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Review Article

Drugs in Discovery Pipeline Targeting Colorectal Cancer Stem Cells

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Abstract

Colorectal Cancer (CRC) is graded in the top three reasons of cancer-related death worldwide. Recently, Cancer Stem Cell (CSC) hypothesis has gained ground in several malignancies including CRC. Chemo and radiation therapy resistance is their most striking consequence for clinical management of cancer. To completely abolish the tumor cells it is vital to target these treatment resistant CSCs. This can be achieved by screening drugs which target and specifically kill Cancer Stem Cells. Here we review studies which suggest that colorectal cancers conform to the CSCS model and are centered on discovery of targeted drugs for their eradication.

Key Words

Colorectal Cancer Stem Cells (CRCSC); Drugs causing cell cycle arrest; Drugs targeting WNT; EMT pathways; Plant derived drugs against colorectal cancer stem cells.

Abbreviations

CSCs	:	Cancer Stem Cells (CSCs)
CRC	:	Colorectal Cancer
SCs	:	Stem Cells
ESCs	:	Embryonic Stem Cells
NSCs	:	Neural Stem Cells
HSCs	:	Hematopoietic Stem Cells
ASCs	:	Adult Stem Cells
LTSCs	:	Long-Term Stem Cells
STSCs	:	Short-Term Stem Cells
AML	:	Acute Myeloid Leukemia
mCRC	:	Metastatic Colorectal Cancer
VEGF	:	Vascular Endothelium Growth Factor
Cmab	:	Cetuximab
Pmab	:	Panitumumab
EGF	:	Epidermal Growth Factor
EGFR	:	Epidermal Growth Factor Receptor
GF	:	Griseofulvin
ND	:	Nocodazole
FZ	:	Frizzle
APC	:	Adenomatous Polyposis Coli
DC	:	Destruction Complex
TCF4	:	Transcription Factor 4
ART	:	Artesunate
NSAID	:	Non Steroidal Anti-Inflammatory Drugs
COX	:	Cyclo Oxygenase
EMT	:	Epithelial to Mesenchymal Transition
SMA	:	Smooth Muscle Actin
FN	:	Fibronectin
CKs	:	Cytokeratin's
HAD	:	Histone Deacetylase
TSA	:	Trichostatin A
VPA	:	Valproic acid
SFN	:	Sulforaphane
TET	:	Tetrandrine
LUT	:	Luteolin
AOM	:	Azomethane

Introduction

Rendering to the carcinogenesis models, cancer cells can originate from any cell of the body and have potential to grow at a specific site or become malignant, presenting unlimited proliferation following multiple mutations. Evidence has

suggested that the capability of cells to initiate a tumor is a unique characteristic of cells with stemness properties. These putative CSCs have been isolated from different cell types including CRC, a significant disease worldwide. Every year over a quarter of million people are observed to be affected by CRCs. The CRC is the second most common cause of cancer relating deaths worldwide with one million new cases diagnosed per year [1-3]. Evidence has shown that the lifetime risk of occurrence of CRC in industrialized nations is approximately 5%, and the lifetime risk of developing an adenoma is 20% [4-6]. When the cancer is only at its primary site, there is a 70% to 90 % chance for acure. However, mortality rate increases in the metastatic state. CRC is rated as one of the top three reasons for cancer-related death worldwide [4]. At ShaukatKhanum Memorial Cancer Hospital and Research Center (Pakistan), colorectal cancers are among the top five malignancies (4.8% of 16,000 cases registered from 1994-2014) (https://www.shaukatkhanum.org.pk/images/skm_img/downloads/pdf/ccrr-1994-2014.pdf).

CSCs have been observed to be resistant to chemotherapy [7] compared to non-CSC populations [8,9] leading towards the increased risk of cancer recurrence. Over the years many drugs have been developed for targeting the CSC population [10]. For this purpose, it is important to identify the regulatory mechanisms and signaling pathways which are involved in CSCs renewal. Hence, these investigations require testing the ability of drugs which kill CSCs to prevent the relapse of disease.

Stem cells

Normal stem cells

Undifferentiated cells possessing the ability to self-renew, generating one daughter cell identical to mother cell (containing self-renewal potential) and the second more specialized cell, are known as stem cells (SCs) [11]. SCs with the property of self-renewal and heterogeneity [12] have various categories with respect to their differentiation pattern. SCs are either totipotent (able to give rise to a new full organism), pluripotent (able to give rise to all tissues of the body except trophoblast of placenta) or multipotent (able to produce all cell types in a certain organ or location) [11]. Embryonic stem cells (ESCs), Neural Stem Cells (NSCs) and Hematopoietic Stem Cells (HSCs) are the best characterized types of SCs [13]. Between the developmental stages (change from totipotent to pluripotent), appoint comes when the cells preserve the state of undifferentiating by self-renewal, known as Adult Stem Cells (ASCs). However these are dedicated to the specific lineages of the organ to which they belong [14]. Studies have shown that ASCs having greater developmental potential, remain quiescent (non-dividing) for long periods of time, in G_0 phase of the cell cycle [14-17], however cellular differentiation asymmetrically takes place when they become activated by a normal need for more cells to maintain tissues, or in drastic conditions (differentiate in symmetric fashion for the maintenance of tissue homeostasis/tissue repair after injury) [18-22]. It has been shown that rapidly regenerating tissues have certain heterogeneity in cell cycle kinetics among SCs [15]. According to cellular division cycles there are two types of SCs present - long-term stem cells LTSCs, having unlimited self-renewal capacity, and short-term stem cells STSCs having limited potential to self-renew [20].

