Published in final edited form as:

Mol Nutr Food Res. 2018 September ; 62(18): e1700913. doi:10.1002/mnfr.201700913.

The Role of Non-coding RNAs and Isothiocyanates in Cancer

Samantha L. Martin¹, Kendra J. Royston¹, and Trygve O. Tollefsbol^{*,1,2,3,4,5}

¹Department of Biology, University of Alabama at Birmingham, 1300 University Boulevard, Birmingham, AL 35294, USA

²Comprehensive Center for Healthy Aging, University of Alabama Birmingham, 1530 3rd Avenue South, Birmingham, AL 35294, USA

³Comprehensive Cancer Center, University of Alabama Birmingham, 1802 6th Avenue South, Birmingham, AL 35294, USA

⁴Nutrition Obesity Research Center, University of Alabama Birmingham, 1675 University Boulevard, Birmingham, AL 35294, USA

⁵Comprehensive Diabetes Center, University of Alabama Birmingham, 1825 University Boulevard, Birmingham, AL 35294, USA

Abstract

Cancer is the second leading cause of mortalities in the United States, only exceeded by heart disease. Current cancer treatments include chemotherapy, surgery and/or radiation. Due to the often harsh effects of current cancer therapies, investigators are focusing their efforts on cancer prevention mediated by dietary phytochemicals. Since the discovery that cancer can be initiated by and progress through both genetic and epigenetic pathways, there has been a significant surge in studies on epigenetic effects mediated by nutritive compounds. Isothiocyanates, naturally occurring molecules found in cruciferous vegetables, have been documented to exhibit many anticarcinogenic activities. Although isothiocyanates have been extensively documented as key players in epigenetic processes such as DNA methylation and histone modifications, their effects on non-coding RNAs is understudied. Non-coding RNAs are molecules that target mRNA production and repress protein translation and are known to be dysregulated in various human malignancies. Studies have used non-coding RNAs as novel targets for exploration in cancer therapy. This review focuses on the exploration of isothiocyanates and their effect on non-coding RNAs in cancer prevention and therapy.

Keywords

Chemoprevention; Epigenetics; Isothiocyanates; Non-coding RNAs

*Address correspondence to: Trygve O. Tollefsbol, CH175, 1300 University Boulevard, Birmingham, AL 35294-1170; Tel: +1-205-934-4573; Fax: 1-205-975-6097; trygve@uab.edu.

Conflict of Interest

The authors declare no financial conflicts of interest.

Introduction

Cancer is a significant health problem globally and in the United States. In 2017, the American Cancer Society (ACS) projects approximately 1.69 million new cancer cases and 600,920 cancer deaths to occur in the United States [1]. The International Agency for Research on Cancer estimates that there were 14.1 million new cancer cases and 8.2 million cancer deaths worldwide in 2017 [2]. It is expected that by the year 2030, the global burden will increase to 21.7 million new cancer cases and 13 million cancer deaths. In addition, it has been suggested that new cancer cases will be significantly higher due in part to the adoption of poor lifestyle habits such as smoking, poor diet and lack of physical activity. Because of this, cancer prevention is increasingly becoming a major public health priority as well as a priority in the scientific field.

Many researchers have turned to epigenetics to answer numerous biologically relevant questions. Epigenetics is the process that involves a change in gene expression without altering the DNA sequence. The field has been linked with a wide variety of illnesses including but not limited to cardiovascular disease [3], type 1 diabetes [4] and cancers of all types [5, 6]. To date, scientists have identified many types of epigenetic processes. The three major epigenetic processes include DNA methylation, histone modification and non-coding RNAs [7]. Although many epigenetic processes naturally occur, they are of particular interest because of their ability to be reversed and influenced by external factors such as environment and lifestyle. While some epigenetic modifications have a role in cellular differentiation and numerous other biological processes, major epigenetic modifications can result in an increase in chronic diseases such as cancer [8, 9]. New and ongoing research is continuously aiding in identifying the role of epigenetics in diseases such as cancer.

