DECORIN INCREASES SURVIVAL AND AMELIORATES DISEASE PHENOTYPE OF COL7A1 HYPOMORPHIC MICE

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BACKGROUND

- Recessive dystrophic epidermolysis bullosa (RDEB) is a rare skin blistering disease due to mutations in the gene *COL7A1* coding for type VII collagen.
- In RDEB, unremitting skin and mucosal blistering leads to chronic wounds, inflammation and fibrosis; these features are responsible for more severe disease complications, such as pseudosyndactyly, esophageal stenosis and squamous cell cancer.
- Recent studies highlighted the role of the TGF-β pathway in modifying RDEB severity, and our previous findings showed that **the proteoglycan decorin (DCN)**, an extracellular matrix component and a biological TGF-β inhibitor, **may influence disease outcome by counteracting TGF-β profibrotic and proinflammatory activity** (Odorisio et al, Hum Mol Gen 2014) .

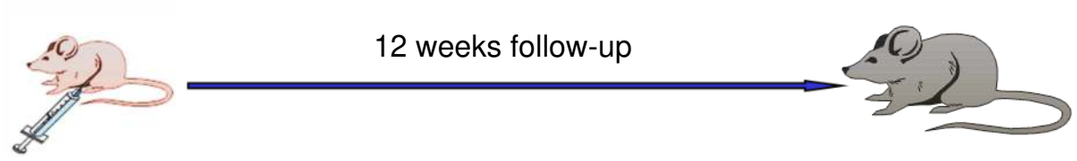
DCN appears as a potential therapeutic candidate to prevent or mitigate disease complications in patients affected with RDEB.

AIM

TO EVALUATE DECORIN POTENTIAL IN IMPROVING RDEB DISEASE PHENOTYPE BY USING A SEVERE RDEB PRECLINICAL MODEL, THE col7a1 HYPOMORPHIC MOUSE

Figure 1

Lentiviral administration



A single intraperitoneal treatment between 7 and 12 days of age:

1) LV-DCN (10^7TU)
2) LV-CTRL (10^7TU)
3) saline solution

Survived mice were sacrified; back skin and forepaws were collected and analyzed

- Newborn Col7a1 hypomorhic mice show a great variability in disease manifestations. They were classified into four groups according to disease severity, and animals from each group were distributed homogeneously among the three treatments.

ELISA for human decorin in the skin

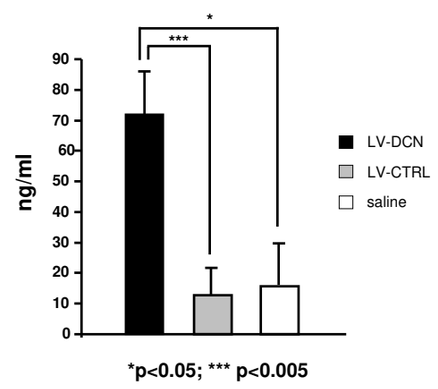
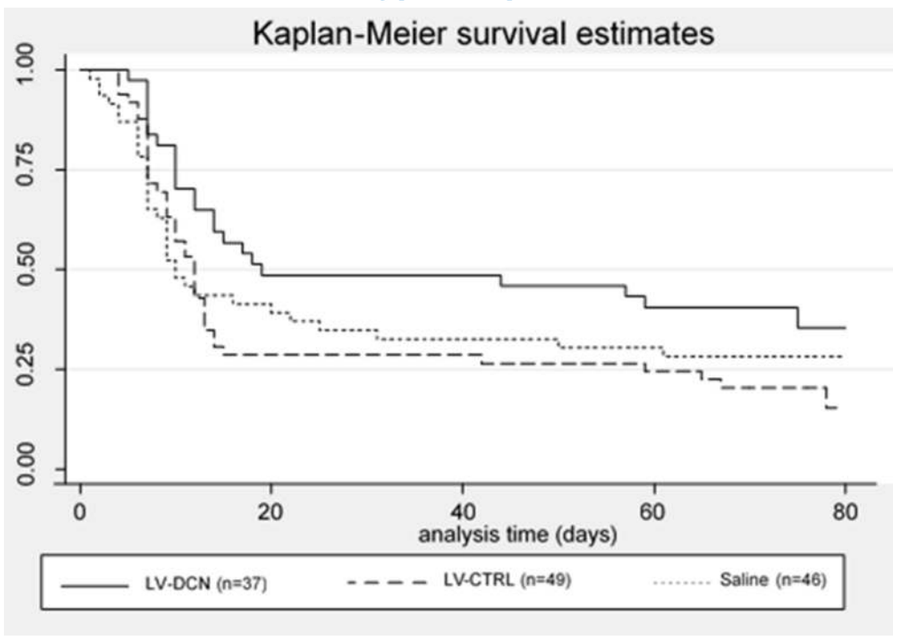


Figure 2

Increased survival of col7a1 hypomorphic mice treated with LV-DCN



median survival time (12 days)	
LV-DCN	19 days
LV-CTRL	12 days
saline	10 days

survival rate (25.9%)	
LV-DCN	40.5%
LV-CTRL	20.4%
saline	28.3%

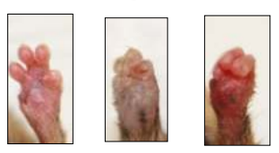
Pairwise comparison (Wilcoxon's test) p-values for survival rate:
LV-DCN vs LV-CTRL, 0.02; LV-DCN vs Saline, 0.04; LV-CTRL vs Saline, 0.78

