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Targeting cancer stem cells as therapeutic approach in the treatment of colorectal cancer

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ABSTRACT

Colorectal cancer is one of the most common cancers globally. A large portion of colorectal cancer patients who are treated with conventional chemotherapy eventually develop local recurrence or metastases. The failure of a complete cure in colorectal cancer patients may be related to the lack of complete eradication of cancer stem cells when using conventional therapy. Colorectal cancer stem cells comprise a small population of tumor cells that possess the properties of rapid proliferation and differentiation. The colorectal cancer stem cells are also phenotypically and molecularly distinct, and resistant to conventional chemo-radiotherapy. Therefore, it is important to identify approaches in combination with conventional therapy for targeting and eradicating cancer cells. The aim of this review was to summarize the main findings of recent studies on targeting colorectal cancer stem cells as a novel therapeutic approach in colorectal cancer treatment.

1. Introduction

Colorectal cancer (CRC) is one of the most common cancers globally with a similar incidence rate in men and women (Kuipers et al., 2015). CRC has an enormous health burden of morbidity and mortality (Kuipers et al., 2015). More than one million new cases of CRC are diagnosed annually globally (Coppedè et al., 2014). Similar conventional chemotherapeutic approach are commonly used for treatment of CRC patients (Patel and Ahnen, 2018; Zhang et al., 2016). In addition to the potential of a poor response, chemotherapy-induced toxicity may limit continuing conventional chemotherapy alone in CRC patients. As a consequence, it is important to identify novel and specific therapeutic approaches in combination with conventional therapy for targeting and eradicating all cancer cells.

There is recent evidence that a small population of cancer cells, which are termed tumor initiating cells with stem cell-like properties, or cancer stem cells, are present in tumors that are responsible for initiating and maintaining tumor growth. Colorectal cancer stem cells (CSCs) are phenotypically and molecularly distinct from the other cells within the tumor; they are resistant to conventional chemo-radiotherapy, and are largely responsible for post-treatment recurrence (Cojoc et al., 2015; Vaiopoulos et al., 2012). With respect to CSCs resistance to conventional therapy, it has been reported that targeted therapy for CSCs eradication can lead to improved survival (Shackleton et al., 2009). We have summarized the main findings of recent studies that have reported the targeting of CSCs as a novel therapeutic approach in CRC treatment.

2. Characteristics of cancer stem cells

It has been shown that tumors are composed of cells with heterogenous properties that can easily adapt to their environment and escape the human immune system. Cancer stem cells have the ability to renew and differentiate. The tumor microenvironment plays an essential role

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in determining the progress and response to treatment of various cancers. The tumor microenvironment can induce conversion of non-CSCs to CSCs. There are many factors considered to be important for this conversion and various studies have linked alteration of tumor microenvironment to the expression of different cancer stem cell markers. These CSC within tumors may be exposed to conditions that are associated with developing genomic instability and hence chemotherapy resistance. The CSCs located in CRC can be identified using different cell surface markers (Vaiopoulos et al., 2012), that can also be used for targeting with various drugs (Dalerba et al., 2007). The well-known surface markers in solid tumors are CD133, CD24, CD44, CD29, ALDH1 and EpCAM (Dalerba et al., 2007; Das et al., 2010; Ricci-Vitiani et al., 2007; Todaro et al., 2010). Leucine-rich repeat-containing G-protein receptor 5 (Lgr5) is another marker that has recently been evaluated in colorectal cancer as well. The core stem cell in colorectal cancerous tissue that is Lgr5 positive, has been shown to play role in tumor initiation, growth and even metastasis (Yun and Lin, 2014). By targeting these markers and eliminating the CSCs, usually the tumor will no longer be able to demonstrate its stem cell-like properties. However recently, it has been proven that targeting a single cancer stem cell will not be effective enough in treating cancer. The best example regarding this latter issue is targeting Lgr5 positive cells. Targeting Lgr5 only temporarily provides beneficial affects, with tumors overcoming this by increasing plasticity of the remaining cancer cells. Although the exact mechanism of this is not known, the specific tumor microenvironment is the main suspect, and further study is needed to prove the 'microenvironment theory' (Novellasdemunt et al., 2015). Tumorigenic signals from the tumor microenvironment and mesenchyme can induce cancer stemness in differentiated cells. This process highlights the role of targeting the microenvironment as well as multitargeting of cancer stem cells in cancerous tissue (Novellasdemunt et al., 2015).

Micro-RNAs are another interesting aspect of CSC in CRC. miRNAs play an important role in suppressing tumor suppressor genes or activating oncogenes. These small RNAs can act as a potential target for therapy, and controlling oncogene expression. The expression of several microRNAs in CSCs are related to specific features. As an example, the expression of miR-21 is seen in undifferentiated CSCs, and is related to resistance to chemotherapy. Targeting such microRNAs may result in a reduced expression of CSC markers and induction of differentiation. These differentiated cells are more prone to response to chemotherapy (Yu et al., 2013).

Hypoxia is a well-known factor involved in angiogenesis during tumor development (Li et al., 2009). Inhibition of apoptosis and reducing oxygen demand is crucial for surviving from hypoxic conditions. Also, hypoxia inducible factors which are produced under hypoxic conditions are responsible for stimulating the development of stem cell like behaviors in solid cancers by inducing genes involved in this process including *JARID1B*, JMJD1A, *HIF-2a*, JMJD2B, *MLL1* and JARID1B (Govaert et al., 2014; Li et al., 2009; Yun and Lin, 2014). Along with hypoxia, Hypoxia-inducible factors (HIFs) are also associated with the development of cancer stem cell behavior (Wang et al., 2015). It is likely that CSC are located in hypoxic areas of tumors (Rajaganeshan et al., 2008).

