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MicroRNAs and their Role in the Pathogenesis of Cervical Cancer

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Abstract

MicroRNAs are tiny, 18-25 nucleotides in length, non-coding RNA molecules preserved throughout evolution. These molecules primarily control gene expression at the post-transcriptional and transcriptional levels. MicroRNAs control target gene expression by a phenomenon known as RNA interference. RNA interference based therapeutics that utilize target gene silencing/degradation by specific microRNAs have potentially enormous advantages over traditional methodologies to treat diseases such as cancers with wide-ranging applicability, precision, and therapeutic selectivity, with decreased adverse side effects. If microRNA profiles can accurately predict malignancies, this technology may be exploited as a tool to surmount diagnostic challenges. This review highlights the successful use of RNA interference inducers against different type of cancers, thereby paving the way for specific therapeutic medicines. Studies have shown the association of microRNA dysregulation with diseases such as cancer. MicroRNAs can function as oncogenes as well as tumor suppressors. Thus, microRNA expression profiles can be used to determine prognosis, predict treatment efficiency and response to drug therapy, as well as patient susceptibility to cancer and metastasis. In addition, they may offer new candidate targets to be exploited for both prognostic and therapeutic strategies in patients with cervical cancer.

Keywords: Carcinogenesis, Cervical cancer, Gene silencing, MicroRNA, RNA interference

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Introduction

Cancer is frequently considered to be a genetic disease caused by alterations in the genetic course that concern a number of oncogenic pathways, resulting in transformation of normal cells to cancerous cells. Researches on gene expression profiling have exposed several alterations which confirm the reality that many genes and pathways play an important role in carcinogenesis. RNA interference (RNAi) technology, based on the use of

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specific microRNAs (miRNAs) can be used to target disease causing mutant genes as well as oncogenes. MicroRNAs have been discovered quite recently. They appear to be strongly associated with cell proliferation and differentiation. In addition, aberrant miRNAs play a significant role in several cancers. These miRNAs are a group of noncoding single-stranded RNAs, approximately 22 nucleotides in length that have emerged as a chief class of small endogenous RNAs which control gene expression post-transcriptionally by base-paring with their target mRNAs. Two types of small RNA molecules, miRNA and small interfering RNA (siRNA), are central to RNA-interference. Small RNAs bind to target mRNA molecules and either increase or decrease their expression. A number of *in vitro* and *in vivo* experiments have successfully used RNAi inducers to develop RNAi based techniques in anti-cancer therapy. Specific miRNAs related to tumorigenesis and tumor expansions have become hot spots for cancer research due to the association of miRNAs with tumor development. Cervical cancer is a multifactorial disease that involves the unusual expression of numerous oncogenes and tumor suppressor genes. Studies in this field have found several genes associated with human cervical cancer. For example, proapoptotic protein Bax expression is regularly lost in carcinomas and p53 plays a critical role in the development of cervical cancer.[1] The focus on known genes has yielded important new information on the role of these miRNAs in cervical cancer biology. Cervical cancer pathogenesis is a multi-step process that includes the transformation of normal cervical epithelium to preneoplastic cervical intraepithelial neoplasia which subsequently transforms to invasive cervical cancer. In this context, there is a well-documented causal relationship between high-risk human papilloma virus (HPV) infection and cervical cancer according to various epidemiologic and functional studies. However HPV infection alone is not sufficient to induce malignant transformation of cells infected with HPV and it has been found that miRNAs are required to induce malignancy in HPV-infected cells. According to research, high-risk human papillomaviruses (HR-HPVs) do not produce any viral miRNAs but lead to upregulation of oncogenic or downregulation of tumor suppressive miRNAs.[2] In addition, there is differential expression of mature miRNAs during the consecutive stages of cervical squamous cell carcinoma.[3] Studies have shown that chromosomal modifications, along with changes in gene expression and promoter methylation are associated with cervical cancer,[4] but little is known about the specific role of miRNAs. The importance of miRNAs in human carcinogenesis is becoming increasingly recognized; the understanding of the potential role of miRNAs in cervical carcinogenesis is still limited but postulated by a number of studies. It has been experimentally verified that some miRNAs play an important role in the hallmarks of cervical cancer such as cell cycle progression, cell death, angiogenesis, and metastasis. These processes are commonly deregulated in cancer, indicating an association of miRNAs with cervical cancer. These observations suggest that many aberrantly expressed miRNAs in cervical cancer could serve as diagnostic and prognostic biomarkers. In this article we summarize the role of miRNAs in cervical cancer, the role of the HR-HPV on miRNA expression, and the possible clinical utility of these miRNAs in diagnosis and treatment of cervical carcinogenesis.

