MicroRNA Regulatory Network in Human Colorectal Cancer

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Abstract: Epigenetic modifications include DNA methylation, histone modifications, and noncoding RNAs containing microRNAs (miRNA). miRNAs are small noncoding RNAs that are 21 to 25 nt in length; they downregulate gene expression during cell development, cell proliferation, cell differentiation, and apoptosis. They play a critical role in human carcinogenesis. Presently, evidences show that miRNAs participate as oncogenic miRNAs or tumor suppressors in the developmental and physiological processes of human colorectal cancer (CRC). Disturbed miRNA expression may be attributable to a mechanism involving multiple factors. In this review, we focus on the colorectal miRNA expression profile and further discuss the miRNA regulatory network involved in the tumorigenesis of human CRC. We, thus, hope to open up new avenues for anticancer therapy based on the epigenetic regulation of miRNA.

Key Words: microRNA, colorectal cancer, epigenesis.

INTRODUCTION

Over the past 10 years, there have been significant developments in the research on small RNAs. Previous studies have heavily focused on gene silencing induced by small noncoding RNAs. In 1998, Dr.Fire and Dr.Mello discovered the mechanism of interference RNA (RNAi) and subsequently won the Nobel Prize in Physiology or Medicine [1]. Similar to siRNAs in some aspects, microRNAs (miRNAs) are approximately 22 nt noncoding RNAs that regulate gene expression in animals, plants, and viruses. The accumulating knowledge on miRNAs has opened a new era in cancer research [2].

Currently, cancer is recognized as both a genetic and an epigenetic disease. Epigenetics is defined as the study of mitotically and meiotically heritable changes in gene expression without changes in the DNA sequence. Epigenetic changes include alterations in DNA methylation, histone modifications, and changes induced by noncoding RNAs, including miRNAs [3]. Due to the high incidence of colorectal cancer (CRC), the pathogenesis, prevention, and treatment of CRC have received considerable attention. There is accumulating evidence that the miRNA expression profile in CRC is abnormal and may contribute to the diagnosis and prognosis of this disease.

In this study, we review recent work on miRNAs in CRC, with particular emphasis on the miRNA regulatory network, in order to identify new avenues for anticancer therapy based on the epigenetic regulation of miRNAs.

miRNA BIOGENESIS

In 1993, the Caenorhabditis elegans heterochronic gene *lin-4* was the first miRNA to be reported. This gene encodes small RNAs with antisense complementarity to lin-14 [4]. The number of miRNA hairpin loci in the miRNA sequence database, namely, miRBase (http://microrna.sanger.ac.uk/), continues to grow rapidly, from 2909 (June 2005; release 7.0) to 9539 (March 2009; release 13.0) in the past 4 years. Over 500 miRNA sequences of the human genome have been annotated in the latest version of the miRBase [5]. miRNAs are involved in cell proliferation, cell differentiation, apoptosis, and organ development, and play a critical roles in the development of infectious diseases, genetic disorders, adipocyte and hematopoietic cell differentiation, insulin secretion, and even heart disease. Certain miRNA have been proven to function as tumor suppressors or proto-oncogenes during tumorigenesis [6-9]. Despite the growing evidence of their significant involvement in cancer, little is known about the exact function and regulation of miRNA expression.

In animals, miRNA genes are transcribed by RNA polymerase II (Pol II) into primary miRNAs (pri-miRNAs) containing a 5′ cap and a poly(A) tail, and subsequently processed in the nucleus by the Rnase III endonuclease Drosha and the double-stranded RNA-binding protein Pasha/DGCR8 into ~70-nt hairpin precursor miRNAs (pre-miRNAs). Next, the pre-miRNAs are exported into the cytoplasm by exportin 5, and subsequently recognized and processed by the RNAse III enzyme Dicer into mature ~22-nt miRNAs. The mature miRNAs are incorporated into an RNA-induced silencing complex (RISC); these mature miRNA strands guide the complex to its mRNA targets. miRNAs induce the degradation of mRNAs and downregulate the target genes with perfect or near-perfect complementarity to the 3′-untranslated regions (UTR) of mRNAs [10-12].

miRNAS IN COLORECTAL CANCER

In 2003, the first study was undertaken to identify changes in miRNA levels in human colorectal neoplasia sample [13]. microRNA expression profile analysis approaches have been

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developed in recent years, and the first profile of miRNA in CRC was published in 2006 using miRNA serial analysis of gene expression (miRAGE) [14,15]. Evidences have shown that miRNAs may function as tumor suppressors or proto-oncogenes during tumorigenesis and may contribute to the diagnosis and prognosis of CRC.

