likely in the later stages of iN reprogramming. In this study, Myt1l marked a minimal number of binding sites despite much optimization effort in ChIP, while its addition to the reprogramming cocktail significantly increased reprogramming efficiency and formation of mature neurons. The low iN conversion rate (~2%–8% depending on cell type) may obscure some interactions among Ascl1 and the supportive transcription factors. Furthermore, it will be interesting to determine how global transcription and epigenetic changes occur in instances when Ascl1 is excluded from the reprogramming cocktail.

Another stimulating question from this study is the existence of trivalent chromatin states or similar unknown chromatin states that enable accessibility to pioneer factors in other transdifferentiation contexts i.e., fibroblasts to hepatocytes and cardiomyocytes, or other known and yet undiscovered conversions (Ladewig et al., 2013). Addressing the binding particulars of how Ascl1 recognizes the trivalent state could be used to predict a more widespread modus operandi of "on target" factors.

Taken as a whole, this study's accomplishments are 2-fold. First, by delving into the mechanism of iN reprogramming, this study has provided more support for the soundness of using the direct conversion method, because binding patterns of essential transcription factors resemble those found naturally, i.e., NPCs. Second, a trivalent chromatin state is uncovered that further underscores the importance of a more complex combinatorial histone code, just like the discovery of bivalent promoters in embryonic stem cells did previously (Bernstein et al., 2006).

REFERENCES

Bernstein, B.E., Mikkelsen, T.S., Xie, X., Kamal, M., Huebert, D.J., Cuff, J., Fry, B., Meissner, A., Wernig, M., Plath, K., et al. (2006). Cell *125*, 315–326.

Davis, R.L., Weintraub, H., and Lassar, A.B. (1987). Cell *51*, 987–1000.

Graf, T. (2011). Cell Stem Cell *9*, 504–516.

Ladewig, J., Koch, P., and Brustle, O. (2013). Nat. Rev. Mol. Cell Biol. *14*, 225–236.

Takahashi, K., and Yamanaka, S. (2006). Cell *126*, 663–676.

Vierbuchen, T., Ostermeier, A., Pang, Z.P., Kokubu, Y., Südhof, T.C., and Wernig, M. (2010). Nature *463*, 1035–1041.

Wapinski, Q.L., Vierbuchen, T., Qu, K., Lee, Y.Q., Chanda, S., Fuentes, D.R., Giresi, P.G., Ng, Y.H., Marro, S., Neff, N.F., et al. (2013). Cell *155*, 621–635.

Xie, H., Ye, M., Feng, R., and Graf, T. (2004). Cell *117*, 663–676.

Yang, N., Ng, Y.H., Pang, Z.P., Südhof, T.C., and Wernig, M. (2011). Cell Stem Cell *9*, 517–525.

Zaret, K.S., and Carroll, J.S. (2011). Genes Dev. *25*, 2227–2241.

Responding to R-Spondin: Slit2 Potentiates Intestinal Regeneration

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Gastrointestinal toxicity of chemoradiotherapy treatment in late-stage cancer patients limits tolerable doses and effectiveness of treatments. Zhou and colleagues in *Nature* (Zhou et al., 2013) suggest that activating Robo/Slit signaling in concert with R-Spondin potentiates Wnt signaling in intestinal stem cells and drives successful intestinal regeneration and recovery.

Over the past 5 years, there has been a dramatic increase in our understanding of intestinal biology, including intestinal stem cells (ISCs), homeostasis, and transformation (reviewed in Barker et al., 2012). Culturing intestinal epithelium as organoids in vitro is now common practice for both the small intestine and colon (reviewed in Barker et al., 2012). This wealth of information suggests that there will be an opportunity to translate this knowledge into therapeutic strategies for regenerative medicine. One key area where our increased understanding should yield important insights is intestinal injury following chemoradiotherapy. Over recent years it has been shown that Wnt signaling is activated during intestinal regeneration (Ashton et al., 2010) and that R-Spondin, a Wnt agonist, can increase proliferation of the intestine and improve recovery after chemoradiotherapy (Kim et al., 2005). Despite this, R-Spondin alone does not appear sufficiently potent to work as a single agent to provide an improved clinical outcome.