Cancer stem cells

CSCs may be the derivatives of normal self-renewing cells after abnormal differentiation pattern or from progenitor cells that might gain oncogenic activity and lose tumor suppressor function, thereby instigating de-differentiation [23-25]. These CSCs go into symmetric fashioned cell division pattern and this evidence was shown by Cicalese's group who worked on an advanced assay with a fluorescent dye by working on breast CSCs [26]. They found that normal cells mostly divide asymmetrically by losing self-renewal potential in cultures as compared to tumor cells which divide in symmetrical way but are nearly immortal, increasing five-folds after every passage [26]. In a tumor, a heterogeneous population of cells is always present and contains mature cells which express differentiation markers reflecting their origin. Furthermore, the cells which don't express surface markers have an immature morphology [27-29].The existence of CSCs was first reported by Bonnet and Dick [30]. They reported that only those cells which have$CD34^+/CD38^-$markers can produce acute myeloid leukemia (AML) in NOD/SCID mice. The first report about existence of CSCs in solid tumor came in 2003, by Al-Hajj et al. [31] who worked on breast cancer [31]. Thus far, CSCs have been found in numerous solid tumors, including lung cancer [32], colorectal cancer [33], prostate cancer [34], brain cancer [35], and melanoma [36]. It has been reported that $CD133^+CD44^+CD166^+EpCAM^+CD24^+$ are the colon cancer markers [33,37-39]. Moreover, within CRC, $CD133^+$ or $CD44^+$ cell subpopulations have been recognized as CSCs, which motivate tumor progress and recurrence [33,38,40-42].

Drugs targeting CRC and colorectal cancer stem cell (CCSCs)

Almost all CRCs start from benign polyps and then slowly progress into malignant tumors [2].Colonoscopy can be used for screening these pre-cancerous polyps, facilitating the detachment before malignant transformation [43]. Evidence indicates that liver is the most common site of Metastatic Colorectal Cancer (mCRC) [44-47]. Typically conventional adjuvant and neo-adjuvant types of chemotherapy is effective [45]. For mCRCFOLFOX (a combination of 5-flourouracil, leucovorin, and oxaliplatin) and FOLFIRI (a combination of 5-flourouracil, leucovorin, and irinotecan) is commonly administered [48]. In neo-adjuvant chemotherapy, anti-angiogenic drugs, such as Bevacizumab (Avastin®; targets the vascular endothelium growth factor, VEGF, pathway) are combined with Cetuximab (Cmab) or panitumumab (Pmab) (target epidermal growth factor (EGF) pathway by acting on

its receptor (EGFR), [47,49,50]. Todaro et al. states that these type of combination therapies work for many patients survival but are not effective for mCRC patient soften resulting in relapse [51]. This may be due to intervention of chemotherapeutic drugs with rapidly growing tumor cells, but not with CSCs, leading to tumor recurrence.

Todaro et al. [52] studied the exposure of oxaliplatin on colon CSCs derived xenografts, and found a decrease in tumor size, however there was a considerable amplification in the proportion of $CD133^+$ cells [52]. They also studied the inhibition of IL-4 signaling transduction pathway with an anti-IL-4 neutralizing antibody or anIL-4 receptor α-antagonist sensitized CSCs to chemotherapeutics through down-regulation of anti-apoptotic proteins, such as cFLIP, Bcl-xL, and PED [52]. Also a number of immune therapies and differentiation therapies have been studied against colon CSCs. Moreover, it has been detected using a tumor sphere assay that Salinomycin a polyether ionophore antibiotic isolated from *Streptomyces albus*, but not oxaliplatin or cisplatin, was able to decrease the CSCs population [53]. Salinomycin causes CCSCs apoptosis by selective target on $CD133^+$ sub-population and decreases malignancy [53]. Although these drugs have been shown to be very effective against signaling pathways specific to CSCs. Particularly, the bio molecules, Salinomycin, parthenolide and biguanide metformin have been reported to produce tumor cell death in various cancers, and may contribute to eradicate cancer more efficiently than compounds which target CSCs or regular cancer cells [54-56].

Drugs targeting CCSC cycle checkpoints

The cell cycle machinery controls cell proliferation, and cancer is a disease of inappropriate cell proliferation [57]. Although many anti-cancer drugs have been discovered, causing inhibition of cell growth at some level, however, CSCs like NSCs undergo G_0, G, S, G_2 and M phase for division with their specific property of self-renewal [58]. Reports say that AEE788 (dual receptor TKI of both EGFR and VEGFR) causes cell cycle arrest in various human cancer models [59,60] including colon cancer cells [61]. In addition, celecoxib has been studied as a COX-2 inhibitor and causes cell cycle arrest on G_0/G_1 phase thus inhibiting the transition to S phase [62]. Valverde et al. [63] used the combination therapy of AEE788 and celecoxib and demonstrated not only enhanced efficacy to inhibit colon cancer cell proliferation, migration and angiogenesis but also reduced colon CSCs sub-population by targeting stemness-related pathways [63].They also showed that AEE788 caused EGFR down-stream signaling thus constraining the cell proliferation and cell cycle arrest at G1 phase. Nonetheless, the antitumor action of this combination therapy is reliant on wild type cell K-Ras status. Another oral antifungal drug, Griseofulvin (GF) at micro molar concentrations, caused cell cycle arrest at G_2/M phase by abnormal microtubule formation, elevation of cyclin B1/cdc2 kinase activity and down-regulation of myt-1 protein expression [64]. The combined drug therapy of Griseofulvin and Nocodazole (ND) was used in athymic mice bearing human colorectal cancer xenografts, which concluded that both drugs combine causes synergistic induction of apoptosis and G_2/M arrest [64]. It has been reported previously that the inhibition mechanism of Wnt/β-catenin signaling down-regulates the expression of cdc25c, cdc2, and cyclinB, resulting in G_2 arrest. Also, the target gene of β-catenin activity, Axin2 play role in mitosis. Axin2 is highly up-regulated in colorectal cancers [65]. Furthermore they reported that NC043 (diterpinoid derivative 15-oxospiramilactone) perhaps regulates the activity of Axin2, leading to cell cycle arrest at G_2/M phase [66].

