HHS Public Access

Author manuscript

Curr Colorectal Cancer Rep. Author manuscript; available in PMC 2019 January 15.

Published in final edited form as:

Curr Colorectal Cancer Rep. 2015 December ; 11(6): 414–421. doi:10.1007/s11888-015-0301-6.

Epigenetic and Immune Regulation of Colorectal Cancer Stem Cells

Amy V. Paschall, B.S. and **Kebin Liu, Ph.D.**

Department of Biochemistry and Molecular Biology, Medical College of Georgia, Georgia Regents University, Augusta, GA 30912, USA. Cancer Center, Georgia Regents University, Augusta, GA 30912. Charlie Norwood VA Medical Center, Augusta, GA 30904, USA

Abstract

Colorectal cancer stem cells (CSCs) were initially considered to be a subset of undifferentiated tumor cells with well-defined phenotypic and molecular markers. However, emerging evidence indicates instead that colorectal CSCs are heterogeneous subsets of tumor cells that are continuously reshaped by the dynamic interactions between genetic, epigenetic, and immune factors in the tumor microenvironment. Thus, the colorectal CSC phenotypes and responsiveness to therapy may not only be a tumor cell-intrinsic feature, but also depend on tumor-extrinsic microenvironmental factors. Furthermore, emerging evidence also implicates colorectal CSCs in potential immune evasion. Therefore, understanding how colorectal CSC-intrinsic mechanisms cooperate with the extrinsic microenvironmental factors to dynamically shape colorectal CSC resistance to chemotherapy and immunotherapy holds great promise for development of targeted CSC therapies of advanced human CRC.

Keywords

Colorectal Cancer Stem Cells; Epigenetics; Chemoresistance; Immune Evasion; DNA Methylation; Histone Methylation; Histone Acetylation; MDSCs; IL-22; Epigenetic Regulation

Introduction

Colorectal cancer is the third highest cause of cancer related deaths in the US. Current statistics by the American Cancer Society show that, while improved screening methods have decreased overall mortality rates of the disease over the past decade, advanced stages of the disease remain problematic for treatment. Over 93,000 new cases for men and women are projected for 2015, while over 49,000 colon cancer-related deaths are estimated for the year. One major factor in colorectal cancer progression is the resistance to conventional therapies, including chemotherapy, radiotherapy, and immunotherapy (1). The mechanism(s) by which colorectal cancer cells develop a resistance to one, some, or all of these therapies is still under investigation. However, one controversial topic has recently been proposed to be involved in the acquisition of multi-therapy resistance: the “stem-like” quality of certain cancer cells (2, 3). Over the last few years, many researchers have clarified the stem-cell

Apaschall@gru.edu, Kliu@gru.edu, Tel: 706-721-9958, Fax: 706-721-6608.

phenotype, particularly with regards to the developing tumor microenvironment (4–11). While the characterization of cancer stem cells (CSC)/cancer-initiating cells according to certain markers remains ambiguous and controversial, we are beginning to understand how cancer stem cells may be responsible, in part, for the persistence of tumors even after conventional therapies. This is particularly relevant in the case of colorectal cancer, as nearly all colorectal cancer patients develop a resistance to 5-Fluoruracil (5-FU), the standard chemotherapy for patients in Stages 2–4 of colorectal cancer. 5-FU acts through the conversion of uracil to fluoronucleotides and inhibiting thymidylate synthase, thereby inducing DNA damage in cells (12). Cells which are rapidly dividing, i.e. cancer cells, do not effectively undergo DNA repair, and thereby undergo cell death. In this way, rapidly-dividing cells are preferentially targeted and normal, slowly-dividing cells will not be as affected. However, as cancer stem cells may be slowly-dividing or otherwise differ in their metabolic rates from differentiated cancer cells, they may be resistant to chemotherapies such as 5-FU, thereby persisting in the tissue and later developing into a recurrent carcinoma (13). A better understanding of how cancer stem cells are regulated, targeted, and differentiated could therefore prove pivotal to overcoming colon cancer recurrence in patients who no longer respond to therapy.

Characteristics of Colorectal Cancer Stem Cells

CD133 was first described as a potential stem cell marker in 2000 (14). Since then, researchers have investigated its potential as a marker for cancer-initiating cells through dilution and transplantation analyses. Human colorectal CSCs were first characterized as $CD133^+$ tumor cells in the heterogeneous CRC population. In a 2007 study, O'Brien et al tested whether certain cellular subsets of human colon cancer were more likely to initiate tumor growth, as opposed to the stochastic model in which every cancer cell is equally capable of forming a tumor (15). Using a NOD/SCID xenotransplant model, the researchers transplanted varying dilutions of human colon cancer cells into mice and determined the tumor-initiating capacity of the cells. Transplantations of $CD133^+$ human colon carcinoma cells initiated tumors at considerably small dilutions (1×10^3 cells/mouse), whereas very few mice developed tumors after receiving $CD133^-$ transplantations at much higher cell densities. The authors therefore concluded that $CD133^+$ human CRC is associated with cancer initiation and resembles the original malignancy *in vivo* (4, 15). Since then, extensive efforts have been devoted to identify additional colorectal CSC markers that further phenotypically and functionally define colorectal CSCs. Certain proteins associated with the epithelial-to-mesenchymal transition (EMT) are most likely linked with the progression of cancer, and thus the cancer stem-cell phenotype. Such markers may include Vimentin (16), EpCam (17), OLFM4 (18), and CEACAM1 (19). Some genes linked to cell cycle and proliferation have also been associated with the stem cell phenotype, such as B7-H1 and p21 (20). Certain CSC markers that may be more specific to CRC include Lgr5 (21, 22), KRT19 (23), FABp2 (24), EphB2 (25), EpCam (26), CD166 (26), ALDH (27–29), DCLK1 (30), CD24 (31, 32), CD26 (33) and CD44 (26, 34), as well as its variant CD44v6 (35).

CSCs have thus far been particularly difficult to define based on phenotypic and functional markers. For example, even though CD133 is widely used as a CSC marker, its suitability as a colorectal CSC phenotypic marker is still controversial (17) (4, 36). Also, characterizing

CSCs in general and colorectal CSCs in particular based on certain phenotypic markers is still ambiguous. However, colorectal CSC markers are often unstable and likely vary depending on tumor stages, the types and timing of therapies and the tumor microenvironment (10, 37) (38, 39). Therefore, the characterization and use of colorectal CSC phenotypic markers should be used as relative and cellular contextual parameters rather than as general properties of the tumors (40).

