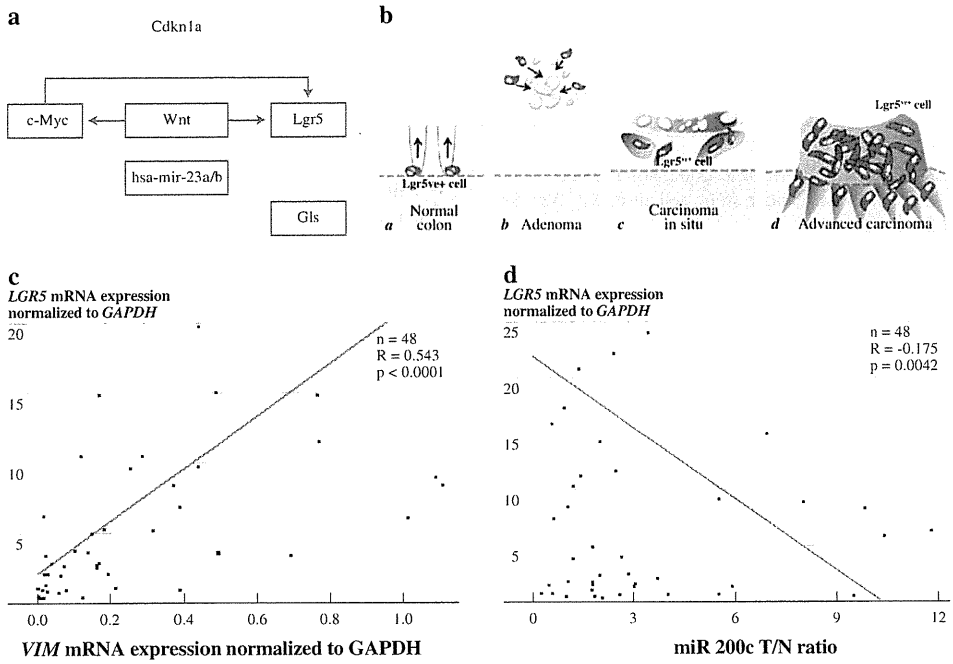

FIG. 5 The proposed schema of the significance of Lgr5^{+ve} CSCs in CRC.[17,18] **a** Schematic representation of the relationships between WNT, LGR5, cMYC, p21CIP1/WAF1/CDKN1A, GLS, and hsa-mir-23a/b family in clinical CRC samples. c-Myc stimulates LGR5 expression, which can be suppressed via the pathway of p21CIP1/WAF1/CDKN1A. cMYC also stimulates LGR5 and indirectly, which is mediated by the inhibition of hsa-mir-23 family; the hsa-mir-23a/b may target the expression of LGR5 and GLS. The stimulatory (*blue*) and suppressive (*red*) pathways are shown. **b** Schematic representation of Lgr5^{+ve} cells in normal and tumor lesions. Lgr5^{+ve} cells locate at basal crypt area of normal colonic mucosa, proliferate upwards, and drop out after terminal differentiation and apoptosis; the process contributes to the homeostasis of colonic epithelium (*a*). In benign adenomas, Lgr5^{+ve} cells are embedded in marginal region and proliferate towards inside. The location of LGR5^{+ve} cells may be resultant of the suppressed apoptosis (*b*). In carcinoma in situ, Lgr5^{+ve} cells lose their polarity and migrate to tumor–host interface (*c*). The alterations of p21CIP1/WAF1/CDKN1A and hsa-mir-23a/b pathways may contribute to the survival of Lgr5^{+ve} CSCs with an increase of glutaminolysis during progression to advanced carcinoma (*d*). **c** Overexpression of *LGR5* was associated with increased expression of the mesenchymal molecule *VIM* in 48 subjects with CRC ($p < 0.0001$, $R = 0.543$). Expression was evaluated by the ratio normalized to *GAPDH* expression. **d** Inverse correlation between *LGR5* and an epithelial inducer, miR-200c ($p < 0.0042$, $R = -0.175$)

would be supposed in cancerous lesions. Increased expression of *LGR5* and *c-MYC* was associated with the expression of the *p21CIP1/WAF1/CDKN1A* gene. An inverse correlation between *p21CIP1/WAF1/CDKN1A* expression and Myc has been reported by an in vitro study, and an in vivo study in mice showed that the oncogenic impact in the intestinal epithelium of direct Myc activation could differ from that of indirect Myc activation via the Wnt/β-catenin pathway.[13,40] The present clinical data reveal the possibility that activation of both *LGR5* and *c-MYC* might mediate transactivation of *p21CIP1/WAF1/CDKN1A* despite tumor proliferation in vivo. As for prognosis, high levels of *LGR5* expression were associated with poor prognosis, significantly so in subjects who underwent radical resection, suggesting that Lgr5^{+ve} cells in unresected lesions affect prognosis.

