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**Emerging role of microRNAs in cancer stem cells: Implications in cancer therapy**

Garg M. Therapeutic applications of miRNAs in cancer

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**Abstract**

A small subset of cancer cells that act as tumor initiating cells or cancer stem cells (CSCs) maintain self-renewal and growth promoting capabilities of cancer and are responsible for drug/treatment resistance, tumor recurrence and metastasis. Due to their potential clinical importance, many researchers have put their efforts over decades to unravel the molecular mechanisms that regulate CSCs functions. MicroRNAs (miRNAs) which are 21-23 nucleotide long, endogenous non-coding RNAs, regulate gene expression through gene silencing at post-transcriptional level by binding to the 3’-untranslated regions or the open reading frames of target genes, thereby result in target mRNA degradation or its translational repression and serve important role in several cellular, physiological and developmental processes. Aberrant miRNAs expression and their implication in CSCs regulation by controlling asymmetric cell division, drug/treatment resistance and metastasis make miRNAs a tool of great therapeutic potential against cancer. Recent advancements on the biological complexities of CSCs, modulation in CSCs properties by miRNA network and development of miRNA based treatment strategies specifically targeting the CSCs as an attractive therapeutic targets for clinical application are being critically analysed.

**Key words:** Cancer stem cells; Drug resistance; Tumor recurrence; MicroRNAs; Cancer therapy

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**Core tip:** Cancer stem cells (CSCs) which are believed to be the prerequisite for metastasis and tumor recurrence, are endowed with ability to undergo symmetric cell division, capacity for self-renewability, long term proliferation and resistance to anti-neoplasic therapeutic drugs. Regulatory characteristics of microRNAs (miRNAs) which are the clusters of non-coding RNA molecules, include widespread changes in gene expression through gene silencing at post-transcriptional level and are dysregulated in human cancer. Over the past two decades, miRNAs have gained widespread attention due to their involvement in acquisition of stem cell-like properties, regulation and reprogramming by cancer cells during cancer progression. Many studies are coming up which document miRNAs as novel therapeutic tool in targeting CSCs functions, sensitizing them to apoptotic effects of anti-cancer drugs, and reducing tumor burden with no relapse in current clinical settings.

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**INTRODUCTION**

Despite high response rates to initial treatments including chemotherapy, radiotherapy or sometimes even after combinational chemotherapies, cancer remains the most lethal disease. Recent technological advancements and animal studies have validated the presence of samll subset of cancer cells among the heterogenous population of tumor cells and are known as cancer stem cells (CSCs) or tumor initiating cells. They have different biological properties, increased tumorgenic potential and are supposed to serve important role in drug resistance, tumor development and its recurrence. Anti-cancer therapeies are being employed on the basis of their ability to shrink tumor cells, but CSCs survive and proliferate owning to an increased intrinsic (*de novo*) or acquired resistance to anti-cancer drugs. This makes the clinical therapy ineffective, leads to tumor recurrence and results in high mortality rates in cancer patients. Decreased experssion of energy dependent transporters to retain drugs inside the cancer cells and increased efficacy of drug response by sensitizing these subset of cancer cells to drug induced apoptosis could be key mechanisms towards improved treatment of cancer. Therefore, there is an urgent need to understand the molecular mechanisms controlling CSCs population and functions to develop effective therapies to eradicate recurrence which is a real threat to complete cancer cure. Emerging evidences have suggested the critial involvement of protein-coding genes as well as non-coding RNAs (ncRNAs) including microRNAs (miRNAs) in various types of cancers and serve important role in CSCs functions. Recent findings on the miRNAs, responsible for the maintenance and regulation of CSCs properties like tumorigenicity and drug resistance, are briefly summarized in the later sections. Gaining further insight into the potential and therapeutic approaches by targeting CSCs using miRNAs as a tool would be helpful for deciphering and designing future strategies for human cancer treatment with better outcome by preventing metastatic progression and tumor recurrence.

**CANCER STEM CELLS**

Cancer stem cells with tumor intiating potential constitute only a fraction of neoplastic cells and were first reported by Lapidot *et al*[1]. These cells are endowed with the capacity of long term proliferation, ability to undergo symmetric cell division and seed new tumors by virtue of their intrinsic self-protection and self-renewal capabilities. Capacity for self-renewal, propagation of differentiated progenitors and the expression of specific stem cell genes are some of the biological properties that are shared by both normal and CSCs. Accumulation of genetic and epigenetic alterations degregulate the basic stem cell biology and distinguish CSCs from the normal stem cells in their chemoresistance, enhanced tumorigeneic and metastatic activities[2]. Recent experimental studies have identified the activation of oncogenes and inactivation of tumor suppressor genes to be responsible for self-renewal ability and pluripotency of CSCs[3]. Concept of heterogeneity among CSCs as stationary and migrating CSCs came into existence with the discovery of process of epitheilal-to-mesencymal transition (EMT). EMT is a crucial event which allows the stationary CSCs at the primary site to loose their epithelial character, morphological transition, and loss of cell-cell adherence. This transition imparts high motility to the stationary CSCs, which is a necessary requirement by migrating CSCs for local invasion and metastatic dissemination. The concept of migrating CSCs and especially EMT as a widespread mechanism of stem cell generation validates the dynamic nature of CSCs and strongly supports the current CSC theory[4,5].

Over the past few years, number of studies have reported the isolation and charecterization of CSCs for hematological malignancies including acute lymphoblastic leukemia, chronic myeloid leukemia, acute myelogenous leukemia, multiple myeloma and also for solid tumors of breast, colon, stomach, brain, lung, liver, skin, prostate, testis, ovary and pancreas[6]. The subpopulation of CSCs are identified, isolated and charecterized on the basis of capacity to form sphere-clusters with a high clonogenic efficiency, a stronger self-renewal ability and much higher levels of cell cycle markers which account for G1-S/G2-M phases progression, side population profile and stemness gene expression.

Stem cell maintenance, survival, self-renewal and differentiation of CSCs are known to be regulated by several signaling pathways and molecules. Notch, Wnt/catenin, Sonic Hedgehog, bone morphogenetic protein, receptor tyrosine kinase and TGF-β signaling pathways are studied to be aberrantly active in CSCs and regulate self-renewal activity, thereby play significant role in tumor initiation and development in various malignancies including breast, colorectal, prostate, pancreatic, glioma, leukemia and colon cancers[7-11].

CSCs are not only the source of initial tumor formation, postoperative recurrence and metastasis but also contribute to chemo/radioresistance. High invasiveness and frequent recurrence due to the presence of inefficient DNA repair mechanisms, which render CSCs highly resistant to chemotherapeutic and ionizing radiations, are the main reasons for treatment failure and recurrent disease[12,13]. Targeting the pathways and mechanisms behind the development of chemo/radioresistance, modifying the niche of cancer cells, regulating the cellular response to damage by modulating apoptosis, cell cycle proliferation, DNA repair, invasion and differentiation functions might help in eliminating CSCs subpopulation and developing therapeutic strategies for complete cancer treatment with no relapse.

MiRNAs have gained widespread attention in modulating the CSCs functions through affecting the expression level of target genes and proteins that are involved in signaling pathways including cell proliferation and cell death. Recent advancements on the role of miRNAs in regulation and maintenance of tumor cells with stem cell properties are discussed in later sections.

**MICRORNAS AND THEIR REGULATORY FUNCTIONS IN CANCER STEM CELLS**

***MicroRNAs and its biogenesis***

The most important advancement over the past recent years is the discovery of non-coding RNA families which are actively transcribed from the genome of many organisms and generate widespread changes by regulating vital biological processes. Among them, miRNAs are the novel small non-protein coding, evolutionarily conserved, 21-23 nucleotide long single-stranded RNA molecules. Many important diverse functions of miRNAs include the regulation of cellular differentiation, proliferation and apoptosis by regulating the stability or translational efficiency of target messenger RNAs which are expressed in tissue-specific or developmental stage-specific manner.

MiRNA-mRNA target recognition due to sequence complementarity of the lin-4 RNA to multiple conserved sites within the lin-14 3’ untranslated region (3’UTR) leads to the discovery of miRNAs way back in 1993 during genome study in Caenorhabditis elegans development[13,14]. MiRNAs are randomly located in mammalian genome and the complex process of biogenesis begins in nucleus with the transcription of polycistronic primary-miRNA (pri-miRNA) transcript by RNA polymerases II and III[15,16]. Hundreds or thousands of nucleotides long pri-miRNA transcripts with one or many stem loops, 5’ capping and 3’ polyadenylation, undergo further cleavage by nuclear microprocessor complex containing enzymes Drosha, RNA III endonuclease and double stranded RNA binding protein DGCR8 (DiGeorge syndrome critical region gene 8)[17,18]. The resulting 70 nucleotide long pre-miRNAs are exported to cytoplasm by exportin 5 and Ran-GTP[19]. Another enzyme, RNA III endonuclease known as Dicer, cleaves hairpin-like pre-miRNA into two complementary fragments and one of which is mature miRNA[20]. Mature miRNA strand is then incorporated into the members of Agronaute (Ago) protein family, which constitutes the catalytic portion of the multi-protein RNA-induced silencing complex (RISC). MiRNAs then direct RISC to target mRNAs which share sequence complementation in seed region that consists of nucleotides at position 2-8 of 5’ end of mature miRNA. Complementation between the seed sequence and 3’UTR of target mRNA results in mRNA transcript degradation while imperfect complementation results translational repression[21]. Nuclear pri-miRNA and cytoplasmic pre-miRNA cleavage steps and mature miRNA: target mRNA recognition sites are the potential therapeutic points against cancer by regulating miRNA processing, its biogenesis and miRNA functions (Figure 1).