PROTO-ONCOGENIC AND TUMOR-SUPPRESSOR miRNAS IN CRC

mir-21

Presently, the only miRNA found to be overexpressed in any type of solid tumor, including colon, breast, lung, prostate, stomach, and endocrine pancreatic tumors, is *mir-21*. This gene is located in the 3′-UTR region of the gene encoding vacuole membrane protein 1 (VMP1) at chromosome 17q23.2, a region frequently observed to be amplified in colon cancer [16]. miRNA expression profiles revealed high levels of *mir-21* expression in colon adenocarcinoma. A high expression level of *mir-21* was associated with lymph node positivity and the development of distant metastases. Therefore, such a high expression level correlated with the metastasis (TNM) stage, poor survival rate and poor therapeutic outcome [17,18]. The mechanism of the *mir-21*-mediated tumorigenesis of CRC needs to be elucidated. Asangani IA *et al.* argued that *mir-21* may posttranscriptionally downregulate the tumor suppressor programmed cell death 4 gene (Pdcd4) and stimulate invasion, intravasation, and metastasis in CRC [19].

mir-17-92

Among the miRNAs with oncogenic potential, *mir-17-92* is the best-studied one. Tumors induced by the *Myc* and *mir-17-92* clusters were more aggressive than those induced only by *Myc*. Lanza G *et al.* reported the existence of differences in miRNA expression between CRC showing microsatellite stability (MSS) and those showing high microsatellite instability (MSI-H). They found that a molecular signature, including 8 miRNAs, could correctly distinguish the MSI-H from the MSS colon cancer samples. Among the differentially expressed miRNAs, various members of the oncogenic *miR-17-92* family were upregulated in MSS cancers. The analysis of the combination of mRNA/miRNA expression signatures may represent an approach for improving biomolecular classification of colon cancer [20]. *miR-17-92* was observed to downregulate the important cell cycle regulators *E2F1* and *RB1* and subsequently cause deregulation of the expression of the downstream targets involved in cell-cycle control that favor cell proliferation over apoptosis. The oncogenic action of the *miR-17-92* cluster is believed to be partially attributable to a defective apoptotic program [21].

miR-143 and miR-145

The transfection of *miR-143* or *miR-145* precursors into cells demonstrated a significant growth inhibition in the human CRC cell lines DLD-1 and SW480 [22]. Real-time PCR examination in CRC tissue revealed that *miR-145* was significantly deregulated and the *miR-143* and *miR-145* expression levels were significantly lower in tumors with maximal diameters > 50 mm [18,23]. *miR-145* has been suggested to target mitogen-activated protein 3 kinase 3 (MAP3K3) and mitogen-activated protein 4 kinase 4 (MAPK4K4) and certain genes with oncogenic functions, such as those encoding MYCN, FOS, YES, and FLI, and cell-cycle promoters such as cyclin D2 and L1. Further, *miR-145* has been demonstrated to inhibit the growth of colon cancer cells by targeting insulin receptor substrate-1. *Erk5* was reported to be the target gene of *miR-143* in DLD-1 cells. Thus, low expression of *miR-143* and *miR-145* could result in an unbalanced signaling cascade, even in the case of MAPK, which could lead to sustained cell proliferation [24,25] (Fig. **1**).

let-7

miRNAs belonging to the *let-7* family of miRNAs may be considered to be candidate tumor suppressors [2]. The growth of DLD-1 human colon cancer cells that express low levels of *let-7* miRNA was found to be inhibited after transfection with *let-7a-1* precursor miRNA; simultaneously, the levels of Ras and c-Myc proteins were lowered. Meanwhile, reduced *let-7* miRNA expression was detected in approximately 30% of human colon cancer tissues and cells [26]. Besides, all-*trans* retinoic acid (ATRA) downregulation of *Ras* and *Bcl-2* was demonstrated to correlate with the *let-7a* miRNA activation [27] (Fig. **1**). These detections suggested possible tumor suppressor roles of *let-7* miRNA.

mir-34a

The introduction of *mir-34a* into the CRC cell lines HCT116 and RKO induced a marked inhibition of cell proliferation; this indicates its possible function as a tumor suppressor in CRC. However, *miR-34a* over- expression has been reported to support cell proliferation in certain cases of cancer. Different molecular mechanisms of carcinogenesis were considered to be the cause for the cell type-dependent association of *miR-34a* overexpression and proliferation. Its regulation has been reported to be associated with several signaling pathways, including *E2F-3*, *RAS*, and *p53* signaling pathways; therefore, E2F-3 proteins could be candidate targets for *miR-34a*. However, the inhibitory effect of *miR-34a* on cell proliferation is not mainly caused by the apoptotic response [28,29].

