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siRNA and apoptosis in breast cancer cells: A minireview

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Abstract: Small interfering RNA (siRNA), is a class of double-stranded RNA at first non-coding RNA molecules, operating within the RNA interference (RNAi) pathway. It interferes with the expression of specific genes with complementary nucleotide sequences by degrading mRNA after transcription, preventing translation. siRNAs have been widely used to study gene function and extensively exploited for their potential therapeutic applications. siRNAs have gained attention as a potential therapeutic reagent due to their ability to inhibit specific genes in cancer cells. Increased resistance to apoptosis is a challenging issue for treatment of many cancers, including breast cancer. It has been recently reported that siRNAs and RNAi technology can be used to increase the apoptotic susceptibility of cancer cells. It has been shown that apoptosis is induced in cancer cells by siRNA-mediated silencing of the livin/ML-IAP/KIAP gene. Association of siRNA with apoptosis via mitochondrial depolarization and caspase-3 activation has been highlighted in cancer cells. Although many aspects of siRNA actions in cancer cells have been revealed through in vivo and in vitro studies, the biological mechanisms underlying siRNA mediated knockdown of gene expression and apoptosis induction in cancer cells are not yet fully understood. The main aim of this review is to investigate the effects of siRNAs on apoptosis induction in breast cancer cells.

Keywords: siRNA, Apoptosis, Breast cancer cell

1 Introduction

1. 1. Breast cancer

Breast cancer is the most common cancer among women (Malthaner et al., 2004; Miller, 1992), accounting for approximately 1% of all breast cancers in men (Giordano, 2018) and the

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second most common cause of cancer-related death (Wiechmann and Kuerer, 2008; Moore et al., 2013). According to the statistics of the World Health Organization, one person out of every 8 to 10 women will get breast cancer, and this figure in the statistics of Iran, there is a possibility that one woman out of every 10 to 15 women will get breast cancer; But the age of breast cancer in Iranian women is at least a decade lower than women in developed countries. The average age of breast cancer diagnosis is 56 years in Western countries and 45 years in Iran (Malthaner et al., 2004; Miller, 1992). Also, the mean age at diagnosis for men is approximately 5 years older than for women (67 years vs. 62 years), including for men who have a first-degree relative with the disease (Giordano, 2018). Breast cancer is one of the cancers that is separated from the breast tissue and develops inside the internal cells of the milk ducts and lobules that deliver milk to this duct, and because it originates from the ducts, it is also known as ductal cancer (Jin et al., 2018). Breast cancer is a highly heterogeneous disease that is caused by the interaction of genetic and environmental risk factors and leads to the progressive accumulation of genetic and epigenetic changes in cancer cells. Although epidemiological evidence emphasizes the existence of risk factors, especially such as age, obesity, alcohol consumption, dealing with estrogen during life, but the existence of a family history of this cancer is considered the strongest risk factor for this disease. Almost 20% of all breast cancers are familial types, and in terms of pathogenesis, they have a specific dependence on the specific predisposing gene for that disease (Gray et al., 2017; Antoniou and Easton, 2006).

The ways to treat breast cancer can be different depending on its type and the stage of the disease and the extent of its spread in the body. Often, people with breast cancer experience multiple treatments. Breast cancer treatment methods are both local and systemic. Local treatments for breast cancer include methods such as surgery and radiotherapy, and its systemic treatments include chemotherapy, hormone therapy, targeted therapy, and immunotherapy (Sledge et al., 2014). Additionally, while advances in cancer treatments have greatly reduced morbidity and mortality in women with breast cancer, they have contributed to an increase in cardiovascular complications and risks. For example: anthracyclines, such as doxorubicin, which are the mainstay of current chemotherapy regimens, are associated with dose-dependent cardiotoxicity, which can be effective in reducing the cardiovascular risks of exercise in a variety of clinical conditions (Varghese et al., 2021). Also, in the treatment of patients with breast cancer, the use of ICB as monotherapy has achieved durable and visible responses in the prescription and use of this method. New findings also indicate that the clinical effect of this method is greater with chemotherapy. In addition, the researchers of this research are evaluating the data of their studies to increase the response to the treatment of this cancer by factors such as: targeted therapy, vaccines, combining ICB with additional chemotherapy agents and local ablative treatments, and also examining the results of breast cancer treatment (Adams et al., 2019).