DNA methylation and histone modifications are two of the most studied and well-characterized epigenetic processes to date [10]. Non-coding RNAs have since gained considerable attention due to their ability to regulate gene expression. A non-coding RNA (ncRNA) is an endogenous RNA molecule that is not translated into a protein [11]. Although non-coding RNA research is still in its infancy, four major types of ncRNAs have attracted the most attention. ncRNAs include microRNAs (miRs), small interfering RNAs (siRNAs), piwi interacting RNAs (piRNAs) and long non-coding RNAs (lncRNAs). In general, ncRNAs, with the exception of lncRNAs, regulate gene expression at the post-transcriptional level and can be divided into two broad groups, short and lncRNAs depending on their size [12]. The first group, short ncRNAs, generally contains a sequence of less than 30 nucleotides [13]. Conversely, the second group, lncRNAs, contain a much larger sequence; generally greater than 200 base pairs in length [14]. Both short and long ncRNAs play a major role in histone modifications, DNA methylation and gene silencing [15] [16].

miRs are the most extensively studied short ncRNAs to date. According to miRs are small ncRNAs approximately 20–25 nucleotides long that inhibit protein translation by typically binding to the untranslated region (UTR) of their target messenger RNA (Figure 1) [17]. In turn, this mechanism results in the degradation of the mRNA which ultimately prevents protein translation. It is estimated by bioinformatic studies that miRs regulate more than 30% of all mammalian genes in many human cancers [18]. In addition to binding to the

UTR region of the target mRNA, miRs also bind to what Bartel has identified as canonical, marginal and atypical sites of which there are 7–8-nt seed-matched sites, 6-nt sites matching the seed region and sites with productive 3' pairing respectively. It is now common knowledge that a single miRNA regulates many target genes, while a single gene is targeted by many miRs [19]. Since the initial discovery of miRs in the early 1990's, miR biology has expanded considerably. Chakraborty and colleagues reported that more than 20 miR-and siRNA based therapeutic clinical trials have been launched; however, only one miR therapeutic has officially entered clinical trials [20]. There are several in the preclinical phases of research that are quickly progressing into clinical trials but further study is needed. In just over two decades, miR biology has provided us with insights into the roles of miRs in many diseases such as but not limited to colorectal cancer (CRC) [21], breast [22] and cervical cancer [23]. More importantly, investigators have found that miRs can act as either tumor suppressors or oncogenes [24]. Functional studies have confirmed that miR-targeted therapeutics may be one of the most effective targets for mediating cancer prevention [25]. More importantly, studies have observed that inhibiting oncomiRs and/or force expressing tumor suppressing miR mimics has led to the development of strategies that may aid in treating many forms of malignancy [26].

siRNAs are a class of double-stranded RNA (dsRNA) molecules approximately 20–25 nucleotides long. Similar to miRs, siRNAs are also members of the endogenous RNA interference pathway (RNAi) and function as complementary nucleotide sequences to inhibit protein translation by degrading mRNA [27]. The major difference between siRNAs and miRs is that the latter has many target genes, while the former must be fully complementary to the mRNA target sequence. Because of this, there is a single target gene for each siRNA, significantly increasing its target specificity. Although siRNAs were initially discovered in plants, their discovery has led to a surge in information in the field of cell biology. Many siRNAs are used to knock down genes by recruiting the dicer enzyme to cut the dsRNA into a single-stranded siRNA which can then be incorporated into the RNA induced silencing complex (RISC) (Figure 1). The single-stranded siRNA binds to target mRNA, which results in the degradation of mRNA and an inhibition of protein translation. siRNAs have made a major impact on the biomedical research industry and are of particular interest in drug discovery because of their high target specificity [28].

piRNAs are the most distinct and understudied ncRNAs to date. Unlike miRs and siRNAs that have nucleotide lengths of 20–25, piRNAs are the largest of the small non-coding RNA molecules with an approximate length of 25–33 nucleotides. piRNAs form protein complexes through directly interacting with piwi proteins and have been documented to protect germline cells from transposons in many different organisms (Figure 1) [29]. Since their initial discovery, much has come to light regarding their function; however, investigators are still unclear about the biogenesis pathway and maturation of these small ncRNAs.