Statsitical methods and profiling studies validate the presence of miRNAs in close proximity to chromosomal breakpoints, cancer associated genomic regions and fragile sites where mutations/deletions can occur. Loss of tumor suppressive miRNAs and increased expression of oncogenic miRNAs enhance the expression of target oncogenes and represse the target tumor suppressor genes respectively. Deregulation of novel miRNAs expression leads to induction of anti-apoptotoic activity, tumor invasion, drug resistance and metastasis and has been correlated with the pathogenesis of cancer. MiRNAs are present in complex regulatory circuits to regulate stem cells function and are being examined to play important role in generation of CSCs, maintenance of enhanced self-renewal capacities of CSCs, pluripotency and their neoplastic transformation into tumors (Figure 2).

***MicroRNAs in maintenance and regulation of CSCs properties***

Many important functions of miRNAs in embryonic development and stem cells regulation in mammals are investigated. Dynamic expression profile of miRNAs in CSCs validates its significant role in controlling the self-renewal ability, pluripotency, diffentiation of progenitor cells, prosurvival and antistress mechanisms (Table 1).

MiRNAs playing role in differentiation processes can either directly suppress the self-renewal state by suppressing the markers of pluripotency including Nanog, POU class 5 homoebox 1 (Pou5f1) also known as Oct4, Kruppel like factor 4 and sex-determining region Y-box containing gene 2 (Sox2, a crucial transcription factor for the maintenance of embryonic stem cell (ESC) pluripotency and the determination of cell fate), or stabilize the differentiated cell fate by targeting the transcripts that are regulated by the pluripotency transcription factors including *Oct4, Sox2, Nanog and Tcf3*[22]. The transfection of let-7c, member of Let-7 family has been shown to rescue the differentiation defects in DGCR8-/- cells by downregulating the stemness genes including *Oct4*, *Sox2* and *Nanog*[22]. Negative feedback regulation between Lin-28, a marker of undifferentiated ESCs and Let-7 family members has been reported in mouse differentiated cells and embryonic carcinoma cells[23]. Reduced patient survival and tumor relapse in human colon adenocarcinomas has been correlated with increased expression of Lin-28[24]. Studies on inhibition of Sox2 and placenta-specific 1 gene by miR-126 has been reported in gastric carcinogenesis[25].

First oncomiR reported as miR-17-92 polycistron has been examined to regulate c-Myc expression and accelerate tumor development in stomach, prostate, pancreatic, colon, lung and breast cancers. Novel stem cell specific miRNAs including miR-290, miR-302/367 and miR-371 clusters are identified in human ESCs and exhibit altered cell cycle profile and inhibit ESCs transition from self-renewal to differentiated state. Members of the miR-302 family has been shown to reprogram human skin carcinoma cells ino pluoripotent ESC-like state[26].

Inhibition of tumor sphere growth *in vitro* and tumor formation *in vivo* upon restoration of miR-34 has been concluded to be associated with the suppression of growth of CSCs with biomarkers of CD44+ and CD133+ in human pancreatic cancer cells. These CSCs are deficient in miR-34 and show higher expression of Bcl-2 and Notch, which are the target genes for tumor suppressor like p53, and are involved in survival and self-renewal of CSCs. MiR-34 has been shown to inhibt the reprogramming through repressing pluripotency genes like *Nanog*, *Sox2* and *N-Myc*[27]. Further research has confirmed that restoration of miR-34 directly regulates Bcl-2 and Notch target genes, activates caspase-3, induces apoptosis, thereby could increase chemotherapeutic and radiotherapeutic sensitivites in pancreatic cancer cells[28,29]. Suppresssion of tumorigenesis *in vitro* has been found to be regulated by miR-134 by downregulating the Notch target proteins and affecting G2/M pahse of cells in human endometrial CSCs[30]. Independent study by Park *et al*[31], 2013 revealed the differential expression of 43 miRNAs and their putative target genes such as *p53*, *ErbB1*, *Notch*, *Wnt*, and TGF-β signaling pathways which are the key regulators for stem cells and are mainly involved in cell death, cellular development, cellular growth, proliferation and maintenance of cancer and stem cell in glioblastoma multiforme (GBM) which is the most aggressive primary brain tumor, and notorious for resistance to chemo/radiotherapy.

Owning to high degree of biochemical specificity and potency shown by miRNAs in regulating the multiple vital pathways that can significantly affect the cancer progression, development of miRNAs as therapeutic molecule in association with anti-cancer drugs provides immense opportunities to counteract chemo/radioresistance and improve treatment outcome in various human cancers. Recent advances in the mechanisms, co-delivery of therapeutic molecules using nano-carriers and various advantages of combinational therapy in elimination of CSCs population and in complete cure of human cancer with no recurrence or metastasis are discussed in the subsequent section.

**COMBINATIONAL THERAPEUTIC APPROACHES TO TARGET CANCER STEM CELLS**

MiRNAs involved in acquisition of stem cell-like properties, regulation and reprogramming by cancer cells during cancer progression are not only exploited as molecular biomarkers to predict the risk of metastasis, systemic treatment resistance, and disease relapse of patients with cancer but are also an important novel therapeutic tools to improve patient-tailored treatments based on the unique signatures of a patient disease. MiRNAs can function as tumor suppressors or oncogenes and thereby either silence or express hundreds of genes and at the same time, where one gene can be targeted by multiple miRNAs. Local administration of miRNA-sponges which are the transcripts having multiple, tandem binding sites to the miRNAs of interest; antagomiRNAs/anti-miRNAs (highly chemically modified miRNA passenger strand) which include strong RNA binding analog locked nucleic acid (LNA) in mixmers with DNA, charged neutral anti-miRNA oligonucleotides like peptide nucleic acid (PNA),phosphorodiamidatemorpholino oligonucleotides, 2’-modified anti-miRNA oligonucleotides such as 2’-O-methyl, 2’-O-methoxyethyl (MOE), 2’-fluoro/2’-methoxyethyl mixmers (2’F/MOE) enhance binding affinity for their RNA targets, steric-block potency and biostability and induce inhibition of endogenous miRNA functions following miRNA- anti-miRNA oligonucleotide binding in a chemistry dependent manner[32,33]. Expression levels of related miRNA families that share the same seed sequence can be effectively reduced by using synthetic mRNAs, which contain multiple binding sites for endogenous miRNAs[34]. MiRNA replacement therapy is another important therapeutic intervention which is based on the systematic delivery of miRNA mimics into the cytoplasm of target cells to gain tumor suppressor functions. Inducing miRNA-mediated biological processes or blocking specific targets either could reverse the development of cancer or delay tumor growth[35]. Besides this, based on the relationship between expression of miRNA-regulated target genes and their reaction to drug therapies, some other miRNAs are explored which can mediate molecular target drugs and regulate chemosensitivities and chemoresistances of cancer. Experimental evidences document that the co-administration of CSCs with either antagomiRNAs/anti-miRNAs or miRNA mimics along with potential anti-cancer drugs can better sensitize cancer cells to promote apoptosis and autophagy, downregulate efflux transporters, revert EMT and inhibit tumor angiogenesis and could be employed as a more popular therapeutic approach for better clinical outcome in the treatment of human malignancies (Figure 3).

Viral vectors like lentiviral/adenovirus vectors are efficient delivery systems for miRNAs but toxicity and immunogenicity are the major hurdles for their clinical use. MiRNAs enclosed into non-viral particles, nanoparticles (NP) to form micelle-like structures for their effective delivery and enhanced stability in circulation. Some of the commonly used NPs for miRNAs delivery include polycationic-liposome hyaluronic acid, neutral lipid 1,2 dioleoyl-sn-glycero-3 phosphatidylcholine, polyethyleneimine, dendrimers, protamine, atelocollagen, poly(lactide-co-glycolide) particles, gold- or silica-based inorganic NPs. Binding of tumor specific ligands to NPs or conjugation with different compounds that have specific affinity with tumor cells ensure tissue-specific targeted delivery of miRNAs[36]. Some of the significant recent work being conducted in cancer cell lines and or animal models to understand the cellular processes that regulate CSCs functions and investigate the therapeutic effects of miRNAs and add new dimensions in cancer treatment, are being discussed here (Table 2).

The effects of knockdown of miR-21 expression include induced apoptosis, reduced cell proliferation, invasion, and colony formation ability of colon tumor cells, inhibited G1/S cell cycle transition and increased sensitivity of cancer cells to 5-Fluorouracil (5-FU) chemotherapy. Direct targeting of human mut S homolog 2 and indirect regulation of the expression of thymidine phosphorylase and dihydropyrimidine dehydrogenase by miR-21 confirms its important role in enhancing the sensitivity of 5-FU resistance of colon cancer cells. Re-emergence of chemotherapy-resistant CSCs could be one of the possible reasons for recurrence in colon cancer[37]. The synergistic effect of increased miR-145 and reduced miR-21 expression which target Wnt/β-catenin signaling pathway along with co-delivery of metformin, in combination with 5-fluorouracil and oxaliplatin (FuOx), has been examined inducing cell death in chemoresistant colon cancer cells, inhibiting colonospheres formation and enhancing colonospheres disintegration in the treatment of recurring colon cancer[38]. The delivery of synthetic Cy5-tagged anti-miR-9 to the resistant GBM cells has been observed to reverse the expression of the drug efflux transporter, P-glycoprotein and stimulate the sensitization of GBM cells to temozolomide, as shown by increased cell death and caspase activity[39]. Experimental studies by Shi *et al*[40], 2014 demonstrate the therapeutic effects of miR-125b inhibitor in enhancing the invasion-prevention activity of temozolomide in glioblastoma stem cells through targeting PIAS3 [protein inhibitor of activated STAT (signal transducer and activator of transcription)], which contributes to reduced STAT3 transcriptional activity and subsequent decreased expression of matrix metalloproteinase-2 (MMP-2) and MMP-9.