3. Targeting of colorectal cancer stem cells

There are various approaches available for targeting CSCs. These approaches can be divided into four main categories and we try to discuss each of these approaches in upcoming sections. Here, we only summarize these approaches. The first approach is the targeting of a specific signaling pathways, cell checkpoints and epigenetic modification. These signaling pathways are most highly expressed in CSCs. The WNT/β-catenin pathway is a well-known signaling pathway in CSCs and is important in maintaining renewal capacities (Fodde and Brabletz, 2007). Targeting this pathway with COX-2 inhibitors has been shown to be a potential method for targeting CSC growth (Valverde et al., 2015). It has been demonstrated that use of Nonsteroidal anti-inflammatory drugs along with a COX2 inhibitor, celecoxib, will prevent polyp formation in familial adenomatous polyposis by inhibiting Wnt/B-cateniin transcription. Other selective Wnt inhibitors are also introduced which can be categorized according to their mode of action. Some of these agents target B-catenin destruction complex while other target nuclear/transcription factor complex or Wnt receptor complex (Novellasdemunt et al., 2015). Dishevelled inhibitors and Tankyrase inhibitors are 2 important B-catenin destruction complex which are being used in their early clinical trial phase in colon cancer (Novellasdemunt et al., 2015). B-catenin/TCF antagonists is an important inhibitors of nuclear/transcription factor complex which target downstream transcription effectors of Wnt pathway. Among Wnt receptor complex inhibitors, Porcupine inhibitors which add a palmitoyl group to Wnt proteins is an important strategy which is still in preclinical phase. Among this group, LGK974 has started the phase-I clinical trial (Novellasdemunt et al., 2015). Regardless of Wnt pathway, Targeting ligands of a specific pathway which have been shown to be important in CSCs development and also, targeting the products of these pathways are the other mechanisms for targeting a CSCs function. One can include epigenetic drugs as emerging therapeutic approach and a potential way of eradicating CSCs (Parizadeh et al., 2018a). The main goal of such approaches will be differentiating CSCs or even arresting their proliferation capacity (Paldino et al., 2014). DNA methyltransferase inhibitors are potent drugs that can affect CSCs differentiation and stop stem cell-like behaviors. Targeting cellular check points which regulate cellular proliferation has been considered as an approach for controlling cellular division (Kim et al., 2018a,2018b; Parizadeh et al., 2018a). Another approach could be targeting CSC by their surface markers this targeting should be specific CSC and can be achieved by different antibodies or even using the body immune system. The manipulation of the immune system for targeting CSCs has also been emerged. This type of immunotherapy benefits from the γδ T lymphocytes. Using γδ T cell activators results in the proliferation of these cells and cytokine release which can further induce CSCs susceptibility to perforins (Todaro et al., 2009). The 2 last strategy is to target the tumor microenvironment or specific MicroRNAs. Hypoxia and inflammation increase the development of CSCs. Using anti angiogenic factors may disrupt the CSCs vascular niche and affect their microenvironment (Tonini et al., 2003). Also, some microRNAs have been reported to be effective regulators of CSCs stemness and stem like behavior which can be targeted by specific therapies.

4. Targeting the signaling pathways in colorectal cancer stem cells

Many studies have shown that there are several developmental pathways in regulating cancer stem cells, especially in colon cancer. The most common pathways include Notch, Hedgehog, Wnt, human epidermal growth factor receptor 2 (EGFR2)–AKT; and cytokines including interleukin (IL)-6 and IL-8 and signal transducers and activators of transcription 3 (STAT3). It has also been shown that signaling pathways (Wnt pathway, NOTCH, STAT) play an important role in resistance to anti-cancer treatment and accelerated repopulation of CSC after or during treatment andlocking the various self-renewal molecules and pathways including BMI1, Wnt, Notch, PTEN and Hedgehog in CSCs appear to be a potential treatment targets (Lin, Li et al., 2011; Zeuner et al., 2014). Knock down of BMI1 cancer cells has resulted in inhibition of cancer stem cells self-renewal. BMI1 is act as an epigenetic modifier of various genes. BMI1 overexpress in some cancers such as colorectal cancer and induces metastasis and increase in tumor grade as well as promoting self-renewal of cancer stem cells (Network, 2012). Also, another regulator of epigenetic processes within CSCs is SIRT1. SIRT1 induce expression of RelB which is an NFkB family member by deactivating NFkB/p65 which will further promote euchromatin silencing. Also, SIRT1 overactivation has been linked to chemotherapy

