THE ROLE OF CYSTEINE CATHEPSINS IN PANCREATIC CANCER

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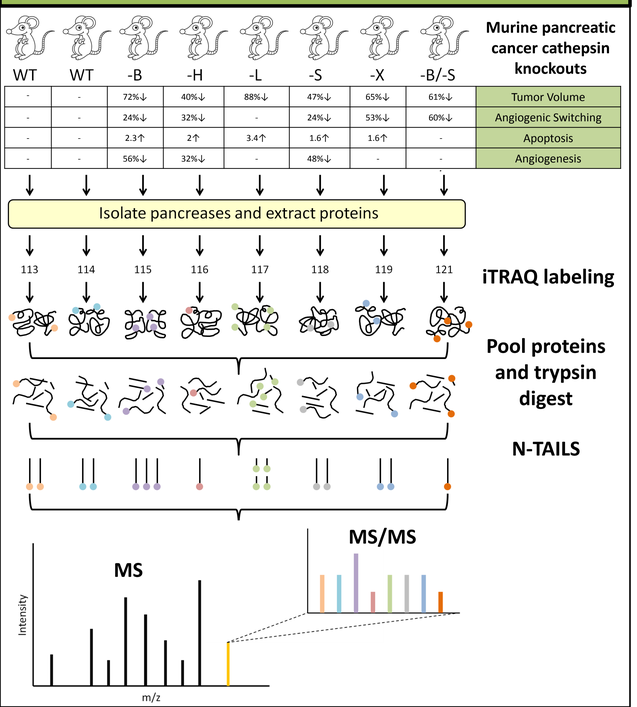
INTRODUCTION

Proteolytic enzymes are integral to the homeostasis of a cell. The cysteine cathepsins participate in various biological processes as both digestive and regulatory proteases. They were first classified as lysosomal proteases, but recent evidence has uncovered nontraditional roles in both the cytosol and nucleus (1). Clinical, molecular and pharmacological studies involving the modulation of these enzymes have revealed a causal role in tumor progression and invasion (2). The identification of cathepsin-specific changes in protein degradation in cancer could lead to the development of better therapies through a targeted approach.

AIM

Quantify changes in the proteome, specifically the differentiated abundance of protein amino termini resulting from six different cathepsin knockouts in a murine pancreatic cancer model.

METHODS



DATA EXPLORATION

Calculation of iTRAQ ratios

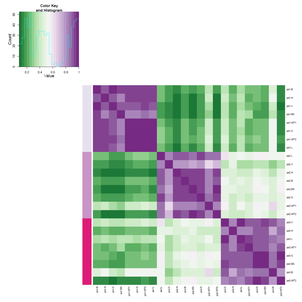
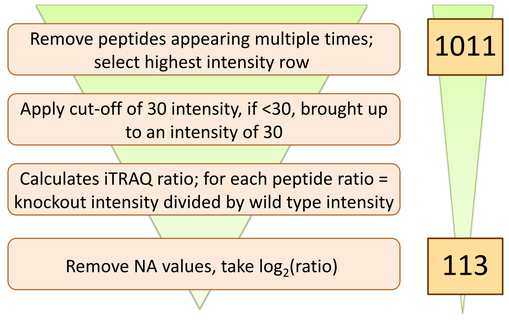


Figure 1. *Robust correlation matrix of peptide intensities demonstrates batch effect between experimental replicates.*



Minimization of batch effect by different denominators in ratios

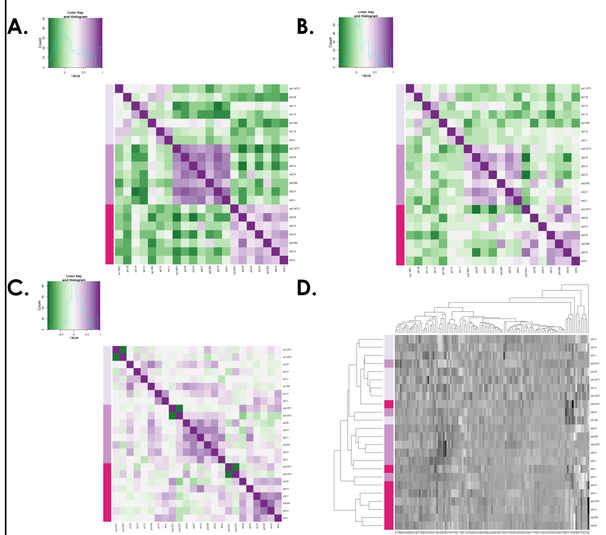


Figure 2. *Calculation of peptide iTRAQ-TAILS ratios demonstrates batch effect between experimental replicates*. **A-C:** Robust correlation matrix of samples; **A.** Ratio calculated with denominator of wt1; **B.** Ratio calculated with denominator of wt2; **C.** Ratio calculated with denominator of the average of wt1 and wt2.
D: Heat map of peptide ratios calculated with denominator of the average of wt1 and wt2. Columns are peptides, rows are samples.

