stress in dopamine (DA) neurons, thereby mimicking Parkinson's disease (PD) pathology. Mechanistically, miR-126 targets a series of factors in PI3K/AKT/GSK-3β and MAPK/ERK signaling pathways and small increases of this miRNA cause a downregulation of these signaling cascades, impairing the effects of neurotrophic and neuroprotective GF, such as IGF-1, NGF, BDNF, and soluble amyloid precursor protein α (sAPPα). In turn, inhibiting miR-126 enhances the actions of GF without disturbing normal neuronal cell function. Our data indicate that miR-126 may play a profound role in neuronal cell survival, at least in part by regulating GF/PI3K/AKT and MAPK/ERK signaling. While its elevation is neurotoxic, its inhibition is neuroprotective, suggesting that targeting this miRNA may have therapeutic potential for neurological and age-related disorders. To experimentally address this concept, we have developed a therapeutic strategy using a nanotechnology approach to prevent disease onset in a mouse model of AD.

743. Minicircles Are Similar to Plasmids in Providing High Level, Long-Term Expression in the Lung

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Background: Many gene therapy applications require persistent transgene expression to treat chronic disease. We have previously developed CpG-free plasmids capable of robust, long-term transgene expression in the mouse lung (Hyde *et al*; 2008 Nat. Biotech. 26:549) and confirmed their utility in clinical trials, where monthly aerosol delivery of plasmid/liposomes to patients with Cystic Fibrosis resulted in a stabilisation of their lung function over a 12-month period (Alton *et al* 2015 Lancet Respir. Med. 3:684). The removal of all CpGs from a plasmid, including the selection of a CpG-free promoter, can be time-consuming, therefore we evaluated which aspects of plasmid design were crucial for long-term transgene expression in the lung, including the use of minicircles instead of conventional plasmids. Results: First, we measured luciferase (Lux) expression in the lungs of BALB/c mice from a CpG-free transgene expression cassette (hCEFI-soLux) compared with a standard CpG-rich (hCEFI-Lux) cassette. Plasmid DNA was complexed with 25 kDa branched polyethylenimine (PEI) and delivered as an aerosol to the lungs of BALB/c mice (n=6 per time-point). Lux activity from the CpG-free hCEFI-soLux cassette was greater than its CpG-rich counterpart ($p < 0.005$) at every time-point (up to 28 days), with no loss of activity over the course of the study. Expression from the expression cassette containing CpGs, however, was not persistent, but declined to between 30% and 2% of respective day 1 levels by day 28 ($p < 0.05$). Next, we performed similar studies using minicircles, where the CpG-free and CpG-rich expression cassettes were manufactured as minicircles with minimal backbone sequences (209bp with 11 CpGs). When the CpG-free cassette was used there was no significant difference between Lux activity obtained from the plasmid and minicircles; in both cases robust expression persisted beyond the duration of the study (28 days). Finally, we investigated whether the detection of CpGs by the Toll-like receptor-9 (TLR9) signalling pathway played a role in the loss of expression from the CpG-rich cassette. The experiments were repeated in TLR9-deficient mice and the results showed that Lux levels were similar to those obtained in BALB/c mice. This indicated that the effect of CpGs on the *in vivo* expression profile (whether in the transgene cassette or backbone) is independent of the TLR9 pathway. Conclusion: These studies indicate that a CpG-free transgene cassette is crucial to achieving high levels of persistent expression in the murine lung, when delivered as a plasmid or minicircle. Minicircles have not yet been evaluated clinically, but they have several advantages over plasmids, including their reduced overall size such that a higher effective dose is delivered. These findings could also be applicable to other organs, such as muscle and liver.

744. Optimizing Conditions for Aptamer Folding Using a High-Throughput Aptamer Fluorescence Binding and Internalization (AFBI) Assay

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Aptamers are small structured RNA or DNA oligonucleotides that bind target molecules with high avidity and affinity. Aptamer selection begins with a hugely complex library of aptamer sequences that are enriched for a specific target using the SELEX process, Systematic Evolution of Ligands by EXponentional enrichment. An important consideration in the SELEX process is the protocol used to fold aptamers into their active conformations. The folding conditions include multiple variables, such as temperature, buffer components, incubation time and aptamer concentration. Aptamer folding protocols vary widely across the aptamer field, and most published folding conditions primarily describe only temperature and folding time. To understand how variations in folding conditions impact aptamer function, we developed a novel high-throughput assay to interrogate the optimal folding conditions of several published aptamers. The Aptamer Fluorescence Binding and Internalization (AFBI) assay is a cell-based platform that uses a 96-well plate format to rapidly and efficiently screen multiple fluorescent-labelled aptamers against hundreds of conditions. The AFBI assay can be applied to rapidly determine aptamer binding constants (Kd) on cells, time course of aptamer internalization and cross reactivity of aptamers against different cell types.

Using the AFBI assay, we screened several different folding parameters against published aptamers. We found that the buffer components contributed significantly more to an aptamer's function than any other examined factor. The concentration of an aptamer during folding was important for some aptamers but not others, including aptamers that originated from the same selection. Most surprising was that most temperature protocols had little impact on aptamer function after folding, the exception being that high temperature (95°C) often attenuated aptamer function. In summary, our data using the AFBI assay revealed that aptamer folding is more dependent on buffer components than the temperature protocol. Furthermore, aptamers derived from the same selection may have different optimal folding conditions. These data demonstrate that optimal aptamer folding protocols need to be more carefully interrogated on a per aptamer basis and reported in detail to allow for efficacious and reproducible results.