Mutation Research 751 (2012) 287–303

Contents lists available at SciVerse ScienceDirect

Mutation Research/Reviews in Mutation Research

journal homepage: www.elsevier.com/locate/reviewsmr
Community address: www.elsevier.com/locate/mutres

Review

MicroRNAs as targets for dietary and pharmacological inhibitors of mutagenesis and carcinogenesis

Alberto Izzotti [a], Cristina Cartiglia [a], Vernon E. Steele [b], Silvio De Flora [a,*]

[a] Department of Health Sciences, University of Genoa, Genoa, 16132, Italy
[b] National Cancer Institute, Rockville, MD 20892, USA

ARTICLE INFO

Article history:
Received 8 March 2012
Received in revised form 28 May 2012
Accepted 29 May 2012
Available online 7 June 2012

Keywords:
MicroRNA
Chemoprevention
Dietary agents
Pharmacological agents

ABSTRACT

MicroRNAs (miRNAs) have been implicated in many biological processes, cancer, and other diseases. In addition, miRNAs are dysregulated following exposure to toxic and genotoxic agents. Here we review studies evaluating modulation of miRNAs by dietary and pharmacological agents, which could potentially be exploited for inhibition of mutagenesis and carcinogenesis. This review covers natural agents, including vitamins, oligoelements, polyphenols, isoflavones, indoles, isothiocyanates, phospholipids, saponins, anthraquinones and polyunsaturated fatty acids, and synthetic agents, including thiols, nuclear receptor agonists, histone deacetylase inhibitors, antiinflammatory drugs, and selective estrogen receptor modulators. As many as 145 miRNAs, involved in the control of a variety of carcinogenesis mechanisms, were modulated by these agents, either individually or in combination. Most studies used cancer cells *in vitro* with the goal of modifying their phenotype by changing miRNA expression profiles. *In vivo* studies evaluated regulation of miRNAs by chemopreventive agents in organs of mice and rats, either untreated or exposed to carcinogens, with the objective of evaluating their safety and efficacy. The tissue specificity of miRNAs could be exploited for the chemoprevention of site-specific cancers, and the study of polymorphic miRNAs is expected to predict the individual response to chemopreventive agents as a tool for developing new prevention strategies.

Contents

* Corresponding author. Tel.: +39 010 3538500; fax: +39 010 3538504.
E-mail address: sdf@unige.it (S. De Flora).

1383-5742/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.mrrev.2012.05.004

1. Introduction

MicroRNAs (miRNAs) are small (18–25 nucleotides), noncoding, single-stranded RNAs, which negatively regulate gene expression either by translational inhibition or exonucleolytic messenger RNA (mRNA) decay [1]. These evolutionary conserved RNAs have been recognized in virtually all species, ranging from viruses to humans [2]. While the information provided by transcriptome analyses is redundant due to the fact that mRNA can be regulated posttranscriptionally, miRNAs regulate a number of genes simultaneously. The miRBase Version 16.0 has 1048 miRNA sequences annotated in the human genome, and miRNAs are believed to target about one-third of human mRNAs [3], a single miRNA targeting approximately 200 transcripts simultaneously [4].

Accordingly, miRNAs have been implicated in almost every biological process, including development, cell cycle regulation, cell growth and differentiation, stress response, and apoptosis [5]. In addition, miRNAs play a role in a variety of diseases [6] and in particular in cancer [7]. MiRNAs are known to be dysregulated as a response to toxic and genotoxic agents [8], including physical agents such as ionizing radiation [9] and UV radiation [10], chemical agents such as benzo[*a*]pyrene [11], hepatotoxicants [12] and drugs [13], and complex mixtures such as cigarette smoke [14,15] and environmental pollutants [16,17].

In the present review article we summarize the findings of a number of studies evaluating modulation of miRNAs by known inhibitors of mutagenesis and carcinogenesis. These inhibitors represent putative cancer chemopreventive agents, as assessed in experimental test systems and sometimes in clinical chemoprevention trials. They include both natural agents, mostly of dietary source, and synthetic agents, mostly used as pharmacological agents, which are analytically discussed in Sections 2 and 3, respectively.

Due to the large variety of mechanisms by which it is possible to inhibit mutagenesis and carcinogenesis [18–20], modulation of miRNAs as an epigenetic response to drugs [21] and dietary agents [22–26] is of particular relevance to understand their mechanism of action and to evaluate their safety and efficacy.

Table 1 summarizes the main findings relative to modulation of miRNAs by putative cancer chemopreventive agents, as inferred from both literature data available in PubMed up to December 2011 and unpublished data from our laboratory. The investigated miRNAs are listed starting from the *let-7* family and continuing with the *miR* series in increasing nomenclature number. Those miRNAs that are identified with the symbol § in Table 1 undergo single nucleotide polymorphisms (SNPs) in humans [27,28]. The tissue specificity indicates the cell type or organ in which each miRNA has the highest expression levels, as reported in the Mirnamap database (mirnamap.mbc.nctu.edu.tw) and literature data. The main functions regulated by each miRNA are inferred from the Mirnamap database (mirnamap.mbc.nctu.edu.tw), the Mirbase database (www.mirbase.org) and from literature data.

The fourth column in Table 1 reports either the cells that were analyzed *in vitro* or the species and organ analyzed *in vivo*. Most *in vitro* studies used cancer cells, mainly of human origin, in which the authors investigated the ability of putative anticancer agents to modulate the expression of miRNAs with the goal of exploring their mechanisms of action and modifying their phenotype. Apart from a couple of studies using human samples and another one using a plant, all other *in vivo* studies used tissues from rats or mice exposed to carcinogens, such as cigarette smoke (CS), vinyl carbamate (VC), and azoxymethane (AOM), or subjected to particular diets, such as vitamin- or choline-deficient diets. Several

Table 1
Denomination and main functions of miRNAs that were have been reported, up to December 2011, to be modulated by natural and synthetic chemopreventive agents in either *in vitro* or *in vivo* studies.

MiRNA	Tissue specificity	Main regulated functions	Analyzed cells (*in vitro*) or species/organ (*in vivo*)	Modulating agent [Ref.]
let-7a§	Lung, cervix, liver	Cell proliferation, k-*Ras* activation, apoptosis	Human leukemia cells	↑Retinoic acid [30]
			Human hepatocarcinoma cells	↑Ellagitannin BJA32515 [51]
			Mouse lung (CS+)	↑PEITC [82,83]
			Mouse lung	↑SAHA [UD]
			Mouse lung (CS+)	↑Budesonide [82]
			Mouse liver	↓Budesonide [82]
			Rat lung (CS+)	↑PEITC [81]
			Rat lung (CS+)	↑Oltipraz + NAC [81]
let-7b	Lung, cervix, kidney	Cell proliferation	Human pancreatic cancer cells	↓Diindolylmethane [78]
			Human pancreatic cancer cells	↓Genistein [78]
			Human lung cancer cells	↑SAHA [93]
			Rat lung (CS+)	↑Oltipraz [81]
			Rat lung (CS+)	↑Oltipraz + NAC [81]
			Rat lung (CS+)	↑PEITC + I3C [81]
let-7c	Lung, kidney	Intercellular adhesion	Human hepatocarcinoma cells	↑EGCG [53]
			Human leukemia cells	↑Retinoic acid [30]
			Human pancreatic cancer cells	↓Genistein [78]
			Human pancreatic cancer cells	↓Diindolylmethane [78]
			Mouse lung (CS+)	↑PEITC [82]
			Rat lung (CS+)	↑PEITC [81]
			Rat lung (CS+)	↑PEITC + I3C [81]
			Rat lung (CS+)	↑Oltipraz [81]
			Rat lung (CS+)	↑Oltipraz + NAC [81]
let-7d	Lung, cervix, prostate, bladder	Cell proliferation	Human leukemia cells	↑Retinoic acid [30]
			Human pancreatic cancer cells	↓Genistein [78]
			Human pancreatic cancer cells	↓Diindolylmethane [78]
			Rat colon (AOM+)	↑PUFA (Fish oil) [86]
let-7e	Lung, cervix	Cell proliferation	Human hepatocarcinoma cells	↑Ellagitannin BJA3121 [52]
			Human pancreatic cancer cells	↓Genistein [78]
			Human pancreatic cancer cells	↓Diindolylmethane [78]
let-7f	Lung, cervix, kidney, liver	Cell proliferation, k-*Ras* activation, angiogenesis	Rat lung (CS+)	↑PEITC + I3C [81]
let-7l	Lung	Cell proliferation	Human colon carcinoma cells	↓SAHA [90]
miR-9	Brain	Apoptosis	Rat fetus central nervous system	↓Retinoic acid [37]
miR-10§	Lung, kidney, breast	Angiogenesis	Human neuroblastoma cells	↑Retinoic acid [36]
			Human embryonic stem cells	↑PUFA [87]
			Mouse fetus brain	↓Folic acid [40]
			Rat lung (CS+)	↑5,6-Benzoflavone [81]
			Rat lung (CS+)	↑Oltipraz [81]
			Rat lung (CS+)	↑Oltipraz + NAC [81]
			Rat lung (CS+)	↑I3C [81]
			Rat lung (CS+)	↑PEITC + I3C [81]
miR-15	Lymphocyte, thymus, lung, liver	Lymphocyte differentiation, apoptosis	Human breast cancer cells	↑Curcumin [58]
			Human leukemia cells	↑Retinoic acid [30]
			Human lymphoma cells	↓SAHA [91]
			Mouse liver (CS+)	↑Budesonide [82]
			Rat colon (AOM+)	↑PUFA (Fish oil) [86]
miR-16§	Lung	Apoptosis	Human hepatocarcinoma cells	↑EGCG [53]
			Human leukemia cells	↑Retinoic acid [30]
miR-17	Lung, kidney, bladder	Tumor suppressor gene *PTEN*, DICER, TGF-beta, c-*Myc*	Human colon cancer cells	↓Resveratrol [62]
			Human prostate cancer cells	↓Resveratrol [68]
			Human leukemia cells	↓Retinoic acid [29]
			Human neuroblastoma cells	↓Retinoic acid [35]
			Human lymphoma cells	↓SAHA [91]
miR-18	Lung, kidney, prostate	Small RNA transcription	Human hepatocarcinoma cells	↑EGCG [53]
			Human lymphoma cells	↓SAHA [91]
miR-20	Bladder, lung, thymus, prostate, kidney	No data available	Rat heart	↑Resveratrol [69,70]
miR-21	Lung, kidney, bladder, liver	Tumor suppressor gene *PTEN*, cell proliferation	Human pancreatic cancer cells	↓Difluorinated curcumin [61]
			Human colon cancer cells	↓Curcumin [60]
			Human colon cancer cells	↓Resveratrol [62]
			Human breast cancer cells	↑Diindolylmethane [79]
			Human breast cancer cells	↑Retinoic acid [32]
			Human breast cancer cells	↓Polyphenon-60 [55]
			Mouse lung (VC+)	↓I3C [80]
			Mouse liver (CS+)	↑Budesonide [82]
			Rat heart	↑Resveratrol [69,70]
			Human breast cancer cells	#Tamoxifen [99]
miR-22	Muscle	Estrogen receptor alpha	Human pancreatic cancer cells	↑Curcumin [57]
			Human colon cancer cells	↑SAHA [90]
miR-23	Ovary, kidney, bladder	Gene transcription	Neuronal cells	↑Retinoic acid [33]
miR-24	Lung, kidney, prostate	Cell differentiation	Human embryonic stem cells	↑PUFA [87]

Table 1 (*Continued*)

MiRNA	Tissue specificity	Main regulated functions	Analyzed cells (*in vitro*) or species/organ (*in vivo*)	Modulating agent [Ref.]
miR-25§	Pancreas, breast, kidney	DICER	Human colon cancer cells	↓Resveratrol [62]
			Human hepatocarcinoma cells	↑EGCG [53]
			Human leukemia cells	↓Retinoic acid [29]
miR-26	Lung, kidney, bladder, cervix, liver	TGF beta	Mouse liver	↓PEITC [82]
			Mouse lung (CS+)	↑PEITC [82]
			Mouse lung (CS+)	↑Budesonide [82]
			Rat lung (CS+)	↑PEITC [81]
			Rat lung (CS+)	↑5,6 benzoflavone [81]
			Rat lung (CS+)	↑Oltipraz[81]
			Rat lung (CS+)	↑NAC [81]
			Rat lung (CS+)	↑Oltipraz + NAC [81]
			Rat lung (CS+)	↑I3C [81]
			Rat lung (CS+)	↑PEITC + I3C [81]
miR-27	Bladder, prostate, cervix, kidney, liver	Tumor suppressor genes, cell proliferation, stress response, protein repair	Human colon cancer cells	↓NO-NSAID GT-094 [97]
			Human breast cancer cells	↓Polyphenon-60 [55]
			Human uveal melanoma cancer cells	↓Genistein [71]
			Mouse liver	↓Budesonide [82]
miR-29	Kidney, lung, heart	Collagen production, inflammation, apoptosis	Human colon cancer cells	↓SAHA [90]
			Human hepatocarcinoma cells	↑Ellagitannin BJA32515 [51]
			Human cardiac cells	↓Pioglitazone [89]
			Rat heart	↓Pioglitazone [89]
			Mouse lung (CS+)	↑PEITC [82]
miR-30§	Kidney, lung	Intercellular adhesion, protein repair, NFκB activation, cell cycle, EGF activation, stem cell recruitment, multidrug resistance	Human lung cancer cells	↓SAHA [93]
			Human hepatocarcinoma cells	↓EGCG [54]
			Human hepatocarcinoma cells	↓Anthocyanidin [54]
			Mouse lung (CS+)	↑PEITC [83]
			Rat lung (CS+)	↑PEITC + I3C [81]
miR-31	Small intestine, kidney, lung	Protein synthesis and secretion, stress response	Mouse lung (VC+)	↓I3C [80]
			Mouse lung (CS+)	↑PEITC [82]
			Mouse lung (CS+)	↑Budesonide [82]
miR-32§		Apoptosis	Human leukemia cells	↑1,25-dihydroxyvitamin D3 [43]
miR-34§	Ovary, prostate, lung	*P53*	Human cancer prostate cells	↑Selenium [49]
			Human pancreatic cancer stem cells	↑SAHA [92]
			Mouse liver	↑PEITC [82]
			Rat lung (CS+)	↑5,6 benzoflavone [81]
			Rat lung (CS+)	↑Oltipraz [81]
			Rat lung (CS+)	↑Oltipraz + NAC [81]
			Rat lung (CS+)	↑I3C [81]
			Rat lung (CS+)	↑PEITC + I3C [81]
miR-92§	Lung	DICER	Human colon cancer cells	↓Resveratrol [62]
			Human lung cancer cells	↓Resveratrol [67]
			Human hepatocarcinoma cells	↑EGCG [53]
miR-99	Cervix, prostate, ovary, kidney, lung	Apoptosis	Rat lung (CS+)	↑PEITC [81]
			Rat lung (CS+)	↑PEITC + I3C [81]
miR-100	Liver, placenta, cervix, lung	Apoptosis	Human colon cancer cells	↓SAHA [90]
			Mouse liver	↓Budesonide [82]
miR-106	Thymus, kidney, bladder, lung, liver, placenta	Cell adhesion, TNF activation, stress response	Prostate cancer cells	↓Resveratrol [68]
			Human lung cancer cells	↓SAHA [93]
			Human colon cancer cells	↓PUFA [88]
			Mouse liver	↓Budesonide [82]
miR-107	Brain, kidney	Intracellular trafficking, apoptosis	Human leukemia cells	↑Retinoic acid [30]
			Rat colon (AOM+)	↑PUFA (Fish oil) [86]
miR-122§	Liver	Stress response, lipid metabolism	Rat liver (choline-def. diet)	↑Folate [38,39]
			Rat liver	↓α-Tocopherol [48]
			Rat liver	↑α-Tocopherol [47]
miR-123	Lung	Angiogenesis, cell proliferation	Rat lung (CS+)	↑NAC [81]
			Rat lung (CS+)	↑Oltipraz [81]
			Rat lung (CS+)	↑Oltipraz + NAC [81]
			Rat lung (CS+)	↑PEITC [81]
			Rat lung (CS+)	↑PEITC + I3C [81]
miR-124	Brain, lung	Gene transcription, apoptosis	Human lung cancer cells	↑SAHA [93]
			Rat fetus central nervous system	↓Retinoic acid [37]
			Rat lung (CS+)	↑PEITC + I3C [81]
miR-125§	Lung, cervix, brain, ovary, prostate, bladder	Oncogene *ERBB*, vitamin D receptor, inflammation, gene transcription	Human melanoma cells	*1,25-dihydroxyvitamin D3 [45,46]
			Rat lung (CS+)	↑I3C [81]
			Mouse lung	↑SAHA [UD]
			Rat liver	↓α-Tocopherol [48]
			Rat liver	↑α-Tocopherol (Vit. E) [47]
			Rat fetus central nervous system	↓Retinoic acid [37]
			Mouse lung (CS+)	↑PEITC [82]
			Mouse liver	↓PEITC [82]
			Mouse liver (CS+)	↑PEITC [81]
			Mouse liver	↓Budesonide [82]
			Mouse liver (CS+)	↑Budesonide [82]
			Mouse lung (CS+)	↑PEITC [82]
			Rat lung (CS+)	↑PEITC + I3C [81]
			Rat lung (CS+)	

