

miR-93 suppresses proliferation and colony formation of human colon cancer stem cells

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Abstract

AIM: To identify differentially expressed microRNAs (miRNAs) in human colon cancer stem cells (SW1116csc) and study their function in SW1116csc proliferation.

METHODS: SW1116csc were isolated from the human colon cancer cell line, SW1116 and cultured in serum-free medium. A miRNA microarray was used to detect differential expression profiles of miRNAs in SW1116csc and SW1116 cells. Real-time quantitative polymerase chain reaction (PCR) was performed to verify the differential expression of candidate miRNAs obtained from the microarray. Target mRNAs of differentially expressed miRNAs were predicted with target prediction tools. miRNA expression plasmids were transfected into SW1116csc using Lipofectamine 2000 reagent. Cell proliferation curves were generated with trypan blue staining, and the colony formation rate of transfected cells was measured with the soft agar colony formation assay. Expression of target mRNAs and proteins from differentially expressed miRNAs were detected using reverse transcription (RT)-PCR and western blotting.

RESULTS: Compared with expression in SW1116 cells, 35 miRNAs (including hsa-miR-192, hsa-miR-29b, hsa-miR-215, hsa-miR-194, hsa-miR-33a and hsa-miR-32) were upregulated more than 1.5-fold, and 11 miRNAs (including hsa-miR-93, hsa-miR-1231, hsa-miRPlus-F1080, hsa-miR-524-3p, hsa-miR-886-3p and hsa-miR-561) were downregulated in SW1116csc. The miRNA microarray results were further validated with quantitative RT-PCR. miR-93 was downregulated, and its predicted mRNA targets included BAMBI, CCND2, CDKN1A, HDAC8, KIF23, MAP3K9, MAP3K11, MYCN, PPARG, TLE4 and ZDHHC1. Overexpressed miR-93 significantly inhibited cell proliferation and colony formation by SW1116csc. Furthermore, miR-93 negatively regulated the mRNA and protein levels of HDAC8 and TLE4.

CONCLUSION: Some miRNAs were differentially expressed during differentiation of SW1116csc into SW1116 cells. miR-93 may inhibit SW1116csc proliferation and colony formation.

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Key words: miR-93; Stem cell; Colon cancer; Expression profile

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INTRODUCTION

Cancer stem cells (CSC) are a sub-population of cancer cells that possess characteristics associated with normal

stem cells, such as self renewal and the ability to differentiate into multiple cell types. CSCs are tumorigenic, in contrast to most cancer cells, which are thought to be non-tumorigenic. The CSC hypothesis infers that if CSCs were eliminated, the tumor would simply regress due to differentiation and cell death. Since the identification and characterization of CSCs in hematological malignancies, an increasing number of studies have described CSCs in solid tumors such as ovarian^[1], colon^[2], lung^[3], breast^[4], liver^[5], melanoma^[6] and pancreatic^[7] tumors, raising the possibility that the CSC hypothesis applies to most neoplastic systems. CSCs are the most critical tumor cell type because they are capable of self renewing, differentiating, and maintaining tumor growth and heterogeneity, and thus play an important role in tumorigenesis and therapeutics.

MicroRNAs (miRNAs) are small noncoding RNAs that posttranscriptionally regulate gene expression. Mature miRNAs can specifically bind to 3' untranslated regions of target cellular mRNA, in turn triggering mRNA degradation or inhibition of translation^[8]. To date, thousands of miRNAs have been identified in the human genome, where they act as key regulators of a wide variety of biological processes including development, cell differentiation, apoptosis, metabolism, and signal transduction^[9]. Consequently, abnormal patterns of miRNAs have been found in various human diseases, most notably cancer^[10]. Recent findings indicate that alterations in the expression of several miRNAs are present in human cancers, suggesting potential roles in carcinogenesis^[11]. Expression of some miRNAs, such as let-7 in human lung cancers^[12], the miR-15a/miR-16-1 cluster in chronic lymphocytic leukemia^[13], and neighboring miR-143/miR-145 in colorectal neoplasia and breast cancer^[14,15], is reduced, suggesting potential tumor suppressor activity. In contrast, other miRNAs, such as the miR-17-92 cluster in human B-cell lymphomas^[16] and miR-155/BIC in Hodgkin lymphoma^[17], are overexpressed, suggesting oncogenic potential.

