Knock-out Experiments on a Neuronal Boolean Model

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Neuronal pathologies in neurodegenerative disease

- Alzheimer's disease is characterized by accumulation of intra-neuronal neurofibrillary tangles through hyperphosphorylated Tau (pTau, Fig.1) and extracellular amyloid beta proteins leading to subsequent neuronal death.
- Healthy neurons are postmitotic, but neurodegenerative disease was shown to trigger neuronal cell cycle re-entry that results in neuronal death rather than division.
- The damaged, ROS-rich microenvironment in AD leads to hyper-phosphorylation of neuronal Cdk5, which:
  - promotes aberrant cell cycle entry by blocking RB and inducing E2F1
  - hyper-phosphorylates Tau, leading to tangles

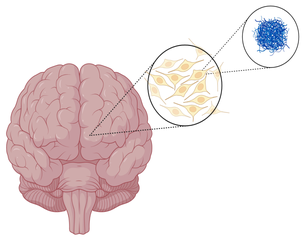


Figure 1. Neurofibrillary tangles in AD.

Research question 1 : How do diseased neurons turn on cell cycle and why does this entry lead to apoptosis?

Neuronal stem cell differentiation vs. renewal

- In response to nerve growth factor (NGF), neuronal progenitors take one of two paths, stochastically chosen:
  - Proliferation and self-renewal of stem-cell pool (less common)
  - Differentiation into neurons (more common)
- In response to epidermal growth factor (EGF), neuronal progenitors generally enter the cell cycle
- The two mutually exclusive fates are controlled by the balance of RAS-MAPK/ERK (RAS) signalling and the PI3-Kinase-ATK-mTOR (PI3K) pathway.
  - RAS signalling induces differentiation
  - PI3K pathway promotes self-renewal

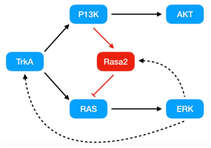


Figure 2. Competing signalling pathways in neuronal progenitor fate determination, modulated by Rasa2.

Research question 2 : How do neuronal progenitor cells entering the differentiation pathway lock down their cell cycle, and how is this linked to higher MAPK signaling?

Method

- Unified Boolean Regulatory network model of neuronal progenitors & neurons (Fig. 3).
- The model includes regulation of cell cycle, apoptosis, neuronal fate commitment and growth signaling in response to NGF / EFG.
- In silico experiments:
  - Stable states of the model corresponding to distinct cell phenotypes (Fig 4)
  - Time courses testing the ability of the model to self-renew / differentiate / enter cell cycle in an AD environment
  - Knockdown and overexpression of key mediators (high Cdk5, pTau, Rasa2)

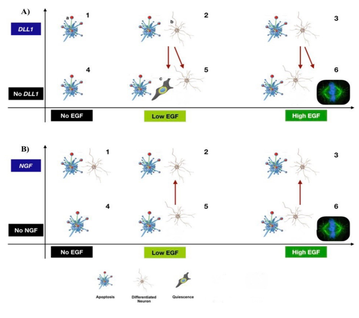
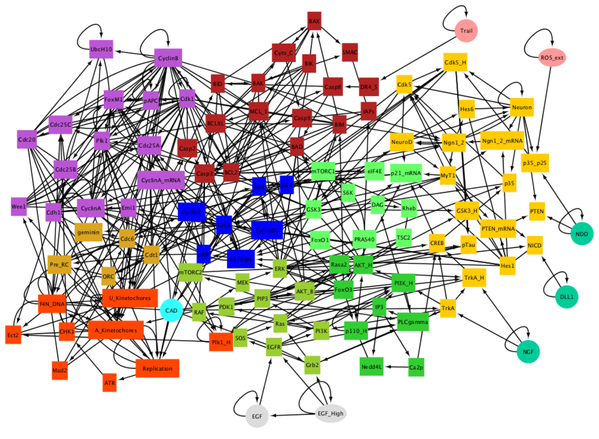


Figure 4. Stable states of the model in a healthy microenvironment. A) Microenvironments with no NGF, varying EGF and DLL1. B) Microenvironments with no DLL1, varying EGF and NGF.

