Utilising Immune System to Tackle Cervical Cancer

A TCR-T Immunotherapy for HPV⁺ Cancers in Development

Yijun Lin
MSci (Hons) Biomedical Sciences, School of Biomedical, Nutritional and Sport Sciences
Contact: Y.Lin@Newcastle.ac.uk Supervisor: Dr Lei Huang

Newcastle University
Institute of Cellular Medicine

1. Introduction

Cervical cancer is a malignant cancer caused by infection from human papilloma virus (HPV). It is the 14th most common cancer in females in the UK. Moreover, incidence of HPV⁺ head and neck cancer is rising as boys are not normally vaccinated against HPV[1]. T cell receptor-T cell (TCR-T) therapy is a promising treatment for this type of cancer, as it allows the production of a large quantity of cancer specific T cells. T cells are a special type of immune cells that are effective at recognising and killing abnormal cells and they achieve this by using their T cell receptors (TCR). However, it is very important for these T cells to only recognise and kill cancer cells but not healthy cells. To achieve this, T cells are isolated from the patient's blood and artificial genes coding for cancer cell specific TCR are introduced into these T cells. Cells are then expanded to produce a large quantity of the same cell, which will destroy cancer cells when transferred back to the patient[2].

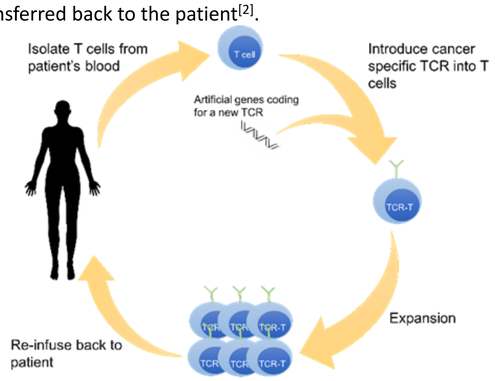


Figure 1. TCR-T therapy overview.

HPV⁺ cancer cells express a surface antigen called E7, which is not expressed by healthy cells. Genetically modified T cells carrying E7-specific TCR can therefore recognise this target and destroy cancer cells[3].

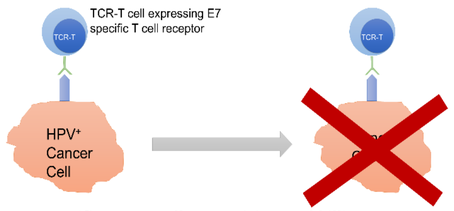


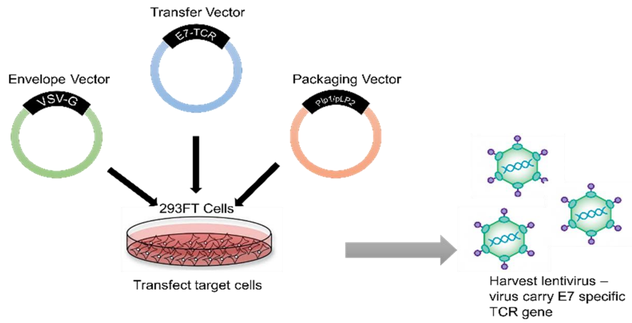
Figure 2. E7-specific TCR-T cell recognising and killing HPV⁺ cancer cell.

2. Aims

- To package gene coding for E7-specific TCR using a lentivirus-based construct.
- To test transferability of packaged TCR gene by introducing into BW CD8 ly2.2 cells (a mouse T cell line that is TCR-negative)

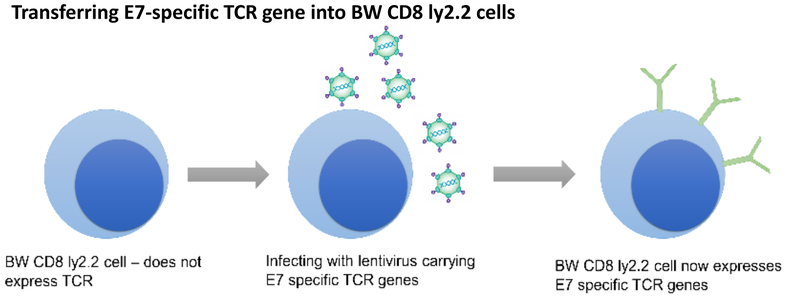
3. Methods

1) Producing lentivirus carrying gene coding for E7-specific TCR



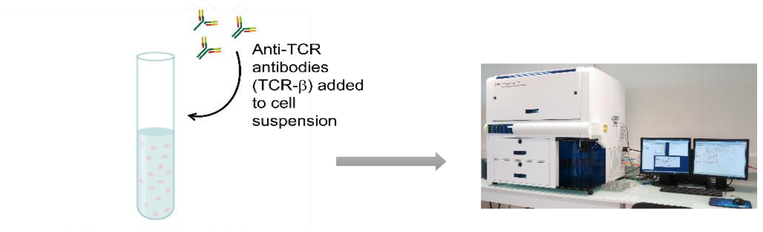
- 293FT is a human cell line capable of replicating and producing retrovirus.
- Lentivirus is a subtype of retrovirus and it has the ability to introduce DNA into cells that they infect - in this study they were used to transfer the E7-specific TCR gene into T cells.
- Three types of vectors were introduced into 293FT cells to produce lentivirus carrying E7-TCR gene:
  1. The transfer vector, which contains gene coding for E7-specific TCR.
  2. The envelope vector, which contains genes coding for the viral envelope (the 'shell' of the virus).
  3. The packaging vector, which is essential for the assembly of the lentivirus.