Table 1 (*Continued*)

MiRNA	Tissue specificity	Main regulated functions	Analyzed cells (*in vitro*) or species/organ (*in vivo*)	Modulating agent [Ref.]
miR-126	Lung, kidney	Gene transcription	Human lung cancer cells	↑SAHA [93]
miR-128	Brain	Cell proliferation, apoptosis	Human glioma cells	↑Ginsenoside Rh2 [84]
miR-129	Brain, lung	Calmodulin transcription activation	Human lung cancer cells	↑SAHA [93]
			Human hepatocarcinoma cells	↓EGCG [53]
miR-130	Kidney, cervix, prostate, lung, liver,	Gene transcription, apoptosis	Mouse lung (VC+)	↓I3C [80]
miR132§	Brain	Gene transcription	Human lung cancer cells	↑SAHA [93]
miR-133	Lung, prostate	Inflammation	Mouse lung	↓Budesonide [82]
miR-135	Kidney, thyroid, lung	*Ras* regulation, cell adhesion	Mouse lung (CS+)	↑PEITC [82]
miR-139	Brain	Cell proliferation, cell differentiation	Human breast cancer cells	↑Trichostatin A [94]
miR-140§	Lung	*P53*	Rat lung (CS+)	↑NAC [81]
			Rat lung (CS+)	↑Oltipraz + NAC [81]
			Rat lung (CS+)	↑PEITC + I3C [81]
miR-141	Placenta, kidney, intestine	Cell proliferation, cancer invasion	Human colon cancer cells	↓SAHA [90]
miR-142	Liver, thymus, spleen, lung	Protein repair, DNA repair, prostaglandin-mediated platelet aggregation	Mouse liver (CS+)	↓PEITC [82]
			Mouse liver	↓Budesonide [82]
miR-145	Prostate, cervix, ovary, bladder, lung	Protein repair, angiogenesis	Rat lung (CS+)	↑PEITC + I3C [81]
miR-146§	Lung	NFκB stress response, inflammation	Human colon fibroblasts	↑Polyphenolic extracts [50]
			Human neural cells	↓Resveratrol [65]
			Human pancreatic cancer cells	↑Genistein [23]
			Human breast cancer cells	↑Trichostatin A [94]
			Mouse lung (VC+)	↓I3C [80]
			Rat lung (CS+)	↑5,6-benzoflavone [81]
			Rat lung (CS+)	↑NAC [81]
			Rat lung (CS+)	↑Oltipraz + NAC [81]
			Rat lung (CS+)	↑PEITC [81]
			Rat lung (CS+)	↑PEITC + I3C [81]
miR-152	Neural tissue	Transcriptional repressor	Human neuroblastoma cells	↑Retinoic acid [34]
miR-153	Liver, brain, lung	Protein repair, protein synthesis, signal transduction	Mouse liver (CS+)	↑PEITC [82]
miR-155	Lung	TGF-beta	Human monocytic cells	↓Resveratrol [63,64]
			Human breast cancer cells	↑Trichostatin A [94]
			Human lymphoma cells	↓SAHA [91]
miR-156	Plants (*Arabidopsis*, *Arachis*)	Anthocyanin accumulation	*Arabidopsis thaliana*	•Anthocyanin [77]
miR-181§	Brain, thymus, kidney, lung	NFκB stress response	Human leukemia cells	↓Vitamin D3 [42]
			Mouse lung	↓PEITC [82]
			Human leukemia cells	↓Retinoic acid [30]
			Human breast cancer cells	#Tamoxifen [99]
miR-182§	Thymus, lung	Inflammation, cell proliferation	Human breast cancer cells	↑2,5-Hydroxyvitamin D3 [44]
miR-183	Lung	Apoptosis, cell adesion	Lung cancer cells	↑SAHA [93]
			Mouse lung	↑SAHA [UD]
miR-186	Bladder	Apoptosis	Human lung cancer cells	↓Curcumin [59]
			Human leukemia cells	↑Retinoic acid [29]
miR-191	Brain, cervix, kidney, lung	Cell proliferation	Rat colon (AOM+)	↑PUFA (Fish oil) [86]
			Rat lung (CS+)	↑PEITC + I3C [81]
miR-192	Intestine, kidney, liver, lung	Cell proliferation, k-*Ras* activation	Rat lung (CS+)	↑PEITC [81]
			Rat lung (CS+)	↑PEITC + I3C [81]
			Rat lung (CS+)	↑Oltipraz + NAC [81]
miR-193	Muscle, lymphocytes	Signal transduction	Human leukemia cells	↓Retinoic acid [29]
miR-194§	Intestine, kidney	Apoptosis, cell proliferation	Human lung cancer cells	↑Resveratrol [67]
miR-195	Cervix, prostate, ovary, bladder, lymphocytes	Small RNA transcription	Human leukemia cells	↓Retinoic acid [29]
miR-196§	Kidney, cervix	TGF-beta	Human colon cancer cells	↓Resveratrol [62]
			Human hepatocarcinoma cells	↓EGCG [53]
			Human pancreatic cancer cells	↓Curcumin [57]
miR-197	Brain	Apoptosis, cell proliferation	Human hepatocarcinoma cells	↓Ellagitannin BJA32515 [51]
			Human hepatocarcinoma cells	↑Anthocyanidin [54]
miR-200§	Kidney, lung, liver	Apoptosis, intracellular trafficking, protein repair	Human pancreatic cancer cells	↓Difluorinated curcumin [61]
			Human pancreatic cancer cells	↓Genistein [78]
			Human pancreatic cancer cells	↓Diindolylmethane [78]
			Human hepatocarcinoma cells	↓EGCG [53]
			Mouse lung (CS+)	↑PEITC [82]
			Mouse liver	↓PEITC [82]
			Mouse liver	↓Budesonide [82]
miR-210	Lung	Hipoxia-inducible factor-1	Mouse and human lung cancer cells	↑EGCG [56]
miR-215	Intestine	Intracellular trafficking, apoptosis	Human leukemia cells	↑Retinoic acid [29]
			Human breast cancer cells	↑Trichostatin A [94]
miR-218§	Lung, kidney, bladder, prostate, liver	Stress response, oncogene k-*Ras* activation, antioxidant	Mouse lung (CS+)	↑Pioglitazone [UD]
			Mouse lung (CS+)	↑Bexarotene + Pioglitazone [UD]
miR-221	Prostate	SERM resistance	Human breast cancer cells	#Tamoxifen [99,98]
			Human breast cancer cells	#Fulvestrant [101]
			Human prostate cancer cells	↓Genistein [85]
			Human colon cancer cells	↓SAHA [90]

Table 1 (*Continued*)

MiRNA	Tissue specificity	Main regulated functions	Analyzed cells (*in vitro*) or species/organ (*in vivo*)	Modulating agent [Ref.]
miR-222§	Prostate, lung, bladder	Angiogenesis, cell proliferation, SERM resistance	Human breast cancer cells	#Tamoxifen [99,98]
			Human breast cancer cells	#Fulvestrant [101]
			Human lymphoblastoid cells	↓Folate [41]
			Human colon cancer cells	↓SAHA [90]
			Human prostate cancer cells	↓Genistein [85]
			Rat lung (CS+)	↑Oltipraz + NAC [81]
			Rat lung (CS+)	↑PEITC [81]
			Rat lung (CS+)	↑PEITC + I3C [81]
miR-223	Spleen, lung	Protein repair, k-*Ras* activation	Human leukemia cells	↑Retinoic acid [29,30]
			Rat lung (CS+)	↑PEITC + I3C [81]
miR-290	Lung	Stem cell marker	Mouse lung (CS+)	↑Bexarotene + Pioglitazone [UD]
miR-292	Liver	Hepatocyte growth factor-induced cell proliferation, angiogenesis	Mouse liver (CS+)	↑PEITC [82]
			Mouse liver (CS+)	↑Budesonide [82]
miR-296§	Muscle, prostate, lung, bladder	Thioredoxin and cysteine synthesis (antioxidants), inflammation	Mouse lung (CS+)	↑Pioglitazone [UD]
			Mouse lung (CS+)	↑Bexarotene + Pioglitazone [UD]
miR-297	Liver	Protein repair, cell cycle	Mouse liver (CS+)	↑PEITC [82]
			Mouse liver (CS+)	↑Budesonide [82]
miR-299	Liver, lung, cervix, testes	NFκB activation, stress response, peroxisome activation	Human lung cancer cells	↑Resveratrol [67]
			Mouse liver (CS+)	↑PEITC [82]
miR-300	Liver,	Protein repair, intracellular trafficking, cell proliferation	Mouse liver	↓Budesonide [82]
miR-302	Lung	Cell adhesion, protein repair, intracellular trafficking, cell proliferation	Mouse lung	↑Myo-inositol [UD]
			Mouse lung	↓SAHA [UD]
miR-320	Bladder, cervix, liver, lung	Protein repair, intracellular trafficking, cell proliferation	Mouse liver	↓Budesonide [82]
miR-322	Liver	Protein repair, cell proliferation	Mouse liver (CS+)	↑PEITC [82]
			Mouse liver (CS+)	↑Budesonide [82]
miR-323	Liver	Peroxisome activation, protein repair	Mouse liver	↓PEITC [82]
miR-324§	Brain, kidney, prostate	Cell proliferation	Rat colon (AOM+)	↑PUFA (Fish oil) [86]
miR-331	Liver	Stress response	Mouse liver	↓PEITC [82]
			Mouse liver	↓Budesonide [82]
miR-335	Lung	Insulin growth factor, cell proliferation, apoptosis	Mouse lung (CS+)	↑Pioglitazone [UD]
			Mouse lung (CS+)	↑Bexarotene + Pioglitazone [UD]
miR-338	Liver	Protein repair, stress response	Human lung cancer cells	↑Resveratrol [67]
			Mouse liver	↓PEITC [82]
			Mouse liver	↓Budesonide [82]
miR-342	Brain, lymphocytes	Stress response, protein repair, SERM resistance	Human breast cancer cells	#Tamoxifen [99,100]
			Human leukemia cells	↑Retinoic acid [30]
			Human hepatocarcinoma cells	↓EGCG [53]
miR-345§	Thyroid, kidney	Intracellular trafficking	Human lung cancer cells	↓SAHA [93]
miR-370	Brain	Apoptosis, inflammation	Human hepatocarcinoma cells	↑Ellagitannin BJA3121 [52]
miR-373§	Liver	Apoptosis, inflammation	Human hepatocarcinoma cells	↓Ellagitannin BJA32515 [51]
			Human hepatocarcinoma cells	↑Ellagitannin BJA3121 [52]
miR-376	Liver	Carbonic anhydrase (antioxidant), peroxisome biogenesis, *P53*, cell cycle progression, signal transduction, apoptosis, intracellular vesicle trafficking	Mouse liver (CS+)	↑PEITC [82]
miR-377	Lung	Angiogenesis	Mouse lung (VC+)	↓I3C [80]
miR-382	Lung, brain	Gene transcription	Human lung cancer cells	↑SAHA [93]
			Mouse lung (CS+)	↑PEITC [82]
			Mouse lung (CS+)	↑Budesonide [82]
miR-409§	No data available	Intracellular trafficking	Human lung cancer cells	↑SAHA [93]
miR-424	Intestine, uterus	No data available	Human colon cancer cells	↑SAHA [90]
miR-452	Prostate, cervix, kidney, lung	Stress response, cell cycle arrest in response to DNA damage	Mouse liver	↑PEITC [82]
miR-463	Lung	Cell proliferation, protein repair, stress response	Mouse lung (CS+)	↑Budesonide [82]
			Mouse liver (CS+)	↑PEITC [82]
miR-466§	Lung, liver	Cell proliferation,, k-*Ras* activation, gene transcription	Mouse lung	↓PEITC [82]
			Mouse liver	↓PEITC [82]
			Mouse liver (CS+)	↑PEITC [82]
			Mouse lung	↑SAHA [UD]
			Mouse liver	↓Budesonide [82]
			Mouse liver (CS+)	↑Budesonide [82]
miR-467	Liver	Cell proliferation, protein synthesis	Mouse liver (CS+)	↑PEITC [82]
			Mouse liver (CS+)	↑Budesonide [82]
miR-470	Liver	k-*Ras* activation, intracellular vesicle trafficking, xenobiotic metabolism	Mouse liver (CS+)	↑PEITC [82]
miR-483	Liver, lung	Protein repair	Mouse liver	↑Budesonide [82]
miR-484	Lung, heart	Apoptosis, cell differentiation	Mouse lung (CS+)	↑Bexarotene + Pioglitazone [UD]
miR-489			Human breast cancer cells	#Tamoxifen [99]
miR-493	Lung	Cell differentiation, cell proliferation	Mouse lung (CS+)	↑Bexarotene [UD]
			Mouse lung (CS+)	↑Bexarotene + Pioglitazone [UD]
miR-509§	Testis, kidney, lung	Cell adhesion, k-*Ras* activation	Mouse lung	↑Myo-inositol [UD]