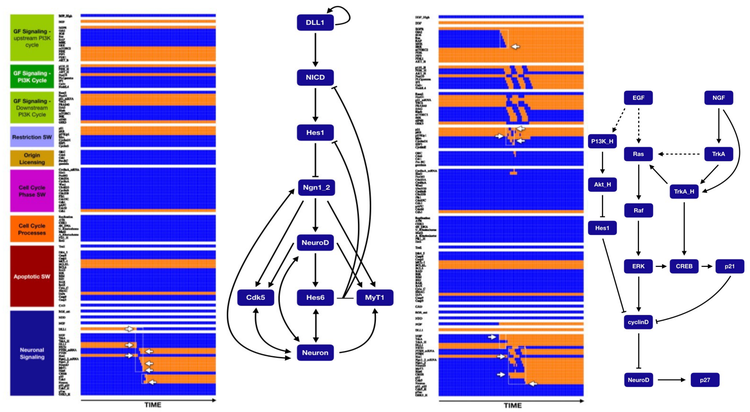
Results



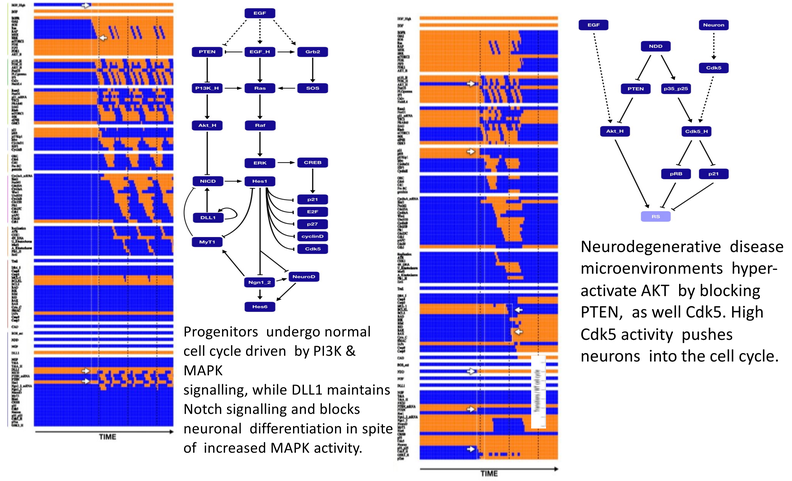
Cell cycle Processes Cell cycle Phase SW Apoptotic Switch Restriction Switch Origin Licensing
GF Signalling(upstream cycle)
Neuronal Signalling Neuronal Microenvironment DNA Fragmentation
GF Signalling (P13K)
GF Signalling (downstream) Death signals and ROS

Figure 3. Modular Boolean model of neuronal self-renewal, differentiation and cell cycle control.

- **Differentiation** induced by loss of DLL1 (left) or exposure to NGF (right)

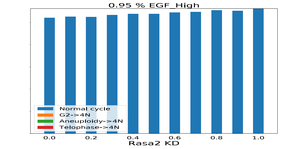


- **EGF-driven cell cycle entry in healthy progenitors vs diseased neurons**

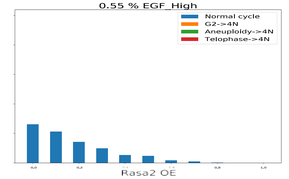


Progenitors undergo normal cell cycle driven by PI3K & MAPK signalling, while DLL1 maintains Notch signalling and blocks neuronal differentiation in spite of increased MAPK activity.

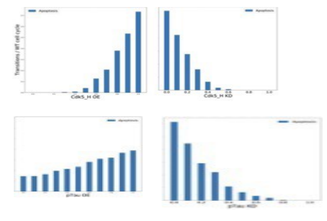
Neurodegenerative disease microenvironments hyper-activate AKT by blocking PTEN, as well Cdk5. High Cdk5 activity pushes neurons into the cell cycle.



- **Rasa2 knockdown** slightly increases proliferation rate in progenitors



- **Constitutively active Rasa2** decreases progenitor proliferation



- Model shows increased vs. decreased apoptosis with hyperactive pTau (top left) vs. pTau knockdown (top right); results are similar in response to Cdk5 hyeractivation and knockdown (bottom panels)

Mode of neuronal death in model is incorrect: it relies on mitotic catastrophe rather than E2F1-mediated apoptosis

Discussion

-Rasa2 knockdown and NDD microenvironment cell death showed that the model needed to be modified further.

- Therefore, to link E2F1 activation in neuronal cell cycle entry to apoptosis, there were additional protein interactions we needed to put in and link with neuronal module and apoptosis module.(see fig.4).
- Tested further knockdown /overexpression phenotypes(Cdk5_H, pTau, Rasa2 and GSK_3 against data matched well with experimental papers.

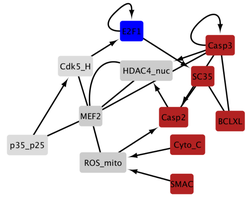


Figure.8. Additional pathway integrated into the Neuronal pathway for accurate depiction of E2F1 mediated apoptosis.

A model was built that was less dependent on the MAPK pathway, making E2F1-mediated apoptosis possible through creating a more robust model

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