Impaired function of ECM1 underlies the pathological disorganization of vascular and basement membrane molecules in lichen sclerosus

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There is growing evidence that vasculopathy-induced hypoxia and oxidative stress enhance fibrosis in sclerotic skin. There are several models used to investigate skin fibrosis based on bleomycin treatment. Although the body temperature is maintained at around 37°C, the temperature of skin, which is facing directly to external environment, is affected by external climate. Since epidermal Langerhans cells reside in upper most layers of skin, they must be affected with temperature shift. But most of the in vitro experiments are conducted at 37°C. In this study, therefore, the phenotypic changes of Langerhans cells were examined using a model cell line, THP-1 cells. Since the skin surface temperature goes down to 25°C when people go in to a room at 10°C, we chose the culture temperature at 29°C, 31°C, and regular 37°C. The proliferation rate declined as temperature went down. The amount of RNA extracted from the same number of cells cultured at lower temperature was smaller than at 37°C. These results suggest that cellular activity is lower at lower temperature. However, when gene expression was examined, the specific genes were detected at lower temperature, including inhibitor of growth family2, B cell anti-translocator gene antiproliferative, while inflammatory genes were not augmented but elongated in IL-10KO compared to WT(24 hours:1.45%, 120 hours: 0.31%). Kaempferol might improve bleomycin-induced fibrosis by reducing oxidative stress, and TNFα in vitro experiments when extraporating to in vivo.

Inhibitory effect of kaempferol on skin fibrosis in systemic sclerosis by the suppression of oxidative stress

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kaempferol on oxidative stress in bleomycin-treated mice and SSc fibroblasts was inhibiting kaempferol treatment with sRNA-knockdown for ECM1 and analyzed transcription profiles by cDNA microarray. Comparison with siRNA-untransfected fibroblasts identified 3,015 differentially expressed genes. Functional annotation assigned that 1,477 upregulated and 1,564 downregulated genes are related to proteins binding to DNA, RNA and proteins, response to external triggers, cation transport and cellular movement, employing in vivo localization and proposed function of ECM1 in the skin narrowed to 49 upregulated genes, including COL4A, laminin, fibronectin, MMPs, CTGF, PDGFA and its receptor, SMAD, and TGFβ receptor. Realtime RT-PCR and ELISA supported the upregulation of paneled genes and corresponding proteins. Laminin 312 and type IV collagen, the representatives upregulated by ECM1 silencing, revealed unique expression pattern on immunohistochemistry using LS skin. Moreover, type VII collagen, which did not satisfy the ECM1 silencing upregulation but showed abnormal expression pattern in the LS skin, bound to ECM1 recombinant protein. Impaired ECM1 function may thus cause increased expression and selective disassembly of basement membrane, vascular, and extracellular matrix molecules, as well as growth factors facilitating fibroblast proliferation, contributing to the LS skin pathology.

Evolution of autoantibody B and T cells in pemphigus patients with Rituximab or corticosteroid regimen treatment

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Rituximab is an autoimmune blistering disease mediated by autoantibodies (Abs) directed against desmosomogens (Dsg). We recently showed that first line treatment with Rituximab (RTX) was more effective than standard oral corticosteroid (CS). To understand the immunological mechanisms that mediate the long-lasting clinical remission (CR) after RTX treatment, we selected the patients with the highest specificity of B cells and T cells helper cells (TFH) by flow cytometry and the number of Dsg IgA Abs Secreting Cell (ASC) by ELISPOT. At Baseline, Dsg+ B cells were detected at a frequency of 0.1-0.6% of total B cells and were depleted after RTX. Flow cytometry and IgG switched memory B cells. Dsg+ IgG ACS were detected at a frequency of 0.08% of total ASC for Dsg1 and 0.1-0.2% for Dsg3. The CS treatment did not influence the frequency nor the phenotype of Dsg+ B cells and Dsg+ ASC, which were detected even in patients in CR. In contrast, RTX induced a significant decrease of IgG switched Dsg+ memory B cells. Accordingly, Dsg+ ASC were no longer detected in patients in CR at M6. Interestingly, Dsg1-specific TFH cells were detected in patients after treatment, with an increased proportion of activated TH2/TH17 cell subsets. Strikingly, the frequency of autoantibody-negative Dsg+ T cells is increased in patients with a clinical response, the intensity of Th17/Th22 decrease suggesting that the response to RTX in pemphigus involves two mechanisms: i) a sustained depletion of IgG switched memory autoantibody B cells leading to the disappearance of Dsg+ B cells and ii) the decrease of Dsg1-specific circulating TFH cells, which is likely involved in the blockage of B cell maturation and the delayed reappearance of Dsg+ memory B cells.

Phenotypic Changes of a Monocyte Cell Line depend on the Culture Temperature

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Knowing the effect of temperature on KC cells, we examined the expression of CD86, which is involved in the blockage of B cell maturation and the delayed reappearance of Dsg+ memory B cells, was examined using a model cell line, THP-1 cells. The proliferation rate declined as temperature went down. The amount of RNA extracted from the same number of cells cultured at lower temperature was smaller than at 37°C. These results suggest that cellular activity is lower at lower temperature. However, when gene expression was examined, the specific genes were detected at lower temperature, including inhibitor of growth family2, B cell anti-translocator gene antiproliferative, while inflammatory genes were not augmented but elongated in IL-10KO compared to WT(24 hours:1.45%, 120 hours: 0.31%). Kaempferol might improve bleomycin-induced fibrosis by reducing oxidative stress, and TNFα in vitro experiments when extraporating to in vivo.

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