Classifying the Microenvironment of Mesothelioma

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Background

- Malignant pleural mesothelioma (MPM) is a rare form of thoracic cancer associated with exposure to asbestos.
- Patients with MPM have a dismal prognosis with a short overall survival.[1]
- Cytokine and chemokine presence within the tumor microenvironment (TME) is reflective of the type of immune response and the permissiveness of the tumor to immune infiltration.

Methods

- Twelve MPM tissue resections snap frozen in liquid nitrogen were lysed using two different detergents:
  - T-PER Tissue Protein Extraction Reagent
  - RIPA buffer
- Assessment of total protein concentration in each sample was tested in duplicate on three colorimetric assays—a Lowry-based assay, a Bradford reagent-based assay, and a BCA protein assay
- Presence of 79 cell signaling factors was assessed in duplicate using a Luminex-based approach.
- Optimal protein concentration for detection was assessed by comparing read quality of 0.5 mg/mL and 1 mg/mL sample concentration.
- Results were compared with gene expression profiling using the Nanostring tumor signaling 360 panel of RNA extracted from the same tumor tissue.

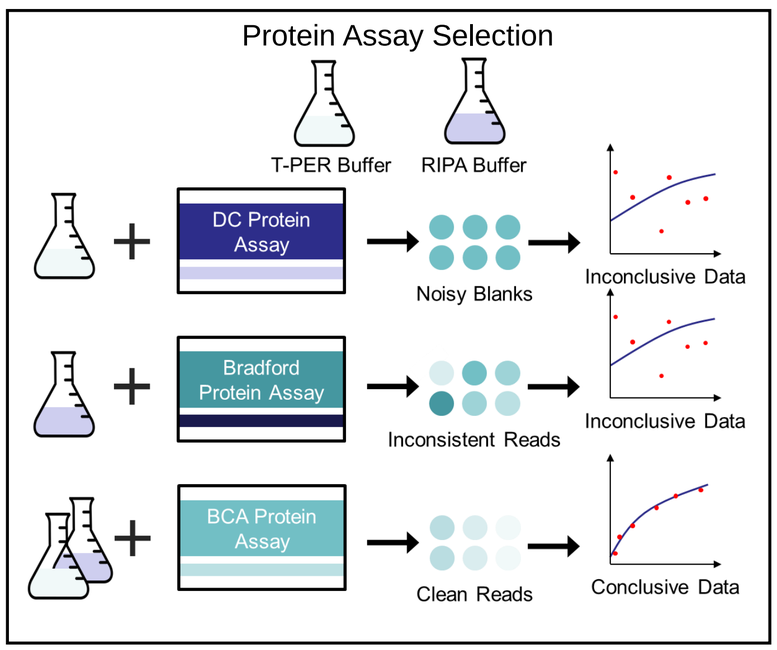

Fig. 1 Overview of experiments run to determine the most fitting assay to measure total sample protein concentration.

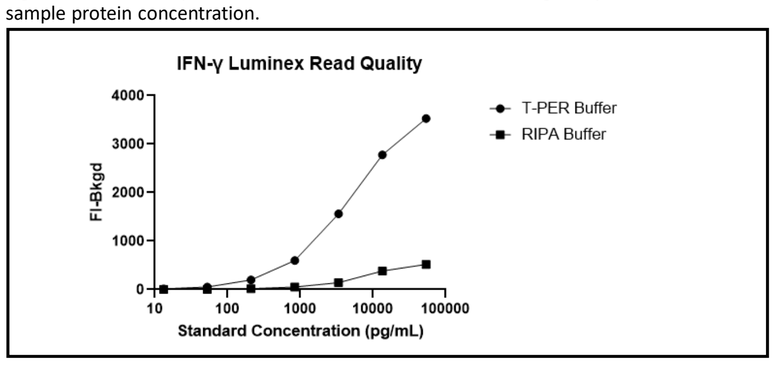

Fig. 2 Comparison of read qualities (signal detected with background subtracted) between different buffers in duplicate used in the Luminex instrument. IFN-γ standard dilutions are shown here as an example. This aligns with expectations as RIPA contains SDS, a protein denaturing agent.