Table 1 (*Continued*)

MiRNA	Tissue specificity	Main regulated functions	Analyzed cells (*in vitro*) or species/organ (*in vivo*)	Modulating agent [Ref.]
miR-526§	Placenta, liver	Cell proliferation, apoptosis, inflammation	Human hepatocarcinoma cells Human hepatocarcinoma cells	↑Ellagitannin BJA3121 [52] ↓EGCG [53]
miR-532	Liver	Gene transcription, apoptosis	Human hepatocarcinoma cells	↑Anthocyanidin [54]
miR-539	Liver	Protein repair, intracellular trafficking	Mouse liver	↑Budesonide [82]
miR-543	Lung	Stress response, inflammation	Mouse lung	↓Myo-inositol [UD]
miR-544	Breast	P53	Human breast cancer cells	↑Trichostatin A [94]
miR-548§	No data available	Gene transcription	Human lung cancer cells	↓SAHA [93]
miR-551	Liver	DNA repair, inflammation, cell proliferation	Mouse liver Mouse liver	↓PEITC [82] ↓Budesonide [82]
miR-582	Prostate, bladder, kidney	Cell proliferation, apoptosis	Human lung cancer cells	↑Resveratrol [67]
miR-592	Intestine, lung	Cell adhesion, insulin growth factor, angiogenesis	Mouse lung	↑Myo-inositol [UD]
miR-622	Lung	Cell proliferation, k-*Ras* activation	Human bronchial cells	↑Resveratrol [66]
miR-638	Adipose tissue, intestine	Cell proliferation, apoptosis	Human lung cancer cells	↑Bostrycin [75]
miR-645	Breast	Gene transcription	Human breast cancer cells	↓Trichostatin A [94]
miR-657	Testes, brain, prostate, cervix, ovary, liver, kidney, lung	Gene transcription	Human breast cancer cells	↓Trichostatin A [94]
miR-660§	Kidney, lung	Protein repair	Human lung cancer cells	↑SAHA [93]
miR-663§	Prostate	TGF-beta	Human colon cancer cells Human leukemia cells	↑Resveratrol [63] ↑Retinoic acid [31]
miR-666	Lung	Protein repair, stress response	Mouse lung	↓PEITC [82]
miR-684	Lung	Signal transduction	Mouse lung	↓SAHA [UD]
miR-687	Liver	Tumor suppression by phosphatidylinositol catabolism, cell proliferation	Mouse liver (CS+) Mouse liver (CS+)	↑PEITC [82] ↑Budesonide [82]
miR-690	Lung	Cell proliferation, cell adhesion	Mouse liver (CS+)	↑Budesonide [82]
miR-697	Liver	Protein repair, intracellular trafficking, cell adhesion	Mouse liver (CS+) Mouse liver (CS+)	↑PEITC [82] ↑Budesonide [82]
miR-706	Lung	Intracellular trafficking, cell motility	Mouse lung	↓PEITC [82]
miR-708	Lung	Stress response, NFκB activation	Mouse lung	↓PEITC [82]
miR-709	Liver	Stress response, inflammation, lysosome activation	Mouse liver (CS+) Mouse liver (CS+)	↑PEITC [82] ↑Budesonide [82]
miR-710	Liver	Cell proliferation, collagen production, k-*Ras* activation	Mouse liver (CS+) Mouse liver (CS+)	↑PEITC [82] ↑Budesonide [82]
miR-715	Lung	No data available	Mouse lung (CS+)	↑Bexarotene + Pioglitazone [UD]
miR-719	Liver	Inflammation	Mouse liver (CS+) Mouse liver (CS+)	↑PEITC [82] ↑Budesonide [82]
miR-742	Liver	Protein repair, stress response	Mouse liver	↑Budesonide [82]
miR-758§	Lung	Cell proliferation, apoptosis	Human lung cancer cells	↑Resveratrol [67]
miR-763	Liver	Cell membrane integrity, peroxisome biogenesis, stress response	Mouse liver	↓Budesonide [82]
miR-764	Lung	Mitochondrial function	Mouse lung Mouse lung Mouse lung (CS+) Mouse lung (CS+)	↓Myo-inositol [UD] ↓SAHA [UD] ↑Pioglitazone [UD] ↑Bexarotene + Pioglitazone [UD]
miR-804	Liver, lung	Cell proliferation, collagen production, k-*Ras* activation	Mouse lung (CS+)	↑PEITC [83]
miR-874	Liver	Protein repair, intracellular vesicle trafficking, cell proliferation, *P53* dependent apoptosis, inflammation, stress response	Mouse liver (CS+)	↑PEITC [82]
miR-876	Lung	No data available	Mouse lung	↑Myo-inositol [UD]
miR-877	Lung	No data available	Human lung cancer cells Human colon cancer cells	↓SAHA [93] ↓SAHA [90]
miR-880	Lung	No data available	Mouse lung	↑Myo-inositol [UD]
miR-883	Liver	Tumor suppressing activity through phosphatidylinositol catabolism, protein synthesis, intracellular vesicle trafficking, protein repair, cell proliferation	Mouse liver (CS+) Mouse liver (CS+)	↑PEITC [82] ↑Budesonide [82]
miR-923	Lung	Cell proliferation, apoptosis	Human lung cancer cells	↑Bostrycin [75]
miR-936	No data available	No data available	Human lung cancer cells	↓SAHA [93]
miR-1224	No data available	No data available	Human hepatocarcinoma cells	↑Anthocyanidin [54]
miR-1296	Prostate, ovary	Cell cycle arrest	Human prostate cancer cells Human ovarian cancer cells	↑Genistein [74] ↑Genistein [73]
miR-1901	Lung	No data available	Mouse lung	↓Myo-inositol [UD]
miR-1953	No data available	No data available	Mouse lung	↑Myo-inositol [UD]

(CS+), mice or rats exposed to cigarette smoke; (AOM+), rats treated with azoxymethane; (VC+), mice treated with vinyl chloride. ↑ upregulation; ↓ downregulation; *Modulation of antitumor effects; • Anthocyanins accumulation in plant is regulated by *miR-156*; #the reported miRNA is responsible for resistance to SERMs. § miRNA undergoing single nucleotide polymorphisms in humans. UD, Izzotti et al., unpublished data.

studies from our laboratory analyzed in parallel miRNA expression in organs of rodents, either unexposed or exposed to CS, in order to evaluate modulation by the investigated agents both of baseline expression profiles and of CS-induced dysregulation. This approach allowed us to predict both safety and efficacy of test agents at the molecular level.

The last column in Table 1 reports, for each miRNA, the results obtained, the investigated agent, and the corresponding reference. The arrows indicate whether modulation of miRNA expression occurred either in the sense of upregulation (upward arrows) or downregulation (downward arrows). The meaning of other symbols is reported in the footnote to the table.

It should be noted that some authors of the reviewed papers did not report all modulated miRNAs but made a selection of those that were evaluated to be more relevant. In general, the selection was made according to three analytical criteria, including (a) a more than two-fold variation, accompanied by a statistically significant difference; (b) inclusion in the highest or lowest quartile of distribution; and (c) confirmation of data by biological or functional analyses.

2. Modulation of miRNA expression by natural agents

2.1. Vitamins and derivatives

2.1.1. Vitamin A metabolites (retinoic acid)

All-*trans*-retinoic acid (RA) is a metabolite of vitamin A (all-*trans*-retinol) responsible for most of its biological effects. RA was tested *in vitro* for the ability to modulate miRNA expression in a variety of human cultured cancer cells, including acute promyelocytic leukemia (APL) cells, estrogen receptor-positive breast cancer (MCF-7) cells, embryonal carcinoma (NT2) cells, and neuroblastoma cells.

In APL cells, RA upregulated the expression of *miR-186*, *miR-215* and *miR-223*, while it downregulated the expression of *miR-17*, *miR-25*, *miR-193*, *miR-195* [29]. In another study using the same cells, RA was found to upregulate the expression of *miR-15a*, *miR-15b*, *miR-16-1*, *let-7a-3*, *let-7c*, *let-7d*, *miR-107*, *miR-223*, and *miR-342*, whereas *miR-181b* was downregulated [30]. Differentiation of APL cells by RA was reported to be mediated by miRNA modulation, mainly involving *miR-663* upregulation [31].

Proliferation of MCF-7 cells was inhibited by RA *via miR-21* upregulation [32]. *MiR-23* was shown to play a critical role in the RA-induced neuronal differentiation of NT2 cells into neural cells [33]. In another study, differentiation of these cells was induced by RA following *miR-152* upregulation [34]. In addition, RA downregulated *miR-17*, which in turn activated the expression of genes involved in neuroblastoma cell differentiation and apoptosis [35], and upregulated *miR-10a* and *miR-10b*, targeting the SR-family splicing factor SFRS1 [36].

RA downregulated the expression of *miR-9*, *miR-124*, and *miR-125b* in the central nervous system of rat fetuses, thereby increasing Bcl2- and P53-related apoptosis and inducing an abnormal development of spinal cord [37].

2.1.2. Vitamin B9 (folate)

In male Fisher rats, a diet deficient in folate, methionine and choline resulted in the formation of hepatocellular carcinoma at 54 weeks of age, in the absence of carcinogen treatment. This process was accompanied by *miR-122* downregulation. Folate replenishment increased *miR-122* levels and was associated with inhibition of liver tumorigenesis [38,39].

Folic acid blocked ethanol-induced teratogenesis in fetal mouse brain through *miR-10a* downregulation [40].

Utilizing blood samples from a population-based case-control study of head and neck squamous cell carcinoma, *miR-222* was identified as being overexpressed in lymphoblastoid cells in culture obtained from subjects with a low folate intake. Folate supplementation in the culture medium restored miRNA levels, which suggests that dietary modulation of miRNA expression is reversible [41].

2.1.3. Vitamin D (calciferol) and derivatives

Vitamin D was found to modulate *in vitro* the expression of miRNAs in cultured human cancer cells. In particular, vitamin D3 downregulated *miR-181* resulting in cell cycle arrest of human myeloid leukemia cells [42]. 1,25-Dihydroxyvitamin D3 markedly induced the expression of *miR-32* in the same cells, leading to Bim targeting and inhibition of AraC-induced apoptosis [43].

In breast epithelial cells (MCF-12F), 2,5-hydroxyvitamin D3, a major vitamin D metabolite, conferred a protective role against cellular stress by modulating P53 and PCNA levels and dysregulating the expression of several miRNAs, among which *miR-182* [44].

The cancer chemopreventive effects of vitamin D are mediated *via* binding with its receptor, whose expression is linked to *miR-125b* [45]. Indeed, malignant melanoma cells expressing the vitamin D receptor respond to the antiproliferative effects of vitamin D3. Endogenous vitamin D receptor-mRNA levels are inversely related with the expression of *miR-125b*, which is involved in the resistance against vitamin D3 antiproliferative effects in melanoma cells [46].

2.1.4. Vitamin E (tocopherol)

In Fisher 344 rats fed for 6 months diets deficient or sufficient in α-tocopherol, the major congenerer of vitamin E, vitamin E-deficiency resulted in reduced liver concentrations of *miR-122a*, which is involved in lipid metabolism, and *miR-125b*, which is involved in inflammation [47]. A review paper published by the same group reported that α-tocopherol downregulates the same miRNAs [48].

2.2. Oligoelements

Sodium selenite activated the P53 pathway and the related miRNA effector *miR-34* in prostate cells. In fact, incubation of $P53^{+/+}$ human prostate cancer cells with selenium triggered induction of *miR-34*, which was associated with a rapid transcriptional activation of *P53* and upregulation of the expression of P53-targeted genes [49].

2.3. Polyphenols

2.3.1. Flavonol-rich extracts

Polyphenolics extracted from *Ilex vomitoria* (yaupon holly) leaves, whose main components are quercetin and kaempferol 3-rutinosides, upregulated *miR-146a*, a negative regulator of proinflammatory NFκB, in human colon fibroblasts (CCD-18Co), and protected these cells from inflammation [50].

2.3.2. Ellagitannins

In human hepatocellular carcinoma HepG2 cells, 1,3,4-tri-O-galloyl-6-O-caffeoyl-β-D-glucopyranose (BJA32515), a natural ellagitannin compound extracted from *Balanophora japonica* Makino, upregulated *let-7a* and *miR-29a* and downregulated *miR-197* and *miR-373*. These miRNA modifications resulted in inhibited cell proliferation and increased apoptosis [51]. In the same cells, 1,3-di-O-galloyl-4,6-(s)-HHDP-b-D-glucopyranose (BJA3121) dysregulated the expression of 25 miRNAs, and in particular upregulated *let-7e*, *miR-370*, *miR-373*, and *miR-526*, thus inhibiting cell proliferation [52].

2.3.3. Epigallocatechin 3-gallate and other green tea polyphenols

HepG2 cells, epigallocatechin 3-gallate (EGCG) treatment altered the expression levels of a total of 61 miRNAs, 13 of which were upregulated and 48 were downregulated. Among them, *miR-16*, which was confirmed to target and to inhibit the antiapoptotic protein Bcl-2, was one of the upregulated miRNAs. This mechanism explains the proapoptotic effect exerted by EGCG. Other miRNAs changed their expression more than 2-fold as a consequence of EGCG treatment, including *let-7c*, *miR-18*, *miR-25*, and *miR-92* (all of them upregulated), and *miR-129*, *miR-196*, *miR-200*, *miR-342*, and *miR-526* (all of them downregulated) [53]. In another study using the same cells treated with EGCG, *miR-30b* was found to be downregulated [54].