miRNAs are emerging as important regulators of cellular differentiation and proliferation and have been implicated in the etiology of a variety of cancers. However, the role of miRNAs in human colon cancer stem cells remains poorly understood. In this study, we screened and identified differential miRNA expression profiles in colon cancer stem cells using a miRNA microarray and studied the function of differentially expressed miRNAs in the proliferation of colon cancer stem cells.

MATERIALS AND METHODS

Cell culture

The human colon cancer cell line (SW1116) was purchased from Cell Bank, Shanghai Institute of Life Science, Chinese Academy of Sciences and maintained in RPMI-1640 medium (Invitrogen, Carlsbad, CA, United States) supplemented with 10% fetal bovine serum (Gibco BRL, United States), penicillin G (1×10^5 U/L), and streptomycin (100 mg/L) in a 50 mL/L CO₂ atmosphere at 37 °C.

SW1116csc (Figure 1) were isolated previously^[18] and maintained in serum-free DMEM/F12 medium supplemented with human recombinant epidermal growth factor (20 µg/L; Invitrogen), human recombinant basic fibroblast growth factor (20 µg/L; Invitrogen), L-glutamine (2 mmol/L), insulin (4 U/L), penicillin G (1×10^5 U/L), and streptomycin (100 mg/L).

Microarray experiments

Total RNA was extracted from SW1116csc and SW1116 cells using Trizol reagent (Invitrogen). The quantity was measured on a spectrophotometer (Ultraspec 2000, Pharmacia Biotech), and the integrity of the RNA was checked on a 1% agarose gel. Low-molecular-weight RNA (< 200 nt) was separated from the total RNA using mirVana miRNA purification columns (Ambion, Austin, TX, United States) for miRCURY™ array microarray (v.13.0) (Exiqon) analysis according to the manufacturer's protocol, which uses the LNA probe to detect miRNA expression. LNA is a high-affinity RNA analog with a bicyclic furanose unit locked in an RNA-mimicking sugar conformation, which results in unprecedented hybridization affinity toward complementary single-stranded RNA molecules. This makes LNA-modified DNA probes ideally suited for RNA targeting. Microarray images were analyzed using an Axon GenePix 4000B microarray scanner (Molecular Devices), and GenePix pro V6.0 (Molecular Devices) was used to read the raw intensity of the image. Average values of the replicate spots of each miRNA were background subtracted, normalized, and subjected to further analysis. Normalization was performed using the signal of U6 snRNA on the chip, and the cutoff value was set to 1000.

Real-time quantitative reverse transcription polymerase chain reaction

For polymerase chain reaction (PCR) of miRNAs, cDNA synthesis was performed with 100 ng total RNA using a SuperScript III Reverse Transcriptase kit (Invitrogen) according to the manufacturer's protocol. The sequences of primers and probes specific for individual miRNAs and the U6 RNA internal control are shown in Table 1. PCR consisted of denaturation at 95 °C followed by 40 cycles of 95 °C for 15 s, 60 °C for 30 s, and 72 °C for 3 s. Thermal cycling and fluorescent monitoring were performed on an ABI 7700 sequence analyzer (PE Biosystems). Relative expression (RE) of the sample gene was calculated using the $\Delta\Delta CT$ method using the formula $RE = 2^{\Delta\Delta CT}$ where $CT = \text{PCR cycle in which the sample fluorescent intensity exceeds that of background}$, $\Delta CT \text{ sample} = CT \text{ sample} - CT \text{ U6 sample}$, $\Delta CT \text{ control} = CT \text{ control} - CT \text{ U6 control}$, and $\Delta\Delta CT = \Delta CT \text{ sample} - \Delta CT \text{ control}$.