Discovery of microRNAs (miRNAs)

In 1993, Victor Ambros initially recognized lin-4 miRNA in *Caenorhabditis elegans (C. elegans)* and concurrently, Gary Ruvkun identified lin-14 gene as the first miRNA target. These two important findings established a new mechanism of post-transcriptional gene regulation. Later, Ruvkun and Horvitz identified another miRNA, let-7 in *C. elegans*, and found that it was conserved across animal phylogeny, including humans. In 2001, three research groups from different countries identified 21-22 nucleotide non-coding small RNA molecules in C. elegans, drosophila, and humans. These single-stranded small RNA

Table 1. MicroRNAs (miRNAs) involved in cervical cancer.

S. No.	MiRNA	Expression	Regulation	Function	Reference
1.	Mir-522*	Upregulated	Cell cycle	Cell cycle:G2/M DNA damage, checkpoint regulation	53
2.	Mir-148a	Upregulated	PTEN, P53INP1 and TP53INP2 genes	Tumor suppressor genes	53
3.	Mir-21	Upregulated	Cell proliferation, apoptosis, and angiogenesis	Pro-proliferation, anti-apoptosis, and proangiogenesis	10
4.	Mir-10a	Upregulated	Cell transformation and progression	Target (HOX) genes	53
5.	Mir-132	Upregulated	Cell transformation and progression	Target (HOX) genes	53
6.	Mir-143	Downregulated	Cell proliferation and angiogenesis	Antiproliferation and antiangiogenesis	2
7.	Mir-145	Downregulated	Cell proliferation and angiogenesis	Antiproliferation and antiangiogenesis	2
8.	Mir-146a	Upregulated	Tumor invasion and metastasis, proliferation	Anti-metastasis and pro-proliferation	2
9.	Mir-218	Downregulated	Tumor invasion and metastasis	Anti-metastasis	2
10.	Mir-424	Downregulated	Angiogenesis	Antiangiogenesis	2

molecules with spatial and temporal expression differed from the formerly reported siRNA detected in the interference pathway and were consequently named miRNAs.[5] Cancer associated genomic regions have been reported to harbor more than 50% of miRNA genes, which suggested that miRNAs could be important in cancer development.[6] Recent studies have shown that miRNAs control many crucial biological activities of a cell, such as proliferation, differentiation and apoptosis.[7] The main function of miRNA is to repress the expression of target mRNA by cleavage or translational silencing, which depends on their complementation with the 3'UTR of target mRNAs.[8] By using high throughput miRNA microarray analysis, compared with the adjacent normal tissues, deregulation of the expression of miRNAs has been reported in different kinds of human cancers,[9,10] including cervical cancer. The aberrant expression of miRNA in cancer indicates a possible function of miRNAs in cancer development. Current evidence indicates that viruses use these miRNAs to manipulate both cellular and viral gene expressions.

Biogenesis of microRNAs (miRNAs)

MicroRNAs are encoded by specific miRNA genes in genomic DNA which are initially transcribed by RNA polymerase II into a stem-loop that is approximately 500 to 3000 bp in length.[11] This initial transcript is known as pri-miRNA. These pri-miRNAs are additionally altered into approximately 60 to 70 hairpin-shaped originator miRNAs (pre-miRNAs) in the nucleus by an enzyme named Drosha (a type of RNase III). Exportin 5 (Exp-5) then exports pre-miRNAs into the cytoplasm where they are cleaved by Dicer to generate 20-24 nucleotide RNA duplexes, one strand of which is loaded into the argonaute-containing RNA-induced silencing complex (RISC). These miRNA-RISC complexes are able to silence target mRNAs via imperfect complementarity with sequences located in the 5′-UTR coding sequences and, most commonly, 3′-UTR. When pre-miRNA is transported to the cytoplasm by the transport protein Exp-5, the pre-miRNA hairpin in the cytoplasm is cleaved into two strands by Dicer (RNase III) with the release of two complementary 22-base nucleotide chains. Subsequent to being processed by the enzyme

