Fli1 deficiency alters the phenotype of dermal dendritic cells, promoting the development of dermal fibrosis in bleomycin-treated mice

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INTRODUCTION :

Systemic sclerosis (SSc) and Friend leukemia virus integration 1 (Fli1)

- SSc is a multisystem autoimmune and vascular disease resulting in fibrosis of the skin and certain internal organs.
- Fli1 is a member of the Ets transcription factor family, the expression of which is broadly suppressed in various types of cells in involved and uninvolved skin of patients with SSc.
- Fli1 haploinsufficiency augments the induction of SSc-like phenotypes in dermal fibroblasts, dermal microvascular endothelial cells, and macrophages in mice treated with bleomycin (BLM).
- Therefore, the molecular analysis based on Fli1 deficiency provides us with a useful clue to know the significance of target molecules in the pathogenesis of SSc.

Skin fibrosis and dendritic cells (DCs)

- Skin fibrosis is generally related to dermal fibroblast activation, Th2/Th17-skewed immune polarization, M2 macrophage differentiation, aberrant B cell activation, increased infiltration of plasmacytoid dendritic cells, increased endothelial ICAM-1 expression, endothelial-to-mesenchymal transition, and epithelial cell activation.
- In particular, immune cells seem to play a central role because immunosuppressants and/or corticosteroids are mostly effective for skin fibrosis to variable degrees in most of skin fibrotic disorders.
- Recently, we have demonstrated a pivotal role of CD11b⁺CD103⁺ dermal dendritic cells (DCs) in the pathological dermal fibrosis of BLM-treated mice, an established animal model of SSc.

Hypothesis and objective

- These results suggest that Fli1 potentially contributes to the function of dermal DCs in SSc, but the detail is not clear. Therefore, we investigate if Fli1 deficiency affects the phenotype of dermal DCs in the pathological dermal fibrosis by utilizing BLM-treated *Fli1*^{+/-} mice.

METHODS :

Mice

Wild-type (WT) (C57BL/6) mice were purchased from Japan SLC. *Fli1*^{+/-} mice were kindly provided by Dennis K. Watson. All mice were healthy and fertile, and did not display evidence of infection or disease. All mice used in this study were female, and mice used in the BLM-induced SSc model were 6-8 weeks old at the beginning of treatment with phosphate buffered saline (PBS) or BLM.

BLM treatment

Two hundred microgram of BLM (Nippon Kayaku) dissolved in PBS or control PBS alone was injected subcutaneously into a single location on the backs of the mice daily. Mice were treated with BLM for 1 week. All studies and procedures were approved by the Committee on Animal Experimentation of the University of Tokyo Graduate School of Medicine.

RNA isolation and real-time polymerase chain reaction (PCR)

Total RNA was isolated from the lower back skin with RNeasy spin columns (Qiagen). One microgram of RNA was reverse transcribed using iScript cDNA Synthesis kits (Bio-Rad). Real-time quantitative PCR was carried out using SYBR Green PCR Master Mix (Life Technologies) on an ABI Prism 7000 system (Life Technologies) in triplicates. The messenger RNA (mRNA) levels were normalized to those of the GAPDH gene.

Flow cytometric analysis

On the day after final injection, lymphocytes from draining lymph nodes and dermis isolated from the lower back skin were obtained. After staining cells for surface and intracellular antigens, cells were analyzed on a FACSVerse flow cytometer (BD Biosciences).

Analysis of ALDH activity at the single-cell level

The presence of cells displaying aldehyde dehydrogenase activity (ALDH⁺ cells) was determined using an ALDEFLUOR staining kit (StemCell Technologies).

Induction of DCs from the mice bone marrow

Bone marrow cells (5x10⁶ cells) suspended in medium were collected from tibias and femurs of WT and *Fli1*^{+/-} mice, and cultured in tissue culture dishes with 10ng/ml GM-CSF (Peprotech). Two-thirds of the medium was replaced on day 3. On days 6 of culture, cells were stimulated by *Escherichia coli*-LPS (0.1 mg/ml; Sigma). On day 7 of culture, most of the non-adherent cells had acquired typical dendritic morphology, and these cells were used.

Statistical analysis

Data are presented as the mean ± SEM. Statistical analysis was carried out using GraphPad Prism. The Mann-Whitney U-test was used for two-group comparison. Statistical significance was defined as a P value of <0.05.

