Next-generation Gene Silencers for Cancer Immunotherapy

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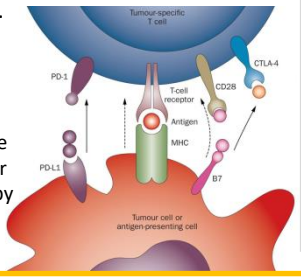
Next-generation Gene Silencers for Cancer Immunotherapy

Introduction

Immune evasion of cancer cells

In the tumor microenvironment, immune evasion entails a reciprocal interaction between cancer cells and T-cells. This leads to the inhibition of the tumor killing ability of T-cells *via* immune checkpoint protein interactions, mainly - PD-1/PD-L1 & CTLA-4/B7.



Hypothesis: Inhibiting immune checkpoint proteins can be used for cancer immunotherapy

Caveats of existing therapies

1) Antibodies
- Adverse side effects, such as autoimmunity

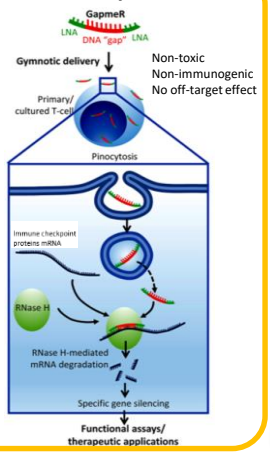
2) Chemical inhibitors
- Non-specificity
- Toxicity

3) siRNA / Peptides
- *In vivo* delivery
- Low stability
- Low bioavailability

WHY?

T-cells are hard-to-transfect!

Conceptualization



Aim

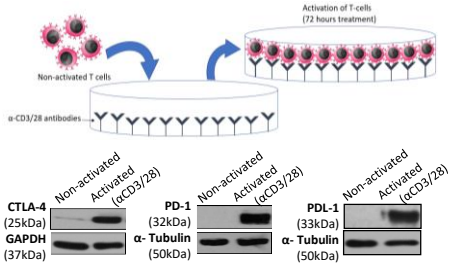
To develop specific Locked Nucleic Acid (LNA) antisense oligonucleotides (GapmeR) targeting immune checkpoint proteins in human primary T-cells.

Methodology

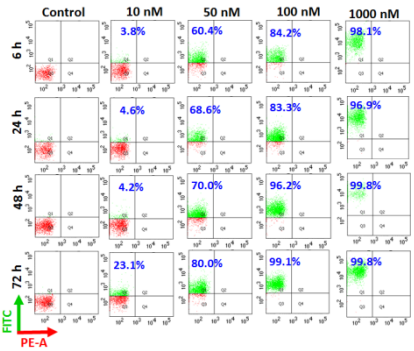
- T-cell isolation and activation
- Design and synthesis of GapmeR
- Fluorescence-activated cell sorting
- Confocal microscopy
- Western immunoblotting
- RT-qPCR

Results

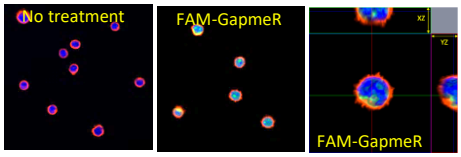
1. The expression levels of immune checkpoint proteins (CTLA-4, PD-1 and PD-L1) **are highly up-regulated in activated primary T-cells.** Cells were activated with immobilized anti-CD3/28 for 72 h and protein expression was analyzed using Western immunoblotting.



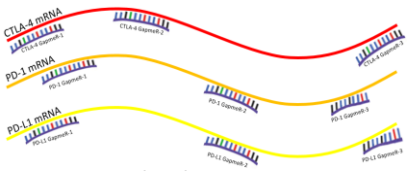
2. GapmeR molecules internalize into human T-cells in a time- and dose-dependent manner. Cells were treated with various doses of FAM-labelled GapmeR for up to 72 h and analyzed using flow cytometry.



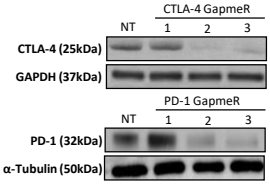
3. GapmeR localizes to the nucleus and cytoplasm in human T-cells. Cells were treated with 500 nM FAM-labelled GapmeR (green) for 48 h, co-stained with Rhodamine-Phalloidin (red) and Hoechst (blue) and imaged using confocal microscopy.



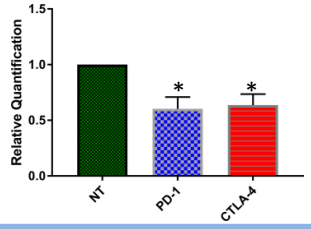
4. Design and synthesis of GapmeR targeting immune checkpoint proteins. Three different constructs targeting various regions of CTLA-4, PD-1 or PDL-1 mRNA were designed using Exiqon's "Design-Tool" software.



5. GapmeR molecules targeting CTLA-4 or PD-1 significantly reduce the expression of corresponding proteins in activated T-cells. Cells were activated (72 h) in presence of GapmeR targeting CTLA-4 or PD-1 and analyzed via Western immunoblotting.



6. GapmeR molecules targeting CTLA-4 (design 2) **or PD-1** (design 3) **significantly reduce their corresponding mRNA levels in human T-cells.** Cells were activated in presence of GapmeR targeting PD-1 or CTLA-4 for 72 h and mRNA levels were analyzed using qRT-PCR.



Conclusion

- We optimized an *in vitro* model using human primary T-cells mimicking tumor micro-environment with the up-regulation of immune checkpoint proteins.
- We developed GapmeR gene silencers targeting CTLA-4 and PD-1 and validated their efficiency.

Future works

- Tumor killing assays; *in vitro/in vivo,* to further validate functional efficacy of GapmeR targeting CTLA-4, PD-1, PDL-1 or multiple combinations.
- Development of nanotechnology enabled controlled-release of GapmeR for *in vivo* applications.

References

1. Fazil MHUT, et al. *Scientific Reports* 2016.
2. Topalian SL, et al. *Cancer Cell* 2015.

Project Title: Next-generation Gene Silencers for Cancer Immunotherapy

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