resistance and inhibition of SIRT1 in SCSs has been shown to be effective in reducing colony growth (Li et al., 2012). Moreover, the role SIRT1 has been studied in the tumor microenvironment which will be discussed further in the last section of this review. Among signaling pathways during CSC development and survival, SHH- and PTCH1-dependent as well as non-canonical hedgehog signaling are crucial for CSC survival. In contrast to hedgehog pathway and its receptor, PTCH1, non-canonical hedgehog pathway is not widely studied. This pathway acts independent of GLI family activation mostly through PTCH1 and independent of SMO or act through SMO. The crosstalk between WNT and hedgehog signaling is important in development of colon cancer. Activation of non-canonical hedgehog signaling will positively regulate WNT signaling and result in stabilization of Beta cathenin which will further active WNT response genes. This fact emphasis the important role of non-canonical hedgehog signaling in CSC survival and made them a potential target for anticancer therapies (Yamamoto et al., 2013). Most patients with colon cancer are sensitive to inhibition of EGFR signaling, however, those carrying KRAS mutations do not respond well to EGFR-targeted therapy. The targeted inhibition of Notch ligand Delta-like ligand (DLL) 4 with neutralizing anti-DLL4 antibodies can reduce the CSC frequency in colon tumor cells with KRAS mutations (Takebe et al., 2011). Excessive Wnt signaling is another important factor in the pathogenesis of CRC (Basu et al., 2016). Mutations in Wnt signaling components lead to deregulation of the Wnt pathway which is crucial for cancer initiation, late-stage cancer, and metastasis (Dalerba et al., 2007). Approximately, 90% of sporadic colorectal cancer cases, are the result of at least one genetic variants in Wnt signaling pathway regulators (Network, 2012). Hence, much of the current research has aimed to identify suitable targets for inhibiting the Wnt signaling pathway. This approach may be beneficial in promoting cellular apoptosis and it can also preventing the CSC's from excessive proliferation and differentiation (Basu et al., 2016; Takebe et al., 2011). The leucine-rich repeat-containing G protein-coupled receptor 5 (Lgr5) promotes Wnt/β-catenin signaling. A higher level of expression of this receptor is correlated with more advanced stages of CRC. The response to treatment with 5-fluorouracil (5-FU) appears to be better when the expression of the Lgr5 is low, and it has been reported that significant reduction of Lgr5 protein expression in samples treated with the phytochemicals Cucurbitacin B and Demethoxycurcumin, that inhibit the Wnt signaling pathway. Cucurbitacin E is a potential inhibitor of Wnt pathway with a lower potency compared to Cucurbitacin B and Demethoxycurcumin (Jansma, 2014). Curcumin has been reported to be effective in inhibiting tumor growth, invasion and metastasis in different tumors including CRC through its interaction with various signaling pathways (Kunnumakkara et al., 2008; Sharma et al., 2004). Yu et al. evaluated the effect of curcumin in elimination of CSCs. Results of this study revealed that anti-cancer therapy with curcumin alone or in combination with FOLFOX regimen (5-FU + oxaliplatin + leucovorin) may be effective treatment in the eradication of CSCs (Yu et al., 2009). Herbal medicines are also now being considered for the treatment of CSCs elimination. For example, Huaier aqueous extract which is a Chinese drug isolated from Trametes robiniophila has been reported to be effective in targeting the Wnt/β-catenin signaling pathway is (Zhang et al., 2013). Wnt signaling also caused down-regulation of HOXA5 in CRC stem cell which prevents differentiation. HOXA5 modulates APCDDI, CXXC4 and NKD1 genes, which are tumor suppressors in colon cancer. Differentiation of cells in CRCs can result from repression of Wnt signaling which is mediated by HOXA5 through pathway antagonists APCDD1, CXXC4, NKD1. These studies suggest that stimulating CSCs differentiation in colon cancer result in a reduction of tumor growth and metastatic progression. Drug-induced differentiation should be considered as a promising approach to eradicate this tumor-enhancing cell population (Ordóñez-Morán et al., 2015). Recent experimental reports suggest that STAT3 dimerisation, phosphorylation and translocation to the nucleus has a significant role in cell cycle progression, proliferation, invasion, and survival in primary CRC cells. The STAT protein family is comprise a group of transcription factors which play a role in relaying extracellular signals. Studies have shown that inhibition of STAT3 results in apoptosis and a reduction in tumor cell invasion. it can also serve as an attractive therapeutic target for colorectal carcinoma. Moreover, a recent study by Lin and coworkers has shown that FLLL32, a curcumin analog, inhibited STAT3 phosphorylation, cell viability, and tumorsphere formation in $ALDH^+/CD133^+$ CSCs. They showed that STAT3 is a therapeutic target in colorectal cancer stem-like cells, and the STAT3-selective inhibitor, FLLL32 might be useful as a new therapeutic reagent targeting cancer stem-like cells or colon cancer-initiating cells in the future (Lin, Li et al., 2011). Indeed, recent finding have suggested that epigenetic changes, including DNA methylation and histone modifications are associated with the Wnt signaling pathway. JMJD2C as a well-known histone demethylase which regulates sphere formation by modulating the recruitment of β-catenin to target genes in CRCs. Affecting sphere-formation ability is related to the tumor formation and the complex interactions between Wnt and Notch pathways has been shown to play role in sphere formation (Yamamoto et al., 2013). Kim and coworkers have shown that JIB-04 can down-regulate the expression of Wnt/β-catenin (Kim et al., 2018a, 2018b). Moreover, this drug also interferes with the interaction between JMJD2 and β-catenin involved in the clonal expansion, self-renewal, and differentiation of human colorectal CSCs. They also found that JIB-04 treatment attenuated tumorsphere initiation and growth, CSC marker expression, and clonogenic proliferation in several CRC cell lines. Based on this study, JIB-04 may be a novel therapeutic agent for colorectal cancer (Kim et al., 2018a, 2018b). A recent report has shown that secreted HH ligands block the function of PATCHED1 (PTCH1), which normally inhibits SMOOTHENED (SMOH). In brief, active SMOH can trigger the activation of the GLI zinc finger transcription factors, and result in GLI1 function, and inhibition of GLI repressors, mostly GLI3R. Experimental evidence shows that the presence of active HH-GLI signaling in epithelial tumor cells of patient-derived primary CRCs. HH-GLI activity in epithelial tumor cells play important roles in tumor growth, recurrence and metastatic growth. This pathway also regulates the behavior of human CRC stem cells in vivo. This finding suggests a crucial role of the HH-GLI signaling in CRCs, and GLI may be an appropriate target for anti-cancer therapies, especially for intractable metastatic cancer (Varnat et al., 2009). It has been suggested that mammalian target of rapamycin (mTOR) acts as a critical modulator of cancerous cell growth and proliferation (Francipane and Lagasse, 2013). Francipane et al. have assessed the effect of Torin-1 as an mTOR inhibitor in CSCs suppression. They found that Torin-1 administration affects CD44, Notch pathway members, Ki67, Lgr5, DLL4 and DLL1 and the CSCs, causing tumor growth suppression in vitro and in vivo (Francipane and Lagasse, 2013). Primary CRC tissues with or without neoadjuvant chemotherapy by FOLFOX6 regimen and 4 CRC cell line (HT-29, SW480, DLD-1 and CACO2) were evaluated by Cai and colleagues. Treatment with rapamycin (a first-generation mTOR inhibitor) and PP242 (a second generation mTOR inhibitor) repressed the stimulation of CSCs. Furthermore, this therapeutic approach suppressed CSCs growth and reduced sphere formation and ALDH activity in CSCs (Cai et al., 2014). There is accumulating evidence that, in addition to its antidiabetic properties, metformin has anti-cancer properties especially in CRC and inhibit tumor cells development and proliferation (Kim, 2014; Zhang et al., 2011). In this regard, Kim et al. assessed the effect of metformin in CSCs of various types CRC cell lines. Metformin showed different effects in different CRC cell lines, via the glutamine metabolic and AMPK-mTOR pathways. Kim et al. also reported that inhibition of glutamine metabolic pathway could enhance the anti-cancer effect of metformin in CSCs (Kim et al., 2018a, 2018b). The Signal transducer and activator of transcription 3 (STAT3) pathway is another important pathways for CSCs. STAT3 is an effective signaling factor which is crucial for cellular proliferation and survival. Recently, it has been demonstrated that this pathways is important in regulating CSC self-renewal and inhibition of this pathway reduces the population of CSCs.

