

134. Generation of Light-Emitting Somatic-Transgenic Mice for Disease Modelling of Hypoxic Ischaemic Encephalopathy

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Hypoxic Ischemic Encephalopathy (HIE), occurs in 2-3 per 1000 live births. 25% of surviving affected newborns will suffer from severe life-long neurological disability. HIE is associated with reactive astrogliosis and inflammation. During this process the activity of STAT3 and NFκB transcription factors is increased, and glial fibrillary acidic protein (GFAP) expression is upregulated in astrocytes.

Light producing transgenic mice, where luciferase expression is controlled by a surrogate promoter or by a minimal promoter downstream of tandem, synthetic, transcription factor binding elements, are used to provide an in vivo readout of disease processes. In this study, we aimed to deliver STAT3 and NFκB activated or GFAP promoter driven luciferase reporter constructs to brains of neonatal mice as a form of assessing the amount of astrogliosis and inflammation in a mouse model of HIE.

A rodent GFAP promoter, and STAT3 and NFκB response elements were each cloned into a lentivirus vector upstream of the genes encoding a codon-optimised firefly luciferase and green fluorescent protein. Lentivirus vector pseudotyped with either gp64 or VSV-g viral envelope glycoproteins were injected intra-cranially into CD1 outbred neonatal (P0) mice, and luciferase expression was monitored continually by whole body bioluminescence imaging of conscious mice. Vector containing NFκB response element was also injected into P0 mice, which underwent HIE surgery at P7.

The two pseudotypes gp64 and VSV-g exhibited different cellular tropisms within the central nervous system; VSV-g predominately targeting neuronal cells whereas gp64 transducing cells of fibrillary astrocytic morphology. Long-term expression of luciferase was observed. Following induction of HIE, the most severely affected mice lost weight. We observed a significant correlation between the luciferase expression 24 hours after surgery and weight 7 days after surgery ($P = 0.0007$) in mice which had received the gp64 pseudotyped NFκB biosensor; the most severely affected HIE mice had the lowest luciferase expression and weight. However, the HIE mice which had received the VSV-g pseudotyped NFκB biosensor failed to show this correlation between the luciferase expression and weight ($P = 0.479$). This indicates that NFκB-controlled luciferase expression in astrocytes is predictive of brain injury. We are now investigating the mechanism of this predictive relationship.

Aptamers

135. Sequence-Engineered mRNA Without Chemical Nucleoside Modifications Enables an Effective Protein Therapy in Large Animals

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Being a transient carrier of genetic information, mRNA could be a versatile, flexible and safe means for protein therapies. While recent findings highlight the enormous therapeutic potential of mRNA, evidence that mRNA-based protein therapies are feasible beyond small animals such as mice is still lacking. Previous studies imply that mRNA therapeutics require chemical nucleoside modifications to obtain sufficient protein expression and avoid activation of the innate immune system. Here, by applying sequence-engineered mRNA we show that chemically unmodified mRNA can achieve those goals as well. Using erythropoietin (EPO) driven production of red blood cells as the biological model, engineered Epo mRNA elicited meaningful physiological responses from mice to non-human primates. Even in pigs of about 20 kg in weight, a single adequate dose of engineered mRNA encapsulated in lipid nanoparticles (LNPs) induced high systemic Epo levels and strong physiological effects (Fig.). Our results demonstrate that sequence-engineered mRNA has the potential to revolutionize human protein therapies.

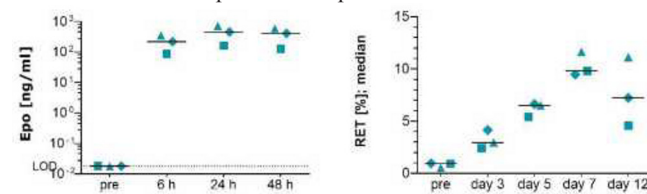


Fig.: Engineered, unmodified Epo mRNA can elicit systemic physiological responses in swine. Animals received LNP encapsulated porcine Epo mRNA by intravenous injection on day 0 and were analyzed for EPO levels and hematological parameters at various times (pre = prevalue before treatment). Protein levels before treatment were below the limit of detection (LOD) of the assay.

136. First-in-Human Study of NS-065/NCNP-01; the Morpholino Based Antisense Oligonucleotide for Exon 53 Skipping in Duchenne Muscular Dystrophy

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Currently, phase 2/3 clinical trials of exon 51 skipping for Duchenne Muscular Dystrophy (DMD) are being conducted and have been shown promising results for NDA. Next to the patients treatable by exon 51 skipping, the patients amenable to exon 53 skipping are the second largest population; therefore a development of exon 53 skipping drug has high priority. National Center of Neurology and Psychiatry, with Kyoto-based pharmaceutical company Nippon Shinyaku, had jointly developed the exon 53 skipping drug since 2009 and started an investigator-initiated clinical trial from June 2013 (UMIN Clinical Trial ID: UMIN000010964, ClinicalTrial.