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RNAi Approaches for Neuroprotection and Regeneration after Brain and Spinal Cord Injury

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This special issue for Brain & Spinal Cord Injury focuses on the development of RNA interference (RNAi) as a therapeutic modality for traumatic brain injury (TBI) and spinal cord injury (SCI). TBI is a leading cause of mortality and morbidity and often causes long-term disabling changes in cognition, sensorimotor function, and personality. SCI is also a devastating clinical disorder that often results in chronic pain, permanent motor disability, and other neurological dysfunctions. Although numerous molecular targets of secondary injury after TBI and SCI have been identified and validated during the past decade, no individual target has been readily druggable and no effective therapy for CNS injury has been established [1-3]. Therefore, there remain important unmet medical needs in the treatments of TBI and SCI.

RNAi, a powerful gene silencing technology, represents a novel therapy applicable to such non-druggable targets [3-11]. Since its discovery in 1998, RNAi has emerged as an important and widely used tool for evaluating target gene function *in vivo* with a great potential for clinical applications. Available technologies for generating RNAi include chemically synthesized small interfering RNA (siRNA) and vector-based shRNA or microRNA systems. The synthetic siRNA has been shown to offer great potential as a novel therapeutic strategy based on the highly specific, safe, and efficient silencing of a target gene. The major issues are the effective delivery methods, the stability of these siRNA inside the *in vivo* system, and the off-target effects such as nonspecific activation of immune system by siRNA including the TLR3 as well as the TLR7 pathways, toll-like immunity, and the induction of α, β interferons. Currently, there are two major kinds of *in vivo* delivery systems: nonviral or viral systems. As one of the nonviral carrier systems, nanoparticle-based nonviral delivery (combinations of liposomes and cationic polymer complexes) for application of siRNA *in vivo* has demonstrated improved stability and more effective silencing with less off-target effects. Lentiviral vectors encoding shRNA have also emerged as promising tools for RNAi therapy. shRNA refers to short hairpin RNA which can be incorporated into viral vectors, in contrast to the double stranded oligonucleotides used for small interfering RNA. Lentiviral shRNAs exhibit several advantages over other viral systems. For instance, lentiviral-shRNA vectors have the ability to infect both dividing and non-dividing cells, including neurons, oligodendrocytes, microglia, and astrocytes. At present, viral vectors encoding shRNAs are the most effective method for RNAi in postmitotic cells, with the long-term gene knockdown and the less immune response [3-11].

Many studies have recently demonstrated the pre-clinical progress in RNAi-based therapeutics on delivery strategies and identification of novel therapeutic targets as well as the *in vivo* stability of these siRNA in cancer and neurodegenerative CNS disorders such as Alzheimer's disease, Parkinson's, Huntington's diseases and amyotrophic lateral sclerosis (ALS) [4,6,9,10]. Remarkably, siRNA/shRNA therapies have begun clinical development [5,9] and the first evidence of successful siRNA-mediated silencing in humans has been reported by Davis et al. [11]. The potential of use of RNAi for humans encourages development of RNAi therapies in the clinical applications. However, up to now, a few studies are reported on the use of RNAi in TBI and SCI [12-15]. These studies demonstrated that: 1) RhoA siRNA inhibited allodynia, preserved white matter, and increased axonal regeneration after contusion SCI in rats [12], 2) lentiviral Lingo-1 shRNA promoted functional recovery and nerve regeneration after SCI [13], 3) siRNA targeting GFAP and vimentin improved acute urinary dysfunction after SCI [14], and 4) siRNA targeting Fzd2 inhibited Wnt5a expression and intracellular Ca^{2+} accumulation after TBI [15].