The cooperative effect of miR-451 (having a target site for SMAD in its promoter region) in combination with imatinib mesylate treatment in dispersal of GBM neurospheres and reduced tumorigenicity in glioblastoma validates this co-treatment as a new potential drug against the stem-cell like characteristics of glioblastoma[41]. The delivery of combinational formulations of gemcitabine and miR-205 conjugated copolymers in pancreatic cancer cells effectively reversed chemoresistance, invasion and migration by inducing apoptosis and inhibiting the growth and proliferation of CSCs[42]. In an another study, synergetic effects of targeted delivery of miR-200c and docetaxel (DOC) by gelatinases-stimuli NPs on inhibition of CSCs and non-CSC cancer cells in gastric cancer has been verified. The treatment of cancer cells with miR-200c/DOC NPs has been shown to significantly enhance the cytotoxicity of DOC, possibly by decreasing tubulin beta 3 Class III (TUBB3) level and reversing EMT, thereby affect tumor cell viability, migration and invasion[43]. Ectopic expression of another tumor suppressor, miR34a has been studied to reduce CSCs properties and increase sensitivity to doxorubicin treatment by directly targeting Notch1 in chemoresistant breast cancer cells[44]. c-Kit has been established as a new direct target of miR-34 where p53-induced miR-34 microRNA family mediated repression of c-Kit *via* a conserved seed-matching sequence in the c-Kit 3’-UTR, result reduced chemoresistance to 5-fluorouracil, reduced migration/invasion and stemness, further proves the hypothesis that this regulation interferes with several c-Kit-mediated effects in colorectal cancer cells[45]. Recent preliminary studies document the tumor growth inhibition in human NSCLC (non-small cell lung cancer) xenografts and KRAS-G12D transgenic mouse model or the elimination of self-renewing breast CSCs upon therapeutic delivery of Let-7 mimics[46,47]. Up-regulation of miR-30e with tumor suppressor functions by downregulating BCR-ABL (Breakpoint cluster region-Abelson murine leukemia viral oncogene homolog 1) expression in chronic myeloid leukemic cells has been examined to render therapeutic efficacy against this disease. Owing to presence of putative target site for miR-30e in the 3’-untranslated region of the *ABL* gene, enforced expression of miR-30e in K562 cells has been shown to suppress proliferation and induce apoptosis of these cells and sensitize them to imatinib treatment[48]. Attenuation of EMT by retaining epithelial cell morphology, reducing the levels of α-smooth muscle actin and increasing the levels of E-cadherin in human kidney proximal tubular epithelial cells (HK-2) has been observed after enforced expression of all the three members of miR-106b-25 cluster (miR-106b, miR-93 and miR-25) in salvianolic acid B[49].

Protective functions of decreased expression of oncogenic miR-21 and increased expression oftumor suppressor miR-138 regulated by α-solanine has been shown in prostate cancer. These therapeutic effects have been experimentally examined to be mediated by suppressing ERK and PI3K-Akt signaling pathways, inhibiting EMT, reducing proliferation and inducing apoptosis[50].

Systemic administration of cationic lipid NP/pre-miR-107 significantly has been shown to retard tumor growth by 45.2% compared to NP/pre-miR-control by decreasing the expression of miR-107 targets including protein kinase Cε, cyclin-dependent kinase 6, and hypoxia-inducible factor 1-β. NP/pre-miR-107 further has been shown to reduce the cancer-initiating cell population and dampen the expression of the core embryonic stem cell transcription factors, Nanog, Oct3/4, and Sox2 and inhibit the clonogenic survival, cell invasion, and cell migration of HNSCC (head and neck squamous cell carcinoma) cells[51].

Overexpression of miR-612 has been shown to suppress the stemness of hepatocellular carcinoma by reducing the number and size of tumorospheres, clone formation in soft agar and relieve the cancer cells from drug resistance to cisplatin and 5-fluorouracil by targeting Wnt/β-catenin signaling, thereby control EMT-associated stem cell-like traits[52].

Diverse cellular pathways that modulate CSCs functions act in concert with various molecular events and promote tumor initiation, progression and metastasis. Over the past recent years, emergence of miRNAs and molecular-targeted therapies has intensified research in the development of miRNA based therapeutic agents for cancer treatment with improved clinical outcome. Preclinical therapeutic data on mechanistic regulation of metastasis by miRNAs from *in vitro* studies need to be reevaluated in genetically engineered animal models which could further help us to provide new insights into clinical applications of miRNAs. Understanding the complex network of biological properties of CSCs, their control by multifunctional miRNAs and potential involvement of miRNAs in the development of anti-cancer drug resistance, reversion of cancer stemness and thereby rendering advanced cancers more susceptible to long term control are some of the open challenges to design novel strategies that can be used in preventive and treatment settings as an adjuvant to current cancer therapeutics.

**CONCLUSIONS**

Cancer stem cells which reside as a subset in many cancers are capable of self-renewal, tumor initiation, recurrence, metastasis, conferring resistance to anti-neoplastic drugs are still considered as huge obstacles on the way to cure cancer. This highlights the need to design strategies and therapeutics that specifically target and kill CSCs to eliminate cancer. Over the past few years, small non-coding RNAs, called miRNAs, have gained widespread attention in molecular biology due to their involvement in DNA translational control, their impression on mRNA and protein expression levels, and their ability to reprogram molecular signaling pathways in cancer. Differential miRNA profile in CSCs make them as potential biomarkers for aggressive tumor biology and therapeutic resistance and their biological specificity in targeting the various properties of CSCs make them strong targets for improving the response to anti-cancer drugs by sensitizing these cells to enhanced apoptotic effects of drugs. Novel anticancer therapies are based on the manipulation of oncogenic or tumor suppressor miRNAs by reducing or increasing their expression levels respectively. Success of using miRNAs as specific drug target in clinically relevant animal models as well as in preclinical development has been tested and determined by normal organ morphology, blood chemistries, serum cytokine levels, significant tumor regression and prolonged survival.

**REFERENCES**

1 **Lapidot T**, Sirard C, Vormoor J, Murdoch B, Hoang T, Caceres-Cortes J, Minden M, Paterson B, Caligiuri MA, Dick JE. A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. *Nature* 1994; **367**: 645-648 [PMID: 7509044 DOI: [10.1038/367645a0](http://dx.doi.org/10.1038/367645a0)]

2 **Colmont CS**, Harding KG, Piguet V, Patel GK. Human skin cancer stem cells: a tale of mice and men. *Exp Dermatol* 2012; **21**: 576-580 [PMID: 22775992 DOI: 10.1111/j.1600-0625.2012.01533.x]

3 **Baccelli I**, Trumpp A.The evolving concept of cancer and metastasis stem cells. *J Cell Biol* 2012; **198**: 281-293 [PMID: 22869594 DOI: 10.1083/jcb.201202014]

4 **Hatina J**. The dynamics of cancer stem cells. *Neoplasma* 2012; **59**: 700-707 [PMID: 22862171 DOI: 10.4149/neo\_2012\_092]

5 **Garg M**. Epithelial-mesenchymal transition - activating transcription factors - multifunctional regulators in cancer. *World J Stem Cells* 2013; **5**: 188-195 [PMID: 24179606 DOI: 10.4252/wjsc.v5.i4.188]

6 **Garg M.** Mismatch repair system: Therapeutic approaches to cancer stem cells. In: Cancer Stem Cells. Singh SR, editor. India: Research Signpost, 2010: 271-291

7 **Jamieson CH**, Ailles LE, Dylla SJ, Muijtjens M, Jones C, Zehnder JL, Gotlib J, Li K, Manz MG, Keating A, Sawyers CL, Weissman IL. Granulocyte-macrophage progenitors as candidate leukemic stem cells in blast-crisis CML. *N Engl J Med* 2004; **351**: 657-667 [PMID: 15306667]

8 **Korkaya H**, Paulson A, Charafe-Jauffret E, Ginestier C, Brown M, Dutcher J, Clouthier SG, Wicha MS. Regulation of mammary stem/progenitor cells by PTEN/Akt/beta-catenin signaling. *PLoS Biol* 2009; **7**: e1000121 [PMID: 19492080 DOI: 10.1371/journal.pbio.1000121]

9 **Yan K**, Yang K, Rich JN. The evolving landscape of glioblastoma stem cells. *Curr Opin Neurol* 2013; **26**: 701-707 [PMID: 24152818 DOI: 10.1097/WCO.0000000000000032]

10 **Takahashi RU**, Miyazaki H, Ochiya T. The role of microRNAs in the regulation of cancer stem cells. *Front Genet* 2014; **4**: 295 [PMID: 24427168 DOI: 10.3389/fgene.2013.00295]

11 **So JY**, Suh N. Targeting cancer stem cells in solid tumors by vitamin D. *J Steroid Biochem Mol Biol* 2015; **148**: 79-85 [PMID: 25460302 DOI: 10.1016/j.jsbmb.2014.10.007]

12 **Garg M**. Potential therapeutic applications of microRNAs in response to DNA damage in cancer stem cells. *J Stem Cells* 2011; **6**: 51-65 [PMID: 22997846]

