

Published in final edited form as:

Mol Cell Pharmacol. 2011 January 1; 3(3): 135–141.

Modulation of MicroRNAs by Chemical Carcinogens and Anticancer Drugs in Human Cancer: Potential Inking to Therapeutic Advantage

Subrata Haldar^{1,2} and Aruna Basu²

¹Dept of Pharmacology, Case Comprehensive Cancer Center, Case Western Reserve University, Cleveland, Ohio

²Center for Biomedical Sciences, MetroHealth Campus, Case Western Reserve University, Cleveland, Ohio

Abstract

The disorder of microRNAs (miRNAs) often referred as ‘micromanagers of gene expression’ has been implicated with a vast array of neoplasms. The discovery establishes an important connection with the etiology, diagnosis and potential therapy of human cancer. Indeed, the wide range of profiling studies enabled to create miRNA signatures of solid tumors as well as cancers of blood origin. MiRNAs have been observed to play a significant role in the regulation of gene expression—a critical aspect of many biological processes, including cell development, differentiation, apoptosis and proliferation. The differential expression levels of miRNAs in tumors and their normal counterpart have enabled scientists to designate their roles as oncomir or tumor suppressor. Interestingly, the diminishment of oncogenic or enhanced levels of tumor suppressor miRNAs (antagomirs) have been reported to modulate the sensitivity of cancer cells to anticancer agents. To the other end, carcinogenic chemicals either possess the ability of silencing beneficial tumor suppressive miRNAs or maintain the augmented levels of their oncogenic counterpart. In this article we provide a comprehensive overview on the modulation of these “micromanaging oligos” by cancer causing as well as cancer preventing agents.

Keywords

MicroRNAs; Chemical carcinogens; Chemopreventive agents; Cancer

Introduction

Cancer is a multi-step process in which normal cells sustain genetic damage over a prolonged period of time. Some of the key characteristics required for a normal cell to acquire full malignant potential comprise of self-sufficiency in growth or insensitivity to growth inhibitory signals. The defects in major regulatory microcircuits which are fundamental for normal homeostasis can lead to transformed cellular traits. MiRNAs are a new class of small non-protein-coding RNAs (ncRNA) that control gene expression post-transcriptionally by binding to various mRNA targets involved with diverse signaling pathways. Thus it is quite likely that this particular set of ncRNA molecules might act as

Correspondence: Dr. Subrata Haldar, R456, Rammelkamp Building, MetroHealth Medical Center, 2500 MetroHealth Drive, Cleveland, OH 44109, USA. Tel. 216-778-1167. Shaldar@metrohealth.org.

Conflicts of Interest

The authors have no potential conflict of interest with respect to the publication of the article.

molecular switches in the intracellular network of cancer cells. Indeed, the expression of miRNA signatures as well as individual miRNAs was observed to be connected with the diagnosis of a variety of human cancers (1–8). The emerging roles of miRNAs as oncogenes or tumor suppressors are prevalent in the literature (2–4, 8–12).

The underproduction or overproduction of small genetic information described as miRNAs in cancer cells compared to normal healthy counterpart is thought to play a significant role in the progression of human malignancies (1–12). This mysterious genetic material with a tiny stretch of approximately 22–25 nucleotides (13) is apparently of great strength and power when aberrantly expressed in uncontrollably growing tumor cells. Cumulative studies reveal that miRNAs may be attractive targets to intervene the progression of human cancer. However the information on the status of oncomirs (oncogenic miRNAs) or tumor suppressor miRNAs have mostly emerged from the analysis of archived tissues of human cancer patients. The knowledge gained out of these studies has definitely been instrumental to establish miRNA signatures as distinct biomarkers for the diagnosis and prognosis of an wide array of epithelial as well as hemapoietic malignancies.

