

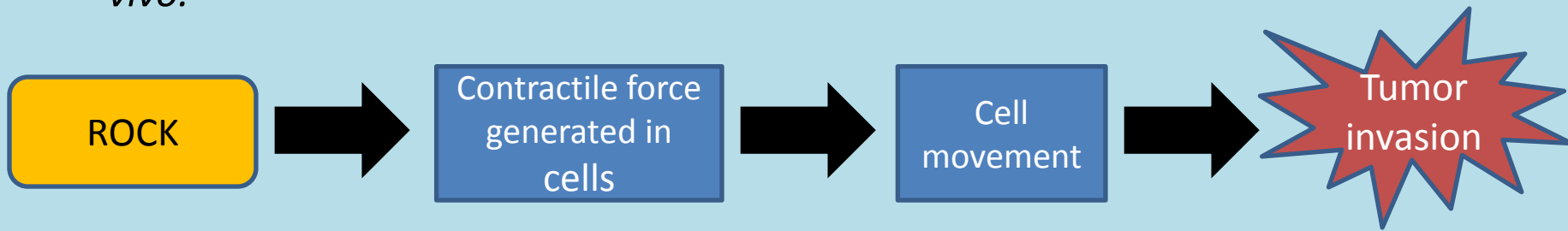
Project Title:
Characterizing the specificity and potency of
putative ROCK inhibitors

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Comprehensive Cancer Center



Background and Goals

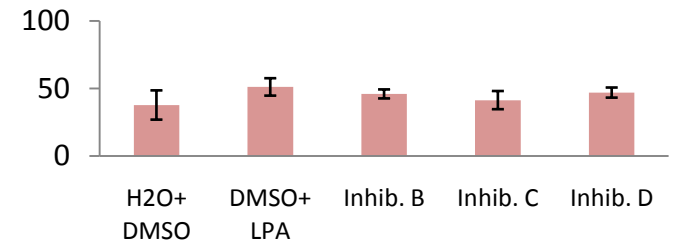
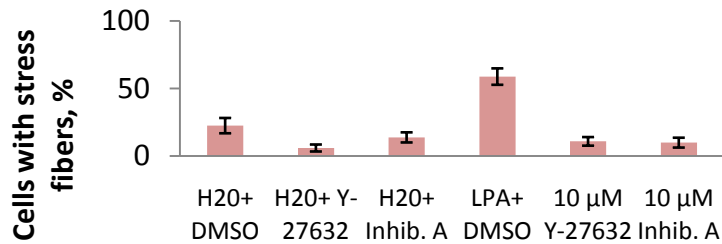
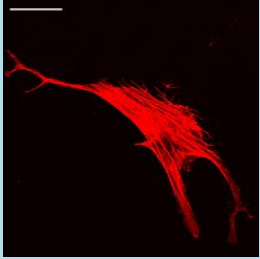
- RhoA is a small GTPase protein that, when misregulated, promotes tumor invasion and metastasis.
- RhoA kinase, also called ROCK, is an important effector of RhoA that normally plays roles in regulating cell shape, tissue differentiation, and smooth muscle contraction.
- There are two gene products, ROCK1 and ROCK2. ROCK1 is involved in cell proliferation while ROCK2 is involved in smooth muscle contraction, making ROCK1 a good target for anti-cancer therapies.
- Y-27632 is a known ROCK inhibitor that reduces tumor cell dissemination *in vivo*.



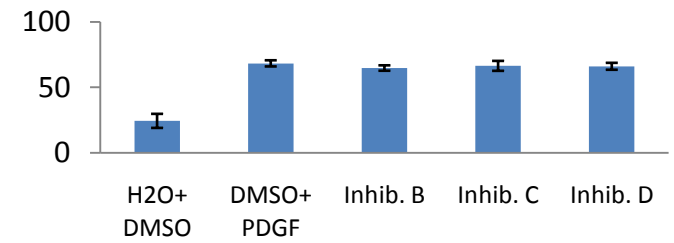
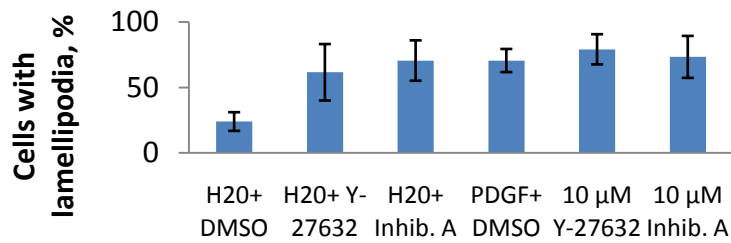
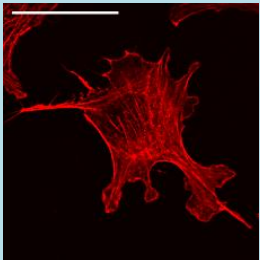
- **GOALS:** To test the specificity and potency of four new putative ROCK inhibitors in NIH 3T3 cells by determining if they selectively block certain cell shape changes: RhoA induction of stress fibers (LPA stimulation) but not Rac or Cdc42 induction of lamellipodia or filopodia (PDGF or bradykinin [BDK] stimulation, respectively).
- **EXPECTED RESULTS:** If the putative ROCK inhibitors are effectively inhibiting the ROCK pathway, a decrease in RhoA-induced stress fibers and possibly an increase in lamellipodia and filopodia should be seen.

Project Results:

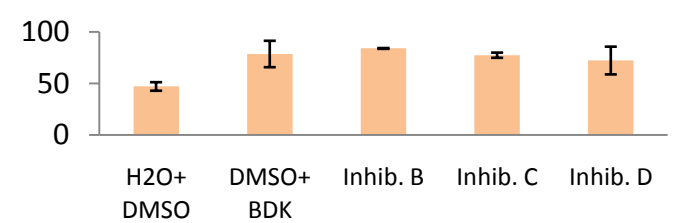
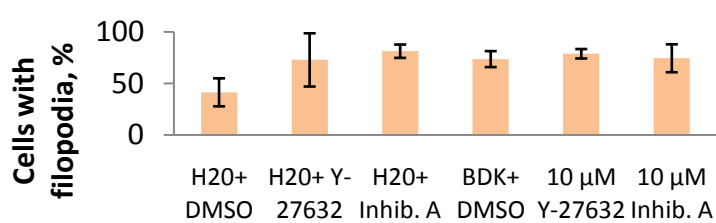
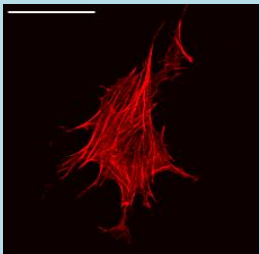
Stress fibers



Lamellipodia



Filopodia



Y-27632 (positive control) and Inhibitor A inhibited RhoA-induced stress fibers in NIH 3T3 cells while increasing lamellipodia and filopodia, suggesting that these inhibitors can block ROCK selectively. Inhibitors B, C, and D were ineffective in this assay, but the cells had become less sensitive to LPA stimulation. The assay should be repeated with cells that are more responsive.