Dicer, the antisense miRNA chain and mRNA target region complementary base pair together to form a complex with argonaute proteins. Argonaute guides the miRNA chain to reach the target sequence on the mRNA and combines with RISC.[12] Next, RISC combines with target mRNA at the 3′-UTR and the miRNA base pairs with the target mRNA, which causes target mRNA cleavage and inhibition of protein expression.[13] miRNAs are also found to regulate a number of oncogenes and tumor suppressor genes. Calin et al. have indicated the presence of about 50% of miRNA genes associated with cancer in the genomic regions.[6] MicroRNAs are most frequently located within intergenic or intronic regions of protein-coding genes, and less commonly within exons or antisense transcripts. MicroRNAs located in introns of genes which have the same orientations as the host transcripts are generally co-transcribed with their host genes, thus their expression levels show strong correlation with their host transcripts. Intergenic miRNAs are transcribed as independent transcription units with their own promoter/transcriptional regulatory region.

Tumor-specific microRNAs (miRNAs)

Ongoing researches in the field of RNAi demonstrate the importance of miRNAs in regulating biological characteristics common to various tumors such as unlimited replication potential, self-growth signals, abnormal apoptosis, insensitivity to anti-growth signals, sustained induction of angiogenesis, and invasion and metastasis organization.[14] Many researchers have identified tumor-specific miRNA signatures that accurately distinguish malignant tumors from various types of benign tissues. They have demonstrated that certain miRNAs are carcinogenic depending on the other gene mutations in the tumors.[15] Regulation of miRNAs in tumor cell lines directly affects cell proliferation and apoptosis. Similarly, a number of studies have identified a link between abnormal miRNA expression, intracellular signal transduction pathway abnormalities, and tumorigenesis. For example, miR-9 is activated by YC/MYCN, which induces cancer metastasis by regulating the metastasis suppressor protein E-cadherin,[16] while miR-449a causes retinoblastoma (Rb)-dependent cell cycle arrest and cellular senescence in prostate cancer.[17] miR-15a/16-1 are the first identified tumor suppressor miRNAs,[18] where the genomic locus of miR-15a and miR-16-1 has been initially reported to be deleted in around 68% of B-cell chronic lymphocytic leukemia patients which resulted in decreased expression of the mir-15a~16-1 cluster.[19] Deletion of this locus and decreased expression of the mir-15a~16-1 cluster have also been reported in several other cancers, including prostate, lung, and pancreatic cancer.[8, 20-22] miR-15a/16-1 inhibit cell growth, tumor growth and angiogenesis, as well as induce apoptosis and cell cycle arrest.[23-25] Interestingly, this miRNA cluster regulates cell cycle progression in many tumor types by targeting two key cyclin genes, CCND1 and CCNE1.[25] The miR-34 family is another example of a tumor suppressor miRNA. This family consists of three miRNAs, miR-34a/b/c, and is encoded by two transcripts. miR-34b and miR-34c share one common transcript, while miR-34a is encoded by its own transcript. miR-34 family members are direct downstream targets of tumor suppressor p53 gene. Their expression is frequently downregulated in different cancers due to p53 mutation.[26] In addition to p53 regulation, the miR-34 family can also be regulated by promoter DNA methylation.[27] miR-34 also functions in p53 signaling due to its transactivation by p53 and can be regulated independently of p53 during oncogene-induced senescence. In this case, miR-34a is transcriptionally regulated by ELK1 that targets the MYC proto-oncogene during BRAF-induced senescence.[28] In non-small cell lung cancer cells, ectopic expression of miR-34a/c enhances TRAIL-induced apoptosis and inhibits tumorigenesis.[29]