lncRNAs are the largest of the non-coding RNAs with approximate base pair lengths greater than 200 [30]. lncRNAs have gained widespread attention and have been attributed to playing a key role in biological regulation and are numerous amounting to several thousand in comparison to the 5000 discovered miRNAs [31, 32]. Functional studies indicate that

aberrant expression levels of lncRNAs are correlated with malignant transformation and many disease processes [33, 34]. lncRNAs are expressed at very low levels in the cell and are poorly conserved between species [35, 36]. Although the molecular functions of lncRNAs are still unclear, investigators continuously highlight their functional roles in cell biology [37, 38]. A subset of lncRNAs called lincRNAs or long intergenic non-coding RNAs have been shown to block protein production by binding to mRNA. These lincRNAs have various roles in the regulation of pluripotency and the prevention of cellular differentiation [39, 40]. A point of intrigue with respect to lnc and lincRNAs is that they have the ability to function as transcriptional regulators acting both locally and distally by regulating the expression of nearby genes as well as the expression of genes across multiple chromosomes, respectively [41].

Isothiocyanates are naturally occurring compounds found in cruciferous vegetables such as watercress, broccoli, kale and broccoli sprouts [42]. Isothiocyanates are widely studied for their chemopreventive properties and have been documented to act through a number of pathways including but not limited to apoptosis, cell cycle, migration invasion and MAPK signaling [43, 44]. It is reported that isothiocyanates are derived from the hydrolysis of glucosinolates [45]. Depending on multiple variables such as climate, cultivation and plant type, the glucosinolate content significantly varies [46]. Notably, isothiocyanates are abundantly present in young plants such as broccoli sprouts or growing leaves [47]. Studies have reported that overcooking and/or boiling cruciferous vegetables could result in glucosinolate content [48]. Isothiocyanates exert many chemotherapeutic properties, but do not cause side effects as seen by chemotherapeutic agents. Importantly, isothiocyanates have also been reported to alter the expression of non-coding RNAs in many forms of malignancy. This review aims to summarize the current state of knowledge of both non-coding RNAs and isothiocyanates in cancer (Table 1).

Isothiocyanates

Sulforaphane

Sulforaphane (SFN), an isothiocyanate derived from glucosinolates in cruciferous vegetables such as broccoli sprouts, cabbage and kale, is one of the most studied isothiocyanates to date [49]. These cruciferous vegetables contain high levels of glucoraphanin which upon mastication is released as SFN by myrosinase, which is also found in the human gut [50]. Evidence suggests that SFN may target epigenetic mechanisms such as histone deacetylase inhibition (HDACi) and genome-wide methylation; however, as depicted in Figure 2, the assessment of SFN-mediated non-coding RNA regulation is a recent area of investigation [51]. ncRNAs have the ability to regulate genome stability and integrity by regulating DNA damage and response (DDR) [52]. The core protein-coding components of DDR pathways are targets of miRs, and are subjected to inhibition in damage response. SFN has been reported to induce DNA double-stranded breaks thereby promoting cellular lethality [53]. Additionally, SFN has also been reported to indirectly alter the Wnt/beta pathway. Recent studies reported that the Wnt/beta-catenin pathway is suppressed by miRNA-34, which SFN has been shown to regulate in breast cancer cells [54, 55]. Studies may reveal that this isothiocyanate increases the presence of miRNA-34 thereby suppressing the self-renewal

network of the Wnt/beta-catenin pathway. Another study reported that lncRNA expression levels are significantly altered by SFN treatments in prostate cancer (PCa) cells [56]. Although multiple lncRNAs were identified as targets of SFN, the authors used lncRNA LINC01116, which has been identified to promote cell proliferation, as a lncRNA representative to elucidate the SFN-mediated changes. A lncRNA siRNA yielded the strongest phenotypic changes in the prostate cancer (PCa) cells, with no visible changes in normal cells. Additionally, physiologically relevant amounts (at least 7 μ M concentration) of SFN resulted in a significant reduction in lncRNA LINC01116.

