between barrier repair and inflammation amelioration following AhR activation in macrophages.