miRNA target prediction

A list of potential miRNA targets was created by combining predicted targets from the mirBase, TargetScan, miRanda, and PicTar target prediction algorithms. Po-

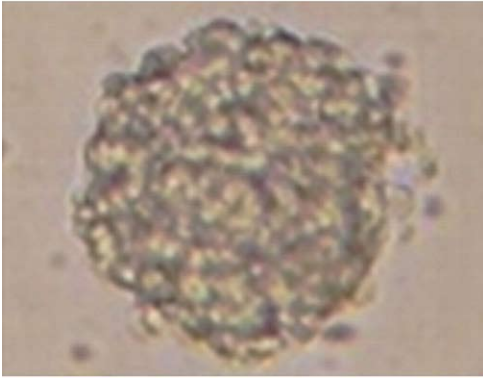


Figure 1 Human colon cancer stem cells grow into clonally derived spheres in serum-free medium.

Table 1 Sequences of primers used in quantitative reverse transcription polymerase chain reaction

Primer	Primer sequence (5'→3')
hsa-miR-93-RT	CTCAACTGGTGTCGTGGAGTCG-GCAATTCAGTTGAGCTACCT
hsa-miR-93-F	ACACTCCAGCTGGGCAAAGTGCTGTTCTGTCG
hsa-miR-1231-RT	CTCAACTGGTGTCGTGGAGTCG-GCAATTCAGTTGAGGCACT
hsa-miR-1231-F	ACACTCCAGCTGGGGTGCTGGGCGGAC
hsa-miR-32-RT	CTCAACTGGTGTCGTGGAGTCG-GCAATTCAGTTGAGTGCAAC
hsa-miR-32-F	ACACTCCAGCTGGGTATTGCACATTACTAA
hsa-miR-33a-RT	CTCAACTGGTGTCGTGGAGTCG-GCAATTCAGTTGAGTGCAAT
hsa-miR-33a-F	ACACTCCAGCTGGGGTGCATTGTAGTTGC
hsa-miR-194-RT	CTCAACTGGTGTCGTGGAGTCG-GCAATTCAGTTGAGTCCACA
hsa-miR-194-F	ACACTCCAGCTGGGTGAACAGCAACTCCA
hsa-miR-215-RT	CTCAACTGGTGTCGTGGAGTCG-GCAATTCAGTTGAGGTCTGT
hsa-miR-215-F	ACACTCCAGCTGGGATGACCTATGAATTG
hsa-miR-29b-RT	CTCAACTGGTGTCGTGGAGTCG-GCAATTCAGTTGAGAACACT
hsa-miR-29b-F	ACACTCCAGCTGGGTAGCACCATTGAAATC
hsa-miR-192-RT	CTCAACTGGTGTCGTGGAGTCG-GCAATTCAGTTGAGGGCTGT
hsa-miR-192-F	ACACTCCAGCTGGGCTGACCTATGAATTG
URP	TGGTGTCGTGGAGTCG
U6	F-CTCGCTTCGGCAGCACAR-AACGCTTCACGAATTTGCGT

URP: Universal reporter primer; RT: Reverse transcription.

tential targets were chosen based on gene function, the number of predicted target sites, and target prediction by multiple algorithms.

Plasmid constructs and transfection

The human miR-93 precursor (CUGGGGGGCUC-CAAAGUGCUGUUCGUGCAGGUAGUGUGAU-UACCCAACCUACUGCUGAGCUAG CACUUCCC-GAGCCCCCGG) was reverse-transcribed and cloned into pSilencer 4.1 (Ambion). The correct plasmid was named pS-miR-93, and the control plasmid (pS-Neg)

consisted of a scrambled sequence (Ambion). The sequence of the pS-miR-93 plasmid was confirmed with DNA sequencing. SW116csc were seeded at 1×10^5 cells/well in a 12-well plate (BD Bioscience). After 48 h, the cells were transfected with 1 μ g/well of pS-miR-93 or pS-Neg using 4 μ L Lipofectamine 2000 reagent (Invitrogen) per well according to the manufacturer's protocol. After 24 h, cells were harvested, and total mRNAs and proteins were extracted.