RESULTS

Peptides do not cluster based on cathepsin knockout

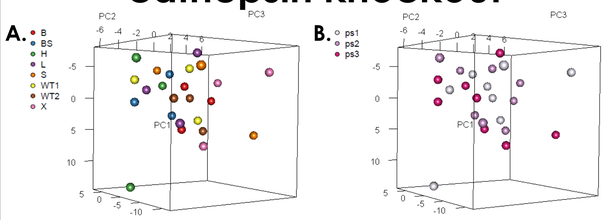


Figure 3. *Principal component analysis reveals peptides do not cluster based on cathepsin knockout, however the experiment based batch effect appears to be removed*. PCA based on first three principal components. Ratios calculated with denominator of the average of wt1 and wt2. **A.** Points coloured by mouse genotype; **B.** Points coloured by experimental replicate.

Differentially identified peptides

Table 1. Differentially abundant peptides as identified by LIMMA.

	μ_{WT}	μ_B	μ_{BS}	μ_H	μ_L	μ_S	μ_X
-1	0	0	0	0	4	0	1
0	113	113	113	112	108	113	111
1	0	0	0	1	1	0	1

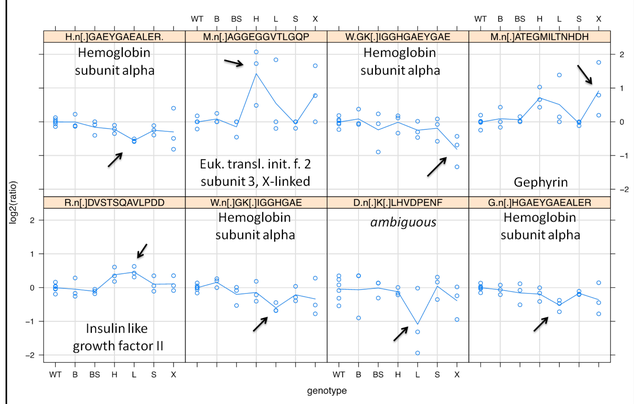


Figure 4. *LIMMA analysis identified differentially abundant peptides between the cathepsin L, H or X knockouts and a baseline of zero, q-value < 0.05*. The LIMMA parameters tested the statistical significance of the means of the $\log_2$ transformed ratios of each condition from zero. Zero was chosen as a baseline because an unaffected peptide would have a ratio = 1 and the $\log_2(1) = 0$. Values > 0 indicate a higher intensity in knock-out compared to wild type and values < 0 indicate a lower intensity in knock-out compared to wild type. A LIMMA model measuring the difference between knockout and wild type was also attempted, but no peptides with a q-value < 0.05 were identified.

Cathepsin B and S interaction

A.

	S -	S +
B -	BS KO	B KO
B +	S KO	WT

B.

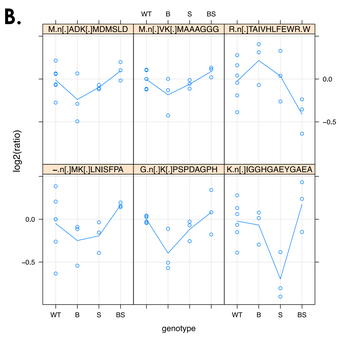


Figure 5. *LIMMA analysis identified no peptides with significant interaction effect between Cathepsin B, S, and double BS knockouts at q-value < 0.05.* **A.** LIMMA model was fitted with two conditions (B present/absent or S present/absent). **B.** The 6 highest scoring peptides for this analysis (none met q <0.05 threshold).

Peptides demonstrate similar abundance trends

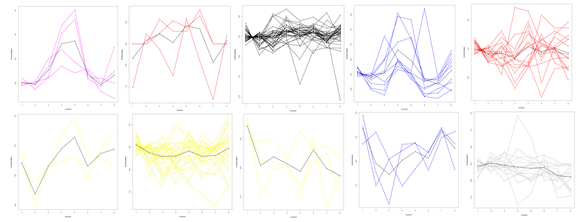


Figure 6. *k-means clustering of peptide iTRAQ-TAILS ratios reveals the presence of similarly abundant peptide trends between mice backgrounds*. Label 1, 2, 3, 4, 5, 6, 7 and 8 are "WT1", "WT2", "B", "H", "X", "BS", "S“ and "L“, respectfully. A k=10 was chosen and the size of each cluster are: 4, 3, 33, 9, 15, 2, 26, 3, 3, and 15.

CONCLUSIONS

- Batch effect between experimental replicates for this data set was minimized utilizing iTRAQ ratios calculated with a denominator of the average intensities of the two wild types
- High variability between biological replicates was observed through PCA
- Some peptides were identified as significantly different between knockouts and wild type

REFERENCES

1) Turk, V. *et al*. (2012). *Biochim Biophys Acta*. **1824**(1):68-88.
2) Mason, S.D. and Joyce, J.A. (2011). *Trends Cell Biol.* **21**(4):228-37.

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