To address this, Zhou and colleagues investigated pathways that may be active within ISCs that could potentiate R-Spondin action and discovered the Slit/Robo pathway as a potential collaborator (Figure 1). They first used fluorescent

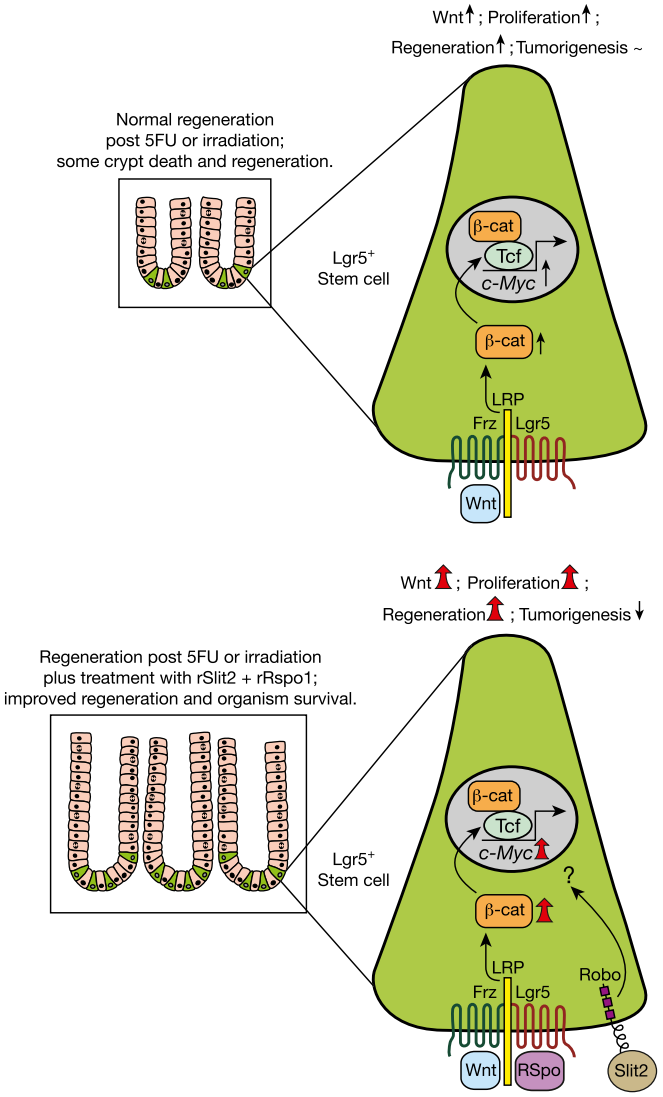

Figure 1. Treatment with rSpon1 and rSlit2 Augments Intestinal Regeneration
Following treatment with 5-FU or irradiation, intestinal crypts are ablated, and large, proliferating crypts appear as the intestine attempts to regenerate. The Wnt signaling pathway is activated during regeneration and upregulates the transcription factor c-Myc, which is required for efficient proliferation. Treatment with rSpon1 and rSlit2 following 5-FU or irradiation acts to boost Wnt signaling in Lgr5$^+$ intestinal stem cells, providing a pulse of proliferation that results in enhanced regeneration, increased organism survival, and reduced tumor formation.

in situ hybridization (FISH) to identify that Slit2 and its receptor Robo1 were expressed in the Lgr5$^+$ stem cells. To determine the functional requirement for Robo/Slit, Zhou et al. examined the intestinal phenotype of *Robo1*$^{+/-}$*; Robo2*$^{+/-}$ mice and found that these mice had truncated villi associated with reduced crypt proliferation and fewer Lgr5$^+$ ISCs and were unable to grow as organoids in vitro. Moreover, treatment with a Robo-blocking antibody could also inhibit proliferation, reduce the number of ISC-marker-expressing cells, and stop organoid growth. Activation of Robo/Slit signaling via the generation of a mouse overexpressing human *Slit2* (Slit2-Tg) in the intestine caused villi hypertrophy associated with an increase in proliferation, an increased number of Lgr5$^+$ cells (and other ISC-marker-positive cells), and larger organoids in vitro.