Drugs targeting Wnt signaling pathway

The activities of the Wnt signaling pathway has been reported to be involved in the regulation of normal stem cells, tumor cells and cancer stem cells [67,68]. For the control of intestinal epithelial stem cell function , Wnt signaling pathway is regulated, in which Wnt binds to Frizzle (FZ), activating one of the two pathways [69]. In first, canonical, β-catenin becomes active which is being regulated by highly conserved proteins (controls cellular proliferation) and in second, non-canonical, Ca^{2+} is involved (pedals cellular movement and polarity) [70-72]. In the canonical/β-catenin pathway, when Wnt signals are absent, the tumor suppressor, adenomatous polyposis coli (APC) and Axin/Axin2 proteins in the destruction complex (DC) (composed of the tumor-suppressor protein APC, glycogen synthase kinase3β and axin), become activated for targeting β-catenin [73,74]. The outcome is serine phosphorylation of β-catenin, recognition by an E3 ubiquitin ligase, and its subsequent degradation [68]. If Wnt signal is present, the kinase activity of DC is blocked and β-catenin remain sun-phosphorylated, resulting in accumulation of β-catenin in the nucleus [68]. Hence, β-catenin remains bound to the transcription factor TCF4 that can activate downstream target genes such as the proto-oncogene MYC promoting entry of the cell into the S-phase of the cell cycle [75]. Evidence suggests that mutations in APC (tumor suppressor gene), serine/threonine residues, scaffolding protein Axin2 or the formation of nuclear TCF/β-catenin complexes results in uncontrolled TCF target gene transcription [76-79].

For colorectal cancers, associated with a hyperactive Wnt/β-catenin pathway, disruption of the signaling pathway provides a target for new anti-cancer drugs [80]. It has been reported that in the development of cancer, the mutation of Wnt/β-catenin components reduce normal ubiquitination and degradation of β-catenin protein [81,82]. Lin et al. [83] used Artesunate (ART) *in vitro* on CLY cell line. They reported that ART treatment caused β-catenin to translocate from the nucleus to adherent junctions of membrane which switched the function of β-catenin from promoting target genes expression to enhancing cell adherence. ART helps in decreasing mRNA level of Wnt/β-catenin target genes such as c-myc and surviving, thus promotes anti-tumor activity [83].

Aspirin (non-steroidal anti-inflammatory drug) constrains β-catenin/TCF4 signaling in colon cancer cells [84-86].

Coxibs (such as celecoxib and rofecoxib) also affect CCSCs independently of COX-2 expression [87]. Tuynman et al. [88] suggested that celecoxib limits c-Met-dependent signaling, resulting in down-regulation of oncogenic Wnt signaling in CRC [88]. Similarly, Salinomycin, has been reported to inhibit CSCs growth in different types of human cancers including CRC, almost certainly by snooping with ABC drug transporters, the Wnt/β-catenin signaling pathway, and other CSC pathways [89].

Nangia-Makker et al. [90] studied the effect of metformin in combination with 5-FU and oxaliplatin (FuOx) on CCSCc by down-regulation of Wnt/β-catenin signaling pathway [90]. An et al. [91] studied the effect of a LP SN (*Lactobacillus plantarum* (LP) supernatant (SN) with combination of 5-FU, and their results showed that LP SN can increase the therapeutic effect of 5-FU for colon cancer, and lessen CCSC by reversing the development of resistance to anti-cancer drugs. So, they concluded that probiotic (a microorganism introduced into the body for its beneficial qualities) substances may be a useful therapeutic alternatives as bio-therapeutics for chemo-resistant CRC [91].

Drugs targeting EMT pathway

The Epithelial-to-Mesenchymal Transition (EMT) program is a diverse series of events that outcomes in the epithelial cells' losing their epithelial characters and obtaining numerous properties of mesenchymal cells (including increased expression of vimentin, Smooth Muscle Actin (SMA), FibroNectin (FN), matrix metalloproteinases and N-cadherin same as shift to fibroblastic morphology in monolayer culture [92-94]. Epithelial cells become more motile and lose their cell to cell polarity and cytoskeletal reorganization because they transit to mesenchymal cells by decreasing expression of epithelial Cytokeratin's (CKs), like CK-8 and CK-18, in addition to reduced expression of cell-to-cell adhesion proteins (e.g., E-cadherin and plankoglobin (leading towards disassembling of adherens junctions and desmosomes respectively) [92-94]. Transcriptional repression of E-cadherin promotors, the proteolytic cleavage of E-cadherin protein and any somatic or chromosomal mutations can be the reason of inactivation of E-cadherin [95].

Throughout cancer development, various stimuli can trigger EMT by secreting many molecules, such as Hedgehog, EGF, hepatocyte growth factor, and members of the TGF-β, Wnt, fibroblast growth factor, and insulin-like growth factor families [96]. Recent studies have highlighted a link between EMT and CSC formation that it is sufficient to endow differentiated normal and cancer cells with stem cell properties. This relationship between EMT and CSCs might have many implications in tumor progression; hence explore the importance of these links in the development of improved antitumor therapies. EMT pathway causes drug resistance in CSCs and ultimately results in tumor relapse [96]. Salinomycin prompts expression of E-cadherin by RNA interference and down-regulates expression of vimentin in HT29 tumorous cell line [53]. Histone deacetylase (HDA) inhibitors like trichostatin A (TSA) and valproic acid (VPA) have been recently reported to induce mesenchymal features in CRC by decrease in E-cadherin and increase in vimentin expression at mRNA and protein levels [97]. A study reported that PS341 (Bortezomib) is first proteasome drug that inhibit mCRC by inhibition of cell proliferation, Epithelial-Mesenchymal Transition (EMT), the expression of stemness-related genes, cell migration and invasiveness [98].