13 **Lee RC**, Feinbaum RL, Ambros V. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. *Cell* 1993; **75**: 843-854 [PMID: 8252621]

14 **Garg M**. MicroRNAs, stem cells and cancer stem cells. *World J Stem Cells* 2012; **4**: 62-70 [PMID: 22993663 DOI: [10.4252/wjsc.v4.i7.62](http://dx.doi.org/10.4252/wjsc.v4.i7.62)]

15 **Lee Y**, Kim M, Han J, Yeom KH, Lee S, Baek SH, Kim VN. MicroRNA genes are transcribed by RNA polymerase II. *EMBO J* 2004; **23**: 4051-4060 [PMID: 15372072 DOI: [10.1038/sj.emboj.7600385](http://dx.doi.org/10.1038/sj.emboj.7600385)]

16 **Borchert GM**, Lanier W, Davidson BL. RNA polymerase III transcribes human microRNAs. *Nat Struct Mol Biol* 2006; **13**: 1097-1101 [PMID: 17099701 DOI: [10.1038/nsmb1167](http://dx.doi.org/10.1038/nsmb1167)]

17 **Cai X**, Hagedorn CH, Cullen BR. Human microRNAs are processed from capped, polyadenylated transcripts that can also function as mRNAs. *RNA* 2004; **10**: 1957-1966 [PMID: 15525708 DOI: [10.1261/rna.7135204](http://dx.doi.org/10.1261/rna.7135204)]

18 **Gregory RI**, Chendrimada TP, Shiekhattar R. MicroRNA biogenesis: isolation and characterization of the microprocessor complex. *Methods Mol Biol* 2006; **342**: 33-47 [PMID: 16957365 DOI: [10.1385/1-59745-123-1:33](http://dx.doi.org/10.1385/1-59745-123-1:33)]

19 **Bohnsack MT**, Czaplinski K, Gorlich D. Exportin 5 is a RanGTP-dependent dsRNA-binding protein that mediates nuclear export of pre-miRNAs. *RNA* 2004; **10**: 185-191 [PMID: 14730017 DOI: [10.1261/rna.5167604](http://dx.doi.org/10.1261/rna.5167604)]

20 **Hutvágner G**, McLachlan J, Pasquinelli AE, Bálint E, Tuschl T, Zamore PD. A cellular function for the RNA-interference enzyme Dicer in the maturation of the let-7 small temporal RNA. *Science* 2001; **293**: 834-838 [PMID: 11452083]

21 **Pratt AJ**, MacRae IJ. The RNA-induced silencing complex: a versatile gene-silencing machine. *J Biol Chem* 2009; **284**: 17897-17901 [PMID: 19342379 DOI: 10.1074/jbc.R900012200]

22 **Melton C**, Judson RL, Blelloch R. Opposing microRNA families regulate self-renewal in mouse embryonic stem cells. *Nature* 2010; **463**: 621-626 [PMID: 20054295 DOI: 10.1038/nature08725]

23 **Yu J**, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, Nie J, Jonsdottir GA, Ruotti V, Stewart R, Slukvin II, Thomson JA. Induced pluripotent stem cell lines derived from human somatic cells. *Science* 2007; **318**: 1917-1920 [PMID: 18029452 DOI: 10.1126/science.1151526]

24 **King CE**, Cuatrecasas M, Castells A, Sepulveda AR, Lee JS, Rustgi AK. LIN28B promotes colon cancer progression and metastasis. *Cancer Res* 2011; **71**: 4260-4268 [PMID: 21512136 DOI: 10.1158/0008-5472.CAN-10-4637]

25 **Otsubo T**, Akiyama Y, Hashimoto Y, Shimada S, Goto K, Yuasa Y. MicroRNA-126 inhibits SOX2 expression and contributes to gastric carcinogenesis. *PLoS One* 2011; **6**: e16617 [PMID: 21304604 DOI: 10.1371/journal.pone.0016617]

26 **Lin SL**, Chang DC, Chang-Lin S, Lin CH, Wu DT, Chen DT, Ying SY. Mir-302 reprograms human skin cancer cells into a pluripotent ES-cell-like state. *RNA* 2008; **14**: 2115-2124 [PMID: 18755840 DOI: 10.1261/rna.1162708]

27 **Choi YJ**, Lin CP, Ho JJ, He X, Okada N, Bu P, Zhong Y, Kim SY, Bennett MJ, Chen C, Ozturk A, Hicks GG, Hannon GJ, He L. miR-34 miRNAs provide a barrier for somatic cell reprogramming. *Nat Cell Biol* 2011; **13**: 1353-1360 [PMID: 22020437 DOI: 10.1038/ncb2366]

28 **Corney DC**, Flesken-Nikitin A, Godwin AK, Wang W, Nikitin AY. MicroRNA-34b and MicroRNA-34c are targets of p53 and cooperate in control of cell proliferation and adhesion-independent growth. *Cancer Res* 2007; **67**: 8433-8438 [PMID: 17823410]

29 **Ji Q**, Hao X, Zhang M, Tang W, Yang M, Li L, Xiang D, Desano JT, Bommer GT, Fan D, Fearon ER, Lawrence TS, Xu L. MicroRNA miR-34 inhibits human pancreatic cancer tumor-initiating cells. *PLoS One* 2009; **4**: e6816 [PMID: 19714243 DOI: 10.1371/journal.pone.0006816]

30 **Gao Y**, Liu T, Huang Y. MicroRNA-134 suppresses endometrial cancer stem cells by targeting POGLUT1 and Notch pathway proteins. *FEBS Lett* 2015; **589**: 207-214 [PMID: 25528443 DOI: 10.1016/j.febslet.2014.12.002]

31 **Park EC**, Kim G, Jung J, Wang K, Lee S, Jeon SS, Lee ZW, Kim SI, Kim S, Oh YT, Shin JH, Jang HS, Choi BO, Kim GH.Differential expression of MicroRNAs in patients with glioblastoma after concomitant chemoradiotherapy. *OMICS* 2013; **17**: 259-268 [PMID: 23586679 DOI: 10.1089/omi.2012.0065]

32 **Garg M**. Targeting microRNAs in epithelial-to-mesenchymal transition-induced cancer stem cells: therapeutic approaches in cancer. *Expert Opin Ther Targets* 2015; **19**: 285-297 [PMID: 25563894 DOI: 10.1517/14728222.2014.975794]

33 [**Torres AG**](http://www.ncbi.nlm.nih.gov/pubmed/?term=Torres%20AG%5BAuthor%5D&cauthor=true&cauthor_uid=21441346), [Fabani MM](http://www.ncbi.nlm.nih.gov/pubmed/?term=Fabani%20MM%5BAuthor%5D&cauthor=true&cauthor_uid=21441346), [Vigorito E](http://www.ncbi.nlm.nih.gov/pubmed/?term=Vigorito%20E%5BAuthor%5D&cauthor=true&cauthor_uid=21441346), [Gait MJ](http://www.ncbi.nlm.nih.gov/pubmed/?term=Gait%20MJ%5BAuthor%5D&cauthor=true&cauthor_uid=21441346). MicroRNA fate upon targeting with anti-miRNA oligonucleotides as revealed by an improved Northern-blot-based method for miRNA detection. [*RNA*](http://www.ncbi.nlm.nih.gov/pubmed/?term=RNA+2011%2C+17+(5)%2C+933-943)2011; **17**: 933-943 [PMID: 21441346 DOI: 10.1261/rna.2533811]

34 **Bader AG**. [miR-34 - a microRNA replacement therapy is headed to the clinic.](http://www.ncbi.nlm.nih.gov/pubmed/22783274) *Front Genet* 2012; **3**:120 [PMID: 22783274 DOI: 10.3389/fgene.2012.00120]

35 [**Bader AG**](http://www.ncbi.nlm.nih.gov/pubmed/?term=Bader%20AG%5BAuthor%5D&cauthor=true&cauthor_uid=20807816), [Brown D](http://www.ncbi.nlm.nih.gov/pubmed/?term=Brown%20D%5BAuthor%5D&cauthor=true&cauthor_uid=20807816), [Winkler M](http://www.ncbi.nlm.nih.gov/pubmed/?term=Winkler%20M%5BAuthor%5D&cauthor=true&cauthor_uid=20807816). The promise of microRNA replacement therapy. [*Cancer Res*](http://www.ncbi.nlm.nih.gov/pubmed/?term=Cancer+Res%2C+2010%2C+70+(18)%2C+7027) 2010; **70**: 7027-7030 [PMID: 20807816 DOI: 10.1158/0008-5472.CAN-10-2010]

36 [**Rothschild SI**](http://www.ncbi.nlm.nih.gov/pubmed/?term=Rothschild%20SI%5BAuthor%5D&cauthor=true&cauthor_uid=26056576).microRNA therapies in cancer**.** [*Mol Cell Ther*](http://www.ncbi.nlm.nih.gov/pubmed/?term=Rochschild+SI%2C+Mol+Can+Ther%2C+2014%2C+2%2C7) 2014; **2**: 7 [PMID: 26056576DOI: 10.1186/2052-8426-2-7]

37 **Deng J**, Lei W, Fu JC, Zhang L, Li JH, Xiong JP. Targeting miR-21 enhances the sensitivity of human colon cancer HT-29 cells to chemoradiotherapy in vitro. *Biochem Biophys Res Commun* 2014; **443**: 789-795 [PMID: 24275137 DOI: 10.1016/j.bbrc.2013.11.064]

