Decoding ERK Regulation of the Cell Cycle To Direct Rational Combinatorial Therapy

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GRAPHICAL ABSTRACT

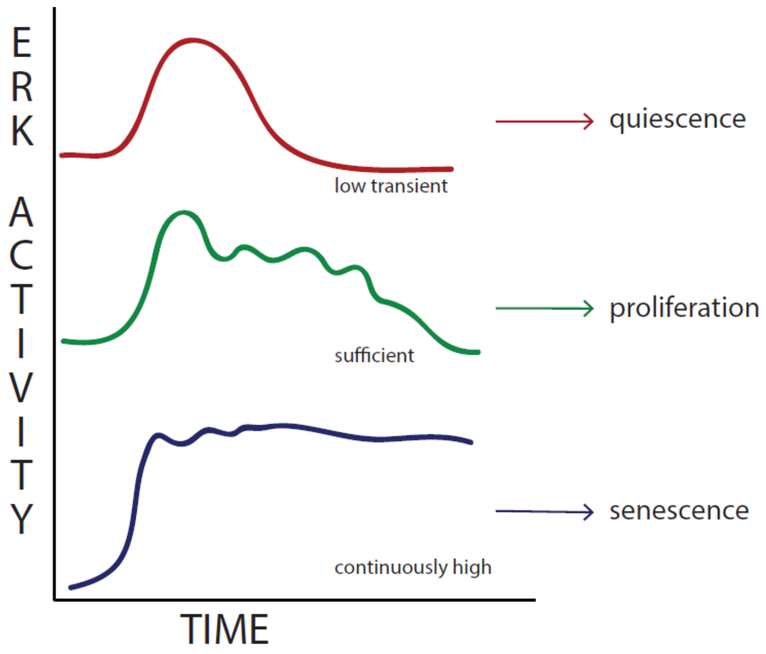

Figure 1. Different ERK patterns induce varying cell fates. This information is essential to optimally combine ERK and CDK inhibitors to decrease cell growth.

INTRODUCTION

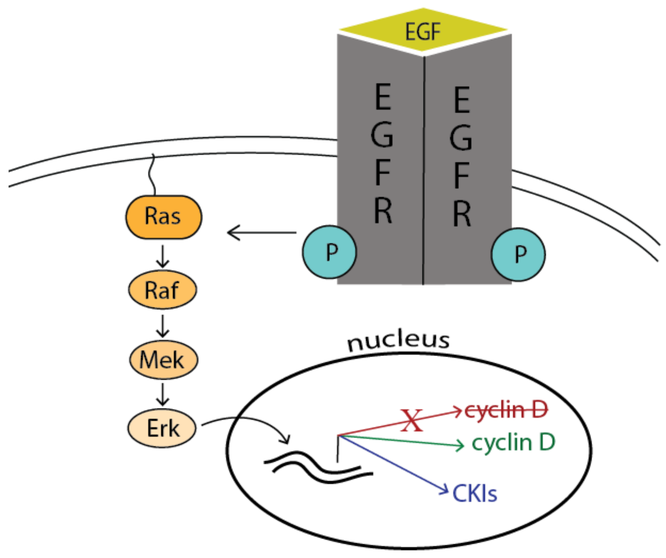

Figure 2. The mitogen-activated protein kinase (MAPK) pathway induces genes controlling either proliferation or senescence.

Cancer cells often carry mutations in the Ras/MAPK pathway that regulate cell cycle events[1] leading to uncontrolled cell proliferation. ERK, the terminal kinase of the MAPK pathway, increases cyclin expression, controlling cell cycle in complex with cyclin dependent kinases (CDKs)[2]. Paradoxically, while ERK is necessary for cell proliferation, sustained, high activity can induce cell senescence[3]. This study investigates ERK activity throughout the cell cycle to inform which synergistic combination of ERK and CDK inhibitors reduces proliferation.

Our hypothesis is that **distinct ERK patterns underlie cell fate decisions** for quiescence, proliferation, or senescence.

