Abstract

Innate immune sensors recognize mRNA. During RNA capping, Cap0 (m7GpppN) is formed as an intermediate. Methylation of Cap0 (m7GpppN) to form Cap1 (m7GpppNp) can be beneficial for mRNA translation as it is recognized by PRRs. In addition, co-transcriptional capping with CleanCap™ (Cap1) helps evade an immune response. Previously, we identified 5-methoxyuridine (5moU) as a promising modification to avoid capping assay that allows direct assessment of mRNA capping. Capping efficiencies as high as 85% were observed. Incorporation of our novel co-transcriptional capping method called CleanCap™ that yields Cap1 with high fidelity and efficiency is an attractive strategy to avoid detection by PRRs and allow maximal translation of the transfected mRNA. For mRNA drugs to achieve high expression in cells or target organs, transfected mRNAs must avoid detection by PRRs. To further explore the ability of CleanCap™ to avoid detection by PRRs, we incorporated 5-methoxyuridine (5moU) or pseudouridine (Ψ) into an mRNA encoding Luciferase. Luciferase mRNAs were fully substituted with these modifications and tested in THP-1 cells. Luciferase Rs and U deleted FLuc mRNA were synthesized and tested in THP-1 cells to assess the effect of the modifications. Five different modifications were evaluated in THP-1 Dual cells, but only the use of 5moU resulted in lower toxicity compared to wild type (wt) mRNA. Luciferase activity in THP-1 cells was measured for HPLC purified and non-HPLC purified mRNAs. If 5moU is not recognized by PRRs, then activity of 5moU should be equivalent in HPLC purified and non-HPLC purified mRNAs. HPLC purification to remove dsRNA reduced toxicity and interferon response. If PseudoU derivatives are not recognized by PRRs, then activity of PseudoU derivatives should be equivalent in HPLC purified and non-HPLC purified mRNAs. This study shows that PseudoU derivatives are not recognized by PRRs.

Conclusions

- We have introduced a number of novel modified bases with interesting translational and immunological properties.
- PseudoU derivatives do not have a risk of insertional mutagenesis and do not alter the activity of Smo mRNA.
- The ability of Smo mRNA to suppress innate immune stimulation is sequence context dependent.
- PseudoU derivatives are not recognized by PRRs.
- Future Directions
  - If PseudoU is not recognized by PRRs, then activity of Smo mRNA should be equivalent in HPLC purified and non-HPLC purified mRNAs.

Contact
Anton McCaffrey
amccaffrey@trilinkbiotech.com

www.trilinkbiotech.com