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Mechanistic Role of MicroRNA in Cancer Chemoprevention by Nonsteroidal Anti-inflammatory Drugs

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Abstract

Over the past several decades, studies have documented the significance of nonsteroidal anti-inflammatory drugs (NSAIDs) on cancer chemoprevention by lowering incidence and slowing down progression of malignant disease, which consequently lead to decline of cancer-related mortality and improvement of disease progression free survival (PFS). Inhibition of cyclooxygenase (COX) has been primarily believed to be the key mechanism responsible for anticancer activity of NSAIDs, while the serious toxicity caused by COX inhibitory effect reduces the enthusiasm to use NSAIDs as chemoprevention agents in the clinic. Recently, more and more studies demonstrate that non-COX inhibitory mechanisms may account for anticancer properties of NSAIDs, at least partially, which potentially support the indication of NSAIDs on cancer chemoprevention. MicroRNAs (miRNAs) are a set of non-coding and small RNA molecules with master regulatory effect on over 30% human genes through the post-transcriptional and translational modulation. Although miRNAs have been reported to be involved in many normal and pathological processes including cell proliferation, apoptosis, differentiation, as well as tumorigenesis, their roles in NSAIDs' properties of cancer chemoprevention have not yet been studied exclusively. Here, we will review the prior studies reporting interactions between miRNAs and COX/non-COX pathways with intent to provide insights into better understanding molecular mechanisms of cancer chemoprevention by NSAIDs.

Keywords

MiRNAs; NSAIDs; Cancer; cyclooxygenase (COX); COX-2; chemoprevention

Introduction

According to the latest world cancer report, the global burden of cancer rose to an estimate of 14 million new cases per year, which is predicted to reach 22 million annually within the next twenty years[1]. Although the cancer burden grows at an alarming pace, fortunately, cancer prevention plays a crucial role in combating the tidal wave of cancer that we have seen coming across the world over the past a few decades. In particular, cancer

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chemoprevention is referred to reversion, suppression, or prevention of either the initial phase of carcinogenesis or the development of neoplastic cells to cancer by the use of natural, synthetic or biologic substances[2,3]. Nonsteroidal anti-inflammatory drugs (NSAIDs) are generally a chemically diverse group of agents with anti-inflammatory, antipyretic, and/or analgesic properties. Intriguingly, numerous reports support the anti-neoplastic role of NSAIDs in various human tumors, such as colon, breast, lung, prostate, gastric, and bladder cancer [4-6], although the molecular mechanisms by which NSAIDs prevent tumorigenesis and progression have not been understood completely.

MiRNAs are a class of noncoding small (averaging 20 nucleotides) RNA molecules that can negatively regulate gene expression through repressing translation or affecting mRNA stability [7]. It is estimated that over 0ne third of human genes are targeted and regulated by miRNAs[8]. Because of their key role in regulation of gene expression, miRNAs have been recognized as “master” regulators of human gene expression, which are responsible for a multitude of normal biological and pathological events, such as differentiation, proliferation, cell growth, apoptosis, and tumorigenesis. In human cancer, miRNAs have been widely reported to be involved in tumor pathogenesis, progression, metastasis, prognosis, and responses of patients to chemotherapy. However, its role in cancer chemoprevention has not been studied systematically. Given that miRNAs are recognized as the master regulators of gene expression and alternation of their putative targets are almost involved in all cellular events, miRNAs could be assumed as a set of mechanistic mediators accounting for the mechanistic basis of NSAIDs' pleiotropic antineoplastic activities. To support this assumption, we will summarize the published literatures by focusing on the studies that can potentially provide insights into the mechanistic role of miRNAs in cancer chemoprevention by NSAIDs.

1. Cancer chemoprevention of NSAIDs

NSAIDs are referred to a chemically diverse family of drugs that are usually used for treatment of a variety of inflammatory conditions and/or relief of pain caused by diseases, such as arthritis. They are emerging as a particularly valuable class of chemopreventative agents based on their documented anti-tumor properties. Previous studies reported that the use of NSAIDs was associated with a reduced colorectal cancer risk and incidence [9,10], and long-term use of low-dose selective NSAIDs, such as aspirin, could reduce risk for colorectal and other solid tumors [6,11,12]. Thereby, it is obvious that NSAIDs play a role in cancer chemoprevention. Over the past decades, numerous studies have explored the mechanisms by which NSAIDs prevent tumorigenesis and progression, while demonstration of cyclooxygenase (COX) inhibition is one of the most significant achievements in the field of study.

