Background

New insights into genetic characteristics of leukemic cells have initiated the development of the chimeric antigen receptor (CAR) T-cell therapy. This type of adoptive cell immunotherapy has been a breakthrough in the treatment of aggressive B-cell lymphoma and B-cell precursor acute lymphoblastic leukemia. (NIH)

CAR T cells are constructed by harvesting mononuclear cells. These cells are usually acquired from a patient’s own blood. T cells are altered genetically or chemically to express a transgene encoded with a tumor-specific CAR. The altered CAR T cells are infused into the patient to multiple and eradicate tumor cells.

Previous CAR T therapies have mixed outcomes.

The development of CD27-CARs to target CD70 shows promising increased safety for CAR-T cell immunotherapy.

Methods

We tested the efficacy of CD27-CARs to target CD70. Through the transformation of 27CAR plasmids in a competent E-coli cell. CD27 functional activity was assessed through the generation of 27-CAR viral particles by transfection. The measurement of viral titration was examined through Flow cytometry test. Following, the isolation of CD70 plasmid DNA from selected clones allows for the desired plasmid to be confirmed through RE (restriction enzyme) digestion. Thus, allowing for a ligand/receptor communication between CD27 and CD70 and the generation of CAR T cells.

-NanoDrop 2000 is used to quantify and assess the purification of DNA, RNA, protein. In these graphs the results were low excluding the last test with the results 258.83 (Nucleic Acid) and 1.8 (260/280). With low results means the CD70 protein needs to be tested with a midiprep protocol

Results

Developed CD27-CARS to target CD70 to promote tumor eradicate

CD70 proteins are highly expressed in T-cell Lymphoma and Leukemia such as AML CD27-CARs are viral particles formulated by infecting host organisms. Particles are functionalized by genetic and chemical manufacturing. The construction of lentiviral vectors constructed from plasmids into 293T cells through transfection, transfection, and transduction.

The measurement of the viral titration showed MOI (multiplicity of infection) to be at its highest at 48 hours. Bi-functional CAR-T cells show increased safety for CAR-T cell immunotherapy and enhanced movement against cancer target antigens to promote tumor eradication.

CD70 antigen to CD27 ligand

The protein encoded by this gene is a cytokine that belongs to the tumor necrosis factor (TNF) ligand family. This cytokine is a ligand for CD27. It is a surface antigen on activated lymphocytes. It induces proliferation of costimulated T cells, enhances the generation of cytolytic T cells, and contributes to T cell activation. This cytokine is also reported to play a role in regulating B-cell activation, cytotoxic function of natural killer cells, and immunoglobulin synthesis. (provided by RefSeq, Jul 2008)

Conclusions

CAR-T treatment for patients with blood tumors has shown promising outcomes. Further study is needed. The optimization of protocols to generate CAR-T cells has the potential to improve the quality of cells and thus, therapies. The immunotherapeutic treatment of immunocompromised patients with CAR T cells is one of the most encouraging adoptive cellular therapy tactics to date in the treatment of leukemia and lymphoma. Promoting the progression of CAR therapies will further adopted cell therapy to cure cancers.

Acknowledgements

Lymphoma/Myeloma Department
Sattva Neelapu Lab
Pappanaicken R Kumar

References