Despite many advances in clinical and therapeutic techniques in recent years, many breast cancer patients still die due to metastasis. Therefore, it is necessary and necessary to create new treatments to overcome the ineffectiveness of the current treatments. Small interfering RNA (siRNA) is a method used to study gene function and treat diseases. Cancer treatment with siRNA method is more efficient, more effective, more specific and less expensive than other gene therapy methods (Fire, 2007; Klug and Cummings, 2003). This method has higher

specificity than other cancer treatment methods such as surgery and chemotherapy (Mansoori et al., 2014).

1. 2. siRNA

Interfering RNAs are non-coding RNAs that affect the regulation of gene expression and are cell controllers that can cause fundamental changes in the DNA molecule, turning off genes and changing their expression levels (Steuerwald et al., 1999). Also, their role in post-transcriptional gene silencing (PTGS) has been determined, and synthetic siRNAs can induce RNAi in mammalian cells (Elbashir et al., 2001), In addition, there have been significant advances in siRNA treatments with organic (carbon-based) and inorganic (non-carbon) nanoparticles that have been successful in delivering drugs to the brain (Eisenstein, 2019); However, human applications of siRNA have significant limitations in its success (Saad et al., 2008). Cellular and molecular etiology of breast cancer indicates that breast cancer is related to the activity of small interfering RNAs (siRNA). In fact, siRNA can be effective in the etiology of breast cancer by regulating gene expression and can be considered as a tool for the treatment of cancers, especially breast cancer (Yu et al., 2014).

1. 2. 1. Mechanism of action of siRNA

The mechanisms of RNA interference cover a wide spectrum, including: down-regulation of mRNA levels (Klein, 2002), establishment and maintenance of heterochromatin (Mocellin et al., 2003), DNA excision (Mason et al., 2002), promoter silencing, developmental control (Dorak, 2007), up-regulation of transcription during the cell cycle (Jorgensen et al., 1996) and transposon silencing (Couzin, 2002). Also, siRNAs can be divided into two groups. A group that is directly derived from double-stranded RNA and performs its action without the need for replication, and another group that requires the primary long double-stranded RNA to be amplified by the RNA-dependent RNA polymerase enzyme in order to have the ability to interfere. In the siRNAs of the first group, the Dicer enzyme cuts the double-stranded RNA from one end and creates a 21-nucleotide fragment. Primary RNAs that undergo Dicer action include viral replication mediators, mRNAs that have folded on themselves and formed a hairpin structure, and double-stranded RNAs that have been experimentally introduced into the cell. The product of Dicer function is an siRNA consisting of two strands of 21 nucleotides, which include 16 nucleotides as base pairs and 2 nucleotides as overhangs at the 3′ end. These siRNAs have a phosphate group at the 5' end and a hydroxyl group at the 3' and 2' ends, which may be changed by s-adenosylmethionine-dependent methyltransferase enzymes such as Han1, and in plants and insects to -o-methyl ′2 and -hydroxy'3 to be converted. Then the double-stranded siRNA is connected to the RISC complex (Ketting, 2011). RISC requires the energy released from the unwinding of the double-stranded siRNA and the conformational change of the initially assembled ribonucleoprotein components. The opening of these coils is done by the activity of Argonate protein, which is one of the components of the RISC complex (Sen and Roy, 2007). Argonaute protein unwinds the double-stranded siRNA at the end with relatively lower energy content. Among the two strands of siRNA, the strand that remains in the RISC protein complex is called the guide strand. The other strand, which is called the passenger strand, is degraded by exonuclease enzymes. The guide strand causes the activation of the Argonat