Cyclooxygenase has two informs referring to COX-1 and COX-2, which are named as principle targets of NSAIDs but with distinct expression patterns and bioactivities [13]. COX-1 is a “housekeeping” gene that is commonly found in the kidney, stomach and platelets. It is responsible for the process of producing physiological prostaglandins (PGs), which are of importance for COX-1's homeostatic functions, such as to maintain the renal blood flow, mediate normal platelet function, and regulate the microcirculation of the gastric

mucosa. COX-2 is mainly expressed in macrophages, leukocytes and fibroblasts [14], but its expression levels are restricted in normal tissues. Intriguingly, COX-2 can be elevated by the inflammatory reaction. Pro-inflammatory cytokines, such as interleukin 1(IL-1) and tumor necrosis factor-alpha(TNF-α), have been shown to induce the expression of COX-2 [15].

Aside from the phenotypes in inflammation, COX increases in some types of human cancers, such as colon cancer [16]. The resistance to apoptosis, or programmed cell death may be involved in the mechanisms underlying the association between COX-2 overexpression and tumorigenic potential[15]. Given evidence in support of its signature in tumorigenesis and cancer progression, COX-2 has been named as the one of the key targets accounting for anticancer activity of NSAIDs [17,18]. This conclusion is also supported by the following studies showing that COX-1/COX-2 specific inhibitors and non-selective COX inhibitors could not only inhibit tumor cell growth, but also reduces tumor metastasis [19]. A recent preclinical study reported that NSAIDs with properties inhibiting COX-2 can effectively prevent tumor metastasis and disease progression through antiangiogenic mechanisms *in vivo* [20], which further supports that use of NSAIDs should be a viable option for cancer patients with advanced diseases.

Regardless of anticancer efficacy, NSAIDs are actually not recommended to be used for cancer chemoprevention in the clinic because of COX inhibition is often associated with potentially fatal gastrointestinal, renal and cardiovascular toxicity[21]. However, numerous studies have reported that COX inhibition does not fully account for its antineoplastic activity [22-28], which implies that alternative mechanisms can be targeted to develop safer and more efficacious derivatives. For example, Piazza's group has made a significant contribution in studies of the non-COX inhibitory mechanism by characterizing cyclic guanosine monophosphate (cGMP) phosphodiesterase (PDE) as a novel target of sulindac for breast and colon cancer prevention [29-33]. They found that sulindac sulfide (SS) can inhibit PDE5 and certain other cGMP degrading isozymes, which increase intracellular cGMP levels that activate protein kinase G (PKG) in breast cancer cells and that this activity is COX-independent[30,31]. These studies provide strong evidence in support of the COX-independent properties of sulindac leading to safer and more efficacious derivatives for breast cancer. Moreover, Wnt/β-catenin, AMPK, NF-kb, and PPARγ signaling pathways are reported to be involved in non-COX anticancer activities of selective NSAIDs[34-38]. Based on these previous accomplishments, we think that better understanding of mechanistic basis of NSAIDs' anticancer activity should be a key step to develop novel, safe, and effective agents to prevent tumorigenesis and cancer progression, while the master regulators of gene expression, miRNAs, may be involved in these underlying mechanisms.

2. MiRNAs and Cancer

MiRNAs are of single-stranded, non-coding sequences and naturally occurring small RNA molecules that bind to 3′-UTR of target genes to repress their expression at the post-transcriptional and translational levels[39,40]. Almost 30% of all human genes are regulated by miRNAs in which each is capable of mediating the expression of several hundreds of cognate messenger RNA targets simultaneously, and over one third of human genes appear to be conserved MiRNA targets[8]. Approximately a couple of thousands of human

MiRNAs have been identified that are involved in many biological processes including apoptosis, proliferation, differentiation, cell death, immune reactions, tumorigenesis, and metastasis [41,42].