Besides, miRNAs may contribute to the diagnosis and prognosis of CRC. A systematic expression analysis of miRNAs from more than 300 samples of various human cancers, including CRC, revealed the possibility of successfully classifying poorly differentiated tumors by using miRNA expression profiles [30]. The *in vivo* significance of miRNAs was evaluated using clinical samples of colorectal neoplasms. The result of the evaluation indicated that *hsa-miR-200c* was significantly associated with patient survival (P = 0.0122) and may be a potential prognostic factor in CRC [31]. The data from quantitative real-time RT-PCR of miRNAs in 110 colon cancer patients showed that *miR-106a* deregulation could be a marker of disease-free survival (DFS) and overall survival (OS) independent of tumor stage [32].

miRNA and Chemotherapeutics in CRC

Given the oncogenic or tumor-suppressor role of miRNAs in CRC, it is essential to investigate whether or not antineoplastic drugs can alter the miRNA gene expression pattern. *In vitro* experiments performed on colon cancer cells

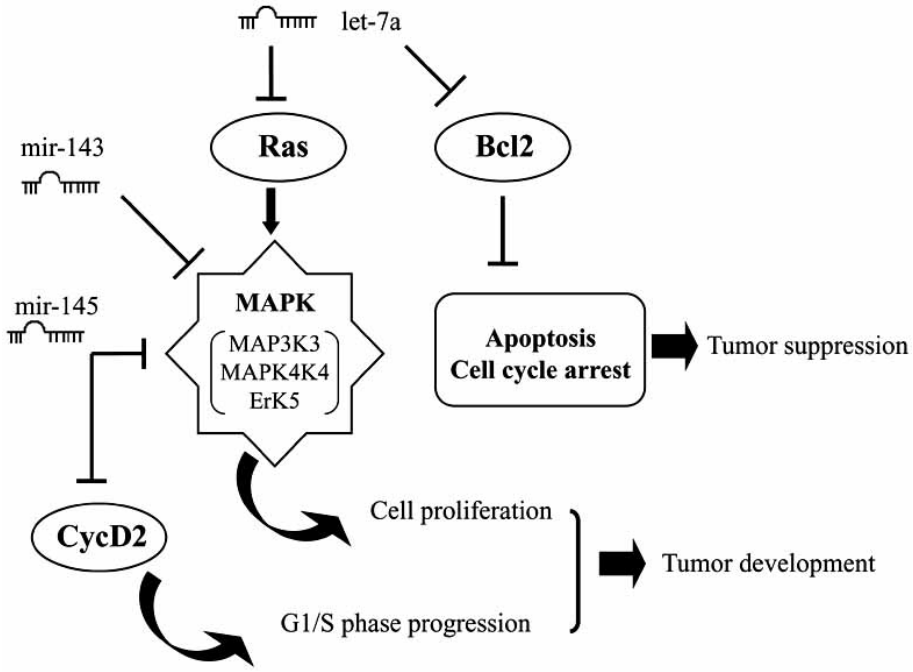

Fig. (1). Simplified scheme showing the probable mechanism of tumor suppressor miRNAs in CRC: The 3 miRNA in red have been identified to be tumor suppressors in CRC. "↓," activation; "⊥," repression/silencing. BCL2, B-cell CLL/lymphoma 2; RAS, Ras oncogene; ERK5, extracellular signal-related kinase 5; MAPK, mitogen-activated protein kinase; Cyc D2, cyclin D2. Ras and Bcl 2 were demonstrated to be downregulated by let-7a . miR-143 and miR-145 could affect the MAPK signaling cascade. miR-145 might target cell-cycle promoters such as cyclin D2.

showed that 5-fluorouracil (5-FU), a conventional antimetabolite largely used in clinical settings, was able to bring about at least a twofold upregulation or downregulation of *miR-19* and *miR-3* gene expression, respectively. The antineoplastic drugs appeared to influence the expression of miRNA genes in a direction that was opposite to that induced by neoplastic transformation [33]. Adriamycin, a DNA-damaging agent, was demonstrated to induce *miR-34a* expression in a p53-dependent manner. Following treatment with adriamycin, the *miR-34a* expression in HCT 116 was upregulated in a time-dependent manner; however, no changes in gene expression were observed in HCT 116 $p53^{-/-}$ cells [28]. Besides, in DLD-1 cells, the level of *mir-143* gradually increased following treatment with alpha-mangostin, a xanthone from the pericarps of mangosteen [34]. These findings supported the hypothesis that antineoplastic drugs could alter miRNA gene expression, and miRNAs may play synergistic roles during chemotherapy for CRC.

