



Guanine residues of precursor microRNA's terminal loop as a potential target for cancer therapy and prevention

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Abstract: MicroRNAs (miRNAs) are small endogenous RNA molecules with diverse biological functions that have been implicated in various human diseases, including cancer. Global downregulation of miRNA expression in different human cancer types, after exposure to cigarette-smoke, or to the hormone estrogen, have been shown to be associated with guanine (G) enrichment in the terminal loops (TLs) of their precursors. Here, we describe the role of G content of TLs in the regulation of miRNA expression, and highlight its potential as a target in cancer prevention and treatment.

Keywords: Guanine; microRNA; cancer; estrogen; cigarette-smoke; oxidative stress; DNA adducts; cruciferous vegetables; DNA repair; acyclovir

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Introduction

MicroRNAs (miRNAs, miRs) are small endogenous noncoding RNA molecules that negatively regulate gene expression affecting many biological processes and diseases, including cancer (1,2). The active and mature miRNA form is ~22 nucleotides (nt) in length, and is the result of a multiple-step process. This begins with the primary long transcript (pri-miRNA) being processed by an RNase, termed Drosha, and its partner protein DiGeorge syndrome chromosomal region 8 (DGCR8), that cut it into a ~70-nt stem loop (SL) precursor (pre-miRNA), which contains the mature miRNA sequence in one of its arms and the less abundant, partially complementary miRNA mature form, in the other arm (3,4). The pre-miRNA is then actively transported by exportin-5 (XPO5) from the nucleus to the cytoplasm, where it is processed by another RNase, termed Dicer (5,6). The result of this second 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 commonly targets it to a 3' untranslated region (3'UTR) of a specific mRNA and leads to its degradation (1).

The precursor miRNA terminal loop (TL) is an important platform for different RNA-binding proteins (RBPs) which operate as activators or repressors of Drosha and Dicer (7). Two of these RBPs are known to selectively regulate miRNA processing by binding to guanine (G) residues-enriched motifs in the TL: miRNAs with the sequence motif GGAG in their TL are regulated through the binding of the RBP Lin28, which interferes with Dicer processing (8), while the sequence AGGGU in the TL promotes miRNA maturation by the K-homology splicing regulatory protein (KSRP) RBP (9). It has recently been shown that modification of KSRP results in the downregulation of a subset of TL G-rich miRNAs, subsequently promoting tumorigenesis (10).

The widespread downregulation of miRNA expression

is a phenomenon observed in many types of human cancer (11-14). A comprehensive repression of miRNA expression has also been reported after exposure to cigarette-smoke (CS) (15-17), and treatment with the hormone estrogen (17 β -estradiol; E2) (18-20). These aforementioned alterations in miRNA expression can occur as a result of changes in the transcription of miRNA genes, as was shown after c-Myc activation (21), miRNA export from the nucleus (22), 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 (23).

Recently, we have found an association between the comprehensive miRNA reduction observed in human cancers and a high TL-G content in their precursors (24,25), as well as a similar G enrichment existing in TLs of downregulated miRNAs after E2 exposure (26). The potential carcinogenic activity of estrogens involves their oxidative metabolism to catechol estrogens and the reactive quinone metabolites forming specific DNA adducts at the N-7 G (27,28). These adducts generate apurinic sites that can be converted into mutations by error-prone repair, which in turn may initiate tumorigenesis (29). Furthermore, oxidative metabolites of estrogens form 8-oxoguanine (8-oxoG); a major product of oxidation damage, which serves as a biomarker of oxidative stress and eventually leads to carcinogenesis (30).

Because G has a lower oxidation potential it is most easily oxidized among the four nucleobases (31). Also, sequences with repeated G bases (GG or GGG) show higher reactivity toward oxidation than isolated G bases (32). Most interestingly, 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 (24-26). Therefore, oxidation and/or adduct formation by carcinogens, such as CS and estrogen metabolites, that react with G/GG/GGG in precursor of tumor suppressor (TS) miRNA TL may contribute to the development of cancer. Herein we suggest several hypotheses and potential ways for the prevention of cancer that may be initiated by interaction of carcinogens with the G content of TS precursor miRNA's TL.

Cellular pathways used to repair G damage

There are various types of DNA repair mechanisms that specialize in removing different kinds of DNA lesions caused by endogenous and environmental insults, thereby

helping to prevent cancer. One example is the base excision repair (BER) system used to repair oxidative lesions caused by reactive oxygen species (ROS) such as 8-oxoG (33,34). Another example is O⁶-methylguanine DNA methyltransferase (O⁶MT) that removes O⁶-alkylguanine adducts and effectively restores the G base in DNA (27).