Cell proliferation assay

SW116csc and pS-miR-93-transfected SW116csc (SWt) cells were seeded at a density of 1×10^4 in 35-mm Petri dishes. Cultured cells stained with trypan blue were observed and counted in triplicate over 6 wk.

Colony formation in soft agar

Cells were disassociated, suspended in medium containing 0.3% agar and 10% serum, and plated onto a bottom layer containing 0.6% agar. The cells were plated at a density of 3×10^4 cells/6-cm dish, and the number of colonies > 0.5 mm in diameter was counted 14 d later.

RT-PCR

Total RNA was extracted, and cDNA was synthesized as above. PCR was performed in a 50- μ L volume containing 1 μ L Taq DNA polymerase (Hua Mei Co.), 5 μ L $10 \times$ buffer, 1 μ L dNTPs (10 mmol/L), 2 μ L primers (10 μ mol/L), and 1 μ L cDNA (0.1 μ g/ μ L). Amplification was performed in a thermal cycler (Perkin-Elmer Co., United States). The PCR products were analyzed and photographed with a gel documentation system (FR-200, Shanghai Fu Ri Bio Co.). For HDAC8, the forward primer used was F: 5'-TGGGCAGTCGCTGGT-3', and the reverse primer was R: 5'-GTGGCTGGGCAGTCATAA-3' (product size: 285 bp). For TLE4, F: 5'-ACTCCCAGTGTGTGCAAG-3', R: 5'-GTTTCTGGCACAATGCACAG-3' (194 bp). For glyceraldehyde-3-phosphate dehydrogenase (GAPDH), F: 5'-TTGGTATCGTGGGAAGGACTCA-3', R: 5'-TGTCATCATATTGTCGAGGTT-3' (270 bp). RT-PCR primers were synthesized by Shanghai Sangon Co.

Western blotting

Cell extracts were prepared in lysis buffer (20 mmol/L Tris-HCl, pH 7.5, 0.1% Triton X-100, 0.5% sodium deoxycholate, 1 mmol/L phenylmethylsulfonyl fluoride, 10 μ g/mL aprotinin, and 10 μ g/mL leupeptin) and centrifuged at $12\,000 \times g$ at 4 $^{\circ}$ C. The total protein concentration was measured using a BCA assay. Cellular extracts containing 50 μ g total proteins were subjected to 10% SDS-PAGE and transferred electrophoretically to polyvinylidene difluoride membranes (Invitrogen). Blots were probed at 4 $^{\circ}$ C overnight with primary antibodies in 5% milk/TBST. The antibodies for western blotting were HDAC8, TLE4 and GAPDH (Santa Cruz Biotechnology, Santa Cruz, CA).