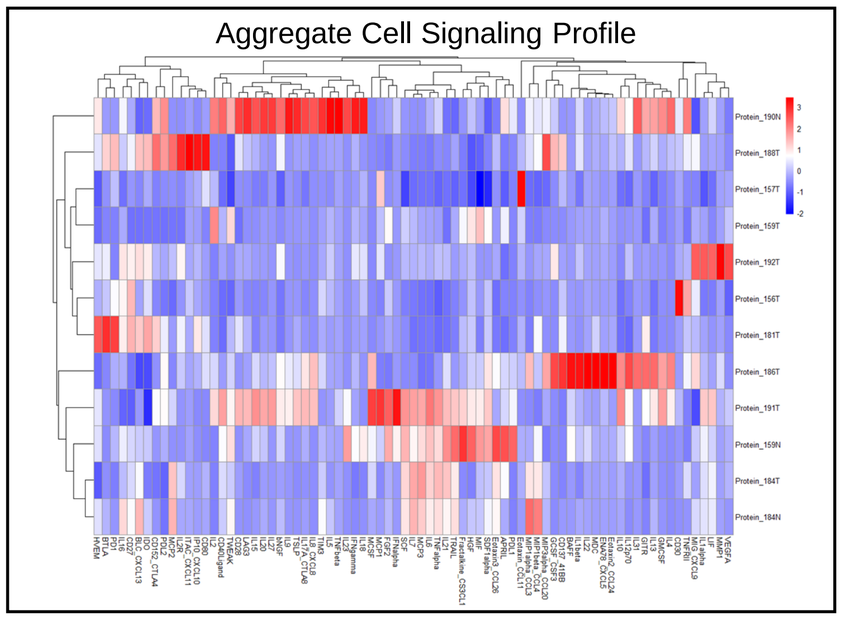

Fig. 3 Overall relative detection of all 79 cell signaling molecules in each of the twelve samples.

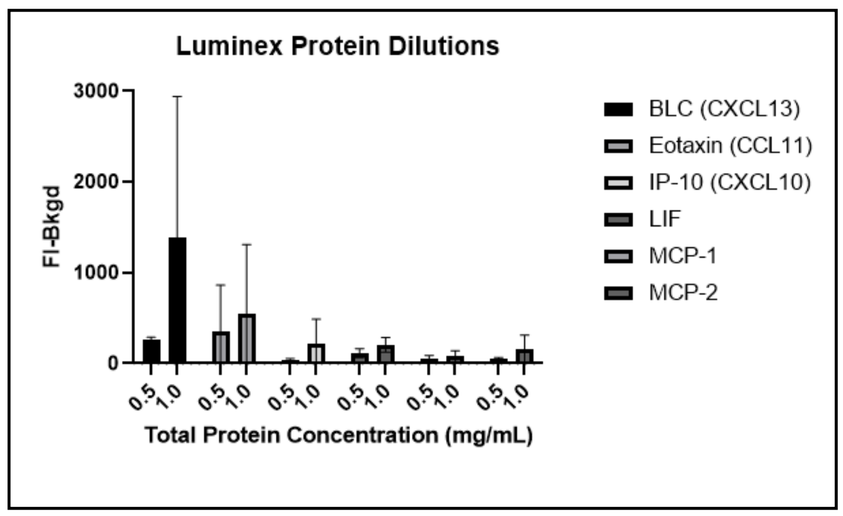

Fig. 4 Comparison of read qualities (signal detected with background subtracted) between different sample dilutions in duplicate used in the Luminex instrument (n=3). In some cases, total protein concentration of 1.0 mg/mL offered substantial improvement in signal quality, justifying its use over 0.5 mg/mL in following experiments.