METHODS

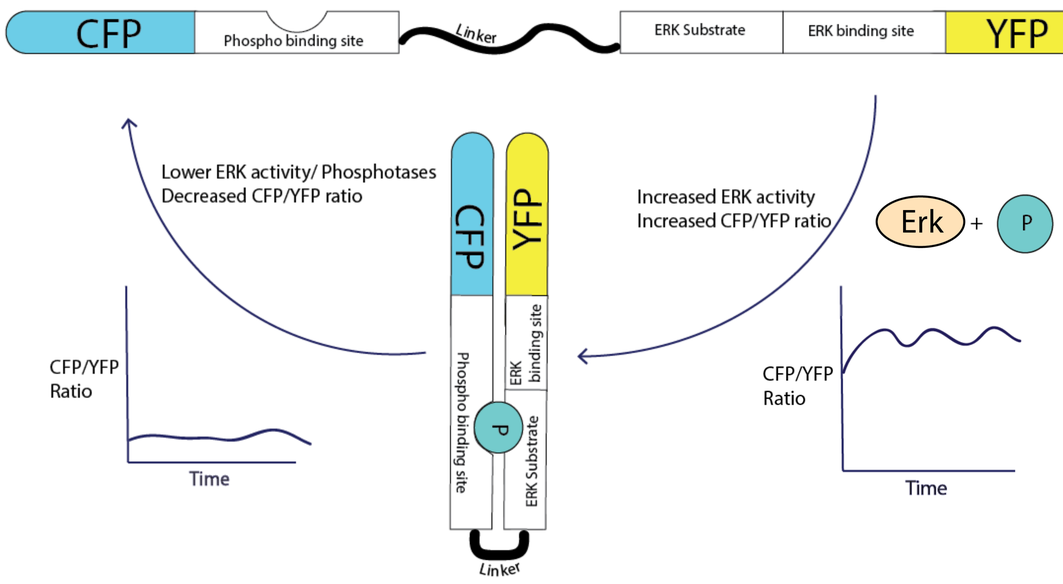

Figure 3. FRET reporter of ERK activity. Upon phosphorylation, the reporter undergoes a conformational change, and energy is transferred from CFP to YFP which is measured as the CFP/YFP ratio.