On the basis of these studies, at present it is believed that the approaches to interfere specific miRNA expression might offer novel therapeutic opportunities for human cancer. In addition, a better comprehension of how these miRNAs participate in the development and progression of the disease such as cancer is warranted. Recently, studies on the effect of anti-cancer drugs or chemopreventive agents on the modulation of miRNA expression profiles have been explored. Our recent investigation to explore whether anticancer agent such as benzyl isothiocyanate can influence the expression of miRNAs reveals its capability to alter the expression of a limited set of miRNAs such as miR-221 and miR-375 in pancreatic cancer (14). To the other end, tobacco carcinogens induced epithelial-mesenchymal transition (EMT) in human lung epithelial cells might be linked to epigenetic silencing of specific miRNAs such as miR-205 and miR-200 (15). Since the environmental exposure to biological, chemical or physical agents has been implicated with human diseases including carcinoma, such studies might come across a relationship between the expression of miRNAs and exposure to environmental carcinogens.

miRNAs and chemical carcinogenesis

Non-coding RNAs (ncRNAs) are thought to be important regulators of gene expression in eukaryotes. Among all the ncRNAs, miRNAs have been extensively studied and the key function of these small double-stranded RNAs is attributed to negatively regulate gene expression. Since the aberrant expression of these tiny regulators has been implicated with the tumorigenesis, scientists thought that uncovering relationships between exposure to environmental carcinogens and expression of miRNAs might help to disclose early mechanisms of this disease. In this respect the report by Tellez et al (15) demonstrating the epigenetic silencing of miR-205 and miR-200 family during carcinogen induced transformation of human lung epithelial cells is quite interesting. In this report, the authors show that exposure of premalignant lung epithelial cells to genotoxic tobacco carcinogens such as benzo (a) pyrenedioloxide (BPDE) or methylnitrosourea (MNU) can indeed induce a multifaceted dedifferentiation program marked by EMT (16). The induction of EMT and stem cell-like properties by these tobacco carcinogens was mediated by DNA methylation to prolong silencing of tumor suppressive miRNAs such as miR-200b, miR-200c and miR-205. Previous reports indicate that this family of miRNAs is indeed a regulator of EMT and tumor aggressiveness including pancreatic cancer (17, 18). It is believed that EMT occurs due to a reciprocal feedback loop in which transcriptional repressors such as *ZEB1* and *ZEB2* repress the transcription of the miR-200 family by binding to E-boxes within their promoter regions (16).

In addition to silencing of EMT specific miRNAs, the expression of miR-10a was also attenuated in malignant human bronchial epithelial cells (HBEC) induced by BPDE with concomitant upregulation of its putative target HOXA1 (19). Thus miR-10a downregulation might be associated with HOXA1 mediated malignant transformation of HBEC. Another study revealed overrepresentation of miR-106a in BPDE induced malignant HBEC by targeting tumor suppressor RB1 (20). Besides studies are evident in the literature identifying carcinogens that can modulate miRNAs involved in regulating genes which are important for DNA repair, proliferation, apoptosis or carcinogen metabolism (21). So far the effect of approximately twenty carcinogens on the regulation of miRNAs was investigated in cultured human cells or in animals (15, 19–29). Of note, genomic analysis of miRNA expression in liver of mice treated with mutagenic carcinogen N-ethyl-N-nitrosourea (ENU) revealed the alteration of many miRNAs including miR-762 (22), which was also shown to be upregulated in DMBA induced oral carcinogenesis in Syrian hamster (23). Interestingly, these reports link miR-762 for the first time with any malignant lesion. Apart from this, the magnitude of expression of both oncomir –21 and –221 were elevated in oral squamous cell carcinoma due to exposure of genotoxic carcinogen DMBA (23).

Azoxymethane (AOM) when injected into Sprague Dawley rats induces colon specific carcinogenesis (25). Davidson et al (25) observed overexpression of miR-34a, miR-132 and miR-223/224 (>10 fold) and downmodulation of miR-192, miR-194, miR-215 and miR-375 (0.32 fold or lower) in AOM induced colon adenocarcinoma compared to normal rat mucosa. This perturbed miRNA expression pattern is comparable with the human colorectal cancer miRNAome (30). Among the AOM induced upregulated miRNAs, miR-34a is known to be a tumor suppressor and plays a role in p53 mediated apoptosis. However, its role in colon carcinogenesis remains to be seen because approximately 75% human colon cancer specimens exhibit significant overexpression of miR-34a (31).

Among the other carcinogens, Vinyl carbamate (VC) induced murine lung tumors display elevated level of miR-21 compared to normal tissue. The oncogenic effect of miR-21 was mediated through a variety of signaling molecules such as PTEN, PDCD4 and RECK (32). Other studies (24) demonstrate that in estrogen receptor (ER) positive breast cancer cells, estradiol (E2) treatment can dysregulate miRNA expression profile. The exposure to E2 results in enhanced expression of miRNAs such as Let-7 family and miR-21, which can deplete the levels of c-MYC and E2F2 proteins (24). At the same time, E2 can also induce the miRNA processing enzyme Dicer. This observation suggests that genesis of ER positive breast cancer might depend on the balance between E2 regulated oncogenic and tumor suppressor miRNAs (24).

