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Figure 1. Results obtained in the validation stage of the three biomarker candidates identified by proteomics. ****, $p < 0.0001$; ***, $p < 0.0005$; **, $p < 0.01$; *, $p < 0.05$.

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THU0219

THE INVOLVEMENT OF MITOCHONDRIAL ACTIVATION VIA GLUTAMINOLYSIS IN HUMAN B CELL DIFFERENTIATION AND ITS RELEVANCE TO THE PATHOGENESIS OF SLE

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Background: B cells play a crucial role in Systemic Lupus Erythematosus (SLE). Recently, "Immunometabolism" attract much attention. Glucose and glutamine are important nutrition for energy production such as ATP in various cells. It has been reported that aerobic glycolysis, glutaminolysis and mitochondrial functions enhanced in cancer cells. However, the involvement of metabolic reprogramming in plasmablast differentiation and its relevance to the pathogenesis of SLE remained elusive.

Objectives: We first investigated the abnormality of mitochondria in B cells from patients with SLE by flow cytometry. Next, B cells were isolated from healthy donors (HDs) and metabolic reprogramming were assessed in vitro.

Methods: First, peripheral blood mononuclear cells (PBMCs) were obtained from age-matched 31 HDs and 29 patients with SLE. The mitochondrial membrane potential was measured with DiOC6 by flow cytometry. In addition, CD19⁺ cells were isolated from HDs and stimulated with CpG (TLR9 ligand) and IFN- α . Change of aerobic glycolysis, glutaminolysis and mitochondrial functions were assessed in the absence of glucose or glutamine and in the addition of metformine, which is known as AMPK activator, in vitro.

Results: We first examined the abnormality of mitochondria in B cells from patients with SLE using DiOC6 as a marker of depolarization-activated mitochondrial membrane. Baseline characteristics of SLE were males: females=1:28, age 40.2 years, disease duration 132.2 months, SLEDAI 6.4 and BILAG 6.2 at the timing of admission due to exacerbation of SLE. The percentage of IgD⁺CD27⁺ memory B cells were higher than those of HDs, while the percentage of IgM memory B cells were lower than that of HDs. The percentage of CD24⁺DiOC6⁺ cells in IgD⁺CD27⁺ memory B cells from patients with SLE were higher than that of HDs. These results indicate that B cells from patients with SLE have the abnormality of differentiation and mitochondrial functions. Next, we assessed the change of aerobic glycolysis, mitochondrial functions and glutaminolysis in the process of plasmablast differentiation in vitro. Stimulation with CpG and IFN- α , 1) enhanced aerobic glycolysis, which was assessed by lactate production. 2) increased the area of cytoplasm including many expanded mitochondrial cristae with slightly wider and loosely organized intermembrane space in electric microscopy, accompanied with ROS production and DiOC6 up-regulation, 3) induced CD27^{hi}CD38^{hi} plasmablasts differentiation and immunoglobulin production. Lactate production was decreased in the absence of both glucose or glutamine. ROS production and DiOC6 expression were decreased in the absence of glutamine, leading to inhibition of plasmablasts differentiation and immunoglobulin production. On the other hand, this tendency was not shown in the absence of glucose. Next, we evaluated oxygen

consumption rate (OCR). OCR was also suppressed in the absence of glutamine. Metformin, abrogated glutamine uptake, resulting in suppression of ROS production, DiOC6 expression, plasmablast differentiation and immunoglobulin production.

Conclusion: These results suggest that mitochondrial activation via glutaminolysis may play an important role in the differentiation from IgD⁺CD27⁺ double negative B cells to plasmablasts and production of immunoglobulins in patients with SLE.

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PEPTIDYLARGININE DEIMINASE 4 DEFICIENCY AMELIORATED A MURINE MODEL OF LUPUS BY REDUCING NEUTROPHIL MIGRATION TO THE KIDNEY

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Background: Peptidylarginine deiminase 4 (PADI4) is reported to play several biological roles in neutrophils, including neutrophil extracellular trap formation, apoptosis, and epigenetic regulation. Neutrophils could play pivotal roles in the pathogenesis of SLE.

Objectives: We set to determine the pathological role of neutrophils and PADI4 in SLE using *Padi4* knock out (KO) mice.

Methods: A murine model of imiquimod (IMQ)-induced lupus was analyzed. A TLR7 agonist, IMQ, was administered topically to the ear skin of B6 wild-type (WT) and *Padi4* KO mice. Proteinuria, spleen weight, serum anti-dsDNA level, frequencies of spleen and renal immune cells, and the histopathological findings of ear skin and kidney, were assessed. Neutrophil migration and adhesion were evaluated by adoptive transfer experiments *in vivo*, and adherence assays *in vitro*.

Results: Compared with IMQ-treated WT (WT-IMQ) mice, *Padi4* KO-IMQ mice showed decreased spleen weight. Moreover, proteinuria, and dermatitis were not exacerbated in the *Padi4* KO-IMQ mice. There was a positive correlation between the frequency of kidney neutrophils and the degree of proteinuria, while significant decreases in kidney neutrophils were noted in the *Padi4* KO-IMQ mice. Notably, the elevation of serum anti-dsDNA levels, or the degree of immune complex deposition in the kidney, showed no significant difference between the WT-IMQ and *Padi4* KO-IMQ mice. In the adoptive transfer experiment, there was a significant decrease in the transferred *Padi4* KO neutrophil infiltrations in the kidney compared with those of WT neutrophils. The frequency of adhesive neutrophils toward ICAM-1 was significantly decreased in the *Padi4* KO neutrophils primed by the TLR7 agonist.

Conclusion: Nephritis was ameliorated in *Padi4* KO-IMQ mice. Our study shed light on the importance of neutrophils in the pathogenesis of SLE, and the suppression of neutrophil function by inhibition of PADI4 will be a unique therapeutic strategy for SLE.

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