Lewinska et al. observed significant changes in more than 90 miRs and significantly inhibited the expression of miR-23b, miR-92b, miR-381 and miR-382 by regulating the AKT/NF-KB, TGF- β /Smad3/p21, TGF- β /Smad and RERG/Ras/ERK pathways respectively. The changes reported were mediated by physiologically relevant doses of SFN [57]. Zhu et al. also documented that SFN significantly inhibited miR-19 by regulating the Wnt/ β -catenin pathway in lung cancer stem cells [58]. Investigators have reported on SFN's ability to exert anti-cancer properties in non-small cell lung cancer cells. Further, they showed that the upregulation of tumor suppressor miR-214 was targeted by the coding region of master regulator, c-MYC. Importantly, the SFN/miR-214 signaling sensitized non-small cell cancer stem cells to cisplatin treatments [59]. SFN has also been documented to possess anti-metastatic effects in non-small cell lung cancer cells. Wang and colleagues observed that SFN significantly decreased miR-616-5p levels which lead to a significant regulation of the GSK3/ β -catenin pathway [60]. SFN has also been shown to dose-dependently increase tumor suppressor miR-200c in oral squamous cell carcinomas and suppress cancer stemness both *in vitro* and *in vivo* [61]. Interestingly, SFN has also shown great promise in upregulating miR-let-7, while significantly inhibiting K-ras alone and in combination with other dietary phytochemicals [62]. Evidence places emphasis on SFN's modulatory ability of miRs indicating it to be an ideal candidate for additional clinical trials.

SFN has also been documented to significantly alter miR expression profiles in nontransformed human epithelial cells [63]. A significant down-regulation of miR-155, an oncogenic miR, has been observed following SFN treatment [64]. Moreover, a significant induction of miR-200c was observed following treatment with SFN in human bladder T24 cancer cells in another study [65]. Importantly, following a micro-array analysis, it was found that 68 miRs were dysregulated in breast ductal carcinoma in situ (DCIS) lesions [66]. Li et al. reported that 72 miRs were dysregulated in the DCIS lesions as compared to control MCF10A cells [67]. Also observed was the finding that independent of histological grade, there was a significant miR-140 loss in all malignancy types. It was reported that there was a major difference in miR-140 expression between normal and cancer stem-like cells. Li and colleagues also reported that treatment with SFN of DCIS tissues resulted in an activation of miR-140 expression [67]. Other studies have also observed that treatment with SFN of DCIS stem-like cells resulted in a significant inhibition of oncogenic miRs miR-21 and miR-29. miR-21, a well-known oncogenic miR, has been reported to be dysregulated in many forms of cancer [68] [69]. Lan and colleagues reported that SFN enhanced the efficacy of temozolomide, a chemotherapeutic agent widely used for the treatment of glioblastomas, by significantly inhibiting miR-21 in glioblastoma cells [70]. It is well known that the

upregulation of miR-21 is associated with a poor prognosis in various cancer forms [71–73]. Because of this, it is apparent that studies altering miR expression are greatly warranted.

Accordingly, we evaluated the effects of SFN on oncogenic miR-21 with physiologically relevant concentrations of SFN on colorectal cancer (CRC) cells [74]. We observed that the treatment of CRC cells with SFN resulted in a significant inhibition of oncogenic miR, miR-21. Additionally, we also observed a significant inhibition of human telomerase reverse transcriptase (hTERT) protein and enzymatic activity, which is elevated in 90% of human cancers. Collectively, these studies suggest that SFN is a vital isothiocyanate in mediating epigenetic regulation via non-coding RNAs.

Benzyl-isothiocyanate

Benzyl-isothiocyanate (BITC) is a member of the Brassica family and is derived from cruciferous vegetables such as cabbage and garden cress [75]. Second to SFN, BITC is one of the most-studied members of the isothiocyanates family. BITC has been documented to inhibit cancer in many animal models [76–79] and BITC has also demonstrated strong anti-cancer effects via HDAC inhibition in pancreatic cancer cells [80]. Although the study of BITC and its regulatory roles in ncRNAs are limited, a few studies have opened avenues for greater exploration. For example, miR-375, known to function as a tumor suppressor in pancreatic cancer cells, was significantly upregulated by BITC treatments [81]. Moreover, these investigators also observed a significant inhibition of oncogenic miR-221 following BITC treatments of pancreatic cancer cells. Although the aforementioned studies are informative and novel, future studies are warranted to investigate the interaction between BITC and ncRNAs.

