METHOD OF *IN SITU* CELL CHARACTERISATION

CROSS-REFERENCES

[0001] This application claims priority to Singapore patent application 10202260245V, filed on 29 November 2022, which is expressly incorporated herein by reference in its entirety, with particular reference to the figures, legends, and claims therein.

FIELD OF THE INVENTION

[0002] The present invention relates generally to the field of molecular and cell biology. In particular, the present invention relates to methods of cell characterisation.

BACKGROUND

[0003] High-dimensional, spatially resolved analysis of intact biological tissue samples promises to transform biomedical research and diagnostics. Recent advancements in single-cell RNA-sequencing (scRNA-seq) make it possible to unbiasedly define cell types reflecting ontogeny, functions, or anatomical locations. However, high-throughput mapping of these cells within intact biological systems is still a technical challenge. Existing methods such as spatial indexing combined with next-generation sequencing has enabled spatial mapping of sequencing reads and *in situ* reconstructions of cell types. However, sequencing-based spatial transcriptomics methods are limited by RNA diffusion and capture efficiency. Alternatively, cell types can also be characterised via imaging-based spatial transcriptomics methods, by targeting RNAs with multiplexed single-molecule Fluorescence *In situ* Hybridisation (FISH) or *in situ* sequencing. Such methods are highly quantitative and scalable to the whole transcriptome (~10,000 genes), but suffer from disadvantages including high non-specific background noises, limitation by molecular crowding, and the requirement of high-resolution microscopes. The imaging-based spatial transcriptomics methods also become increasingly laborious with larger number of targets. Another approach for spatial mapping of cells is multiplexed immunostaining or spatial proteomics. While the increased copy number of proteins compared to RNAs may lead to an increase in detection robustness, antibody panels are more costly, less flexible, with poor scalability.

[0004] Therefore, what is needed is a technology that enables easy, efficient and a scalable method for spatial characterisation of cells within the context of normal tissue physiology or disease microenvironment. Furthermore, other desirable features and characteristics will become apparent from the subsequent detailed description and the appended claims, taken in conjunction with the accompanying drawings referred to herein.

SUMMARY

[0005] In one aspect, the present disclosure refers to a method of characterizing cells in a biological sample in situ, comprising: a. contacting the biological sample with a plurality of probes that bind to

ribonucleic acid (RNA) transcripts of a plurality of pre-determined genes, wherein each probe comprises i) a detectable label, and ii) a domain that binds specifically to a ribonucleic acid transcript of one of the pre-determined genes; wherein a signal is emitted when the probe binds to the ribonucleic acid transcript; b. detecting a combination or plurality of emitted signals from the plurality of probes; and c. characterizing the cells based on the combination or plurality of emitted signals.

[0006] In another aspect, the present disclosure refers to a method to determine the prognosis of a subject suffering from cancer, comprising: a. obtaining a sample of the subject; b. characterizing one or more cancer cells in the sample using the method of any one of claims 1 to 13 to determine the stage of the cancer; and c. determining the prognosis based on the stage of the cancer.

[0007] In another aspect, the present disclosure refers to a kit for characterising cells in a biological sample in situ comprising: a plurality of probes that bind to ribonucleic acid (RNA) transcripts of a plurality of pre-determined genes; wherein each probe comprises i) a detectable label, and ii) a domain that binds specifically to a ribonucleic acid transcript of one of the pre-determined genes, and instructions for use.

[0008] In another aspect, the present disclosure refers to a kit for characterizing a colorectal cancer in a biological sample in situ comprising: a plurality of probes that bind to ribonucleic acid (RNA) transcripts of a plurality of pre-determined genes, wherein the plurality of pre-determined genes is selected from the genes listed in Table 6 (6a) – (6d); wherein each probe comprises: i) a detectable label, and ii) a domain that binds specifically to a ribonucleic acid transcript of the plurality of pre-determined genes, and instructions for use.

[0008a] In one aspect, provided herein is a method of characterizing cells in a biological sample in situ, comprising:

a. contacting the biological sample with a plurality of probes that bind to ribonucleic acid (RNA) transcripts of a plurality of pre-determined genes, wherein the plurality of pre-determined genes comprises at least one gene and at least one other gene that show coordinated changes in their expression levels, and

   wherein each probe comprises:

      i) an identical detectable label, and

      ii) a domain that binds specifically to a ribonucleic acid transcript of one of the pre-determined genes;

   wherein a signal is emitted when the probe binds to the ribonucleic acid transcript;

b. detecting a combination or plurality of emitted signals from the plurality of probes; and

c. characterizing the cells based on the combination or plurality of emitted signals.

[0008b] In another aspect, provided herein is a kit for characterising cells in a biological sample *in situ* comprising:

a plurality of probes that bind to ribonucleic acid (RNA) transcripts of a plurality of pre-determined genes; wherein the plurality of pre-determined genes comprises at least one gene and at least one other gene that show coordinated changes in their expression levels, and wherein each probe comprises

i) an identical detectable label, and

ii) a domain that binds specifically to a ribonucleic acid transcript of one of the pre-determined genes, and instructions for use.

[0008c] In another aspect, provided herein is a kit for characterizing a colorectal cancer in a biological sample *in situ* comprising:

a plurality of probes that bind to ribonucleic acid (RNA) transcripts of a plurality of pre-determined genes, wherein the plurality of pre-determined genes is selected from the genes listed in Table 6 (6a) – (6d);

wherein each probe comprises:

i) an identical detectable label, and

ii) a domain that binds specifically to a ribonucleic acid transcript of the plurality of pre-determined genes, and instructions for use.

BRIEF DESCRIPTION OF THE DRAWINGS

[0009] **Figure 1** provides a schematic overview of the *in situ* hybridisation (ISH) method as described herein for characterisation of cells. The method as described herein can be used for accurate mapping of cell types without disrupting the tissue architecture. As described herein, the method is a sensitive, robust, and scalable *in situ* hybridisation (ISH)-based spatial transcriptomics method that profiles single cells using multiple co-regulated genes. As used herein, co-regulated genes refers to genes that show coordinated changes in the gene expression level, i.e. covarying genes. As shown in **Figure 1A**, co-regulated genes are spatially co-localized in the same cells within a tissue, which allows designing of hybridisation probes to target a large set of genes for reliable detection of a cell population of interest. **Figure 1A** provides a cell-by-gene count matrix from single-cell RNA sequencing (scRNA-seq). The matrix is used to cluster cell types, which are characterized by their unique gene expression profiles (for example, genes A-D are grouped for one cluster of cells and genes E-I are grouped as a different cluster). **Figure 1B** provides a graphical illustration of the identification of groups of correlated genes from the reference scRNA-seq data. Genes that show coordinated changes in expression levels with each other are spatially co-localized in the same cells within a tissue. Based on the groups of correlated genes