In human breast cancer MCF-7 cells, 23 miRNAs were differentially expressed after treatment with polyphenon-60, a green tea extract. These miRNAs included *miR-21* and *miR-27*, which were found to be downregulated. These two miRNAs had previously been identified as being overexpressed in these cells, with *miR-21* specifically implicated in the downregulation of the tumor suppressor gene tropomyosin-1 [55].

In mouse and human lung cancer cells in culture, EGCG specifically upregulated the expression of *miR-210*, a major miRNA modulating the hypoxya-inducible factor 1α (HIF-1α) pathway. The EGCG-induced upregulation of *miR-210* stabilized HIF-1α by inhibiting its ubiquitination and subsequent proteasome degradation in lung cancer cell lines, thus leading to reduced cell proliferation rate and anchorage-independent growth [56].

2.3.4. Curcumin and analogues

Curcumin (diferuloylmethane) is a flavonoid derived from the rhizome of *Curcuma longa*. *In vitro*, curcumin altered miRNA expression in human pancreatic cancer cells (PxBC-3) by upregulating *miR-22*, whose predicted targets were estrogen receptor 1 and transcription factor Sp1. On the other hand, *miR-196*, an oncogenic miRNA involved in gastric cancer, was significantly downregulated after curcumin treatment [57].

In human breast cancer cells (MCF-7), curcumin reduced the expression of Bcl-2 by upregulating *miR-15a* and *miR-15b* [58].

In addition, alterations in miRNA expression were detected in curcumin-treated lung cancer A549 cells, including a significant downregulation of *miR-186*, whose targets include caspase-10. These results demonstrate that curcumin induces A549 cell apoptosis through a miRNA-mediated pathway [59].

In colorectal cancer cells (Rko and HCT116), curcumin inhibited the transcriptional regulation of *miR-21 via* AP-1 and suppressed cell proliferation, tumor growth, invasion and *in vivo* metastasis, and stabilized the expression of the tumor suppressor gene *Pdcd4* in colorectal cancer cells tested in the chorioallantonic membrane invasion assay [60].

Due to the low bioavailability of curcumin *in vivo*, the synthetic analogue difluorinated curcumin (CDF) was evaluated in the pancreatic cancer cells AsPC-1 and MIAPaCa-2. Curcumin and its CDF analogue, either alone or in combination, attenuated the expression of *miR-200* and *miR-21*, leading to induction of the tumor suppressor gene *phosphatase and tensin homolog* (*PTEN*), which negatively regulates the intracellular levels of phosphatidylinositol-3,4,5-trisphosphate thereby preventing cells from growing and dividing too rapidly. In the same cell lines, CDF attenuated cancer stem cell markers *via* changes in *miR-21* and *miR-200* [61].

2.3.5. Resveratrol and analogues

A series of *in vitro* studies were carried out with resveratrol, a stilbenoid that is found in the skin of red grapes and other fruits, and its analogues. In colon cancer cells (SW480), resveratrol downregulated several oncogenic miRNAs, including *miR-17*, *miR-21*, *miR-25*, *miR-92a*, *miR-196a*, thereby mediating the regulation of *Dicer*, *PDCD4*, and *PTEN*. Resveratrol upregulated *miR-663*, a tumor suppressor miRNA inhibiting TGFβ [62]. In monocytic cells, resveratrol induced a *miR-663*-dependent effect targeting activator protein-1 (AP-1) through the *Jun* signaling pathway. Interestingly, resveratrol also impaired the upregulation of oncogenic *miR-155* in a *miR-663*-dependent manner [63]. On the whole, *miR-21*, *miR-155* and *miR-663* were recognized as the main miRNAs regulated by resveratrol [64].

Treatment of human neural cells with the resveratrol analogue CAY10512 downregulated *miR-146a*, whose targets include complement factor H. *MiR-146* is upregulated in the brain of Alzheimer's disease patients causing repression of complement factor H, a potent anti-inflammatory mediator [65]. These findings provide evidence that miRNA regulation plays a major role in the antiinflammatory effects of resveratrol.

In human benzo[*a*]pyrene-transformed bronchial epithelial cells (16HBE-T), resveratrol upregulated *miR-622*, recognizing k-*Ras* as a target. *MiR-622* upregulation inhibited cell proliferation, inducing G0 growth arrest and suppressing the ability of 16HBE-T cells to form colonies *in vitro* and to develop tumors in nude mice. k-*Ras* messenger RNA was predicted as a putative *miR-622* target [66].

Resveratrol treatment altered miRNA expression in human non-small cell lung cancer cells (A549), with 26 of the 753 analyzed miRNAs that exhibited greater than 2-fold expression changes in resveratrol-treated cells relative to their expression levels in untreated cells. Six of the resveratrol-modulated miRNAs showed greater than 20-fold changes in expression. These included *miR-92a* (downregulated) and *miR-194*, *miR-299*, *miR-338, miR-582*, and *miR-758* (all of them upregulated). Target genes of resveratrol-regulated miRNAs are related to apoptosis, cell cycle regulation, cell proliferation, and differentiation [67].

In prostate cancer cells (PCa), resveratrol downregulated 23 miRNAs and upregulated 28 miRNAs. The downregulated miRNAs included *miR-17-92* and *miR-106ab* clusters, having well recognized oncogenic properties, while the upregulated miRNAs included several tumor suppressors, some of them targeting *PTEN* [68].

In an *in vivo* ischemia/reperfusion rat model, resveratrol pretreatment restored the expression pattern of miRNAs close to the control levels in the ischemic heart. The upregulated miRNAs included *miR-20b* and *miR-21* (antiangiogenic), which are implicated in cardiac remodeling. These data suggest that resveratrol exerts a significant cardioprotection through miRNA modulation [69,70].

2.4. Isoflavones

2.4.1. Genistein

Genistein is an isoflavone isolated from soybean. *In vitro*, genistein downregulated the expression of *miR-27a* and inhibited cell growth of human uveal melanoma cells (C918). The growth of these cells *in vivo* was significantly inhibited by genistein administration to Balb/C nu/nu mice carrying xenografts of uveal melanoma cancer cells [71].

Genistein upregulated *miR-146a* in human pancreatic cancer cells and inhibited their invasive potential by downregulating EGFR, NFκB, IRAK-1, and MTA-2 [72].

In ovarian cancer cells (UL-3A, UL-313), genistein modulated 53 miRNAs, which resulted in the induction of estrogen receptor expression and cell growth rate decrease [73].

In human prostate cancer cells (PC3) treated with genistein, the minichromosome maintenance gene *MCM2*, involved in DNA replication and commonly dysregulated in cancer cells, was downregulated through *miR-1296* modulation. Genistein induced

the expression of *miR-1296* by up to five-fold, along with cell cycle arrest in S-phase [74]. In the same cells, genistein upregulated the tumor suppressor gene ARHI by downregulating *miR-221* and *222* [75]. In human prostate cancer cell lines (PC-3, DU145, and LNCaP), genistein variously modulated miRNA expression profile [76].

2.4.2. Anthocyanin

In *Arabidopsis thaliana*, anthocyanin accumulation is under the regulation of *miR156*. At least one of the *miR-156* targets, the squamosa promoter binding protein-like-9, negatively regulates anthocyanin accumulation by directly preventing expression of anthocyanin biosynthetic genes [77]. These results provide a potential target for manipulation of anthocyanin content in plants.

In hepatoma HepG2 cells, grape seed proanthocyanidin or cocoa proanthocyanidin extracts downregulated *miR-30b* and upregulated *miR-197*, *miR-532*, and *miR-1224* [54].

2.5. Indoles

Several studies investigated the miRNA modulating activity of indole-3-carbinol (I3C), found in cruciferous vegetables, and its *in vivo* dimeric product 3,3′-diindolylmethane (DIM).

In human pancreatic cancer cell lines (MiaPaCa-2, Panc-1 and Aspc-1), resistant to gemcitabine, DIM and a mixture of other genistein isoflavones downregulated the expression of *let-7b*, *let-7c*, *let-7d*, *let-7e*, *miR-200b*, and *miR-200c*, thereby reversing in part the malignant phenotype and inhibiting cancer cell growth [78].

The effect DIM on miRNA expression was investigated in both estrogen-dependent MCF-7 and estrogen receptor negative, *P53* mutant human breast cancer MDA-MB-468 cells. DIM dose dependently inhibited the proliferation of both cells. In addition, an *in vivo* xenograft model showed that DIM strongly inhibited the development of human breast tumors. DIM increased *miR-21* expression causing a downregulation of Cdc25A, which resulted in inhibition of breast cancer cell proliferation. Thus, DIM was able to stop the cell cycle progression of human breast cancer cells regardless of their estrogen-dependence and *P53* status [79].

I3C and DIM reversed VC-induced dysregulation of several miRNAs in the lung of female A/J mice. *MiR-21*, *miR-31*, *miR-130a*, *miR-146b*, and *miR-377* were consistently upregulated, while *miR-1* and *miR-143* were downregulated in lung cancer as compared to normal lungs. Moreover, the upregulation of *miR-21*, *miR-31*, *miR-130a*, *miR-146b*, and *miR-377* observed in VC-treated animals was abrogated by I3C treatment, suggesting that I3C could inhibit the expression of these oncogenic miRNAs. PTEN, PDCD4, and RECK were potential targets of *miR-21*, and I3C upregulated these tumor suppressor genes though inhibition of *miR-21* [80].

In CS-exposed rats, I3C restored in lung the expression of downregulated miRNAs targeting P53 functions (*miR-34b*), TGF-β expression (*miR-26a*), ERBB2 activation (*miR-125a*), and angiogenesis (*miR-10a*) [81].

2.6. Isothiocyanates

Phenethyl isothiocyanate (PEITC), a naturally occurring phytochemical, was evaluated for the ability to modulate the expression of miRNAs, after administration with the diet, in the lung and liver of rodents, either unexposed or exposed to CS.

In the lung of CS-free mice, PEITC decreased the expression of *miR-181*, *miR-466a*, *miR-666*, *miR-706* (2.1-fold), and *miR-708*. In addition, PEITC was effective in counteracting miRNA alterations induced by CS for *let-7a*, *let-7c*, *miR-26*, *miR-29*, *miR-31*, *miR-125*, *miR-135*, *miR-200*, and *miR-382* [82].

In the liver of the same mice, PEITC decreased the expression of miR-26a, *miR-125a*, *miR-142*, *miR-200*, *miR-323*, *miR-331*, *miR-338*, *miR-466*, *miR-551*, and increased the expression of *miR-34c*, *miR-299*, *miR-452*. In addition, PEITC was effective in counteracting miRNA alterations induced by CS in mouse liver for *miR-125b*, *miR-153*, *miR-292*, *miR-297*, *miR-322*, *miR-376b*, *miR-463*, *miR-466*, *miR-467*, *miR-470*, *miR-687*, *miR-697*, *miR-709*, *miR-710*, *miR-719*, *miR-874*, and *miR-883* [82].

In a separate study PEITC was able to counteract miRNA alterations induced by CS in mouse lung tissue, either normal or affected by pneumonia, but not in lung cancer tissue. The protected miRNAs were *let-7a*, *miR-30*, and *miR-804* [83].

The effect of PEITC on miRNA alterations induced by CS in rat lung was evaluated by Izzotti et al. [81]. Of the five dietary agents tested, PEITC was the most effective in restoring CS-downregulated miRNAs. Major PEITC-induced miRNA targets were *let-7a*, *let-7c*, *miR-26a*, *miR-99b*, *miR-123*, *miR-125b*, *miR-146*, *miR-192*, and *miR-222*.

2.7. Combination of phenethyl isothiocyanate and indole-3-carbinol

Modulation of miRNAs by PEITC was increased by its combination with I3C. In fact, in the lung of CS-exposed rats treated with both PEITC and I3C, in addition to the miRNAs that were individually modulated by each compound, *let-7b*, *let-7f*, *miR-30*, *miR-124*, *miR-140*, *miR-145*, *miR-191*, and *miR-223* were significantly upregulated [81].

2.8. Phospholipids

Myo-inositol is widely occurring in nature and food, and is present in all living cells. Dietary myo-inositol significantly upregulated *miR-302*, *miR-509*, *miR-592*, *miR-876*, *miR-880*, and *miR-1953*, and downregulated *miR-543*, *miR-764*, and *miR-1901* in mouse lung. A parallel effect on proteins targeted by these miRNAs was observed for cyclin-dependent kinase inhibitor 1A involved in the cell cycle (*miR-302*), Rho GTPase activating protein 1 involved in signal transduction (*miR-509*), epidermal growth factor receptor pathway substrate 8 involved in cell differentiation (*miR-543*), nuclear receptor subfamily 3 involved in inflammatory responses, cellular proliferation and differentiation (*miR-543*), insulin receptor involved in metabolic functions (*miR-592*), and serine/threonine kinase 24 involved in stress response (*miR-880*) [Izzotti et al., unpublished data].

2.9. Saponins

The triterpene saponin ginsenoside Rh2, extracted from the traditional medical plant ginseng, upregulated 14 miRNAs and downregulated 12 miRNAs in human glioma cells (U251, T98MG and A172). In particular, upregulation of *miR-128* appears to be responsible for the antiproliferative effects of Rh2 in glioma cells [84].

2.10. Anthraquinones

The anthracenedione bostrycin, belonging to the large family of anthraquinones and isolated from marine fungi, upregulated *miR-638* and *miR-923* in human lung adenocarcinoma cells (A549). This effect resulted in downregulation of the PI3K/AKT pathway, thus playing a role in induction of cell cycle arrest and apoptosis in bostrycin-treated cells [85].

2.11. Polyunsaturated fatty acids

Polyunsaturated fatty acids (PUFA) contained in fish oil, administered to AOM-treated rats, inhibited colon cancer appearance and progression by increasing *let-7d*, *mir-15*, *miR-107*, *miR-191*, and *miR-324* expression [86].

Sodium butyrate, a short chain fatty acid inhibiting histone deacetylase, contained in goat and buffalo cheese, regulated endodermal differentiation by upregulating *miR-10* and *miR-24* in cultured human embryonic stem cells [87].

In human colon cancer cells butyrate inhibited cancer cell proliferation by inhibiting *miR-106* thus inducing *P21* expression [88].