Targeting STAT3 by flavonoid morin and MST-312 in CD133 and CD44 positive cancer cell population in breast and colorectal cancers has shown valuable evidence about improving the prognosis of such cancers(Chung et al., 2016).

Beside cellular pathways, cellular checkpoints are also important players in tumorigenesis. Our cells have some mechanisms to overcome different stresses and damages to their genetic content. Cellular checkpoint can be considered mostly as cell cycle breaks which are important in decision making of a single cell. After an induced arrest by cellular check points, a cell will decide to undergo a repair or chose to undergo apoptosis and death. However, cancer cells mostly have abnormal checkpoint system (Kim et al., 2018a, 2018b). The proficiency of cancer stem cell in using checkpoint system for survival has complicated the therapy in most cancers. As mentioned earlier, tumors are consisted of a heterogenous population of cancerous cells (Maugeri-Saccà et al., 2012). When a cancerous tissue undergoes anti-cancer therapy including chemo or radiotherapy, DNA damage usually occur. Those cells which fail to repair themselves will undergo apoptosis. Other cells which are mostly CSCs, alter their checkpoint systems and upregulate Chk1-Chk2, extend their G phase and scape apoptosis and continue to proliferate. So, developing drugs which are able to target such checkpoints will overcome this stem cell like behavior of some cancer cells (Zhai et al., 2015). Some Chk1-Chk2 inhibitors including UCN-01 could significantly reduce CD133 positive cells population in colorectal cancer (Gallmeier et al., 2011). Considering all these pathways involved in tumorogenesis and colorectal cancer development, a major concern is still prominent in the literature. Most of these pathways are crucial for normal cell development and targeting such pathways may result in alteration in normal cellular function. Moreover, the cross talk between different pathways should also be taken to account. Such limitation has should be considered prior to taking a potential targeting mechanisms in clinical settings.

