Guanine content of precursor microRNA's terminal loop and its association with cancer

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Abstract: MicroRNAs (miRNAs) are small noncoding segments of RNA that negatively regulate gene expression at the post-transcriptional level and fine-tune gene functions. Several lines of evidence suggest that terminal loops (TLs) of miRNAs are important features determining their processing efficiency. A global repression in miRNAs expression in different types of human tumors, after exposure to cigarette-smoke (CS), or to the hormone estrogen, was shown to be associated with guanine (G) enrichment in the TLs of their precursors. In this review we summarize the results that show the relation of TLs G content to the regulation of miRNA maturation and function, suggest a new G-dependent miRNA-related model of carcinogenesis, and specify several dietary phytochemicals that can be used for its prevention.

Keywords: Guanine; microRNA (miRNA); cancer; estrogen; cigarette-smoke (CS); c-Myc; DNA adducts; cruciferous vegetables; Phenethyl Isothiocyanate; Indole-3-Carbinol; Sulforaphane; Resveratrol

Background

MicroRNAs (miRNAs) are endogenous ~22-nucleotides (nt) RNA molecules, that negatively regulate gene expression at the post-transcriptional level and have a role in networking and fine-tuning gene expression in the cell (1). The miRNA maturation process begins with the primary long transcript (pri-miRNA), which is first processed by an RNase, termed Drosha, that cuts it into ~70-nt stem-loop (SL) precursor (pre-miRNA), containing the mature miRNA sequence in one of its arms and the less abundant partially complementary miRNA mature form in the other arm (2,3). After the first processing step, pre-miRNA is actively transported by exportin-5 (XPO5) from the nucleus to the cytoplasm, where it is processed by another RNase, termed Dicer (4,5). The result of this processing event is a double stranded RNA, where one of its strands is incorporated into

the argonaute (Ago) protein of the RNA-induced silencing complex (RISC) that targets it to a 3' untranslated region (3'UTR) of a specific mRNA and leads to its degradation (1).

A comprehensive reduction in miRNA was commonly observed in human cancers, where miRNAs showed lower expression levels in tumors and cancer cell lines compared with normal tissues (6-9). A widespread repression of miRNA expression has also been reported after exposure to cigarette-smoke (CS) (10-12), treatment with the hormone estrogen (13-15), and c-Myc activation (16), and the observed global downregulation of miRNAs was also inversely correlated with their predicted targets. These aforementioned alterations in miRNA expression can occur as a result of affecting the transcription of miRNA genes (16), miRNA export from the nucleus (17), or at any stage of the miRNA maturation process by modulation of key regulators or components of the miRNA biogenesis

pathway, including the microprocessor complex Drosha-DGCR8, and Dicer (18).

Guanine enrichment in TL sequences of cancer/estrogen/Myc-repressed miRNAs

There are several indications for the importance of guanine (G) content in miRNAs TL sequences to the regulation of their biogenesis and function. Izzotti and Pulliero showed in their study that the G content of the TLs of miRNAs which are involved in stress response, is higher than the G content of the other miRNAs (19). We have recently found an association between the widespread miRNAs reduction that is observed in human cancers and a high TL G content in their precursors (20,21). Using bioinformatic analysis of zebrafish, mouse, and human breast cancer cell lines, we also showed that similar G enrichment exists in TLs of downregulated miRNAs after estrogen (17β-estradiol; E2) exposure (22), and most striking was the observation that of the different G combinations in TL sequences of both cancer and E2-repressed miRNAs, the relative enrichment of double G (GG) and triple G (GGG) was especially dominant (21). Remarkably, this phenomenon is also observed when looking at the ten most c-Myc-repressed miRNAs of a human B cells model (16), where six of them are also common to cancer-repressed miRNAs (miR-15a, miR-24, miR-29a, miR-29c, miR-125b, miR-195) (20), and the other four to E2-repressed miRNAs (miR-23a, miR-23b, miR-26a, miR-27a) (22). The transcription factor c-Myc physically interacts with estrogen receptor alpha (ERα) and is recruited to estrogen-responsive genes (23). Indeed, estrogen can cause cellular growth, proliferation and cancer by inducing oncogenes such as c-Myc (24). Therefore, part of the global miRNA downregulation that is observed after exposure to estrogen, might be attributed to c-Myc regulation. It is interesting to note, however, that c-Myc activation was also shown to be associated with an altered estrogen metabolism (25). The potential carcinogenic activity of estrogen involves the oxidative metabolism of estrogens to catechol estrogens and the reactive quinone metabolites, that form specific DNA adducts at the *N*-7 G (26,27), and it was shown that miRNAs are even more sensitive than DNA to the formation of G-adducts (19). These adducts generate apurinic sites that can be converted into mutations by error-prone repair, which in turn may initiate tumorigenesis (28). Also, oxidative metabolites of estrogens can react with DNA to form 8-oxo-dG (8-Oxo-2'-deoxyguanosine); the most frequent DNA oxidative