Colorectal CSCs in Chemoresistance and Immune Evasion

Intrinsic and acquired resistance to chemotherapy and resultant tumor recurrence and metastasis to distant organs, primarily to the liver, accounts for over 90% of human CRC mortality (41). Like other types of human cancer cells, human CRC cells are now considered to be highly dynamic heterogeneous populations that are continuously reshaped by epigenetic and microenvironmental factors (5, 42, 43). Therefore, a conventional idea is that chemotherapeutic agents such as 5-FU kill sensitive tumor cells to selectively enrich the resistant subsets of tumor cells. Because 5-FU preferentially targets rapidly dividing tumor cells, quiescent colorectal CSCs may be spared by the chemotherapy, resulting in a relative increase in the CSCs due to the selective survival of the CSC subset. Indeed, several studies have shown that 5-FU therapy selectively enrich subsets of CRC cells with colorectal CSC phenotypes, including $CD133^+$, $CD44^+$ and $Lgr5^+$ colorectal CSCs (3, 13, 44, 45). In a recent study gastric cancer cell lines were treated with increasing concentrations of 5-FU (24). Cells that survived this treatment were assessed for their stem-cell-like properties, using CD133, CD326, and CD44 as potential biomarkers. These 5-FU resistant cells were shown to have increased self-renewal capabilities as well as increased expression of CD133, CD326, and B lymphoma Mo-MLV insertion region 1 (BMI1), which have all been associated with the cancer stem cell phenotype. Colorectal CSCs persistence has been not only associated with drug resistance, but resistance to radiation as well. A 2012 study by Hua et al describes how a particular subset of intestinal stem cells is radioresistant. Using Lgr5-lacZ mice as a model for studying crypt base columnar cells, the researchers show that this particular subset of intestinal stem cells repair DNA damage more quickly than differentiated intestinal cells after mice received radiation with 12 Gy (46).

One key mechanism of the anti-tumor response *in vivo* is immune surveillance. While the idea of tumor immunity was once controversial, the emerging clinical success of PD-1/PD-L1-based checkpoint blockade immunotherapy in human cancer patients has highlighted the critical importance of cancer immune surveillance in protection of the host against cancer (47–50) Cancer vaccines and adoptive T cell therapy are just some of the ways researchers have been using the immune system to control tumor growth. Like chemotherapy, the immune system, while suppressing tumor cells through its cytotoxic effector mechanisms, also imposes a selective pressure on the target tumor to enrich immune escape variants (51). However, whether these immune escape variants resemble CSCs is not clear. The process of immune anti-tumor surveillance can be compromised when immune cells themselves are regulated, exhausted, or otherwise functionally impaired. This can occur in several ways: tumor cells may down-regulate pro-death cell surface signaling molecules such as Fas to avoid triggering cytotoxic T lymphocyte (CTL)-mediated killing (52); tumor cells may also produce certain factors that promote activity of regulatory T cells (Tregs) or myeloid-derived

suppressor cells (MDSCs) that inhibit CTL activity. Inflammatory cells—including MDSCs—may even directly affect tumor cell activity and growth through the production of certain cytokines (39, 53–55). In one recent study, researchers investigated the direct effects of MDSCs on the stem cell phenotype of cancer cells and performed xenograft transplantation of human ovarian cancer cells into immunodeficient mice to determine the minimum cell number needed to induce tumor growth. However, when these ovarian cancer cells were co-cultured with MDSCs before transplantation, the number of cells needed for tumor induction was significantly decreased. These observations suggest that MDSCs condition and enrich the cancer stem-like tumor-initiating cell populations (56). Certain tumor cells can, in turn, promote MDSC accumulation through the production of myeloid-stimulating cytokines such as GM-CSF (57, 58). Thus, deregulated immune surveillance can promote tumor persistence and recurrence through promotion of cancer stem cells. The impact of the immune system on colorectal CSCs is still unclear.

Epigenetic Regulation of CSCs

Epigenetic modifications play vital roles in transcriptional regulation of key genes associated with cancer and stem cells (59–61). Epigenetic modifications are reversible changes to the structure of DNA regions through chemical additions to histones or to the DNA itself. Such modifications include DNA methylation, histone acetylation, histone methylation, and ubiquitination, among many others. Epigenetic regulation can also include gene-regulating RNAs, such as microRNAs. These epigenetic regulating factors can thus determine if certain pro-proliferative, anti-apoptotic, or other genes associated with the cancer phenotype are up- or down-regulated, particularly in response to certain drugs or other stimuli which promote epigenetic enzyme activity, such as DNA methyltransferases (DNMTs) or histone deacetylases (HDACs) (61, 62). Epigenetic regulation may also be involved in the enrichment of chemoresistant sub-populations of cancer cells, particularly in the colon where the process of colonic precursors to fully differentiated colon endothelial cells must be tightly regulated (45, 62). Any modifications to the genome that promote transcription of pro-proliferative genes could thereby promote colon carcinomas. Therefore, genes associated with the cancer stem-like phenotype could be key targets for epigenetic drug therapy (62–65).

DNA methylation in particular is an epigenetic modification that has been highly associated with almost all types of human cancers. DNA methylation in humans occurs through the action of DNMTs, including DNMT1, DNMT3a, and DNMT3b (66). These enzymes would therefore be considered ideal molecular targets for preventing DNA hypermethylation, which can prevent transcription of key anti-cancer genes. Decitabine is a DNMT inhibitor currently approved by the FDA for certain blood disorders, and has been shown to promote apoptosis of cancer cells, including human colon carcinoma cells (66, 67). DNA hypermethylation can also affect cell cycle genes, whereby the developing cancer cells can escape checkpoint blockade and senescence (68). As such, DNA hypermethylation plays a key role in the development of stemness of colon cancer cells (69).

In a recent study, DNMT1 was shown to be associated with cancer stem cell enrichment *in vivo*. Researchers tested DNA methylation and expression of DNMT1 in cancer stem cells *in*

vivo and observed that sphere-forming tumor cells express higher levels of DNMT1(70). Using a DNMT1 deletion model, studies showed that $DNMT1^{\Delta/\Delta}$ mice expressed fewer stem cell markers such as $CD49^{+}CD24^{-}$ cells in the mammary tissues. $DNMT1^{\Delta/\Delta}$ tumor cell transplantation in mice also had a much lower percentage of tumor incidence, suggesting inhibition or ablation of cancer-initiation stem cells in these populations (70). Another recent study has also identified DNA methylation patterns in cancer stem cells in a mouse tumor model. Glioblastoma-derived stem cells were cultured in stem cell-specific medium; tumorispheres were then isolated and used for high throughput screening and DNA methylation changes were analyzed. Cell lines derived from the glioblastoma stem cells had much higher patterns of DNA methylation at key tumor suppressor genes including PENK, GATA6, and TES compared to the parent glioblastoma cell lines (71).