3. Modulation of miRNA expression by synthetic agents

3.1. Metabolic inducers (beta-naphthoflavone)

Beta-naphthoflavone, or 5,6-benzoflavone, is a synthetic flavonoid that acts as a potent inducer of P4501A enzyme and agonist of the arylhydrocarbon receptor. This agent significantly fully counteracted CS-induced *miR-10a* downregulation in rat lung by increasing its expression up to 5.2-fold and restoring the same expression level detected in sham-exposed rats. In the same experimental model, beta-naphthoflavone increased the expression of *miR-26a*, *miR-34c*, and *miR-146* in CS-exposed rats as compared with rats exposed to CS, in the absence of chemopreventive agents. However, their expression was still lower than that detected in sham-exposed rats [81].

3.2. Thiols and derivatives

3.2.1. N-Acetyl-L-cysteine

The thiol *N*-acetyl-L-cysteine (NAC), an analogue and precursor of reduced glutathione (GSH), given with the drinking water, counteracted the CS-induced downregulation of *miR-26a*, *miR-123*, *miR-140*, and *miR-146* in rat lung [81].

3.2.2. Oltipraz

The dithiolthione oltipraz significantly counteracted CS-induced downregulation of miRNAs by increasing the expression of *let-7b*, *let-7c*, *miR-10*, *miR-26*, *miR-34*, and *miR-123* in rat lung [81].

3.2.3. Combination of N-acetyl-L-cysteine and oltipraz

The effect of oltipraz on miRNA expression was increased by its combination with NAC. In fact, in addition to the miRNAs modulated by the individual agents, oltipraz + NAC upregulated *let-7a*, *miR-192*, and *miR-222* in the lung of CS-exposed rats [81].

3.3. Nuclear receptor agonists

3.3.1. Bexarotene

Bexarotene, also known as Targretin, is a retinoid X receptor (RXR) agonist. In mouse lung, bexarotene alone did not significantly alter miRNA expression profiles. In the same organ, bexarotene was effective in counteracting the CS-induced downregulation of pulmonary *miR-493*. In parallel, antibody microarray analyses indicated that two *miR-493*-targeted proteins were modulated by bexarotene, including keratin pan, involved in cell differentiation, and cell division cyclin 27, involved in the cell cycle [Izzotti et al., unpublished data].

3.3.2. Pioglitazone

Pioglitazone is a peroxisome proliferator-activated receptor (PPAR)-gamma agonist. *In vivo* pioglitazone protected the rats against myocardial ischemia–reperfusion injury by downregulating *miR-29a* and *miR-29c* levels in the heart. The same finding was obtained *in vitro* in cardiac H9c2 cells. Downregulation of *miR-29* by pioglitazone protected H9c2 cells from simulated ischemia–reperfusion injury, as indicated by an increased cell survival and decreased caspase-3 activity. In contrast, overexpressing *miR-29* promoted apoptosis and completely blocked the protective effect of pioglitazone. Antagomirs against *miR-29a* or *miR-29c* significantly reduced myocardial infarct size and apoptosis in hearts subjected to ischemia–reperfusion injury. Western blot analyses demonstrated that Mcl-2, an anti-apoptotic Bcl-2 family member, was increased by *miR-29* inhibition [89].

Dietary pioglitazone did not significantly affect lung miRNA profiles in mouse lung. However, this agent was effective in counteracting CS-induced alterations for *miR-218*, *miR-296*, *miR-335*, and *miR-764*. Parallel antibody microarray analyses indicated that lung proteins targeted by pioglitazone-modulated miRNAs were also modified in their expression. They included thioredoxin and Ras proteins, targets for *miR-218*; insulin-like growth factor, target for *miR-335*; and peroxisomal D3,D2-enoyl-CoA isomerase, target for *miR-764* [Izzotti et al., unpublished data].

3.3.3. Combination of bexarotene and pioglitazone

Combination of bexarotene and pioglitazone effectively protected the mouse lung from CS-induced miRNA downregulation. In fact, the number of downregulated miRNAs in CS-exposed mice was 79, out of a total of 694 tested, whereas one miRNA only was downregulated in CS-exposed mice treated with bexarotene + pioglitazone. The most potently upregulated miRNAs by this combination of chemopreventive agents were *miR-290*, *miR-484*, and *miR-715* [Izzotti et al., unpublished data].

3.4. Histone deacetylase inhibitors

3.4.1. Suberoylanilide hydroxamic acid (SAHA)

The histone deacetylase inhibitor (HDACi) suberoylanilide hydroxamic acid (SAHA), or Vorinostat, markedly altered the expression of 31 miRNAs in human colon carcinoma cells (HCT116) as well as downstream targets affecting cell cycle, apoptosis, and differentiation. In particular, SAHA downregulated *let-7l*, *miR-29*, *miR-100*, *miR-141*, *miR-221*, *miR-222*, and *miR-877*, while it upregulated *miR-22* and *miR-424* [90].

In human lymphoma cells (L540), SAHA downregulated the c-*Myc*-related miRNAs *miR-17-3p*, *miR-17-5*, and *miR-18* as well as *miR-15b and miR-155*, which are not c-*Myc*-regulated [91].

SAHA was also found to restore the expression of *miR-34a* in human pancreatic cancer stem cells, which provides mechanistic insights and therapeutic targets for pancreatic cancers [92].

In human non-small cell lung cancer cells (A549), SAHA dysregulated the expression of 64 miRNAs having several target genes related to angiogenesis, apoptosis, chromatin modification, cell proliferation and differentiation. The miRNAs that were upregulated more than 4-fold included *let-7b*, *miR-124*, *miR-126*, *miR-129-3p*, *miR-132*, *miR-382*, *miR-409-3p*, and *miR-660*. The miRNAs that were downregulated more than 4-fold included *miR-30c-1*, *miR-30e*, *miR-106a*, *miR-345*, *miR-548c-3p*, *miR-877*, and *miR-936* [93].

In vivo, the dietary administration of SAHA to mice modulated miRNA expression in the lung. In particular, SAHA upregulated *let-7a, miR-125b*, *miR-183*, and *miR-466*, and downregulated *miR-302*, *miR-684*, and *miR-764* [Izzotti et al., unpublished data]. Interestingly, *let-7* and *miR-183* had also been found to be upregulated by SAHA in lung cancer cells *in vitro* [93]. Parallel analyses detected the modulation by SAHA of miRNA target proteins in mouse lung, including cyclin-dependent kinase inhibitor 1A (P21), a P53-dependent negative regulator of the cell cycle targeted by *miR-302* and *miR-106*; integrin beta-1, regulating cell adhesion, targeted by *miR-183*; protein phosphatase 2, implicated in the negative control of cell growth and division, targeted by *miR-183*; importin beta-1, playing a role in signal transduction, targeted by *miR-684*; histone deacetylase 3, regulating epigenetic repression, targeted by *miR-125* and *miR-466* [Izzotti et al., unpublished data].

3.4.2. Trichostatin

The HDACi trichostatin A altered the expression profile of miRNA signatures in the apoptosis-resistant breast carcinoma cell line (MCF-7TN-R). Trichostatin A induced significant upregulation of 22 miRNAs and downregulation of 10 miRNAs. Among them, the most remarkably upregulated miRNAs were *miR-139*, *miR-146*, *miR-155*, *miR-215*, and *miR-544*, and the most remarkably downregulated miRNAs were *miR-645* and *miR-657*. These results demonstrate that the anticancer activity of trichostatin A is correlated with alteration of miRNA expression profiles [94].

3.4.3. LAQ824

The HDACi LAQ824 produced a dramatic alteration in miRNA profiles in human breast cancer cells (SKBr3), 22 miRNAs being upregulated and 5 miRNAs being downregulated [95].

3.5. Anti-inflammatory agents

3.5.1. Glucocorticoids

The modulation of miRNAs by the glucocorticoid budesonide, given with the diet, was examined in the lung and liver either in CS-free mice or CS-exposed mice. In mouse lung, budesonide alone decreased the expression of one miRNA only (*miR-133*), while it exerted a more remarkable effect in the liver by downregulating 14 miRNAs and upregulating 3 miRNAs. The downregulated miRNAs included *let-7a*, *miR-27a*, *miR-100*, *miR-106b*, *miR-125a*, *miR-142*, *miR-200b*, *miR-300*, *miR-320*, *miR-331*, *miR-338*, *miR-466a*, *miR-551*, and *miR-763*. The upregulated miRNAs included *miR-483*, *miR-539*, *miR-742*. In the lung of CS-exposed mice, budesonide was effective in counteracting CS-related miRNA alterations for 5 miRNAs, including *let-7a*, *miR-26a*, *miR-31*, *miR-382*, and *miR-463*. In the liver of the same mice, budesonide was effective in counteracting CS-related miRNA alterations induced by CS for 15 miRNAs, including *miR-15a*, *miR-21*, *miR-125b*, *miR-292*, *miR-297*, *miR-322*, *miR-466*, *miR-467*, *miR-687*, *miR-690*, *miR-697*, *miR-709*, *miR-710*, *miR-719*, and *miR-883* [82].

In humans, the expression of 227 miRNAs was examined in airway biopsies obtained from normal and mild asthmatic patients. MiRNA profiles were analyzed before and after 1 month of treatment with inhaled budesonide. No significant difference was detected in the expression of all 227 analyzed miRNAs, irrespective of treatment with budesonide. These results suggest that changes in miRNA lung expression are not involved in the anti-inflammatory action of the corticosteroid budesonide in asthmatic patients [96].

3.5.2. Nonsteroidal antiinflammatory drugs (NSAIDs)

Ethyl-2-((2,3-bis(nitroxy)propyl)disulfanyl benzoate (GT-094) is a nitric oxide donor NSAID (NO-NSAID) that is expected to undergo rapid thiol/disulfide exchange with protein sulfhydryl groups leading to NSAID (thiosalycylate) release. Using human colon cancer cells (RK0 and SW480), GT-094 was found to downregulate *miR-27a*, which in part may be responsible for the anticancer activity of this agent [97].

3.6. Selective estrogen receptor modulators (SERMs)

3.6.1. Tamoxifen

Mir-221 and *miR-222* have consistently been implicated in the resistance to tamoxifen in breast cancer. These two miRNAs were found to be elevated in estrogen receptor alfa (ERα)-negative breast cancer cells as compared to ERα-positive cells, which suggest a role in the regulation of ERα expression [98]. Using breast cancer cells (MCF-7), either sensitive or resistant to tamoxifen, *mir-221, miR-222* and *miR-181* were found to have an increased expression in tamoxifen resistant cells, whereas *miR-21*, *miR-342* and *miR-489* had a decreased expression. In addition, this study demonstrated a relationship between *mir-221* and *miR-222* expression and HER2/neu oncoprotein overexpression in primary breast cancer cells [99]. Another study suggested that *miR-342* regulates tamoxifen response in breast cancer cell lines *in vitro*, and clinical data indicated a relationship between reduced *miR-342* expression and tamoxifen resistance [100].

3.6.2. Fulvestrant

An increased expression of *mir-221* and *miR-222* was found to play a role also in acquired resistance toward fulvestrant, a SERM antagonist used in hormone-sensitive breast cancers following failure of previous tamoxifen or aromatase inhibitor therapies [101].

4. Discussion

4.1. MiRNAs as targets for chemopreventive agents

The results reported in Table 1 provide evidence that many chemopreventive agents, belonging to various chemical classes and functional families, are able to modulate the expression of miRNAs in experimental test systems, either *in vitro* or *in vivo*. A total of as many as 148 miRNAs were found to be modulated in the studies reported in Table 1. Interestingly, several miRNAs were targeted by multiple chemopreventive agents. In particular, 7 of the miRNAs investigated in the cited studies, including *let-7a*, *miR-21*, *miR-26*, *miR-34*, *miR-125*, *miR-146*, and *miR-200*, were targeted by at least 5 chemopreventive agents each. These miRNAs play important roles in controlling several mechanisms that are involved in various stages of the carcinogenesis process, such as inflammation, stress response, cell proliferation, apoptosis, oncogene activation (k-*Ras*, *TGF*, *ERBB2*), modulation of oncosoppressor genes (*PTEN*, *P53*), and signal transduction pathways. It is conceivable that certain miRNAs may represent preferential targets for chemopreventives and may conveniently be used as indicators of efficacy of anticancer agents. Furthermore, it may be hypothesized that, in the future, miRNA themselves or anti-miRNA oligonucleotides may be used to suppress cancer development.

Opposite expressional directions of miRNA modulation by the same chemopreventive agents were reported in few cases, for instance with α-tocopherol, budesonide, and PEITC (see Table 1). In some cases, these findings may be related to noise in the omics studies. However, in other cases they may be due to the fact that the same miRNA was tested in different organs, which involves different pharmacokinetic and metabolic patterns. As an example, the bifunctional metabolic inducer PEITC exerted different effects in liver and lung [82].

4.2. Analysis of miRNAs for evaluating the safety and efficacy of chemopreventive agents

The majority of the studies reported in Table 1 used cancer cell lines *in vitro* with the goal of evaluating the anticancer effects of test agents and other properties, such as occurrence of resistance to drugs, through modulation of miRNA expression. The main drawback of this methodology is that cancer cells are less sensitive than differentiated cells to miRNA modulation by chemopreventive agents, as assessed by comparing lung cancer tissue and the surrounding healthy tissue in mice treated with either PEITC or NAC [83].

In vivo approaches not only take into account pharmacokinetic and metabolic features of test compounds but also appear to be more appropriate to evaluate genuine cancer preventive effects of dietary and pharmacological agents. In our opinion, such a goal should be pursued by evaluating both the ability of test agents to

affect baseline miRNA expression profiles, as an indicator of their safety, and their ability to inhibit miRNA alterations caused by carcinogens, as an indicator of their efficacy. Table 1 reports a number of examples of application of this kind of protocol in mice and rats, either unexposed or exposed to CS [81–83, plus unpublished data].

4.3. Rationale for designing combinations of chemopreventive agents by miRNA analysis

The therapy of the most important diseases (*e.g.*, cancer, cardiovascular diseases, AIDS, *etc.*) involves the combination of different drugs. Likewise, application of a "combined chemoprevention" strategy is particularly promising. However, it is difficult to evaluate how the combined agents may interact in terms both of safety and efficacy. The rationale for designing a proper combination is to use agents having different and possibly complementary mechanisms of action. Most chemopreventive agents have pleiotropic properties and work *via* multiple mechanisms [18–20]. Since each miRNA targets a number of transcripts simultaneously, evaluation of miRNA expression provides a convenient tool for assessing the outcome of combinations of different agents at the molecular level.