protein from the RISC complex, and this protein, with Rnase activity, cuts the target sequence into a position that is complementary to nucleotides 10 and 11 from the 5' end side of siRNA (Grewal and Elgin, 2007). Plants, fungi and some animals, such as Cenorhabditis elegans, have an RNA-dependent RNA polymerase (RdRp) enzyme, which has the ability to synthesize complementary single-stranded RNA without the need for primers. RdRp's involved in the process of RNA interference make secondary siRNA by copying from the target mRNA. The secondary siRNA is then cut by a Dicer enzyme and exerts its regulatory action on other mRNAs. In worms, secondary siRNA is created directly by transcription without the need for intermediary double-stranded RNA and Dicer enzyme. This type of secondary siRNA has two or three phosphate groups at the 5' end, while the secondary siRNA that undergoes Dicer action has one phosphate group. siRNAs in most cases cause a decrease in gene expression at the post-transcriptional stage, but through a mechanism that is completely unknown, they can sometimes increase the transcription or translation of mRNA with the help of specific Argonaute proteins. siRNAs that perform gene silencing directly in the nucleus; It has been identified in plants, but the presence and mechanism of this type of siRNAs in animals and especially mammals is still controversial (Collins and Cheng, 2006). Interfering RNA represents a mechanism that nature has built to protect the genome. The molecular mechanism of small interfering RNA can target any gene with high specificity and efficiency. Today, siRNAs can have many applications in living and laboratory environments and in therapeutic fields, such as overcoming diseases dependent on genes (de Fougerolles et al., 2007) (Figure 1).