CAUSES OF DISTURBED miRNA EXPRESSION

To date, little is known about the causes of disturbed miRNA expression. Such a disturbance may be involved in a mixed mechanism that involves epigenomics, processing, chromosomal abnormalities, and so on.

Proto-Oncogenes and Tumor Suppressors

Mounting evidence has shown that miRNA is controlled by proto-oncogenes and tumor suppressors. The regulation of miRNAs by p53 is one of the most important discovery in recent years. The three-member *miRNA-34* family was reported to be directly regulated by p53 and involved in p53 tumour suppressor network [28,35,36]. *miR-34b/c* cooperate with p53 in suppressing proliferation and adhesion-independent growth of human ovarian carcinoma cells [37]. In CRC cells, the transcription driven from the *miR-34b/c* CpG island could be stimulated by p53 [38]. Besides, the expression levels of a number of miRNAs in human CRC cell line were affected by wt-p53. Global sequence analysis revealed that over 46% of the miRNA promoters contain potential p53-binding sites; this suggested that these miRNAs might potentially be directly regulated by wt-p53 [39]. Recently, studies have shown a direct role of c-Myc in activation of the *miR-17-92* cluster [40].

Epigenomics

Changes induced by noncoding RNAs, including miRNAs, are recognized as epigenetic alterations. Interestingly, recent evidence has suggested that other epigenetic alterations such as DNA methylation and histone modifications regulate miRNA expression during tumorigenesis. On comparing the miRNA expression profiles of the HCT 116 cell line and DNA methyltransferase 1 and 3b (DNMT1 and DNMT3b) double knockout cell line, Han *et al.* found that approximately 10% of all miRNAs were regulated by DNA methylation [41]. The silencing of *miR-342* in CRC was achieved through CpG-island methylation upstream of the host gene *evl* [42]; *miR-124a* was proven to undergo transcriptional inactivation by CpG-island hypermethylation in human tumors with different cell types [43]. As the targets of p53 mentioned before, *miR-34b/c* were epigenetically silenced in CRC, which was associated with CpG island meth-

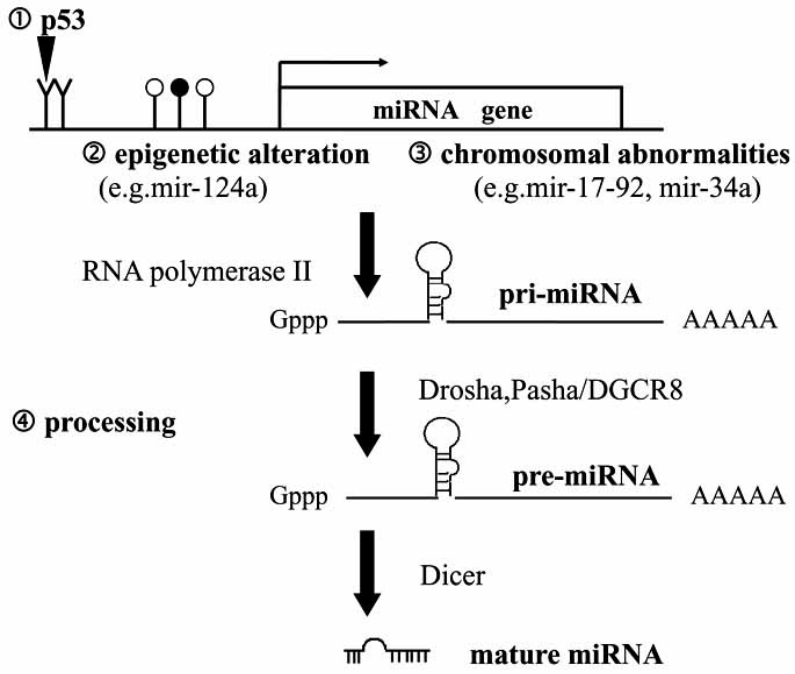

Fig. (2). Simplified scheme showing the probable causes for disturbed miRNA expression: (1) Conventional tumor suppressors such as p53 may bind to the potential p53-binding sites in miRNA promoters and directly regulate their transcription. (2) Several miRNAs in CRC may undergo transcriptional inactivation by CpG-island hypermethlyation. (3) Chromosome translocation or heterozygous deletion may lead to the down- or upregulation of miRNA. (4) Changes in any of the processing steps may result in deregulated miRNA expression in CRC.