Table 2 Differentially expressed miRNAs in colon cancer stem cells

miRNA name	SW1116csc	SW1116	Fold change
	image intensity	image intensity	
hsa-miR-93	0.46	8.13	0.06
hsa-miR-1231	0.02	0.08	0.22
hsa-miRPlus-F1080	0.01	0.05	0.23
hsa-miR-524-3p	0.05	0.16	0.34
hsa-miR-886-3p	3.34	8.37	0.40
hsa-miR-561	0.02	0.04	0.45
hsa-miR-497	0.01	0.03	0.51
hsa-miR-23a	11.92	22.51	0.53
hsa-miR-886-5p	2.89	5.18	0.56
hsa-miRPlus-A1087	0.47	0.79	0.59
hsa-miRPlus-E1170	30.71	49.93	0.61
hsa-miRPlus-E1102	0.42	0.28	1.50
hsa-miR-138-2	0.39	0.26	1.50
hsa-miR-31	0.45	0.30	1.52
hsa-miR-17	2.50	1.64	1.52
hsa-miR-374a	5.40	3.55	1.52
hsa-miR-424	3.38	2.21	1.53
hsa-miRPlus-F1181	0.64	0.42	1.53
hsa-miRPlus-E1238	0.28	0.18	1.54
hsa-miR-542-3p	0.38	0.24	1.56
hsa-miR-582-3p	0.27	0.17	1.57
hsa-miR-584	0.28	0.17	1.61
hsa-miR-522	0.29	0.18	1.64
hsa-miR-590-5p	0.41	0.25	1.67
hsa-miR-487b	2.88	1.72	1.68
hsa-miR-29c	1.53	0.89	1.72
hsa-miR-96	2.39	1.34	1.78
hsa-miR-193a-3p	22.24	12.50	1.78
hsa-miR-20a	0.74	0.41	1.79
hsa-miR-301a	2.28	1.27	1.80
hsa-miRPlus-E1106	0.56	0.31	1.82
hsa-miR-30e	1.21	0.65	1.86
hsa-miR-874	0.20	0.10	1.99
hsa-miR-19a	17.38	8.71	2.00
hsa-miR-519a	1.73	0.77	2.24
hsa-miRPlus-A1065	0.30	0.13	2.32
hsa-miR-521	0.94	0.40	2.36
hsa-miR-876-5p	0.23	0.10	2.37
hsa-miR-493	0.28	0.11	2.53
hsa-miR-101	4.92	1.28	3.85
hsa-miR-32	1.89	0.35	5.48
hsa-miR-33a	3.97	0.64	6.16
hsa-miR-194	0.79	0.11	7.11
hsa-miR-215	0.97	0.11	9.09
hsa-miR-29b	3.00	0.33	9.20
hsa-miR-192	1.47	0.14	10.67

Statistical analysis

All data are presented as the mean \pm SE. SPSS for Windows (version 15.0, SPSS Inc., United States) was used for statistical analysis. *P* values < 0.05 were considered statistically significant.

RESULTS

miRNA expression profiling with miRNA microarray

We used an miRNA microarray to evaluate miRNA expression profiles of colon cancer stem cells (SW1116csc) and differentiated colon cancer cells (SW1116). Analysis of data derived from the miRCURYTM array microarray indicated that compared with SW1116 cells, there

were 46 differentially expressed miRNAs in SW1116csc. Among them, 35 miRNAs (including hsa-miR-192, hsa-miR-29b, hsa-miR-215, hsa-miR-194, hsa-miR-33a and hsa-miR-32) were overexpressed more than 1.5-fold and 11 miRNAs (including hsa-miR-93, hsa-miR-1231, hsa-miRPlus-F1080, hsa-miR-524-3p, hsa-miR-886-3p and hsa-miR-561) were downregulated (Table 2). Expression of miR-93 in SW1116csc was decreased by 16.7 times, and we chose this miRNA for further study.

Validation of microarray results using quantitative PCR

To validate our microarray results, we performed quantitative PCR with some of the miRNAs that were differentially expressed in SW1116csc according to the microarray data. We selected miR-93, miR-1231, miR-32, miR-33a, miR-194, miR-215, miR-29b and miR-192 for quantitative PCR. The quantitative PCR results indicated that the expression of miR-93 and miR-1231 was decreased, whereas the expression of miR-32, miR-33a, miR-194, miR-215, miR-29b and miR-192 was significantly increased in SW1116csc cells (Figure 2). The quantitative PCR result was in agreement with that of the microarray.

Prediction of miR-93 target gene

By combining predicted targets generated with the mir-Base, TargetScan, miRanda and PicTar target prediction algorithms, potential targets of miR-93 were chosen. The stem relevant mRNA targets included BAMBI, CCND2, CDKN1A, HDAC8, KIF23, MAP3K9, MAP3K11, MYCN, PPARD, TLE4, and ZDHHC1.

