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Role of RNAi in Inhibition of Angiogenesis: A Review

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Abstract
Uncontrolled developments of new blood vessels take place in cancer and many other life threatening diseases. Currently, RNA interference has boosted the therapeutic prospects for inhibiting gene expression responsible for increased and uncontrolled angiogenesis in disease conditions. Small interference RNA is a fast way for analysis of gene operations by masking their function phenotype. Angiogenesis is a complex mechanism with the involvement of many different protein factors. The application of siRNA enables the rapid analysis of different pathways and recognition of new target genes. So the Initial research on the curative effects of small interfering RNA in the angiogenesis process has demonstrated that this new class of drug takes great potential for curative innovation. There are two strategies in this regard: the use of “unmodified” or the use of “complexed”; “targeted and/or protected nucleic acids”. The major problem for the clinical use will be to control the off-targets effects and the transient property of the sequence specific effect of silencing. To tackle the targeted distribution to cells which is involved in the different stages of angiogenesis process will be another problem. This article will briefly discuss in-vitro and in-vivo “angiogenesis inhibition” related perspective in complex pathological conditions.
Keywords: RNAi; Angiogenesis inhibition; siRNA, shRNA.

INTRODUCTION

Angiogenesis is the practice of developing new blood capillaries from already existing blood vessels. Different gens products produced in specific sequence from different cells are responsible for this process. The RNAi acts as a key to activate the cell death machinery (Leulier *et al.,* 2006). In recent research, it provides revolutions to the study of gene expressions in mammals, their functions and role in onset of a disease. It is valid to detect and get access to a disease. RNAi is used for site specific sequence splicing or silencing, so, it is discovered effective with lentivirus in the process of animal transformation and also knockdown the gene of interest. RNAi is involved in formulation structure of genetic targeted drug for Schistosomes, by inhibiting the protein kinase C that affect BI2536, which target the eggs of worm (Guidi *et al.,* 2015).

RNAi target the unwanted mRNAs of animals and plants by splicing or translational repression (Bartel *et al.*, 2009). These small RNAi molecules are integral part of different classes, which effect equilibra and conformational status in different ways (Shimaoka *et al.,* 2003). It inhibits prostate cancer by targeting EZH2 and reduces the amount of EZH2 protein in cells (Varambally *et al.,* 2002). RNAi inhibit the HIV-1 replication by blocking cell's or viral pathway (Martinez *et al.,* 2002). During onset of GLI-mediated transcription, RNAi participate in final steps of Hedgehog pathway (Lauth *et al.,* 2007). Betanodavirus B2 is also an RNAi and it targets the nucleus of cell and accumulates in the last step of viral infection (Fenner *et al.,* 2006). Normally Synthetic siRNA contained 2'-O-methyl guanosine or uridine nucleosides in the same strand. In case of non-inflammatory siRNA have less than 20% modification (Judge et al. 2006). RNAi is involved in synthesis of antiviral therapeutics. Chemical modification of siRNA inhibits gene expression without affecting its ability (van Rij *et al.,* 2006).

Use of RNAi

Double stranded RNAi is widely used to silence the expression of genes in C. Elegans, Drosophila and even plants (Fire *et al.,* 1998). It inhibits the expression of endogenous as well as transfected genes. The silencing process that it triggers in mammalian cells is gene-specific (Bernstein *et al.,* 2001). This response has found its applications to explore the gene structure and function. It has ability to knock down as well as knock down the gene expression (Weinert *et al.,* 1998). The transfection of mammalian cell or tissues with long double stranded RNAs become the cause of inducing antiviral interferon response that result in gene silencing or in some cases even cell death (Dorsett *et al.,* 2004). Gene-silencing method of RNAi involves the sequence specific degradation of mRNA (Figure 1). SiRNA has now found its applications in therapeutics. It is regarded to have clinical potential in number of diseases including cancer, viral infections and many neurodegenerative disorders (Xu, *et al.,* 2004).

