学位論文内容の要旨
（Summary of dissertation）

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HSP47 siRNA 含有ビタミン A 結合リポソームは慢性移植片対宿主病の皮膚線維化を改善する
(Vitamin A-coupled liposomes containing siRNA against HSP47 ameliorate skin fibrosis in chronic graft-versus-host disease)

Introduction: Chronic graft-versus-host disease (GVHD) after allogeneic hematopoietic stem cell transplantation is characterized by multiple-organ fibrosis and often refractory to conventional immunosuppressive therapies, profoundly affecting quality of life of SCT survivors. Heat shock protein 47 (HSP47), a collagen specific molecular chaperone, plays a critical role in collagen synthesis in myofibroblasts. Vitamin A-coupled liposomes carrying HSP47 siRNA (VA-lip HSP47) have been shown to deliver HSP47 siRNA to pathogenic myofibroblasts and ameliorate mouse liver cirrhosis. We tested if VA-lip HSP47 could inhibit collagen synthesis in myofibroblasts and ameliorate skin fibrosis in chronic GVHD, using a well-established murine model of cutaneous chronic GVHD.

Methods: VA-lip HSP47 was provided by Nitto Denko Corporation (Osaka, Japan). BALB/c mice were exposed to a single dose of 6 Gy total body irradiation, followed by intravenous injection with 8×10^6 bone marrow cells plus 2.5×10^7 splenocytes from minor histocompatibility antigen mismatched B10.D2 or syngeneic BALB/c donors on day 0. Recipient mice were intravenously injected with 4.5 mg/kg VA-lip HSP47 or vehicle thrice a week from day +1 to +41 after bone marrow transplantation (BMT). To deplete macrophages, a group of recipients were intraperitoneally injected with 0.5 mg/body of anti-CSF1 receptor monoclonal antibodies (αCSF1R) thrice a week from day +7 after BMT. Skin samples were harvested on day +42. For in vitro analysis, primary fibroblasts isolated from mouse skin were stimulated by 5 ng/ml TGF-β in the presence or absence of 50 nM of VA-lip HSP47 or VA-lip containing scrambled siRNA. HSP47 expressions were measured by quantitative PCR.

Results: Masson’s trichrome staining of the skin sections demonstrated skin thickening with massive fibrosis of the dermis 42 days after BMT. Quantitative collagen assay confirmed collagen volume was significantly increased in the skin of allogeneic recipients compared to syngeneic controls. Immunofluorescent study showed myofibroblasts expressing both alpha-smooth muscle actin (α-SMA) and

HSP47 were accumulated in the dermis of allogeneic animals, while these cells were hardly detected in the skin of syngeneic controls. Macrophage-depletion with repeated administration of αCSF1R dramatically reduced HSP47+ myofibroblasts, suggesting a critical role of macrophages in differentiation of HSP47+ myofibroblasts. In vitro culture showed VA-lip HSP47 knocked down HSP47 in mouse skin myofibroblasts, while VA-lip carrying scrambled siRNA did not alter HSP47 expression. To confirm in vivo distribution of VA-lip HSP47 specifically to the fibrotic skin lesion, mice with bleomycin-induced localized skin fibrosis were injected with VA-lip HSP47 labeled with fluorescent dye. We confirmed VA-lip HSP47 was distributed specifically to fibrotic skin, whereas no VA-lip HSP47 particle was found in non-fibrotic skin of the same mouse. Next, recipients of allogeneic BMT were treated with VA-lip HSP47 from day +1 to +41. VA-lip HSP47 knocked down HSP47 expression in the skin of allogeneic mice, resulting in reduced deposition of collagen and skin thickness. In contrast, VA-lip HSP47 had no effects on collagen volumes in the skin of naïve mice. Finally, allogeneic recipients were injected with VA-lip HSP47 thrice a week from day +21 to test if VA-lip HSP47 could promote regression of established skin fibrosis in chronic GVHD. Skin thickness and collagen volume on day +42 were again reduced in allogeneic mice treated with VA-lip HSP47 compared to allogeneic controls, indicating that VA-lip HSP47 is a promising therapeutic agent for established skin fibrosis. Because VA-lip HSP47 did not affect donor T-cell expansion in the spleen and systemic GVHD scores after allogeneic BMT, VA-lip HSP47 ameliorated skin fibrosis independently of systemic immunosuppression.

Conclusion: VA-lip HSP47 abrogates HSP47 expression in pathogenic myofibroblasts in fibrotic skin lesion of chronic GVHD without affecting collagen synthesis in the normal skin. VA-lip HSP47 is a safe and promising anti-fibrotic agent for skin fibrosis in chronic GVHD without inducing systemic immunosuppression.