RNAscope ISH

Advanced RNA-ISH for Detection of RNA in FFPE Tissues

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This application note describes major techniques to detect RNA biomarkers in FFPE tissues including RNAscope ISH, a revolutionary technology that has been shown to be successful in FFPE tissues as old as 25 years. Using spatial multiplexing with ISH-approaches in BioChain breast carcinoma FFPE tissue sections, medium to high spatial heterogeneity of HER2 gene expression was revealed in equivocally tested tumor tissues.

Introduction

Did you know cancer remains the second most common cause of death in the US? More than 1.8 million new cancer cases and 606,520 deaths are expected in the US, in 2020 alone. That is about 4,950 new cases and 1,600 deaths each day (American Cancer Society Inc.).

FFPE (Formalin-Fixed Paraffin-Embedded) is the most cost-effective long-term preservation technique for biopsy samples and majority of the biobanks store tumor tissues as FFPE tissues. With the advantages of long-term storage and durability these tissues can be used for cancer research, diagnostics and personalized medicine. These studies are made possible by detecting biological signals called biomarkers that include DNA, RNA and proteins. Biomarker profiles are often complex and unique for individual patients and cancer types.

Major Techniques for RNA Detection

Early quantitative methods such as Northern blotting, qRT-PCR, digital PCR, microarrays and high-throughput sequencing require RNA extraction from the sample. However, new developments allow for in situ detection and quantification. These techniques include in situ hybridization (ISH) based techniques such as FISH, FISH with barcoding, RNAscope; in situ sequencing and patterned barcoded microarrays.

These in situ techniques offer the advantage of analysis of cell subpopulations over extraction-based methods and in situ sequencing can also provide information on large number of transcripts. However, ISH based methods are preferred in clinical settings since it is a routine procedure.

RNA ISH

Basic workflow of RNA in situ hybridization includes the following steps:

1. Fix cells
2. Permeabilize
3. Hybridize target specific probe to target RNA
4. Signal amplification using pre-amplifiers, amplifiers and labeled (fluorescent/ chromogenic enzyme) probes
5. Signal detection

RNAscope ISH - a revolutionized ISH

RNAscope is a proven advanced ISH technology for detection of target RNA in intact cells, which aims at amplification of target-specific signals using proprietary probe design (Advanced Cell Diagnostics, Inc.). With over 2900 peer reviewed publications since 2011, it is becoming widely accepted by researchers especially in cancer research. The technology can be used for detection of single target, multiple (up to 12) targets as well as for RNA-protein co-detection. It allows spatial mapping of mRNA, splice variants, highly homologous sequences and point mutations in a wide variety of tissues including BioChain's FFPE tissues *(https://www.biochain.com/products/tissue/ffpe-tissue-section/)*.

What makes RNAscope a revolution?

1. **Unique** - It uses two independent **double Z probes** that need to hybridize to the target RNA sequence in tandem for the signal to be amplified.
2. **Specificity** - This ensures high specificity since the likelihood of two independent probes hybridizing to a non-specific target next to each other is little.
3. **Reduces background noise** since nonspecific hybridization is not amplified.
4. **Sensitive** - Only three double Z probes are enough to detect signal from a single RNA molecule.
5. **Robust** - 20 double Z probes provide **robustness against partial target RNA accessibility/ degradation**.
6. **FFPE Tissue samples as old as 25 years** have been successfully used for RNA detection using RNAscope technology (*https://acdbio.com/innovations-rnascope*).

BioChain offers a wide variety of FFPE tissues, arrays as well as sections, that can be combined with this revolutionary technology to assist you in your research (*https://www.biochain.com/products/tissue/*).

Spatially multiplexed RNA ISH

Voith von Voithenberg et al. (2020) have demonstrated use of **microfluidic probe delivery system** which allows for localized delivery on nanoliter scale and thus spatial multiplexing and detection of multiple RNA targets in FFPE tissues obtained from **BioChain**. This technique enables study of spatial heterogeneity of cancers and evaluation of target RNA along with internal control and complex cancer biomarker panels on a single tissue section.

