Cell Reports

Article

Single-Cell Transcriptomics of Traced Epidermal and Hair Follicle Stem Cells Reveals Rapid Adaptations during Wound Healing

Graphical Abstract



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In Brief

Joost et al. elucidate how skin stem cells from different niches respond upon injury. Single-cell transcriptomics revealed that Lgr5 and Lgr6 progeny molecularly converge during wound healing. Instant cell adaptations of Lgr5 cells within their original niche permit interactions with the wound environment, an ability Lgr6 cells already possess before wounding.

Published 2018





Pasparakis M et al Nat Rev Immunol. 2014 PMID: 24722477





- different stem cell reservoirs in adult mouse HF
- timing of SC activation & subsequent long-term contribution to re-epithelialized wound area varies
- majority of Krt15 progeny from bulge SC rapidly lost following wound healing
- progeny from Sox9, Gli1, Lrig1 SCs in bulge/isthmus, JZ contribute to **long-term regeneration**

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so far two hypothesis of stem cell (SC) contribution to wound healing:

Stochastic model

 fraction of retained SC progeny = proportional to initial contribution

Selective model

 specific SC progeny are retained within regenerated area (at expense of others)

Schepeler et al Development. PMID: 24961797



key method: single cell RNA sequencing

Bulk seq



scRNA seq





key method: single cell RNA sequencing







Technology - 10x GenomicsKlas KatharinaSingle-Cell Profiling (RNA-Seq and ATAC-Seq) | Iowa Institute of Human Genetics (uiowa.edu)



- breeding of tomato-mice to cre-recombinase harbouring mice \rightarrow STOP cassette deleted in offspring in the *cre*-expressing tissue/cells \rightarrow tdTomato (red) fluorescence
- Tamoxifen treatment (7wks) induces cre-expression
- Post wound; tomato traced cells were sorted and sequenced

Experimental workflow and sampling strategy





Spatial distribution of traced cells during wound healing



- sign. difference in temporospatial distribution
 - Lgr6 at leading edge (wound front) from beginning on
 - Lgr5 progeny reached WF after 3-5 days
 - Lgr5 progeny less abundant in closed wound/remodeled wound

Comparing transcriptome profiles of sorted Lgr5/6 from unwounded skin



- Lgr6 showed almost exclusively interfollicular epidermis (IFE)-like signature
- Only few Lgr6 showed isthmus-like signature

Identification of activated vs. non-activated Lgr5/6 cells

D Wound cell selection in Lgr5[™] samples

E Wound cell selection in Lgr6[™] samples (compared to both IFE and isthmus control)





Profiling transcriptional heterogeneity of Lgr5/Lgr6 A t-SNE visualization of wound cells Lgr5-EGFP-Ires-CreERT2 / tdTomato (Lgr5TM) Lgr6-EGFP-Ires-CreERT2 / tdTomato (Lgr6TM)

- Lgr5 & Lgr6 cells did not only cluster by time point (PWI)
 - each cluster contained different wound healing time points









A and B not necessarily imply temporal order but expression of early wound signature



Profiling transcriptional heterogeneity of Lgr5/Lgr6 SC progeny

C Barplot visualization of gene expression in single cells



- Several marker genes converged in Lgr5 and Lgr6 cells
- Lgr5 cells showed higher expression of outer bulge markers

Identification of gene signature switch upon Wound response A Pseudotimeline of Lgr5^{TOM} cells based on outer bulge or IFE basal identity B Expression of outer bulge markers in Lgr5^{TOM} cells along pseudotime

- Lgr5 but not Lgr6 cell span entire expression axis from bulge to IFE
 → Indicating gradual change in cellular identity
- Genes enriched in outer bulge = downregulated over wound healing course
- Most basal IFE markers = induced in Lgr5 during wound healing + remained high

 Certain genes already upregulated to acute injury; others due to location change from niche



Molecular interactions of Lgr5/Lgr6 SC progeny in homeostasis & early wound response

Crosstalk between epidermis & wound environment = central in orchestrating cell recruitment into wound \rightarrow re-epithelialization

Combination of **sequencing data** of early wound cell populations **+ bulk seq** containing stromal and epithelial woundparts

→ Identification of potential receptorligand interactions between activated wound cells & environment





Molecular interactions of Lgr5/Lgr6 SC progeny in homeostasis & early wound response

- Primarily receptors expressed in epithelial cells ligands originating from wound environment
- Striking difference in # receptors expressed in Lgr5 & Lgr6 cells

# receptors	Lgr5	Lgr6
Before wounding	10	45
upon injury	39	48

 \rightarrow Lgr5 cells capable of gaining competence to react to signals from wound environment

B Potential interactions between Lgr5[™] and Lgr6[™] control and early wound cells (state 1) and the wound environment (1 day PWI)