Plant Derived drug therapies

Natural extracts causing CCSC cycle arrest

Resveratrol is a naturally occurring polyphenol (derived from grapes) [99,100] with cancer chemo-preventive properties [101]. The effect of this compound on human colonic adeno carcinoma cell line CACO-2 is an increase of apoptotic activity of cells after 24 and 48h of treatment with 200 μmol/L resveratrol, by measuring caspase-3 activity. A disrupted cell cycle progression from S to G_2 phase was observed up to 50 μmol/L concentration (cell cycle arrest), while higher concentrations led to reversal of S-phase arrest. Furthermore, down-regulation of cyclin D_1/CDK_4 protein complex was also observed [102]. Another group studied the effect of Resveratrol on HCT116 colon cancer cell line, and they concluded that a dose dependent activity of this compound on CSC of CRC, and the cell cycle arrest at G0/G1 phase with promotion of cell apoptosis [103]. Sulforaphane (SFN) (naturally occurring Isothiocyanate, found in cruciferous vegetables) has also anti-tumoral properties causing cell cycle arrest at G_2/ M phase in HT29 human colon cancer cell line, correlated with greater than before expression of cyclin A and B1 [104-106]. A number of studies have also reported that sulforaphane may target CSCs in different types of cancer through modulation of NF-κB, SHH, epithelial-mesenchymal transition and Wnt/β-catenin pathways [107]. Flavonoids, present in many fruits and vegetables, have been comprehensively studied [108-112]. It has been reported that flavonoids (like Quercetin) inhibits cell cycle progression at G1/S phase by effect on tyrosine kinase as well as other kinase activities [113-115] and another Genistein arrest cell cycle at G2/M phase by specific inhibition of tyrosine kinase activity [116]. Quercetin has also been reported to obstruct G2/M phase of CSCs [117].

Plant derived drug therapies against Wnt pathway

A natural product bis benzyl isoquinoline alkaloid tetrandrine (TET) exhibits anti-cancer activity against colon cancer cell line HCT116. TET effectively targets Wnt/β-catenin signaling pathway in human colon cancer cells by inhibiting the activity of TCF/LEF reporter, TOP-Luc and Myc/Max-Luc of a well characterized downstream target c-Myc. TET's synergizes with 5-FU in an anti-proliferation effect on human

colon cancer cells [118]. Luteolin (LUT), a flavonoid, which inhibited colon carcinoma by reducing Azomethane (AOM)-induced cell proliferation by the involvement of key components of Wnt signaling pathway, β-catenin, GSK-3β enzyme and cyclin D1 [119]. Similarly, Fisetin (flavonoid) treatment resulted in down-regulation of COX2 protein expression and Wnt-signaling activity through down-regulation of β-catenin and TCF4 (T cell factor 4) and decreased the expression of target genes such as cyclin D1 and matrix metalloproteinase-7. Fisetin treatment suppresses the growth of colon cancer cells by inhibition of COX2 and Wnt/EGFR/NF-κB signaling pathways [120]. Curcumin and piperinehave also been described to inhibit Wnt/β-catenin pathway in CSCs [121].

Plant derived drug therapies against EMT pathway

It has been reported previously, that Curcumin (derivative of rhizomes of plant *curcuma longa*) and its analogues have been shown to be effective in dropping tumor relapse by targeting the CSC population on signaling pathways (Wnt/β-catenin, Notch and Hedgehog) and EMT at multiple levels [122]. Resveratrol has also been reported to suppress EMT trough TGF-β1/ SMADS signaling pathway mediated SNAIL/ E-cadherin expression [123].

Conclusion

Targeted therapy by identifying new targets in colorectal cancer stem cells followed by discovery of novel drugs to directly kill these resistant cell populations is required to eradicate the disease completely.