All together, cumulative data in the field indicate that miRNAs might play significant role in all stages of chemical carcinogenesis that include initiation as well as advancement of the disease. The alteration in the magnitude of miRNAs is observed in cancer targeting tissues following acute or chronic exposure of carcinogens. To the contrary, noncarcinogens are without any effect on the modulation of miRNA levels. In a nutshell, the knowledge on specific miRNA profiles and the overlapping miRNAs altered due to the exposure of a variety of carcinogens should be helpful to identify them as biomarkers of chemical carcinogenicity.

Modulation of microRNAs by anticancer agents

The chemosensitivity and chemoresistance of anticancer agents are usually regulated by multiple signal transduction pathways. The modulation of drug activity has been widely studied at the DNA, RNA and protein levels (33, 34). Recent emerging evidence suggest the regulatory roles of miRNAs on the efficacy of anticancer agents in human cancer cells (14,

32, 35–43). Several groups have explored the effect of anti-cancer drugs and chemopreventive agents (that modulate cell proliferation, apoptosis and angiogenesis) on miRNA expression profiles. Interestingly, the potential role of miRNAs, such as let-7i, miR-16 and miR-21 was discovered in the anticancer drug response when tested in NCI-60 human cancer cell lines (35, 36).

Previously, Scott *et al.* (38) demonstrated that proapoptotic dose of histone deacetylase inhibitor (HDACi) can downregulate let-7 as well as miR-27a/27b miRNA, which are aberrantly expressed in breast cancer cells. In this report, authors have identified two putative targets of miR-27a/27b such as RYBP/DEDAF (an apoptotic facilitator) and ZBTB10/RINZF (a Sp1 repressor) by antisense suppression of miR-27a /27b. The elevated expression of these genes was also noted in breast cancer cells exposed to HDACi thus suggesting the post-transcriptional effects of HDACi in mediating their anticancer activity. Notably, 3' UTR of ZBTB10/RINZF gene also contains putative target sequence of let7 miRNA (38). Thus the enhanced level of ZBTB10/RINZF might emerge from the concerted effect of down-regulation of both miR-27 and let-7 by HDACi.

In addition, all-trans-retinoic acid (ATRA) treatment of leukemic cells resulted in the differential expression of a number of miRNAs, including let-7 and miR-16 family (39). More importantly, Weidhaas *et al* (40) suggested that microRNAs might be potential targets for altering resistance to cytotoxic anticancer therapy. The authors reported here that a radiosensitive state of lung cancer cells can be created when the select let-7 family of miRNAs is overexpressed *in vitro*. Strikingly, *in vivo* in a *Caenorhabditis elegans* model of radiation-induced cell death, decreasing the levels of let-7 family members can result in radioresistance.

To the other end the evidences of the adaptation of miRNA circuits by dietary components to exert anticancer effect are also evolving (41). Epigallocatechin gallate (EGCG) is one of the key chemopreventive polyphenols of green tea (42). In our laboratory, we previously observed that EGCG induces mitochondrial membrane depolarization and caspase-dependent apoptosis in pancreatic cancer cells. In addition, EGCG invokes Bax oligomerization and facilitates cytochrome c release into from mitochondria to cytosol. We also demonstrated cross talk between extrinsic and intrinsic cell death pathway during EGCG mediated apoptosis in pancreatic cancer cells (42). The studies on the effect of EGCG on miRNA profile were lately undertaken in hepatocellular carcinoma (43). The results from this investigation suggest the importance of miRNAs in the regulation of proapoptotic effect of EGCG. The chemopreventive action of EGCG, has been shown to be mediated by the upregulation of miR-16 and down regulation of antiapoptotic protein Bcl-2 in hepatocellular carcinoma cells (43). Indeed Bcl-2 was previously shown to be one of the major targets of miR-16 in chronic lymphocytic leukemia (10).