Three combinations are reported in Table 1, including pioglitazone + bexarotene, NAC + oltipraz, and PEITC + I3C. Pioglitazone is a synthetic ligand of peroxisome proliferator-activator receptor-gamma (PPAR-gamma) and bexarotene is a synthetic agonist of retinoid X receptor (RXR), which is an obligate heterodimeric partner for other nuclear receptors, including PPAR [102]. NAC is characterized by a variety of protective properties but its primary mechanisms are nucleophilicity and antioxidant activity as a scavenger of ROS [103], whereas a major mechanism for oltipraz is the induction of electrophile detoxification enzymes [104]. PEITC is a typical phase II activity inducer [105], whereas I3C has a broad spectrum of anticancer properties, including its ability to interfere with multiple oncogenic signaling pathways that govern cell cycle progression, survival, invasion, and other aggressive phenotypes of cancer cells [106]. In addition, the data reported in Table 1 may be useful to design novel combinations of chemopreventive agents. For instance, SAHA inhibit the expression of *miR-221* and *miR-222*, which are consistently associated with resistance to SERMs. Accordingly, a combination of SAHA and SERMs could be proposed to prevent resistance to SERMs.

4.4. Mechanisms of miRNA modulation by chemopreventive agents

For genotoxic agents, such as ionizing radiation, alteration of miRNA expression has been ascribed to the fact that P53 interacts with the Drosha/DGCR8 processing complex through an association with RNA helicase p68, which modulates the processing of pri-miRNAs to pre-miRNAs [9]. According to these data, DNA damage modulates miRNA expression *via* a P53-dependent mechanism [107]. The number of components of the miRNA processing machinery serving as direct transcriptional targets for *P53* in response to DNA damage has been expanding by also including the endoribonuclease Dicer [108]. The central role of Dicer in the cellular response to UV induced damage is established [10]. However, in case of treatment with chemopreventive agents, it is unlikely that miRNA expression is modulated through DNA damage and *P53* activation. Recent bioinformatic analyses indicate that Dicer, the enzyme involved in the cytoplasmic phase of miRNA maturation, is a preferential cytoplasmic target for mutagens. In particular, the binding affinity of 25 mutagens for each Dicer's RNase III domain was estimated by calculating the global contact-energy and the number of intermolecular contacts. The mutagens tested form stable complexes with Dicer, which are more stable than those formed by Dicer with its natural substrate, *i.e.*, pre-miRNAs [109]. These data indicate that mutagens affect miRNA maturation by competing with pre-miRNA for Dicer binding. This is a short-term adaptive response of the cell to mutagen exposure resulting in maturation blockage for miRNAs acting as negative regulators of genes involved in stress response. However, the long-term alteration of miRNA maturation resulting from long-term exposure constitutes a stimulus toward carcinogenesis [110].

It should be noted that bioinformatic models indicate that Dicer binding by mutagens is non-covalent and involves low-energy. Accordingly, the Dicer catalytic sites are not irreversibly blocked but just change their affinity for specific substrates depending on oligonucleotidic sequences. This explains why only few selected miRNAs are affected by Dicer regulation depending on their specific structures. As an example, those miRNAs that are enriched in guanine in their terminal hairpin, such as those belonging to the *let-7* family, are highly sensitive to miRNA alterations induced by mutagens.

The same bioinformatic approach revealed that also chemopreventive agents are characterized by affinity for Dicer. Indeed, it was reported that isothiocyanates and I3C show high Dicer affinity [109]. Further analyses using the same approach indicated that resveratrol, EGCG, I3C, and beta-naphthoflavone display Dicer affinity by binding the catalytic site of Dicer sub-units (Fig. 1). These findings indicate that chemopreventive agents may compete with mutagens for Dicer binding. According to this view, chemopreventive agents act through hormetic effects sharing the same molecular effect of mutagens at the Dicer epigenetic level thereby competing with them for the activation of adverse mechanisms such as the alteration of miRNA expression.

4.5. Polymorphic miRNAs. A nutrigenomic/pharmacogenomic approach to cancer prevention?

A number of miRNAs targeted by chemopreventive agents, identified with the symbol § in Table 1, undergo SNPs. This feature also applies to frequently modulated miRNAs, such as *let-7a*, *miR-34*, *miR-125*, *miR-146*, and *miR-200*. For example, a G/U SNP at nucleotide 8 of *miR-125* gene has been reported to downregulate maturation of this miRNA [111]. The *miR-125* genetic targets include the *ERBB2* proto-oncogene encoding for the EGF receptor, which is highly expressed in carcinomas. *MiR-125* genes, located in the 11q23–q24 region, are frequently deleted in lung cancer [112]. This miRNA was strongly downregulated by CS in rat lung [14,15]. *MiR-125* was also downregulated in the airway epithelium of smoking humans [113]. Several chemopreventive agents, including NAC, oltipraz, I3C and PEITC, inhibited the CS-induced downregulation of *miR-125* in rat lung [81]. Insofar the role of miRNA SNPs for predicting cancer risks has been estimated to be low [27,114]. Further studies are needed to establish the impact of miRNA polymorphisms on safety and efficacy of chemopreventive agents. It is conceivable that miRNA polymorphisms could be important for explaining the interindividual variability in the response to the protective effects of pharmacologic and dietary agents, according to a pharmacogenomic/nutrigenomic approach. Interindividual variability is one of the main factors affecting the outcome of cancer chemoprevention trials in humans.

4.6. Tissue specificity of miRNAs and chemoprevention of site-specific cancers

A further issue is the tissue specificity of miRNAs, which is reported in Table 1 next to the identification of each miRNA. This information could be useful to address the clinical use of dietary and pharmacological agents for the prevention of site-specific cancers. In fact, a given type of cancer is expected to be more

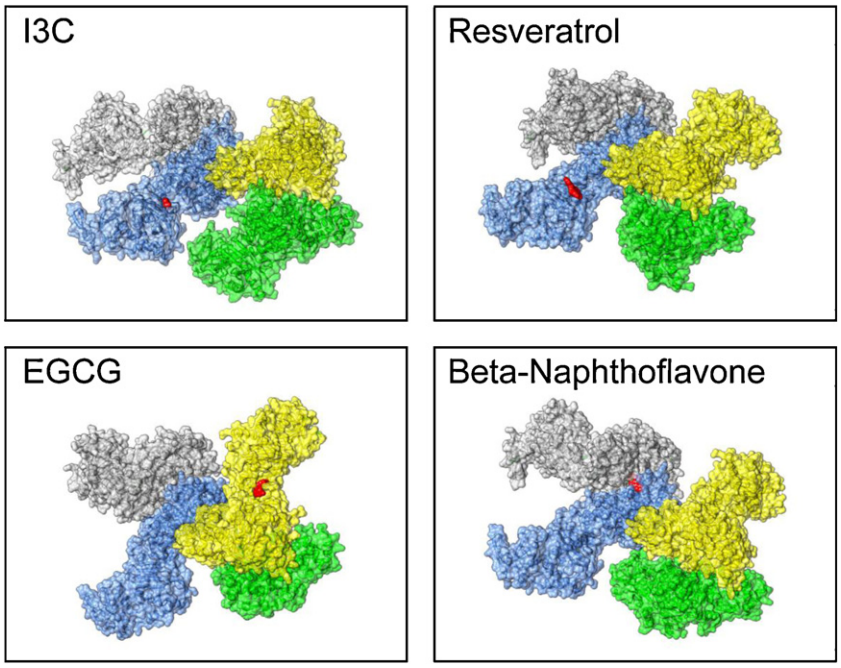

Fig. 1. Bioinformatic analysis showing the binding sites of 4 chemopreventive agents to Dicer. The Dicer 3D structure is rotated in each panel to show the chemopreventive agent binding site. The colors identify the agent (in red) and the Dicer subunits (yellow, sub-unit A; green, sub-unit B; grey, sub-unit C; blue, sub-unit D).

effectively prevented by agents that are able to modulate miRNA profiles in the target organ. For instance, chemopreventive agents modulating miRNAs of the *let-7* family, which are highly expressed in the lung and are considered to be major players in lung cancer development [115], could be proposed for the prevention of lung cancer. Those modulating *miR-122* could be proposed for the prevention of liver cancer. In fact, functional and molecular studies have uncovered mechanisms that link deregulated *miR-122* to pathways associated with hepatocellular carcinoma [116], to such an extent that an increase of this miRNA in serum has been proposed as a novel noninvasive biomarker for the detection of this cancer in healthy subjects [117].

5. Conclusions

A continuously expanding literature covers the issue of miRNA involvement in response to dietary and pharmacological agents. The present article reports the data relative to 31 agents, either natural or synthetic, which are known to behave as inhibitors of mutagenesis and carcinogenesis and are regarded as potential cancer chemopreventive agents. The majority of the studies reviewed, however, evaluated the effects of test agents on miRNA expression profiles in cultured cancer cell lines rather than their actual role in cancer chemoprevention. On the other hand, studies in mice and rats evaluated either the ability of test agents to alter the baseline expression of miRNAs and/or their ability to counteract miRNA alterations induced by carcinogens. In this way, it is possible to predict the *in vivo* effects of chemopreventive agents both in terms of safety and efficacy. In previous studies, we pursued a similar objective by evaluating transcriptome and proteome profiles in organs of rodents treated with carcinogens and/or chemopreventive agents [118,119]. However, mRNAs analysis gives redundant information, whereas proteome analysis just covers a large minority of the existing proteins.

The data generated by using animal models are likely to bear relevance to the human situation because the miRNA machinery is well conserved among species. Indeed, the miRNA alterations induced by CS in the lung of mice and rats are similar to those observed in the airway epithelium of smoking humans [120]. Accordingly, miRNA analysis in preclinical models may be useful to identify those chemopreventive agents that are worthy of being assayed in clinical trials as well as to select the identity of miRNAs to be analyzed as intermediate biomarkers. This task might be pursued in humans by minimally invasive procedures, due to the fact that miRNAs are released from target organs to the blood [121].

In conclusion, it is conceivable that miRNA analysis will become an important tool for developing new strategies for the prevention of cancer and other mutation-related diseases.

Conflict of interest statement

None.

Acknowledgments

This work was supported by the U.S. National Cancer Institute (NCI, contract NO1-CN53301) and by the Associazione Italiana per la Ricerca sul Cancro (AIRC, grant no. 8909). We thank Dr. Patrizio Arrigo (National Research Council, Genoa, Italy) for providing 3D Dicer structures.

References

[1] V.N. Kim, J.W. Nam, Genomics of microRNA, Trends Genet. 22 (2006) 165–173.
[2] E. Berezikov, E. Cuppen, R.H. Plasterk, Approaches to microRNA discovery, Nat. Genet. 38 (2006) S2–S7.
[3] D.P. Bartel, MicroRNAs: genomics, biogenesis, mechanism, and function, Cell 116 (2004) 281–297.
[4] J. Brennecke, A. Stark, R.B. Russell, S.M. Cohen, Principles of microRNA-target recognition, PLoS Biol. 3 (2005) e85.
[5] G. Di Leva, C.M. Croce, Roles of small RNAs in tumor formation, Trends Mol. Med. 16 (2010) 257–267.
[6] J. Couzin, MicroRNAs make big impression in disease after disease, Science 319 (2008) 1782–1784.
[7] C.M. Croce, Causes and consequences of microRNA dysregulation in cancer, Nat. Rev. Genet. 10 (2009) 704–714.
[8] M.D. Wouters, D.C. van Gent, J.H. Hoeijmakers, J. Pothof, MicroRNAs, the DNA damage response and cancer, Mutat. Res. 717 (2011) 54–66.