2) Transferring E7-specific TCR gene into BW CD8 ly2.2 cells



- BW CD8 ly2.2 is a mouse T cell line that is TCR-negative.
- These cells were used in this study to test whether the E7-specific TCR gene had been successfully packaged.

3) Analysis using flow cytometry to verify outcome



4. Results

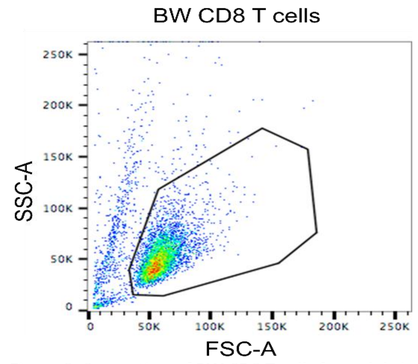


Figure 3. Separation of BW CD8 T cells from debris. Plotting side scatter (SSC-A) against forward scatter (FSC-A) allows separation of cells from debris and impurities.

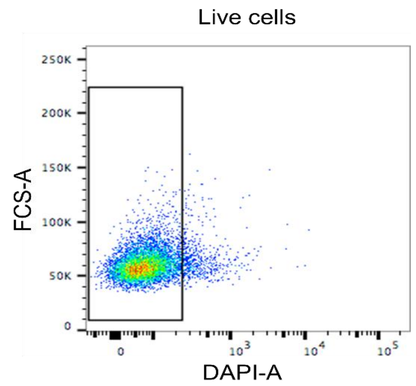


Figure 4. Identification of live cells. DAPI is a dye which stains for dead cells. Live cells that are not stained by DAPI are gated out.

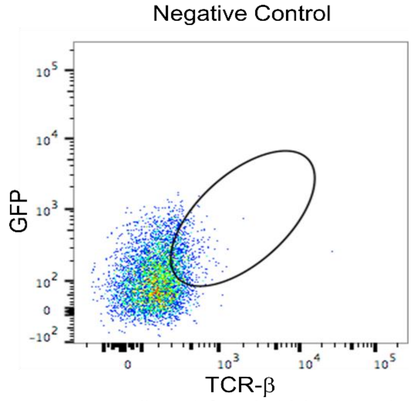


Figure 5. BW CD8 T cells not infected with lentivirus do not express TCR. The E7-TCR vector contains a gene that codes for green fluorescence protein (GFP). Non-infected cells are both TCR- and GFP-negative.

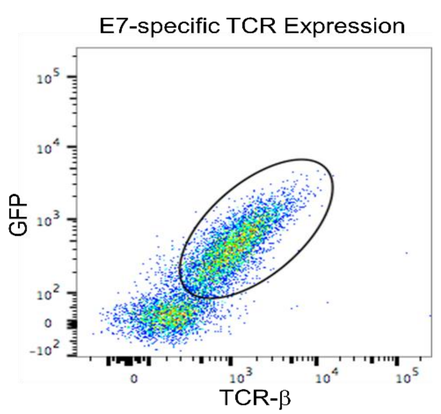


Figure 6. BW CD8 T cells infected with lentivirus show TCR expression. As shown by the gating, majority of infected cells express TCR and GFP.

5. Conclusion

- Lentivirus-based approach was effective in packaging E7-TCR gene.
- Transferring the E7-TCR gene to BW CD8 T cells was successful and the gene was expressed in majority of cells tested.
- This means that the approach used in this study was effective in producing cancer cell specific T cells for TCR-T therapy.

6. Future Work

- The next step would be to test the functionality of the engineered T cells to see if they are effective at killing cancer cells. One way to do this would be using MHC tetramers with E7 antigen to test if they can be recognised by the TCR-T cells.
- The TCR-T approach can also be investigated further on human T cells in culture or in preclinical models.

7. Acknowledgement

I'd like to thank Dr Lei Huang for his supervision and guidance, Dr Henrique Lemos and Dr Rong Ou for their assistance throughout my project. In addition, thanks to Newcastle University for providing the funding.

References: 1. Cancer Research UK. https://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/cervical-cancer/incidence [cited 17/10/2019]. 2. Jiang X et al, Cancer Letters, 462(2019)23-32. 3. Jin BY et al, JCI Insight, 2018;3(8):e99488