Recent Study Published in Nature Unravels a Novel Pathway for Stem Cell Self-Renewal

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– 10x Genomics® Single-Cell 3' Solution used to discover a unique self-renewal and priming mechanism of intestinal stem cells –

PLEASANTON, Calif. – May 3, 2017 – 10x Genomics, a company focused on enabling the mastery of biology by accelerating genomic discovery, today announced publication of an article in the journal *Nature* of a collaborative research study with researchers at the Stanford University School of Medicine. The article entitled, "Non-equivalence of Wnt and R-spondin ligands during Lgr5+ intestinal stem-cell self-renewal," utilizes the 10x Genomics' Single Cell 3' Solution for single-cell RNA-seq (scRNA-seq) to unravel the priming and self-renewal mechanisms of intestinal stem cells (ISCs).

The renewal and differentiation of Lgr5+ ISCs is critical to the continuous regeneration of the epithelial lining of the gut, which enables us to absorb nutrients and provides a barrier to protect us from the external environment. Disruptions in this process can lead to or worsen human intestinal disorders such as inflammatory bowel disease (IBD), gastrointestinal cancer and Celiac disease.

This carefully regulated process occurs within a stem-cell niche called the intestinal crypt, and depends on Wnt signaling, which can be turned up by Wnt and R-spondin (RSPO) ligands. The authors sought to identify the unique functional roles of Wnt and RSPO ligands for regulating Lgr5+ ISCs and the relative contributions of both ligands to *in vivo* Wnt signaling and stem-cell biology.

The authors were able to show using *in vivo* experiments that Wnt and RSPO are not redundant signals. RSPO was shown to expand stem cell number. Although Wnt was needed to maintain Lgr5+ ISCs in the presence of RSPO, Wnt was not sufficient to induce additional numbers of Lgr5+ ISCs above a certain threshold, demonstrating that RSPO, and not Wnt, establishes the set point for Lgr5+ ISC number. The authors performed single-cell RNA-seq to definitively show that the signaling contributions of Wnt and RSPO elicited distinct effects on ISCs, by fully characterizing the expression profile for each unique cellular subtype on a cell-by-cell basis upon perturbation of those signals in vivo.

By characterizing gene expression from 13,102 single cells, Yan and colleagues were able to show that Lgr5- control cells represented differentiated cell types of the small intestinal lineages, including Paneth, goblet, enteroendocrine, enterocyte, pre-enterocyte, and tuft cells. The Lgr5+ cells consisted of three cellular sub-populations, corresponding to cycling stem cells, non-cycling stem cells, and transit amplifying cells. The authors were able to further show that these three distinct sub-populations of Lgr5+ cells were each uniquely affected by perturbations in Wnt and RSPO signaling, conclusively demonstrating that Wnts are priming factors that enable stem cells to be competent by expressing RSPO receptors on their cell surface, whereas RSPOs are actual self-renewal factors that expand stem cell number.

"Single-cell analysis provided conclusive evidence for the unique roles of Wnt and RSPO signaling to their respective function, either co-operatively priming Lgr5+ cells for competency, or for RSPO-mediated self-renewal." said Grace Zheng, Ph.D., research scientist at 10x Genomics. "This powerfully illustrates the utility of single-cell RNA-seq to monitor discrete stem-cell states and their dynamic perturbation. To do this with any other technology would have been extremely cumbersome, if not impossible."

"We are very excited about this result, and it opens up the possibility that analogous multi-tiered regulation by priming and self-renewal factors may be a generalized property of stem cells across other organ systems, either through Wnt and RSPO or functionally equivalent stem-cell niche components," said Ben Hindson, Ph.D., president, co-founder, and chief scientific officer of 10x Genomics. "This could have wide reaching implications for stem-cell research and potentially yield new insight towards therapeutic applications in the future."

The lead author of the study is Kelley Yan, M.D. Ph.D., lead author of the study, formerly a postdoctoral fellow at Stanford and now an Assistant Professor of Medicine and of Genetics and Development in the Columbia Center for Human Development at Columbia University Medical Center. The senior author is Calvin Kuo, M.D. Ph.D., Professor of Medicine at Stanford.

The article entitled, "Non-equivalence of Wnt and R-spondin ligands during Lgr5+ intestinal stem-cell self-renewal," can be accessed from *Nature* online: http://dx.doi.org/10.1038/nature22313

For more information about this research, visit the Kuo Lab at Stanford:http://kuolab.stanford.edu/index.html

About 10x Genomics

10x Genomics is changing the definition of sequencing by providing an innovative genomics platform that dramatically upgrades the capabilities of existing sequencing technologies. This is achieved through a combination of new microfluidic science, chemistry and bioinformatics. By implementing GemCode Technology within the Chromium System, researchers can now, for the first time, find new structural variants, haplotypes and other valuable genomic information with comprehensive workflows for Single Cell, Genome, Exome and *de novo* Assembly applications that incorporate their pre-existing sequencing technologies.

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