5. Targeting microRNAs in colorectal cancer stem cells

microRNA (miR or miRNA) is one of the epigenetic mechanisms that has critical role in the development of CSCs and may be important in overcoming chemoresistance and enhancing sensitivity of this type of cells to chemotherapy (Fesler et al., 2017). It has been reported that there is a balance between autophagy and apoptosis in CSCs which is crucial for maintaining stem cell-like properties in these cells. miRNAs can influence two critical processes, autophagy and apoptosis, by which CSCs resist chemotherapy (Fesler et al., 2017). There is growing evidence that miRNAs are involved in tumor invasion and metastasis of CRC. miRNAs are small, non-coding RNAs that regulate the expression of specific target genes in mammalian cells by repressing translation (Parizadeh et al., 2018b). Indeed, dysregulation of miRNA leads to disruption in the balance of cell signaling and growth processes in cancers. It has been reported that changes in miRNA expression is associated with CRC tumorigenesis and progression. In addition miRs may be able to regulate several properties of CSCs (Fesler et al., 2017). Aberrant expression of specific miRNA might be employed as potential prognostic and predictive markers in CRC (Ji et al., 2014). Hongdan and coworkers have reported that miR-3120-5p expression was significantly increased in $CD133^+$ and Lgr5-positive ($Lgr5^+$) CSCs, suggesting that miR-3120-5p promotes stem cell-like properties and invasiveness of colon cancer cells by reducing Axin2 expression which is a regulator of Wnt signaling. Furthermore, Axin2 is crucial component for phosphorylation of β-catenin by GSK-3b that is required for its ubiquitination and targeting for the ubiquitin-proteasome pathway. Therefore it has been suggested that inhibition of miR-3120-5p has the potential to improve CRC treatment (Hongdan and Feng, 2018). Another study established the role of miR-34 as a tumor suppressor which regulates multiple developmental cell-fate mechanisms, including the differentiation of embryonic stem cells and somatic cell reprogramming. Also, this miRNA causes reduced Notch protein levels that plays crucial role in cell-fate determination during development and oncogenesis. Analysis of early-stage dividing CSC showed that an increase in miR-34a levels was associated with a reduction in both symmetric CSC-CSC division and asymmetric division, resulting in fewer CSC daughter cells and more non-CSC daughter cell (Bu et al., 2013). miR-451 targets ATP-binding cassette (ABCB1) and macrophage migration inhibitory factor (MIF). High levels of ABCB1 lead to more efficient efflux of anti-colon cancer drugs such as the irinotecan-derived SN38 metabolite. MIF increases the expression of cyclooxygenase-2 (COX-2) that can prevent b-catenin degradation in Wnt signaling which is strongly associated with stemness and self-renewal of CSCs. It has been shown that miR451 modulated several genes that thereby affect a response to irronectan based therapy in patients with CRC. It has been suggested that miR- might be a useful prognostic marker and a target for new therapies in the treatment of CRC (Bitarte et al., 2011). An experimental study conducted by Xu et al. in stem cell-like side population (SP) cells in colorectal cancer revealed that miR 326 plays an inhibitory role in the development of drug resistance and cell invasion by targeting ABCG2 and matrix metalloproteinase (MMP) 16. The authors report that the inhibition of invasion associated with miR-328 might be correlate to MMP16, a member of the MMP family that causes degradation of type III collagen, gelatin, fibronectin and laminin-1, and that can enhance growth and invasiveness of cancer cells. (Xu et al., 2012). miR-215 has been reported as significant therapeutic effector of anticancer molecular pathways, which is involved in p53 signaling and also plays a role in inhibition of cancer stem cells. In fact, it is an effector of transcription factor caudal-type homeobox 1 (CDX1) that is a crucial regulator of differentiation in the normal colon and in colorectal relation with cancer (CRC). It has been shown that CDX1 is negatively correlated with Bmi1, a marker of stem cells that is important target of miR-215. Moreover, miR-215 can be mediated by binding of CDX1 to its promoter that results in regulation of differentiation in the colon (Jones et al., 2015). The overexpression of hsa-miR-140-5p inhibits cell invasion, proliferation, and induced cell cycle arrest in CRCs. it has been shown that hsa-miR-140-5p plays a role in reducing invasiveness of CRC cell lines by suppressing of Smad2 expression, which is a direct mediator of TGF-β signaling and can also be activated by Nodal pathway to promote colorectal CSCs self-renewal, CRC progression and also its carcinogenesis. Furthermore, hsa-miR-140-5p targets smad2 and ATG12 that are involved in autophagy. Also expression levels of CTSB and CTSS are reduced by hsa-miR-140-5p. CTSS and CTSB are plays an important role in autophagy and degradation damaged organelles. Regardless of the effect on autophagy, CTSB level which can be affected by ectopic expression of miR-140-5p can activate TGF-B pathway. So, disruption of autophagy by alteration in mir-140-5p will result in autophagy disruption and indirectly affect CSCs survival (Zhai et al., 2015). Autophagy is a feature of CSCs that protects them against microenvironment stress, essential for CSCs maintenance, including hypoxia and starvation. It has been showed that hsa-miR-140-5p has an indirect regulatory role in TGF-β signaling pathway through CTSB. Hsa-miR-140-5p results in direct killing of CSCs in vitro by disruption of autophagy and ectopic expression (Zhai et al., 2015). The effect of ectopic expression of transfer RNA-derived RNA fragments (tRF)/miR-1280 in CRC has been investigated by Huang and colleagues. They have reported that tRF/miR-1280 interacts with JAG2 in Notch pathways, leading inhibitions of CSCs (Huang et al., 2017). Toden et al. evaluated the effect of Epigallocatechin-3-gallate (EGCG) that is present in green tea. It was found to enhance sensitization of CSCs to 5-FU and repression of sphere formation resulted from treatment with EGCG. EGCG as found to increase the expression of suppressive miRNAs and decreased Notch1, Ezh2, Suz12 and Bmi1 expression (Toden et al., 2016). Another promising agent with anti-cancer effect on CSCs was suggested by Sam and his colleagues. Treating CSCs isolated from CRC cell line (HCT-116) with Methyl-3-pentyl-6-methoxyprodigiosene, an agent isolated from Serratia marcescens, which targets the expression of tumor suppressor miR-16-1, Caspase-3 and also proto-oncogen survivin has resulted in