[9] H.I. Suzuki, K. Yamagata, K. Sugimoto, T. Iwamoto, S. Kato, K. Miyazono, Modulation of microRNA processing by p53, Nature 460 (2009) 529–533.
[10] J. Pothof, N.S. Verkaik, W. van IJcken, E.A. Wiemer, V.T. Ta, G.T. van der Horst, N.G. Jaspers, D.C. van Gent, J.H. Hoeijmakers, S.P. Persengiev, MicroRNA-mediated gene silencing modulates the UV-induced DNA-damage response, EMBO J. 28 (2009) 2090–2099.
[11] H. Duan, Y. Jiang, H. Zhang, Y. Wu, MiR-320 and miR-494 affect cell cycles of primary murine bronchial epithelial cells exposed to benzo[*a*]pyrene, Toxicol. In Vitro 24 (2010) 928–935.
[12] B.K. Elamin, E. Callegari, L. Gramantieri, S. Sabbioni, M. Negrini, MicroRNA response to environmental mutagens in liver, Mutat. Res. 717 (2011) 67–76.
[13] C. Lema, M.J. Cunningham, MicroRNAs and their implications in toxicological research, Toxicol. Lett. 198 (2010) 100–105.
[14] A. Izzotti, G. Calin, P. Arrigo, V.E. Steele, C. Croce, S. De Flora, Downregulation of microRNA expression in the lung of rats exposed to cigarette smoke, FASEB J. 23 (2009) 806–812.
[15] A. Izzotti, G.A. Calin, V.E. Steele, C.M. Croce, S. De Flora, Relationship of microRNA expression in mouse lung with age and exposure to cigarette smoke and light, FASEB J. 23 (2009) 3243–3250.
[16] M.J. Jardim, MicroRNAs: implications for air pollution research, Mutat. Res. 1 (2011) 38–45.
[17] E. Sonkoly, A. Pivarcsi, MicroRNAs in inflammation and response to injuries induced by environmental pollution, Mutat. Res. 717 (2011) 46–53.
[18] S. De Flora, C. Ramel, Mechanisms of inhibitor of mutagenesis and carcinogenesis. Classification and overview, Mutat. Res. 202 (1988) 285–306.
[19] G.J. Kelloff, C.W. Boone, V.E. Steele, J.A. Crowell, R.A. Lubet, P. Greenwald, E.T. Hawk, J.R. Fay, C.C. Sigman, Mechanistic considerations in the evaluation of chemopreventive data, IARC Sci. Publ. 139 (1996) 203–219.
[20] S. De Flora, L.R. Ferguson, Overview of mechanisms of cancer chemopreventive agents, Mutat. Res. 591 (2005) 8–15.
[21] W. Zhang, M.E. Dolan, Emerging role of microRNAs in drug response, Curr. Opin. Mol. Ther. 12 (2010) 695–702.
[22] C.D. Davis, S.A. Ross, Evidence for dietary regulation of microRNA expression in cancer cells, Nutr. Rev. 66 (2008) 477–482.
[23] Y. Li, D. Kong, Z. Wang, F.H. Sarkar, Regulation of microRNAs by natural agents: an emerging field in chemoprevention and chemotherapy research, Pharm. Res. 27 (2010) 1027–1041.
[24] A. Link, F. Balaguer, A. Goel, Cancer chemoprevention by dietary polyphenols: promising role for epigenetics, Biochem. Pharmacol. 80 (2010) 1771–1792.
[25] S. Reuter, S.C. Gupta, B. Park, A. Goel, B.B. Aggarwal, Epigenetic changes induced by curcumin and other natural compounds, Genes Nutr. 6 (2011) 93–108.
[26] M.A. Parasramka, E. Ho, D.E. Williams, R.H. Dashwood, MicroRNAs, diet, and cancer: new mechanistic insights on the epigenetic actions of phytochemicals, Mol. Carcinog. 51 (2012) 213–230.
[27] L.J. Chin, E. Ratner, S. Leng, R. Zhai, S. Nallur, I. Babar, R.U. Muller, E. Straka, L. Su, E.A. Burki, R.E. Crowell, R. Patel, T. Kulkarni, R. Homer, D. Zelterman, K.K. Kidd, Y. Zhu, D.C. Christiani, S.A. Belinsky, F.J. Slack, J.B. Weidhaas, A SNP in a let-7 microRNA complementary site in the KRAS 3′ untranslated region increases non-small cell lung cancer risk, Cancer Res. 68 (2008) 8535–8540.
[28] J. Gong, Y. Tong, H.M. Zhang, K. Wang, T. Hu, G. Shan, J. Sun, A.Y. Guo, Genome-wide identification of SNPs in microRNA genes and the SNP effects on microRNA target binding and biogenesis, Hum. Mutat. 33 (2011) 254–263.
[29] A. Rossi, O.F. D'Urso, G. Gatto, P. Poltronieri, M. Ferracin, P. Remondelli, M. Negrini, M.G. Caporaso, S. Bonatti, M. Mallardo, Non-coding RNAs change their expression profile after retinoid induced differentiation of the promyelocytic cell line NB4, BMC Res. Notes 3 (2010) 24.
[30] R. Garzon, F. Pichiorri, T. Palumbo, M. Visentini, R. Aqeilan, A. Cimmino, H. Wang, H. Sun, S. Volinia, H. Alder, G.A. Calin, C.G. Liu, M. Andreeff, C.M. Croce, MicroRNA gene expression during retinoic acid-induced differentiation of human acute promyelocytic leukemia, Oncogene 26 (2007) 4148–4157.
[31] P. Jian, Z.W. Li, T.Y. Fang, W. Jian, Z. Zhuan, L.X. Mei, W.S. Yan, N. Jian, Retinoic acid induces HL-60 cell differentiation via the upregulation of miR-663, J. Hematol. Oncol. 4 (2011) 20.
[32] M. Terao, M. Fratelli, M. Kurosaki, A. Zanetti, V. Guarnaccia, G. Paroni, A. Tsykin, M. Lupi, M. Gianni, G.J. Goodall, E. Garattini, Induction of miR-21 by retinoic acid in estrogen receptor-positive breast carcinoma cells: biological correlates and molecular targets, J. Biol. Chem. 286 (2011) 4027–4042.
[33] H. Kawasaki, K. Taira, Functional analysis of microRNAs during the retinoic acid-induced neuronal differentiation of human NT2 cells, Nucleic Acids Res. Suppl. 3 (2003) 243–244.
[34] S. Das, N. Foley, K. Bryan, K.M. Watters, I. Bray, D.M. Murphy, P.G. Buckley, R.L. Stallings, MicroRNA mediates DNA demethylation events triggered by retinoic acid during neuroblastoma cell differentiation, Cancer Res. 70 (2010) 7874–7881.
[35] N.J. Beveridge, P.A. Tooney, A.P. Carroll, N. Tran, M.J. Cairns, Down-regulation of miR-17 family expression in response to retinoic acid induced neuronal differentiation, Cell Signal. 21 (2009) 1837–1845.
[36] S. Meseguer, G. Mudduluru, J.M. Escamilla, H. Allgayer, D. Barettino, MicroRNAs-10a and -10b contribute to retinoic acid-induced differentiation of neuroblastoma cells and target the alternative splicing regulatory factor SFRS1 (SF2/ASF), J. Biol. Chem. 286 (2011) 4150–4156.
[37] J.J. Zhao, D.G. Sun, J. Wang, S.R. Liu, C.Y. Zhang, M.X. Zhu, X. Ma, Retinoic acid downregulates microRNAs to induce abnormal development of spinal cord in spina bifida rat model, Childs Nerv. Syst. 24 (2008) 485–492.
[38] H. Kutay, S. Bai, J. Datta, T. Motiwala, I. Pogribny, W. Frankel, S.T. Jacob, K. Ghoshal, Downregulation of miR-122 in the rodent and human hepatocellular carcinomas, J. Cell. Biochem. 99 (2006) 671–678.
[39] I.P. Pogribny, V.P. Tryndyak, S.A. Ross, F.A. Beland, Differential expression of microRNAs during hepatocarcinogenesis induced by methyl deficiency in rats, Nutr Rev. 66 (Suppl. 1) (2008) S33–S35.
[40] L.L. Wang, Z. Zhang, Q. Li, R. Yang, X. Pei, Y. Xu, J. Wang, S.F. Zhou, Y. Li, Ethanol exposure induces differential microRNA and target gene expression and teratogenic effects which can be suppressed by folic acid supplementation, Hum. Reprod. 24 (2009) 562–579.
[41] C.J. Marsit, K. Eddy, K.T. Kelsey, MicroRNA responses to cellular stress, Cancer Res. 66 (2006) 10843–10848.
[42] X. Wang, E. Gocek, C.G. Liu, G.P. Studzinski, MicroRNAs181 regulate the expression of p27Kip1 in human myeloid leukemia cells induced to differentiate by 1,25-dihydroxyvitamin D3, Cell Cycle 8 (2009) 736–741.
[43] E. Gocek, X. Wang, X. Liu, C.G. Liu, G.P. Studzinski, MicroRNA-32 upregulation by 1,25-dihydroxyvitamin D3 in human myeloid leukemia cells leads to Bim targeting and inhibition of AraC-induced apoptosis, Cancer Res. 71 (2011) 6230–6239.
[44] X. Peng, A. Vaishnav, G. Murillo, F. Alimirah, K.E. Torres, R.G. Mehta, Protection against cellular stress by 25-hydroxyvitamin D3 in breast epithelial cells, J. Cell. Biochem. 110 (2010) 1324–1333.
[45] T. Mohri, M. Nakajima, S. Takagi, S. Komagata, T. Yokoi, MicroRNA regulates human vitamin D receptor, Int. J. Cancer 125 (2009) 1328–1333.
[46] S. Essa, N. Denzer, U. Mahlknecht, R. Klein, E.M. Collnot, W. Tilgen, J. Reichrath, VDR microRNA expression and epigenetic silencing of vitamin D signaling in melanoma cells, J. Steroid Biochem. Mol. Biol. 121 (2010) 110–113.
[47] S. Gaedicke, X. Zhang, C. Schmelzer, Y. Lou, F. Doering, J. Frank, G. Rimbach, Vitamin E dependent microRNA regulation in rat liver, FEBS Lett. 582 (2008) 3542–3546.
[48] G. Rimbach, J. Moehring, P. Huebbe, J.K. Lodge, Gene-regulatory activity of alpha-tocopherol, Molecules 15 (2010) 1746–1761.
[49] S. Sarveswaran, J. Liroff, Z. Zhou, A.Y. Nikitin, J. Ghosh, Selenite triggers rapid transcriptional activation of p53, and p53-mediated apoptosis in prostate cancer cells: implication for the treatment of early-stage prostate cancer, Int. J. Oncol. 36 (2010) 1419–1428.
[50] G.D. Noratto, Y. Kim, S.T. Talcott, S.U. Mertens-Talcott, Flavonol-rich fractions of yaupon holly leaves (*Ilex vomitoria*, Aquifoliaceae) induce microRNA-146a and have anti-inflammatory and chemopreventive effects in intestinal myofibroblast CCD-18Co cells, Fitoterapia 82 (2011) 557–569.
[51] R.T. Ai, S.Y. Wu, X.Y. Wen, W. Xu, L. Lv, J.J. Rao, S.G. Wu, 1,3,4-Tri-O-galloyl-6-O-caffeoyl-β-D-glucopyranose, a new anti-proliferative ellagitannin, regulates the expression of microRNAs in HepG(2) cancer cells, Nan Fang Yi Ke Da Xue Xue Bao 31 (2011) 1641–1648.
[52] X.Y. Wen, S.Y. Wu, Z.Q. Li, Z.Q. Liu, J.J. Zhang, G.F. Wang, Z.H. Jiang, S.G. Wu, Ellagitannin (BJA3121), an anti-proliferative natural polyphenol compound, can regulate the expression of MiRNAs in HepG2 cancer cells, Phytother. Res. 23 (2009) 778–784.
[53] W.P. Tsang, T.T. Kwok, Epigallocatechin gallate up-regulation of miR-16 and induction of apoptosis in human cancer cells, J. Nutr. Biochem. 21 (2010) 140–146.
[54] A. Arola-Arnal, C. Bladé, Proanthocyanidins modulate microRNA expression in human HepG2 cells, PLoS One 6 (2011) e25982.
[55] L.N. Fix, M. Shah, T. Efferth, M.A. Farwell, B. Zhang, MicroRNA expression profile of MCF-7 human breast cancer cells and the effect of green tea polyphenon-60, Cancer Genomics Proteomics 7 (2010) 261–277.
[56] H. Wang, S. Bian, C.S. Yang, Green tea polyphenol EGCG suppresses lung cancer cell growth through upregulating miR-210 expression caused by stabilizingHIF-1{alpha}, Carcinogenesis 32 (2011) 1181–1189.
[57] M. Sun, Z. Estrov, Y. Ji, K.R. Coombes, D.H. Harris, R. Kurzrock, Curcumin (diferuloylmethane) alters the expression profiles of microRNAs in human pancreatic cancer cells, Mol. Cancer Ther. 7 (2008) 464–473.
[58] J. Yang, Y. Cao, J. Sun, Y. Zhang, Curcumin reduces the expression of Bcl-2 by upregulating miR-15a and miR-16 in MCF-7 cells, Med. Oncol. 27 (2010) 1114–1118.
[59] J. Zhang, Y. Du, C. Wu, X. Ren, X. Ti, J. Shi, F. Zhao, H. Yin, Curcumin promotes apoptosis in human lung adenocarcinoma cells through miR-186* signaling pathway, Oncol. Rep. 24 (2010) 1217–1223.
[60] G. Mudduluru, J.N. George-William, S. Muppala, I.A. Asangani, R. Kumarswamy, L.D. Nelson, H. Allgayer, Curcumin regulates miR-21 expression and inhibits invasion and metastasis in colorectal cancer, Biosci. Rep. 31 (2011) 185–197.
[61] B. Bao, S. Ali, D. Kong, S.H. Sarkar, Z. Wang, S. Banerjee, A. Aboukameel, S. Padhye, P.A. Philip, F.H. Sarkar, Anti-tumor activity of a novel compound-CDF is mediated by regulating miR-21, miR-200, and PTEN in pancreatic cancer, PLoS One 6 (2011) e17850.
[62] E. Tili, J.J. Michaille, H. Alder, S. Volinia, D. Delmas, N. Latruffe, C.M. Croce, Resveratrol modulates the levels of microRNAs targeting genes encoding tumor-suppressors and effectors of TGFbeta signaling pathway in SW480 cells, Biochem. Pharmacol. 80 (2010) 2057–2065.
[63] E. Tili, J.J. Michaille, B. Adair, H. Alder, E. Limagne, C. Taccioli, M. Ferracin, D. Delmas, N. Latruffe, C.M. Croce, Resveratrol decreases the levels of miR-155 by upregulating miR-663, a microRNA targeting JunB and JunD, Carcinogenesis 31 (2010) 1561–1566.
[64] E. Tili, J.J. Michaille, Resveratrol, microRNAs, inflammation, and cancer, J. Nucleic Acids (2011), http://dx.doi.org/10.4061/2011/102431 (Epub Aug 10).