The link between DNA methylation and CSCs has also been investigated specifically in colon cancer stem cells. One study investigated the expression of tumor suppressor gene spinophilin on cancer stem cell markers such as CD133, as well as the ability of human colon cancer cells to form spheres in stem cell-conditioned medium and of these stem cells to resist treatment with 5-FU (72). After treatment with Decitabine, tumor cells were sensitized to 5-FU therapy and less likely to form stem cell-like spheres in culture through increased expression of the tumor suppressor gene. Similarly, in another study (73), knockout of DNMT1 in human colon carcinoma cells showed decreases in $CD44^{+}CD24^{+}$ cell numbers as well as impaired tumor-initiating capabilities compared to the parental tumor cells. These researchers also utilized transient DNMT1 knockdown using siRNA, and saw that short-term inhibition of DNMT1 activity was sufficient to disrupt the cancer-initiating potential of the stem cell-like subset of human colon carcinoma cells (73). Furthermore, DNMT3a expression was shown to be higher in colon adenomas than in normal mucosal tissue, and the highest DNMT3a level was detected in $Lgr5^{+}$ colon cancer stem cell-like cells (74). These observations thus suggest that DNMT3a is also upregulated in $Lgr5^{+}$ progenitor cancer cells. Consequently, conditional deletion of DNMT3a inhibited the capacity of these cells to form tumors in the colons of immunodeficient mice (74). Overall, these studies demonstrated a key role of DNMTs in CSC development.

Histone modification is another example of an epigenetic mechanism that regulates transcription of genes associated with cancer progression, differentiation and stemness(75–77). Histone lysine deacetylases (HDAC) and histone acetyltransferases (HAT) modify the lysine side chain of chromatin histones to modulate gene expression through contradictory mechanisms (78, 79). HDACs are highly expressed in cancer cells that are often associated with decreased acetylation of chromatin histones, transcriptionally inactive chromatin conformation, and loss of gene expression (80, 81). Several HDAC inhibitors, such as Vorinostat, have been approved for patient treatment by the FDA and are also commonly used in cancer research. Other HDAC inhibitors such as MGCD0103 are currently undergoing clinical trials. Cancer cells use the coordinated DNA hypermethylation and histone de-acetylation to silence the expression of tumor suppressor genes, thereby controlling cross-talk networks between the two epigenetic mechanisms in regulation of gene expression to their advantage (67, 82). This phenomenon is best demonstrated by the epigenetic silencing of the cell death receptor Fas. Fas is a death receptor in tumor cells. The host immune system uses FasL of the cytotoxic T lymphocytes (CTLs) to engage Fas to

induce tumor cell death as a key mechanism of its cancer immune surveillance (83). Fas is highly expressed in normal human colon epithelial cells but is diminished in colon carcinoma cells, whereas complete silencing of Fas is often see in the metastatic human colon carcinoma cells *in vivo* (84, 85). Thus human colon carcinoma may use silencing of Fas to evade the host cancer immune surveillance to advance the disease (52). Treatment of the metastatic human colon carcinoma cells with Decitabine and Vorinostat cooperatively increased Fas expression and sensitized the tumor cells to FasL-induced apoptosis *in vitro* and CTL-mediated tumor suppression *in vivo* (67). HADCs have been shown to regulate intestinal stem cell homeostasis, and inhibition of histone de-acetylation by HDAC inhibitors inhibits the ability of cancer-initiating cells to form spheres in stem cell conditioned media (76). However, the role of histone acetylation in regulation of colorectal CSC stemness remains an area to be explored.

Histone methylation can also affect whether or not certain genes are transcribed, though its classification as a transcriptionally active or repressive state may be dependent on the cell type, as well as the particular lysine residue that is affected. Histone methyltransferases (HMTases) have been shown to be upregulated in certain types of cancers (86). The Enhancer of Zeste homolog 2 (EZH2) enzyme is a HMTase known to be involved in different signaling pathways and gene regulatory pathways associated with colon cancer. This regulation may have direct and/or indirect effects on tumor progression. For instance, the epidermal growth factor (EGF) and its receptor (EGFR) are involved in pro-proliferative signaling pathways in multiple cancer types, including colon cancer. Research has recently shown that inhibition of this pathway can be enhanced by concomitant inhibition of EZH2 using a specific HMTase inhibitor, UNC1999 (87). Interestingly, HMTases seem to act in concert with histone acetylation and DNA methylation to regulate tumor suppressor genes such as Fas in human colon cancer cells (52),suggesting multifaceted epigenetic regulation of tumor suppressor genes in human colon cancer (52, 67). HMTase regulation can also affect pathways associated with stem cell-like phenotypes. EZH2 has been shown to be highly upregulated in poorly differentiated breast cancer stem cells through increased H3K27 methylation (88). Further research has shown that, not only repressive histone methylation against tumor-suppressive pathways, but also active histone methylation marks at stem cell genes can affect cancer progression. H3K4me3 is associated with transcriptional activation, and has been shown to be enriched at promoter regions of certain stem cell genes, including NANOG, OCT4, and MYC (89). The implications of histone methylation regulation of colon cancer stem cells have yet to be determined.

Regulation of CSCs by Tumor Microenrionmental Factors

Emerging evidence indicates that CSCs are continuously shaped by the tumor microenvironmental factors (39, 42, 43, 90). These factors may include cytokines IL17A, IL22 and TGFβ, tumor stromal factors BMP antagonists, MM proteases, hepatocyte growth factor (HGF), osteopontin (OPN) and Jagged 1 (38, 43, 56). Immune cells, including T cells and MDSCs, comprise some of the major populations of cells in the tumor microenvironment (91). In addition, immune modulatory molecules such as IL17A, IL22 and IL10 are often present in the tumor microenvironment (20, 38, 39, 56). Epigenetic mechanisms are therefore expected to interfere with interactions between CSCs and immune

cells in the tumor microenvironment (38, 92). Epigenetic regulation of MDSCs has not been well-studied. However, histone acetylation has recently been implicated in the differentiation and suppressive phenotype of MDSCs *in vivo* (93). Specifically, when HDAC11 is knocked out in mice and these animals are challenged with tumor cells, MDSCs from the spleens of these mice are shown to have a more inhibitory effect on IFNγ production in T cells, which correlates with the production of immune suppressive cytokine IL10 (93). The co-localization of MDSCs and CSCs in the tumor microenvironment and the resultant cancer persistence and progression suggest a close relationship between MDSCs and CSCs. In colon cancer, epigenetic and immune regulation of cancer can work both ways: immune cells can induce epigenetic changes that promote colon cancer stemness (39, 92). Indeed, a recent study showed that MDSCs triggered miRNA-101 (miR101) expression in cancer cells. miR-101 subsequently represses the co-repressor CtBP2. CtBP2 directly targeted stem cell core genes, resulting in increased cancer cell stemness and increasing metastatic and tumorigenic potential (56). Therefore, the MDSCs-miR101-CtBP2-stem cell core genes axis extrinsically controls cancer stemness to impact patient outcome.