ylation [38]. However, it should be noted that not all miRNAs are regulated by epigenomics. For instance, the treatment of CRC with chromatin remodeling drugs were previously reported to alter the expression level of tumor suppressor genes in the CRC cell lines, Colo320 and SW1116 [44]. B-cell lines did not show an upregulation of the expressions of *miR-143* and *miR-145*. Thus, an epigenetic change does not seem to be responsible for the low expression levels of *miR-143* and *miR-145* in the B cells [24]. Since the regulation of miRNA expression by DNA methylation is complex, further studies on the methylation patterns of miRNA in CRC are warranted in the near future.

Chromosomal Abnormalities

Chromosome translocation between chromosome 22 and chromosome 9 results in the production of a fusion protein tyrosine kinase known as BCR-ABL; the *miR-17-92* cluster is one of the BCR-ABL expression-dependent genes [45]. Recently, it was reported that heterozygous deletion of chromosome 1p36 in human primary neuroblastomas led to the down-regulation of *miR-34a*. Further, the same genomic region is frequently deleted in colon cancers [46].

Processing

As previously mentioned, the long primary transcripts transcribed by RNA polymerase II are processed by Drosha and Dicer to form mature miRNAs. Therefore, changes in any step or enzyme during tumorigenesis in CRC may result in deregulated miRNA expression (Fig. **2**).

Extensive research on the regulatory network of miRNAs in colorectal cancer is essential.

CLINICAL SIGNIFICANCE AND FUTURE DIRECTIONS

miRNA could be a good candidate as biomarkers of CRC diagnosis and prognosis. Meanwhile, miRNA-based cancer therapies could be new strategies for CRC treatment. Patient-specific therapeutic "drugs" for individuals could be designed according to the specific abnormalities in the miRNA genes in their colorectal tumors. Knocking down the overexpressed oncogenic miRNA or restoring the activity of silenced tumor suppressor miRNAs in cancer cells might contribute to tumor killing. In addition, an attempt could be made to identify new avenues for anticancer therapy based on the epigenetic regulation of miRNA. Further, it would be necessary to investigate if this could be a novel antiCRC strategy for restoring the activity of hypermethylation-silenced miRNA genes with tumor-suppressor functions in CRC. To date, therapy with epigenetic drugs has been based on the conventional protein-coding tumor-suppressor genes. With the identification of a greater number of epigenetically silenced tumor-suppressor miRNA genes in human cancer, the epigenetic silencing of these miRNAs will become a prospective target for the development of novel epigenetic drugs. We expect further research and development in this direction of epigenetic drug development to happen in the near future.

REFERENCES

[1] Fire, A.; Xu, S.; Montgomery, M.K.; Kostas, S.A.; Driver, S.E.; Mello, C.C. Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature*, **1998**, *391*, 806-11.

[2] Blenkiron, C.; Miska, E.A. miRNAs in cancer: approaches, aetiology, diagnostics and therapy. *Hum. Mol. Genet.*, **2007**, *16*, R106-13.

[3] Egger, G.; Liang, G.; Aparicio, A.; Jones, P.A. Epigenetics in human disease and prospects for epigenetic therapy. *Nature*, **2004**, *429*, 457-63.

[4] Lee, R.C.; Feinbaum, R.L.; Ambros, V. The *C.elegans* heterochronic gene *lin-4* encodes small RNAs with antisense complementarity to *lin-14*. *Cell*, **1993**, *75*, 843-54.

[5] Griffiths-Jones, S.; Saini H.K.; van Dongen, S.; Enright, A.J. miRBase: tools for microRNA genomics. *Nucleic Acids Res.*, **2008**, *36*, D154-8.

[6] Lin, S.L.; Chang, S.J.; Ying, S.Y. First *in vivo* evidence of microRNA-induced fragile X mental retardation syndrome. *Mol. Psychiatry*, **2006**, *11*, 616-7.

[7] van Rooij, E.; Sutherland, L.B.; Liu, N.; Williams, A.H.; McAnally, J.; Gerard, R.D.; Richardson, J.A.; Olson, E.N. A signature pattern of stress-responsive microRNAs that can evoke cardiac hypertrophy and heart failure. *Proc. Natl. Acad. Sci. USA*, **2006**, *103*, 18255-60.