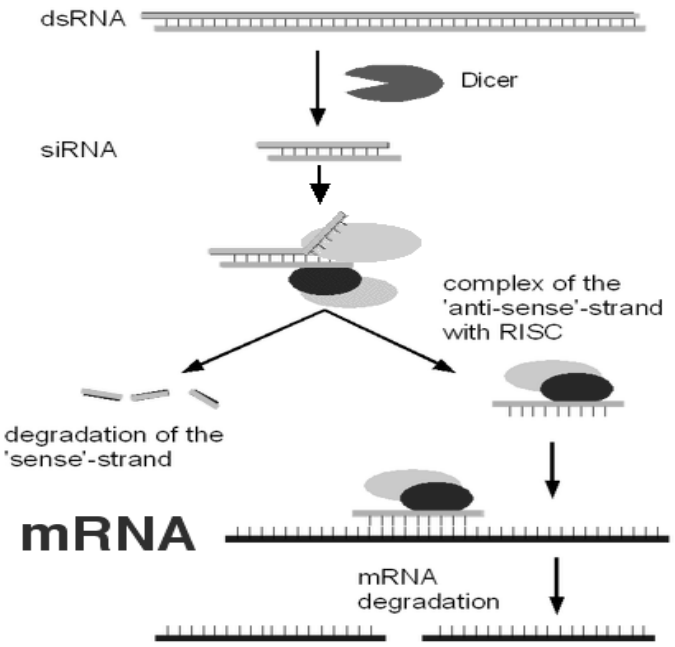

Fig. 1. Schematic Diagram of RNAi

Angiogenesis

Angiogenesis is the mechanism in which new blood vessels are formed from pre-existing vessels. It has found a number of applications in pathological process including inflammation, muscular degeneration, tumor growth and formation of tumor blood vessels (Griffioen *et al.,* 2000). Different inducing angiogenic proteins are involved along with various growth factors i.e. nPAR, VEGFs and MMPs expression enhanced (Figure 2). This changes the normal vessels to actively proliferating vessels that infiltrate the host tissues.

Once tissues are injured or diseased, they continue to produce pro-angiogenic factors which then diffuse into neighboring tissues. It then binds to its specific receptors present on ECs surface and activate them (Suhardja *et al.,* 2003). This leads to the production of several enzymes that are secreted and dissolve basement membrane. ECs then proliferate and begin to migrate towards the area where surface proteins have produced the pro-angiogenic factors (Jin and Varner, 2004). ECs close and form a lumen. Subsequently, these small individual tubes connect with each other to form blood vessels that are truly functional. The structural support is provided to the new capillary by

the penetration of specialized cells (Gerhardt and Betsholtz, 2003). Thus, by knowing the mechanism many therapeutic interventions can be made possible to inhibit these processes.

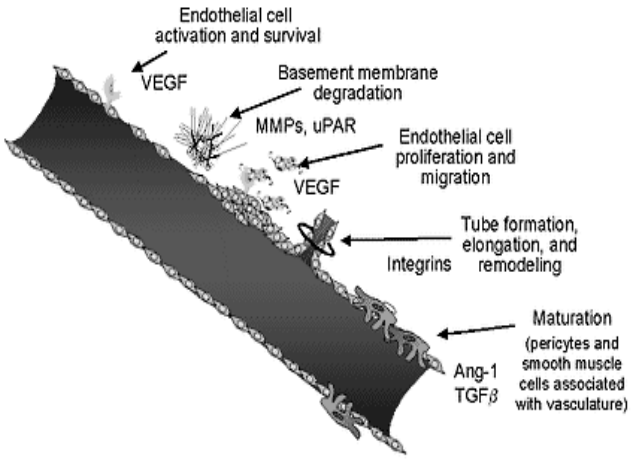

Fig. 2. The Angiogenic process (Griffioen et al., 2000)