Allyl isothiocyanate

Another major isothiocyanate that has been studied in relation to cancer treatment and prevention is allyl isothiocyanate (AITC) which is the hydrolysis product of sinigrin [82]. This compound is commonly referred to as mustard oil and is responsible for the pungent taste of horseradish, mustard and plants from the Brassicaceae family. According to estimates, approximately 90% of this compound is absorbed upon consumption [83] and has been shown to have anticancer properties in a number of studies. For example, Lai and colleagues found AITC to be capable of inhibiting migratory ability of colorectal cancer cells (CRCs) [84]. Treatment with AITC in CRCs resulted in the down-regulation of matrix metalloproteinase-2 (MMP-2), MMP-9 and mitogen-activated protein kinases (MAPKs) (p-JNK, p-ERK and p-p38) at the protein level which suggests that AITC mediates the MAPK and MMP-2/–9 signaling pathways thereby impeding the metastatic potential of these cells. Other investigators have reported AITC to induce reactive oxygen species while decreasing mitochondrial membrane potential in breast cancer cells [85]. They further examined genes involved in the apoptotic pathway including caspase-8–9 and –3 and revealed this natural compound to be instrumental in their upregulation. Interestingly, both cell lines studied showed decreases in the anti-apoptotic *Bcl-2* and increases in the proapoptotic *Bax*. However, the estrogen receptor α positive (ER α +) MCF-7 cells appeared to undergo apoptosis via the Endo G signaling pathway whereas the triple-negative MDA-MB-231 breast cancer cells achieved apoptosis through the GADD153 pathway. Similarly, Geng and

associates report AITC's induction of apoptosis and apoptotic pathway genes in bladder cancer [86]. Further, AITC caused *Bcl-2* phosphorylation thereby nullifying *Bcl-2*'s protective properties by allowing lipid peroxidation. miR-30a-5p is considered a tumor suppressor in multiple cancer types due to its repression [87, 88]. Studies have indicated that this miR can be induced through the introduction of AITC. Recent studies observed a significant AITC-mediated induction of miR-30a-5p liver and colon cancer cells [89, 90]. In 2012, Wagner and colleagues reported AITC to be capable of decreasing TNF α and pro-inflammatory markers [91]. They also show an attenuation of miR-155 levels in LPS-stimulated RAW264.7 macrophages. Others showed that hydrated mustard seed powder caused apoptosis and G2/M phase arrest in bladder cancer cell lines proving the bioavailability of AITC as well as its anticancer potential [92]. Though much research has been conducted examining the efficacy of this compound, more study of its epigenetic potential in cancer seems warranted, especially considering AITC's bioavailability and its efficacy in promoting cancer cell death. PEIT

Phenylethyl isothiocyanate

Phenylethyl isothiocyanate (PEITC), derived predominantly from watercress, is known for its roles in histone modification and DNA methylation [93]. A recent study conducted by Izzotti and colleagues examined miRs through microarray analysis and found that well over 500 exhibited an increased gene expression in the presence of environmental cigarette smoke [94]. PEITC protected the lung from environmental cigarette smoke-induced alterations of miR expression but exhibited some adverse effects in liver. More recently, the Kong group analyzed PEITC's anti-cancer roles in prostate cancer [95]. They report that its suppression of miR-194 is linked to the down-regulation of oncogenic MMP-9 and MMP-2 suggesting PEITC reduces prostate cancer cell invasiveness and metastatic potential through targeting BMP1.

