ABSTRACT

Nurse, Nathan P. Ph.D., Purdue University, December 2016. Engineering Chromatin Vectors for Gene Therapy Applications. Major Professor: Chongli Yuan.

Gene therapy has the potential to treat a wide variety of inherited and acquired genetic diseases through the delivery of DNA encoding a protein that can provide a therapeutic effect to the diseased cells. The full potential of gene therapy has yet to be realized due to a myriad of safety and efficacy impediments. Chief among these efficacy impediments is the inability to achieve high levels of sustainable protein production from the delivered transgenes. Silencing of these transgenes via epigenetic mechanisms is a major contributor to this lack of sustainability. Upon entry into the nucleus, the delivered transgene becomes complexed with host cell histone octamers. These histone octamers proceed to be post-translationally modified, in particular on the histone tails, in a manner to induce silencing of the transgene. We hypothesized that this epigenetic silencing due to histone modifications could be countered by co-delivering, with the transgene, histone octamers containing modifications associated with active gene expression. The focus of this work was therefore to investigate the ability of modified and unmodified histones to influence transgene behavior in the nucleus.

As chromatin structure can dictate the transcription state of a gene by mediating DNA accessibility, we first investigated the role that H3 and H4 tails play in controlling the

structure of nucleosome core particles and nucleosome arrays. We found that the H3 tail partakes in intra- and inter-nucleosomal interactions that serve to modulate both short range and long range chromatin compaction. We found that the H4 tail also partakes in interactions that compact chromatin, but to a lesser extent than what was seen for H3 tails. These results suggested that H3 tail modifications could be of primary importance for influencing transgene behavior.

Since histone proteins function in the nucleus not only as histone octamers but also as dimers and tetramers we investigated the effects of co-delivery of transgenes with unmodified histone dimers, tetramers, and octamers. We found that co-delivery with dimers lead to an initial increase in transgene expression efficiency but this increase did not persist over time. We also found that the co-delivery with histone octamers led to decreased transgene degradation, suggesting that they remain complexed to the transgene over the observed time period and could be good candidates to effect long term transgene behavior.

After identifying octamers as good candidates for influencing transgene behavior, we co-delivered pDNA with unmodified or modified (H3K9,14Q, H4K16Q, H3Kc4me3, or H3Kc9me3) octamers and observed their delivery independent effects on transgene expression. Transgenes with H3K9,14Q, H4K16Q, or H3Kc9me3 modified octamers all exhibited increased transgene expression efficiency compared transgenes with unmodified octamers. Additionally, the fold increase for these modified octamers over unmodified was sustained during the observed time window. This suggests that octamer modifications can serve an advantageous tool that can be employed to improve the long term behavior of transgenes for gene therapy applications.