2.1 MiRNA biogenesis

The biogenesis of miRNAs is similar to other RNA molecules starting from DNA transcription. MiRNAs are primarily transcribed by RNA polymerase II (pol II) as long primary transcripts known as primary-miRNAs (pri-miRNAs). These transcripts are often spliced and located within their intronic segments. Additionally, pri-miRNAs contains multiple miRNA sequences and within itself can fold back into stem-loop precursors of approximately 60-80 nucleotides, known as the miRNAs precursor (pre-miRNAs). During the process, the hairpins are recognized and excised from pri-miRNAs in the nucleus by the microprocessor complex formed by the RNase III enzyme Drosha and its binding partner DGCR8[43]. Then pre-miRNAs are rapidly exported to the cytoplasmic by the nuclear export factor - exportin 5[44,45]. And the cytoplasmic processing of pre-miRNAs into double- stranded miRNA duplexes by Dicer, a member of the RNase III superfamily of bidentate nucleases. Further processing to mature miRNAs is through a large protein complex, the RNA-induced silencing complex (RISC), which includes the Argonaute proteins as core components. Thereafter, only one strand binds with the RISC stably to become the mature miRNAs to regulate the expression of target genes. The other strand, also known as the passenger strand or miRNA*, is disposed by two alternative mechanisms: mRNA cleavage or translational repression. When it is loaded into the RISC, the only human Ago protein capable of cleaving target mRNAs, the passenger strand may be cleaved; RISC containing any Ago protein may remove miRNA* that does not require[42,45-47]. The process of miRNA biogenesis and their central function in regulation of gene expression are shown as Fig. 1.

2.2 Anti-neoplastic activity of MiRNAs

MiRNAs play a key regulatory role in the pathological processes in addition to their curial functions in normal cellular events, such as development, cell proliferation, cell differentiation, and apoptosis[41,48,49]. To date, more than 11,000 literatures have been recorded in PubMed under the key words of “miRNA and cancer.” The first direct study reporting the significance of miRNAs in cancer was published by Calin et al a decade ago [50]. They found that a 30kb deletion within the chromosome 13q14 where miR15 and miR16 are located is correlated to the incidence of B-cell chronic lymphocytic leukemia (B-CLL) [50].

Following this pilot study, scientists and physicians have made significant progress on studies of miRNA and human diseases including cancer in basic, translational, and clinical researches. For example, Song *et al* reported that miR-200b could inhibit tumor cell growth and migration by suppressing ZEB1 and sequentially augment E-cadherin *in vivo* [51]. Lu et al used bead-based flow cytometric miRNA expression profiling method to implement a systematic expression analysis of 217 mammalian miRNAs in 334 human samples. Their results showed the aberrant expression patterns of oncogenic and tumor suppressor miRNAs in human tumor tissues when compared to normal tissues, which suggested the potential of

miRNA as biomarkers for disease diagnosis[52]. The prognostic values of miRNAs in cancer have also been identified in variety of human tumors. For example, in addition to miR-200c that was reported to be a novel prognostic marker in colorectal cancer by our lab[53], miRNAs are also found to target the tumor suppressor genes to promote hematopoietic stem cell self-renewal and transformation[54]. In this study, overexpression of miR-22 inhibiting the tumor suppressor TET2 was demonstrated to be responsible for the poor clinical outcomes in myelodysplastic syndrome (MDS) and leukemia [54]. A recent study reported that the assays featured by multiple miRNA signatures can not only discriminate hepatocellular carcinoma (HCC) from normal tissues, but also can predict HCC patients' survival[55]. Development of bioinformatics and technology provides new insights into study of miRNA and human diseases. For example, the cancer miRNA regulatory network (http://cmrn.systemsbiology.net/) is built to show the interaction of miRNAs with 2,240 genes based on 46 cancer transcriptome profiling studies. Searching this database is able to quickly identify candidate miRNAs that correlate to selective diseases.

