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Dopamine receptor type 2 (DRD2) inhibits non-functioning human pituitary tumor-derived cell line HP75 migration through ROCK-mediated cofilin inactivation

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Neurosurgery is the treatment of choice of non-functioning pituitary tumors (NFPAs), but its success is strongly affected by local invasion. Medical therapy is still under debate, although the use of cabergoline results in tumor shrinkage in a subset of patients. We recently demonstrated that dopamine receptor DRD2 agonist BIM53097 exerts cytostatic and cytotoxic effects in cultured cells from NFPAs, but no data are present in literature about DRD2 mediated effects on pituitary cell migration and invasion. A key protein involved in cell migration and invasion is the actin binding protein cofilin (CFL1), whose activity is negatively regulated by phosphorylation at Ser3 by LIM kinases (LIMK), and LIMK is upstream regulated by ROCK.

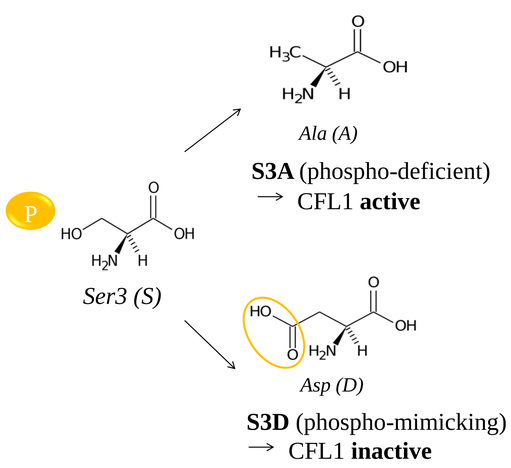
The aim of this study was to evaluate the effect of BIM53097 on migration of the non-functioning human pituitary tumor-derived cell line HP75, and to investigate the molecular mechanism involved focusing on the role of CFL1.

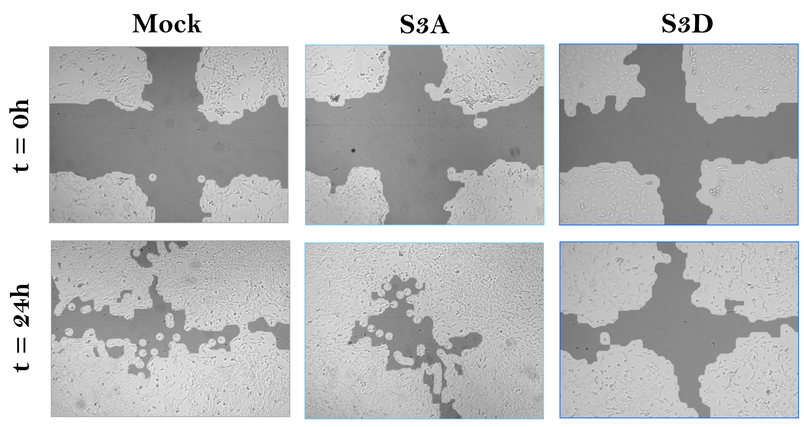
Materials and Methods

- ✓ **Wound Healing Assay**: Wound Healing assays were performed on confluent monolayers of HP75 cells, by scraping off an area of cells with a p200 tip. BIM53097 1µM was added after the scratch. Images were taken immediately and 24h after scratch. To measure cell migration we used the automated image analysis software tool "TScratch" (www.chaton.ethz.ch/software).
- ✓ **Western Blot**: Samples were separated on SDS-PAGE, and the proteins were detected by Western Blotting using antibodies against Cofilin (Cell Signaling), and P-Cofilin (Cell Signaling). The ratio of immunoblotting signalling intensity was measured using NIH ImageJ software.
- ✓ **Transient cells transfection:** Transient transfections of HP75 cells with cofilin mutants S3A or S3D were performed using the transfection reagent Lipofectamine2000, according to the instruction of the manufacturer.

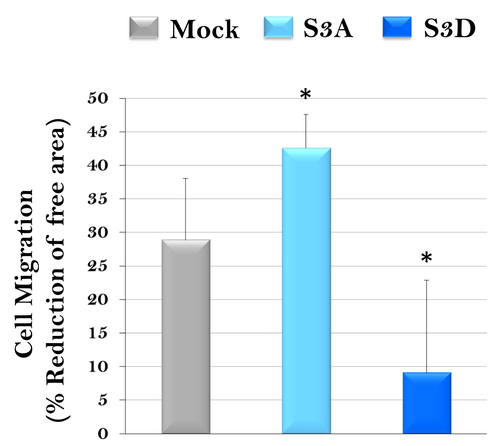
Cofilin promotes HP75 cell migration

To evaluate the role of cofilin phosphorylation on cell migration, we prepared a phospho-mimicking and a phospho-deficient mutants of cofilin (S3D or S3A, respectively). We transfected HP75 cells with these constructs and performed Wound Healing assays.



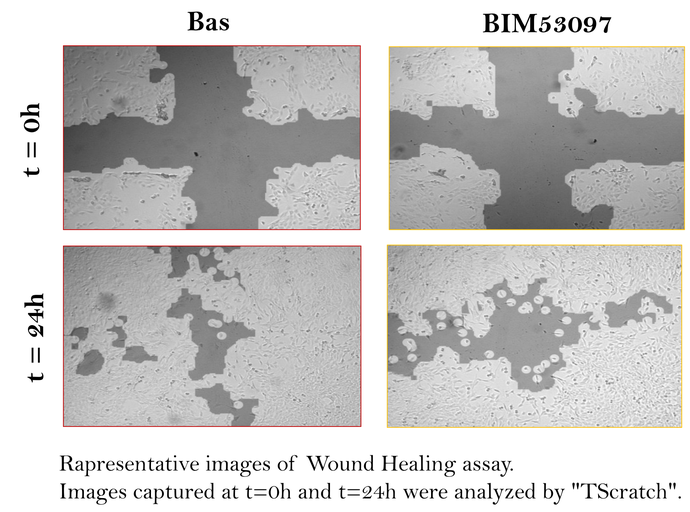


Rapresentative images of Wound Healing assay.
Images captured at t=0h and t= 24h were analyzed by "TScratch" software.