Altered microRNA (miRNA) signatures in cervical cancer

Cervical cancer is a malignant neoplasm arising from cells that originate in the cervix uteri. It is

the third most common cancer type among women worldwide. More than 90% of cervical cancers are due to infection and subsequent transformation of cervical cells by specific HPV subtypes.[29] Persistence infection by HPV subtype, such as HPV-16 and 18 appears to be a necessary but not sufficient factor in the development of cervical cancers.[30] With the application of the Pap smear test and HPV vaccine, the incidence and mortality of cervical cancer have declined approximately 50% in the United States over the past three decades, however it remains a serious health threat. In cervical cancer, integration of HPV genome into the host chromosome(s) leads to over expression of viral oncoproteins E6 and E7 that can bind to cellular tumor suppressor p53 and pRB, respectively, which interfere with cell cycle control and DNA repair mechanism.[30] In addition, persistent expression of E6 and E7 can accumulate genetic mutations and promote genetic instability. Several molecular cytogenetic studies have mentioned that specific recurrent chromosome aberrations such as the gain of chromosome 3q in cervical cancer[31] implies that the genes residing in these chromosome regions are likely to play important roles in cervical tumorigenesis.[32,33] Altered miRNA expression profiles are reported in cervical cancer (Table 1). Importantly, overexpression of miR-21 and miR-205 have been consistently observed in different studies, which supports the importance of these miRNAs in cervical tumorigenesis.[8,10] However, the functional roles of these deregulated miRNAs are not fully understood. According to a number of studies, each cancer tissue has a specific miRNA signature and miRNA based cancer classification is a very effective and potential tool in the therapeutic context.[34] MicroRNA expression signatures are promising biomarkers for cervical cancer prognosis, which suggests that miRNA expression patterns may serve as potential biomarkers for pre-invasive cervical disease and potential therapeutic targets. Keeping in view the immense impact of miRNAs expressional profile in cancer biology, surveys of miRNA expression patterns in cervical cancer suggest that miRNAs play a major role in cervical cancer pathogenesis and progression beyond HPV.[35] Expression profiling of miRNA in cervical cancer cell lines have found that out of 174 reported miRNAs, the miR-21, miR-24, miR-27a, and miR-205 miRNAs were most abundant in cervical cancer or cervical intraepithelial neoplasia derived cell lines.[8] Another study suggested that overexpressions of miR-17-5p, miR-20a, miR-21, miR-92, miR-106a, and miR-155 could be considered a miRNA signature of solid tumors.[36] There were 18 upregulated miRNAs in solid tumors and 15 that downregulated in cervical cancer tissues. The increased expressions of miR-15b, miR-16, miR-146a, miR-155, and miR-223 observed in cervical cancer tissues have been implicated in the development of other human cancers. MicroRNA array analysis for age-matched normal cervix and cervical cancer tissues in combination with Northern blot verification identified some downregulated miRNAs in cervical cancer tissues. Apart from these, functional studies have shown that both miR-143 and miR-145 are suppressive to cell growth and their upregulation can be helpful for targeting tumorigenesis. According to Lee at al., miR-199a could be a new potential therapeutic target for future cervical cancer therapy.[9]

Post-transcriptional repression by microRNAs (miRNAs)

Computational studies followed by investigational justification highlighted the significance of the seed region in miRNA induced gene silencing. Such studies have shown important preservation of matches to the miRNA seed region or, in some cases, deficiency of miRNA seed matches.[9] MicroRNAs regulate gene expression at the post-transcriptional level by definite inhibition of transformation or orientation of mRNA cleavage. MicroRNA duplexes are integrated into the protein complex RISC and, after unwinding, they modernize the complex to create an active RISC.[37] This suggests that the appearance of miRNA target genes can be fine-tuned in animals by changing concentrations or identities contained by cells. Compared to animals,

plant miRNAs are more absolutely paired to their target RNA and utilize RNA cleavage more often than transformation suppression as the most important silencing mechanism. Although complementarity with targets has been established in plants, in animals the recognition of presumed targets is more complicated.[38] MicroRNA expression profiling studies in cases with cervical cancer have shown that miR-9, miR-21, miR-200a, miR-218, and miR-203 were found to be appreciably associated with cancer survival. Among these five miRNAs, three differentially expressed (upregulation of miR-21 and downregulation of miR-203 and miR-218) in cervical cancers compared with the normal cervix.[9,10] An expression profusion analysis showed that both normal and cancerous cervical tissues had abundant expressions of miR-23a, miR-23b, let-7a, let-7c, and let-7d. High expressions of miR-26a, miR-29a, miR-99a, miR-100, miR-125b, miR-143, miR-145, miR195, and miR-199a were only found in normal cervical tissues. High expressions of miR-16, miR-21, miR-205, and let-7f were only observed in cervical cancer tissues. Among the upregulated miRNAs in normal cervical tissues, expressions of miR-143 and miR-145 showed more than 2.7-fold reductions in cervical cancer tissues.[39]

