Antibody Colocalization Microarray (ACM): A Scalable, Cross-Reactivity-Free Nano-ELISA Platform for Proteomic Studies

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1. Introduction

Multiplex sandwich immunoassays (MSI) suffer from cross-reactivity leading to false positive signal

- In gold-standard ELISA, specificity is afforded by using matched antibody pairs, and a single analyte is measured
- In multiplexed immunoassays, capture antibodies are spotted on a surface, and detection antibodies are applied as a mixture
- Number of possible cross-reactivity interactions increases exponentially with the number of targets being measured
- Scaling – increasing the number of targets – is difficult

2. Validation

- Validation of measurements made with ACM by measuring 20 individual patient samples with leptin ELISA and ACM
- Sensitivity is similar to ELISA and ACM
- Specificity is identical to that of ELISA
- Common cross-reactivity interactions are avoided

3. Measurement of clinical samples

Breast cancer patients
- Measurement of 36 targets in serum of 15 breast cancer patients and 11 normal controls
- Six targets demonstrated statistically significant difference
- Clusters based on six targets leads to 100% classification (discovery cohort)

Severe traumatic brain injury patients
- Patients with severe traumatic brain injuries at the Montreal General Hospital
- Cerebral microdialysis catheter (cut-off 100 kDa) implanted into brain tissue
- Serum, plasma, and cerebrospinal fluid collected from patients, all collected for 3 days
- Goal: find biomarkers of prognosis and predictors of secondary brain injury (brain swelling)

Mice with human breast cancer
- Immunodeficient mice are injected with human breast cancer cells in the mammary fat pads
- Development of breast cancer is monitored over four weeks
- Serum samples are collected weekly and 50 targets are measured using the SnapChip, a more portable version of ACM
- Measured targets are mostly produced by breast cancer cells (human-specific)
- Anatomical progression of cancer is correlated to levels of targets

4. Conclusion

- Cross-reactivity interactions plague commercial multiplexed sandwich immunoassays
- Antibody colocalization microarray (ACM) avoids those cross-reactivity interactions by physically separating detection antibodies during assay
- ACM has been used to measure serum and other biological samples from patients suffering from breast cancer and severe traumatic brain injury, as well as serum from mice injected with human cancer cells
- Increases the number of targets on ACM as simple as finding a validated ELISA antibody pair
- Current limitation is acquiring validated ELISA matched antibody pairs to add to ACM

5. Future work

- Increase number of targets on ACM
- Study post-translational modifications such as glycosylation and phosphorylation
- Improve portability with SnapChip (figure 8) and bead-based assay format
- Possibility of adding animal-specific targets

6. Acknowledgments

7. References