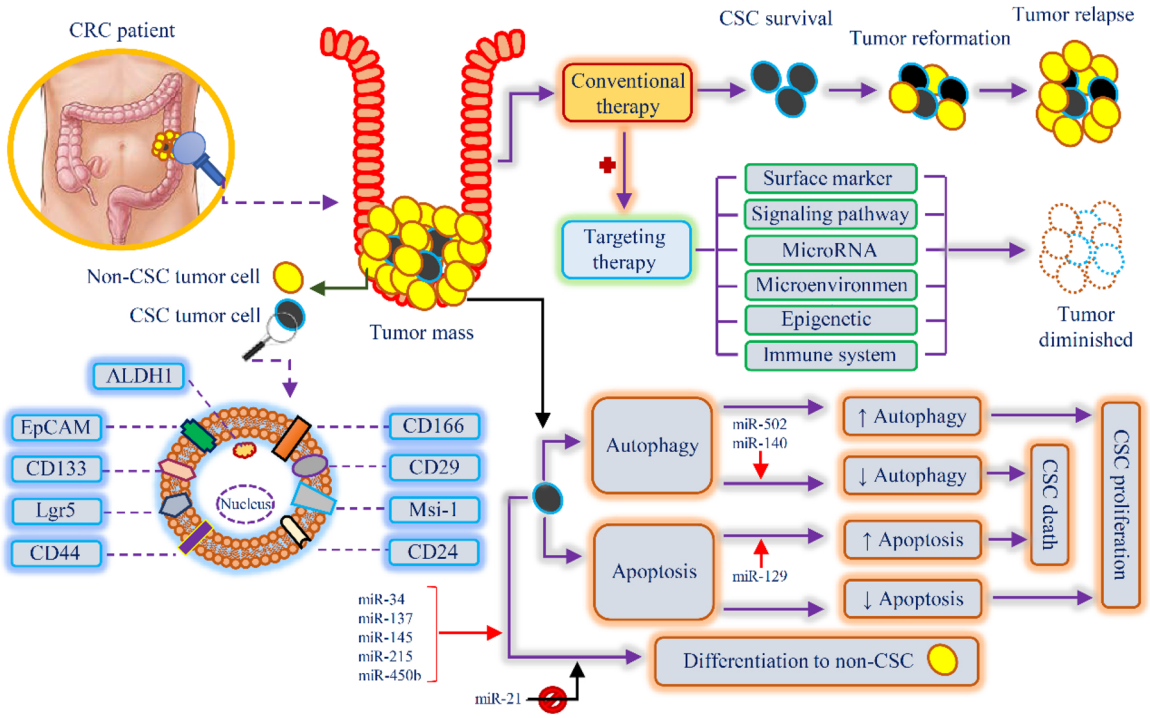

Fig. 1. Illustration of the heterogeneous nature of colorectal cancer (CRC). The colorectal cancer stem cell (CSC) is the main cancerous cell which shows stemness behaviors. While conventional therapies has mainly focused on eliminating CRC cells; however those treatment which fail to eliminate the CSCs will be unsuccessful and the tumor will not be eliminated completely. Targeted therapy approach in combination with conventional therapy will result in more favorable results. CSCs have various specific surface markers which are candidates in targeted therapy. Alongside with targeting tumor specific molecular pathways, tumor microenvironment, altering the epigenetic nature of CSC and using immune system for more specific fighting against CSCs, micro-RNAs are also a good potential target which are illustrated in more detail in this figure.

significant reduction in growth-rate and induce apoptosis in CSCs (Sam et al., 2016) (Fig. 1).

6. Targeting cell surface markers in colorectal cancer stem cells

Membrane proteins are cell surface markers that may be specific for cancer stem cells and may be important for diagnostics and disease staging purposes, as well as determination of patient prognosis, prediction of therapeutic response and treatment selection. There is substantial evidence that colorectal cancer stem cell markers can act as effective therapeutic targets (Cojoc et al., 2015). Lgr5 is a seven-transmembrane protein of the class A Rhodopsin-like family of GPCRs. Lgr5 acts as stem cell/progenitor hierarchies of CRC tissue, as well as a CSC marker and a marker for drug resistance. Lgr5 overexpression has been shown to be important in CRC progression as a result of its role in promoting the Wnt signaling pathway. Lgr5 binds to RSPO protein and prevents the degradation of the Wnt receptor, Frizzled and LRP5/6 by ligases RNF43/ZNRF3. Therefore $Lgr5^{+}$ cells may be considered to be important in the development of CSCs. (Kobayashi et al., 2012). CD44, a trans-membrane glycoprotein, is another marker that is involved in many cellular processes, including growth, survival, differentiation and motility. Indeed, it contributes as adhesion molecule in cancer cell migration and matrix adhesion in response to the cellular microenvironment, enhancing cellular aggregation and tumor cell growth. It has been showed that CD133 is also a cell surface trans-membrane glycoprotein which exists in the cholesterol-rich domain of lipid rafts. Investigation of CD44 and CD133 role in CSC showed that CD44 is a robust marker and has functional importance for CSC Initiation. Therefore CD44 might be a candidate for targeted therapy to eradicate cancer (Du et al., 2008). In contrast to this finding experimental result showed that CD133 is currently the most relevant single marker and CD44 is has less prognostic value than CD133. Moreover, CD44 alone may be insufficient as a marker for Co-CSCs, since it requires several cofactors to be relevant for CRC progression. Combined analysis of CD133, CD44, and CD166 might be of superior prognostic power than CD133 alone. Therefore, these markers might be useful in the diagnosis of different colorectal cancer stages (Horst et al., 2009). Nevertheless a meta-analysis has shown that CD133 expression is associated with prognosis of colorectal cancer patients (Sahlberg et al., 2014). Amanda and colleagues have suggested that ST6Gal-I may be a novel marker. It is glycosyltransferase for adding the negatively charged sugar, sialic acid, in a α2-6 linkage to the termini of *N*-glycans. ST6Gal-I overexpression promotes cell migration as result of sialylation of the b1-integrin receptor. Moreover ST6Gal-I might regulate ability of apoptosis by α2-6 sialylation of Fas reduces apoptotic signaling and TNFR1 death receptor. ST6Gal-I also lead to sialylation of CD45 that prevents CD45 internalization and protects T cells from apoptosis. It has been found that ST6Gal-I can also act as a key negative regulator of galectin-dependent apoptosis which mediates cell death. Based on this findings ST6Gal-I appears to be involved in maintaining some aspect of stem-like cell behavior, therefore it may be used as targeted therapy (Swindall et al., 2013). The prognostic role of CD166, CD44 s, EpCAM has been shown in CRC. Reducing CD44 s, CD166 and EpCAM membranous expression, which result in a reduction in the adhesion function, correlates with aggressive tumor-related features. Therefore, loss of expression of these markers indicates a more aggressive tumor phenotype such as tumor invasiveness, progression and infiltrating tumor growth pattern (Lugli et al., 2010). Xiang et al. have used CRC cell line (HT-29) and mice model of CRC for evaluating the efficacy of CSC elimination by combining doxorubicin with EpCAM aptamer which is a novel drug delivery system for drug targeting against CSCs surface marker. Results showed more effective drug concentration and retention in CSCs. Although many studies have reported doxorubicin is almost ineffective in CSCs eradication, in this study Xiang et al. showed that this therapeutic approach resulted in 30-fold reduction in the CSC frequency as well as at least 3-fold reduction tumor cells proliferation and improvement in

Table 1
Summary of the most relevant in vitro studies investigating targeting microRNAs in colorectal cancer stem cells.