In addition to miR101, several other miRNAs, including miR215, miR218 and miR328, have been shown to promote colorectal CSCs (94–97). The transcription factor CDX1 is a key regulator of differentiation in the normal colon, as well as in CRC. CDX1 activates transcription of enterocyte genes (97). Jones *et al* recently determined that miR-215 acts as a direct transcriptional target of CDX1 in CRC cells. miR-215 expression is depleted in FACS-enriched cancer stem cells. Furthermore, miR-215 mediates the repression of cell cycle and stemness genes downstream of CDX1, including gene BMI, to promote stemness and self-renewal of colon CSCs (94). However, whether these miRNAs can be regulated by tumor-infiltrating immune cells such as MDSCs in the tumor microenvironment is unknown.

IL-22$^+$ immune cells have been shown to protect developing colon tumors. Co-culture of IL22$^+$ T cells with colon cancer cells in stem cell-specific media showed increased sphere formation. IL22$^+$ cells were also shown to activate the STAT3 transcription pathway and upregulate expression of an HMTase, disruptor of telomeric silencing 1-like (DOT1L) (92). DOT1L methylates H3K79, and was shown to be associated with the transcription of several stem cell-associated genes, including NANOG and SOX2. The interplay of immune modulators and epigenetic regulators is thus an important consideration in the understanding of colon cancer stem cells and resistance to therapy.

Conclusion

Recent studies have firmly linked colorectal CSCs to self-renewal, tumor initiation, chemoresistance and radioresistance, providing a strong rationale to develop targeted therapy to suppress colorectal CSCs in cancer therapy. However, emerging experimental data from both colorectal cancer patients and genetic and cancer mouse models indicate that colorectal CSCs are highly heterogeneous and exhibit dynamic plasticity. This heterogeneousity and plasticity are apparently not only mediated by genetic and epigenetic factors, but as we know now also continuously reshaped by microenvironmental factors such as tumor-infiltrating MDSCs and T cells (Fig. 1). Various colorectal CSC-defining phenotypic and functional markers have been identified. However, these markers are

unstable and potentially tumor stage and cellular context-dependent. More recently, immune checkpoint blockade immunotherapy has renewed hope for controlling human cancer by showing durable efficacy in many types of human cancers in the last few years. However, outside MSI-H CRC, which accounts for only about 4% of advanced CSC patients, CSC stands out as one of the few cancers types where PD-1, PD-L1, and CTLA-4 inhibition immunotherapy has been unsuccessful. What distinguishes CRC from other tumor types immunologically is unclear. Considering the functions of immune cells in shaping colorectal CSC stemness and the key role of colorectal CSCs in chemoresistance and immune evasion, colorectal CSCs may be a significant contributing factor of the failure of immune checkpoint immunotherapy in human CRC patients. Therefore, further elucidation the genetic, epigenetic and immunological mechanisms underlying colorectal CSC homeostasis will provide the foundation for development of targeted therapy not only for overcoming CSC chemo- and radioresiatance, but also for enhancing the efficacy of immune checkpoint blockade immunotherapy for human CSC patients.

References

1. Holohan C, Van Schaeybroeck S, Longley DB, Johnston PG. Cancer drug resistance: an evolving paradigm. Nat Rev Cancer. 2013;13:714–26. [PubMed: 24060863]
2. Vidal SJ, Rodriguez-Bravo V, Galsky M, Cordon-Cardo C, Domingo-Domenech J. Targeting cancer stem cells to suppress acquired chemotherapy resistance. Oncogene. 2014;33:4451–63. [PubMed: 24096485]
3. Dallas NA, Xia L, Fan F, Gray MJ, Gaur P, van Buren G, 2nd, et al. Chemoresistant colorectal cancer cells, the cancer stem cell phenotype, and increased sensitivity to insulin-like growth factor-I receptor inhibition. Cancer Res. 2009;69:1951–7. [PubMed: 19244128]
4. Ricci-Vitiani L, Lombardi DG, Pilozzi E, Biffoni M, Todaro M, Peschle C, et al. Identification and expansion of human colon-cancer-initiating cells. Nature. 2007;445:111–5. [PubMed: 17122771]
5. Kreso A, van Galen P, Pedley NM, Lima-Fernandes E, Frelin C, Davis T, et al. Self-renewal as a therapeutic target in human colorectal cancer. Nat Med. 2014;20:29–36. [PubMed: 24292392]
6. Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. Nature. 2001;414:105–11. [PubMed: 11689955]
7. Beck B, Blanpain C. Unravelling cancer stem cell potential. Nat Rev Cancer. 2013;13:727–38. [PubMed: 24060864]
8. Barker N, van de Wetering M, Clevers H. The intestinal stem cell. Genes Dev. 2008;22:1856–64. [PubMed: 18628392]
9. Barker N, van Es JH, Kuipers J, Kujala P, van den Born M, Cozijnsen M, et al. Identification of stem cells in small intestine and colon by marker gene Lgr5. Nature. 2007;449:1003–7. [PubMed: 17934449]
10. Medema JP, Vermeulen L. Microenvironmental regulation of stem cells in intestinal homeostasis and cancer. Nature. 2011;474:318–26. [PubMed: 21677748]
11. Vaiopoulos AG, Kostakis ID, Koutsilieris M, Papavassiliou AG. Colorectal cancer stem cells. Stem Cells. 2012;30:363–71. [PubMed: 22232074]
12. Longley DB, Harkin DP, Johnston PG. 5-fluorouracil: mechanisms of action and clinical strategies. Nat Rev Cancer. 2003;3:330–8. [PubMed: 12724731]
13. Touil Y, Igoudjil W, Corvaisier M, Dessein AF, Vandomme J, Monte D, et al. Colon cancer cells escape 5FU chemotherapy-induced cell death by entering stemness and quiescence associated with the c-Yes/YAP axis. Clin Cancer Res. 2014;20:837–46. [PubMed: 24323901]
14. Uchida N, Buck DW, He D, Reitsma MJ, Masek M, Phan TV, et al. Direct isolation of human central nervous system stem cells. Proc Natl Acad Sci U S A. 2000;97:14720–5. [PubMed: 11121071]