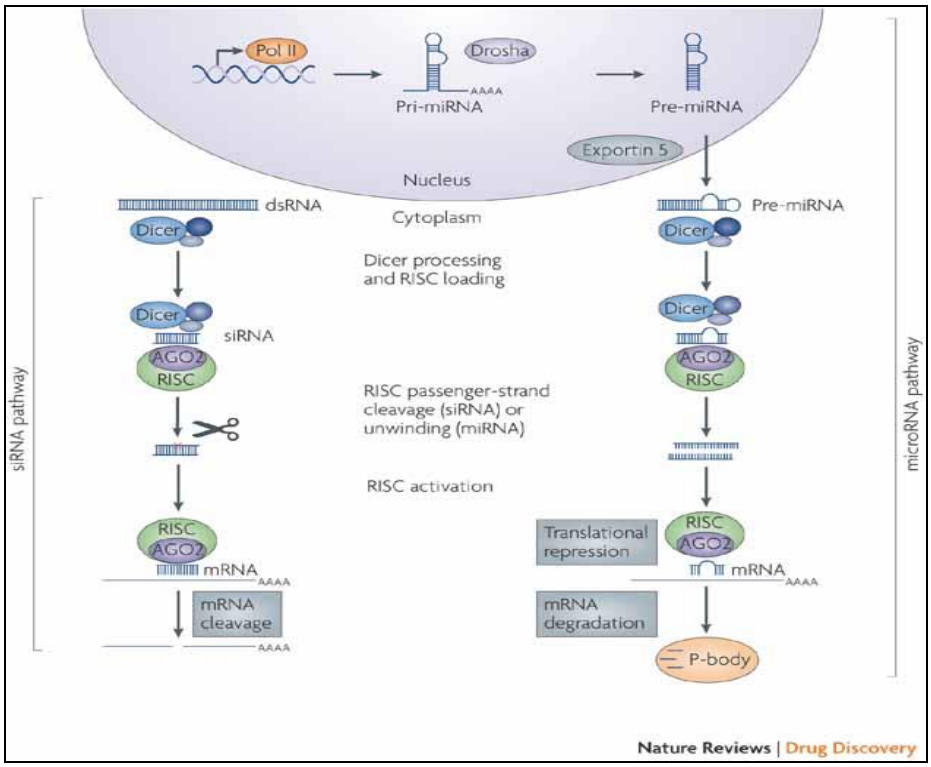

Figure 1. Mechanism of action of siRNA and microRNA

1. 3. Apoptosis

Apoptosis, or programmed death, is a highly regulated pathway responsible for the elimination of redundant, old, and damaged cells. The distinctive and important morphological features of apoptosis include: cell membrane wrinkling, chromatin condensation, loss of position of organelles in the cytoplasm, DNA fragmentation and, finally, apoptotic bodies are created. These apoptotic bodies are quickly identified, engulfed and removed by phagocytes. In physiological conditions, the changes that occur in the cytoplasmic membrane of the cell can be recognized by phagocytes and cause the ingestion of these apoptotic bodies (Armitage, 1998). Also, during the process of apoptosis, inflammatory reaction does not occur, which is due to three reasons: 1- apoptotic cells do not release their contents into the environment- 2- they are quickly phagocytized by macrophages in the environment- 3- phagocytic cells do not produce inflammatory cytokines (Armitage, 1998) and Also, unlike necrosis, a type of traumatic cell death caused by acute cell damage, apoptosis is a very controlled and regulated process that has advantages during the life cycle of the organism. Also, apoptosis produces apoptotic bodies from cell fragments that phagocytes can destroy and digest before spilling the cell contents to the surrounding cells and damaging them (Alberts et al., 2008). Also, the intracellular apoptosis signal starts in response to stress (Wang et al., 2022), which may lead to programmed cell death and suicide. Also, binding of nuclear receptors by glucocorticoids, heat, radiation, nutrient deprivation, viral infection, hypoxia (Cotran et al., 2004), increased intracellular concentration of free fatty acids (Hardy et al., 2003) and the increase of intracellular calcium concentration (Mattson and Chan, 2003; Uğuz et al., 2009.), can cause the release of intracellular apoptosis signals by a damaged cell by damaging the membrane. Also, some cellular components such as poly ADP ribose polymerase may help to regulate apoptosis (Chiarugi and Moskowitz, 2002). In addition, in experimental investigations of apoptosis, single cell fluctuations due to stress have also been observed (Goldstein et al., 2000; Lee et al., 2010).

1. 3. 1. Molecular mechanisms of apoptosis

In addition to key morphological changes, complex molecular mechanisms and processes are also involved. Apoptosis is activated by two important pathways. One pathway is through death receptors, which is called the external pathway, and caspases 8 and 10 play a role in it, and the other pathway is the mitochondrial pathway or the internal pathway, in which caspase 9 plays a role. Caspases 3, 6, and 7 are activated as a result of the activation of the intrinsic and extrinsic pathway of apoptosis. The activation of caspases leads to the breakdown of enzymes involved in DNA repair, including: polyADP ribopolymerase (PARP), DNA-dependent protein kinase (DNA-pk), chromatin breakdown by CAD-scaspase-activated DNAs and breakdown of cellular skeleton units. and membrane proteins by proteins that bind to Ca2+, which ultimately leads to apoptotic cells, and these bodies are phagocytized by macrophages. One of the most important factors in the process of apoptosis is the P53 gene, the P53 gene is a tumor suppressor gene and is strongly involved in human and animal carcinogenesis. Today, P53 gene mutations are the most well known genetic changes in people with cancer. The process of apoptosis is related to the induction of the P53 gene and is under the control of the antiapoptotic gene bc1-2 (World Health Organization, 1982) (Figure 2).