In vitro inhibition of angiogenesis

On synthesis of DNA in angiogenesis as tool siRNA has been used to cut off stimulatory pathways of VEGF (Kranenburg *et al.,* 2004). Due to sphingosine-kinase silencing VEGF unabled to stimulate Ras-GTP or phosphorylated ERK proteins; an important step in VEGF pathway and leads to initiation of DNA synthesis (Mousa and Mousa, 2004). Using SiRNA, the inhibition of Ets-2 stopped amino peptidase N expression on stimulated ECs (Wall and Shi, 2003). As ECs trigger the capillaries formation, so by inhibiting Ets-2 we can inhibit angiogenesis. Two antiangiogenic compounds such as endostatin and fumagillin were analyzed by using differential gene expression analysis. Three genes were identified i.e. DOC1, TC1 and KLF4 whose expression was effected by both endostatin and fumagillin therapy (Griffioen and Molema, 2000). It showed that TC1 and KLF4 expression were not induced in response to endostatin due to the silencing of DOC1 (Grant *et al.,* 2004). But after fumagillin treatment, the DOC1 silencing did not affect TC1 and KLF4 expression induction (Middleton *et al.,* 2004). These results show a role for DOC1 upstream from KLF4 and TC1 in angiogenic impacts stimulated by endostatin (Bian *et al.,* 2004). These examples show the use of RNAi in indicating pathways and mediators which play a vital role in the angiogenic cascade (Sato, 2003). Figure 3 explains the transfection of cells and exogenously synthesis of siRNA or shRNA.

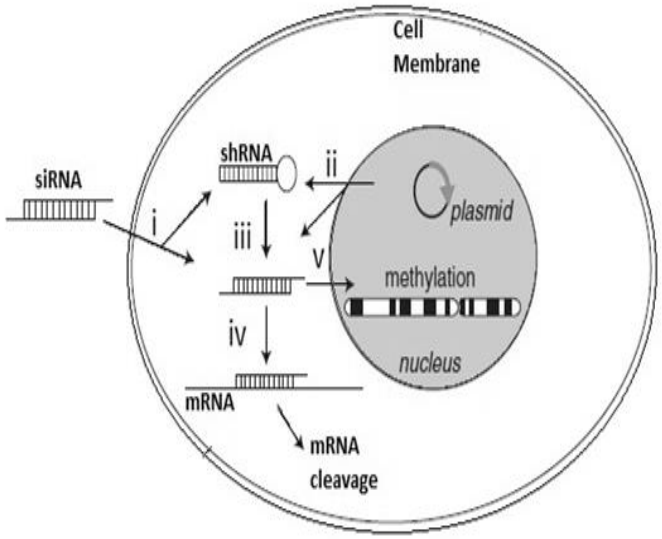

Fig. 3. Transfection of Cells and Exogenously Synthesis of siRNA or shRNA

RNAi is also used to identify novel target proteins which are involved in the process of angiogenesis or may be beneficial for curative innovations (Tsopanoglou and Maragoudakis, 2004). The novel protein disulfide isomerase expressed in tumor ECs named endo-PDI. The normal PDI acted as a survival factor for ECs in normal oxygen pressure and under hypoxic conditions (Seiki *et al.,* 2003). The endo-PDI only did so under hypoxia which is determined by using siRNA (Gerhardt and Betsholtz, 2003). The inhibition of endo-PDI cause the loss of proteins involved in the prevention of angiogenesis. So the endo-PDI may be a novel antiangiogenic target (Scavelli *et al.,* 2004). These studies represent the impressiveness and ability of using RNAi to identify novel target proteins which can be used to inhibit angiogenesis (Sowter *et al.,* 2004).

In vivo inhibition of angiogenesis using siRNA

SiRNA is a type of RNA interference. Its target is to decrease the angiogenesis and suppress the activity of tumor growth in cancer. Vasohibin 1 is a factor that works as a regulator to promote the angiogenesis and its activity is induced in endothelial cells by a stimulator known as vascular endothelial growth factor VEGF (Watanabe *et al.,* 2004). RNA Interference uses the retroviral procedures for the delivery of siRNA in tumor cells, systematically at low dosage in saline treatment result in suppressing or inhibiting the expression of VEGF (Brummelkamp *et al,* 2002; Lewis *et al.,* 2002).

