Single-cell analysis of skin immune cells reveals an Angptl4-ifi202b axis that regulates monocyte differentiation during wound healing

(Nicole Tong, LKC, Rachit Agrwal, LKC, Yao Yimin, LKC)

INTRODUCTION

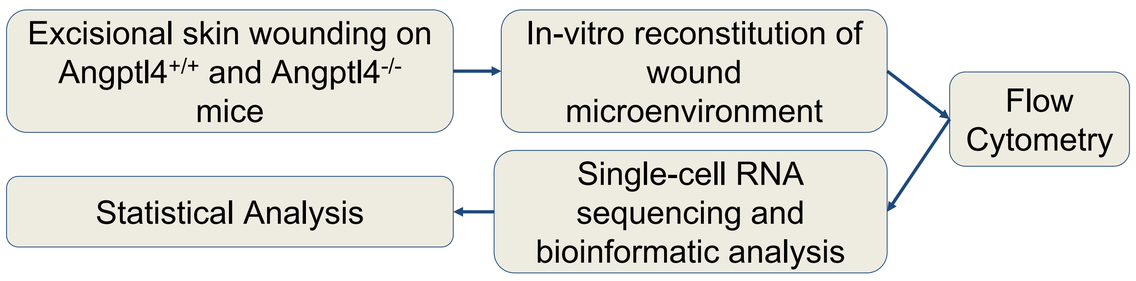
Normal wound healing aims to re-establish a new epithelial barrier following injury (1). It was believed that inflammation in wound healing was phasic, with each phase being completed before the next phase begins, namely from neutrophils to macrophages to fibroblasts to collagen remodelling. However, chronic wounds remain in an inflammatory state due to a postponed, incomplete, or uncoordinated healing process (2). This is caused by disruptions in cell-cell and cell-matrix communication mediated by chemokines and matricellular proteins. As such, further studies were required to better understand the complexities involved in wound healing

One such matricellular protein is Angiopoietin-like 4 protein (Angptl4) which has an involvement in inflammatory processes. However, its precise role remains unclear.

RESEARCH OBJECTIVE

This study examines the immune cell landscape of excisional wounds in wild-type (Angptl4$^{+/+}$) and Angptl4$^{-/-}$ mice

METHODS



DISCUSSION

The dynamics between neutrophils and macrophages are important in the transition from inflammation to the proliferative phase of wound healing. This dynamic is affected by Angptl4 deficiency. Our analyses revealed prolonged neutrophil persistence with markedly reduced mature macrophages in Angptl4-/- mice, which arose from impaired monocyte to macrophage differentiation. It also points to an impaired transition from inflammation to the proliferative phase of wound healing in Angptl4-/- wounds.

An in vitro reconstituted wound microenvironment study showed that wound fluid derived from Angptl4+/+ wounds could stimulate monocyte to macrophage differentiation, regardless of monocyte genotype. This could make new grounds for novel treatment options for chronic wounds.

FUTURE APPLICATIONS

Our current study identified a role for Angptl4 in the acute inflammatory response to injury. It is also conceivable that Angptl4 also plays a distinct role in adaptive immunity involving T and B cells, which were underrepresented in our skin wounding model, and may be better studied in other chronic inflammatory models like atherosclerosis or arthritis. Of particular interest is SLE. Treatment strategies against SLE generally involve immunosuppression that poorly addresses disease manifestations. It is widely established that genetic predisposition has a key role in susceptibility to SLE.

RESULTS

Angptl4 deficiency portends prolonged inflammation in wounds

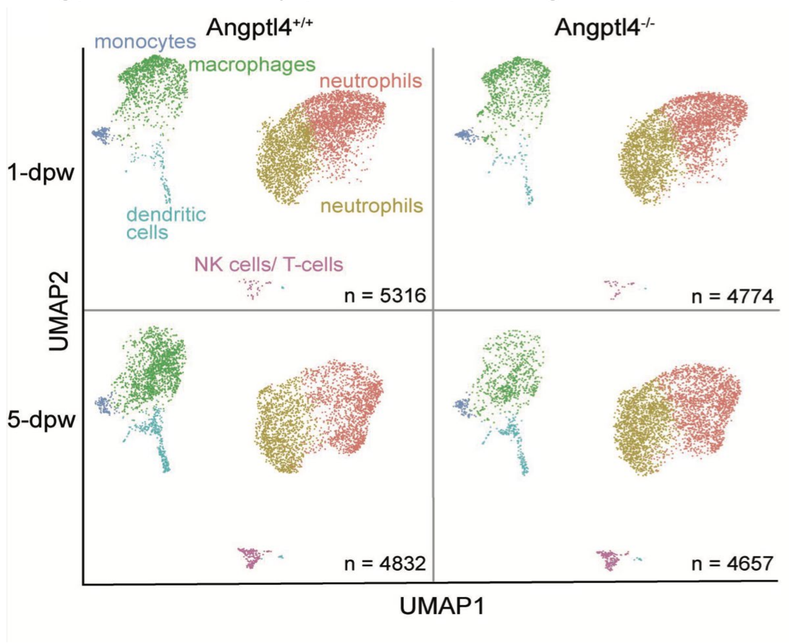


Fig 1. Population of neutrophils and macrophages 5 days post wound, showing less macrophages and more neutrophils in Angplt4 deficient mice

Angptl4 deficiency results in impaired monocyte differentiation during wound healing

	% of infiltrated CD45^{+} cells	1-dpw		5-dpw	
Cluster	**Cell type**	Angptl4$^{+/+}$	Angptl4$^{-/-}$	Angptl4$^{+/+}$	Angptl4$^{-/-}$
0	neutrophils	39.95	42.73	30.11	39.49
1	neutrophils	33.13	34.67	25.50	35.60
2	macrophages	21.61	18.06	30.69	13.64
3	dendritic cells	1.90	1.76	7.16	4.49
4	monocytes	2.73	2.09	2.30	2.30
5	NK cells/T cells	0.68	0.69	4.22	3.97

Fig 2. Percentages of different cells populations 1 day and 5 days post wound showing Angptl4$^{-/-}$ wounds having a decrease in the number of monocyte-differentiated macrophages

Angptl4 regulates the expression of interferon-activated gene 202b via S6K, FAK, and CDK signalling networks.