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References

1. Nelson H, Petrelli N, Carlin A, Couture J, Fleshman J, et al. (2001) Guidelines 2000 for colon and rectal cancer surgery. J Natl Cancer Inst 93: 583-596.
2. Sancho E, Batlle E, Clevers H (2004) Signaling pathways in intestinal development and cancer. Annu Rev Cell Dev Biol 20: 695-723.
3. Siegel RL, Miller KD, and Jemal A, (2016) Cancer statistics. CA cancer journal for clinicians 66: 7-30.
4. Sears CL, Garrett WS (2014) Microbes, microbiota, and colon cancer. Cell Host Microbe 15: 317-328.
5. Rasool S, Kadla SA, Rasool V, Ganai BA (2013) A comparative overview of general risk factors associated with the incidence of colorectal cancer. Tumour Biol 34: 2469-2476.
6. Esteban-Jurado C, Garre P, Vila M, Lozano JJ, Pristoupilova A, et al. (2014) New genes emerging for colorectal cancer predisposition. World J Gastroenterol 20: 1961-1971.
7. Dean M, Fojo T, Bates S (2005) Tumour stem cells and drug resistance. Nat Rev Cancer 5: 275-284.
8. LaBarge MA (2010) The difficulty of targeting cancer stem cell niches. Clin Cancer Res 16: 3121-3129.
9. Wang Z, Li Y, Ahmad A, Azmi AS, Kong D, et al. (2010) Targeting miRNAs involved in cancer stem cell and EMT regulation: An emerging concept in overcoming drug resistance. Drug Resist Updat 13: 109-118.
10. Chen K, Huang YH, Chen JL (2013) Understanding and targeting cancer stem cells: therapeutic implications and challenges. Acta Pharmacol Sin 34: 732-740.
11. Lin H, Schagat T (1997) Neuroblasts: a model for the asymmetric division of stem cells. Trends Genet 13: 33-39.
12. Al-Hajj M, Clarke MF (2004) Self-renewal and solid tumor stem cells. Oncogene 23: 7274-7282.
13. Ramalho-Santos M, Yoon S, Matsuzaki Y, Mulligan RC, Melton DA (2002) "Stemness": transcriptional profiling of embryonic and adult stem cells. Science 298: 597-600.
14. Krause DS (2002) Plasticity of marrow-derived stem cells. Gene Ther 9: 754-758.
15. Fuchs E (2009) The tortoise and the hair: slow-cycling cells in the stem cell race. Cell 137: 811-819.
16. Kiel MJ, He S, Ashkenazi R, Gentry SN, Teta M, et al. (2007) Haematopoietic stem cells do not asymmetrically segregate chromosomes or retain BrdU. Nature 449: 238-242.
17. Passegué E, Wagers AJ, Giuriato S, Anderson WC, Weissman IL (2005) Global analysis of proliferation and cell cycle gene expression in the regulation of hematopoietic stem and progenitor cell fates. J Exp Med 202: 1599-1611.
18. Morshead CM, Reynolds BA, Craig CG, McBurney MW, Staines WA, et al. (1994) Neural stem cells in the adult mammalian forebrain: a relatively quiescent subpopulation of subependymal cells. Neuron 13: 1071-1082.
19. Zhang R, Zhang Z, Zhang C, Zhang L, Robin A, et al. (2004) Stroke transiently increases sub ventricular zone cell division from asymmetric to symmetric and increases neuronal differentiation in the adult rat. J Neurosci 24: 5810-5815.
20. Weissman IL (2000) Stem cells: units of development, units of regeneration, and units in evolution. Cell 100: 157-168.
21. Holtzer H (1978) Cell lineages, stem cells and the 'quantal'cell cycle concept. Stem cells and tissue homeostasis 1-27.
22. Leblond CP (1964) Classification of Cell Populations on the Basis Of Their Proliferative Behavior. Natl Cancer Inst Monogr 14: 119-150.
23. Vicente-Dueñas C, Gutiérrez de Diego J, Rodríguez FD, Jiménez R, Cobaleda C (2009) The role of cellular plasticity in cancer development. Curr Med Chem 16: 3676-3685.
24. Singh A, Settleman J (2010) EMT, cancer stem cells and drug resistance: an emerging axis of evil in the war on cancer. Oncogene 29: 4741-4751.
25. Ito M, Yang Z, Andl T, Cui C, Kim N, et al. (2007) Wnt-dependent de novo hair follicle regeneration in adult mouse skin after wounding. Nature 447: 316-320.
26. Cicalese A, Bonizzi G, Pasi CE, Faretta M, Ronzoni S, et al. (2009) The tumor suppressor p53 regulates polarity of self-renewing divisions in mammary stem cells. Cell 138: 1083-1095.
27. Fidler IJ, Kripke ML (1977) Metastasis results from preexisting variant cells within a malignant tumor. Science 197: 893-895.
28. Fidler IJ, Hart IR (1982) Biological diversity in metastatic neoplasms: origins and implications. Science 217: 998-1003.
29. Heppner GH (1984) Tumor heterogeneity. Cancer Res 44: 2259-2265.
30. Bonnet D, Dick JE (1997) Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat Med 3: 730-737.
31. Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF (2003) Prospective identification of tumorigenic breast cancer cells. Proc Natl Acad Sci USA 100: 3983-3988.

32. Kim CF, Jackson EL, Woolfenden AE, Lawrence S, Babar I, et al. (2005) Identification of bronchioalveolar stem cells in normal lung and lung cancer. Cell 121: 823-835.

33. Dalerba P, Dylla SJ, Park IK, Liu R, Wang X, et al. (2007) Phenotypic characterization of human colorectal cancer stem cells. Proc Natl Acad Sci U S A 104: 10158-10163.

34. Collins AT, Berry PA, Hyde C, Stower MJ, Maitland NJ (2005) Prospective identification of tumorigenic prostate cancer stem cells. Cancer Res 65: 10946-10951.

35. Marsden CG, Wright MJ, Pochampally R, Rowan BG (2009) Breast tumor-initiating cells isolated from patient core biopsies for study of hormone action. Methods Mol Biol 590: 363-375.

36. Fang D, Nguyen TK, Leishear K, Finko R, Kulp AN, et al. (2005) A tumorigenic subpopulation with stem cell properties in melanomas. Cancer Res 65: 9328-9337.

37. Yeung TM, Gandhi SC, Wilding JL, Muschel R, Bodmer WF (2010) Cancer stem cells from colorectal cancer-derived cell lines. Proc Natl Acad Sci U S A 107: 3722-3727.

38. O'Brien CA, Pollett A, Gallinger S, Dick JE (2007) A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. Nature 445: 106-110.

39. Du L, Wang H, He L, Zhang J, Ni B, et al. (2008) CD44 is of functional importance for colorectal cancer stem cells. Clin Cancer Res 14: 6751-6760.

40. Ricci-Vitiani L, Lombardi DG, Pilozzi E, Biffoni M, Todaro M, et al. (2007) Identification and expansion of human colon-cancer-initiating cells. Nature 445: 111-115.

41. Ieta K, Tanaka F, Haraguchi N, Kita Y, Sakashita H, et al. (2008) Biological and genetic characteristics of tumor-initiating cells in colon cancer. Ann Surg Oncol 15: 638-648.