Case study: Voith von Voithenberg et al., 2020

Here, the authors combined spatial multiplexing with ISH-approaches based on signal amplification, with bright field detection and with PPFE tissue sections obtained from BioChain. Using **Breast carcinoma tissue sections from BioChain** (*https://www.biochain.com/product/cancerseq-plus-paraffin-tissue-tumor-sections-breast-14/*), they analyzed the expression of HER2 with internal positive and negative controls (ActB, dapB) as well as predictive biomarker panels (ER, PgR, HER2) in a spatially multiplexed manner on single mammary carcinoma sections (Figure 1). They revealed medium to high spatial heterogeneity of HER2 gene expression in equivocally tested tumor tissues.

Case study: Baggio et al., 2018

Here, the authors studied Glucagonlike peptide-1 receptor (GLP-1R) expression in the human heart using GLP-1R–directed antisera, qPCR, RT-PCR to detect full-length mRNA transcripts, and ISH. Using **human RNA samples from BioChain** (*https://www.biochain.com/products/rna/total-rna-rna/?fwp_species=human*), they studied relative GLP1R mRNA expression in human tissues (Figure 2). To determine the localization of GLP1R expression in the human heart, the authors studied GLP1R expression in heart samples available from two different human tissue biobanks and assessed the sensitivity and specificity of two GLP-1R antisera known to recognize the human GLP-1R. The authors reported consistent detection of GLP1R mRNA transcripts in biopsy samples obtained from all four chambers from 15 different human hearts.

