Proteomic profiling of blood serum during porcine full thickness wound healing

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Background

Acute wounds heal in an orderly and efficient manner characterized by four distinct, but overlapping phases: hemostasis, inflammation, proliferation, and remodeling[1]. Specific signaling pathways regulate the transition through wound healing phases; dysregulation of these pathways contributes to pathological healing.

Proteomics enables comparative analysis of blood biomarkers during wound healing. By first understanding acute wounds, new therapies can be identified to enhance wound healing.

Goal: understand proteomic profile of blood serum during porcine acute wound healing

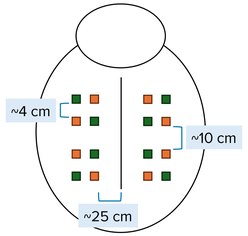

Figure 1. Full thickness wounds (2 x 2 cm) were created by scalpel. Gauze (green) or Tegaderm (orange) were used as a primary dressing.

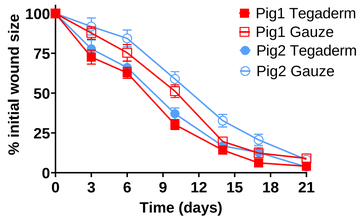

Figure 2. Wounds (n=8) closed at a comparable rate between animals and between primary dressing. Female adult Yorkshire pigs were studied.

SomaScan Assay

Somalogic aptamer-based technology was used for proteomic analysis[2].

- Blood was collected in EDTA tubes during wound healing at all timepoints
- After blood clotting, serum was isolated and stored at -80°C until analysis
- SomaScan 11k Assay was conducted at Somalogic Headquarters in Boulder, CO
  - RFU values underwent adaptive normalization
- Quality control was completed in Somalogic DataDelve platform and then exported for further processing.

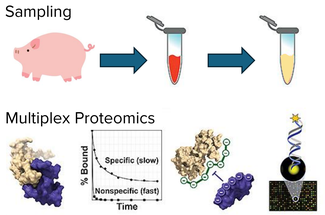

Figure 3. Blood serum was collected from pigs at regular intervals during wound healing. Serum was evaluated using a porcine-specific SomaScan Assay.

Data analysis pipeline

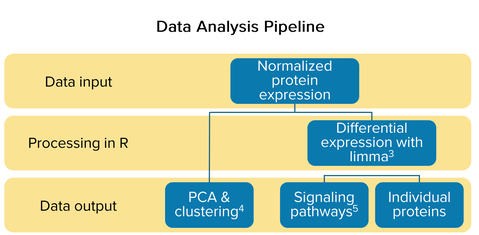

Figure 4. Analysis pipeline was adapted from an established RNAseq pipeline using normalized expression data as the input.

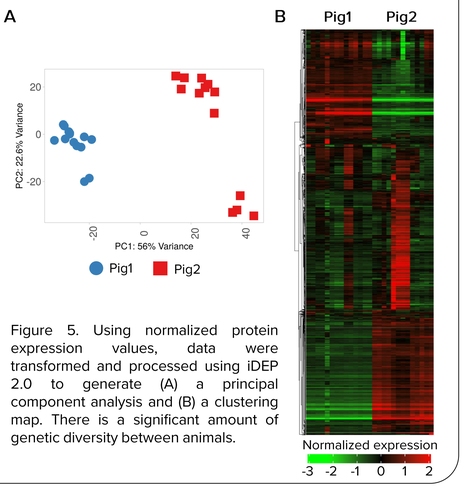

Figure 5. Using normalized protein expression values, data were transformed and processed using iDEP 2.0 to generate (A) a principal component analysis and (B) a clustering map. There is a significant amount of genetic diversity between animals.

Signaling pathway analysis

ReactomeGSA is an open source, peer-reviewed pathway database.

- Identified significantly enriched pathways between data sets
- A gene set analysis algorithm (as implemented in limma) was used

 # Significant pathways identified vs. Day 0

	Day 3	Day 6	Day 10	Day 14
Upregulated	20	8	34	92
Downregulated	3	0	14	6

*No significant pathways identified at day 17 and 21

Reactome analysis identified a number of significantly enriched pathways Table 1 (above). Table 2 (right) list the pathways that are enriched at day 3 vs day 0.