42. Haraguchi N, Ohkuma M, Sakashita H, Matsuzaki S, Tanaka F, et al. (2008) CD133+ CD44+ population efficiently enriches colon cancer initiating cells. Annals of surgical oncology 15: 2927-2933.

43. Young PE, Womeldorph CM (2013) Colonoscopy for colorectal cancer screening. J Cancer 4: 217-226.

44. Fong Y, Cohen AM, Fortner JG, Enker WE, Turnbull AD, et al. (1997) Liver resection for colorectal metastases. J Clin Oncol 15: 938-946.

45. Lubezky N, Geva R, Shmueli E, Nakache R, Klausner JM, et al. (2009) Is there a survival benefit to neoadjuvant versus adjuvant chemotherapy, combined with surgery for resectable colorectal liver metastases?. World journal of surgery 33: 1028-1034.

46. Simmonds P, Primrose J, Colquitt J, Garden O, Poston G, et al. (2006) Surgical resection of hepatic metastases from colorectal cancer: a systematic review of published studies., Brit J Cancer 94: 982-999.

47. Weiss L, Grundmann E, Torhorst J, Hartveit F, Moberg I, et al. (1986) Haematogenous metastatic patterns in colonic carcinoma: an analysis of 1541 necropsies. J Pathol 150: 195-203.

48. Lenz HJ (2008) First-line combination treatment of colorectal cancer with hepatic metastases: choosing a targeted agent. Cancer Treat Rev 34: S3-S7.

49. Cunningham D, Humblet Y, Siena S, Khayat D, Bleiberg H, et al. (2004) Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. N Engl J Med 351: 337-345.

50. Hurwitz H, Fehrenbacher L, Novotny W, Cartwright T, Hainsworth J, et al. (2004) Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med 350: 2335-2342.

51. Todaro M, Francipane MG, Medema JP, Stassi G (2010) Colon cancer stem cells: promise of targeted therapy. Gastroenterology 138: 2151-2162.

52. Todaro M, Alea MP, Di Stefano AB, Cammareri P, Vermeulen L, et al. (2007) Colon cancer stem cells dictate tumor growth and resist cell death by production of interleukin-4. Cell Stem Cell 1: 389-402.

53. Dong TT, Zhou HM, Wang LL, Feng B, Lv B, et al. (2011) Salinomycin selectively targets 'CD133+' cell subpopulations and decreases malignant traits in colorectal cancer lines. Ann Surg Oncol 18: 1797-1804.

54. Naujokat C, Fuchs D, Opelz G (2010) Salinomycin in cancer: A new mission for an old agent. Mol Med Rep 3: 555-559.

55. Mathema VB, Koh YS, Thakuri BC, Sillanpää M (2012) Parthenolide, a sesquiterpene lactone, expresses multiple anti-cancer and anti-inflammatory activities. Inflammation 35: 560-565.

56. Kourelis TV, Siegel RD (2012) Metformin and cancer: new applications for an old drug. Med Oncol 29: 1314-1327.

57. Kastan MB, Bartek J (2004) Cell-cycle checkpoints and cancer. Nature 432: 316-323.

58. Velasco-Velázquez MA, Yu Z, Jiao X, Pestell RG (2009) Cancer stem cells and the cell cycle: targeting the drive behind breast cancer. Expert Rev Anticancer Ther 9: 275-279.

59. Park YW, Younes MN, Jasser SA, Yigitbasi OG, Zhou G, et al. (2005) AEE788, a dual tyrosine kinase receptor inhibitor, induces endothelial cell apoptosis in human cutaneous squamous cell carcinoma xenografts in nude mice. Clinical cancer research 11: 1963-1973.

60. Barbarroja N, Torres LA, Rodriguez-Ariza A, Valverde-Estepa A, Lopez-Sanchez LM, et al. (2010) AEE788 is a vascular endothelial growth factor receptor tyrosine kinase inhibitor with antiproliferative and proapoptotic effects in acute myeloid leukemia. Experimental hematology 38: 641-652.

61. Venkatesan P, Bhutia SK, Singh AK, Das SK, Dash R, et al. (2012) AEE788 potentiates celecoxib-induced growth inhibition and apoptosis in human colon cancer cells. Life Sci 91: 789-799.

62. Grösch S, Tegeder I, Niederberger E, Bräutigam L, Geisslinger G (2001) COX-2 independent induction of cell cycle arrest and apoptosis in colon cancer cells by the selective COX-2 inhibitor celecoxib. FASEB J 15: 2742-2744.

63. Valverde A, Peñarando J, Cañas A, López-Sánchez LM, Conde F, et al. (2015) Simultaneous inhibition of EGFR/VEGFR and cyclooxygenase-2 targets stemness-related pathways in colorectal cancer cells. PloS one 10: 0131363.

64. Ho YS, Duh JS, Jeng JH, Wang YJ, Liang YC, et al. (2001) Griseofulvin potentiates antitumorigenesis effects of nocodazole through induction of apoptosis and G_2/M cell cycle arrest in human colorectal cancer cells. International journal of cancer 91: 393-401.

65. Lustig B, Jerchow B, Sachs M, Weiler S, Pietsch T, et al. (2002) Negative feedback loop of Wnt signaling through upregulation of conductin/axin2 in colorectal and liver tumors. Mol Cell Biol 22: 1184-1193.

66. Wang W, Liu H, Wang S, Hao X, Li L (2011) A diterpenoid derivative 15-oxospiramilactone inhibits Wnt/Î²-catenin signaling and colon cancer cell tumor genesis. Cell Res 21: 730-740.

67. Valkenburg KC, Graveel CR, Zylstra-Diegel CR, Zhong Z, Williams BO (2011) Wnt/Î²-catenin Signaling in Normal and Cancer Stem Cells. Cancers (Basel) 3: 2050-2079.