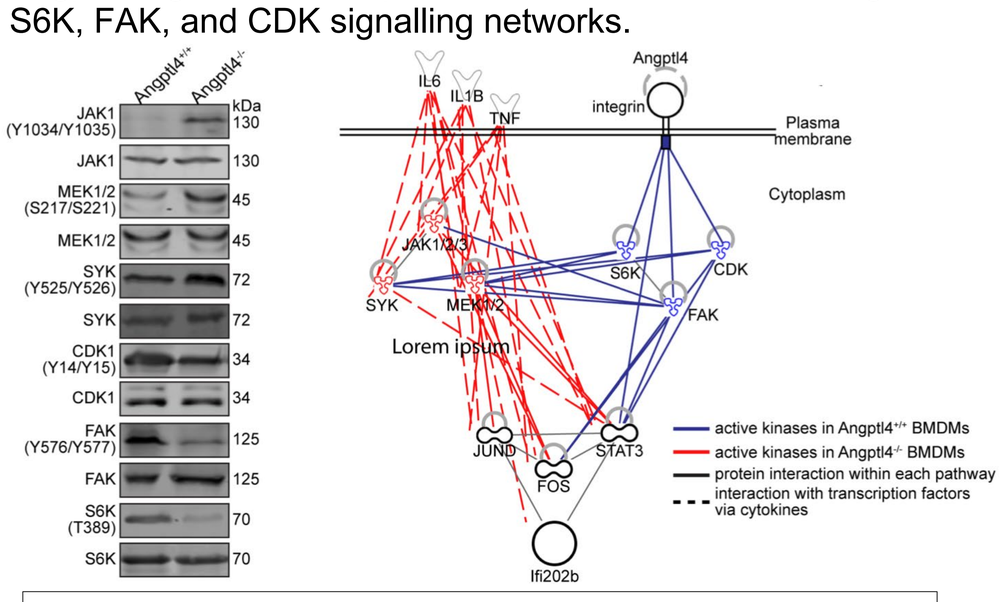


Fig 3. In the presence of Angptl4, high S6K, FAK, and CDK activation that suppressed the expression of ifi202b was observed. Increased phospho-activated Jak1/2/3, Mek1/2, and Syk kinases in Angptl4-/- BMDMs that activate Jun, Fos, and Stat3 transcription factors were also observed

Ifi202b regulates monocyte differentiation to macrophages.

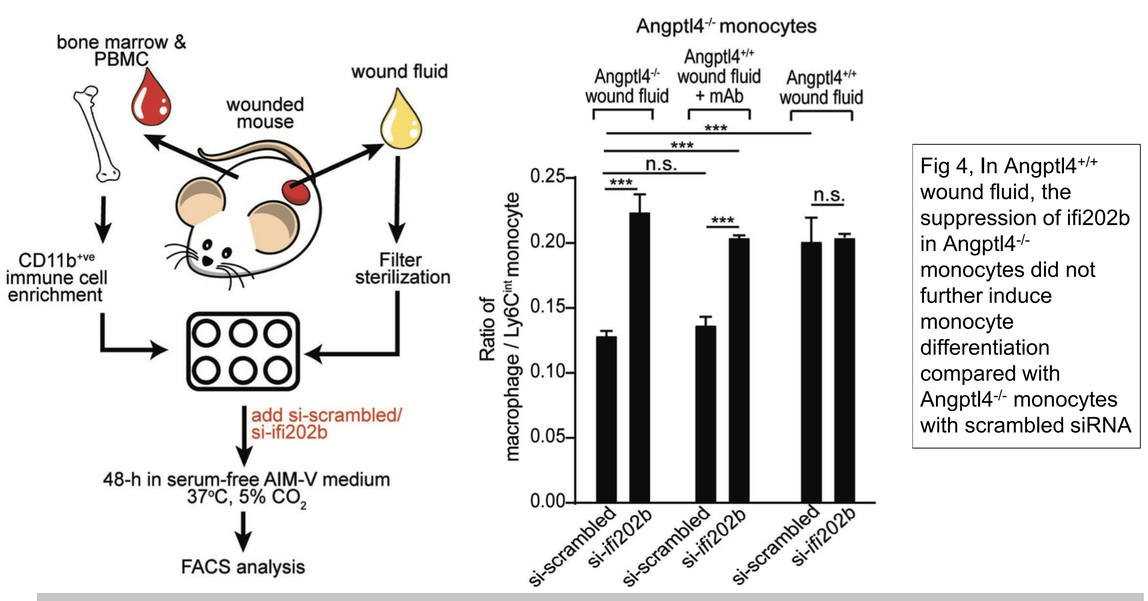


Fig 4, In Angptl4$^{+/+}$ wound fluid, the suppression of ifi202b in Angptl4$^{-/-}$ monocytes did not further induce monocyte differentiation compared with Angptl4$^{-/-}$ monocytes with scrambled siRNA

REFERENCES

1. Canedo-Dorantes L, Canedo-Ayala M. Skin Acute Wound Healing: A Comprehensive Review. Int J Inflam. 2019;2019:3706315.
2. Eming SA, Krieg T, Davidson JM. Inflammation in wound repair: molecular and cellular mechanisms. J Invest Dermatol. 2007;127(3):514-25.