Diabetic Complications Consortium

Application Title: Simultaneous spatial mapping of RNA and protein targets in human kidney tissue

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1. Project Accomplishments:

There is a lack of cost-effective, scalable, and robust clinical assays to map the spatial distribution of RNA and protein components relevant to diabetic injury in human cells and tissues. Though delayed by the COVID-19 pandemic, we were able to successfully develop a colorimetric method to detect multiple RNA targets in human mesangial cells and kidney tissues. The platform is flexible enough to detect DNA targets and oligo-labeled antibodies. We are currently testing a) multiplexed RNA and DNA detection, b) serial detection of multiple RNA species using different colorimetric readouts and c) simultaneous detection of protein and RNA targets.

2. Specific Aims:

Specific Aim: To create a simple and inexpensive method for multiplexed imaging of genes and proteins of interest in human kidney cells and tissues.

Results: While RNA-ISH has been used to study human and animal models of diabetic kidney injury, the most widely used commercial platform, RNAscope is cost limiting (~$40-50/slide), if applied a large number of samples. Furthermore, RNAscope is "black box" and does not reveal the identity or sequence of the probe oligos. Therefore, **routine application of a streamlined and inexpensive workflow, would increase the adoption of RNA-ISH for clinical purposes and for validation of single cell RNA-seq studies on tissue specimens**. Moreover, a universal set of reagents that could be used for colorimetric and/or fluorescent detection and that are rapid and cheap to design and synthesize would increase the flexibility of the platform and ensure assay readiness for future pandemic pathogens.

To meet this need, we built upon our recently developed Signal Amplification by Exchange Reaction (SABER) technology and combined it with colorimetric detection to detect multiple RNA species in cultured human cells (**Figure 1**). In Step 1, the probes will be extended by primer extension reaction (PER) in vitro. In Step 2, cultured cells/tissue sections are treated with H_2O_2 to block endogenous peroxidases with H_2O_2 and SABER probes are applied. After overnight hybridization, in Step 3, the probes will be washed off and oligo-conjugated horseradish peroxidase (HRP) that is complementary to the extended probes will be applied. In the last Step 4, the probes will be detected with DAB development, dehydrated and mounted in xylene containing mounting medium. We estimate the cost for this workflow to be ~$4-5/slide.

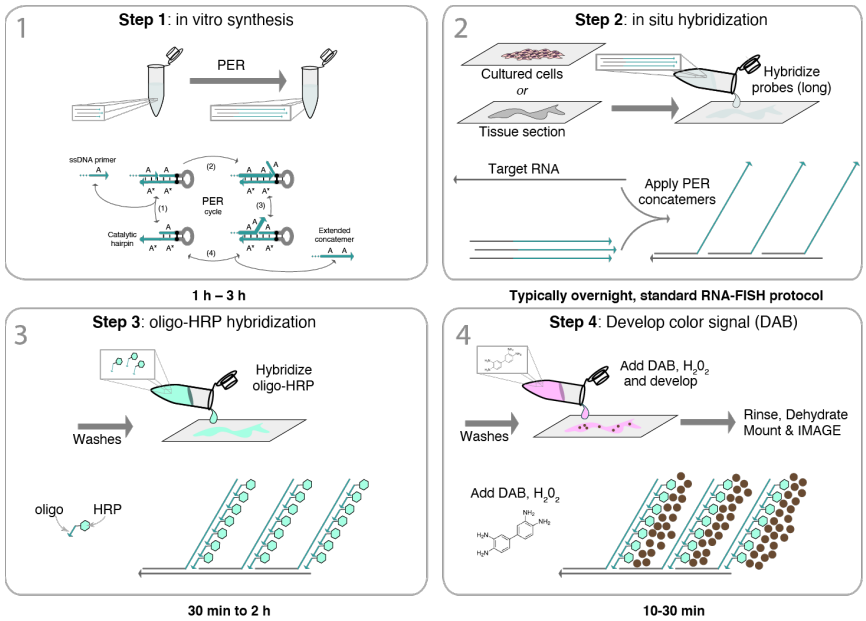

Figure 1. Colorimetric RNA-ISH using SABER. Please see text for details.

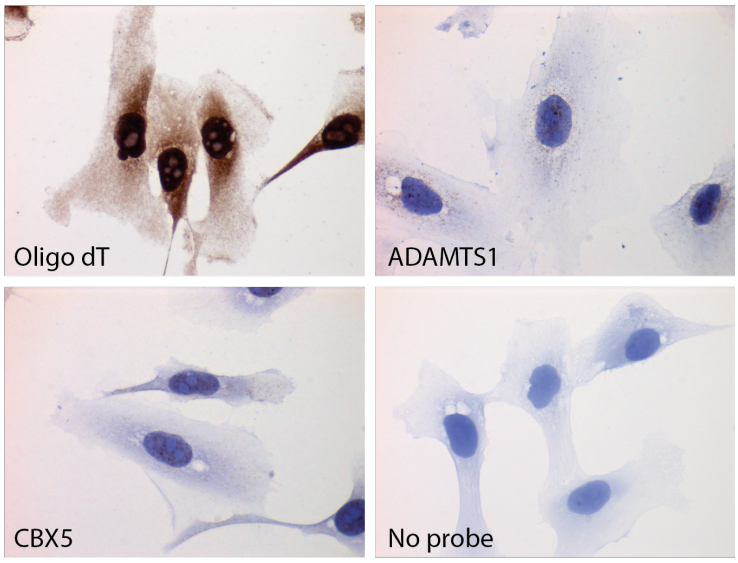

Figure 2. Colorimetric RNA-ISH for various targets in cultured human mesangial cells.

We tested detection of high and low abundance RNA species with distinct localization patterns in cultured conditionally immortalized human mesangial cells. Polyadenylated mRNA was readily detected using a SABER probe against oligo dT and shows a distinct exclusion from nucleoli. Gene-specific SABER probes targeting ADAMTS1 and CBX1 showed cytoplasmic and nucleoplasmic signal and occasional large nuclear spots corresponding to sites of active transcription. Omission of the SABER probe demonstrated no specific signal and low background.

Ongoing experiments and future directions: We are currently adapting SABER-ISH for detection of DNA target sequences. Future work will also incorporate oligo-conjugated antibodies to detect protein epitopes in cultured cells and tissue sections.

3. Publications:

Manuscript is in preparation. None currently published.