Editorial

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Small interfering RNA (siRNA): a hope for the loss-of-function studies in anesthesiology?

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Since the recombinant plasmid was first introduced into E.coli by Cohen et al. [1] in 1973, genetic engineering (modification) has been developing rapidly. Genetic modifications in vivo or in vitro, which have an effect on the expression of specific proteins, such as channel, receptor, enzyme, and signals, are actively applied in the researches that highlight the relationship between the effects of anesthetics and functions of specific proteins [2-5]. The loss-of-function studies that control the function of proteins, are very important in clarifying the mechanism of action of anesthetics. To achieve this, post-translational proteins are directly inhibited by pharmacological inhibitors [6,7], or are indirectly suppressed by transforming DNA construct via mutation or recombination, which is known as transgenic knockout technique [8]. After the creation of knockout mice by Capecchi et al. in 1989, transgenic knockout techniques are not only available in vitro, but are also available in vivo animal studies (In 2007, Capecchi MR won a nobel prize in appreciation for creating transgenic knockout mice). Although the gene knockout technique has several advantages over conventional laboratory techniques, including a long-lasting and a definitive specific gene-silencing effect, it is not easy to get a genetic engineering technique and initially it is expensive to purchase a gene knockout animal. After the discovery of RNA interference (RNAi) that is induced by short sequence-specific double stranded RNA (dsRNA) ranging in size from 20 to 25 nucleotides in length, and that targets the complementary sites on mRNA, exogenous synthetic small interfering RNA (siRNA) could be used to induce post-transcriptional gene silencing and that was first applied in mammalian cells in 2001 [9]. Because well-designed siRNA can induce suppression of any gene through forming the RNA- induced silencing complex (RISC) in the cytoplasm, the research for therapeutic application of siRNA against cancer, viral infection, and neurodegenerative disease are now being performed and clinical trials are also being carried out [10,11]. Moreover, if methods for effective in vivo delivery of siRNAs into the target tissue or organ are developed, anesthesiologists could easily achieve control over the tissue or organ-specific expression of individual target proteins used in the research. It is known that systemically or locally delivered siRNA induces a temporary gene expression knockdown effect by up to 90% from 48 hours to 3 weeks in animal experiments for eyes, brain, spinal cord, lungs, subcutaneous tissue, vagina, skin, isolated tumor, heart et al. [10-12]. But systemically or locally administered siRNA is easily degraded during the phase of delivery to the target cells by endogenous enzymes or phagocytosis, and its permeation through the negatively charged hydrophobic cellular membranes is difficult because of its high molecular weight (~13 kDa) and its too negatively charged character. Also, the cytoplasmic concentration of transfected siRNA is reduced as cell divisions progress and, cell types and pericellular factors influencing cell division may influence the gene silencing effects over time [13]. In general, the small size of the siRNA is considered not to provoke cytosolic dsRNA-mediated interferon response. But, it is reported that transfected siRNA is linked to the production of interferon and interleukin in some cell types (plasmacytoid dentritic cells, macrophages), that may be related to immunotoxicity. And, it is also known that a siRNA could degrade not only the targeted

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mRNAs but also the unintended mRNAs with sequence homology with the siRNA, which is called the "off-target effect". As a result, there is a chance of inaccurate results in the experiments undertaken using the suppression of a specific protein and even for assessing the cytotoxicity. To correct such shortcomings, it is recommended that the administered doses of siRNA should be reduced while constructing the design of experiments for successful delivery of siRNA into a target cell or tissue. And, the nucleotide modifications of siRNA can be applied in order to prevent the 'off-target effect' [13]. Ki et al. [14] in this month's journal found the lowest concentration of siRNA against a specific protein that could not only induce effective transfection of siRNA but also show maximum RNAi-mediated gene silencing while not demonstrating cytotoxicity, both in the cultured astrocytes and microglial cells [14]. Identifying the lowest effective concentration of siRNA is desirable for minimizing the adverse reactions and also for improving the cost-effectiveness for researchers who are always confronted with the problem of lack of financial support for experiments. Although Ki et al. [14] have clarified that each cell type has its own characteristic for determining the lowest concentration of siRNA against a specific protein at which the targeted mRNA is effectively silenced (5 nM for astrocytes, 20 nM for microglial cells), it was not determined whether that concentration of siRNA in each cell is related to the 'off-target effect' (knockdown of untargeted proteins). Tschuch et al. [15] reported that 5 nM siRNA against green fluorescent protein (GFP), which was the protein used in the study by Ki et al. [14], can suppress the expression of the other unintended proteins (CYLD and SOAT) in HeLa and HEK cells. This fact implies that untargeted proteins may also be suppressed by 20 nM siRNA against GFP, the lowest concentration suppressing the target gene expression both in the astrocytes and microglial cells in the study by Ki et al. [14], and thereby the accuracy of results of the study may be adversely affected. Thus before performing research using the procedure for siRNA silencing of expression of a specific protein, it is necessary not only to find out the lowest concentration at which a target protein could be controlled, but also to investigate the concentration of siRNA at which cellular apoptosis and expression of stress-response proteins are not triggered [16]. Ki et al. [14] tried to estimate the degree of cytotoxicity at the measured concentration of siRNA using the MTT assay, by which cell viability can be assessed. At present, control of protein expression by siRNA-induced transcriptional gene silencing is on the rise in the field of laboratory research. But, siRNA can provoke 'off-target effect' and immunomodulation, and the time course of siRNA-induced gene silencing is still unclear. So, results of the study may be adversely affected. Further research is needed to confirm the accuracy of results of the RNA interference experiments using siRNA.

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