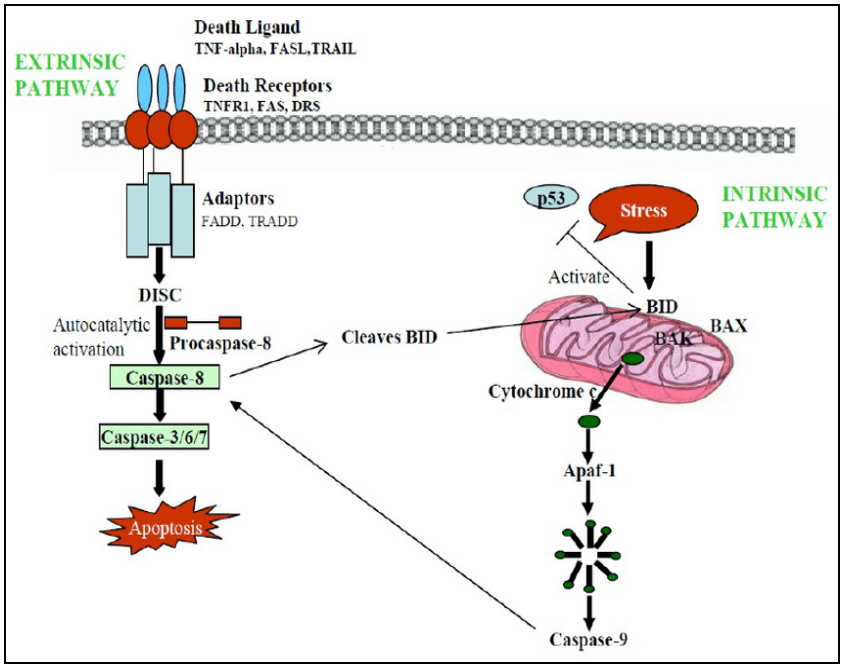

Figure 2. Intrinsic and extrinsic pathways of apoptosis

1. 4. SiRNA and apoptosis in cancer cells

Studies conducted in the last five years have shown that there are two main cell-intrinsic pathways to induce apoptosis; one is initiated by binding of cell surface death receptors and the other involves the release of cytochrome c from the mitochondria (Kaufmann and Earnshaw, 2000). Most cytotoxic anticancer agents induce apoptosis. Since the same mutations that suppress apoptosis during tumor development also reduce the sensitivity of the treatment, apoptosis provides a conceptual framework for linking cancer genetics with cancer treatment (Lowe and Lin, 2000). Thus, inactivation of the apoptosis pathway allows cancer cells to grow. But if the apoptotic pathway causes the inactivation of a tumor that leads to cell death due to DNA damage, or it is the same pathway or overlaps with it, most cancers are expected to be resistant to apoptosis in response to DNA damage (Brown and Attardi, 2005). In this regard, studies have shown that treating colorectal tumor cells with cyclopamine, a known inhibitor of Hedgehog (Hh) signaling. In this study, it was found that cyclopamine treatment induces apoptosis in adenoma- and cancer-derived cell lines, which can be partially released by further stimulation of Hh signaling. Also, the researchers of this study showed that autocrine Hh signaling can increase the survival of ectopic cells in colorectal tumor cells, which may be a new target for the treatment of colon cancer using drugs such as cyclopamine (Qualtrough et al., 2004). Also, the results of the study show that the destruction of cyclin D1b suppresses the malignant phenotypes of human bladder cancer cells by inducing apoptosis and suppressing the stemness of cancer cells and epithelial-mesenchymal transition. As a result, the use of cyclin D1b siRNA can provide a new treatment for bladder cancers that express cyclin D1b (Kim et al., 2018). Also, research stated that the most suitable way to improve the treatment of ovarian cancer is the use of RNAi and nanotechnology, which help to overcome chemical resistance. Also, siRNA can be helpful to destroy certain genes when they enter the cytosol, and in-vivo

RNA interference (RNAi) inhibits the mRNA expression responsible for the translation of those genes, which has been successful in the treatment of this cancer (Rehman et al., 2022).

1. 4. 1. Overexpression of anti-apoptotic protein bcl-xL

Researchers in lung cancer have found that it potentially often contributes to tumor development, progression, and drug resistance was designed to override the block of apoptosis in lung adenocarcinoma and small cell lung cancer cells induced by overexpression of an antisense oligodeoxynucleotide targeting a sequence unique to the bcl-xL coding region. These findings suggest that bcl-xL is a more important survival factor for lung adenocarcinomas and suggest the use of oligonucleotide 4259 for the treatment of this major subtype of lung cancer (Leech et al., 2000). On the other hand, microRNAs play an important role in cell growth, differentiation, proliferation and apoptosis. They can act as tumor suppressors or oncogenes. In a study, it was found that miR-192 induces cell apoptosis through the caspase pathway, which is the direct target of miR-192 in retinoblastoma-1. RB1-siRNA treatment inhibited cell proliferation and induced cell apoptosis in lung cancer cells (Feng et al., 2011). Also, studies on lung cancer have shown that APE1 may play an important role in radiation-induced angiogenesis, and administration of Ad5/F35-APE1 siRNA during radiation therapy can be a powerful adjuvant therapeutic approach to increase radiotherapy response, effectively eliminate metastasis, and improve the efficacy of radiation therapy. for NSCLC (Gu et al., 2013). In colorectal cancer, where β1-integrin overexpression is known to be the main factor, they showed that siRNA/HNP complexes in combination with Regorafenib/HNPs were identified as the most effective treatment to reduce β1-integrin gene expression and increase the rate of apoptosis in resistant cells. As a result, combined treatment using the siRNA/HNP and Regorafenib/HNP complex can reduce integrin-β1 gene expression and thus induce apoptosis, which may potentially induce drug sensitivity (Zhiani et al., 2021). Studies have shown that increased expression of STAT5A and STAT5B plays a significant role in the development of leukemia, in which leukemia cells acquire the ability to proliferate uncontrolled and angiogenesis. At the same time, these cells gain the ability to escape from apoptosis and the host's immune system. Studies also showed that apoptosis was significantly induced in leukemia cells treated with siRNA compared to cells treated with ODN oligonucleotides. As a result, compared to ODN treatment, siRNA applications were more effective in terms of gene silencing based on the amount of apoptosis and mRNA suppression, and finally, siRNA application can be a new and alternative treatment method as a supportive treatment in CML patients (Kaymaz et al., 2013).