Inhibition of cell proliferation and colony formation of SW1116csc by pS-miR-93 transfection

We tested for differences in the proliferation rate between SW1116csc, pS-Neg-transfected SW1116csc (SWcon), and pS-miR-93-transfected SW1116csc (SWt). The cells were examined from week 1 to week 7 after seeding. As shown in Figure 3, there was a difference in the growth rate between SWcon and SWt cells. SWt cells grew slowly and showed growth inhibition after week 3. The self-renewing capacity of SWt cells was also examined with the colony formation assay. When plated at a density of 100 cells/well, SW1116csc and SWcon cells generated a greater mean number of tumor spheres (72.3 ± 4.2 and 64.5 ± 3.6 , respectively) than did SWt cells (19.6 ± 2.1) (Figure 4).

Overexpression of miR-93 reduces the mRNA and protein levels of HDAC8 and TLE4

To provide additional evidence for the role of miR-93 in inhibition of SW1116csc proliferation and colony formation, we examined the effect of miR-93 on the expression of HDAC8 and TLE4. As shown in Figure 5, miR-93 mimics significantly attenuated the mRNA and protein levels of HDAC8 and TLE4.

DISCUSSION

Mature functional miRNAs of approximately 22 nucleo-

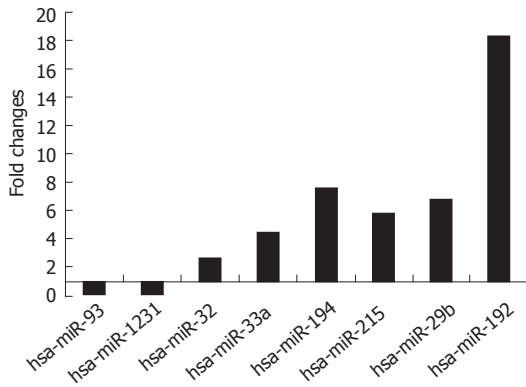


Figure 2 Expression levels of select miRNAs in SW1116csc as measured with quantitative reverse transcription polymerase chain reaction.

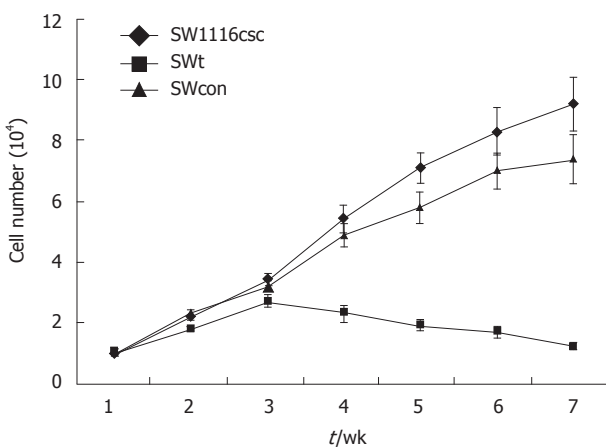


Figure 3 Growth curves of SW1116csc, SWcon cells, and SWt cells. mean \pm SD are shown.

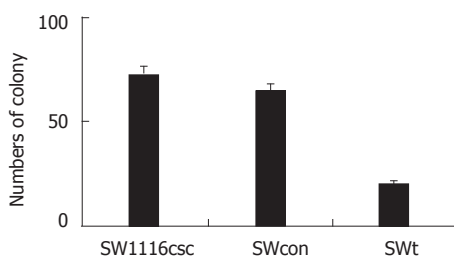


Figure 4 Colony formation after incubation of 100 separate cells for 14 d. mean \pm SD are shown.

tides that are generated from long primary miRNA transcripts control gene expression at the posttranscriptional level by degrading or repressing target mRNAs. Some miRNAs aberrantly expressed in cancer have been well documented^[19,20]. These miRNAs regulate the expression of signaling molecules such as cytokines, growth factors, transcription factors, and proapoptotic and antiapoptotic molecules. Recently, miRNAs were found to play a role in the differentiation of stem cells. Proper regulation of differentiation of stem cells is crucial to normal development and the avoidance of cancer^[21]. However, the differential expression of miRNAs in human colon cancer