Through different biochemical and biophysical pathways characteristic of cancer cells, tumor cells adopt high glycolysis and decreased respiration in the presence of oxygen, which is known as the Warburg effect.[41] The ability of cells to grow during hypoxia results in part from activation of c-Myc.[41] Glutaminolysis by Gls catabolizes glutamine, which is transported into proliferating cells and is a major source of energy and nitrogen for biosynthesis as well as a carbon substrate for anabolic processes in cancer cells.[19] The present study also indicated that *GLS* which is directly controlled by c-Myc, also targets *LGR5*, indicating that c-Myc plays a role in the glutaminolysis of *Lgr5*$^{+ve}$ CSCs. *LGR5* high-expression tumors also have a mesenchymal character such as high expression of *Vimentin* and low expression of miR-200c, indicating that *Lgr5*$^{+ve}$ CSCs play a key role in migration and metastasis in cancer cells.

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REFERENCES

1. Markowitz SD, Bertagnolli MM. Molecular origins of cancer: molecular basis of colorectal cancer. *N Engl J Med.* 2009; 361:2449–60.
2. Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. *Nature.* 2001;414:105–11.
3. Dick JE. Looking ahead in cancer stem cell research. *Nat Biotechnol.* 2009;27:44–6.
4. Vermeulen L, Todaro M, de Sousa Mello F, et al. Single-cell cloning of colon cancer stem cells reveals a multi-lineage differentiation capacity. *Proc Natl Acad Sci USA.* 2008;105: 13427–32.
5. Dalerba P, Dylla SJ, Park IK, et al. Phenotypic characterization of human colorectal cancer stem cells. *Proc Natl Acad Sci USA.* 2007;104:10158–63.
6. Haraguchi N, Ohkuma M, Sakashita H, et al. CD133+CD44+ population efficiently enriches colon cancer initiating cells. *Ann Surg Oncol.* 2008;15:2927–33.
7. Yasuda H, Tanaka K, Saigusa S, et al. Elevated CD133, but not VEGF or EGFR, as a predictive marker of distant recurrence after preoperative chemoradiotherapy in rectal cancer. *Oncol Rep.* 2009;22:709–17.
8. Horst D, Scheel SK, Liebmann S, et al. The cancer stem cell marker CD133 has high prognostic impact but unknown functional relevance for the metastasis of human colon cancer. *J Pathol.* 2009;219:427–34.
9. Saigusa S, Tanaka K, Toiyama Y, et al. Correlation of CD133, OCT4, and SOX2 in rectal cancer and their association with distant recurrence after chemoradiotherapy. *Ann Surg Oncol.* 2009;16:3488–98.
10. Weichert W, Denkert C, Burkhardt M, et al. Cytoplasmic CD24 expression in colorectal cancer independently correlates with shortened patient survival. *Clin Cancer Res.* 2005;11:6574–81.
11. Ahmed MA, Al-Attar A, Kim J, et al. CD24 shows early upregulation and nuclear expression but is not a prognostic marker in colorectal cancer. *J Clin Pathol.* 2009;62:1117–22.
12. Choi D, Lee HW, Hur KY, et al. Cancer stem cell markers CD133 and CD24 correlate with invasiveness and differentiation in colorectal adenocarcinoma. *World J Gastroenterol.* 2009;15: 2258–64.
13. van de Wetering M, Sancho E, Verweij C, et al. The beta-catenin/ TCF-4 complex imposes a crypt progenitor phenotype on colorectal cancer cells. *Cell.* 2002;111:241–50.
14. Barker N, van Es JH, Kuipers J, et al. Identification of stem cells in small intestine and colon by marker gene Lgr5. *Nature.* 2007;449:1003–7.
15. Jaks V, Barker N, Kasper M, et al. Lgr5 marks cycling, yet long-lived, hair follicle stem cells. *Nat Genet.* 2008;40:1291–9.
16. Tuupanen S, Turunen M, Lehtonen R, et al. The common colorectal cancer predisposition SNP rs6983267 at chromosome 8q24 confers potential to enhanced Wnt signaling. *Nat Genet.* 2009;41: 885–90.
17. Haegebarth A, Clevers H. Wnt signaling, lgr5, and stem cells in the intestine and skin. *Am J Pathol.* 2009;174:715–21.