Figure 3

Increased digit length and reduced digit loss in LV-DCN-treated mice

Digit lenght (cm)

	baseline	12 weeks	p-value
LV-DCN	0.26 (±0.03)	0.32 (±0.04)	0.03
LV-CTRL	0.26 (±0.02)	0.26 (±0.03)	0.78
saline	0.24 (±0.06)	0.23 (±0.03)	0.12



Digit loss

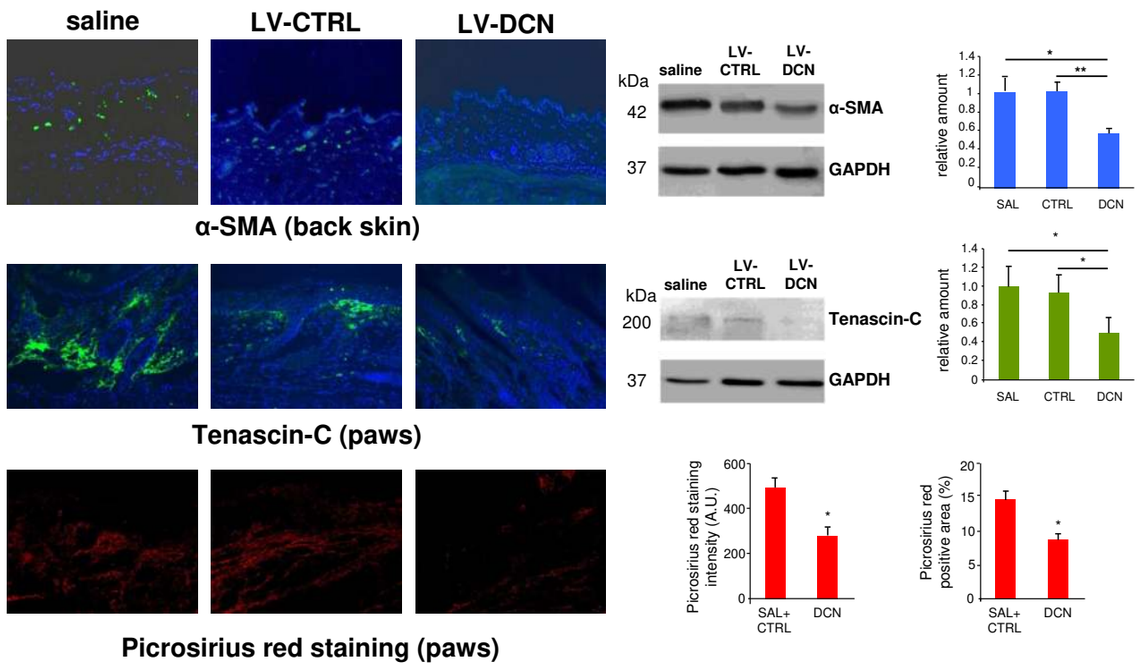
	DCN (%)	CTRL (%)	saline (%)
no	9 (64.3)	2 (22.2)	1 (8.3)
yes	5 (35.7)	7 (77.8)	11 (92.7)
Total	14	9	12

DCN vs saline, p = 0.005

- At the endpoint, only col7a1 hypomorphic mice treated with LV-DCN exhibit a significant increase in digit length.
- A reduction in digit loss was observed in col7a1 hypomorphic mice treated with LV-DCN as compared to control treatments.

Figure 4

Reduced fibrosis in the skin of LV-DCN-treated mice

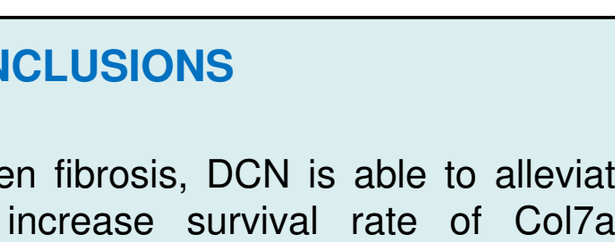


- As markers for tissue fibrosis, α-smooth muscle actin (α-SMA, expressed by myofibroblasts) and Tenascin-C (an extracellular matrix glycoprotein promoting fibrosis via Toll-like receptor 4 binding) were analyzed by immunofluorescence in forepaws, and by immunofluorescence and western blot in back skin (n= saline 8; LV-CTRL 6; LV-DCN 9).
- The expression of α-SMA and Tenascin-C was significantly reduced in both back skin and paws in LV-DCN treated mice as compared to controls.
- Staining by picrosirius red to visualize collagen networks/bundles under polarized light was reduced in skin samples from the forepaws of LV-DCN treated mice; the reduction was significant when control values were pooled and analyzed against LV-DCN values. However, this reduction was not evident in skin samples from back skin. (*p<0.05; **p<0.01).

Figure 5

Reduced TGF-β signaling in the skin of LV-DCN-treated mice

- TGF-β signaling was analyzed by detecting the activation of SMAD2 (phosphorylated SMAD2, p-SMAD2).
- The analysis of back skin protein extracts by western blot revealed reduced amount of p-SMAD2 in LV-DCN-treated mice as compared to controls (*p<0.05).



n: saline 6; LV-CTRL 5; LV-DCN 7

CONCLUSIONS

- By contrasting TGF-β-driven fibrosis, DCN is able to alleviate disease symptoms and increase survival rate of Col7a1 hypomorphic mice.
- Our data corroborate the results obtained by systemically treating col7a1 mice with losartan, a drug interfering with TGF-β signaling (Nyström et al, EMBO Mol Med 2015). These findings advocate clinical testing of anti TGF-β pathway agents for symptom-relief therapies in RDEB.

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