3. MiRNAs, a new player in cancer chemoprevention by NSAIDs

NSAIDs have been proposed as chemopreventive agents for variable human tumors, although the mechanisms of cancer chemoprevention by NSAIDs have not been understood completely [56,57]. As discussed above, COX and non-COX inhibitory pathways have been documented as two of the most important mechanisms accounting for the basis of action [56]; however, it has not been concluded that these known pathways can fully address the pleiotropic activities of NSAIDs in cancer prevention. Given the properties of targeting multiple genes by which miRNAs influence variety of signaling pathways, impaired miRNA regulation could contribute to the development of cancer and other diseases.

COX-2 has been reported to play an important role in tumorigenesis[58], and overexpression of COX-2 in varieties of cancers has been demonstrated, such as breast cancer[59], colon cancer[58], lung cancer[60], prostate cancer[61,62] and other tumors[63,64]. As discussed above, COX-2 is named as one of the most important targets responsible for the anticancer activity of NSAIDs. Thereby, any miRNAs that are able to trigger COX-2 may be involved in the COX dependent mechanism of NSAIDs anticancer activity. There are only several studies reporting the inhibition of COX-2 signaling by miRNAs[65-67], but few of them are related to human cancer[67]. A latest study reported that miR-101 was downregulated in the cisplatin-resistant human bladder cancer cells T24/CDDP, while overexpression of miR-101 rescued the anticancer activity of cisplatin. The luciferase reporter assay demonstrated that miR-101 could directly target 3′-UTR of COX-2 gene and silence of COX-2 by siRNAs could simulate the phenotype led by overexpression of miR-101[67]. In addition, a study demonstrated that celecoxib, a COX-2 selective inhibitor, could regulate the expression of miRNA-29c in human gastric cancer cells [68]. In this study, miR-29c showed the tumor suppressor signature; whereas COX-2 inhibition of miR-29c might be responsible for progression of gastric cancer [68].

The NSAID sulindac has been shown to display strong efficacy for the treatment of precancerous lesions in patients with familial adenomatous polyposis (FAP) by reducing adenoma size and number by as much as 60-70%[69]. These observations are consistent

with a large number of preclinical studies that have shown the ability of sulindac and other NSAIDs to inhibit tumorigenesis in various experimental animal models involving either early or late stage disease[70-73]. In one of our recent publications, we reported that sulindac sulfide (SS) can potently inhibit the invasion of human breast and colon tumor cells at concentrations less than those required to inhibit tumor cell growth *in vitro*[74]. This inhibitory activity by SS was found to be associated with significant changes in miRNA expression, implying that miRNAs may be involved in the pleiotropic anticancer activity of sulindac against tumor progression and metastasis [74]. Using microarray analysis, we found that SS treatment could alter the expression of 132 miRNAs (17 up and 115 down) in human HCT116 colon tumor cells, in which several have been previously reported to promote tumor metastasis and invasion, such as miR-10b, miR-17, miR-21, and miR-9. We further demonstrated that the mechanism of NSAIDs in control of these oncogenic miRNAs is through the NF-κB signaling. When SS inhibits the entrance of NF-κB to the nucleus, its transcription activity upregulating these oncogenic miRNAs is thereby decreased [74]. Thus, our results support a non-COX-inhibitory mechanism involving miRNAs that is responsible for anti-invasive activity of sulindac. The mechanistic basis of miRNA accounting for anticancer activity of NSAIDs is summarized in Fig. 2.

4. Summary

The characteristic of miRNAs has been implicated in the pathogenesis of variable human cancer types and their potential as as diagnostic markers, therapeutic targets and prognostic indicator have been intensively studied [75]; however, it mechanistic role in cancer chemoprevention by NSAIDs has not been well studied. Given the signature targeting over 30% human genes that are involved in most of cellular events, miRNAs have potentials to address the pleiotropic antineoplastic activities of NSAIDs. In support of this assumption, our recent study reported that downregulation of selective oncogenic miRNAs by sulidac through NF-κB signaling is responsible for anti-invasive activity of this NSAID, which is a novel non-COX inhibitory mechanism. In addition, a recent review article by Yiannakopoulou proposes that targeting epigenetic processes and miRNA expression by aspirin and other NSAIDs may be a novel strategy with potential efficacy for cancer therapy and prevention[76]. Altogether, to better understand mechanistic roles of miRNAs in cancer chemoprevention by NSAIDs can provide novel insights into development of novel agents with better efficacy and safety.