Given this striking functional role of Slit/Robo signaling, Zhou and colleagues next investigated the interrelationship between Slit/Robo and R-Spondin. Surprisingly, they demonstrated that rSlit2 could not only substitute for rSpon1 but functionally cooperated with rSpon1 to promote organoid growth. In vivo, the number of intestinal Lgr5$^+$ cells was significantly increased when mice were injected with rSpon1 and rSlit2 together, while injection of a Robo1-blocking antibody reduced the number of Lgr5$^+$ cells. Given that Lgr5 and many of the other ISC markers are Wnt target genes, these data suggest that R-Spondin and Slit2 can cooperate to increase intestinal stem cell function via upregulation of Wnt signaling. However, it is worth noting that Slit2 has previously been shown to inhibit nuclear accumulation of β-catenin (Tseng et al., 2010) and therefore a very important future study is to determine how R-Spondin and Robo-Slit signaling cooperate mechanistically to increase intestinal stem cells.

Finally, Zhou et al. demonstrated the clinical application of their findings by attempting to reduce the massive impact that chemoradiotherapy has on the intestinal epithelium by promoting the regeneration process. First, wild-type mice were exposed to 5-FU or a lethal dose of irradiation and then treated with a 3 day pulse of rSpon1 and rSlit2. In both experiments the treatment with rSpon1 and rSlit2 significantly increased intestinal regeneration, was associated with an increase in Lgr5$^+$ cells and proliferating cells in the crypts, and extended life span.

A major concern with upregulating Wnt signaling for regenerative medicine is the possibility of promoting tumorigenesis. Deregulation of Wnt signaling has been shown to both initiate cancer and drive progression. The role of Wnt signaling for tumorigenesis is particularly evident in CRC, where 80% of tumors carry mutations in the Wnt signaling pathway. These mutations are predominantly in Apc, a cytoplasmic protein that targets the cotranscription factor β-catenin for destruction and thus restrains the Wnt pathway. A direct concern for increasing Wnt signaling is that recent studies have shown that other pathways such as RAC and NF-KB, which increased the expression of the Wnt-driven ISC signature, can also expand the cell of origin of colorectal cancer through increased dedifferentiation (Myant et al., 2013;

Schwitalla et al., 2013). To address this Zhou and colleagues administered a 3 day pulse of rSpon1 and rSlit2 to $Apc^{Min/+}$ mice treated with DSS to accelerate intestinal tumor formation and exposed to the chemotherapy agent 5-FU. Remarkably, not only was life span significantly increased, but this treatment was associated with a marked decrease in the number of intestinal tumors. In this experiment, the 5-FU only group died before the end of therapy, and thus it's hard to know whether the reduction of tumorigenesis in the rSpon1 and rSlit2 mice was comparable with what would have been observed with 5-FU alone. However, it is tempting to speculate that these results might support the "just right" model for Wnt signaling during tumor formation. This model suggests that there is an optimal window of Wnt signaling required for tumorigenesis and that very high Wnt signaling, as induced here by rSpon1 and rSlit2, is actually cytotoxic and kills transformed cells (Albuquerque et al., 2002; Méniel et al., 2013). Further work is required to determine the effect of this combination on cancer cells and normal cells; many other epithelial cells, e.g., skin, express both Lgr4–6 receptors and thus could be activated if they also express the ROBO receptor. Moreover, recent studies have shown the SLIT/ROBO pathway as a key new axis identified by the TGCA sequencing consortia (Biankin et al., 2012); thus, the impact of these mutations would be important to ascertain if patients undergoing chemoradiotherapy were to be cotreated with rSpon1 and rSlit2.