15. 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. [PubMed: 17122772]
16. McInroy L, Maatta A. Down-regulation of vimentin expression inhibits carcinoma cell migration and adhesion. Biochem Biophys Res Commun. 2007;360:109–14. [PubMed: 17585878]
17. Shmelkov SV, Butler JM, Hooper AT, Hormigo A, Kushner J, Milde T, et al. CD133 expression is not restricted to stem cells, and both CD133+ and CD133- metastatic colon cancer cells initiate tumors. J Clin Invest. 2008;118:2111–20. [PubMed: 18497886]
18. van der Flier LG, Haegebarth A, Stange DE, van de Wetering M, Clevers H. OLFM4 is a robust marker for stem cells in human intestine and marks a subset of colorectal cancer cells. Gastroenterology. 2009;137:15–7. [PubMed: 19450592]
19. Stokrova J, Sloncova E, Sovova V, Kucerova D, Zila V, Tureckova J, et al. Characterization of four clones derived from human adenocarcinoma cell line, HT29, and analysis of their response to sodium butyrate. Int J Oncol. 2006;28:559–65. [PubMed: 16391813]
20. Bardhan K, Paschall AV, Yang D, Chen MR, Simon PS, Bhutia YD, et al. IFNgamma induces DNA methylation-silenced GPR109A expression via pSTAT1/p300 and H3K18 acetylation in colon cancer. Cancer Immunol Res. 2015.
21. Kemper K, Prasetyanti PR, De Lau W, Rodermond H, Clevers H, Medema JP. Monoclonal antibodies against Lgr5 identify human colorectal cancer stem cells. Stem Cells. 2012;30:2378–86. [PubMed: 22969042]
22. Bini F, Frati A, Garcia-Gil M, Battistini C, Granado M, Martinesi M, et al. New signalling pathway involved in the anti-proliferative action of vitamin D(3) and its analogues in human neuroblastoma cells. A role for ceramide kinase. Neuropharmacology. 2012;63:524–37. [PubMed: 22579669]
23. Asfaha S, Hayakawa Y, Muley A, Stokes S, Graham TA, Ericksen RE, et al. Krt19(+)/Lgr5(−) Cells Are Radioresistant Cancer-Initiating Stem Cells in the Colon and Intestine. Cell Stem Cell. 2015;16:627–38. [PubMed: 26046762]
24. Cao L, Kuratnik A, Xu W, Gibson JD, Kolling Ft, Falcone ER, et al. Development of intestinal organoids as tissue surrogates: cell composition and the epigenetic control of differentiation. Mol Carcinog. 2015;54:189–202. [PubMed: 24115167]
25. Jung P, Sato T, Merlos-Suarez A, Barriga FM, Iglesias M, Rossell D, et al. Isolation and in vitro expansion of human colonic stem cells. Nat Med. 2011;17:1225–7. [PubMed: 21892181]
26. Dalerba P, Dylla SJ, Park IK, Liu R, Wang X, Cho RW, et al. Phenotypic characterization of human colorectal cancer stem cells. Proc Natl Acad Sci U S A. 2007;104:10158–63. [PubMed: 17548814]
27. Carpentino JE, Hynes MJ, Appelman HD, Zheng T, Steindler DA, Scott EW, et al. Aldehyde dehydrogenase-expressing colon stem cells contribute to tumorigenesis in the transition from colitis to cancer. Cancer Res. 2009;69:8208–15. [PubMed: 19808966]
28. Hynes MJ, Huang KM, Huang EH. Review paper: Implications of the "cancer stem cell" hypothesis on murine models of colon cancer and colitis-associated cancer. Vet Pathol. 2009;46:819–35. [PubMed: 19429978]
29. Huang EH, Hynes MJ, Zhang T, Ginestier C, Dontu G, Appelman H, et al. Aldehyde dehydrogenase 1 is a marker for normal and malignant human colonic stem cells (SC) and tracks SC overpopulation during colon tumorigenesis. Cancer Res. 2009;69:3382–9. [PubMed: 19336570]
30. Nakanishi Y, Seno H, Fukuoka A, Ueo T, Yamaga Y, Maruno T, et al. Dclk1 distinguishes between tumor and normal stem cells in the intestine. Nat Genet. 2013;45:98–103. [PubMed: 23202126]
31. Wang F, Scoville D, He XC, Mahe MM, Box A, Perry JM, et al. Isolation and characterization of intestinal stem cells based on surface marker combinations and colony-formation assay. Gastroenterology. 2013;145:383–95 e1–21. [PubMed: 23644405]
32. King JB, von Furstenberg RJ, Smith BJ, McNaughton KK, Galanko JA, Henning SJ. CD24 can be used to isolate Lgr5+ putative colonic epithelial stem cells in mice. Am J Physiol Gastrointest Liver Physiol. 2012;303:G443–52. [PubMed: 22723265]
33. Pang R, Law WL, Chu AC, Poon JT, Lam CS, Chow AK, et al. A subpopulation of CD26+ cancer stem cells with metastatic capacity in human colorectal cancer. Cell Stem Cell. 2010;6:603–15. [PubMed: 20569697]