Curcumin, a flavinoid derived from the rhizome of *Curcuma longa* can exert various pharmacologic effects on cancer related signaling mediators (44). Sun *et al* (37) reported that antitumor effect of curcumin could be mediated by modulation of miRNA expression (37). Here authors presented experimental evidences showing the ability of Curcumin to upregulate miR-22 and attenuate pancreatic cancer cell proliferation by targeting Sp1 transcription factor and estrogen receptor 1 (ESR1). Apparently Sp proteins are critical for growth and metastasis of diverse tumor types due to their capacity of regulating cell cycle and vascular endothelial growth factor (VEGF) gene expression (45). Moreover, ESR1 is also known to be one of the important therapeutic targets in breast and pancreatic cancer (46, 47).

Resveratrol, a natural antioxidant is the constituent of a variety of plants that include grapes, mulberries and peanuts (48). At present, this compound with anti-inflammatory properties is at the stage of preclinical studies for human cancer prevention (48). Interestingly, experimental evidences indicate that the protective properties of resveratrol might be attributed at least in part from its competence to adapt the miRNA profiles in cells. In human SW480 colon cancer cells, resveratrol treatment can result in the enhancement of the level of a tumor suppressor miR-663 that targets TGF β 1 transcripts. In a nutshell, resveratrol mediated upregulation of multiple components of the TGF β signaling pathway (TGF β receptors type I and type II) decreases the transcriptional activity of SMADs, the main effectors of the canonical TGF β pathway. In contrast, due to resveratrol treatment, the expression of several oncogenic miRNAs is compromised. Such diminishment results in increased level of Dicer 1 (cytoplasmic RNase III which converts precursor miRNA to matured miRNA), tumor suppressor factors such as PDCD4 or PTEN and the effectors of TGF β signaling pathway.

In a separate report Tili *et al* (50) also observed the upregulation of miR-663 in human monocytic cells as well as in human blood monocytes when treated with resveratrol. Here, authors show that, in human monocytic cells as well as in human blood monocytes, resveratrol upregulates miR-663, a microRNA potentially targets multiple genes implicated in the immune response. In human acute monocytic leukemia cell line, miR-663 decreases endogenous activator protein-1 (AP-1) activity and impairs its upregulation by lipopolysaccharides (LPS), at least in part by directly targeting JunB and JunD transcripts. More importantly, resveratrol has the ability to intervene the upregulation of miR-155 by LPS in a miR-663-dependent manner. Several miRNAs, and especially miR11 155, play an essential role in both the innate and adaptive immune response. Since the microenvironment of most neoplastic tissue is comprised of inflammatory component and miR-155 is upregulated in many cancers, these findings suggest that manipulating miR-663 levels may help to optimize the use of resveratrol as both an anti-inflammatory and anticancer agent against malignancies associated with high levels of miR-155.

Soy isoflavones such as genistein (51) is recognized to possess the ability to inhibit cancer cell growth and metastasis. Li *et al* (51) undertook the approach whether genistein can affect the expression of miRNAs in gemcitabine resistant pancreatic cancer cells. Primarily, they noted the significant down regulation of miR-200b, miR-200c, and members of let-7 family in gemcitabine resistant cells when compared with their counterpart. Moreover gemcitabine resistant pancreatic cancer cells exhibited EMT characteristics with higher levels of mesenchymal markers such as vimentin and ZEB1 (15–18). The treatment of gemcitabine resistant pancreatic cancer cells with isoflavone can reverse EMT phenotype to epithelial morphology by restoring miR-200 level. Soy isoflavone also could induce tumor suppressor let-7 to attenuate cancer cell proliferation. In particular, the reprogramming ability of genistein to revert EMT to epithelial morphology should be further explored for novel therapies for pancreatic cancer.

The epidemiological studies indicate a clear correlation between greater consumption of cruciferous vegetables and lower incidence of cancer occurrence (52). As a result, cruciferous vegetables, a rich source of glucosinolates, have been of great interest for potential use in the chemoprevention of cancer. The glucosinolates are degraded into isothiocyanates by enzymatic action of plant specific myrosinase or intestinal flora myrosinase in the body (53). Previous reports demonstrate that isothiocyanates rapidly accumulate within cells and intracellular concentration of these agents can reach millimolar levels (54).