Notably, miR-141 expression was also down-regulated by PEITC in prostate cancer cells [96]. It was shown that PEITC is involved in the inhibition of androgen receptor transcriptional activity, which when mutated causes prostate cancer cell progression. In another study, miR-135a was shown to be upregulated by PEITC thereby promoting cell death in rat C6 glioma cells [97]. This same study reports the activation of apoptotic pathway genes caspase-9 and -3. Jutooru and colleagues observed that PEITC reduces the expression of miR-27a, miR-20a and miR-17-5p while inducing the transcriptional repressors ZBTB-10, 4 and 34 thereby down-regulating Sp1, Sp3 and Sp4 in pancreatic cancer cells [98]. A number of additional effects resulting in cancer cell growth inhibition invoked by PEITC treatment were also described. Recently, Yu and colleagues observed that treatment with PEITC reduced p300, CBP-associated factor (PCAF), a vital regulator of the androgen receptor [99]. They found that the PEITC-mediated- PCAF inhibition was mediated by significant upregulation of miR-17 in prostate cancer cells. PEITC is an isothiocyanate that has been shown to target multiple pathways that affect a wide variety of miRs, ultimately affecting many mRNA targets. While much of the current literature focuses on PEITC and its indirect effects of miRs, the role that PEITC plays on directly modulating miRs remains unclear. Investigating the effects of PEITC and other vital isothiocyanates on miR modulation is greatly warranted.

Conclusion

The isothiocyanates are excellent candidates for cancer prevention and adjuvant therapy and exciting information on the efficacy of these compounds in cancer and their modulation of non-coding RNAs are emerging. It is apparent that isothiocyanates have underlying mechanisms by which they impede cancer cell progression and disease processes are epigenetically regulated by non-coding RNAs including miRs and long lncRNAs. Isothiocyanates have shown much promise in cancer cell growth inhibition, although it is apparent that considerable more studies elucidating the molecular mechanisms involved in the regulation of tumor cells or lack thereof are warranted.

Acknowledgments

This work supported by grants from the National Cancer Institute (R01 CA178441; R01 CA204346) and the American Institute for Cancer Research (316184).

Abbreviations:

ACS	American Cancer Society
AITC	Allyl isothiocyanate
BITC	Benzyl-isothiocyanate
CRC	Colorectal cancer
DCIS	Ductal carcinoma in situ
dsRNA	Double-stranded RNA
ERα+	Estrogen receptor alpha positive
HDACi	Histone deacetylase inhibitor
hTERT	Human telomerase reverse transcriptase
lncRNAs	Long non-coding RNAs
MAPK	Mitogen-activated protein kinases
miR	microRNA
MMP	Matrix metalloproteinase
ncRNA	Non-coding RNA
PCa	Prostate cancer
PEITC	Phenylethyl isothiocyanate
piRNA	piwi interacting RNAs
RISC	RNA induced silencing complex
RNAi	RNA interference

SFN	Sulforaphane
siRNA	short interfering RNA
UTR	Untranslated region

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Figure 1. Small non-coding RNA biogenesis.

Depiction of the biogenesis of small ncRNA. A) Biogenesis of siRNA. B) Biogenesis of miRNA. C) Biogenesis of piRNA.

Figure 2. Isothiocyanates and non-coding RNA modulation.
 Illustration of the ability of isothiocyanates to modulate ncRNAs in cancer.

Table 1.**Isothiocyanates and cancer.**

Examples of the major isothiocyanates SFN, AITC, BITC and PEITC that have been reported to treat various forms of cancers.

Isothiocyanate	Chemical Structure	Cancer Affected	Food Source
Sulforaphane		Bladder [41], prostate [38], colon cancer [49], ductal carcinoma [42], and DCIS stem-like cells [43]	Cabbage Broccoli Sprouts Broccoli
Allyl Isothiocyanate (AITC)		Colorectal [61], [65], breast [62] and liver cancer [63]	Broccoli Cabbage Mustard Greens Broccoli Sprouts
Benzyl Isothiocyanate (BITC)		Pancreatic cancer [56] [58]	Cabbage Garden Cress
Phenyl Isothiocyanate (PEITC)		Liver [69], prostate [71], glioma, pancreatic cancer [73]	Watercress

Examples of the major isothiocyanates SFN, AITC, BITC and PEITC that have been reported to treat various forms of cancers.