Author (Ref)	Main investigated miRNA(s)	Targeting factor(S)	Main findings
(Bu et al., 2013)	miR-34a	Notch1	miR-34a reduces Notch protein levels and regulates CSCs division
(Bitarte et al., 2011)	miR-451	ABCB1, MIF	miR-451 decreased both genes and it cause response to irronectan based therapy in CRC patients
(Hwang et al., 2014)	miR-146a	Numb	miR-146a promotes symmetrical cell division of CSCs
(Bu et al., 2016)	miR-34a	Numb	miR-34a regulates division of CSCs
(Xu et al., 2012)	miR-328	ABCG2, MMP16	miR-328 plays role inhibitory drug resistant and cell invasion by targeting of ABCG2 and MMP16
(Jones et al., 2015)	miR-215	Bmi1	miR-215 reduces stem-like qualities of CSCs
(Zhai et al., 2015)	hsa-miR-140-5p	smad2, ATG12	Hsa-miR-140-5p disrupts autophagy in CSCs
(Hongdan and Feng, 2018)	miR-3120-5p	Axin2	Inhibition of miR-3120-5p has the potential to improve colon cancer treatment, serving as treatment targets
(Huang et al., 2017)	tRF/miR-1280	JAG2	tRF/miR-1280 suppresses CSCs and metastasis
(Karaayvaz et al., 2013)	miR-129	BCL2	miR-129 increases apoptosis in CSCs
(Fesler et al., 2014)	miR-129	BCL2	miR-129 increases apoptosis in CSCs
(Zhai et al., 2013)	miR-502	RAB1B	miR-502 restrains tumor growth and inhibits autophagy
(Sakaguchi et al., 2016)	miR-137	DCLK1	miR-137 reduce tumorigenicity of CSCs
(Le Rolle et al., 2016)	miR-145	SOX2	miR-145 increases differentiation and reduces tumorigenicity of CSCs
(Yu et al., 2015)	miR-145, miR-21	SOX2	miR-145 increases differentiation and reduces tumorigenicity of CSCs, however miR-21 induce opposite effects on CSCs
(Jin et al., 2016)	miR-450b	SOX2	miR-450b enhances chemosensitivity of CSCs to 5-fluorouracil
(Cantini et al., 2015)	miR-194, miR-200b, miR-203 and miR-429	Bmi1, ZEB1	These miRs Suppress the stem subtype in CRC
(Mukohyama et al., 2017)	miR-221	Quaking-5	miR-221 Knockdown represses clonogenicity of CSCs

CRC: colorectal cancer; miR: microRNA; CSCs: colorectal cancer stem cells; MMP16: matrix metalloproteinase 16.

survival compared with those treated with similar drug dose without aptamer (Xiang et al., 2017). Wang et al. used oxaliplatin encapsulated in another drug delivery carrier, chitosan, and found that anti-cancer therapy by this novel formulation could be able to be effective in elimination of both tumor cells and CSC compared with using free oxaliplatin (Wang et al., 2011). Ortiz et al. have proposed a combination of gene therapy combined with 5-FU as a chemotherapeutic agent. They used biodegradable nanocarriers as a drug delivery system for treating CSCs in CRC apoptosis and chemoresistant SW480 human cell line. Poly (ε-caprolactone) was used as a nanocarrier of 5-FU combined with cytotoxic suicide gene E which could successfully bring synergic antiprolifrative effect (Ortiz et al., 2012). Liu et al. reported that in CRC using combination therapy with paclitaxel and siRNA which could be able to target CD133 and novel siRNA delivery agent (composed of polyethylene glycol + cationic oligomer + lipid-based component) resulted in significantly enhanced chemosensitivity of CSCs of paclitaxel (Liu et al., 2009). Sahlberg and coworkers reached interesting findings related to CD133, CD44 role in CRCs radiation resistance. CD44 has been shown to interact with EGFR, HER2, HER3 and HER4. CD44 is believed to play a role in regulating and maintaining cancer stem cells by EGFR which mediate by downstream signaling via the Phospho-inositol 3 kinase (PI3K)/AKT pathway. AKT is also taking a part in epithelial to mesenchymal transition (EMT) signaling pathway which leads to increased motility, reduced intercellular adhesion, tumor progression and malignant transformation. It has also been shown that colon cancer cells with a high expression of CD133/CD44 lead to EMT after long-term culture. Indeed AKT with CD133 colon cancer cell presented higher resistance to chemotherapeutics. In contrary CD44 has negative correlation with AKT which is a result of regulating role of CD44 isoforms being able to activate or suppress the activation of AKT. Radiation lead to increasing expression of AKT and CD133, and a reduction in CD44 expression in colorectal cancer cells. Functional study on three cell lines demonstrated that CD133/CD44 highly expressing cells were more resistant to radiation and had a higher expression of AKT. Also this finding suggested combinations of inhibitors against AKT and CD44 could be used to avoid negative feed-back loops associated with AKT inhibitors which may cause the cancer cells to survive treatment (Sahlberg et al., 2014). Investigation of targeted colon cancer therapy demonstrated that $Lgr5^+$ cell transient to $Lgr5^-$ cell by treat of anticancer agent HLA-DMA, and EREG. Also $Lgr5^-$ converted to an $Lgr5^+$ state in the absence of the drug. Moreover this data suggested the anti-EREG antibody exhibited antitumor activity against tumors derived from the $Lgr5^+$ cells therefore, using the anti-EREG antibody, might be an option for CSC targeting therapy (Kobayashi et al., 2012). SIRT6, deacetylates histones H3 (H3k9, H3K56) is reported as potential application in CRC therapy that mediate by its role in regulating cancer cell metabolism and promotion cancer cell apoptosis. Results has been suggested SIRT6 inhibits cell proliferation and CDC25 A expression through deacetylation H3K9 at the CDC25 A promoter in $CD133^+$ colorectal CSCs (Liu et al., 2018). Aldehyde dehydrogenase 1 (ALDH1), a detoxifying enzyme that oxidizes intracellular aldehydes has been reported as potential CSCl marker which involved in the resistance to alkylating enzymes Lin et al. investigated the anti-cancer effect of GO-Y030 which is a new curcumin analogue on CSCs from four CRC cell lines (HT-29, HCT-116, SW480 and DLD-1). CD133/ALDH-positive were isolated by using flow cytometry. These subpopulation of CSCs express higher level of phosphorylated STAT3. Lin et al. found that GO-Y030 act as inhibitor of CSC viability and STAT3 phosphorylation and therefore induce CSCs apoptosis. In addition, using this newly developed curcumin in the mouse model confirmed these findings (Lin et al., 2011a, 2011b). Similarly, another novel curcumin analogue investigated by Kanwar and colleagues. In this study after identification CSCs from CRC cell lines (HT-29 and HCT-116) which were resistant to 5-FU and oxaliplatin treatment with combination of difluorinated-curcumin plus 5-FU and oxaliplatin was done. Results showed that this formulation of curcumin together with oxaliplatin and 5-FU was more effective than curcumin in proliferation inhibition and apoptosis induction of CSCs (Kanwar et al., 2011). It is important to note that targeting cell markers has its own drawbacks, which relates to their specificity; for example in considering the targeting of CD133 that is expressed in the lung, brain and colorectal cancer. This marker is not only expressed in CSCs and both CD133 positive and negative cancer cells can develop cancer(Basu et al., 2016; Novellasdemunt et al., 2015) (Table 1).

