Advanced Cell Diagnostics

Characterizing LGR5 stem cells in colorectal adenomas and carcinomas

Overcoming the limitations of antibody-based detection methods

Ann-Marie Baker, PhD

Center for Tumor Biology, Barts Cancer Institute, Barts and the London School of Medicine and Dentistry, Queen Mary University of London

Address for Correspondence: John Vane Science Centre, Charterhouse Square, London EC1M 6BQ, United Kingdom

email: a.m.c.baker@qmul.ac.uk

"Using the RNAscope assay probe against LGR5 mRNA has allowed us to study the localization of LGR5 expression without relying on an antibody"

Having a specific and reliable antibody targeted to a particular protein of interest is key when using antibody-based detection methods. However, if the reliability of an antibody is subject to doubt then so too are experimental results and conclusions. Dr Ann-Marie Baker from the Center for Tumor Biology, Barts Cancer Institute, spoke to ACD about her research into the role of the Leucine-rich repeat-containing G-protein coupled receptor 5 (LGR5) protein in stem cells of colorectal adenomas and carcinomas. Dr Baker employed *in situ* hybridization with RNAscope® technology to overcome the reliability issues associated with anti-LGR5 antibodies.

Can you tell us about the main focus of your research?

I am interested in studying stem cell dynamics in healthy and tumor tissue, particularly in colorectal cancer. The receptor LGR5 is accepted as one of the best putative stem cell markers currently known, and it has been heavily studied in mouse models. However, relatively little is known about its expression patterns in human tissues.
It has been suggested that LGR5-positive stem-like cells are responsible for tumor recurrence and drug resistance, and they therefore represent an important therapeutic target. One of the challenges in this field has been the lack of a specific and reliable antibody against the LGR5 protein, so it has been necessary to utilize alternative methods of detection. Using the RNAscope assay probe against *LGR5* mRNA has allowed us to study the localization of *LGR5* expression without relying on an antibody.

What discoveries have you made using RNAscope?

We have used RNAscope assay probes to publish the first comparison of the *LGR5* stem cell architecture between the two pathways of colorectal carcinogenesis[1]: the conventional pathway and the serrated pathway in which serrated polyps replace the conventional adenoma as the precursor lesion to colorectal cancer. Interestingly we found that serrated adenomas retain a stem cell niche, albeit with an expansion of stem cell number, whereas conventional adenomas widely express *LGR5* but with no evidence of a stem cell niche. Furthermore, we have shown that established carcinomas of all stages generally revert to a compartmentalization of the stem cell population.

Your discoveries have relied on analyzing spatial localization. How does the need for this information influence which techniques you use?

It is crucial that we understand the spatial organization of solid tumors as it can give insight into how the tumor has grown and evolved. However, when we digest tissue and prepare the DNA for a method such as real time PCR we lose this valuable spatial information. In effect we see an 'average' of what is going on in the tumor, and we lose the information on the cellular composition and degree of heterogeneity that may be present.

1 - **Characterization of LGR5 stem cells in colorectal adenomas and carcinomas.** Baker A.M., et al. (2015). Sci. Rep.; 5:8654.

"I would certainly recommend RNAscope technology to researcheds wanting to examine a target of interest for which there is not a reliable antibody available"

The RNAscope assay has enabled us to demonstrate that like murine adenomas, human adenomas of the serrated pathway certainly retain a cellular hierarchy resembling that of a normal crypt, with the *LGR5*-expressing stem cell compartment at the base. In contrast, we see that conventional adenomas usually show very little evidence of cellular hierarchy, and that *LGR5*-positive cells are widespread throughout the glands.

LGR5 is reported to positively regulate invasion and metastasis of gastric cancer. Do your experiments in colorectal cancer come to the same conclusion?

I used the RNAscope *in situ* hybridization protocol to detect and quantify *LGR5* mRNA in colorectal cancers, and found that invasive areas had very high levels of *LGR5* mRNA. This suggests that it is able to support an invasive phenotype. Further experiments would be necessary to determine the mechanism by which *LGR5* may be supporting invasion in colorectal cancer.

Has lack of quality antibodies caused any problems in past research?

Immunohistochemistry is of course heavily reliant on the antibody being specific for the target of interest. The specificity of antibodies against LGR5 has been the subject of some controversy, therefore the results of such experiments must be interpreted with caution.

What does RNAscope ISH provide you, and what features influenced your decision to choose this over other methods?

RNAscope has provided me with a unique opportunity to examine the expression of *LGR5* mRNA in a highly sensitive and specific manner. It also has the advantage of using non-radioactive probes, and a one-day workflow. Furthermore, the ACD technical support that I received has been excellent.

Have you struggled with detecting degraded RNA in FFPE samples with alternative methods before? Have you made use of the ability of RNAscope to detect degraded RNA?

In particular, when performing longitudinal analyses we are heavily dependent on archival FFPE samples, which are variable in quality and can be highly degraded. I have found the RNAscope protocol to be generally effective on many of these archival samples, and therefore we can gain valuable insight into gene expression in these poor quality samples.

What do you see for the future of using RNAscope technology in your research?

I will continue to use RNAscope probes in my research, and look forward to examining other targets of interest in archival FFPE samples. In fact, I would certainly recommend RNAscope technology to researchers wanting to examine a target of interest for which there is not a reliable antibody available.

To read additional Researcher in the Spotlight articles, visit acdbio.com/researcherspotlight

Advanced Cell Diagnostics

www.acdbio.com
California, USA