34. Cao F, Townsend EC, Karatas H, Xu J, Li L, Lee S, et al. Targeting MLL1 H3K4 methyltransferase activity in mixed-lineage leukemia. Mol Cell. 2014;53:247–61. [PubMed: 24389101]
35. Todaro M, Gaggianesi M, Catalano V, Benfante A, Iovino F, Biffoni M, et al. CD44v6 is a marker of constitutive and reprogrammed cancer stem cells driving colon cancer metastasis. Cell Stem Cell. 2014;14:342–56. [PubMed: 24607406]
36. Welte Y, Davies C, Schafer R, Regenbrecht CR. Patient derived cell culture and isolation of CD133(+) putative cancer stem cells from melanoma. J Vis Exp. 2013:e50200. [PubMed: 23525090]
37. Kobayashi S, Yamada-Okabe H, Suzuki M, Natori O, Kato A, Matsubara K, et al. LGR5-positive colon cancer stem cells interconvert with drug-resistant LGR5-negative cells and are capable of tumor reconstitution. Stem Cells. 2012;30:2631–44. [PubMed: 23081779]
38. Kryczek I, Lin Y, Nagarsheth N, Peng D, Zhao L, Zhao E, et al. IL-22(+)CD4(+) T cells promote colorectal cancer stemness via STAT3 transcription factor activation and induction of the methyltransferase DOT1L. Immunity. 2014;40:772–84. [PubMed: 24816405]
39. Lotti F, Jarrar AM, Pai RK, Hitomi M, Lathia J, Mace A, et al. Chemotherapy activates cancer-associated fibroblasts to maintain colorectal cancer-initiating cells by IL-17A. J Exp Med. 2013;210:2851–72. [PubMed: 24323355]
40. Zeuner A, Todaro M, Stassi G, De Maria R. Colorectal cancer stem cells: from the crypt to the clinic. Cell Stem Cell. 2014;15:692–705. [PubMed: 25479747]
41. Longley DB, Johnston PG. Molecular mechanisms of drug resistance. J Pathol. 2005;205:275–92. [PubMed: 15641020]
42. Kreso A, Dick JE. Evolution of the cancer stem cell model. Cell Stem Cell. 2014;14:275–91. [PubMed: 24607403]
43. Biswas S, Davis H, Irshad S, Sandberg T, Worthley D, Leedham S. Microenvironmental control of stem cell fate in intestinal homeostasis and disease. J Pathol. 2015.
44. Hsu HC, Liu YS, Tseng KC, Hsu CL, Liang Y, Yang TS, et al. Overexpression of Lgr5 correlates with resistance to 5-FU-based chemotherapy in colorectal cancer. Int J Colorectal Dis. 2013;28:1535–46. [PubMed: 23784051]
45. Humeniuk R, Mishra PJ, Bertino JR, Banerjee D. Epigenetic reversal of acquired resistance to 5-fluorouracil treatment. Mol Cancer Ther. 2009;8:1045–54. [PubMed: 19383845]
46. Arthur JC, Perez-Chanona E, Muhlbauer M, Tomkovich S, Uronis JM, Fan TJ, et al. Intestinal Inflammation Targets Cancer-Inducing Activity of the Microbiota. Science. 2012.
47. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med. 2012;366:2443–54. [PubMed: 22658127]
48. Brahmer JR, Tykodi SS, Chow LQ, Hwu WJ, Topalian SL, Hwu P, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. N Engl J Med. 2012;366:2455–65. [PubMed: 22658128]
49. Topalian SL, Sznol M, McDermott DF, Kluger HM, Carvajal RD, Sharfman WH, et al. Survival, durable tumor remission, and long-term safety in patients with advanced melanoma receiving nivolumab. J Clin Oncol. 2014;32:1020–30. [PubMed: 24590637]
50. Hamid O, Robert C, Daud A, Hodi FS, Hwu WJ, Kefford R, et al. Safety and tumor responses with lambrolizumab (anti-PD-1) in melanoma. N Engl J Med. 2013;369:134–44. [PubMed: 23724846]
51. Liu K, Caldwell SA, Greeneltch KM, Yang D, Abrams SI. CTL Adoptive Immunotherapy Concurrently Mediates Tumor Regression and Tumor Escape. J Immunol. 2006;176:3374–82. [PubMed: 16517705]
52. Paschall AV, Yang D, Lu C, Choi JH, Li X, Liu F, et al. H3K9 Trimethylation Silences Fas Expression To Confer Colon Carcinoma Immune Escape and 5-Fluorouracil Chemoresistance. J Immunol. 2015.
53. Paschall AV, Zhang R, Qi CF, Bardhan K, Peng L, Lu G, et al. IFN Regulatory Factor 8 Represses GM-CSF Expression in T Cells To Affect Myeloid Cell Lineage Differentiation. J Immunol. 2015;194:2369–79. [PubMed: 25646302]
54. Gabrilovich DI, Ostrand-Rosenberg S, Bronte V. Coordinated regulation of myeloid cells by tumours. Nat Rev Immunol. 2012;12:253–68. [PubMed: 22437938]

55. Ostrand-Rosenberg S Looking to the future of cancer immunotherapy: many questions to answer and many therapeutic opportunities. Cancer Immunol Immunother. 2013;62:1–2. [PubMed: 23254777]
56. Cui TX, Kryczek I, Zhao L, Zhao E, Kuick R, Roh MH, et al. Myeloid-derived suppressor cells enhance stemness of cancer cells by inducing microRNA101 and suppressing the corepressor CtBP2. Immunity. 2013;39:611–21. [PubMed: 24012420]
57. Bayne LJ, Beatty GL, Jhala N, Clark CE, Rhim AD, Stanger BZ, et al. Tumor-derived granulocyte-macrophage colony-stimulating factor regulates myeloid inflammation and T cell immunity in pancreatic cancer. Cancer Cell. 2012;21:822–35. [PubMed: 22698406]
58. Pylayeva-Gupta Y, Lee KE, Hajdu CH, Miller G, Bar-Sagi D. Oncogenic Kras-induced GM-CSF production promotes the development of pancreatic neoplasia. Cancer Cell. 2012;21:836–47. [PubMed: 22698407]
59. Karimi MM, Goyal P, Maksakova IA, Bilenky M, Leung D, Tang JX, et al. DNA methylation and SETDB1/H3K9me3 regulate predominantly distinct sets of genes, retroelements, and chimeric transcripts in mESCs. Cell Stem Cell. 2011;8:676–87. [PubMed: 21624812]
60. Klajic J, Busato F, Edvardsen H, Touleimat N, Fleischer T, Bukholm I, et al. DNA methylation status of key cell-cycle regulators such as CDKNA2/p16 and CCNA1 correlates with treatment response to doxorubicin and 5-fluorouracil in locally advanced breast tumors. Clin Cancer Res. 2014;20:6357–66. [PubMed: 25294903]
61. Vedeld HM, Skotheim RI, Lothe RA, Lind GE. The recently suggested intestinal cancer stem cell marker DCLK1 is an epigenetic biomarker for colorectal cancer. Epigenetics. 2014;9:346–50. [PubMed: 24384857]
62. Easwaran H, Tsai HC, Baylin SB. Cancer epigenetics: tumor heterogeneity, plasticity of stem-like states, and drug resistance. Mol Cell. 2014;54:716–27. [PubMed: 24905005]
63. Bardhan K, Liu K. Epigenetics and colorectal cancer pathogenesis. Cancers (Basel). 2013;5:676–713. [PubMed: 24216997]
64. Huang K, Fan G. Epigenetic modifications in distinction: histone versus DNA methylation in ESCs. Cell Stem Cell. 2011;8:604–5. [PubMed: 21624801]
65. Crea F, Nobili S, Paolicchi E, Perrone G, Napoli C, Landini I, et al. Epigenetics and chemoresistance in colorectal cancer: an opportunity for treatment tailoring and novel therapeutic strategies. Drug Resist Updat. 2011;14:280–96. [PubMed: 21955833]
66. Subramaniam D, Thombre R, Dhar A, Anant S. DNA Methyltransferases: A Novel Target for Prevention and Therapy. Front Oncol. 2014;4:80. [PubMed: 24822169]
67. Yang D, Torres CM, Bardhan K, Zimmerman M, McGaha TL, Liu K. Decitabine and vorinostat cooperate to sensitize colon carcinoma cells to fas ligand-induced apoptosis in vitro and tumor suppression in vivo. J Immunol. 2012;188:4441–9. [PubMed: 22461695]
68. Fang JY, Chen YX, Lu J, Lu R, Yang L, Zhu HY, et al. Epigenetic modification regulates both expression of tumor-associated genes and cell cycle progressing in human colon cancer cell lines: Colo-320 and SW1116. Cell Res. 2004;14:217–26. [PubMed: 15225415]
69. Kodach LL, Jacobs RJ, Voorneveld PW, Wildenberg ME, Verspaget HW, van Wezel T, et al. Statins augment the chemosensitivity of colorectal cancer cells inducing epigenetic reprogramming and reducing colorectal cancer cell ‘stemness’ via the bone morphogenetic protein pathway. Gut. 2011;60:1544–53. [PubMed: 21551187]
70. Pathania R, Ramachandran S, Elangovan S, Padia R, Yang P, Cinghu S, et al. DNMT1 is essential for mammary and cancer stem cell maintenance and tumorigenesis. Nat Commun. 2015;6:6910. [PubMed: 25908435]
71. Lee EJ, Rath P, Liu J, Ryu D, Pei L, Noonepalle SK, et al. Identification of Global DNA Methylation Signatures in Glioblastoma-Derived Cancer Stem Cells. J Genet Genomics. 2015;42:355–71. [PubMed: 26233891]
72. Ress AL, Stiegelbauer V, Schwarzenbacher D, Deutsch A, Perakis S, Ling H, et al. Spinophilin expression determines cellular growth, cancer stemness and 5-flourouracil resistance in colorectal cancer. Oncotarget. 2014;5:8492–502. [PubMed: 25261368]