In our recent studies [16-18], the effects of siRNA-mediated knockdown of individual calpain 1 or ERK2, the two key secondary damage mechanisms after CNS injury, were investigated in rat model of contusive SCI. To investigate the hypothesis that ERK2 knockdown using RNAi provides a novel therapeutic strategy for SCI, synthetic siRNA and lentiviral ERK2 shRNA were utilized to knockdown ERK2 expression in the spinal cord following SCI. Pre-injury intrathecal administration of chemically stabilized ERK2 siRNA complexed with lipofectamine 2000 or *in vivo* jetPEI (polyethylenimine) significantly reduced excitotoxic injury-induced apoptosis or contusive SCI-induced tissue damage in spinal cord. jetPEI is widely used for siRNA delivery *in vivo,* with low cytotoxicity, and is currently being used for human clinical trials (www.polyplustransfection.com). Importantly, the PEI complexation provides almost complete protection against enzymatic or nonenzymatic degradation of chemically unmodified siRNA molecules. Reduction of spinal ERK2 expression by direct intraspinal delivery of lentiviral ERK2 shRNA 1 week pre-injury resulted in a significant improvement in locomotor function, total tissue sparing, white matter sparing, and gray matter sparing 6 weeks following severe contusive SCI. To evaluate whether calpain 1 knockdown by lentiviral shRNA would reduce pathological damage and functional deficits after SCI, we developed a shRNA therapy via convection enhanced delivery (CED, the pressure-driven continuous injections) of lentiviral vectors encoding calpain 1 shRNA (LV-CAPN1 shRNA). The ability of LV-CAPN1 shRNA to knockdown calpain 1 was confirmed in rat NRK cells using Northern and Western blot analysis. LV-CAPN1shRNA or LV-mismatch control shRNA (LV-control shRNA) were administered at 1 week pre-injury by CED at spinal cord level T10 to investigate the neuroprotective effects against thoracic contusion SCI. Intraspinal administration of the lentiviral particles resulted in transgene expression, visualized by eGFP, in spinal tissue at 2 weeks after infection. Calpain 1 protein levels were reduced by 54% at T10 2 weeks after shRNA-mediated knockdown, compared with the LVcontrol group, while calpain 2 levels were unchanged. Intraspinal administration of LVCAPN1shRNA 1 week pre-injury resulted in a significant improvement in locomotor function

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over 6 weeks postinjury, compared with LV-control administration. Histological analysis of spinal cord sections indicated that pre-injury intraspinal administration of LV-CAPN1shRNA significantly reduced spinal lesion volume and improved total tissue sparing, white matter sparing, and gray matter sparing. In these two lentiviral shRNA studies, the most differences between the CED-mediated delivery and direct intraspinal microinjection of lentiviral shRNA are the spread level and the magnitude of eGFP expression achieved. In direct microinjection method, lentivirus can transduce spinal cells, but not spread away from the injection site, thus resulting in a much localized effect. To reduce the spread of secondary damage after SCI, multiple intraspinal injections of lentiviral shRNA at the injury epicenter are needed as shown in previous study [17]. In CED-mediated delivery method, lentiviruses were able to transduce spinal cells with much more spread level and much higher magnitude of eGFP expression. The results suggest that delivery of lentiviral shRNA through CED significantly increased transduction efficacy compared with direct microinjection [18].

Together, these RNAi studies support that RNAi approaches targeting secondary damage mechanisms represents a potential therapeutics for neuroprotection and regeneration after traumatic TBI and SCI.

This special issue of Journal of Spine including review articles and original reports will explore the pre-clinical experiments regarding the development of RNAi therapies including siRNA, shRNA, and siRNA nanotechnology, discuss the challenges and opportunities of RNAi technology as applied to the treatment of TBI and SCI, and present novel approaches to overcome siRNA delivery challenges, prevent the off-target effects, and increase resistance to nuclease degradation. I believe that this Special Issue will add novel information about RNAi therapies for TBI and SCI. I hope that the readers of Journal of Spine will gain new insight into the development of RNAi therapies for CNS injury. I wish that this Special Issue will call attention to the potential and importance of RNAi approach for treating CNS injury within neurotrauma scientific community.

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