38 **Nangia-Makker P**, Yu Y, Vasudevan A, Farhana L, Rajendra SG, Levi E, Majumdar AP. Metformin: a potential therapeutic agent for recurrent colon cancer. *PLoS One* 2014; **9**: e84369 [PMID: 24465408 DOI: 10.1371/journal.pone.0084369]

39 **Munoz JL**, Bliss SA, Greco SJ, Ramkissoon SH, Ligon KL, Rameshwar P. Delivery of Functional Anti-miR-9 by Mesenchymal Stem Cell-derived Exosomes to Glioblastoma Multiforme Cells Conferred Chemosensitivity. *Mol Ther Nucleic Acids* 2013; **2**: e126 [PMID: 24084846 DOI: 10.1038/mtna.2013.60]

40 **Shi L**, Wan Y, Sun G, Zhang S, Wang Z, Zeng Y. miR-125b inhibitor may enhance the invasion-prevention activity of temozolomide in glioblastoma stem cells by targeting PIAS3. *BioDrugs* 2014; **28**: 41-54 [PMID: 23857508 DOI: 10.1007/s40259-013-0053-2]

41 **Gal H**, Pandi G, Kanner AA, Ram Z, Lithwick-Yanai G, Amariglio N, Rechavi G, Givol D. MIR-451 and Imatinib mesylate inhibit tumor growth of Glioblastoma stem cells. *Biochem Biophys Res Commun* 2008; **376**: 86-90 [PMID: 18765229 DOI: 10.1016/j.bbrc.2008.08.107]

42 **Mittal A**, Chitkara D, Behrman SW, Mahato RI. Efficacy of gemcitabine conjugated and miRNA-205 complexed micelles for treatment of advanced pancreatic cancer. *Biomaterials* 2014; **35**: 7077-7087 [PMID: 24836307 DOI: 10.1016/j.biomaterials.2014.04.053]

43 **Liu Q**, Li RT, Qian HQ, Wei J, Xie L, Shen J, Yang M, Qian XP, Yu LX, Jiang XQ, Liu BR. Targeted delivery of miR-200c/DOC to inhibit cancer stem cells and cancer cells by the gelatinases-stimuli nanoparticles. *Biomaterials* 2013; **34**: 7191-7203 [PMID: 23806972 DOI: 10.1016/j.biomaterials.2013.06.004]

44 **Park EY**, Chang E, Lee EJ, Lee HW, Kang HG, Chun KH, Woo YM, Kong HK, Ko JY, Suzuki H, Song E, Park JH. Targeting of miR34a-NOTCH1 axis reduced breast cancer stemness and chemoresistance. *Cancer Res* 2014; **74**: 7573-7582 [PMID: 25368020 DOI: 10.1158/0008-5472.CAN-14-1140]

45 **Siemens H**, Jackstadt R, Kaller M, Hermeking H. Repression of c-Kit by p53 is mediated by miR-34 and is associated with reduced chemoresistance, migration and stemness. *Oncotarget* 2013; **4**: 1399-1415 [PMID: 24009080]

46 [**Trang P**](http://www.ncbi.nlm.nih.gov/pubmed/?term=Trang%20P%5BAuthor%5D&cauthor=true&cauthor_uid=19966857), [Medina PP](http://www.ncbi.nlm.nih.gov/pubmed/?term=Medina%20PP%5BAuthor%5D&cauthor=true&cauthor_uid=19966857), [Wiggins JF](http://www.ncbi.nlm.nih.gov/pubmed/?term=Wiggins%20JF%5BAuthor%5D&cauthor=true&cauthor_uid=19966857), [Ruffino L](http://www.ncbi.nlm.nih.gov/pubmed/?term=Ruffino%20L%5BAuthor%5D&cauthor=true&cauthor_uid=19966857), [Kelnar K](http://www.ncbi.nlm.nih.gov/pubmed/?term=Kelnar%20K%5BAuthor%5D&cauthor=true&cauthor_uid=19966857), [Omotola M](http://www.ncbi.nlm.nih.gov/pubmed/?term=Omotola%20M%5BAuthor%5D&cauthor=true&cauthor_uid=19966857), [Homer R](http://www.ncbi.nlm.nih.gov/pubmed/?term=Homer%20R%5BAuthor%5D&cauthor=true&cauthor_uid=19966857), [Brown D](http://www.ncbi.nlm.nih.gov/pubmed/?term=Brown%20D%5BAuthor%5D&cauthor=true&cauthor_uid=19966857), [Bader AG](http://www.ncbi.nlm.nih.gov/pubmed/?term=Bader%20AG%5BAuthor%5D&cauthor=true&cauthor_uid=19966857), [Weidhaas JB](http://www.ncbi.nlm.nih.gov/pubmed/?term=Weidhaas%20JB%5BAuthor%5D&cauthor=true&cauthor_uid=19966857), [Slack FJ](http://www.ncbi.nlm.nih.gov/pubmed/?term=Slack%20FJ%5BAuthor%5D&cauthor=true&cauthor_uid=19966857). Regression of murine lung tumors by the let-7 microRNA. [*Oncogene*](http://www.ncbi.nlm.nih.gov/pubmed/?term=Oncogene+2010%2C+29%2C+1580) 2010; **29**: 1580-1587 [PMID: 19966857 DOI: 10.1038/onc.2009.445]

47 [**Esquela-Kerscher A**](http://www.ncbi.nlm.nih.gov/pubmed/?term=Esquela-Kerscher%20A%5BAuthor%5D&cauthor=true&cauthor_uid=18344688), [Trang P](http://www.ncbi.nlm.nih.gov/pubmed/?term=Trang%20P%5BAuthor%5D&cauthor=true&cauthor_uid=18344688), [Wiggins JF](http://www.ncbi.nlm.nih.gov/pubmed/?term=Wiggins%20JF%5BAuthor%5D&cauthor=true&cauthor_uid=18344688), [Patrawala L](http://www.ncbi.nlm.nih.gov/pubmed/?term=Patrawala%20L%5BAuthor%5D&cauthor=true&cauthor_uid=18344688), [Cheng A](http://www.ncbi.nlm.nih.gov/pubmed/?term=Cheng%20A%5BAuthor%5D&cauthor=true&cauthor_uid=18344688), [Ford L](http://www.ncbi.nlm.nih.gov/pubmed/?term=Ford%20L%5BAuthor%5D&cauthor=true&cauthor_uid=18344688), [Weidhaas JB](http://www.ncbi.nlm.nih.gov/pubmed/?term=Weidhaas%20JB%5BAuthor%5D&cauthor=true&cauthor_uid=18344688), [Brown D](http://www.ncbi.nlm.nih.gov/pubmed/?term=Brown%20D%5BAuthor%5D&cauthor=true&cauthor_uid=18344688), [Bader AG](http://www.ncbi.nlm.nih.gov/pubmed/?term=Bader%20AG%5BAuthor%5D&cauthor=true&cauthor_uid=18344688), [Slack FJ](http://www.ncbi.nlm.nih.gov/pubmed/?term=Slack%20FJ%5BAuthor%5D&cauthor=true&cauthor_uid=18344688). The let-7 microRNA reduces tumor growth in mouse models of lung cancer. [*Cell Cycle*](http://www.ncbi.nlm.nih.gov/pubmed/?term=cell+cycle+2008%2C+7%3B759) 2008; **7**: 759-764 [PMID: 18344688]

48 **Hershkovitz-Rokah O**, Modai S, Pasmanik-Chor M, Toren A, Shomron N, Raanani P, Shpilberg O, Granot G. MiR-30e induces apoptosis and sensitizes K562 cells to imatinib treatment *via* regulation of the BCR-ABL protein. *Cancer Lett* 2015; **356**: 597-605 [PMID: 25305453 DOI: 10.1016/j.canlet.2014.10.006]

49 [**Tang Q**](http://www.ncbi.nlm.nih.gov/pubmed/?term=Tang%20Q%5BAuthor%5D&cauthor=true&cauthor_uid=25094038), [Zhong H](http://www.ncbi.nlm.nih.gov/pubmed/?term=Zhong%20H%5BAuthor%5D&cauthor=true&cauthor_uid=25094038), [Xie F](http://www.ncbi.nlm.nih.gov/pubmed/?term=Xie%20F%5BAuthor%5D&cauthor=true&cauthor_uid=25094038), [Xie J](http://www.ncbi.nlm.nih.gov/pubmed/?term=Xie%20J%5BAuthor%5D&cauthor=true&cauthor_uid=25094038), [Chen H](http://www.ncbi.nlm.nih.gov/pubmed/?term=Chen%20H%5BAuthor%5D&cauthor=true&cauthor_uid=25094038), [Yao G](http://www.ncbi.nlm.nih.gov/pubmed/?term=Yao%20G%5BAuthor%5D&cauthor=true&cauthor_uid=25094038). Expression of miR-106b-25 induced by salvianolic acid B inhibits epithelial-to-mesenchymal transition in HK-2 cells. [*Eur J Pharmacol*](http://www.ncbi.nlm.nih.gov/pubmed/?term=Tang+Q%2C+Eur+J+Pharmoacol%2C+2014%2C) 2014; **741**: 97-103 [PMID: 25094038 DOI: 0.1016/j.ejphar.2014.07.051]