RESULTS :

Figure 1. Fli1 loss reduced RALDH1 expression in the skin of BLM-treated mice

mRNA level of *Raldh1* was significantly lower in BLM-treated *Fli1*^{+/-} mice than in BLM-treated WT mice. n=4-5 per group. AU=arbitrary unit. *p<0.05.

Figure 2. *Fli1*^{+/-} Dermal DCs decreased the RALDH activity in BLM-treated mice

Flow cytometric analysis of dermis (a) and skin-draining lymph nodes (b) showed Fli1 loss decreased the RALDH activity among dermal DCs of BLM-treated mice. Also, RALDH was predominantly expressed by CD103⁺CD11b⁺ dermal DCs. Furthermore, Mean fluorescence intensity (MFI) of RALDH was decreased in dermis (a) of BLM-treated *Fli1*^{+/-} mice than in BLM-treated WT mice. n=6-13 per group. *p<0.05, **p<0.01.

Figure 3. Fli1 loss decreased the proportion of Tregs in BLM-treated mice

Flow cytometric analysis of dermis (a) and skin-draining lymph nodes (b) showed the proportion of IFN- γ , IL-4, and IL-17A producing CD4⁺ T cells were not altered, while the proportion of regulatory T cells (Tregs) was decreased in BLM-treated *Fli1*^{+/-} mice compared with BLM-treated WT mice. n=5-9 per group. *p<0.05, ***p<0.001.

Figure 4. Migration makers were increased in mature bone marrow-derived DCs (BMDCs) of *Fli1*^{+/-} mice

Flow cytometric analysis showed the increased expression of migration makers, CCR7 and CXCR4, in *Fli1*^{+/-} BMDCs compared with WT BMDCs. n=4 per group. *p<0.05.

DISCUSSION :

- One week after BLM injection, the mRNA levels of *Raldh1*, which encodes a cytosolic enzyme RALDH1 that oxidizes retinaldehyde to retinoic acid, were decreased in *Fli1*^{+/-} mice compared to WT mice. Also, the number of RALDH-producing DCs was decreased in the dermis and skin-draining lymph nodes of BLM-treated *Fli1*^{+/-} mice. Furthermore, MFI of RALDH was decreased in dermis of BLM-treated *Fli1*^{+/-} mice than in BLM-treated WT mice. Consistent with our previous data, RALDH was preferentially expressed by CD11b⁺CD103⁺ DCs. These results suggest that Fli1 loss decreases RALDH1 activity of CD11b⁺CD103⁺ DCs under a BLM treatment.
- The proportion of Tregs was decreased in the dermis and skin-draining lymph nodes of BLM-treated *Fli1*^{+/-} mice, while the proportion of IFN- γ , IL-4, and IL-17A producing CD4⁺ T cells were not altered. This is probably because the pre-treatment of PMA and ionomycin for flow cytometric analysis of intracellular proteins overcame the Treg-dependent suppression of effector CD4⁺ T cells. This notion is supported by our previous *in vitro* data demonstrating that mRNA expression of *Ifng*, *Il4*, and *Il17a* is increased in the lesional skin of BLM-treated *Fli1*^{+/-} mice compared with BLM-treated WT mice.
- The expression of both CCR7 and CXCR4, the migration markers, was increased in *Fli1*^{+/-} BMDCs compared with WT BMDCs. Given that BLM-induced fibrosis is enhanced in *Fli1*^{+/-} mice, the current data collectively suggest that dermal DCs are activated, but lack the induction of RALDH1 expression to terminate inflammation in BLM-treated *Fli1*^{+/-} mice.

CONCLUSION :

- In conclusion, taken together with evidence that retinoic acid produced by CD11b⁺CD103⁺ dermal DCs promotes the differentiation of Tregs, these results indicate that Fli1 deficiency activates dermal DCs, but selectively suppresses RALDH1 production in the CD11b⁺CD103⁺ dermal DC subset, leading to the attenuation of Treg development and the subsequent promotion of dermal fibrosis in BLM-treated *Fli1*^{+/-} mice.
- This study provides a new insight into the elucidation of the mechanism underlying the pathological dermal fibrosis, including that of SSc.