How do mutations give rise to neuroblastoma cancer stem cells?

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Background

Neuroblastoma is a common cancer in children and is marked by a diverse range of outcomes and behaviours. The high-risk cases of this disease are responsible for 15% of cancer-related mortality in this population **[1,2]**. It is believed that cancer stem cells are responsible for the heterogeneous composition of a neuroblastoma. The neural crest, which is comprised of multipotent cells, is a transient structure in the vertebrate embryo. Mutations of the genes called MYCN and ALK transform these progenitor cells into the malignant cancer stem cells. One hypothesis is that the transformation occurs because the mutations favour symmetric cell division (SCD) over asymmetric cell division (ACD) **[2]**. In this project, we attempted to link the mutations to the two modes of cell division mechanistically using tools from graph theory.

Methods

1. Intracellular mechanisms

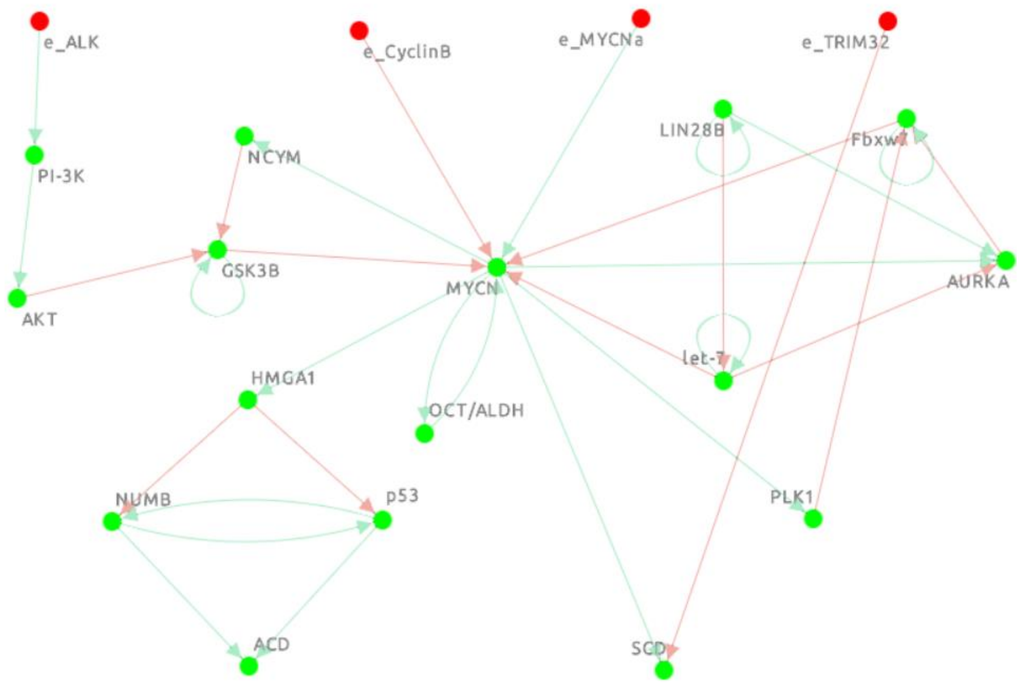

Figure 1**: Intracellular mechanisms regulating the balance between SCD and ACD. Network with 20 nodes and 32 edges. CellColective was used to generate this diagram **[1, 2].

2. Boolean network

We converted the intracellular regulatory mechanisms into a Boolean network, where each node is modelled as a Boolean network.

16 combinations of four input nodes (red nodes).

65536 states, each defined by 16 output nodes (green nodes).

3. State space analysis

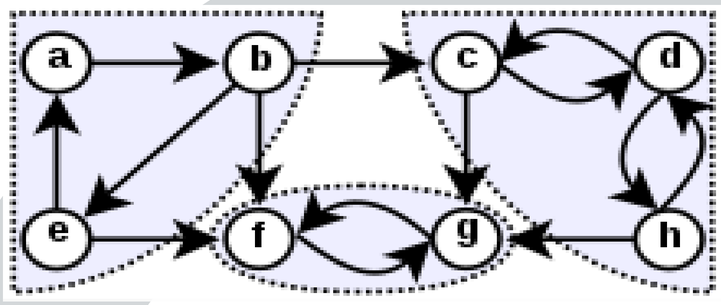

***Figure 2**: Graph with strongly connected components (SCCs) circled **[3]**.*

Tarjan's strongly connected components algorithm found the ergodic sets for each combination of input nodes.