50 [**Shen KH**](http://www.ncbi.nlm.nih.gov/pubmed/?term=Shen%20KH%5BAuthor%5D&cauthor=true&cauthor_uid=25116803), [Liao AC](http://www.ncbi.nlm.nih.gov/pubmed/?term=Liao%20AC%5BAuthor%5D&cauthor=true&cauthor_uid=25116803), [Hung JH](http://www.ncbi.nlm.nih.gov/pubmed/?term=Hung%20JH%5BAuthor%5D&cauthor=true&cauthor_uid=25116803), [Lee WJ](http://www.ncbi.nlm.nih.gov/pubmed/?term=Lee%20WJ%5BAuthor%5D&cauthor=true&cauthor_uid=25116803), [Hu KC](http://www.ncbi.nlm.nih.gov/pubmed/?term=Hu%20KC%5BAuthor%5D&cauthor=true&cauthor_uid=25116803), [Lin PT](http://www.ncbi.nlm.nih.gov/pubmed/?term=Lin%20PT%5BAuthor%5D&cauthor=true&cauthor_uid=25116803), [Liao RF](http://www.ncbi.nlm.nih.gov/pubmed/?term=Liao%20RF%5BAuthor%5D&cauthor=true&cauthor_uid=25116803), [Chen PS](http://www.ncbi.nlm.nih.gov/pubmed/?term=Chen%20PS%5BAuthor%5D&cauthor=true&cauthor_uid=25116803). α-Solanine inhibits invasion of human prostate cancer cell by suppressing epithelial-mesenchymal transition and MMPs expression. [*Molecules*](http://www.ncbi.nlm.nih.gov/pubmed/?term=molecules+2014%2C+19+(8)%2C+11896) 2014; **19**: 11896-11914 [PMID: 25116803 DOI: 10.3390/molecules190811896]

51 **Piao L**, Zhang M, Datta J, Xie X, Su T, Li H, Teknos TN, Pan Q. Lipid-based nanoparticle delivery of Pre-miR-107 inhibits the tumorigenicity of head and neck squamous cell carcinoma. *Mol Ther* 2012; **20**: 1261-1269 [PMID: 22491216 DOI: 10.1038/mt.2012.67]

52 **Tang J**, Tao ZH, Wen D, Wan JL, Liu DL, Zhang S, Cui JF, Sun HC, Wang L, Zhou J, Fan J, Wu WZ. MiR-612 suppresses the stemness of liver cancer *via* Wnt/β-catenin signaling. *Biochem Biophys Res Commun* 2014; **447**: 210-215 [PMID: 24704424 DOI: 10.1016/j.bbrc.2014.03.135]

53 **Hasegawa S**, Eguchi H, Nagano H, Konno M, Tomimaru Y, Wada H, Hama N, Kawamoto K, Kobayashi S, Nishida N, Koseki J, Nishimura T, Gotoh N, Ohno S, Yabuta N, Nojima H, Mori M, Doki Y, Ishii H. MicroRNA-1246 expression associated with CCNG2-mediated chemoresistance and stemness in pancreatic cancer. *Br J Cancer* 2014; **111**: 1572-1580 [PMID: 25117811 DOI: 10.1038/bjc.2014.454]

54 **Hwang-Verslues WW**, Chang PH, Wei PC, Yang CY, Huang CK, Kuo WH, Shew JY, Chang KJ, Lee EY, Lee WH. miR-495 is upregulated by E12/E47 in breast cancer stem cells, and promotes oncogenesis and hypoxia resistance *via* downregulation of E-cadherin and REDD1. *Oncogene* 2011; **30**: 2463-2474 [PMID: 21258409 DOI: 10.1038/onc.2010.618]

55 **Zhou AD**, Diao LT, Xu H, Xiao ZD, Li JH, Zhou H, Qu LH. β-Catenin/LEF1 transactivates the microRNA-371-373 cluster that modulates the Wnt/β-catenin-signaling pathway. *Oncogene* 2012; **31**: 2968-2978 [PMID: 22020335 DOI: 10.1038/onc.2011.461]

56 **Cairo S**, Wang Y, de Reyniès A, Duroure K, Dahan J, Redon MJ, Fabre M, McClelland M, Wang XW, Croce CM, Buendia MA. Stem cell-like micro-RNA signature driven by Myc in aggressive liver cancer. *Proc Natl Acad Sci USA* 2010; **107**: 20471-20476 [PMID: 21059911 DOI: 10.1073/pnas.1009009107]

57 **Xia H**, Ooi LL, Hui KM. MicroRNA-216a/217-induced epithelial-mesenchymal transition targets PTEN and SMAD7 to promote drug resistance and recurrence of liver cancer. *Hepatology* 2013; **58**: 629-641 [PMID: 23471579 DOI: 10.1002/hep.26369]

58 **Bao B**, Ali S, Ahmad A, Azmi AS, Li Y, Banerjee S, Kong D, Sethi S, Aboukameel A, Padhye SB, Sarkar FH. Hypoxia-induced aggressiveness of pancreatic cancer cells is due to increased expression of VEGF, IL-6 and miR-21, which can be attenuated by CDF treatment. *PLoS One* 2012; **7**: e50165 [PMID: 23272057 DOI: 10.1371/journal.pone.0050165]

59 **Xu W**, Ji J, Xu Y, Liu Y, Shi L, Liu Y, Lu X, Zhao Y, Luo F, Wang B, Jiang R, Zhang J, Liu Q. MicroRNA-191, by promoting the EMT and increasing CSC-like properties, is involved in neoplastic and metastatic properties of transformed human bronchial epithelial cells. *Mol Carcinog* 2015; **54** Suppl 1: E148-E161 [PMID: 25252218 DOI: 10.1002/mc.22221]

60 **Ma S**, Tang KH, Chan YP, Lee TK, Kwan PS, Castilho A, Ng I, Man K, Wong N, To KF, Zheng BJ, Lai PB, Lo CM, Chan KW, Guan XY. miR-130b Promotes CD133(+) liver tumor-initiating cell growth and self-renewal *via* tumor protein 53-induced nuclear protein 1. *Cell Stem Cell* 2010; **7**: 694-707 [PMID: 21112564 DOI: 10.1016/j.stem.2010.11.010]

61 **Han YC**, Park CY, Bhagat G, Zhang J, Wang Y, Fan JB, Liu M, Zou Y, Weissman IL, Gu H. microRNA-29a induces aberrant self-renewal capacity in hematopoietic progenitors, biased myeloid development, and acute myeloid leukemia. *J Exp Med* 2010; **207**: 475-489 [PMID: 20212066 DOI: 10.1084/jem.20090831]

62 **Turchi L**, Debruyne DN, Almairac F, Virolle V, Fareh M, Neirijnck Y, Burel-Vandenbos F, Paquis P, Junier MP, Van Obberghen-Schilling E, Chneiweiss H, Virolle T. Tumorigenic potential of miR-18A\* in glioma initiating cells requires NOTCH-1 signaling. *Stem Cells* 2013; **31**: 1252-1265 [PMID: 23533157 DOI: 10.1002/stem.1373]

63 **Sureban SM**, May R, Qu D, Weygant N, Chandrakesan P, Ali N, Lightfoot SA, Pantazis P, Rao CV, Postier RG, Houchen CW. DCLK1 regulates pluripotency and angiogenic factors *via* microRNA-dependent mechanisms in pancreatic cancer. *PLoS One* 2013; **8**: e73940 [PMID: 24040120 DOI: 10.1371/journal.pone.0073940]

64 **Xi S**, Xu H, Shan J, Tao Y, Hong JA, Inchauste S, Zhang M, Kunst TF, Mercedes L, Schrump DS. Cigarette smoke mediates epigenetic repression of miR-487b during pulmonary carcinogenesis. *J Clin Invest* 2013; **123**: 1241-1261 [PMID: 23426183 DOI: 10.1172/JCI61271]

65 **Babashah S**, Sadeghizadeh M, Hajifathali A, Tavirani MR, Zomorod MS, Ghadiani M, Soleimani M. Targeting of the signal transducer Smo links microRNA-326 to the oncogenic Hedgehog pathway in CD34+ CML stem/progenitor cells. *Int J Cancer* 2013; **133**: 579-589 [PMID: 23341351 DOI: 10.1002/ijc.28043]

66 **Ying Z**, Li Y, Wu J, Zhu X, Yang Y, Tian H, Li W, Hu B, Cheng SY, Li M. Loss of miR-204 expression enhances glioma migration and stem cell-like phenotype. *Cancer Res* 2013; **73**: 990-999 [PMID: 23204229 DOI: 10.1158/0008-5472.CAN-12-2895]

67 **Lu Y**, Lu J, Li X, Zhu H, Fan X, Zhu S, Wang Y, Guo Q, Wang L, Huang Y, Zhu M, Wang Z. MiR-200a inhibits epithelial-mesenchymal transition of pancreatic cancer stem cell. *BMC Cancer* 2014; **14**: 85 [PMID: 24521357 DOI: 10.1186/1471-2407-14-85]

68 **Wang Y**, Yu Y, Tsuyada A, Ren X, Wu X, Stubblefield K, Rankin-Gee EK, Wang SE. Transforming growth factor-β regulates the sphere-initiating stem cell-like feature in breast cancer through miRNA-181 and ATM. *Oncogene* 2011; **30**: 1470-1480 [PMID: 21102523 DOI: 10.1038/onc.2010.531]

69 **Morris VA**, Zhang A, Yang T, Stirewalt DL, Ramamurthy R, Meshinchi S, Oehler VG. MicroRNA-150 expression induces myeloid differentiation of human acute leukemia cells and normal hematopoietic progenitors. *PLoS One* 2013; **8**: e75815 [PMID: 24086639 DOI: 10.1371/journal.pone.0075815]