RESULTS

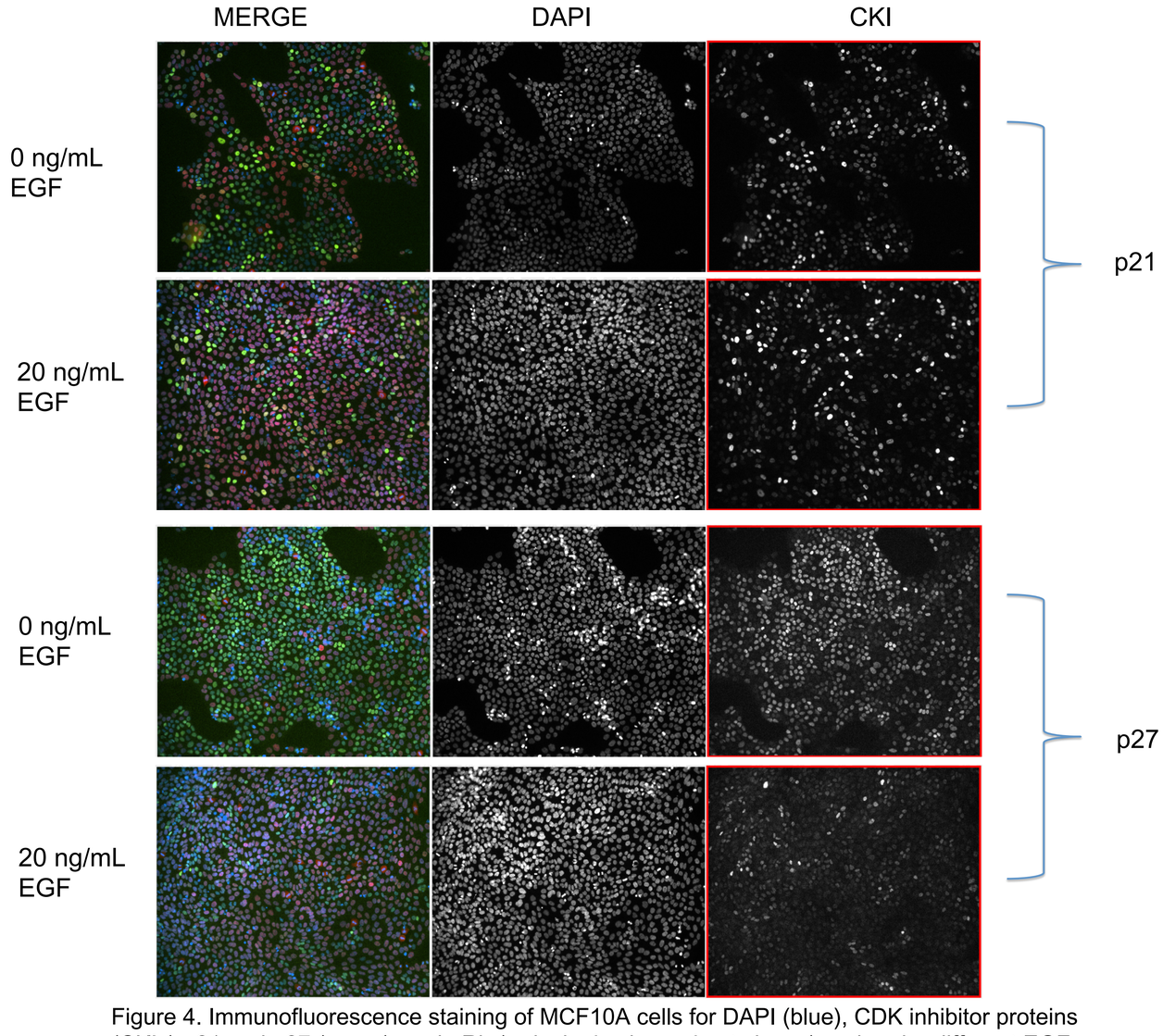

Figure 4. Immunofluorescence staining of MCF10A cells for DAPI (blue), CDK inhibitor proteins (CKIs) p21 and p27 (green), and pRb (red: single channel not shown) and under different EGF stimulation (0.1ng/ml EGF staining not shown) .

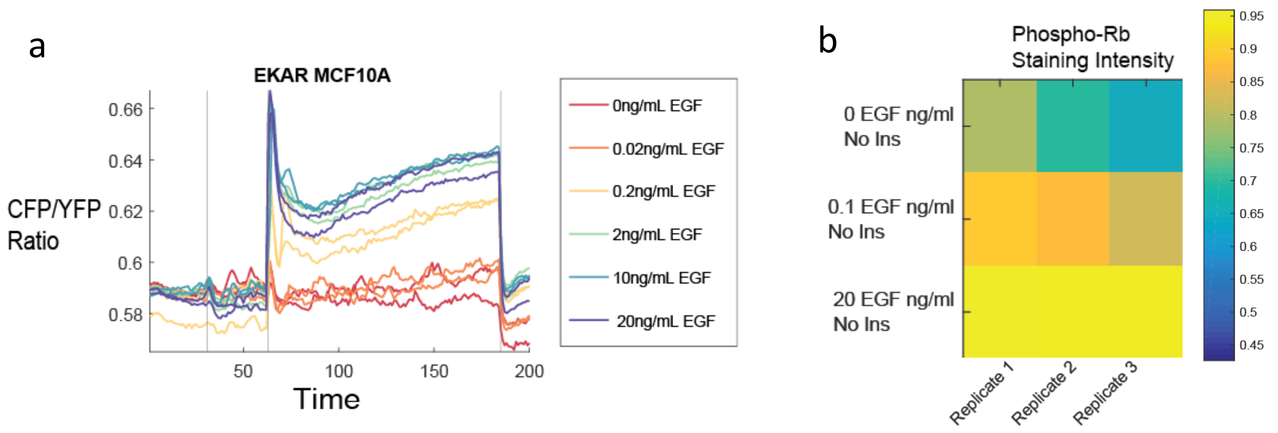

Figure 5. Increasing EGF concentrations stimulate higher ERK activity and more cell proliferation. (a) Mean single-cell traces of ERK activity; vertical lines indicate addition of treatments. The first line indicates spikes of empty media, the second is addition of EGF, and the third is the MEK inhibitor PD0325901. (b) Quantification of pRb from Fig. 4, an indication of cell proliferation, n=3.

CONCLUSIONS

- Increasing EGF stimulation induces both higher ERK activity and cell proliferation via pRb staining
- Response of cell cycle-dependent kinase inhibitor (CKI) proteins to EGF stimulation varies according to each family member
  - p21 levels are highly heterogeneous
  - p27 levels decrease with EGF

FUTURE DIRECTIONS

- Establish a cell line with dual reporters for EKAR and a G1/S phase transition marker for computational alignment of cell cycle phase to quantify ERK activity that promotes cell cycle progression, quiescence, or senescence in live cells
- This data will ultimately be used to identify the single-cell pharmacodynamics to inform synergistic MAPK and CDK inhibition that maximally reduces tumor growth

CITATIONS

1. Dhillon, A.S., *et al.* “MAP kinase signaling pathways in Cancer.” *Oncogene* (2007).
2. Malumbres, M. and Barbacid, M. “Cell cycle, CDKs and cancer: a changing paradigm.” *Nature Reviews Cancer* (2009).
3. Lin, A.W., *et al.* “Premature senescence involving p53 and p16 is activated in response to constitutive MEK/MAPK mitogenic signaling.” *Genes & Development* (1998).

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