damage, which eventually leads to carcinogenesis (29), because G has lower oxidation potential and is most easily oxidized among the four DNA bases (30). Most interestingly, experimental studies have shown that sequences with repeated G bases (GG or GGG) show higher reactivity toward oxidation than isolated G bases (31).

Alterations of G nucleotides in pre-miRNA TL affect miRNA expression level

Several successive studies, conducted by the Chen's group, have shown that G substitution in pre-miRNAs TLs disrupt their maturation process. In their study, Liu *et al.* demonstrated that pre-miRNA loop nucleotides play an important role in controlling the biological activity of miRNAs (32). Specifically, substitution of nucleotides GG to CC in the pre-miRNA loop of miR-181a-1 reduced the activity of mir-181a-1 on T cell development by 70%, and cells transfected with this mutant expressed significantly less mature miR-181a (32). Similarly, using a GG to CC loop mutant of let-7 pre-miRNA resulted in a significant reduction in mature miRNA expression levels and in the activity of target gene repression (33,34). Together, the results of these studies show that terminal loop (TL) mutagenesis of GG affects miRNA level and function, which seems to be caused by alterations of loop sequence and/or structure.

G-enriched motifs in miRNA TL sequences affect miRNA processing

Findings suggest that the miRNA TL is an important platform for different RNA-binding proteins (RBPs) that act as activators or repressors of Drosha and Dicer processing, and selectivity regulate miRNAs by binding to G-enriched motifs in the RNA TLs of their precursors (35). It was shown that miRNAs with the tetra-nucleotide sequence motif GGAG in their TL were regulated through binding of the RBP Lin28, which interferes with Dicer processing (36), and that the sequence AGGGU in the TL mediates regulation of miRNA biogenesis by the KSRP RBP (37). In their study, García-Mayoral *et al.* described the complete analysis of the RNA-binding potential of the four KH domains of KSRP and showed that the KH3 domain can recognize a G-rich sequence (38). Insertion of an isolated G led to a 5-fold increase in KH3 affinity, whereas insertion of a GG element led to a further 4-fold increase (38). Interestingly, KH3 binding
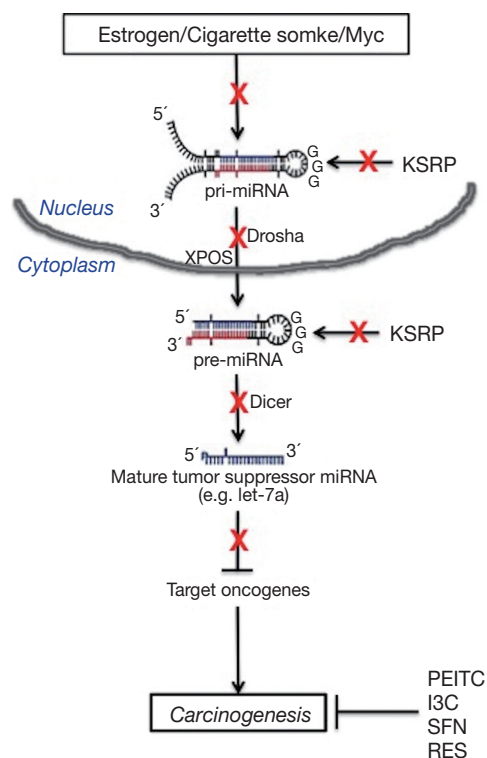
Figure 1 A model summarizing the miRNA biogenesis pathway and its possible regulation through G enrichment in miRNAs TLs. Disruption of transcription and processing of tumor suppressor miRNAs (denoted by the red crosses) by Estrogen/Cigarette smoke/Myc may lead to carcinogenesis, while phytochemical compounds (PEITC, I3C, SFN, RES) may potentially prevent it.