A number of lines of evidence indicate that there are several possible cellular repair mechanisms to cope with RNA damage (35). For example, it has been previously shown that the repair of damaged bases in RNA can be executed by several members of the AlkB family of enzymes, by a unique oxidative demethylation repair mechanism that removes methyl adducts (36). Thus, activation of such cellular defense mechanisms may represent a possible therapeutic direction for repairing various adducts (37), including potential G adducts in miRNA TLs.

The potential use of G analogs for cancer prevention

Several guanosine analogs, such as acyclovir (ACV), are widely used for the treatment of herpesvirus infections and also as antitumor agents for the combined chemotherapy of cancer (38). These drugs compete with deoxyguanosine triphosphate (dGTP) as a substrate for viral DNA polymerase in herpesvirus-infected human cells, resulting in early chain termination and inhibit virus DNA synthesis and replication (39). ACV is a nucleic acid analog made from guanosine (also known as acycloguanosine), which has incomplete cyclic sugar ribose where the carbons at the 3' and 4' positions are missing, while its G moiety is left intact (40). Therefore, introduction of ACV into cells may cause competition for carcinogens binding with the natural G nucleotides, and may reduce oxidatively-generated damage to cellular RNA and nuclear DNA. Notably, a recent study has shown a potent anti-cancer effect by ACV (41). ACV treatment of MCF7 breast cancer cells decreased their growth and proliferation rate, inhibiting both colony formation ability and cell invasion capacity (41). The exact role of ACV in these anti-cancer effects is currently unknown (41), however, it may potentially involve protection of G-rich TS miRNAs.

Restoration of TL G-enriched TS miRNA expression

As mentioned above, the global miRNA repression observed in cancer has been found to be associated with

G enrichment in the TLs of their precursors. Whether this repression is caused by the carcinogen's effects on the miRNA processing machinery (42), or through changes in the expression or deletion of miRNA encoding genes (21,43), the result is the downregulation of TS miRs (e.g., let-7, miR-34) which probably contributes to neoplastic transformation by allowing an increased expression of their target oncogenes [e.g., Kirsten rat sarcoma viral oncogene homolog (KRAS), epidermal growth factor receptor (EGFR)]. Intriguingly, we have recently revealed that the pre-miRNAs TLs of TS miRs are predominantly G enriched (44).

A therapeutic approach for systemically delivering synthetic TS miR mimics have been demonstrated, including several studies showing that restoring the oncosuppressor activity of miR-34a can successfully inhibit tumor growth (45,46). Interestingly, miR-34a precursor TL has a relatively high G content (35% G enrichment). Another potential candidate for such an intervention is miR-218, which is downregulated and acts as a TS miR in various types of human cancers, including lung cancer (47), where it is shown to inhibit cell proliferation and migration by targeting the EGFR oncogene (48). MiR-218 is the most differentially expressed miRNA in the airway epithelium of smokers, with its expression being 4-fold down-regulated (16). Its predicted targets, such as V-Maf Avian Musculoaponeurotic Fibrosarcoma Oncogene Homolog G (MAFG), EGFR-coamplified and overexpressed protein (ECOP), and LIM and SH3 protein 1 (LASP-1), are significantly overrepresented among the genes upregulated in the bronchial epithelium of smokers (49). Moreover, the pre-miRNA's TL of miR-218 is remarkably G-enriched (43% G enrichment) (44).

Fruits and vegetables and their phytochemicals used for cancer chemoprevention

The preventative and therapeutic effects of using fruit

and vegetables and their dietary phytochemicals against various types of cancer are well documented (50,51). Amongst them, cruciferous vegetables have been extensively studied and are especially known for their cancer chemopreventive compounds; phenethyl isothiocyanate (PEITC), sulforaphane (SFN), and indole-3-carbinol (I3C) (52,53). Administration of PEITC and I3C attenuated the CS-induced downregulation of miRNAs (54), which were shown to have a high G content in their TLs (42). Furthermore, PEITC was shown to significantly inhibit the formation of the xenoestrogen bisphenol A (BPA)-induced DNA adducts in mice (55).

Another compound with known antioxidant and chemopreventive activities is the dietary polyphenol derived from grapes, Resveratrol (RES) (56). Multiple studies have shown that RES prevents cancer initiation by blocking oxidation of catechol estrogens to their quinones and estrogen-DNA adducts formation (57-60). Further, both RES and SFN were shown to induce protective phase II enzymes activity, resulting in reduction of estrogen-induced DNA damage (61). Thus, increasing fruits and vegetables (including cruciferous) intake in the diet seems to be a simple and effective way for cancer prevention (62).