In in-vivo inhibition, mostky the target of siRNA is vasohibin 2 (stimulate the sprouting form of angiogenesis)

by changing the expression of its mRNA to knock down it efficiently. Hence, the growth of tumor would be retarded (Kimura *et al.,* 2009;, Xue *et al.,*2013; Takahashi *et al.,* 2012; Koyanagi *et al.,* 2013). Angiogenesis also have the requirement for Sphingosine 1-phosphate SP1. It is a growth regulator of vascular system in embryo. For its suppression, RNA interference uses multiplex si-RNA. It specifically targets the different regions of mRNA transcript which is over expressed in tumor cells (Chae *et al.,* 2004). In case of mice tumor N2A in-vivo inhibition, mice were treated with siRNA injected intravenously after every 3 days. It leads to the reduction of growth rate of tumor because siRNA target the mice murine VEGFR2 by suppressing the function of forming the new blood vessels.

RNAi-based anti-angiogenesis therapeutics

RNA interference is an endogenous mechanism for the powerful and precise inhibition of gene expression which can be applied to target specific gene in specific cell type. Ongoing research indicates promising and milestone result of this approach.

siRNA targeting VEGF for cancer treatment

Cancer growth can be halted either by activating exogenous/endogenous anti-angiogenesis factors or to introducing inhibitors to cease the pro-angiogenic factors activities. Ali et al. (2015) demonstrated that point mutation at MDM2-SNP309 from T to G may be responsible for hepatocellular carcinoma (HCC) in patients with HCV. siRNA is a powerful candidate for both of above mentioned properties. However, their efficiency in this regard can only be interpreted through in-vivo clinical trials that how they are introduced in host cell and and with which possible draw backs (Lu and Woodle, 2005). Cationic carrier VEGF-siRNA has been reported with promising result suppressing angiogenesis in tumour in different research (Takei *et al.,* 2004).

siRNA targeting VEGF to treat ocular *neovascularization* diseases

Uncontrolled blood capillaries development in different diseased conditions like ocular neovascularization, age-related macular degeneration and diabetic retinopathy which ultimately results in blindness. Nano particle associated anti-VEGF siRNA was delivered in mouse with promising results suppressing angiogenesis in these physiological conditions (Takei *et al.,* 2004).

siRNA meditated anti-angiogenesis for rheumatoid arthritis

Even though no efficacy data have been reported using siRNA to reverse rheumatoid arthritis (RA) pathology in animal disease models, the potential application is very promising; the down regulation of RA-causing cytokines and their receptors and of VEGF and its signaling factors, either individually or with siRNAs in combination, represents a novel approach for the treatment of RA (Huang *et al.,* 2016).

CONCLUSION

Pathways that include critical pathological conditions involving angiogenic cascade can be analyzed through the RNAi process. It also involves the detection of novel targeted proteins to make their use in therapeutic intercession. This method has broad range of applications because of its specificity and potential for high range of throughput applications. As far as therapeutic applications are concerned, the exceptional efficiency of RNAi and probability of combining siRNA for the sake of enhancing silencing effects are remarkable features. Since the siRNA have been discovered, recognized significance of RNAi has increased. The examination of siRNA-mediated retardation of angiogenesis involving animal model system demonstrates the brilliant future as concerned with the therapeutic significance of siRNA. Currently siRNA mediated angiogenesis has shown very promising results in clinical trials. Potential draw backs can be overcome and efficacy can be improved by in-depth understanding of mechanism. The future challenges would be to direct the perspicacious heterogeneity of enteric coatings though developing therapeutic intercession for siRNA to different stages of angiogenesis.

CONFLICT OF INTEREST

The authors declare that no competing interests exist.

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