1. 5. SiRNA and apoptosis in breast cancer cells

In a study, they investigated the cellular and molecular changes caused by taurine (Tau) amino acid, which led to the induction of apoptosis in human breast cancer cell lines MCF-7 and MDA-MB-231. In this study, they showed that Tau increased apoptosis in human breast cancer cells by inducing the expression of apoptosis-modulating proteins (PUMA) and inhibited tumor growth in nude mice, and increased Bax and Bcl-2 protein expression and decreased activity. It becomes caspase-3. Collectively, these results indicate that Tau is a strong candidate for breast cancer chemotherapy through increased PUMA expression independent of p53 status

(Zhang et al., 2015). The researchers also investigated the effects of genistein (a prominent isoflavonoid in soy products) as a breast cancer-reducing agent, on cell growth and apoptosis-related gene expression in MDA-MB-231 breast cancer cells. Investigated that as a result, Genistein inhibits the growth of MDA-MB-231 breast cancer cells. It also regulates the expression of apoptosis-related genes and induces apoptosis through a p53-independent pathway. In this regard, the researchers of this study stated that Genistein is an effective chemical or therapeutic agent against breast cancer (Li et al., 1999). By studying the combined effect of low-dose doxorubicin and siRNA inhibition of telomerase on breast cancer cells, they found that, when used individually, apoptosis induction was rapid and potent in both treatments, and when these two treatments were combined, the induction of apoptosis increased. It was found and stability was observed in breast cancer cells. As a result of the combination of siRNA-hTERT and 0.5 μM double doxorubicin, more number of cancer cells were destroyed, which indicates the cumulative effect of these two treatments. This study demonstrated the potential of telomerase inhibition as an effective treatment for breast cancer. And when used together with doxorubicin, it can also enhance the cytotoxic effect of the drug on breast cancer cells (Dong et al., 2009). In addition, the results of another study indicate that Stat3, which is active as a transcription family activator in breast cancer, showed that by reducing its expression by siRNA, it decreases the expression of Bcl-xL and survivin in MDA-MB-231 cells, and also by activating caspases 3, 8, 9 and cleaving PARP1, it leads to Fas-mediated intrinsic apoptosis pathway. Therefore, targeting Stat3 signaling using siRNA may serve as a novel therapeutic strategy for the treatment of breast cancers that express activated Stat3 (Kunigal et al., 2009) and other research showed that the transfection of MDA-MB-468 breast cancer cells by specific siRNA significantly decreased the expression level of CXCR4 Snail-1 gene and significantly increased the expression of miR-143. The results of this study have shown that eliminating the diagnostic markers of breast cancer cells by specific siRNA can successfully reduce the proliferation and invasion of breast cancer cells. Also, the snail-1 gene can be involved in the invasion and metastasis of breast cancer, and by knocking down this gene with specific siRNA, the invasion and metastasis of this cancer can be significantly reduced (Sattarivand and Mohammadzadeh, 2022). The research results showed that the production of IL-10 may be a new escape mechanism for breast cancer cells to prevent destruction by the immune system. Silencing the IL-10 gene reduces its regulation and the expression of the PI3K/AKT and Bcl2 genes and it also increases the expression of Bbc3 cleavage, BAX-caspase-3 and caspase-3 (Alotaibi et al., 2018).

2 Conclusion

In total, the results of this research showed that according to the studies conducted on siRNAs that lead to a decrease in BCL-xl expression and survival in MDA-MB-468 cells, inhibition of telomerase, increased apoptosis of cancer cells, It indicates that these types of RNAs can help a lot in the direction of strategic and targeted breast cancer treatments and also, as mentioned in this review, They can be a suitable therapeutic target for the treatment of cancers, especially breast cancer, and further studies in the future can help in this research.

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Conflict of interests

The author has no conflicts of interests to declare.

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