docks KSRP to the GGG-containing TL of a subset of miRNAs and promotes their maturation (37). Of note, our results revealed a high enrichment for the sequence motif GGAG in TLs of cancer and E2-repressed miRNAs (20,22). Moreover, also a significant enrichment of the GGG motif in TLs of these miRNAs was observed (21). Therefore, G-adducts formation that disrupts binding of KSRP to the TL might be a possible cause for the observed cancer and E2-repressed miRNAs. Indeed, it was recently shown that modification of KSRP resulting in the downregulation of a subset of TL G-rich miRNAs and promoting tumorigenesis (39).

Dietary anti-estrogenic phytochemical compounds used for cancer chemoprevention

It is well known that dietary phytochemicals from vegetables and fruits exhibit chemopreventive activities against various types of cancer (40). Administration of the dietary agents

Phenethyl Isothiocyanate (PEITC) and Indole-3-Carbinol (I3C), two major components of cruciferous vegetables, attenuated the cigarette smoke-induced downregulation of miRNA expression (41). The combined treatment with PEITC and I3C had profound effects on almost all CS-downregulated miRNAs and their expression even exceeded the baseline situation (41). PEITC, which has both chemopreventive and chemotherapeutic effects (42), was also the most effective agent in inhibition of CS-related cytogenetic damage, transcriptome alterations, and lung tumorigenesis (43-45). Also, I3C and its condensation product 3,3'-diindolylmethane (DIM) exhibited potent anti-tumor effects with negligible levels of toxicity in a wide range of human cancer cells, including lung cancer (46). Interestingly, both PEITC and I3C have proved to be anti-estrogenic compounds and inhibited ERalpha expression (47-51). Furthermore, PEITC was shown to significantly inhibit the formation of the xenoestrogen bisphenol A (BPA)-induced DNA adducts in mice (52).

Another compound that has anti-estrogenic effects is the dietary polyphenol derived from grapes, Resveratrol (RES). This natural product is known as an antioxidant and antimutagen, with cancer chemopreventive activity (53).

The Cavalieri-Rogan's groups have described in multiple reports that RES decreases estrogen metabolism, and prevents estrogen-DNA adduct formation (54-57). In these studies, RES was shown to block the oxidation of catechol estrogens to their quinones and their reaction with DNA, and by this way to prevent cancer initiation (27). Both RES and Sulforaphane (SFN), additional isothiocyanate of cruciferous, were shown to induce protective phase II enzymes activity, resulting in reduction of estrogen-induced DNA damage (58).

Conclusions

Taken together, the above results suggest that G content, especially GG and GGG, in miRNA TL sequences, may have some important role in the carcinogenic process induced by estrogen and c-Myc. As mentioned before, the mechanisms of estrogen carcinogenesis include unbalanced estrogen metabolism and the formation of G-adducts, which can also potentially be formed in miRNA TLs. This raises the possibility that G oxidation and/or formation of G-adducts in TLs may lead to the extensive downregulation of tumor suppressor miRNAs, which will then cause induction of their target oncogenes, and commit cells towards carcinogenesis (*Figure 1*).

Indeed, several of the repressed miRNAs were shown to function as tumor suppressors (21), and were upregulated by dietary phytochemical agents such as PEITC, I3C and RES (59).

Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/jxym.2018.09.01). The author has no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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