MicroRNAs (miRNAs) in the prognosis of cervical cancer

MicroRNAs in mammalian cells have an average half-life of approximately 5 days (10 times more than regular mRNAs).[39] Small RNAs are resistant to degradation by RNase, high temperatures, extreme pH, and freeze-thaw cycles. These characteristics of miRNAs make them suitable to serve as prognostic biomarkers for diseases. The miRNA expression profile can discriminate between normal tissue and tumor tissue. This differential expression can also be used as a prognostic marker for clinical diagnosis of human cancers.[9] Calin et al. in a study of chronic lymphocytic leukemia have reported a miRNA expression signature composed of 13 mature miRNAs associated with prognostic factors and disease progression.[6] Rodolakis et al. observed a strong association between overexpression of miR-21 and liver metastasis in pancreatic endocrine tumors and pancreatic acinar cell carcinomas.[32] In lung squamous cell carcinoma, miR-155, let-7, and miR-146a can be used for prognosis.[42] Given the significant association of miRNA expression profiles with carcinogenesis, it is likely that a selected set of miRNAs can be used to build a predictive model for cervical cancer prognosis. Hu et al. have proposed that the level of tumor suppressors miR-200a and miR-9 could predict survival in cervical cancer patients.[43] It was suggested that miR-200a and miR-9 could play important regulatory roles in cervical cancer control because miR-200a likely decreased the metastatic potential of cervical cancer cells by coordination of suppression of multiple genes which included cell motility. miR-9 could potentially be involved in tumor control that blocked the high metabolic rate important for rapid proliferation of cervical cancer cells.[44] An association existed between high levels of the oncogenic miR-210 with disease recurrence and short overall survival in head and neck squamous cell carcinoma. This miRNA also upregulated in cervical cancer.[45] Several authors reported an association between increased miR-127 and miR-21 expressions with cervical carcinogenesis and lymph node metastasis.[9] In contrast, low levels of miR-218 and miR-424 indicated a good prognosis in cervical cancer. Thus, it could be concluded that many aberrantly expressed miRNAs in cervical cancer could serve as prognostic biomarkers. The development of a biomarker panel may be useful in determining which patients with cervical dysplasia are likely to progress to more invasive disease, and it may also be a useful prognostic indicator in patients with invasive cancer. However, more studies are necessary to determine the diagnostic and prognostic implications of many differentially expressed miRNAs in cervical cancer.[46]

Targeting cervical cancer

Cancer is a complex genetic disease which involves a multitude of oncogenic pathways.

Many oncogenes and tumor suppressor genes play an important role in carcinogenesis. Cancer associated genes which are not mutated but overexpressed in a majority of cancers can also be potential targets in RNAi based therapeutics. HPV is a virus that belongs to the Papillomavirus family. HPV infection is a cause for most cases of cervical cancer. RNAi technology has been used to target HPV associated genes as potential targets against cervical cancer. Specific siRNAs that targeted HPV E6 gene expression have resulted in the accumulation of p53 protein, which led to reduced cell growth whereas silencing of the HPV E7 gene by siRNAs resulted in apoptosis. Cancer associated genes which are not mutated but overexpressed in a majority of cancers can also be potential targets in RNAi based techniques.[47] Clusterin, an anti-apoptotic gene expressed in most cancers, was successfully targeted by specific siRNAs.[48]