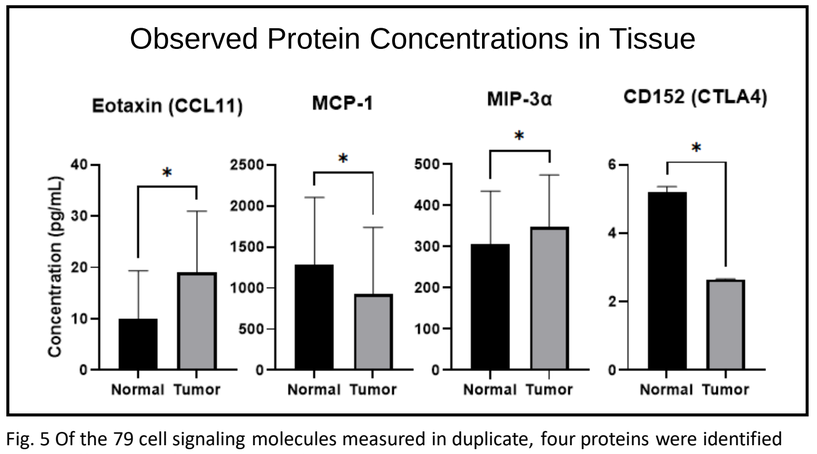

Fig. 5 Of the 79 cell signaling molecules measured in duplicate, four proteins were identified which differed significantly ($p<0.05$) in concentration between normal and tumor tissue (n=2). One sample of each tissue type was derived from one patient per group, therefore statistical analysis was carried out in a paired fashion.

References

1) Maaike Van Gerwen, Naomi Alpert, Andrea Wolf, Nisha Ohri, Erik Lewis, Kenneth E Rosenzweig, Raja Flores, Emanuela Taioli, Prognostic factors of survival in patients with malignant pleural mesothelioma: an analysis of the National Cancer Database, *Carcinogenesis*, Volume 40, Issue 4, April 2019, Pages 529–536, https://doi.org/10.1093/carcin/bgz004

Results

- Total protein concentration was found most consistently using a BCA protein assay
- T-PER reagent samples showed higher read quality
- One mg/mL total sample protein concentration demonstrated high resolution while minimizing sample used
- Presence of **Eotaxin** and **MIP-3α** was found to be significantly higher in tumor tissue as compared to tumor-associated normal tissue
- Presence of **MCP-1** and **CTLA4** was found to be significantly lower in tumor tissue as compared to tumor-associated normal tissue
- No correlation has yet been detected between protein isolation and RNA expression data.

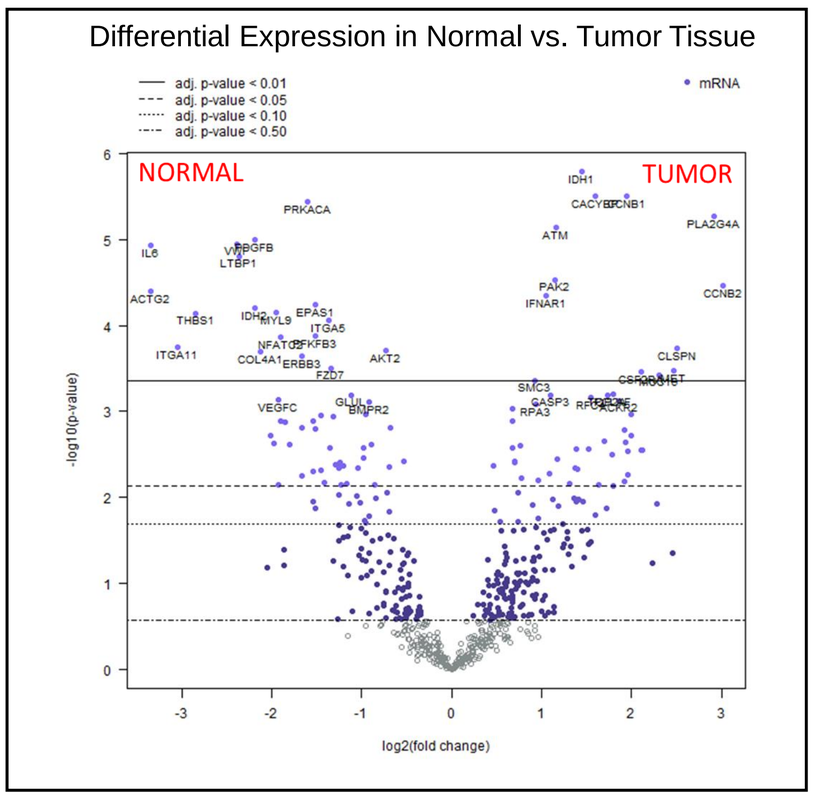

Fig. 6 Volcano plot depicting differential mRNA expression levels of cell signaling molecules derived from the same MPM tissue samples.

Conclusions

- Detection of proteins within primary tumor tissue using a Luminex system was optimized.
- Immune-related proteins possibly associated with tumor tissue as compared to tumor-associated normal tissue were identified.
- More samples are required to determine significant differences in presence of additional cell signaling molecules.
- Analysis is ongoing to determine associations with cytokine presence and tumor thickness as well as correlations with gene expression data.