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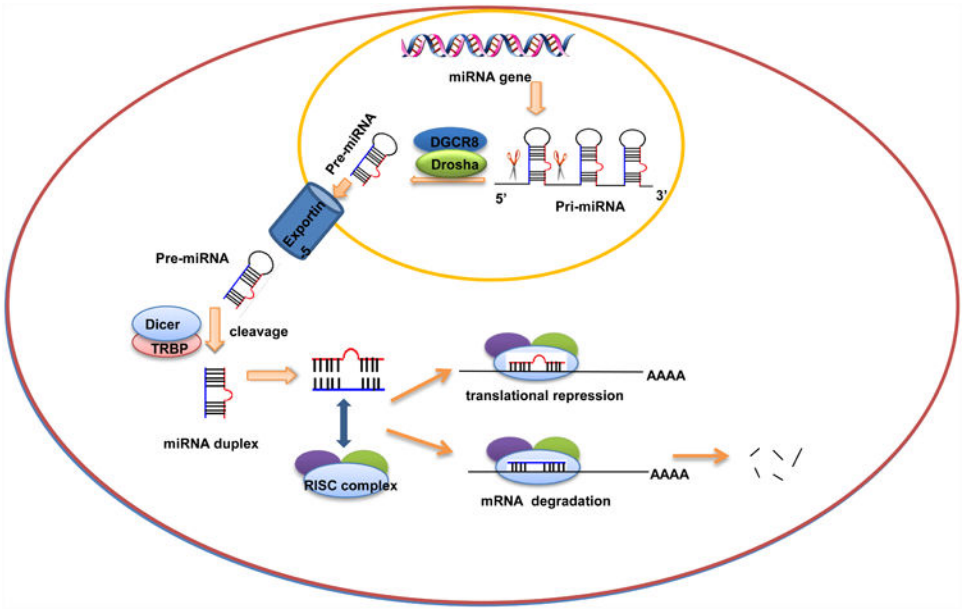

Figure 1.
MiRNA biogenesis and mechanism of action in regulation of gene expression.

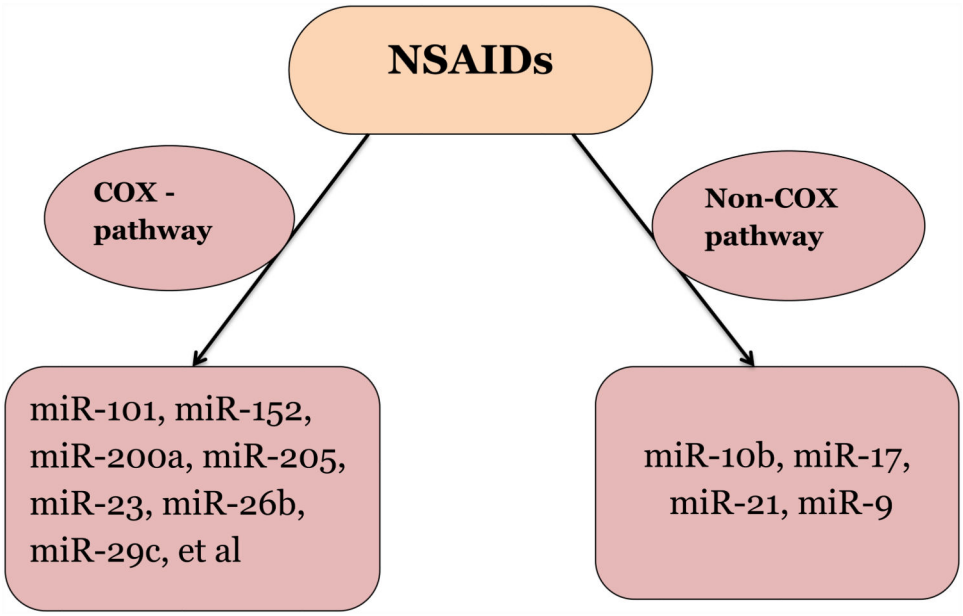

Figure 2. Mechanistic basis of miRNA accounting for anticancer activity of NSAIDs