Implementation on Bessemer HPC cluster. Total computation time over a week on one node with six CPUs and 16GB of RAM.

Results

Input Combinations				ACD = 0	ACD = 1	ACD = 0	ACD = 1	
ALK	MYCNa	Cyclin B	TRIM32	SCD = 0	SCD = 0	SCD = 1	SCD = 1	Total
0	0	0	0	5	10	4	0	19
0	0	0	1	5	10	4	0	19
0	0	1	0	5	10	0	0	15
0	0	1	1	5	10	0	0	15
0	1	0	0	3	6	2	0	11
0	1	0	1	3	6	2	0	11
0	1	1	0	3	6	2	0	11
0	1	1	1	3	6	2	0	11
1	0	0	0	5	10	4	0	19
1	0	0	1	5	10	4	0	19
1	0	1	0	5	10	0	0	15
1	0	1	1	5	10	0	0	15
1	1	0	0	3	6	2	0	11
1	1	0	1	3	6	2	0	11
1	1	1	0	3	6	2	0	11
1	1	1	1	3	6	2	0	11

***Figure 3**: Heat map summarising and classifying ergodic sets.*

- A heat map was produced to summarise and classify the ergodic sets associated with the 16 combinations of inputs.
- Four types of ergodic sets within this classification scheme: ACD and SCD off, ACD on and SCD off, ACD off and SCD on, and ACD and SCD on.
- In this Boolean network, an ergodic set does not contain states wherein both ACD and SCD are on.
- Each combination of inputs is associated with more than 10 ergodic sets. Cell division is a complex phenomenon.

- Bias towards symmetric cell division (SCD) in each ergodic set.
- Ergodic sets with all inputs inactive are tiny: one or two states. Hard to access.
- ALK activation and MYCN amplification favour SCD. Cyclin B and TRIM32 oppose their effects.

ALK	MYCNa	Cyclin B	TRIM32	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12	E13	E14	E15	E16	E17	E18	E19
0	0	0	0	1.00	1.00	0.67	0.67	0.50	0.50	0.50	0.50	0.50	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0	0	0	1	0.67	0.67	0.60	0.60	0.50	0.50	0.50	0.50	0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0	0	1	0	0.50	0.50	0.50	0.50	0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00				
0	0	1	1	0.50	0.50	0.50	0.50	0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00				
0	1	0	0	1.00	1.00	0.50	0.50	0.50	0.00	0.00	0.00	0.00	0.00	0.00								
0	1	0	1	0.67	0.67	0.50	0.50	0.50	0.00	0.00	0.00	0.00	0.00	0.00								
0	1	1	0	0.67	0.67	0.50	0.50	0.50	0.00	0.00	0.00	0.00	0.00	0.00								
0	1	1	1	0.60	0.60	0.50	0.50	0.50	0.00	0.00	0.00	0.00	0.00	0.00								
1	0	0	0	1.00	1.00	0.67	0.67	0.50	0.50	0.50	0.50	0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	0	0	1	0.67	0.67	0.60	0.60	0.50	0.50	0.50	0.50	0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	0	1	0	0.50	0.50	0.50	0.50	0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00				
1	0	1	1	0.50	0.50	0.50	0.50	0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00				
1	1	0	0	1.00	1.00	0.50	0.50	0.50	0.00	0.00	0.00	0.00	0.00	0.00								
1	1	0	1	0.67	0.67	0.50	0.50	0.50	0.00	0.00	0.00	0.00	0.00	0.00								
1	1	1	0	0.64	0.64	0.50	0.50	0.50	0.00	0.00	0.00	0.00	0.00	0.00								
1	1	1	1	0.60	0.60	0.50	0.50	0.50	0.00	0.00	0.00	0.00	0.00	0.00								

***Figure 4**: Heat map presenting the bias towards symmetric cell division (SCD) in each ergodic set.*

Discussion

Based on our state space analysis, MYCN amplification and ALK activation do favor symmetric cell division, in agreement with the hypothesis in the literature. Drugs enhancing Cyclin B and TRIM32 can potentially restore the balance in cell division.

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References

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The University Of Sheffield.

Sheffield Teaching Hospitals NHS Foundation Trust

Sheffield Children's NHS Foundation Trust