In our laboratory, we have observed that multitarget anticancer agent, benzyl isothiocyanate (BITC), can exert antitumor effect in human pancreatic cancer cells by perturbing cell cycle and inducing apoptotic cell death (55). Since it is thought that the approaches to interfere specific miRNA expression might offer novel therapeutic opportunities for human cancer, we were interested to examine whether BITC can adapt miRNA expression in pancreatic cancer cells. We focused our investigation on a restricted number of miRNAs which were altered in pancreatic ductal adenocarcinoma (PDAC) compared to adjacent benign pancreatic tissue or normal pancreas. Experimental evidences reveal that some of the miRNAs which are significantly deregulated in pancreatic cancers can be modulated by BITC. The single-tube TaqMan miRNA q-PCR assay discovered that matured miR-375 (underexpressed in pancreatic cancer patients) was remarkably up regulated due to BITC treatment of cultured pancreatic cancer cells. To the contrary, the expression level of oncogenic miR-221 (overexpressed in pancreatic tumor samples) was noted to be diminished under identical conditions. Importantly, the ratio of *miR-221: miR-375* is thought to bear high discriminatory potential in distinguishing tumor from normal tissue (14, 56).

To summarize we have demonstrated that the expression of putative oncogenic microRNA such as miR-221 and tumor suppressor miR-375 can be altered upon BITC treatment of pancreatic ductal adenocarcinoma cells. We also identify that ectopic overexpression of miR-375 mimic or enforced silencing of miR-221 in cultured pancreatic cancer cells attenuates cell viability and sensitizes antiproliferative action of BITC. Gene expression microarray analysis and real time quantitative PCR identified Insulin like growth factor binding protein 5 (IGFBP5) and Caveolin-1 as putative novel targets of miR-375. IGFBP5 and Caveolin-1 are of particular interest because of their potential link with the aggressiveness of pancreatic cancer (57, 58). To the other end, the expression of p27, a target of miR-221 (59) is elevated in BITC treated pancreatic cancer cells. Another important aspect of our investigation is the underexpression of miR-375 with simultaneous overexpression of miR-221 in KRasG12D driven PanIN developed in pancreatic cancer mouse model. Correlatively, elevated expression of IGFBP5 and CAV-1 was evident in mouse pancreas with preneoplastic lesions in which the expression of miR-375 wanes. Taken together, our findings suggest that anticancer agent BITC might target the expression of tumor suppressor miR-375 and oncomir miR-221 to switch hyperproliferative pancreatic cancer cells to hypoproliferative state.

Perspectives

MicroRNAs play a crucial regulatory role in normal and dysfunctional cellular processes. From tumor progression and viral host interactions, to immune response and stem cell fate determination, miRNAs are quickly growing in importance as the "master regulators" in cell cycle processes. Given that microRNAs can have multiple target genes and can concurrently decrease the levels of genes with different biological functions, the exogenous expression or silencing of microRNAs should have profound inhibitory effect on tumor growth. With the help of cutting edge technologies scientists have discovered the tissue specific expression patterns of miRNAs in human cancers. However, it is a daunting task to target enormous amount of miRNAs usually reported in profiling studies. We absolutely need to focus on a limited set of miRNAs which represent the signature of early events in carcinogenesis. In that respect further studies to pinpoint the miRNAs-necessary in the transformation of premalignant cells to malignant state. Using preclinical models the discovery and characterization of noncoding RNAs in precancerous lesions as well as in the process of tumor initiating stem cells should be of special interest to scientific community. It will be worth pursuing to determine whether these miRNAs can be modulated by chemopreventive or chemotherapeutic agents in tissue microenvironment of preclinical animal models. Thus more comprehensive information on noncoding RNAs arising from these preclinical studies

should be highly beneficial for reprogramming miRNA networks in human cancer. Future efforts should be directed towards the exploration of synergistic effect of antagomirs and target mimicry on the growth of cancer cells *in vitro* and *in vivo*. Such strategy of modulating miRNA by simultaneously targeting diverse molecular components to intervene excessive cellular growth might be instrumental for developing an effective nonconventional therapy for human cancer.

Acknowledgments

The authors' work cited in this article was supported by the National Cancer Institute grants CA137476 (A. Basu) and CA 149785 (S. Halдар)