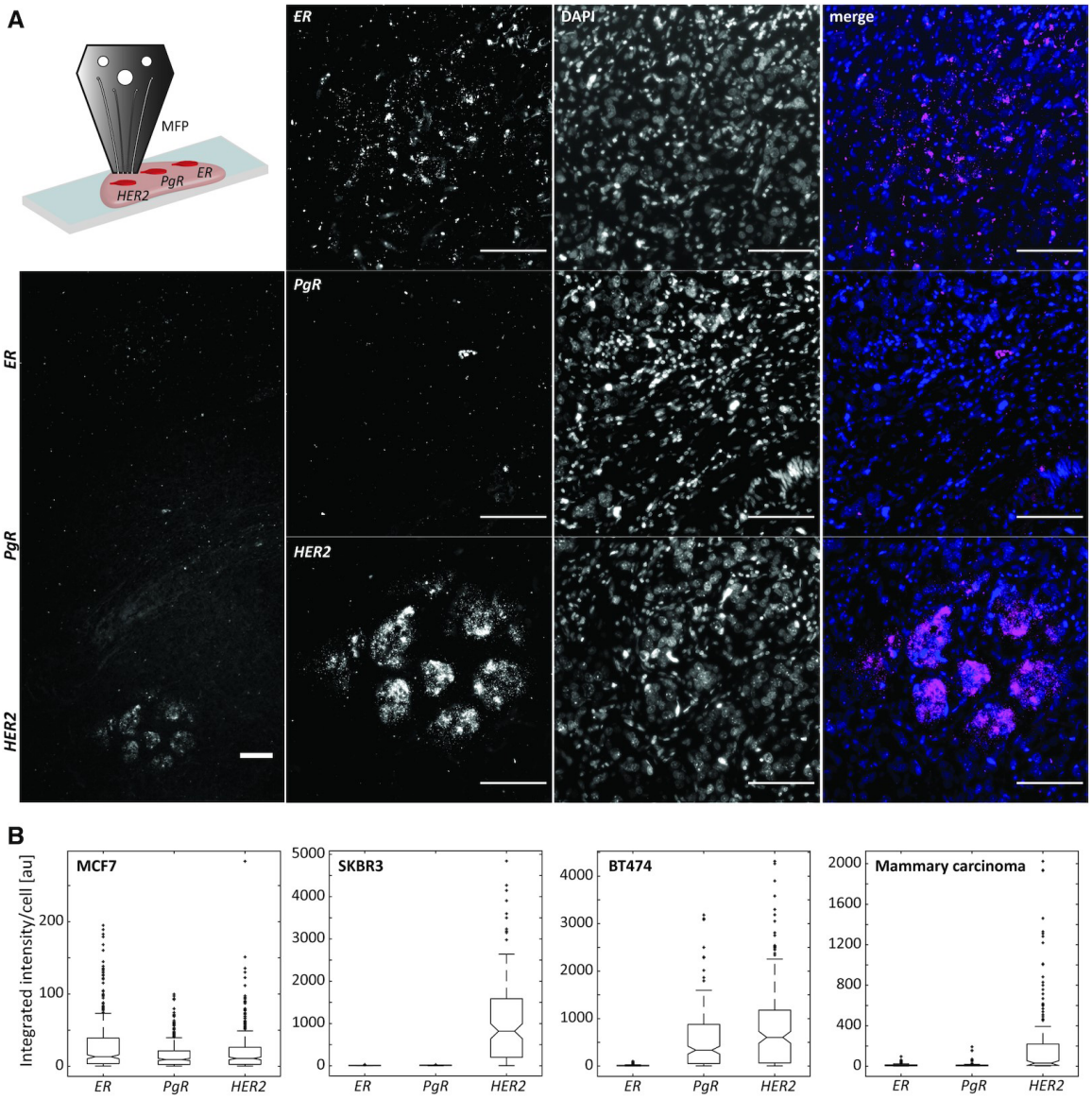

Figure 1. Single color multiplexed RNA-ISH for the detection of breast cancer biomarkers. (A) The primary probes for ER, PgR, and HER2 were delivered to spatially distinct regions of a mammary carcinoma section using a microfluidic chip (schematic in upper left corner). Fluorescence images of the signal detected for the breast cancer biomarkers ER, PgR and HER2 in a mammary carcinoma section. Scale bar: 100 µm. (B) Fluorescence intensity of FISH signals detected for ER, PgR and HER2 integrated per sectioned cell for FFPE sections of the breast cancer cell lines MCF7, SKBR3 and BT474 as well as the mammary carcinoma from (A). 500 ms excitation.