73. Morita R, Hirohashi Y, Suzuki H, Takahashi A, Tamura Y, Kanaseki T, et al. DNA methyltransferase 1 is essential for initiation of the colon cancers. Exp Mol Pathol. 2013;94:322–9. [PubMed: 23064049]
74. Weis B, Schmidt J, Maamar H, Raj A, Lin H, Toth C, et al. Inhibition of intestinal tumor formation by deletion of the DNA methyltransferase 3a. Oncogene. 2015;34:1822–30. [PubMed: 24837369]
75. Rugg-Gunn PJ, Cox BJ, Ralston A, Rossant J. Distinct histone modifications in stem cell lines and tissue lineages from the early mouse embryo. Proc Natl Acad Sci U S A. 2010;107:10783–90. [PubMed: 20479220]
76. Sikandar S, Dizon D, Shen X, Li Z, Besterman J, Lipkin SM. The class I HDAC inhibitor MGCD0103 induces cell cycle arrest and apoptosis in colon cancer initiating cells by upregulating Dickkopf-1 and non-canonical Wnt signaling. Oncotarget. 2010;1:596–605. [PubMed: 21317455]
77. Zimberlin CD, Lancini C, Sno R, Rosekrans SL, McLean CM, Vlaming H, et al. HDAC1 and HDAC2 collectively regulate intestinal stem cell homeostasis. FASEB J. 2015;29:2070–80. [PubMed: 25648995]
78. Lin YY, Kiihl S, Suhail Y, Liu SY, Chou YH, Kuang Z, et al. Functional dissection of lysine deacetylases reveals that HDAC1 and p300 regulate AMPK. Nature. 2012;482:251–5. [PubMed: 22318606]
79. Kouzarides T Chromatin modifications and their function. Cell. 2007;128:693–705. [PubMed: 17320507]
80. Eberharter A, Becker PB. Histone acetylation: a switch between repressive and permissive chromatin. Second in review series on chromatin dynamics. EMBO Rep. 2002;3:224–9. [PubMed: 11882541]
81. Kim JH, Workman JL. Histone acetylation in heterochromatin assembly. Genes Dev. 2010;24:738–40. [PubMed: 20395362]
82. Brinkman AB, Gu H, Bartels SJ, Zhang Y, Matarese F, Simmer F, et al. Sequential ChIP-bisulfite sequencing enables direct genome-scale investigation of chromatin and DNA methylation cross-talk. Genome Res. 2012;22:1128–38. [PubMed: 22466170]
83. Afshar-Sterle S, Zotos D, Bernard NJ, Scherger AK, Rodling L, Alsop AE, et al. Fas ligand-mediated immune surveillance by T cells is essential for the control of spontaneous B cell lymphomas. Nat Med. 2014;20:283–90. [PubMed: 24487434]
84. Koshkina NV, Khanna C, Mendoza A, Guan H, DeLauter L, Kleinerman ES. Fas-negative osteosarcoma tumor cells are selected during metastasis to the lungs: the role of the Fas pathway in the metastatic process of osteosarcoma. Mol Cancer Res. 2007;5:991–9. [PubMed: 17951400]
85. Moller P, Koretz K, Leithauser F, Bruderlein S, Henne C, Quentmeier A, et al. Expression of APO-1 (CD95), a member of the NGF/TNF receptor superfamily, in normal and neoplastic colon epithelium. Int J Cancer. 1994;57:371–7. [PubMed: 8168998]
86. Greer EL, Shi Y. Histone methylation: a dynamic mark in health, disease and inheritance. Nat Rev Genet. 2012;13:343–57. [PubMed: 22473383]
87. Katona BW, Liu Y, Ma A, Jin J, Hua X. EZH2 inhibition enhances the efficacy of an EGFR inhibitor in suppressing colon cancer cells. Cancer Biol Ther. 2014;15:1677–87. [PubMed: 25535899]
88. Wu J, Crowe DL. The histone methyltransferase EZH2 promotes mammary stem and luminal progenitor cell expansion, metastasis and inhibits estrogen receptor-positive cellular differentiation in a model of basal breast cancer. Oncol Rep. 2015;34:455–60. [PubMed: 25998860]
89. Wang XQ, Ng RK, Ming X, Zhang W, Chen L, Chu AC, et al. Epigenetic regulation of pluripotent genes mediates stem cell features in human hepatocellular carcinoma and cancer cell lines. PLoS One. 2013;8:e72435. [PubMed: 24023739]
90. Wang D, Fu L, Sun H, Guo L, DuBois RN. Prostaglandin E Promotes Colorectal Cancer Stem Cell Expansion and Metastasis in Mice. Gastroenterology. 2015.
91. Messmer MN, Netherby CS, Banik D, Abrams SI. Tumor-induced myeloid dysfunction and its implications for cancer immunotherapy. Cancer Immunol Immun. 2015;64:1–13.
92. Koltsova EK, Grivennikov SI. IL-22 gets to the stem of colorectal cancer. Immunity. 2014;40:639–41. [PubMed: 24837100]