References

1. Croce CM. Causes and consequences of microRNA dysregulation in cancer. *Nat Rev Genetics*. 2009; 10:704–714. [PubMed: 19763153]
2. Bhardwaj A, Singh S, Singh AP. MicroRNA-based cancer therapeutics: big hope from small RNAs. *Mol Cell Pharmacol*. 2010; 2:213–219. [PubMed: 21289871]
3. Negrini M, Nicoloso MS, Calin GA. MicroRNAs and cancer--new paradigms in molecular oncology. *Curr Opin Cell Biol*. 2009; 21:470–479. [PubMed: 19411171]
4. Dalmay T, Edwards DR. MicroRNAs and the hallmarks of cancer. *Oncogene*. 2006; 25:6170–6175. [PubMed: 17028596]
5. Bloomston M, Frankel WL, Petrocca F, et al. MicroRNA expression patterns to differentiate pancreatic adenocarcinoma from normal pancreas and chronic pancreatitis. *JAMA*. 2007; 297:1901–1908. [PubMed: 17473300]
6. Szafranska AE, Davison TS, John J, et al. MicroRNA expression alterations are linked to tumorigenesis and non-neoplastic processes in pancreatic ductal adenocarcinoma. *Oncogene*. 2007; 26:4442–4452. [PubMed: 17237814]
7. Lee EJ, Gusev Y, Jiang J, et al. Expression profiling identifies microRNA signature in pancreatic cancer. *Int J Cancer*. 2007; 120:1046–1054. [PubMed: 17149698]
8. Basu A, Jiang X, Negrini M, Halдар S. MicroRNA-mediated regulation of pancreatic cancer cell proliferation. *Oncol Lett*. 2010; 1:565–568.
9. Kent OA, Mendell JT. A small piece in cancer puzzle: microRNAs as tumor suppressors and oncogenes. *Oncogene*. 2006; 25:6188–6196. [PubMed: 17028598]
10. Cimmino A, Calin GA, Fabri M, et al. miR-15 and miR-16 induce apoptosis by targeting BCL2. *Proc Natl Acad Sci USA*. 2005; 102:13944–13949. [PubMed: 16166262]
11. Zhao JJ, Lin J, Yang H, Kong W, He L, Ma X, Coppola D, Cheng JQ. MicroRNA-221/222 negatively regulates estrogen receptor α is associated with tamoxifen resistance in breast cancer. *J Biol Chem*. 2008; 283:31079–31086. [PubMed: 18790736]
12. Fornari F, Gramantieri L, Ferracin M, et al. miR-221 controls CDKN1C/p57 and CDKN1B/p27 expression in human hepatocellular carcinoma. *Oncogene*. 2008; 27:5651–5661. [PubMed: 18521080]
13. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell*. 2004; 116:281–297. [PubMed: 14744438]
14. Basu A, Alder H, Khiyami A, et al. MicroRNA-375 and microRNA-221: Potential noncoding RNAs associated with antiproliferative activity of benzyl isothiocyanate in pancreatic cancer. *Genes Cancer*. 2011; 2:108–119. [PubMed: 21779484]
15. Tellez CS, Juri DE, Do K, et al. EMT and stem cell-like properties associated with miR-205 and miR-200 epigenetic silencing are early manifestations during carcinogen-induced transformation of human lung epithelial cells. *Cancer Res*. 2011; 71:3087–3097. [PubMed: 21363915]
16. Mani SA, Guo W, Liao M-J, et al. The epithelial-mesenchymal transition generates cells with properties of stem cells. *Cell*. 2008; 133:704–715. [PubMed: 18485877]
17. Gregory PA, Bracken CP, Bert AG, Goodall GJ. MicroRNAs as regulators of epithelial-mesenchymal transition. *Cell Cycle*. 2008; 7:3112–3118. [PubMed: 18927505]