Key ligand-receptor interactions

- Most commonly found epithelial receptors for wound signals:
 - Cd44
 - Alpha & beta integrins
- Thbs1 = Itgb1 interaction partner
 - Highest expression in newly forming wound matrix (1 day PWI)

- → Lgr6 cells in IFE = already primed for interactions with wound environment
- → Lgr5 cells actively shape their responsiveness upon wounding adaptation starts within 24hrs upon wounding while still residing in natural niche

A Receptor-ligand pairs with the wound environment (1 day PWI) shared by Lgr5^{TOM} and Lgr6^{TOM} early wound cells (state 1)

Ligands induced in the global wound environment Receptors induced in Lgr5^{TOM} / Lgr6^{TOM} wound cells



Ligands induced in Lgr5^{TOM} / Lgr6^{TOM} wound cells Receptors induced in the global wound environment





RNA-FISH staining of wound-induced receptors and ligands in early wounds (1 day PWI)

C





Profiling common wound-healing transcriptome signature

- Gene enrichment analysis revealed enrichment in both Lgr5 & Lgr6 progeny
- Early wound-cell state associated with basic cellphysiological processes
 - Energy production
 - Cell cycle
 - Migration
 - mRNA synthesis
- Later wound-cell states associated with
 - Ribosome formation
 - Translation
 - Protein processing
- Only NFkB significantly enriched in Lgr5 state 0
 => probably cells that remained in bulge but sensed injury

A Functional signatures induced in Lgr5^{TOM} and Lgr6^{TOM} wound cell states

	TNF sig	naling via NFκB [Halli	mark]								
Steroid biosynthesis [KEGG]											
RNA pol III transcription [Reactome]											11
Myc targets [Hallmark]											
mTORC1 signaling [Hallmark]											
	Proteasome [KEGG]										
IFE basal layer [Joost et al. 2016]											.
Dest	tabilisatio	on of mRNA by Auf1 [F	Reactome]								
Prefoldin mediated transfer of substrate to CCT/TriC [Reactome]											.
Protein folding [Reactome]											.
Regulation of ornithine decarboxylase (ODC) [Reactome]											.
Metabolis	sm of am	nino acids and derivati	ves [Reactome]							
Signaling by Wnt [Reactome]											11
E	ER-phag	josome pathway [Rea	ctome]								
	Cardiac	muscle contraction [K	EGG]								
Cyt	osolic tR	RNA aminoacylation [R	eactome]								.
M/G1 transition [Reactome]											
Mitotic M / M-G1 phase [Reactome]											
	Ś	S phase [Reactome]									
TCA cycle and respiratory electron transport [Reactome]											
mRNA Splicing [Reactome]											
Metabolism of nucleotides [Reactome]											
Processing of capped intron-containing pre-mRNA [Reactome]											
	Mi	smatch repair [KEGG]]								
Activation of mRNA [Reactome]											
SRP-dependent protein targeting to membrane [Reactome]											
3-UTR mediated translational regulation [Reactome]											
	Tr	ranslation [Reactome]									
Reactive	e oxyger	n species (ROS) pathv	vay [Hallmark]								
IF	FE supra	abasal layer [Joost et a	al. 2016]								
					0	~	2	~	2	8	
RNA metabolism		Migration / Cell contraction				Lgr5 ^{TOM}		Lgr6 ^{TOM}		м	
Protein metabolism		Cell cycle			0.05						max
Energy metabolism		Epidermis (Joost et al. 2016)		n.s.		Enrichment in Lgr5 [™] populations [Q-value - normalized]					
Intermediate metabolism		Miscellaneous		n.s.	0.05	; Enrichment in Lgr6™ populations [O-value - normalized]					





Convergent & niche-specific gene expression

- Lgr5[™] and Lgr6[™] Lgr5[™] unique Lgr6[™] unique Krt6a S100a6 Stfa Hspb1 Sprr Tom Nme1 Krt17 BC100530 migration lfitm3 Epcam B2m Gm541 Pkm Tpm' Cspg4 Sprr2a1 loca Arpc Ldha Tm4sf Sprr2a2 loc S100a11 BC11709 Lrrfip1 Ndufa4 Tnfrsf12a Fabp5 Cfl1 Amd2 Lama3 Expression [log² - PP] Expression [log² - PP]
- В Comparison of Lgr5^{TOM} and Lgr6^{TOM} wound cell states 1



cornified envelope

С Immunostaining of STFA1 in Lgr6^{TOM} and Lgr5^{TOM} cells (1 day PWI)



Stfa1 = cysteine proteinase inhibitor



Lgr6

High number of "shared genes" in Lgr5 &

conclusion

- Lgr5 & Lgr6 exhibit at each time point high degree of cellular heterogeneity
- Rapid receptor remodeling in Lgr5 cells
 → Early upregulation of receptors could serve as "initial priming" rendering Lgr5 cells susceptible for migration
- Lgr6 cells = already primed for potential interaction with wound environment
- Transient lineage adaptation during wound repair



Thank you! 😳