Because of the mentioned associations of miRNAs with cancer development and progression, it is now believed that these small regulatory RNAs could serve as targets for anticancer gene therapies.[49] Antisense molecules can inhibit the activity of oncogenic miRNAs. They have been tested for their efficacy in reducing miRNA activity on reporter genes that contain miRNA-binding sites.[50] Antagomirs represent a new class of oligonucleotides designed to antagonize specific miRNAs. Thus, alternative approaches toward the targeting of miRNAs in novel cancer therapies include the use of viral vectors, small molecules or compounds to restore the expression of these lost miRNAs and simultaneously repress the expression of the corresponding target gene. In a recent study, inhibition of miR-200c using an anti-miRNA 2'-0-methyl oligonucleotide has been shown to result in increased expression of one of the targets, transcription factor TCF8. The potential anti-tumor applications of RNAi based techniques offer great hope in designing effective anti-cancer therapies. Expressions of various miRNAs are up- or downregulated in cervical cancer. These expression levels can increase or decrease sensitivity to chemotherapy and radiotherapy. Studies have shown that expression patterns of 25 miRNAs, including miR-138, miR-210, and miR-744, altered the sensitivity to drugs such as 1′S-1′-acetoxychavicol and cisplatin which inhibit the growth of cancer. Thus, miRNAs may have an important role in the response to chemotherapy. Lei et al. have found that miR-155 negatively regulates the epithelial growth factor-induced epithelial-mesenchymal transition, inhibits proliferation, metastasis, invasion and increases sensitivity to cisplatin.[50] miR-214 upregulates expression of Bax, caspase-9, caspase-8, and caspase-3, enhances apoptosis, inhibits cell growth, and increases sensitivity to cisplatin by silencing Bcl2l2 expression.[52] miR-218 induces apoptosis, suppresses tumor growth, and increases sensitivity to cisplatin through the AKT-mTOR signaling pathway in cervical cancer cells. Other miRNAs are implicated in resistance to chemotherapy and radiotherapy. miR-375 contributes to acquisition of resistance to paclitaxel in cervical cancer cells, while other studies have shown that miR-181a increases cellular resistance to radiotherapy through negative regulation of proapoptotic protein kinase, which suppresses radiation-induced apoptosis and decreases the block in transition from G2 phase to M phase of cell division. If overexpression of a specific miRNA causes resistance to chemotherapy or radiotherapy, this resistance may be reduced by inhibiting miRNA function. Similarly, if downregulation of miRNA causes resistance, this may be improved by supplementation with miRNA. Therefore, new combination therapy of miRNA inhibitors or supplementation with chemotherapy or radiotherapy may be developed. Such treatment approaches using miRNAs with distinct expression patterns can be particularly useful in personalized treatment and molecular targeted therapy for cervical cancer.

Conclusion

Cancer is a complex disease that involves a network of oncogenic pathways. These pathways are regulated by a number of genes which can be

used as potential targets using RNAi based techniques. The discovery of miRNAs has provided a new perspective on regulation of gene expression which play an integral role in regulating an array of fundamental biological processes whose alterations lead to disease outcome, including cervical cancer. Numerous *in vitro* studies have reported that the use of miRNAs in gene silencing and targeting via RNAi can bring about cell death in cancer cell lines. Studies show an association of miRNA dysregulation with diseases such as cancer since miRNA can function as oncogenes as well as tumor suppressors. Thus, the miRNA expression profiles can be used to determine prognosis, predict treatment efficiency, response to drug therapy, and patient susceptibility to cancer and metastasis. The effectiveness of RNAi in cancer therapy is bound to increase as novel, efficient delivery methods are devised that offer accurate delivery of specific miRNAs to the target system. With the advent of RNAi based gene therapy, it is possible to combat cancer at the molecular level and open new avenues for an effective anticancer therapy. HPV modulates the expression of numerous cellular miRNAs that are likely to contribute to viral pathogenesis. Surprisingly, recent studies have led to the identification of cellular miRNA regulation by HPV oncoproteins and viral regulation of tumor suppressor miRNA expressions. The data provide evidence that this intimate interplay of oncoproteins and miRNAs can disclose new ways for cancer diagnosis, evaluation of prognosis, and therapy. The interplay between viral oncoproteins and miRNAs can be used as early biomarkers in cervical cancer and potentially used to determine disease prognosis. A deeper understanding of molecular pathways that involve miRNA interplay can provide great insight in the initiation and progression of cervical cancer and is essential for the advancement of therapeutic treatments. However, future studies are needed to determine the function, transcription targets, and mechanisms by which many miRNAs regulate the cellular events within both normal and cervical cancer tissues. The results of these studies can give better information on the role of miRNAs as biomarkers, prognosis, and therapy of cervical cancer.

Conflict of Interest:

No conflict of interest is declared.

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