70 **Pramanik D**, Campbell NR, Karikari C, Chivukula R, Kent OA, Mendell JT, Maitra A. Restitution of tumor suppressor microRNAs using a systemic nanovector inhibits pancreatic cancer growth in mice. *Mol Cancer Ther* 2011; **10**: 1470-1480 [PMID: 21622730 DOI: 10.1158/1535-7163.MCT-11-0152]

71 **Godlewski J**, Nowicki MO, Bronisz A, Williams S, Otsuki A, Nuovo G, Raychaudhury A, Newton HB, Chiocca EA, Lawler S. Targeting of the Bmi-1 oncogene/stem cell renewal factor by microRNA-128 inhibits glioma proliferation and self-renewal. *Cancer Res* 2008; **68**: 9125-9130 [PMID: 19010882 DOI: 10.1158/0008-5472.CAN-08-2629]

72 **Collet G**, Skrzypek K, Grillon C, Matejuk A, El Hafni-Rahbi B, Lamerant-Fayel N, Kieda C. Hypoxia control to normalize pathologic angiogenesis: potential role for endothelial precursor cells and miRNAs regulation. *Vascul Pharmacol* 2012; **56**: 252-261 [PMID: 22446152 DOI: 10.1016/j.vph.2012.03.001]

73 **Di Fiore R**, Drago-Ferrante R, Pentimalli F, Di Marzo D, Forte IM, D'Anneo A, Carlisi D, De Blasio A, Giuliano M, Tesoriere G, Giordano A, Vento R. MicroRNA-29b-1 impairs in vitro cell proliferation, self‑renewal and chemoresistance of human osteosarcoma 3AB-OS cancer stem cells. *Int J Oncol* 2014; **45**: 2013-2023 [PMID: 25174983 DOI: 10.3892/ijo.2014.2618]

74 **Scheibner KA**, Teaboldt B, Hauer MC, Chen X, Cherukuri S, Guo Y, Kelley SM, Liu Z, Baer MR, Heimfeld S, Civin CI. MiR-27a functions as a tumor suppressor in acute leukemia by regulating 14-3-3θ. *PLoS One* 2012; **7**: e50895 [PMID: 23236401 DOI: 10.1371/journal.pone.0050895]

75 **Geng J**, Luo H, Pu Y, Zhou Z, Wu X, Xu W, Yang Z. Methylation mediated silencing of miR-23b expression and its role in glioma stem cells. *Neurosci Lett* 2012; **528**: 185-189 [PMID: 22982144 DOI: 10.1016/j.neulet.2012.08.055]

76 **Chen J**, Fu X, Wan Y, Wang Z, Jiang D, Shi L. miR-125b inhibitor enhance the chemosensitivity of glioblastoma stem cells to temozolomide by targeting Bak1. *Tumour Biol* 2014; **35**: 6293-6302 [PMID: 24643683 DOI: 10.1007/s13277-014-1821-4]

77 **Hwang WL**, Jiang JK, Yang SH, Huang TS, Lan HY, Teng HW, Yang CY, Tsai YP, Lin CH, Wang HW, Yang MH. MicroRNA-146a directs the symmetric division of Snail-dominant colorectal cancer stem cells. *Nat Cell Biol* 2014; **16**: 268-280 [PMID: 24561623 DOI: 10.1038/ncb2910]

**P- Reviewer:** Jun Y, Kiselev SL, Papaccio G **S- Editor:** Gong XM

**L- Editor:E- Editor:**

**Table 1 Some of the potential microRNAs involved in regulation of cancer stem cells**

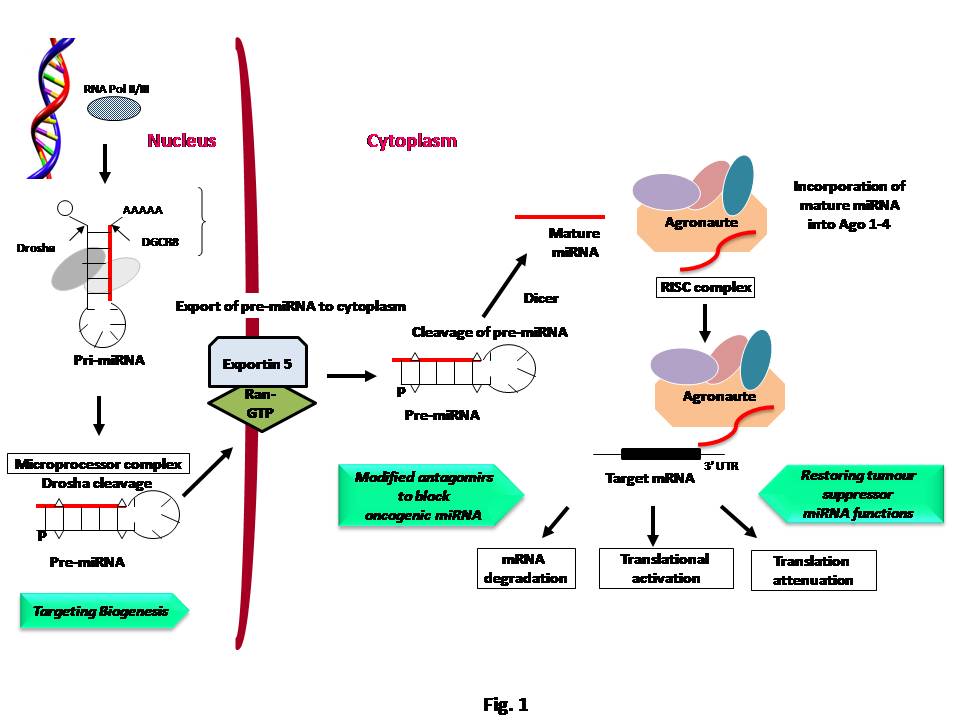
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| --- | --- | --- | --- |
| **MicroRNAs** | **Transcript target** | **Relevance to cancer progression and its biological functions** | **Ref.** |
| **Upregulated microRNAs** | | | |
| miR-1246 | CCNG2 | Pancreatic cancer: Induces chemoresistance and CSC-like properties | [53] |
| miR-495 | E-cadherin and REDD1 | Breast cancer: Promotes oncogenesis and hypoxia resistance | [54] |
| miR-371-373 cluster | Wnt/β-catenin, DKK1  Myc | Many cancers: Promotes cell growth and invasive activity  Liver cancer: Regulates the properties of CSCs | [55,56] |
| miR-216a/217 | PTEN and SMAD7 | Hepatocellular carcinoma: Increased proliferation, migration and metastatic ability | [57] |
| miR-210 | Nanog, Oct4 and EZH2 | Pancreatic cancer: Increased cell migration and invasion | [58] |
| miR-191 | BASP1, Wnt/β-catenin | Lung cancer: Increased migratory potential and neoplastic properties | [59] |
| miR-130b | P53-induced nuclear protein 1 | Acute myeloid leukemia: Regulates hematopoietic stem cells | [60] |
| miR-29a | P53-induced nuclear protein 1 | Acute myeloid leukemia: Regulates hematopoietic stem cells | [61] |
| miR-21 | Nanog, Oct4 and EZH2 | Pancreatic cancer: Increased cell migration and invasion | [58] |
| miR-18 | DLL4, inhibitor of Notch signaling | Glioma: Promotes tumorigenic potential of GSCs | [62] |
| **Downregulated microRNAs** | | | |
| Let-7 | Lin-28 | Colon adenocarcinomas: Promotes cell migration, invasion and transforms immortalized colonic epithelial cells  Pancreatic cancer: Increased pluripotency | [24,63] |
| miR-487b | SUZ12, BMI1, WNT5A, MYC, and KRAS | Lung cancer: Increased proliferation and invasion | [64] |
| miR-451 | SMAD 3 and 4 | GBM: Controls GBM stem cells differentiation | [41] |
| miR-326 | Hh smoothened signal transducer | Chronic myeloid leukemia: Increased cell proliferation and decreased apoptosis | [65] |
| miR-204 | Sox4 and Ephrin receptor EphB2 | Glioma: Involved in GSCs self-renewal and invasion | [66] |
| miR-200 family  miR-200a | VEGFR1, VEGFR2 and EMT-related transcription factors ZEB1, ZEB2, SNAIL and SLUG  N-cadherin, ZEB1,vimentin,  E-cadherin | Pancreatic cancer: Regulates CSCs properties  Pancreatic cancer: Increased cell migration and invasion | [63]  [67] |
| miR-181 | ATM | Breast cancer: Regulates the properties of CSCs | [68] |
| miR-150 | MYb | Acute myeloid leukemia: Blocking of myeloid differentiation | [69] |
| miR-143/145 cluster | KRAS2 and its downstream effector RREB1 | Pancreatic cancer: Regulates CSCs survival | [70] |
| miR-145 | OCT4, SOX2, NANOG, KLF4 as well as KRAS and RREB1 | Pancreatic cancer: Increased pluripotency | [63] |
| miR-128 | Histone methylation [H3K27me(3)], Akt phosphorylation, p21(CIP1) Bmi-1 | Glioma: Increased self-renewal and proliferation | [71] |
| miR-107 | Nanog, Oct3/4, and Sox2 | Head and neck squamous cell carcinoma: Increased CSC proliferation | [72] |
| miR-100/let-7a-2/miR-125b-1 cluster | Myc | Liver cancer: Regulates the properties of CSCs | [56] |
| miR-34 family | Notch and Bcl-2 | Pancreatic cancer: Involved in self-renewal of CSCs | [[28,29] |
| miR-29b-1 | CD133, N-Myc, CCND2, E2F1 and E2F2, Bcl-2, IAP-2, Oct3/4, Sox2 and Nanog | Osteosarcoma: Increased proliferation, self-renewal and chemoresistance | [73] |
| miR-27a | 14-3-3θ, Bax and Bad | Acute leukemia: Regulate apoptosis | [74] |
| miR-23b | Cell cycle arrest | Glioma: Inhibits proliferation | [75] |

CCNG2: Cyclin-G2; DKK1: Dickkopf-1; PTEN: Phosphatase and tensin homolog; BASP: Brain abundant, membrane attached signal protein; ATM: [Ataxia telangiectasia mutated;](http://en.wikipedia.org/wiki/Ataxia_telangiectasia_mutated) Myb: Myeloblastosis protooncogene protein; RREB1: Ras-responsive element binding protein-1; IAP-2: Inhibitor of apoptosis 2; CCND2: Cyclin D2; GSC: Glioma stem cell; GBM: Glioblastoma; CSC: Cancer stem cell.