18. Haque I, Mehta S, Majumder M, et al. Cyr61/CCN1 signaling is critical for epithelial-mesenchymal transition and stemness and promotes pancreatic carcinogenesis. *Mol Cancer*. 2011; 10:8. [PubMed: 21232118]
19. Shen YL, Jiang YG, Greenlee AR, et al. MicroRNA expression profiles and miR-10a target in anti-benzo[a] pyrene-7,8-diol-9, 10-epoxide-transformed human 16HBE cells. *Biomed Environ Sci*. 2009; 22:14–21. [PubMed: 19462682]
20. Jiyang Y, Wu Y, Greenlee AR, et al. miR-106a-mediated malignant transformation of cells induced by anti-benzo[a]pyrene-*trans*-7,8-diol-9,10-epoxide. *Toxicol. Sci*. 2011; 119:50–60. [PubMed: 20889678]
21. Chen T. The role of microRNA in chemical carcinogenesis. *J Environ Sci Health C Environ Carcinog Ecotoxicol Rev*. 2010; 28:89–124. [PubMed: 20552498]
22. Li Z, Branham WS, Dial SL, et al. Genomic analysis of microRNA time-course expression in liver of mice treated with genotoxic carcinogen N-ethyl-N-nitrosourea. *BMC Genomics*. 2010; 11:609. [PubMed: 21029445]
23. Yu T, Wang XY, Gong RG, et al. The expression profile of microRNAs in a model of 7,12-dimethyl-benz[a] anthracene-induced oral carcinogenesis in Syrian hamster. *J Exp Clin Cancer Res*. 2009; 28:64. [PubMed: 19435529]
24. Bhat-Naksharti P, Wang G, Collins NR, et al. Estradiol-regulated microRNAs control estradiol response in breast cancer cells. *Nucleic Acids Res*. 2009; 37:4850–4861. [PubMed: 19528081]
25. Davidson LA, Wang N, Shah MS, et al. n-3 Polyunsaturated fatty acids modulate carcinogen-directed non-coding microRNA signatures in rat colon. *Carcinogenesis*. 2009; 30:2077–2084. [PubMed: 19825969]
26. Izzotti A, Calin GA, Arrigo P, et al. Downregulation of microRNA expression in the lungs of rats exposed to cigarette smoke. *FASEB J*. 2009; 23:806–812. [PubMed: 18952709]
27. Schembri F, Sridhar S, Perdomo C, et al. MicroRNAs as modulators of smoking induced gene expression changes in human airway epithelium. *Proc Natl Acad Sci USA*. 2009; 106:2319–2324. [PubMed: 19168627]
28. Hsy PY, Deatherage DE, Rodriguez BA, et al. Xenoestrogen-induced epigenetic repression of microRNA-9-3 in breast epithelial cells. *Cancer Res*. 2009; 69:5936–5945. [PubMed: 19549897]
29. Moffat ID, Bourtros PC, Celius T, et al. MicroRNAs in adult rodent liver are refractory to dioxin treatment. *Toxicol Sci*. 2007; 99:470–487. [PubMed: 17698510]
30. Schetter AJ, Leung SY, Sohn JJ, et al. MicroRNA expression profiles associated with prognosis and therapeutic outcome in colon adenocarcinoma. *JAMA*. 2008; 299:425–436. [PubMed: 18230780]
31. Raver-Shapira N, Marciano E, Meiri E, et al. Transcriptional activation of miR-34a contributes to p53-mediated apoptosis. *Mol Cell*. 2007; 26:731–743. [PubMed: 17540598]
32. Melkamu T, Zhang X, Tan J, Zeng Y, Kassie F. Alteration of microRNA expression in vinyl-carbamate-induced mouse lung tumors and modulation by the chemopreventive agent indole-3-carbinol. *Carcinogenesis*. 2010; 31:252–258. [PubMed: 19748927]
33. Dai Z, Barbacioru C, Huang Y, Sadee W. Prediction of anticancer drug potency from expression of genes involved in growth factor signaling. *Pharm. Res*. 2006; 23:336–349. [PubMed: 16425089]
34. Dai Z, Huang Y, Sadee W. Growth factor signaling and resistance to cancer chemotherapy. *Curr Top Med Chem*. 2004; 4:1347–1356. [PubMed: 15379649]
35. Blower PE, Chung J-H, Verducci JS, et al. MicroRNAs modulate the chemosensitivity of tumor cells. *Mol Cancer Ther*. 2008; 7:1–9. [PubMed: 18187804]
36. Liu H, D'Andrade P, Fulmer-Smentek S, et al. mRNA and microRNA expression profiles of the NCI-60 integrated with drug activities. *Mol Cancer Ther*. 2010; 9:1080–1095. [PubMed: 20442302]
37. Sun M, Estrov Z, Ji Y, et al. Curcumin (diferuloylmethane) alters the expression profiles of microRNAs in human pancreatic cancer cells. *Mol. Cancer Ther*. 2008; 7:464–473. [PubMed: 18347134]
38. Scott GK, Mattie MD, Berger CE, et al. Rapid alteration of microRNA levels by histone deacetylase inhibition. *Cancer Res*. 2006; 66:1277–1281. [PubMed: 16452179]