93. Sahakian E, Powers JJ, Chen J, Deng SL, Cheng F, Distler A, et al. Histone deacetylase 11: A novel epigenetic regulator of myeloid derived suppressor cell expansion and function. Mol Immunol. 2015;63:579–85. [PubMed: 25155994]

94. Jones MF, Hara T, Francis P, Li XL, Bilke S, Zhu Y, et al. The CDX1-microRNA-215 axis regulates colorectal cancer stem cell differentiation. Proc Natl Acad Sci U S A. 2015;112:E1550–8. [PubMed: 25775580]

95. He X, Dong Y, Wu CW, Zhao Z, Ng SS, Chan FK, et al. MicroRNA-218 inhibits cell cycle progression and promotes apoptosis in colon cancer by downregulating BMI1 polycomb ring finger oncogene. Mol Med. 2012;18:1491–8.

96. Xu XT, Xu Q, Tong JL, Zhu MM, Nie F, Chen X, et al. MicroRNA expression profiling identifies miR-328 regulates cancer stem cell-like SP cells in colorectal cancer. Br J Cancer. 2012;106:1320–30. [PubMed: 22453125]

97. Li T, Lu YY, Zhao XD, Guo HQ, Liu CH, Li H, et al. MicroRNA-296–5p increases proliferation in gastric cancer through repression of Caudal-related homeobox 1. Oncogene. 2014;33:783–93. [PubMed: 23353818]

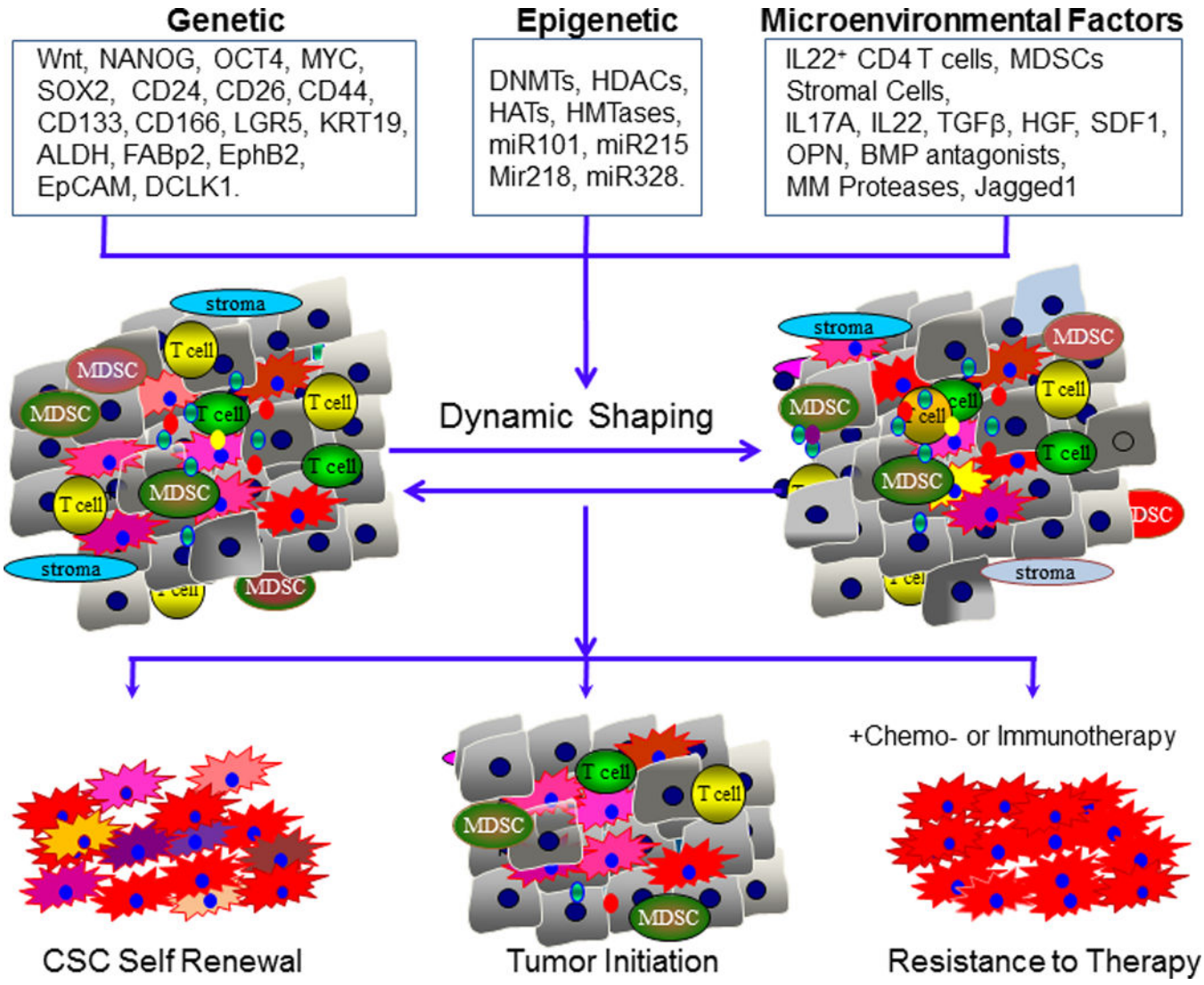

Figure 1. Model of Colorectal CSCs.

Colorectal CSCs are subsets of tumor cells that are heterogeneous and exhibits high plasticity in the tumor microenviroment. The CSC phenotypes are unstable and are continuously reshaped by genetic, epigenetic and microenvironmental factors in the tumor microenvironment. Colorectal CSCs possess self-renewal and tumor initiation potential. Chemotherapy and immunotherapy may apply a selection pressure to eliminate sensitive cells to selectively enrich a subset of the slowly dividing or quiescent CSCs.