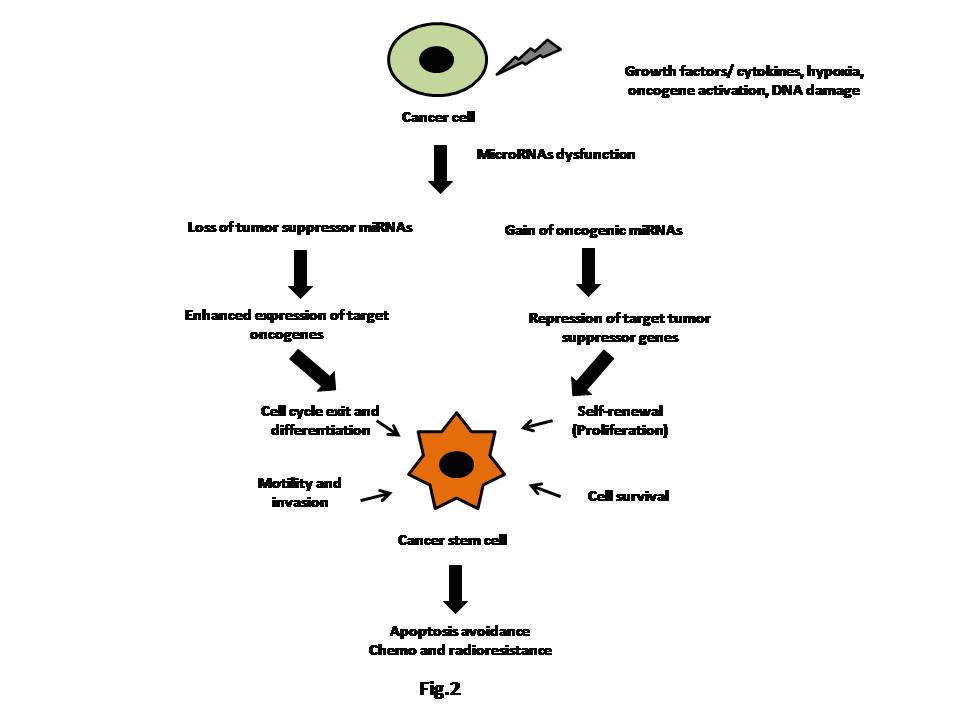
**Table 2 Targeting cancer stem cells with specific microRNAs as a potential combinational therapeutic strategy in human cancer**

|  |  |  |  |
| --- | --- | --- | --- |
| **Co-delivery of microRNAs/ anti-cancer drug** | **Biological functions** | **Cancer type** | **Ref.** |
| **Oncogenic microRNAs** | | | |
| miR-9/ temozolomide | Inhibit the expression of drug efflux transporter, P-glycoprotein | Glioblastoma multiforme | [39] |
| miR-21/ metformin, 5-Fluorouracil and Oxaliplatin | Target Wnt/β-catenin | Colon cancer | [38] |
| miR-125b/temozolomide | Target PIAS3, which contributes to reduced STAT3 transcriptional activity and subsequent decreased expression of MMP-2 and -9 | Glioblastoma | [40] |
| miR-125b/temozolomide | Target pro-apoptotic Bcl-2 antagonist killer 1 (Bak1) and sensitize CSCs to temozolomide induced apoptosis | Glioblastoma | [76] |
| **Tumor suppressor microRNAs** | | | |
| miR-612/cisplatin,  5-Fluorouracil | Target Wnt/β-catenin, regulate EMT and inhibit cell proliferation, migration, invasion, and metastasis | Liver cancer | [48] |
| miR-205/ gemcitabine | Decreased tumor cell population and increased apoptosis | Pancreatic cancer | [42] |
| miR-200c/ docetaxel | Reduced TUBB3 level, and reversed EMT | Gastric cancer | [43] |
| miR-146a/cetuximab | Target Numb to stabilize β-catenin, regulate EMT, direct ACD-to-SCD switch | Colorectal cancer | [77] |
| miR-145/ metformin,  5-Fluorouracil and Oxaliplatin | Target Wnt/β-catenin | Colon cancer | [38] |
| miR-34 family/ 5-Fluorouracil | Repression of c-Kit by p53 | Colorectal cancer | [45] |
| miR-34a/doxorubicin | Target Notch1 and reduce cancer stem cell properties | Breast cancer | [44] |

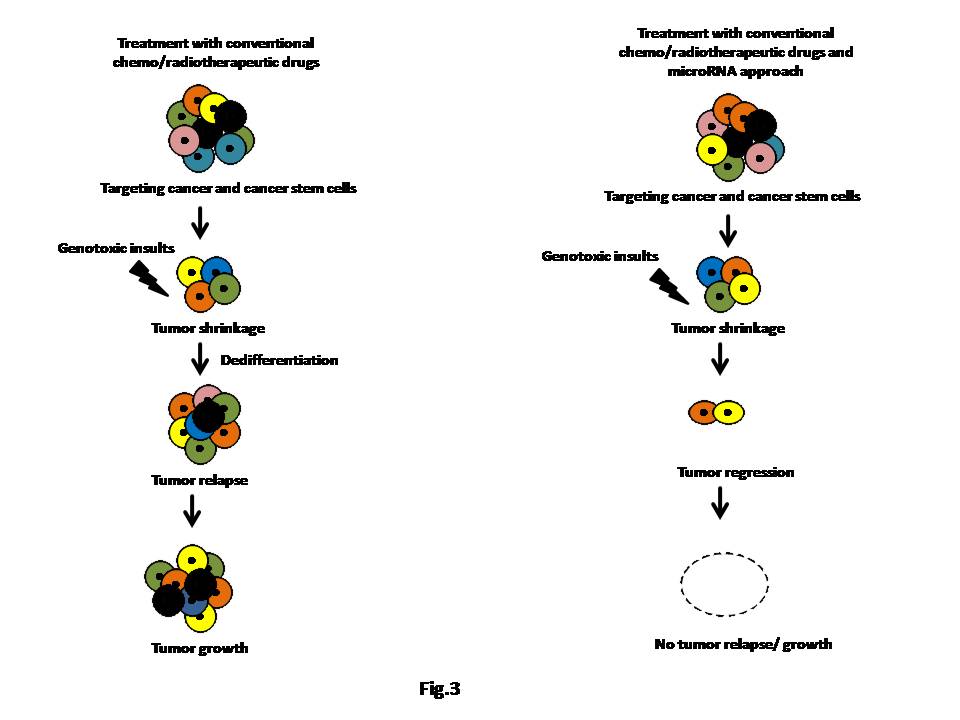
STAT: Signal transducer and activator of transcription; PIAS3: Protein inhibitor of activated STAT; MMP: Matrix metalloproteinase; ACD: Asymmetrical cell division; SCD: Symmetrical cell division; EMT: Epitheilal-to-mesencymal transition; CSC: Cancer stem cell; MMP: Matrix metalloproteinase; TUBB3: Tubulin beta class III.



**Figure 1 MicroRNA biogenesis, activity and potential therapeutic targets.** Mature miRNAs are generated from hairpin-like longer primary transcripts by two sequential processing steps mediated by a nuclear (Drosha) and a cytoplasmic (Dicer) RNAase III endonuclease. MiRNA then directs RNA-induced silencing complex (RISC) to target mRNAs which share sequence complementation between seed region at 5’ end of mature miRNA and 3’ untranslated region (3’UTR) of target mRNA. Stability of miRNA:mRNA recognition and degree of complementation leads to translational activation but more commonly mRNA degradation and translational repression. Potential therapeutic target sites are shown which include steps during miRNA biogenesis and target mRNA recognition.



**Figure 2 MicroRNAs affect and regulate the key properties of cancer stem cells.** Tumor microenvironment influences the maintenance and properties of cancer stem cells (CSCs). Secreted growth factors and cytokines from cancer cells, hypoxia, oncogene activation, DNA damage, errors in DNA repair and aberrant microRNA expression modify cancer cell functions by increasing the self-renewability, cell cycle exit and differentiation, epitheilal-to-mesencymal transition, migration, invasion and survival rate in CSCs thus promote cancer progression and or tumor recurrence.



**Figure 3 Effective novel tumor therapies.** Conventional chemo- and radio-therapeutic drugs target the bulk of tumor cell population and lead to tumor shrinkage. Cancer stem cells however develop resistance to these drugs and as a result of genotoxic insults, tumor cells relapse. MicroRNA approach along with the chemo and radiotherapeutic drugs as a novel treatment strategy target transit amplifying cells as well as differentiated cancer cells and induce differentiation in cancer stem cells thereby remove the bulk of tumor and also the source of cancer.