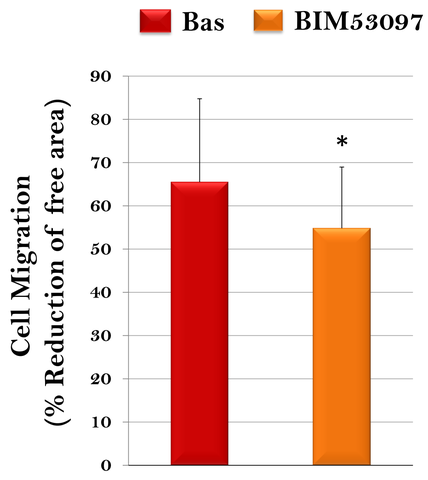


We observed decrease or increase in S3D or S3A expressing cell migration, respectively (9% and 42%, respectively, vs 29% migration of mock transfected cells), suggesting that phosphorylation status of cofilin is causally related to cell migration.

DRD2 agonist inhibits HP75 cell migration



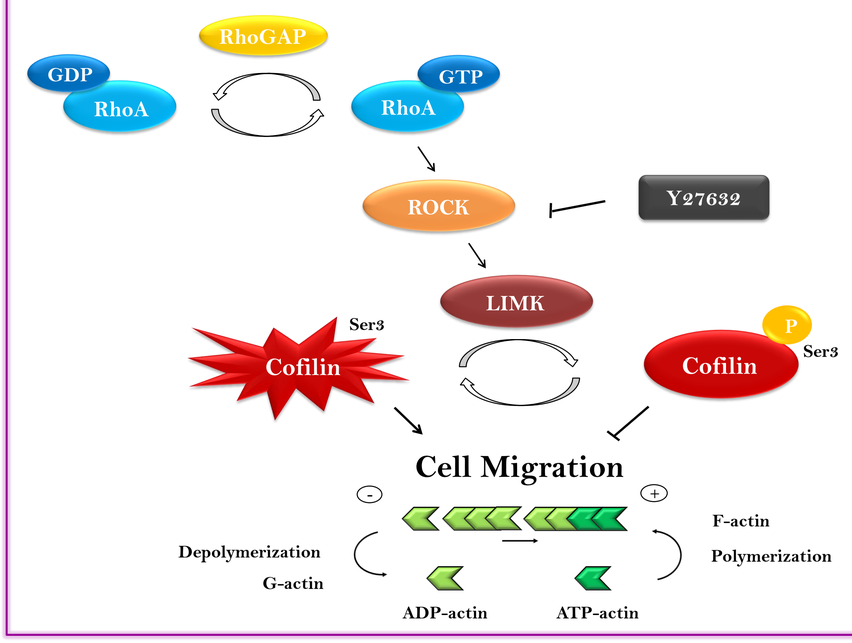
Rapresentative images of Wound Healing assay.
Images captured at t=0h and t=24h were analyzed by "TScratch".



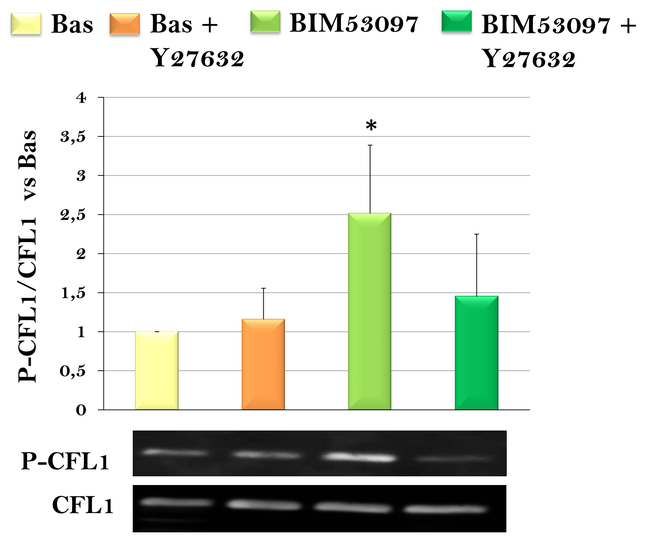
24h incubation with 1µM BIM53097 reduces HP75 cell migration (54% migration vs 65% in control cells, expressed as a % reduction of the free area at 24h vs 0h, $p<0.05$ vs control).

DRD2 agonist induces an increase of cofilin phosphorylation rate through ROCK activation

Cofilin is an actin binding protein involved in the reorganization of cytoskeleton; its activity is negatively regulated by phosphorylation at Ser3 by LIMK. Active (de-phosphorylated) cofilin stimulates severance and depolymerization of actin filaments promoting actin turn-over and cell migration.



It has been evaluated DRD2 agonist effect on phosphorylation level of cofilin in HP75 cells.



Western blot analysis performed with anti phospho-cofilin antibody revealed that 10 min DRD2 agonist treatment induced a 2.5 fold increase cofilin phoshorylation, and this effect being reversed by ROCK inhibitor, Y27632.

Conclusions

- ➢ DRD2 agonist reduces HP75 cell migration
- ➢ The molecular mechanism involves ROCK-dependent phosphorylation of cofilin