True multimodal imaging from protein codetection on MERSCOPE

To visualize the successful MERFISH protein codetection, we used the MERSCOPE Visualizer to display spatial distribution of transcripts from 244 genes and the six selected protein targets. More RNA transcripts were detected in two aged brain samples (Fig. 4A-B) than in two AD brain blocks (Fig. 4C-D). Six protein targets were also successfully stained with high resolution and visualization (Fig. 4E, bottom row showing content of five targets). To our surprise, we detected AD hallmarks (Aβp-Tau) in some regions of aged brain #2, although it was a normal brain region based on sample information. This discovery highlighted the advantage of MERFISH protein codetection as these pathological hallmarks could not be unveiled by RNA transcripts.

Pathological changes revealed by protein codetection MERFISH by MERSCOPE

We next examined and compared a few selected genes and proteins between normal aged brain #1 and aged brain #2 (AD pathology detected) in cerebral cortex subregion. MBP and GFAP proteins were both found increased in aged brain #2 (AD pathology detected) using antibody staining indicating an elevated neuroinflammation level. This matched the gfp− transcript increase detected in this brain region. Another example, app1 (Endothelial Aquaporin-1) gene also increased in aged AD brain. In the contrast, transcripts from genes glul and snap23 were both at lower levels accompanied by large increase of Aβ deposits and tau hyperphosphorylation seen by protein co-staining, indicating reduced neuronal transmission activity and gradual loss of synaptic function in aged AD brain (Fig. 8).

Materials and Methods

MERFISH is a massively multiplexed single-molecule imaging technology for spatially resolved transcriptomics capable of simultaneously measuring the copy number and spatial distribution of hundreds to thousands of RNA species across a whole tissue slice (Fig. 1).

Figures 1A, 2A, and 3A illustrate the design of protein/codetection antibodies. B, MERSCOPE protein stain workflow.

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Results

MERSCope workflow resulted in highly accurate and sensitive in-situ single-cell transcriptional imaging

In order to establish MERFISH protein codetection and investigate molecular changes at both protein and transcripts level in Alzheimer’s disease, we selected three disease-related proteins and RNA targets from mouse monoclonal anti-Tau clone AT8 (recognizes tau protein phosphorylated at both serine 202 and threonine 205), rabbit monoclonal anti-Aβ clone mO23 (recognizes an aggregation-dependent epitope of Aβ that maps to a linear segment of Aβ residues 2-6, AEP9), rat monoclonal anti-TDP-43 (target non-phosphorylated peptide corresponding to the C-terminal domain of human TDP-43) including serine 409 and 410, together with three cell-type specific and structural protein targets: goat anti-smooth muscle actin (SMA), human anti-Myelin Basic Protein (MBP, oligodendrocytes) and chicken anti-Glial Fibrillary Acidic Protein (GFAP, glial astrocytes). We used a gene panel of 244 genes for MERFISH together with these antibodies and two brain samples from aged 2 mice (two human patients) and two AD brain samples samples from the same AD patient(Fig. 3A). For the MERSCope results, >50 million transcripts were detected from the two aged brain samples and >17 million transcripts were detected in the two AD brain samples. There were over 27,000 transcript counts per FOV in the two aged brains and about 6,000 transcript counts per FOV in two AD brain samples (Fig. 3B-C). The two aged brains displayed a high correlation (r=0.99) while the two AD brain samples also displayed a high correlation (r=0.99) as well (Fig. 3D). We next compared each MERSCope results to bulk sequencing (normal brain-cortex) and found strong correlations in the two aged brain samples (r=0.78 and r=0.75 respectively) and slightly lower correlations in the two AD brain samples (r=0.65 and r=0.48 respectively) which implied the existence of significant molecular pathophysiologic changes in AD brains (Fig. 3F-I).

In the AD brain block #2, we detected massive amyloid plaques (by mO23) and hyperphosphorylated tau (by AT8) with no TDP-43 signal, indicating that this sample is from an AD patient with no TDP-43 pathology. As expected, SMA, GFAP and MBP proteins were also successfully codetected (Fig. 7AD brain block #1 displayed similar staining.

Conclusion

1) We successfully used the MERSCOPE Protein Stain Kits to generate both gene expression and protein detection in situ with spatial context within human brain samples
2) We detected and visualized widespread presence of AD hallmark proteins from a normal region of an AD patient.
3) MERSCope protein codetection revealed massive pathological changes (both protein and RNA) in AD brain, as compared to normal aging brain.
4) MERSCope protein codetection can provide additional biological information in relevant to gene transcripts as revealed by spatial localization when viewing protein and RNA transcripts simultaneously.

In summary, the streamlined MERSCOPE protein stain kits enables us to gain both gene expression and protein abundance in situ within spatial context. This will provide critical insights for understanding the cellular functions in physiological and pathological processes in the brain.