Significant Pathway at Day 3 vs. Day 0	Entities found	Entities total	Entities Ratio	Entities pValue	Entities FDR	Direction
RAB geranylgeranylation	45	68	0.004	7.97E-09	0.0000202	Up
FLT3 signaling through SRC family kinases	6	6	0	0.00000479	0.00606	Up
Signaling by SCF-KIT	37	51	0.003	0.0000114	0.00898	Up
Negative regulation of TCF-dependent signaling by WNT ligand antagonists	12	15	0.001	0.0000142	0.00898	Down
GPVI-mediated activation cascade	21	43	0.003	0.0000217	0.011	Up
PECAM1 interactions	6	14	0.001	0.0000369	0.0156	Up
Signaling by the B Cell Receptor (BCR)	67	176	0.011	0.0000477	0.0173	Up
Gene and protein expression by JAK-STAT signaling after Interleukin-12 stimulation	57	73	0.005	0.0000805	0.0255	Up
Regulation of signaling by CBL	16	24	0.002	0.0001	0.0281	Up
Interleukin-12 signaling	64	84	0.005	0.000119	0.0283	Up
Nef and signal transduction	6	9	0.001	0.000123	0.0283	Up
Antigen activates B Cell Receptor (BCR) leading to generation of second messengers	21	103	0.007	0.000191	0.0402	Up
Interleukin-3, Interleukin-5 and GM-CSF signaling	36	50	0.003	0.000218	0.0408	Up
ECM proteoglycans	53	79	0.005	0.000226	0.0408	Down
Signaling by CSF1 (M-CSF) in myeloid cells	20	41	0.003	0.000256	0.0431	Up
Golgi Cisternae Pericentriolar Stack Reorganization	11	17	0.001	0.000292	0.0454	Up
Co-inhibition by PD-1	8	33	0.002	0.000323	0.0454	Up
Disinhibition of SNARE formation	5	10	0.001	0.000323	0.0454	Up
Signaling by KIT in disease	15	28	0.002	0.000368	0.0462	Up
Signaling by phosphorylated juxtamembrane, extracellular and kinase domain KIT mutants	15	28	0.002	0.000368	0.0462	Up
G2/M DNA replication checkpoint	7	9	0.001	0.000383	0.0462	Up
Signaling by LRP5 mutants	6	6	0	0.000405	0.0466	Down
Co-inhibition by BTLA	4	7	0	0.00043	0.0473	Up

Individual proteins of interest

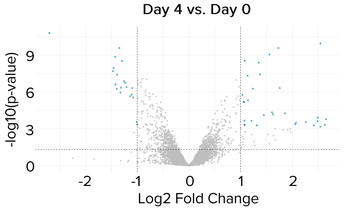

Figure 6. Volcano plot highlighting differentially expressed proteins at Day 4 vs. Day 0. Thresholds were set at Log2 Fold Change > ±1 and –log10(p-value=0.05) for exploratory research.

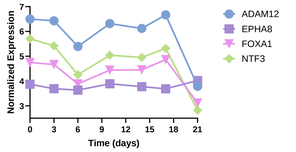

Figure 7. Voom transformed, normalized expression of select proteins during wound healing (n=3-5).

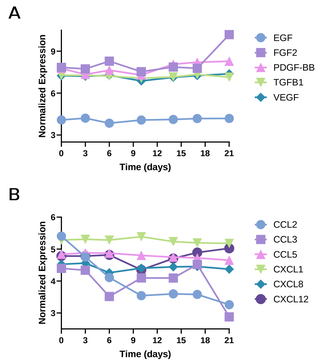

Figure 8. Voom transformed, normalized expression of (A) growth factors and (B) chemokines during wound healing (n=3-5).

Conclusions

- Blood serum may be used for proteomic profiling, but wound exudate may be more accurate
- Due to genetic diversity between pigs, larger sample sizes or baseline normalization will be required for quantitative assessments
- RNAseq data pipelines may be adapted for proteomic data sets

References

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