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Simultaneous Detection of Different RNAs Using a Novel Branched DNA *in situ* Hybridization Method

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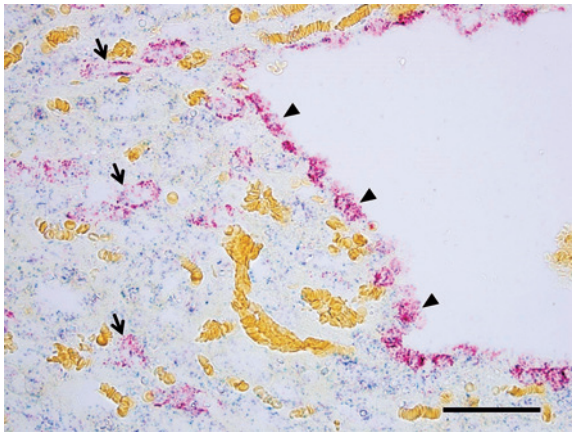

Fig. 1

In situ hybridization (ISH) is an effective method used to ascertain the histological localization of DNA and RNA targets[1]. The QuantiGene ViewRNA ISH Tissue 2-Plex Assay (Affymetrix, Santa Clara, CA), which is based on branched DNA signal amplification techniques, is highly sensitive and can simultaneously detect two target messenger RNAs (mRNAs) *in situ* within formalin-fixed, paraffin-embedded tissue sections[2,3]. Using this ISH method, two different mRNAs, including ubiquitin C and secreted phosphoprotein 1 mRNAs were visualized in the same tissue sections of the rat kidney (**Fig. 1**, red, secreted phosphoprotein 1 mRNA; blue, ubiquitin C (UBC) mRNA). Non-coding RNA (ncRNA) is a functional RNA molecule that does not translate into proteins, and ISH is the only method available for visualizing the expression of this molecule in tissue specimens. Long ncRNAs (lncRNAs) have more than 200 base pairs that regulate the transcription of mRNAs via attachment to mRNAs or chromosomes. H19 is a member of lncRNAs and is highly expressed in fetus and various cancers. In human pancreatic cancer tissues, H19 was detected in the cytoplasm of cancer cells using branched DNA ISH (**Fig. 2A**, each signal indicates one H19 RNA copy). Double staining of H19 ncRNA (**Fig. 2B**, red) and UBC mRNA (blue) revealed that UBC mRNA is expressed in all cell types; however, expression of H19 ncRNA is restricted to pancreatic cancer cells. In conclusion, the ISH method can be effectively used for simultaneous detection of two RNAs, including mRNA and lncRNA.

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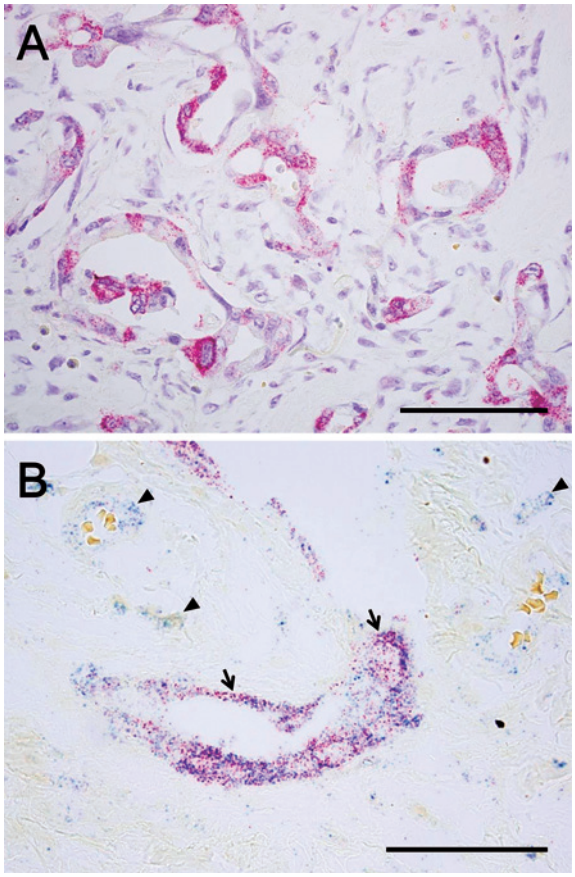

Fig. 2

Fig. 1 Image of branched DNA *in situ* hybridization for two target messenger RNAs. Ubiquitin C mRNA was expressed in the cytoplasm of all cell types in rat kidney tissues, while secreted phosphoprotein 1 mRNA expression was restricted to distal renal tubular epithelium **(arrows)** and transitional epithelium **(arrowheads)** of the renal pelvis. Blue signal, ubiquitin C mRNA; red signal, secreted phosphoprotein 1 mRNA; yellow color, red blood cells. Scale bar, 50 μm.

Fig. 2 Long non-coding RNA H19 expression in human pancreatic cancer cells. H19 ncRNA was expressed in pancreatic cancer cells forming tubular structures, but was not expressed in the adjacent stromal cells (A: counter stain, hematoxylin). In double staining target H19 ncRNA and ubiquitin C mRNA, H19 was expressed in cancer cells **(arrows)**; however, ubiquitin C was expressed in both cancer as well as stromal cells **(arrowheads)** (B). Red signal, H19 non-coding RNA; blue signal, ubiquitin C mRNA. Scale bar, 50 μm.

References

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