[65] W.J. Lukiw, Y. Zhao, J.G. Cui, An NF-kappaB-sensitive micro RNA-146a-mediated inflammatory circuit in Alzheimer disease and in stressed human brain cells, J. Biol. Chem. 283 (2008) 31315–31322.
[66] Z. Han, Q. Yang, B. Liu, J. Wu, Y. Li, C. Yang, Y. Jiang, MicroRNA-622 functions as a tumor suppressor by targeting K-Ras and enhancing the anticarcinogenic effect of resveratrol, Carcinogenesis 33 (2012) 131–139.
[67] S. Bae, E.M. Lee, H.J. Cha, K. Kim, Y. Yoon, H. Lee, J. Kim, Y.J. Kim, H.G. Lee, H.K. Jeung, Y.H. Min, S. An, Resveratrol alters microRNA expression profiles in A549 human non-small cell lung cancer cells, Mol. Cells 32 (2011) 243–249.
[68] S. Dhar, C. Hicks, A.S. Levenson, Resveratrol and prostate cancer: promising role for microRNAs, Mol. Nutr. Food Res. 55 (2011) 1219–1229.
[69] P. Mukhopadhyay, S. Mukherjee, K. Ahsan, A. Bagchi, P. Pacher, D.K. Das, Restoration of altered microRNA expression in the ischemic heart with resveratrol, PLoS One 5 (2010) e15705.
[70] P. Mukhopadhyay, S. Das, N. Gorbunov, M.K. Ahsan, H. Otani, P. Pacher, D.K. Das, Modulation of microRNA 20b with resveratrol and longevinex is linked with their potent anti-angiogenic action in the ischemic myocardium and synergestic effects of resveratrol and γ-tocotrienol, J. Cell. Mol. Med. (2011), http://dx.doi.org/10.1111/j.1582-4934.2011.01480 (Epub ahead of print).
[71] Q. Sun, R. Cong, H. Yan, H. Gu, Y. Zeng, N. Liu, J. Chen, B. Wang, Genistein inhibits growth of human uveal melanoma cells and affects microRNA-27a and target gene expression, Oncol. Rep. 22 (2009) 563–567.
[72] Y. Li, F.H. Sarkar, Down-regulation of invasion and angiogenesisrelated genes identified by cDNA microarray analysis of PC3 prostate cancer cells treated with genistein, Cancer Lett. 186 (2002) 157–164.
[73] L.P. Parker, D.D. Taylor, J. Kesterson, D.S. Metzinger, C. Gercel-Taylor, Modulation of microRNA associated with ovarian cancer cells by genistein, Eur. J. Gynaecol. Oncol. 30 (2009) 616–621.
[74] S. Majid, A.A. Dar, S. Saini, Y. Chen, V. Shahryari, J. Liu, M.S. Zaman, H. Hirata, S. Yamamura, K. Ueno, Y. Tanaka, R. Dahiya, Regulation of minichromosome maintenance gene family by microRNA-1296 and genistein in prostate cancer, Cancer Res. 70 (2010) 2809–2818.
[75] Y. Chen, M.S. Zaman, G. Deng, S. Majid, S. Saini, J. Liu, Y. Tanaka, R. Dahiya, MicroRNAs 221/222 and genistein-mediated regulation of ARHI tumor suppressor gene in prostate cancer, Cancer Prev. Res. 4 (2011) 76–86.
[76] N. Rabiau, H.K. Trraf, M. Adjakly, R. Bosviel, L. Guy, L. Fontana, Y.J. Bignon, D.J. Bernard-Gallon, miRNAs differentially expressed in prostate cancer cell lines after soy treatment, In Vivo 25 (2011) 917–921.
[77] J.Y. Gou, F.F. Felippes, C.J. Liu, D. Weigel, J.W. Wang, Negative regulation of anthocyanin biosynthesis in Arabidopsis by a miR156-targeted SPL transcription factor, Plant Cell 23 (2011) 1512–1522.
[78] Y. Li, T.G. VandenBoom 2nd, D. Kong, Z. Wang, S. Ali, P.A. Philip, F.H. Sarkar, 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. 69 (2009) 6704–6712.
[79] Y. Jin, X. Zou, X. Feng, 3,3′-Diindolylmethane negatively regulates Cdc25A and induces a G2/M arrest by modulation of microRNA 21 in human breast cancer cells, Anticancer Drugs 21 (2010) 814–822 (Retraction in: Anticancer Drugs 22 (2011) 303).
[80] T. Melkamu, X. Zhang, J. Tan, Y. Zeng, F. Kassie, Alteration of microRNA expression in vinyl carbamate-induced mouse lung tumors and modulation by the chemopreventive agent indole-3-carbinol, Carcinogenesis 31 (2010) 252–258.
[81] A. Izzotti, G.A. Calin, V.E. Steele, C. Cartiglia, M. Longobardi, C.M. Croce, S. De Flora, Chemoprevention of cigarette smoke-induced alterations of MicroRNA expression in rat lungs, Cancer Prev. Res. 3 (2010) 62–72.
[82] A. Izzotti, P. Larghero, C. Cartiglia, M. Longobardi, U. Pfeffer, V.E. Steele, S. De Flora, Modulation of microRNA expression by budesonide, phenethyl isothiocyanate and cigarette smoke in mouse liver and lung, Carcinogenesis 31 (2010) 894–901.
[83] A. Izzotti, P. Larghero, R. Balansky, U. Pfeffer, V.E. Steele, S. De Flora, Interplay between histopathological alterations, cigarette smoke and chemopreventive agents in defining microRNA profiles in mouse lung, Mutat. Res. 717 (2011) 17–24.
[84] N. Wu, G.C. Wu, R. Hu, M. Li, H. Feng, Ginsenoside Rh2 inhibits glioma cell proliferation by targeting microRNA-128, Acta Pharmacol. Sin. 32 (2011) 345–353.
[85] W.S. Chen, J.N. Hou, Y.B. Guo, H.L. Yang, C.M. Xie, Y.C. Lin, Z.G. She, Bostrycin inhibits proliferation of human lung carcinoma A549 cells via downregulation of the PI3K/Akt pathway, J Exp. Clin. Cancer Res. 8 (2011) 30, 17.
[86] L.A. Davidson, N. Wang, M.S. Shah, J.R. Lupton, I. Ivanov, R.S. Chapkin, n-3 Polyunsaturated fatty acids modulate carcinogen-directed non-coding microRNA signatures in rat colon, Carcinogenesis 30 (2009) 2077-84.S.
[87] G. Tzur, A. Levy, E. Meiri, O. Barad, Y. Spector, Z. Bentwich, L. Mizrahi, M. Katzenellenbogen, E. Ben-Shushan, B.E. Reubinoff, E. Galun, MicroRNA expression patterns and function in endodermal differentiation of human embryonic stem cells, PLoS One 3 (2008) e3726.
[88] S. Hu, T.S. Dong, S.R. Dalal, F. Wu, M. Bissonnette, J.H. Kwon, E.B. Chang, The microbe-derived short chain fatty acid butyrate targets miRNA-dependent p21 gene expression in human colon cancer, PLoS One 6 (2011) e16221.
[89] Y. Ye, Z. Hu, Y. Lin, C. Zhang, J.R. Perez-Polo, Downregulation of microRNA-29 by antisense inhibitors and a PPAR-gamma agonist protects against myocardial ischaemia–reperfusion injury, Cardiovasc. Res. 187 (2010) 535–544.
[90] S. Shin, E.M. Lee, H.J. Cha, S. Bae, J.H. Jung, S.M. Lee, Y. Yoon, H. Lee, S. Kim, H. Kim, S.J. Lee, I.C. Park, Y.W. Jin, S. An, MicroRNAs that respond to histone deacetylase inhibitor SAHA and p53 in HCT116 human colon carcinoma cells, Int. J. Oncol. 35 (2009) 1343–1352.
[91] L. Kretzner, A. Scuto, P.M. Dino, C.M. Kowolik, J. Wu, P. Ventura, R. Jove, S.J. Forman, Y. Yen, M.H. Kirschbaum, Combining histone deacetylase inhibitor vorinostat with aurora kinase inhibitors enhances lymphoma cell killing with repression of c-Myc, hTERT, and microRNA levels, Cancer Res. 71 (2011) 3912–3920.
[92] D. Nalls, S.N. Tang, M. Rodova, R.K. Srivastava, S. Shankar, Targeting epigenetic regulation of miR-34a for treatment of pancreatic cancer by inhibition of pancreatic cancer stem cells, PLoS One 6 (2011) e24099.
[93] E.M. Lee, S. Shin, H.J. Cha, Y. Yoon, S. Bae, J.H. Jung, S.M. Lee, S.J. Lee, I.C. Park, Y.W. Jin, S. An, Suberoylanilide hydroxamic acid (SAHA) changes microRNA expression profiles in A549 human non-small cell lung cancer cells, Int. J. Mol. Med. 24 (2009) 45–50.
[94] L.V. Rhodes, A.M. Nitschke, H.C. Segar, E.C. Martin, J.L. Driver, S. Elliott, S.Y. Nam, M. Li, K.P. Nephew, M.E. Burow, B.M. Collins-Burow, The histone deacetylase inhibitor trichostatin A alters microRNA expression profiles in apoptosis-resistant breast cancer cells, Oncol. Rep. 27 (2012) 10–16.
[95] G.K. Scott, M.D. Mattie, C.E. Berger, S.C. Benz, C.C. Benz, Rapid alteration of microRNA levels by histone deacetylase inhibition, Cancer Res. 66 (2006) 1277–1281.
[96] A.E. Williams, H. Larner-Svensson, M.M. Perry, G.A. Campbell, S.E. Herrick, I.M. Adcock, J.S. Erjefalt, K.F. Chung, M.A. Lindsay, MicroRNA expression profiling in mild asthmatic human airways and effect of corticosteroid therapy, PLoS One 4 (2009) e5889.
[97] S.S. Pathi, I. Jutooru, G. Chadalapaka, S. Sreevalsan, S. Anand, G.R. Thatcher, S. Safe, GT-094, a NO-NSAID, inhibits colon cancer cell growth by activation of a reactive oxygen species-microRNA-27a: ZBTB10-specificity protein pathway, Mol. Cancer Res. 9 (2011) 195–202.
[98] J.J. Zhao, J. Lin, H. Yang, W. Kong, L. He, X. Ma, D. Coppola, J.Q. Cheng, MicroRNA-221/222 negatively regulates estrogen receptor alpha and is associated with tamoxifen resistance in breast cancer, J. Biol. Chem. 283 (2008) 31079–31086.
[99] T.E. Miller, K. Ghoshal, B. Ramaswamy, S. Roy, J. Datta, C.L. Shapiro, S. Jacob, S. Majumder, MicroRNA-221/222 confers tamoxifen resistance in breast cancer by targeting p27Kip1, J. Biol. Chem. 283 (2008) 29897–29903.
[100] D.M. Cittelly, P.M. Das, N.S. Spoelstra, S.M. Edgerton, J.K. Richer, A.D. Thor, F.E. Jones, Downregulation of miR-342 is associated with tamoxifen resistant breast tumors, Mol. Cancer 9 (2010) 317.
[101] X. Rao, G. Di Leva, M. Li, F. Fang, C. Devlin, C. Hartman-Frey, M.E. Burow, M. Ivan, C.M. Croce, K.P. Nephew, MicroRNA-221/222 confers breast cancer fulvestrant resistance by regulating multiple signaling pathways, Oncogene 30 (2011) 1082–1097.
[102] O. Ziouzenkova, J. Plutzky, Retinoid metabolism and nuclear receptor responses: new insights into coordinated regulation of the PPAR–RXR complex, FEBS Lett. 582 (2008) 32–38.
[103] S. De Flora, A. Izzotti, F. D'Agostini, R.M. Balansky, Mechanisms of N-acetylcysteine in the prevention of DNA damage and cancer, with special reference to smoking-related end-points, Carcinogenesis 22 (2001) 999–1013.
[104] T.W. Kensler, P.A. Egner, P.M. Dolan, J.D. Groopman, B.D. Roebuck, Mechanism of protection against aflatoxin tumorigenicity in rats fed 5-(2-pyrazinyl)-4-methyl-1,2-dithiol-3-thione (oltipraz) and related 1,2-dithiol-3-thiones and 1,2-dithiol-3-ones, Cancer Res. 47 (1987) 4271–4277.
[105] S. De Flora, F. D'Agostini, R. Balansky, A. Camoirano, C. Bennicelli, M. Bagnasco, C. Cartiglia, E. Tampa, M.G. Longobardi, R.A. Lubet, A. Izzotti, Modulation of cigarette smoke-related end-points in mutagenesis and carcinogenesis, Mutat. Res. 523-524 (2003) 237–252.
[106] J.R. Weng, H.A. Omar, S.K. Kulp, C.S. Chen, Pharmacological exploitation of indole-3-carbinol to develop potent antitumor agents, Mini Rev. Med. Chem. 10 (2010) 398–404.
[107] L. Boominathan, The tumor suppressors p53, p63, and p73 are regulators of microRNA processing complex, PLoS One 5 (2010) e10615.
[108] H. Hu, R.A. Gatti, MicroRNAs: new players in the DNA damage response, J. Mol. Cell Biol. 3 (2011) 151–158.
[109] M. Ligorio, A. Izzotti, A. Pulliero, P. Arrigo, Mutagens interfere with microRNA maturation by inhibiting DICER. An in silico biology analysis, Mutat. Res. 717 (2011) 116–128.
[110] A. Izzotti, P. Larghero, M. Longobardi, C. Cartiglia, A. Camoirano, V.E. Steele, S. De Flora, Dose-responsiveness and persistence of microRNA expression alterations induced by cigarette smoke in mouse lung, Mutat. Res. 717 (2011) 9–16.
[111] R. Duan, C. Pak, P. Jin, Single nucleotide polymorphism associated with mature miR-125a alters the processing of pri-miRNA, Hum. Mol. Genet. 16 (2007) 1124–1131.
[112] G.A. Calin, C. Sevignani, C.D. Dumitru, T. Hyslop, E. Noch, S. Yendamuri, M. Shimizu, S. Rattan, F. Bullrich, M. Negrini, C.M. Croce, Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers, Proc. Natl. Acad. Sci. U.S.A. 101 (2004) 2999–3004.
[113] F. Schembri, S. Sridhar, C. Perdomo, A.M. Gustafson, X. Zhang, A. Ergun, J. Lu, G. Liu, X. Zhang, J. Bowers, C. Vaziri, K. Ott, K. Sensinger, J.J. Collins, J.S. Brody, R. Getts, M.E. Lenburg, A. Spira, MicroRNAs as modulators of smoking-induced gene expression changes in human airway epithelium, Proc. Natl. Acad. Sci. U.S.A. 106 (2009) 2319–2324.
[114] Z. Hu, J. Chen, T. Tian, X. Zhou, H. Gu, L. Xu, Y. Zeng, R. Miao, G. Jin, H. Ma, Y. Chen, H. Shen, Genetic variants of miRNA sequences and non-small cell lung cancer survival, J. Clin. Invest. 118 (2008) 2600–2608.
[115] H. Osada, T. Takahashi, Let-7 and miR-17-92: small-sized major players in lung cancer development, Cancer Sci. 102 (2011) 9–17.
[116] M. Negrini, L. Gramantieri, S. Sabbioni, C.M. Croce, MicroRNA involvement in hepatocellular carcinoma, Anticancer Agents Med. Chem. 11 (2011) 500–521.

[117] P. Qi, S.Q. Cheng, H. Wang, N. Li, Y.F. Chen, C.F. Gao, Serum microRNAs as biomarkers for hepatocellular carcinoma in Chinese patients with chronic hepatitis B virus infection, PLoS One 6 (2011) e28486.
[118] A. Izzotti, M. Bagnasco, C. Cartiglia, M. Longobardi, A. Camoirano, E. Tampa, R.A. Lubet, S. De Flora, Modulation of multigene expression and proteome profiles by chemopreventive agents, Mutat. Res. 591 (2005) 212–223.
[119] A. Izzotti, M. Bagnasco, C. Cartiglia, M. Longobardi, R.M. Balansky, A. Merello, R.A. Lubet, S. De Flora, Chemoprevention of genome, transcriptome, and proteome alterations induced by cigarette smoke in rat lung, Eur. J. Cancer 41 (2005) 1864–1874.
[120] C. Perdomo, A. Spira, F. Schembri, MiRNAs as regulators of the response to inhaled environmental toxins and airway carcinogenesis, Mutat. Res. 717 (2011) 32–37.
[121] A. Etheridge, I. Lee, L. Hood, D. Galas, K. Wang, Extracellular microRNA: a new source of biomarkers, Mutat. Res. 717 (2011) 85–90.