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DYRK1A and the Cell Cycle

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Background

DREAM is a key regulator of cell cycle quiescence

- DREAM (**D**P1, the **R**b-related protein p130, **E**2F4, **a**nd the **M**uvB core complex)
- Multi-subunit repressor complex of cell cycle-promoting genes
- Switch from DREAM to BMYB-MuvB (MMB) is highly regulated

DYRK1A *(*dual specificity tyrosine-(Y)-phosphorylation regulated **kinase** 1A)

- nervous system development, cell growth and division, and neuronal differentiation
- Cytogenetic Location: 21q22.13
- **Putative tumor suppressor** and important regulator of many proteins through its kinase activity
- DYRK1A phosphorylates Serine residue 28 on LIN52 of the MuvB core, allowing p130 to bind to LIN52. **Necessary for formation of DREAM complex.**

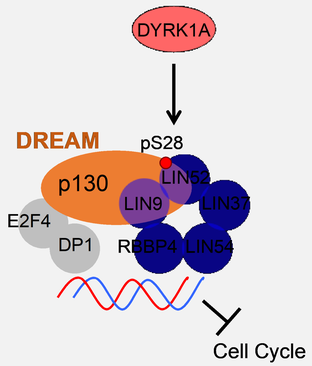


Image from Larisa Litovchick, Md., PhD.

Lab observation: T98G *DYRK1A-KO* cells appear slightly smaller, and *proliferate more slowly* than control cells.

Unexpected phenotype. *DYRK1A* is a **putative tumor suppressor** through phosphorylation of LIN52 (required for binding of p130 for DREAM assembly).

Research Questions: Is there **slower, encumbered DNA synthesis** happening in DYRK1A-KO cell lines to explain the **slower proliferation** observed in T98G cells? What is the underlying mechanism of the delayed proliferation of the DYRK1A-KO cells?

Methods

Cell Line: T98G cells

- Human astrocytes, glioblastoma multiforme cells (originally derived from a 61 year-old Caucasian male)
- Hyperpentaploid, and typically carry three copies of the *DYRK1A* gene

Two mutant lines used in these experiments:

- CRISPR ***DYRK1A-KO*** lines (labeled as 3.1.A6 and 4.1.A1)
- Grown in Dulbecco's Modified Eagle Medium (DMEM) + FBS, + penicillin/streptomycin, + glutamax. Starvation media in these experiments contained 0% FBS

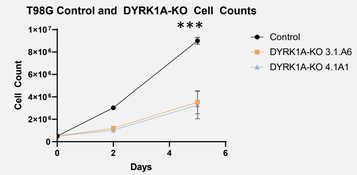
•**Experimental protocol** for starvation and release of cells back into cell cycle: plate equal numbers of cells → replace media with starvation media for 48 hours → Induces cells to enter quiescence/temporary arrest in G1/G0 → Release cells back into the cell cycle through addition of 15% FBS → Collect samples at 0, 16, and 22 hr timepoints.

•Three methods used to gauge cell cycle progression in T98G cells following starvation and release:

- •Cell cycle FACS using propidium iodide (Canto flow cytometry)
- •Western blot for cell cycle (especially S phase) protein markers
- •EdU (Ethynyl deoxyuridine) staining to measure active DNA synthesis across time points

Results

Figure 1*: Using student's T tests between each group (using JMP statistical software) both DYRK1A-KO lines' 5 day cell count means were significantly different from the control line mean cell count (p=0.001 for both mutant lines).*



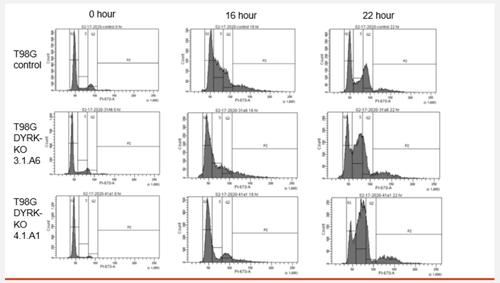
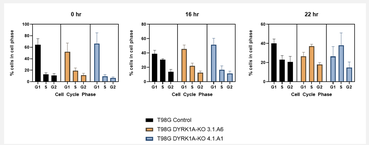


Figure 2*: T98G and T98G DYRK1A-KO lines were starved and then released back into the cell cycle. WT T98G proceeds through cell cycle checkpoints normally, while DYRK1A-KO lags, entering S phase at 22 hours. Quantification on right panel. N=3 biological repeats.*



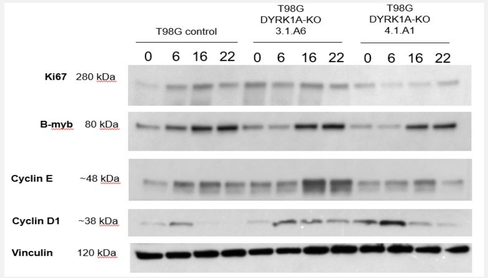
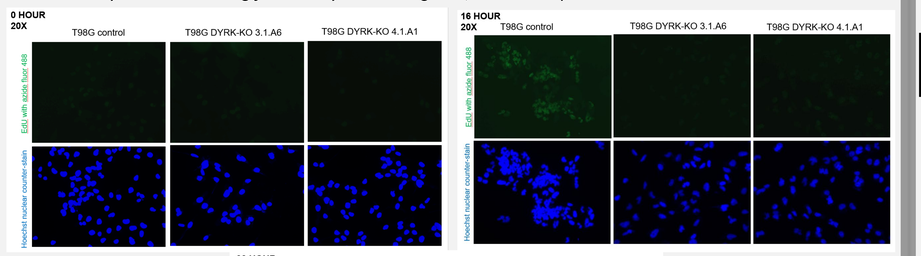


Figure 3*: Cell Cycle protein markers after starvation and release at 0-22 hr timepoints. N=3 biological repeats.*

Figure 4*: EdU staining (30 minute pulse) for T98G control and DYRK1A-KO cells at 0, 16, 22 hr in the cell cycle. EdU staining for DNA synthesis in green, and lower panels Hoechst nuclear stain.*



Discussion

Conclusions

DYRK1A T98G mutants are growing more slowly, entering and proceeding through S phase more slowly than control T98G cells. This is evidenced by:

- FACS cell cycle data: *DYRK1A-KO* cells enter S phase around the 22 hour mark rather than 16-18 hours.
- EdU staining: *DYRK1A-KO* cells do not begin to complete active DNA synthesis until ~22 hrs
- Cell Cycle markers with WB: Cyclin E, B-myb accumulates later in the cell cycle in KO lines

***DYRK1A* may be having an unexpected, but significant effect on the cell cycle.** Further investigation is needed to fully elucidate and understand the roles of *DYRK1A* in cell cycle regulation and arrest.

Future Directions

- Quantification of EdU staining, EdU Cell Cycle FACS
- Probing for additional cell cycle markers (Cyclin A, Cyclin B for S/G2 transition)
- Delving deeper into what is happening with Ki67 and DYRK1A, potentially IP at various time points in the cell cycle for Ki67/DYRK1A interaction
  - Additionally, Ki67 staining in T98G control and *DYRK1A* mutant cells at time points throughout the cell cycle following synchronization
- Cell line-dependent observation? Use of additional cell lines for repeated experiments (Litovchick lab currently works with multiple non-cancer and cancer lines with *DYRK1A-KO)*

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