Conclusions

Endogenous and exogenous carcinogens may oxidize and form adducts at the G (especially GG and GGG) content of TS miRNAs TLs. The resulted G lesions may cause extensive repression of TS miRNAs, leading to the induction of their target oncogenes and carcinogenesis, while several potential methods may be used for its prevention (*Figure 1*). Once the molecular mechanisms of global miRNA downregulation during tumorigenesis is fully elucidated, it can lead to the development of novel strategies for combating cancer. Revealing the role of G content of precursor miRNAs TL in these processes appears to be a promising direction towards this goal.

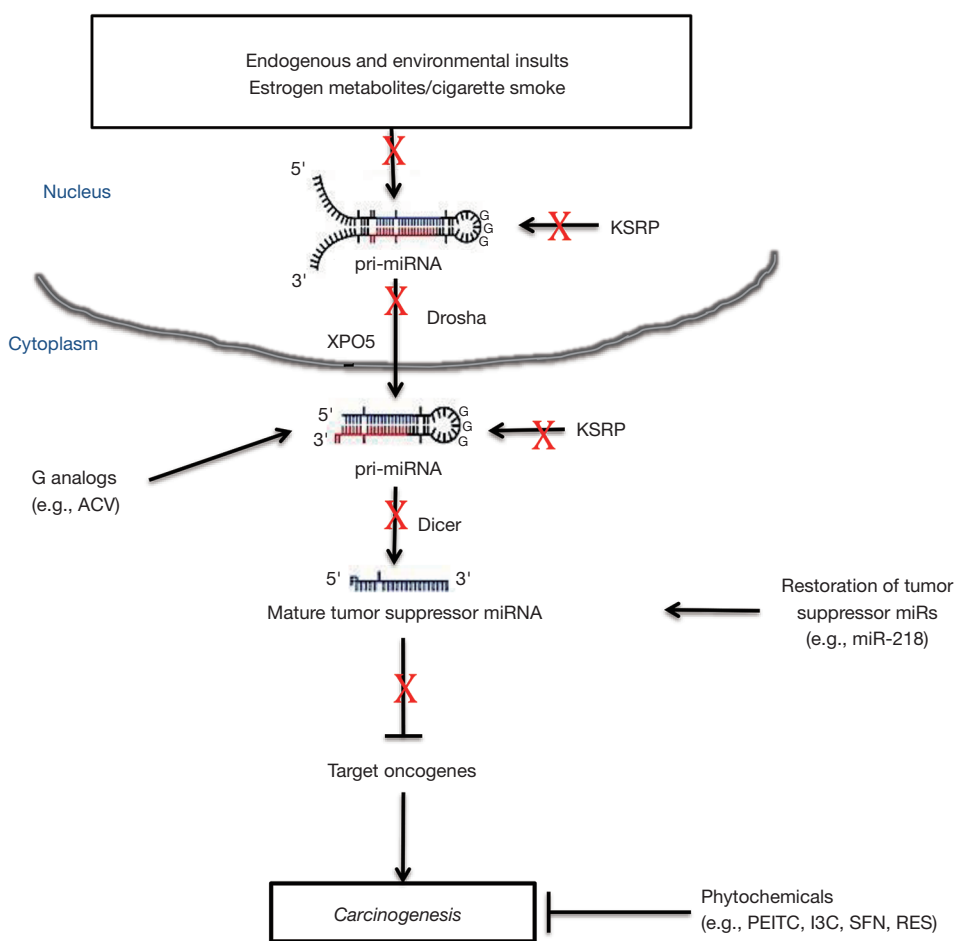


Figure 1 A flowchart describing the miRNA biogenesis pathway and its possible modulation through the guanine (G) content in terminal loops (TLs). Disruption of transcription and the processing of tumor suppressor (TS) miRNAs (denoted by the red crosses) by endogenous and environmental carcinogens, such as estrogens/cigarette-smoke (CS), may lead to carcinogenesis, while introduction of TS miRs/G analogs/phytochemical compounds, may potentially prevent it. ACV, acyclovir; KSRP, K-homology splicing regulatory protein; XPO5, exportin-5; PEITC, phenethyl isothiocyanate; SFN, sulforaphane; RES, resveratrol; I3C, indole-3-carbinol.

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Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at <http://dx.doi.org/10.21037/pcm.2019.01.02>). The authors have 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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