[8] Calin, G.A.; Croce, C.M. MicroRNA-cancer connection: The beginning of a new tale. *Cancer Res.*, **2006**, *66*, 7390-4.

[9] Iwai, N.; Naraba, H. Polymorphisms in human pre-miRNAs. *Biochem. Biophys. Res. Commun.*, **2005**, *331*, 1439-44.

[10] Cho, W.C. OncomiRs: the discovery and progress of microRNAs in cancers. *Mol. Cancer*, **2007**, *6*, 60.

[11] Zanette, D.L.; Rivadavia, F.; Molfetta, G.A.; Barbuzano, F.G.; Proto-Siqueira, R.; Silva-Jr, W.A.; Falcão, R.P.; Zago, M.A. miRNA expression profiles in chronic lymphocytic and acute lymphocytic leukemia. *Braz. J. Med. Biol. Res.*, **2007**, *40*, 1435-40.

[12] Lawrie, C.H. MicroRNAs and haematology: small molecules, big function. *Br. J. Haematol.*, **2007**, *137*, 503-12.

[13] Michael, M.Z.; O' Connor, S.M.; van Holst Pellekaan, N.G.; Young, G.P.; James, R.J. Reduced accumulation of specific microRNAs in colorectal neoplasia. *Mol. Cancer. Res.*, **2003**, *1*, 882-91.

[14] Cummins, J.M.; He, Y.; Leary, R.J.; Pagliarini, R.; Diaz, L.A.; Jr Sjoblom, T.; Barad, O.; Bentwich, Z.; Szafranska, A.E.; Labourier, E.; Raymond, C.K.; Roberts, B.S.; Juhl, H.; Kinzler, K.W.; Vogelstein, B.; Velculescu, V.E. The colorectal microRNAome. *Proc. Natl. Acad. Sci. USA*, **2006**, *103*, 3687-92.

[15] Xi, Y.; Nakajima, G.; Gavin, E.; Morris, C.G.; Kudo, K.; Hayashi, K.; Ju, J. Systematic analysis of microRNA expression of RNA extracted from fresh frozen and formalin-fixed paraffin-embedded samples. *RNA*, **2007**, *13*, 1668-74.

[16] Calin, G.A.; Croce, C.M. Chromosomal rearrangements and microRNAs: a new cancer link with clinical implications. *J. Clin. Invest.*, **2007**, *117*, 2059-66.

[17] Schetter, A.J.; Leung, S.Y.; Sohn, J.J.; Zanetti, K.A.; Bowman, E.D.; Yanaihara, N.; Yuen, S.T.; Chan, T.L.; Kwong, D.L.; Au, G.K.; Liu, C.G.; Calin, G.A.; Croce, C.M.; Harris, C.C. MicroRNA expression profiles associated with prognosis and therapeutic outcome in colon adenocar cinoma. *JAMA*, **2008**, *299*, 425-36.

[18] Slaby, O.; Svoboda, M.; Fabian, P.; Smerdova, T.; Knoflickova, D.; Bednarikova, M.; Nenutil, R.; Vyzula, R. Altered expression of miR-21, miR-31, miR-143 and miR-145 is related to clinicopathologic features of colorectal cancer. *Oncology*, **2007**, *72*, 397-402.

[19] Asangani, I.A.; Rasheed, S.A.; Nikolova, D.A.; Leupold, J.H.; Colburn, N.H.; Post, S.; Allgayer, H. MicroRNA-21(miR-21) post-transcriptionally downregulates tumor suppressor Pdcd4 and stimulates invasion,intravasation and metastasis in colorectal cancer. *Oncogene*, **2008**, *27*, 2128-36.

[20] Lanza, G.; Ferracin, M.; Gafà, R.; Veronese, A.; Spizzo, R.; Pichiorri, F.; Liu, C.G.; Calin, G.A.; Croce, C.M.; Negrini,M. mRNA/microRNA gene expression profile in microsatellite unstable colorectal cancer. *Mol. Cancer*, **2007**, *6*, 54.

[21] Kovalchuk, O.; Tryndyak, V.P.; Montgomery, B.; Boyko, A.; Kutanzi, K.; Zemp, F.; Warbritton, A.R.; Latendresse, J.R.; Kovalchuk, I.; Beland, F.A.; Pogribny, I.P. Estrogen-induced rat breast carcinogenesis is characterized by alterations in DNA methylation, histone modifications and aberrant microRNA expression. *Cell Cycle*, **2007**, *6*, 2010-8.