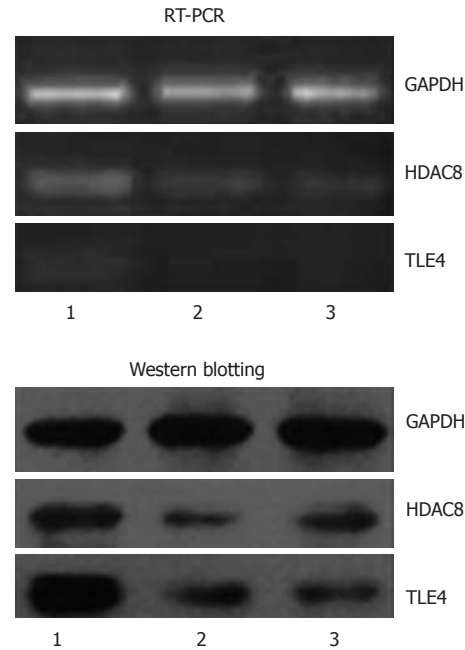


Figure 5 Expression of HDAC8 and TLE4 mRNA and protein in SW1116csc, SW1116 cells and SWt cells. 1: SW1116csc; 2: SW1116 cells; 3: SWt cells. Glyceraldehyde-3-phosphate dehydrogenase was evaluated as an internal control. RT-PCR: Reverse transcription polymerase chain reaction; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase.

stem cells has not been addressed.

Here we analyzed the differential expression of miRNAs in human colon cancer stem cells and differentiated colon cancer cells. We identified 46 differentially expressed miRNAs in SW1116csc. We observed miRNAs that were upregulated and others that were downregulated. The miR-93 expression level was significantly lower in SW1116csc than in SW1116 cells, indicating that miR-93 may be involved in the development or replication of colon CSCs.

One of the key characteristics of stem cells is their ability to divide for long periods of time when most other cells are quiescent^[22]. Because the function of miR-93 in colon CSCs was unknown, we identified the biological effects of miR-93 on colon CSCs when its expression in these cells was upregulated by examining the role of miR-93 on cellular growth and proliferation of transfected SW1116csc. Overexpression of miR-93 consistently inhibited cell proliferation and colony formation of SW1116csc. To the best of our knowledge, this study is the first to demonstrate the differential expression of miRNAs in colon CSCs and the effect of miR-93 on colon CSCs. However, the underlying mechanisms of these effects are not completely understood. Using miRNA target prediction tools and RT-PCR, we found that inhibition of overexpressed miR-93 probably affects cell proliferation and colony formation of colon cancer stem cells by targeting HDAC8 and TLE4.

Histone deacetylase (HDAC) enzymes are a family of proteins with complex, multifunctional roles *in vivo*, including transcriptional regulation, regulation of tubulin

and cytoskeletal function, control of cardiac cell growth, regulation of thymocyte development, and facilitation of DNA repair^[23]. HDAC enzymes function in part to control the acetylation state of nucleosomal histones, thereby regulating transcription. More recently, however, it has been shown that HDAC enzymes have many non-histone acetylation targets as well, including tubulin, heat-shock proteins, and a variety of transcription factors such as p53 and NF- κ B subunit p65^[24,25].

HDAC8 is a newly identified HDAC that was cloned and characterized in 2000. It is a novel marker of smooth muscle differentiation and is expressed at low levels in normal white blood cells but overexpressed in some malignant hematological cell lines^[26]. cDNA microarray analysis suggests that there is differential expression of HDAC8 between mammary tumors and normal lactating mammary glands and that it may play a key regulatory role in mammary gland tumorigenesis^[27]. Here we found that the expression level of HDAC8 in human colon cancer cells was high. Thus, accumulating data increase our understanding of the role of abnormally elevated HDAC8 activity in the pathogenesis of tumors.

