Limited expression of non-integrating CpG-free plasmid is associated with increased nucleosome enrichment

ABSTRACT

CpG-free pDNA was reported to facilitate sustained transgene expression with minimal inflammation in vivo as compared to CpG-containing pDNA. However, the expression potential and impact of CpG-free pDNA in in vitro model have never been described. Hence, in this study, we analyzed the transgene expression profiles of CpG-free pDNA in vitro to determine the influence of CpG depletion from the transgene. We found that in contrast to the published in vivo studies, CpG-free pDNA expressed a significantly lower level of luciferase than CpG-rich pDNA in several human cell lines. By comparing novel CpG-free pDNA carrying CpG-free GFP (pZGFP: 0 CpG) to CpG-rich GFP (pRGFP: 60 CpGs), we further showed that the discrepancy was not influenced by external factors such as gene transfer agent, cell species, cell type, and cytotoxicity. Moreover, pZGFP exhibited reduced expression despite having equal gene dosage as pRGFP. Analysis of mRNA distribution revealed that the mRNA export of pZGFP and pRGFP was similar; however, the steady state mRNA level of pZGFP was significantly lower. Upon further investigation, we found that the CpG-free transgene in non-integrating CpG-free pDNA backbone acquired increased nucleosome enrichment as compared with CpG-rich transgene, which may explain the observed reduced level of steady state mRNA. Our findings suggest that nucleosome enrichment could regulate non-integrating CpG-free pDNA expression and has implications on pDNA design.