7. Targeting the colorectal cancer stem cells microenvironment

As mentioned before, tumor cells have their specific niche and surrounding cells which are necessary for their survival. This microenvironment has some specific features that are necessary for tumor

development. Chronic Inflammatory status that is produced by inflammatory cytokines along with hypoxia are the main findings of this microenvironment. The next prominent finding in the tumoral bed, in addition to hypoxia and inflammation, is a dynamic cell population. Cancer associated fibroblasts are specific kind of fibroblasts that can arise from healthy or tumoral cells. These cells produces many different cytokines including fibroblast growth factor (FGF), epidermal growth factor (EGF), transforming growth factor-β (TGF-β) and metalloproteinases (MMP) (Bhowmick et al., 2004). TGF-β is the most important secreted factor by tumor associated fibroblasts. Increased levels of TGF-B which usually caused by TGF-B receptor mutations will induce angiogenesis and immune system dysregulation which is mandatory for tumor growth and development (Muñoz et al., 2006). As same as TGF-B, HGF is another important cytokine which is important for both maintaining a proper microenvironment and maintain CSC features. HGF can affect B-catenin activity which is a crucial player in Wnt signaling pathway in stemness of CSCs (Vermeulen et al., 2010). As mentioned earlier, SIRT1 is also effective in providing a suitable tumor micro-enviroment for tumor growth and somehow help cancer cells to survive hypoxia. SIRT1 will control angiogenesis by deacetylating FOXO1 intracellular domain and also activating nitric oxide synthesis which will further improve angiogenesis and vascular functions (Wang and Chen, 2013). Regardless of tumor cells that have specific activated and inactivated pathways, other cells in tumor bed including tumor associated fibroblasts will also secret different interleukins. Presence of inflammatory cytokines including IL-6 and IL-8 are necessary for activating NF-kB pathway. Enhancing CSC self-renewal because of this cytokine flow is the result of this activation. By blocking IL-6 and IL-8 signaling pathways the tumor size will decrease and the tumor will become more sensitive to chemotherapy (Korkaya et al., 2011). Along with these cytokines, some immunosuppressive agents such as IL-4 and COX-2 are also present in tumor microenvironment. It has been reported that targeting COX-2 by COX-2 inhibitors will result in macrophage phenotype change. These macrophage will become from a Pro-tumor associated macrophage to anti-tumor associated macrophage which tend to present antigen and killing capacities beside producing immunosuppressive chemokine and inducing cellular proliferation and angiogenesis (Erreni et al., 2011). Providing more specific targeted therapy by use of engineered T cells are also a new therapeutic issue. By knowing the specific marker of CSCs, these engendered immune cells can specifically target CSCs (Beard et al., 2014). Targeting tumor microenvironment has been recently become an interesting topic and still needs further research. It is noteworthy that targeting tumor microenvironment may also target other non-cancerous tissue. Also, restricting the microenvironment issue to only targeting tumor vasculature seems not to be specific enough and targeting more detailed mechanisms with greater specificity for tumoral tissue is necessary.

8. Conclusion

Although there is a rapid growing trend toward identification and targeting cancer stem cells, there remain many unanswered questions about the CSCs. Understanding the signaling pathway that modulate tumor maintenance could serves as a major purpose to improve cancer therapies. Moreover, a number of molecules and gene signatures that underlie stemness future of CSC-CRC have been recognized, and future investigation should be considered to detect and target other key dysregulated pathways (e.g., Wnt, c-Met) driving self-renewal in the CSC subset that force them to go in the asymmetric division. This could provide an effective way to overcome the conventional therapies side effects and suppress mortality rate due to relapse of patients. Furthermore, some studies are working for targeting metabolic properties of CSCs, specifically, anaerobic glycolysis which might be reduced by stem-like cells in some cancer types. In turn novel technical approaches in studying specific metabolic activities such as oxidative phosphorylation, proton production or high resolution global metabolome profiling have been developed for evaluation of the bioenergetics of tumor cells, although substantial experimental merits are needed for accurate metadata during experimental progress. Thus deciphering the CSC metabolism and its underlying molecular and cellular pathways could lead to the detection of novel therapeutic targets, which could provide a novel approach to elucidate efficient routes to eradicate the most malignant cancer cells. Given these potential implications of CSC metabolism, a profound understanding of nutrient homeostasis in malignant and non-cancerous stem-like cells is of great interest to improve personalized patient care.

Conflict of interest

The authors have no conflict of interest to disclose

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