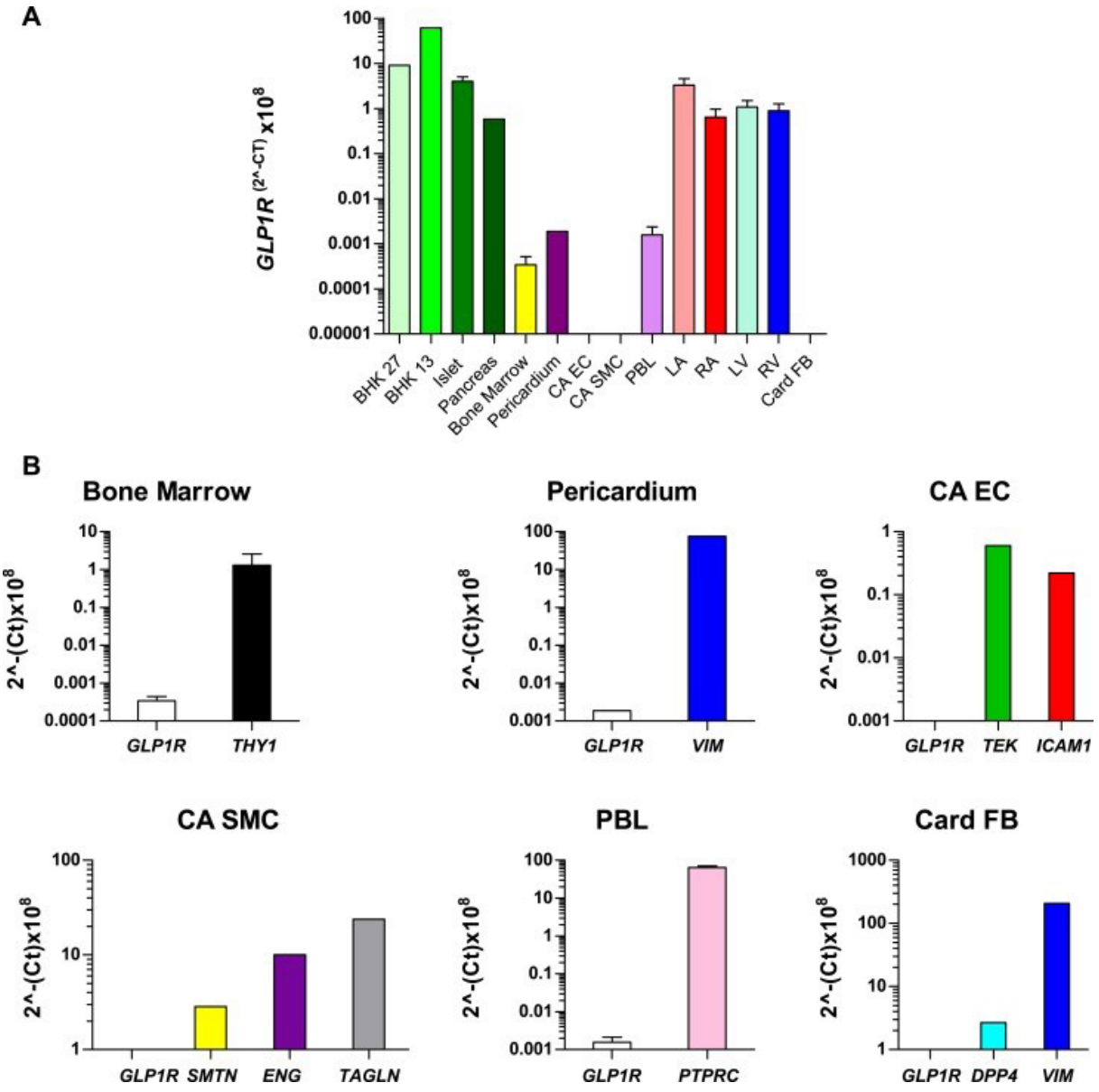

Figure 2. GLP1R mRNA transcript levels in the human heart are comparable to those in human pancreas and islets. (A) GLP1R mRNA levels were measured via qPCR analysis in multiple human tissues and in transfected BHK cells that express low levels of the human GLP-1R. For data represented without standard error bars, each single RNA sample was analyzed in duplicate; for isolates depicted with error bars, peripheral blood lymphocyte samples were analyzed in duplicate from two different sources, and at least three different samples were analyzed in duplicate by qPCR for RNAs from islet, bone marrow, left atria (LA), right atria (RA), left ventricle (LV), and right ventricle (RV). (B) qPCR analysis of GLP1R and tissue- or cell-type–specific gene expression in the indicated samples as confirmation of RNA/cDNA integrity.

BioChain offers diverse, convenient and published products for Biomarker research

BioChain offers diverse products and custom services to facilitate clinical and biopharmaceutical research including, but not limited to, tissues as well as proteins, DNA and RNA isolated from these tissues.

For instance, paraffin tissue sections are ideal for studying cellular localization of DNA, RNA and protein markers using immunohistochemistry and ISH (*https://www.biochain.com/products/tissue/ffpe-tissue-section/*).

Tissue microarrays (TMAs) comprising of dozens of tissue cores, ranging from normal to tumors of different stages, fixed on a single slide are time and resource efficient in histological analyses of multiple cancerous tissues (*https://www.biochain.com/products/tissue/ffpe-tissue-array/#more*).

RNA products and extraction kits are suitable for gene expression, microarray, transcriptomics, and RNA sequencing based studies (*https://www.biochain.com/products/rna/*).

References

American Cancer Society. Cancer Facts & Figures 2020. Atlanta: American Cancer Society; 2020.

Acdbio.com. 2020. FFPE | In Situ Hybridization, RNA-ISH | Acdbio. [online] Available at: <https://acdbio.com/innovation/ffpe> [Accessed 24 September 2020].

Baggio, L. L., Yusta, B., Mulvihill, E. E., Cao, X., Streutker, C. J., Butany, J., ... & Drucker, D. J. (2018). GLP-1 receptor expression within the human heart. Endocrinology, 159(4), 1570-1584.

Voith von Voithenberg, L., Fomitcheva Khartchenko, A., Huber, D., Schraml, P., & Kaigala, G. V. (2020). Spatially multiplexed RNA in situ hybridization to reveal tumor heterogeneity. Nucleic acids research, 48(3), e17-e17.

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