Groucho (Gro) or Transducin-like Enhancer of Split (TLE) proteins constitute a family of highly conserved cofactors for transcription. They act as non-DNA-binding corepressors and are recruited to promoters *via* interaction with a DNA binding partner. Gro corepressor proteins interact with multiple transcription factors and thus affect different signaling pathways^[28]. They contact histones and recruit HDACs, thereby altering local chromatin structure. Gro corepressor proteins confer repressing functions on binding partners with an activating potential^[29].

The Gro/TLE family of corepressors interacts with at least five families of transcription factors and plays critical roles in *Drosophila* and vertebrate development. During B-lymphocyte differentiation, TLEs mediate the repressive effect of Pax5 *via* recruitment by Pu.1, limiting alternative cell fates^[30]. However, the ability of Gro/TLEs to interact with other signaling pathways suggests a potentially broader role for Gro/TLEs in both normal and malignant hematopoiesis. Members of the Gro/TLE family of corepressors bind to all known Tcf/LEF complexes and act as inhibitors of Wnt/ β -catenin signaling^[31,32], a pathway implicated in expansion and self-renewal of the hematopoietic stem cell compartment^[33]. Similarly, Gro/TLEs inhibit NF- κ B signaling^[34], a pathway constitutively activated in acute myeloblastic leukemia and thought to play an important role in hematopoietic cell proliferation, survival, and chemoresistance. *Gro/TLE* gene family members are also key effectors of Notch signaling, a pathway implicated in HSC fate determination and self-renewal^[35]. The high expression of TLE4 in human colon CSCs indicates interactions with Wnt, NF- κ B, or Notch signaling and suggests that TLE4 may be involved in the proliferation and differentiation of colon CSCs.

In summary, our study suggests that during the course of colon CSC differentiation towards colon cancer cells,

some miRNAs are differentially expressed. miR-93 is one of the miRNAs that was downregulated, and overexpressed miR-93 significantly inhibited cell proliferation and colony formation by colon CSCs. Furthermore, overexpression of miR-93 negatively regulated mRNA and protein expression of HDAC8 and TLE4. The inhibition of cell proliferation by miR-93 in colon CSCs may occur by targeting HDAC8 and TLE4.

COMMENTS

Background

The cancer stem cell (CSC) hypothesis is currently at the center of a rapidly evolving field, involving a change of perspective on the development and treatment of cancers. However, research has been hampered by the lack of distinct molecular markers of CSCs.

Research frontiers

Since the identification and characterization of CSCs in hematological malignancies, an increasing number of studies have described CSCs in solid tumors such as ovarian, colon, lung, breast, liver, melanoma and pancreatic tumors, suggesting that the CSC hypothesis applies to most neoplastic systems.

Innovations and breakthroughs

Previously isolated SW1116csc were compared with SW1116 cells. The authors identified 35 miRNAs that were upregulated and 11 miRNAs that were downregulated in SW1116csc. Overexpressed miR-93 significantly inhibited cell proliferation and colony formation of SW1116csc. Furthermore, miR-93 negatively regulated the mRNA and protein expression levels of HDAC8 and TLE4.

Applications

The study of CSCs has important implications for future cancer treatment and therapies. The CSC hypothesis states that if the CSCs were eliminated, the tumor would simply regress due to differentiation and cell death. By selectively targeting CSCs, it may be possible to treat patients with aggressive, non-resectable tumors and prevent the tumor from metastasizing.

Terminology

CSCs are a sub-population of cancer cells that possess characteristics associated with normal stem cells, such as self renewal and the ability to differentiate into multiple cell types. CSCs are tumorigenic, in contrast to most cancer cells, which are thought to be non-tumorigenic. CSCs persist in tumors as a distinct population and cause relapse and metastasis by giving rise to new tumors.

Peer review

The authors presented an original work about colon CSCs and identified a potentially new pathway that could be targeted for colon cancer management.

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