18. Brabletz T, Jung A, Spaderna S, Hlubek F, Kirchner T. Migrating cancer stem cells—an integrated concept of malignant tumour progression. *Nat Rev Cancer.* 2005;5:744–9.
19. Gao P, Tchernyshyov I, Chang TC, et al. c-Myc suppression of miR-23a/b enhances mitochondrial glutaminase expression and glutamine metabolism. *Nature.* 2009;458:762–5.
20. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell.* 2004;116:281–97.
21. Friedman RC, Farh KK, Burge CB, Bartel DP. Most mammalian mRNAs are conserved targets of microRNAs. *Genome Res.* 2009;19:92–105.
22. He L, Thomson JM, Hemann MT, et al. A microRNA polycistron as a potential human oncogene. *Nature.* 2005;435:828–33.
23. Mariadason JM, Bordonaro M, Aslam F, et al. Down-regulation of beta-catenin TCF signaling is linked to colonic epithelial cell differentiation. *Cancer Res.* 2001;61:3465–71.
24. Conacci-Sorrell M, Simcha I, Ben-Yedidia T, et al. Autoregulation of E-cadherin expression by cadherin-cadherin interactions: the roles of beta-catenin signaling, Slug, and MAPK. *J Cell Biol.* 2003;163:847–57.
25. Wellner U, Schubert J, Burk UC, et al. The EMT-activator ZEB1 promotes tumorigenicity by repressing stemness-inhibiting microRNAs. *Nat Cell Biol.* 2009;11:1487–95.
26. Furth J, Kahn MC. The transmission of leukemia of mice with a single cells. *Am J Cancer.* 1937;31:276–82.
27. Lapidot T, Sirard C, Vormoor J, et al. A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. *Nature.* 1994;367:645–8.
28. Bonnet D, Dick JE. Human acute leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nat Med.* 1997;3:730–37.
29. Wulf GG, Wang RY, Kuehnle I, et al. A leukemic stem cell with intrinsic drug efflux capacity in acute myeloid leukemia. *Blood.* 2001;98:1166–173.
30. Prince ME, Sivanandan R, Kaczorowski A, et al. Identification of a subpopulation of cells with cancer stem cell properties in head and neck squamous cell carcinoma. *Proc Natl Acad Sci USA.* 2007;104:973–8.
31. Haraguchi N, Utsunomiya T, Inoue H, et al. Characterization of a side population of cancer cells from human gastrointestinal system. *Stem Cells.* 2006;24:506–13.
32. Ricci-Vitiani L, Lombardi DG, Pilozzi E, et al. Identification and expansion of human colon-cancer-initiating cells. *Nature.* 2007; 445:111–5.
33. O'Brien CA, Pollett A, Gallinger S, Dick JE. A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. *Nature.* 2007;445:106–10.
34. Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. *Proc Natl Acad Sci USA.* 2003;100:3983–88.
35. Piccirillo SG, Reynolds BA, Zanetti N, et al. Bone morphogenetic proteins inhibit the tumorigenic potential of human brain tumour-initiating cells. *Nature.* 2006;444:761–5.
36. Bao S, Wu Q, McLendon RE, et al. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. *Nature.* 2006;444:756–60.
37. Tan BT, Park CY, Ailles LE, Weissman IL. The cancer stem cell hypothesis: a work in progress. *Lab Invest.* 2006;86:1203–7.
38. Hsu SY, Liang, SG, Hsueh AJ. Characterization of two LGR genes homologous to gonadotropin and thyrotropin receptors with extracellular leucine-rich repeats and a G protein-coupled, seven-transmembrane region. *Mol Endocrinol.* 1998;12:1830–45.
39. Reya T, Clevers H. Wnt signalling in stem cells and cancer. *Nature.* 2005;434:843–50.
40. Finch AJ, Soucek L, Junttila MR, Swigart LB, Evan GI. Acute overexpression of Myc in intestinal epithelium recapitulates some but not all the changes elicited by Wnt/beta-catenin pathway activation. *Mol Cell Biol.* 2009;29:5306–15.
41. Gordan JD, Thompson, CB, Simon MC. HIF and c-Myc: sibling rivals for control of cancer cell metabolism and proliferation. *Cancer Cell.* 2007;12:108–13.