Tracking the Migration of T-cell Acute Lymphoblastic Leukemia Cells in Mice: Determining the Role of CCR7 in Central Nervous System Invasion

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Introduction

T-cell acute lymphoblastic leukemia (T-ALL) is a blood cancer commonly found in children and adolescents that can invade the central nervous system (CNS). Inside the CNS, T-ALL cells become inaccessible to chemotherapies that circulate in the blood, necessitating the direct application of harmful drugs and ionizing radiation to the CNS. CCR7 binding to one of its ligands, C-C motif chemokine ligand 19 (CCL19), is required for T-ALL invasion of the CNS. Ultimately, our goal is to develop pharmaceuticals to inhibit CCR7 and prevent T-ALL from invading the CNS. This study focuses on tagging T-ALL cells to allow us to monitor their migration in a mouse model of T-ALL.

Objective

Express luciferase/tdTomato construct in T-ALL cells ± CCR7 expression to allow us to locate the cells during progression of T-ALL in living animals and in fixed tissue samples.

Methodology

Cloning: Generate pLenti backbone and the Luc2-P2A-tdTomato insert

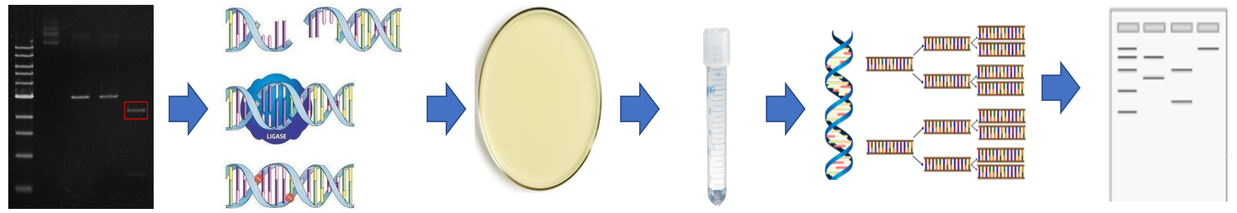
Lentivirus protocol
Day 1: Plate HEK293T cell line
Day 2: Transfect cells with pCDH-EF1-Luc2-P2A-tdTomato and packaging vectors
Day 3: Wash cells
Day 4-6: Collect supernatant and concentrate lentivirus

Transduce T-ALL cell lines
Day 1: Transduce T-ALL cell lines (CEM, DND41, Hut78) with different concentrations of lentivirus
Day 2: Wash cells
Day 5: Assess transduction efficiency

Flow Cytometry: Analyze tdTomato expressing cells

Fluorescence microscopy: Use to detect tdTomato expressing cells

Cell Dilutions: Expand single cells to isolate clones expressing fluorescent cells

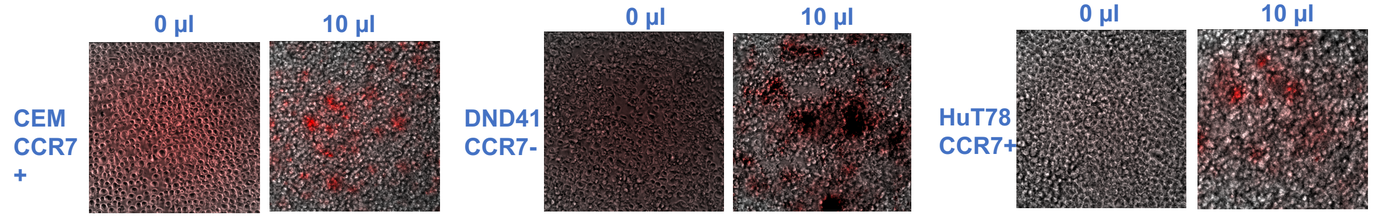


Results

Flow Cytometry

CEM		DND41		HuT78	
0µl FL2 INT Median	10µl FL2 INT Median	0µl FL2 INT Median	10µl FL2 INT Median	0µl FL2 I NT Median	10µl FL2 INT Median
1.22	1.28	1.56	1.09	1.01	0.80

Transduction



Cell-line	# of wells that grew	# of wells that glow
CEM	11	1

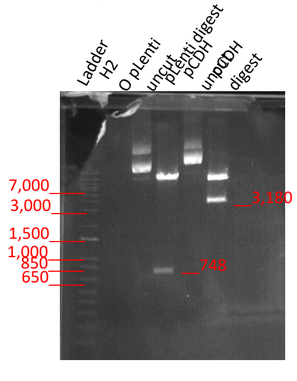
Cell-line	# of wells that grew	# of wells that glow
DND41	1	0

Cell-line	# of wells that grew	# of wells that glow
HuT78	0	0

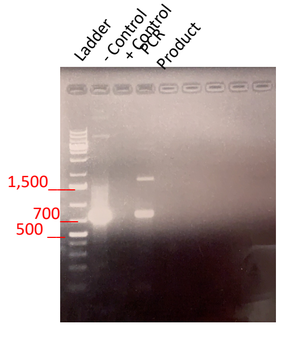
Cell Clones

G1	1	2	3	4	5	6	7	8	9	10	11	12
A	C 48	11	8	5	13	13	7	11	8	10	6	5
B	7	5	4	5	11	3	4	7	2	4	3	7
C	15	G2 D	20	5	78	71	5	1	94	9	100	191
D	13	63	68	135	13	C 11	H 40	1				
E	G3 H 4	1	4	3	0	4	4	2	6	3	4	6
F	4	14	15	4	4	8	12	5	7	6	18	6
G	8	27										
H	D 281	183	1	203	62	8						

Gel Digest



Gel Electrophoresis



Conclusions

- Flow cytometry was not useful to isolate cells since we lacked the appropriate laser to excite fluorophore
- Fluorescence microscopy allowed us to identify tdTomato positive cells compared to the background media fluorescence
- Cell expansion in a 96-well plate was successful to obtain fluorescent cell colonies of CEM and DND41 cells

Future Directions

- Track the localization of T-ALL (CEM, DND41 and HuT78) cells by fluorescent/luciferase imaging in animals to determine if CNS infiltration persists in the presence of CCR7 antagonists.

References

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2. “Retroviral Transduction of T Cells and T Cell Precursors.” *T-Cell Development: Methods and Protocols*, by Rémy Bosselut and Melanie S. Vacchio, vol. 1323, Humana Press, 2016, pp. 99–108.

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