68. Reya T, Clevers H (2005) Wnt signaling in stem cells and cancer. Nature 434: 843-850.

69. Tuna M, Amos CI, (2011) The Role of Micro RNAs in Regulating Cancer Stem Cells. INTECH Open Access Publisher.

70. Reya T, Morrison SJ, Clarke MF, Weissman IL (2001) Stem cells, cancer, and cancer stem cells. Nature 414: 105-111.

71. Katoh M, Katoh M (2007) WNT signaling pathway and stem cell signaling network. Clin Cancer Res 13: 4042-4045.

72. Kohn AD, Moon RT (2005) Wnt and calcium signaling: beta-catenin-independent pathways. Cell Calcium 38: 439-446.

73. Amit S, Hatzubai A, Birman Y, Andersen JS, Ben-Shushan E, et al. (2002) Axin-mediated CKI phosphorylation of beta-catenin at Ser 45: a molecular switch for the Wnt pathway. Genes Dev 16: 1066-1076.

74. Liu C, Li Y, Semenov M, Han C, Baeg GH, et al. (2002) Control of beta-catenin phosphorylation/degradation by a dual-kinase mechanism. Cell 108: 837-847.

75. He TC, Sparks AB, Rago C, Hermeking H, Zawel L, et al. (1998) Identification of c-MYC as a target of the APC pathway. Science 281: 1509-1512.

76. Näthke IS (2004) The adenomatous polyposis coli protein: the Achilles heel of the gut epithelium. Annu Rev Cell Dev Biol 20: 337-366.

77. Morin PJ, Sparks AB, Korinek V, Barker N, Clevers H, et al. (1997) Activation of beta-catenin-Tcf signaling in colon cancer by mutations in beta-catenin or APC. Science 275: 1787-1790.

78. Liu W, Dong X, Mai M, Seelan RS, Taniguchi K, et al. (2000) Mutations in AXIN2 cause colorectal cancer with defective mismatch repair by activating beta-catenin/TCF signalling. Nat Genet 26: 146-147.

79. Fearon ER, Vogelstein B (1990) A genetic model for colorectal tumorigenesis. Cell 61: 759-767.

80. Dihlmann S, von Knebel Doeberitz M (2005) Wnt/beta-catenin-pathway as a molecular target for future anti-cancer therapeutics. Int J Cancer 113: 515-524.

81. Behrens J, Lustig B (2004) The Wnt connection to tumorigenesis. Int J Dev Biol 48: 477-487.

82. Willert K, Jones KA (2006) Wnt signaling: is the party in the nucleus? Genes Dev 20: 1394-1404.

83. Li LN, Zhang HD, Yuan SJ, Tian ZY, Wang L, et al. (2007) Artesunate attenuates the growth of human colorectal carcinoma and inhibits hyperactive Wnt/beta-catenin pathway. Int J Cancer 121: 1360-1365.

84. Dihlmann S, Siermann A, von Knebel Doeberitz M (2001) The nonsteroidal anti-inflammatory drugs aspirin and indomethacin attenuate beta-catenin/TCF-4 signaling. Oncogene 20: 645-653.

85. Nath N, Kashfi K, Chen J, Rigas B, (2003) Nitric oxide-donating aspirin inhibits β-catenin/T cell factor (TCF) signaling in SW480 colon cancer cells by disrupting the nuclear β-catenin–TCF association. Proceedings of the National Academy of Sciences 100: 12584-12589.

86. Dashwood WM, Orner GA, Dashwood RH, (2002) Inhibition of β-catenin/Tcf activity by white tea, green tea, and epigallocatechin-3-gallate (EGCG): minor contribution of H 2 O 2 at physiologically relevant EGCG concentrations. Biochemical and biophysical research communications 296: 584-588.

87. Smith ML, Hawcroft G, Hull M, (2000) The effect of non-steroidal anti-inflammatory drugs on human colorectal cancer cells: evidence of different mechanisms of action. European journal of cancer 36: 664-674.

88. Tuynman JB, Vermeulen L, Boon EM, Kemper K, Zwinderman AH, et al. (2008) Cyclooxygenase-2 inhibition inhibits c-Met kinase activity and Wnt activity in colon cancer. Cancer Res 68: 1213-1220.

89. Naujokat C, Steinhart R (2012) Salinomycin as a drug for targeting human cancer stem cells. J Biomed Biotechnol 2012: 950658.

90. Nangia-Makker P, Yu Y, Vasudevan A, Farhana L, Rajendra SG, et al. (2014) Metformin: a potential therapeutic agent for recurrent colon cancer. PLoS One 9: e84369.

91. An J, Ha EM (2016) Combination therapy of Lactobacillus plantarum supernatant and 5-fluouracil increases chemo-sensitivity in colorectal cancer cells. J Microbiol Biotechnol .

92. Hay ED (1995) An overview of epithelio-mesenchymal transformation. Acta Anat (Basel) 154: 8-20.

93. Shook D, Keller R (2003) Mechanisms, mechanics and function of epithelial-mesenchymal transitions in early development. Mech Dev 120: 1351-1383.

94. Thiery JP (2003) Epithelial-mesenchymal transitions in development and pathologies. Curr Opin Cell Biol 15: 740-746.

95. Agiostratidou G, Hulit J, Phillips GR, Hazan RB, (2007) Differential cadherin expression: potential markers for epithelial to mesenchymal transformation during tumor progression. Journal of mammary gland biology and neoplasia 12: 127-133.

96. Moustakas A, Heldin CH, (2007) Signaling networks guiding epithelial–mesenchymal transitions during embryogenesis and cancer progression., Cancer science 98: 1512-1520.