39. Garzon R, Pichiorri F, Palumbo T, et al. MicroRNA gene expression during retinoic acid-induced differentiation of human acute promyelocytic leukemia. *Oncogene*. 2007; 26:4148–4157. [PubMed: 17260024]
40. Weidhaas JB, Babar I, Nallur P, et al. MicroRNAs as potential agents to alter resistance to cytotoxic anticancer therapy. *Cancer Res*. 2007; 67:11111–11116. [PubMed: 18056433]
41. Davis CD, Ross SA. Evidence for dietary regulation of microRNA expression in cancer cells. *Nutr Rev*. 2008; 582:3542–3546.
42. Qanungo S, Das M, Haldar S, Basu A. Epigallocatechin-3-gallate induces mitochondrial membrane depolarization and caspase-dependent apoptosis in pancreatic cancer cells. *Carcinogenesis*. 2005; 26:958–967. [PubMed: 15705601]
43. Tsang WP, Kwok TT. Epigallocatechin gallate up-regulation of miR-16 and induction of apoptosis in human cancer cells. *J Nutr Biochem*. 2010; 21:140–146. [PubMed: 19269153]
44. Aggarwal BB, Kumar A, Bharti AC. Anticancer potential of curcumin: preclinical and clinical studies. *Anticancer Res*. 2003; 23:363–398. [PubMed: 12680238]
45. Safe S, Abdelrahim M. Sp transcription factor family and its role in cancer. *Eur J Cancer*. 2005; 41:2438–2448. [PubMed: 16209919]
46. Hoist F, Stahl PR, Ruiz C, et al. Estrogen receptor α (ESR1) gene amplification is frequent in breast cancer. *Nat Genet*. 2007; 39:655–660. [PubMed: 17417639]
47. Satake M, Sawai H, Go VL, et al. Estrogen receptors in pancreatic tumors. *Pancreas*. 2006; 33:119–127. [PubMed: 16868476]
48. Kundu JK, Surh YJ. Cancer chemopreventive and therapeutic potential of resveratrol: mechanistic perspectives. *Cancer Lett*. 2008; 269:243–261. [PubMed: 18550275]
49. Tili E, Michaille JJ, Alder H, et al. Resveratrol modulates the levels of microRNAs targeting genes encoding tumor-suppressors and effectors of TGF β signaling pathway in SW480 cells. *Biochem Pharmacol*. 2010; 80:2057–2065. [PubMed: 20637737]
50. Tili E, Michaille JJ, Adair B, et al. Resveratrol decreases the levels of miR-155 by upregulating miR-663, a microRNA targeting JunB and JunD. *Carcinogenesis*. 2010; 31:1561–1566. [PubMed: 20622002]
51. Li Y, VanderBoom TG, Kong D, et al. Up regulation of miR-200 and let-7 by natural agents leads to the reversal of epithelial-to-mesenchymal transition in gemcitabine-resistant pancreatic cancer cells. *Cancer Res*. 2009; 69:6704–6712. [PubMed: 19654291]
52. Bianchini F, Vainio H. Isothiocyanates in cancer prevention. *Drug Metabolism Reviews*. 2004; 36:655–667. [PubMed: 15554241]
53. Holst B, Williamson G. A critical review of the bioavailability of glucosinolates and related compounds. *Natural product Reports*. 2004; 21:425–447. [PubMed: 15162227]
54. Zhang Y. Molecular mechanism of rapid cellular accumulation of anticarcinogenic isothiocyanates. *Carcinogenesis*. 2001; 22:425–431. [PubMed: 11238182]
55. Basu A, Haldar S. Antiproliferative and proapoptotic effects of benzyl isothiocyanate on human pancreatic cancer cells is linked to death receptor activation and RasGAP/Rac1 down modulation. *Int J Oncol*. 2009; 35:593–599. [PubMed: 19639179]
56. Avissar M, Christensen BC, Kelsey KT, et al. MicroRNA expression is predictive of head and neck squamous cell carcinoma. *Clin Cancer Res*. 2009; 15:2850–2855. [PubMed: 19351747]
57. Johnson SK, Haun RS. Insulin-like growth factor binding protein-5 influences pancreatic cancer cell growth. *World J Gastroenterol*. 2009; 15:3355–3366. [PubMed: 19610136]
58. Tanase CP. Caveolin-1: a marker for pancreatic cancer diagnosis. *Expert Rev Mol Diagn*. 2008; 8:395–404. [PubMed: 18598222]
59. Formari F, Gramantieri L, Ferracin M, et al. miR-221 controls CDKN1C/p57 and CDKN1B/p27 expression in human hepatocellular carcinoma. *Oncogene*. 2008; 27:5651–5661. [PubMed: 18521080]