[22] Akao, Y.; Nakagawa, Y.; Naoe, T. MicroRNAs *143* and *145* are possible common onco-microRNAs in human cancers. *Oncol. Rep.*, **2006**, *16*, 845-50.

[23] Bandrés, E.; Cubedo, E.; Agirre, X.; Malumbres, R.; Zárate, R.; Ramirez, N.; Abajo, A.; Navarro, A.; Moreno, I.; Monzó, M.; García-Foncillas, J. Identification by Real-time PCR of 13 mature microRNAs differentially expressed in colorectal cancer and non-tumoral tissues. *Mol. Cancer*, **2006**, *19*, 29.

[24] Akao, Y.; Nakagawa, Y.; Kitade, Y.; Kinoshita, T.; Naoe, T. Downregulation of microRNAs-143 and -145 in B-cell malignancies. *Cancer Sci.*, **2007**, *98*, 1914-20.

[25] Shi, B.; Sepp-Lorenzino, L.; Prisco, M.; Linsley, P.; deAngelis, T.; Baserga, R. MicroRNA 145 targets the insulin receptor substrate-1 and inhibits the growth of colon cancer cells. *J. Biol. Chem.*, **2007**, *282*, 32582-90.

[26] Akao, Y.; Nakagawa, Y.; Naoe, T. let-7 microRNA functions as a potential growth suppressor in human colon cancer cells. *Biol. Pharm. Bull.*, **2006**, *29*, 903-6.

[27] Garzon, R.; Pichiorri, F.; Palumbo, T.; Visentini, M.; Aqeilan, R.; Cimmino, A.; Wang, H.; Sun, H.; Volinia, S.; Alder, H.; Calin, G.A.; Liu, C.G.; Andreeff, M.; Croce, C.M. MicroRNA gene expression during retinoic acid-induced differentiation of human acute promyelocytic leukemia. *Oncogene*, **2007**, *26*, 4148-57.

[28] Tazawa, H.; Tsuchiya, N.; Izumiya, M.; Nakagama, H. Tumor-suppressive miR-34a induces senescence-like growth arrest through modulation of the E2F pathway in human colon cancer cells. *Proc. Natl. Acad. Sci. U.S.A.*, **2007**, *104*, 15472-7.

[29] Dutta, K.K.; Zhong, Y.; Liu, Y.T.; Yamada, T.; Akatsuka, S.; Hu, Q.; Yoshihara, M.; Ohara, H.; Takehashi, M.; Shinohara, T.; Masutani, H.; Onuki, J.; Toyokuni, S. Association of microRNA-34a overexpression with proliferation is cell type-dependent. *Cancer Sci.*, **2007**, *98*, 1845-52.

[30] Lu, J.; Getz, G.; Miska, E.A.; Alvarez-Saavedra, E.; Lamb, J.; Peck, D.; Sweet-Cordero, A.; Ebert, B.L.; Mak, R.H.; Ferrando, A.A.; Downing, J.R.; Jacks, T.; Horvitz, H.R.; Golub, T.R. MicroRNA expression profiles classify human cancers. *Nature*, **2005**, *435*, 834-8.

[31] Xi, Y.; Formentini, A.; Chien, M.; Weir, D.B.; Russo, J.J.; Ju, J.; Kornmann, M.; Ju, J. Prognostic values of microRNAs in colorectal Cancer. *Biomark Insights*, **2006**, *2*, 113-21.

[32] Díaz, R.; Silva, J.; García, J.M.; Lorenzo, Y.; García, V.; Peña, C.; Rodríguez, R.; Muñoz, C.; García, F.; Bonilla, F.; Domínguez, G. Deregulated expression of miR-106a predicts survival in human colon cancer patients. *Genes Chromosomes Cancer*, **2008**, *47*, 794-802.

[33] Rossi, L.; Bonmassar, E.; Faraoni, I. Modification of miR gene expression pattern in human colon cancer cells following exposure to 5-fluorouracil *in vitro*. *Pharmacol. Res.*, **2007**, *56*, 248-53.

[34] Nakagawa, Y.; Iinuma, M.; Naoe, T.; Nozawa, Y.; Akao, Y. Characterized mechanism of alpha-mangostin-induced cell death: casepase-independent apoptosis with release of endonuclease-G from mitochondria and increased miR-143 expression in human colorectal cancer DLD-1 cells. *Bioorg. Med. Chem.*, **2007**, *15*, 5620-9.