In summary, this work holds exciting translational promise to alleviate the severe side effects of chemoradiotherapy. Moreover, the key concern of a promotion of tumorigenesis was not found in this preliminary study, and there is even a possibility that there might be a beneficial effect by overactivating the Wnt signaling pathway in tumor cells during chemotherapy. Finally, given the fact that Lgr5 has recently been found to be expressed on many regenerating epithelial cells, e.g., liver, it is possible that if the Slit/Robo pathway is also active in these cells, there could be broad implications for the use of rSpon1/rSlit2 treatment for regenerative medicine.

REFERENCES

Albuquerque, C., Breukel, C., van der Luijt, R., Fidalgo, P., Lage, P., Slors, F.J., Leitão, C.N., Fodde, R., and Smits, R. (2002). Hum. Mol. Genet. *11*, 1549–1560.

Ashton, G.H., Morton, J.P., Myant, K., Phesse, T.J., Ridgway, R.A., Marsh, V., Wilkins, J.A., Athineos, D., Muncan, V., Kemp, R., et al. (2010). Dev. Cell *19*, 259–269.

Barker, N., van Oudenaarden, A., and Clevers, H. (2012). Cell Stem Cell *11*, 452–460.

Biankin, A.V., Waddell, N., Kassahn, K.S., Gingras, M.C., Muthuswamy, L.B., Johns, A.L., Miller, D.K., Wilson, P.J., Patch, A.M., Wu, J., et al.; Australian Pancreatic Cancer Genome Initiative. (2012). Nature *491*, 399–405.

Kim, K.A., Kakitani, M., Zhao, J., Oshima, T., Tang, T., Binnerts, M., Liu, Y., Boyle, B., Park, E., Emtage, P., et al. (2005). Science *309*, 1256–1259.

Méniel, V., Song, F., Phesse, T., Young, M., Poetz, O., Parry, L., Jenkins, J.R., Williams, G.T., Dunwoodie, S.L., Watson, A., and Clarke, A.R. (2013). PLoS Genet. *9*, e1003638.

Myant, K.B., Cammareri, P., McGhee, E.J., Ridgway, R.A., Huels, D.J., Cordero, J.B., Schwitalla, S., Kalna, G., Ogg, E.L., Athineos, D., et al. (2013). Cell Stem Cell *12*, 761–773.

Schwitalla, S., Fingerle, A.A., Cammareri, P., Nebelsiek, T., Göktuna, S.I., Ziegler, P.K., Canli, O., Heijmans, J., Huels, D.J., Moreaux, G., et al. (2013). Cell *152*, 25–38.

Tseng, R.C., Lee, S.H., Hsu, H.S., Chen, B.H., Tsai, W.C., Tzao, C., and Wang, Y.C. (2010). Cancer Res. *70*, 543–551.

Zhou, W.J., Geng, Z.H., Spence, J.R., and Geng, J.G. (2013). Nature *501*, 107–111.

A New Image of the Hematopoietic Stem Cell Vascular Niche

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The microenvironment within the bone marrow that maintains hematopoietic stem cell (HSC) quiescence is the subject of intense study. In a recent *Nature* paper, Kunisaki et al. combine imaging techniques and computational modeling to define a novel arteriolar niche for quiescent HSCs within the bone marrow.

The hematopoietic stem cell (HSC) niche is a spatially confined unit within the bone marrow cavity that uniquely regulates the choice between HSC quiescence and proliferation (Schofield, 1978). In such microenvironments, HSCs receive regulatory signals from neighboring or adjacent cells, the extracellular matrix, and soluble factors. During the past decade, the existence of at least two subcompartments of the HSC niche has been considered. The endosteal niche,