INTRODUCTION

Immunohistochemistry (IHC) is a well-known and well-characterized technique useful for profiling proteins in cancerous tissue samples for clinical diagnosis, prognosis, subtyping and treatment selection. However, this technique is long, laborious, and limited by its need for large tissue samples and large quantities of antibodies. Its clinical application is further hindered by its use of single biomarkers, as it is widely accepted that a panel of multiple proteins is required for clinical cancer tissue profiling.

To overcome this, my project aims to multiplex IHC in order to identify several biomarkers within cancer tissue samples. By using snap chip technology, multiple proteins can be detected using minute amount of reagents, without cross contamination. An aqueous two-phase system composing of polyethylene glycol (PEG) and antibodies diluted in dextran, is used to provide an aqueous environment for antibody-antigen binding to take place.

METHODOLOGY

By using snap chip technology, an array of pre-spotted antibodies can be transferred from chip-to-chip using minute amount of reagents and without cross contamination. An aqueous two-phase system composed of polyethylene glycol (PEG) and antibodies diluted in dextran, is used to provide an aqueous environment for antibody-antigen binding to take place.

OPTIMIZING AQUEOUS-TWO PHASE SYSTEM FOR ANTIBODY TRANSFER VIA SNAP CHIP

- Optimal antibody concentration of negative isotype control, IgG2B, and cancer biomarker, TIMP1, was determined.
- Four rows of each, at various concentrations, were spotted.
- Little to no signal is detected from IgG2B – indicating very little non-specific binding.
- TIMP1 has increasing signal for increasing concentration of protein.

Calculation Optimal Antibody Concentration

- Optimal antibody concentration was determined using Array Pro Analyzer.
- The net intensity was used to calculate average signal to noise ratio for corresponding IgG2B and TIMP1 concentrations.
- As seen, 400 µg/mL of primary antibody yielded the greatest signal to noise.

Figure 1: Snap chip antibody transfer completed using a two phase aqueous system using IgG2B and TIMP1, at concentrations varying between 50 µg/mL and 400 µg/mL.

Figure 2: Determining the optimal antibody concentration for the snap chip two phase aqueous system by comparing the signal to noise ratio. It is evident 400 µg/mL is the optimal primary antibody concentration.

COMPARING CONVENTIONAL AND SNAP CHIP ASSAYS

- Snap chip system was verified by comparison of snap chip assay and conventional assay using consecutively cut tissue samples.
- The tissues were stained with DAPI and viewed under the confocal microscope using both red and UV light.
- Overlays were generated of the DAPI staining and IHC results.
- The tissue integrity is comparable and confirms the potential of snap chip for clinical application.

Figure 3: Comparing tissue integrity of the snap chip two phase aqueous IHC and conventional IHC protocols. Images were taken using 20X objective on the confocal microscope.

Testing Storage of Antibodies Spotted on Transfer Slide

- To explore clinical potential, a transfer slide was pre-spotted with diluted antibodies and stored in -20°C freezer overnight.
- Overnight storage produced comparable results to freshly spotted antibody transfer slides in terms of signal to noise.
- This shows promising results for prolonged storage.

Figure 4: Testing overnight storage of TIMP-1 antibody spotted transfer slide stored at -20°C. Antibodies were spotted onto fluorosilane slide diluted in 5% dextran, 10% glycerol and PBS to a final concentration of 400 µg/mL.

CONCLUSIONS & FUTURE WORK

Overall, the aqueous two-phase system for the snap chip assay has been successfully applied for detecting protein overexpression in FFPE tissue slides.
- The assay conditions have been optimized, where the optimal antibody concentration, blocking treatment and hydrogen peroxide treatment were determined.
- It was concluded that the tissue structure is unaffected by this snap chip protocol by comparison with conventional IHC.
- Potential for long-term storage was confirmed.
- It can be concluded that this system has potential to be used in both a clinical setting or research laboratories, without access to microarray spotters, in order to test multiple proteins on FFPE slides. Future work will apply to conducting assays on clinical samples previously evaluated by histologists to validate snap chip diagnosis.

REFERENCES

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