[35] He, L.; He, X.; Lim, L.P.; de Stanchina, E.; Xuan, Z.; Liang, Y.; Xue, W.; Zender, L.; Magnus, J.; Ridzon, D.; Jackson, A.L.; Linsley, P.S.; Chen, C.; Lowe, S.W.; Cleary, M.A.; Hannon, G.J. A microRNA component of the p53 tumour suppressor network. *Nature*, **2007**, *447*, 1130-4.

[36] Bommer, G.T.; Gerin, I.; Feng, Y.; Kaczorowski, A.J.; Kuick, R.; Love, R.E.; Zhai, Y.; Giordano, T.J.; Qin, Z.S.; Moore, B.B.; MacDougald, O.A.; Cho, K.R.; Fearon, E.R. p53-mediated activation of miRNA34 candidate tumor-suppressor genes. *Curr. Biol.*, **2007**, *17*, 1298-307.

[37] Corney, D.C.; Flesken-Nikitin, A.; Godwin, A.K.; Wang, W.; Nikitin, A.Y. MicroRNA-34b and MicroRNA-34c are targets of p53 and cooperate in control of cell proliferation and adhesion-independent growth. *Cancer Res.*, **2007**, *67*, 8433-8.

[38] Toyota, M.; Suzuki, H.; Sasaki, Y.; Maruyama, R.; Imai, K.; Shinomura, Y.; Tokino, T. Epigenetic silencing of microRNA-34b/c and B-cell translocation gene 4 is associated with CpG island methylation in colorectal cancer. *Cancer Res.*, **2008**, *68*, 4123-32.

[39] Xi, Y.; Shalgi, R.; Fodstad, O.; Pilpel, Y.; Ju, J. Differentially regulated micro-RNAs and actively translated messenger RNA tran-

scripts by tumor suppressor p53 in colon cancer. *Clin. Cancer Res.*, **2006**, *12*, 2014-24.

[40] O'Donnell, K.A.; Wentzel, E.A.; Zeller, K.I.; Dang, C.V.; Mendell, J.T. c-Myc-regulated microRNAs modulate E2F1 expression. *Nature*, **2005**, *435*, 839-43.

[41] Han, L.; Witmer, P.D.; Casey, E.; Valle, D.; Sukumar, S. DNA Methylation Regulates MicroRNA Expression. *Cancer Biol. Ther.*, **2007**, *6*, 1284-8.

[42] Grady, W.M.; Parkin, R.K.; Mitchell, P.S.; Lee, J.H.; Kim, Y.H.; Tsuchiya, K.D.; Washington, M.K.; Paraskeva, C.; Willson, J.K.; Kaz, A.M.; Kroh, E.M.; Allen, A.; Fritz, B.R.; Markowitz, S.D.; Tewari, M. Epigenetic silencing of the intronic microRNA hsa-miR-342 and its host gene EVL in colorectal cancer. *Oncogene*, **2008**, *27*, 3880-8.

[43] Lujambio, A.; Ropero, S.; Ballestar, E.; Fraga, M.F.; Cerrato, C.; Setién, F.; Casado, S.; Suarez-Gauthier, A.; Sanchez-Cespedes, M.; Git, A.; Spiteri, I.; Das, P.P.; Caldas, C.; Miska, E.; Esteller, M. Genetic unmasking of an epigenetically silenced microRNA in human cancer cells. *Cancer Res.*, **2007**, *67*, 1424-9.

[44] Fang, J.Y.; Chen, Y.X.; Lu, J.; Lu, R.; Yang, L.; Zhu, H.Y.; Gu, W.Q.; Lu, L.G. Epigenetic modification regulates both expression of tumor-associated genes and cell cycle progressing in human colon cancer cell lines: Colo-320 and SW1116. *Cell Res.*, **2004**, *14*, 217-26.

[45] Venturini, L.; Battmer, K.; Castoldi, M.; Schultheis, B.; Hochhaus, A.; Muckenthaler, M.U.; anser, A.; Eder, M.; Scherr, M. Expression of the miR-17-92 polycistron in chronic myeloid leukemia (CML) CD34+ cells. *Blood*, **2007**, *109*, 4399-405.

[46] Welch, C.; Chen, Y.; Stallings, R.L. MicroRNA-34a functions as a potential tumor suppressor by inducing apoptosis in neuroblastoma cells. *Oncogene*, **2007**, *26*, 5017-22.

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