97. Ji M, Lee EJ, Kim KB, Kim Y, Sung R, et al. (2015) HDAC inhibitors induce epithelial-mesenchymal transition in colon carcinoma cells. Oncol Rep 33: 2299-2308.

98. Yang Z, Liu S (2016) PS341 inhibits hepatocellular and colorectal cancer cells through the FOXO3/CTNNB1 signaling pathway. Sci Rep 6: 22090.

99. Frémont L (2000) Biological effects of resveratrol. Life Sci 66: 663-673.

100. Li Y, Wicha MS, Schwartz SJ, Sun D (2011) Implications of cancer stem cell theory for cancer chemoprevention by natural dietary compounds. J Nutr Biochem 22: 799-806.

101. Jang M, Cai L, Udeani GO, Slowing KV, Thomas CF, et al. (1997) Cancer chemo preventive activity of resveratrol, a natural product derived from grapes. Science 275: 218-220.

102. Wolter F, Akoglu B, Clausnitzer A, Stein J, (2001) Downregulation of the cyclin D1/Cdk4 complex occurs during resveratrol-induced cell cycle arrest in colon cancer cell lines. J Nutri 131: 2197-2203.

103. Yang J, Liu J, Lyu X, Fei S (2015) Resveratrol inhibits cell proliferation and up-regulates MICA/B expression in human colon cancer stem cells. Chin J Cell Mol Immu 31: 889-893.

104. Thornalley PJ (2002) Isothiocyanates: mechanism of cancer chemopreventive action. Anticancer Drugs 13: 331-338.

105. Krul C, Humblot C, Philippe C, Vermeulen M, van Nuenen M, et al. (2002) Metabolism of sinigrin (2-propenyl glucosinolate) by the human colonic microflora in a dynamic in vitro large-intestinal model. Carcinogenesis 23: 1009-1016.

106. Gamet-Payrastre L, Li P, Lumeau S, Cassar G, Dupont MA, et al. (2000) Sulforaphane, a naturally occurring isothiocyanate, induces cell cycle arrest and apoptosis in HT29 human colon cancer cells. Cancer research 60: 1426-1433.

107. Li Y, Zhang T (2013) Targeting cancer stem cells with sulforaphane, a dietary component from broccoli and broccoli sprouts. Future Oncol 9: 1097-1103.

108. Ambrose AM, Robbins DJ, Deeds F (1952) Comparative toxicites of quercetin and quercitrin. J Am Pharm Assoc Am Pharm Assoc 41: 119-122.

109. Bjeldanes LF, Chang GW (1977) Mutagenic activity of quercetin and related compounds. Science 197: 577-578.

110. Kim JH, Kim SH, Alfieri AA, Young CW (1984) Quercetin, an inhibitor of lactate transport and a hyperthermic sensitizer of HeLa cells. Cancer Res 44: 102-106.

111. MacGregor JT (1986) Genetic toxicology of dietary flavonoids. Prog Clin Biol Res 206: 33-43.

112. Suolinna EM, Buchsbaum RN, Racker E (1975) The effect of flavonoids on aerobic glycolysis and growth of tumor cells. Cancer Res 35: 1865-1872.

113. Hosokawa N, Hosokawa Y, Sakai T, Yoshida M, Marui N, et al. (1990) Inhibitory effect of quercetin on the synthesis of a possibly cell-cycle-related 17-kDa protein, in human colon cancer cells. International Journal of Cancer 45: 1119-1124.

114. End DW, Look RA, Shaffer NL, Balles EA, Persico FJ, (1987) Non-selective inhibition of mammalian protein kinases by flavinoids *in vitro*. Research communications in chemical pathology and pharmacology 56: 75-86.

115. Graziani Y, Chayoth R, Karny N, Feldman B, Levy J, (1982) Regulation of protein kinases activity by quercetin in Ehrlich ascites tumor cells. Biochimica et Biophysica Acta 714: 415-421.

116. Matsukawa Y, Marui N, Sakai T, Satomi Y, Yoshida M, et al. (1993) Genistein arrests cell cycle progression at G2-M. Cancer Res 53: 1328-1331.

117. Shan BE, Wang MX, Li RQ, (2009) Quercetin inhibit human SW480 colon cancer growth in association with inhibition of cyclin D1 and survivin expression through Wnt/β-catenin signaling pathway. Cancer investigation 27: 604-612.

118. He BC, Gao JL, Zhang BQ, Luo Q, Shi Q, et al. (2011) Tetrandrine inhibits Wnt/ß-catenin signaling and suppresses tumor growth of human colorectal cancer. Molecular pharmacology 79: 211-219.

119. Ashokkumar P, Sudhandiran G, (2011) Luteolin inhibits cell proliferation during Azoxymethane-induced experimental colon carcinogenesis via Wnt/β-catenin pathway. Investigational new drugs 29: 273-284.

120. Suh Y, Afaq F, Johnson JJ, Mukhtar H (2009) A plant flavonoid fisetin induces apoptosis in colon cancer cells by inhibition of COX2 and Wnt/EGFR/NF-kappaB-signaling pathways. Carcinogenesis 30: 300-307.

121. Prud'homme GJ (2012) Cancer stem cells and novel targets for antitumor strategies. Curr Pharm Des 18: 2838-2849.

122. Ramasamy TS, Ayob AZ, Myint HHL, Thiagarajah S, Amini F, (2015) Targeting colorectal cancer stem cells using curcumin and curcumin analogues: insights into the mechanism of the therapeutic efficacy. Cancer Cell International 15: 1-15.

123. Ji Q, Liu X, Han Z, Zhou L, Sui H, et al. (2015) Resveratrol suppresses epithelial-to-mesenchymal transition in colorectal cancer through TGF-beta1/Smads signaling pathway mediated Snail/E-cadherin expression. BMC Cancer 15: 97.