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Identification of CD146 as a novel molecular actor involved in systemic sclerosis

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Short summary : We highlight for the first time that CD146/sCD146 is involved in fibrotic process during SSc. sCD146 could thus constitute a new biomarker to assess disease activity, and potentially a new target for therapeutic applications.

Key words: CD146; soluble CD146; Systemic sclerosis; Fibrosis; Biomarker; Disease activity

To the Editor:

Systemic sclerosis (SSc) is a fibrosing autoimmune disorder characterized by vascular damage, excessive fibrosis in the skin and internal organs, and immune dysfunction.¹ Pulmonary fibrosis and pulmonary arterial hypertension are the most serious complications and currently constitute the major causes of death. The identification of biomarkers for the presence and progression of clinical complications in SSc helps to clarify pathogenic mechanisms, assess disease activity and offer new hopes for treatment. CD146, an adhesion molecule located essentially in the vascular system, is also present as a soluble form (sCD146) in bloodstream.² Because of its role in endothelial integrity and inflammatory response,^{3,4} two processes modulated in SSc, we hypothesized that CD146/sCD146 could represent a new molecular actor involved in SSc pathogenesis.

Sera from 50 SSc patients admitted to internal medicine departments in Marseille (South of France) were analyzed (**Table E1**). All patients fulfilled the 2013ACR/EULAR Classification Criteria for Scleroderma⁵ and were then sub classified according to LeRoy et al criteria.⁶ All samples came from a declared Biobank (DC 2012-1704) with respect of ethical directives (see **Table E1** and **Methods** section in this article's Online Repository).

Soluble CD146 levels were significantly higher in patients with SSc than in healthy controls (867.1 ± 32.3 vs 497.7 ± 16.5 ; $p < 0.001$) (**Fig 1A**). Regarding clinical association, we found a significant association between lowest sCD146 levels and digital gangrene ($n=5$; $p=0.03$) as well as pulmonary fibrosis ($n=20$; $p=0.04$), two severe manifestations of the disease, whereas no significant association was found between sCD146 levels and Raynaud's phenomenon ($n=29$), digital scars / ulcers ($n=16$). (**Fig 1B, C**). Sera available 12 months later for 15 patients showed a significant association ($p=0.03$) between the diminution of sCD146 levels and aggravation of the disease attested by digital necrosis, pulmonary arterial hypertension and pericarditis (**Fig 1D**). To investigate whether sCD146 is active in serum of patients, we examined its effect on endothelial cell proliferation. Results showed a significant correlation between seric CD146 levels and cell proliferation ($p=0.021$) and a significant decrease in cell proliferation when sCD146 was immunodepleted ($p < 0.0001$) (**Fig E1**) (see **Fig E1** in

this article's Online Repository). Thus, sCD146 present in the sera of patients with SSc is biologically active and may represent a protective biomarker of the disease.

To further examine the impact of CD146 we used the bleomycin-induced skin fibrosis model in WT and CD146 deficient mice. In this study, we used 1 μ g of bleomycin/mouse. At this dose, no modification was obtained in dermal thickness under bleomycin or control conditions in WT mice ($p=0.64$; **Fig 2A, B, G**). In contrast in CD146KO mice, dermal thickness was significantly higher after bleomycin treatment than in control conditions ($p=0.0002$; **Fig 2C, D, G**) showing that this concentration was sufficient to induce skin fibrosis in CD146KO mice but not in WT mice. In the same way, epidermal thickness was also increased after bleomycin treatment in comparison to control conditions in CD146KO mice ($p=0.04$), whereas no effect of bleomycin treatment was observed in WT mice ($p=0.63$; **Fig 2H**). Altogether results showed a highly susceptibility of CD146KO mice to develop skin fibrosis after bleomycin injection. Interestingly, injection of sCD146 in bleomycin-treated CD146KO mice showed a significant reduction of dermal and epidermal thickness as compared to bleomycin condition ($p=0.003$ and $p<0.0001$, respectively; **Fig 2E to H**). Thus, our results suggest that sCD146 protects bleomycin-treated CD146KO mice from fibrosis development.

Up to date, SSc-specific autoantibodies and cutaneous sub-classification are the most useful biomarkers for diagnosis and predicting clinical features. If several biomarkers such as ICAM-1 or E-selectin were proposed with a potential prognostic interest,⁷ none of them are used in clinical practice. Our results highlighted the role of CD146/sCD146 in SSc because: (i) sCD146 levels were increased in SSc patients compared to controls, (ii) severe manifestations of the disease, digital gangrene and pulmonary fibrosis, were associated with low sCD146 levels in SSc patients, (iii) mice lacking CD146 were highly susceptible to develop skin fibrosis, (iv) in this KO model, skin fibrosis development can be prevented by sCD146 injection. Altogether our data suggest that patients producing high and sustained levels of sCD146 may be protected against the disease complications. Up to now, sCD146 has been mainly described in human serum as an endothelial biomarker modulated in inflammatory diseases. In inflammatory bowel diseases, we found an association between sCD146 levels and disease activity.⁸ Indeed, low level of sCD146 was associated with the active form of

the disease and an upregulation of sCD146 occurred after anti-TNF treatment and a favourable disease evolution.⁸ Similarly in the present study, unfavorable evolution of SSc was associated with a decrease of sCD146 levels. In addition, vascular ischemic complication (digital gangrene) and pulmonary fibrosis were associated with low sCD146 levels. Although a prospective study is necessary to confirm our conclusion, high sCD146 levels could constitute a good prognostic marker of the disease. Along this line, we showed that CD146KO mice were strongly sensitive to bleomycin-induced skin fibrosis as compared to WT mice. The proof that this effect is CD146-dependent is given by the absence of fibrosis development after sCD146 injection in this animal model. Fibrosis, the hallmark of SSc, is characterized by the proliferation of fibroblasts, their differentiation in myofibroblasts and an excessive extracellular matrix accumulation. Many cytokines such as TGF- β , VEGF, and endothelin-1 are considered as key mediators in this process.⁹ Our study underlines for the first time that CD146/sCD146 may also regulate fibrotic process during scleroderma. Further studies will be conducted to analyze the specific role of CD146/sCD146 in the fibrotic process and interactions with the other pathways.

In conclusion, our data led us to propose CD146/sCD146 as a novel biomarker in SSc for the assessment of the disease activity. Mechanistic studies will now be necessary to delineate the precise role of CD146/sCD146 in the fibrotic process and to further investigate the potential interest of the molecule for therapeutic applications.

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FIGURE LEGENDS

FIG 1. A high and sustained soluble CD146 level in SSc patients is associated with a favourable evolution of the disease.

- A:** sCD146 levels significantly higher in SSc than in controls ($p < 0.001$).
- B:** Significant association between pulmonary fibrosis and low sCD146 levels ($p = 0.04$).
- C:** Significant association between digital gangrene and low sCD146 levels ($p = 0.03$).
- D:** Significant association between diminution of sCD146 levels and disease aggravation ($p = 0.03$).

FIG 2. Mice lacking CD146 are highly susceptible to develop skin fibrosis and soluble CD146 injection prevents bleomycin-induced skin fibrosis in CD146KO mice.

A to F: Wild type (**A, B**) and CD146KO mice (**C to F**) injected with vehicle (**A, C and E**), bleomycin (**B, D**) or bleomycin with sCD146 (**F**). Arrows illustrate dermal thickness. Scale bar: 100µm.

G and H: Significant increase of dermal (**G**) and epidermal thickness (**H**) after bleomycin injection in CD146KO mice prevented by sCD146 injection.