UNIVERSITY of MARYLAND
SCHOOL OF PHARMACY

Effects of FRS targeted compounds on Regulation of AP-1 Proteins in Human Airway Smooth Muscle Cells

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Introduction

- Hyperproliferation of human airway smooth muscle (HASM) cells leads to increased cell mass causing airway obstruction in inflammatory diseases.(Fig. 1)
- ERK1/2 signaling pathway plays a major role in HASM cells proliferation by regulating AP-1 proteins and this represents a potential drug target. (Fig. 2)
- Current ERK1/2 Inhibitors target the ATP binding site and often result in toxicity and lead to acquire a drug resistance.
- We identified a novel function-selective inhibitor, SF-3-030, that binds near a region (F-recruitment site), that mediates interactions with proteins that form the AP-1 complex, and this site is distinct from the ATP-binding site. (Fig. 2)

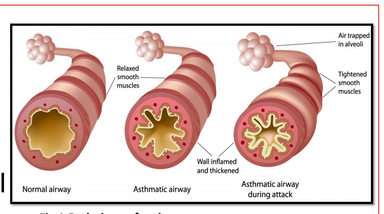

Fig 1:Pathology of asthma.

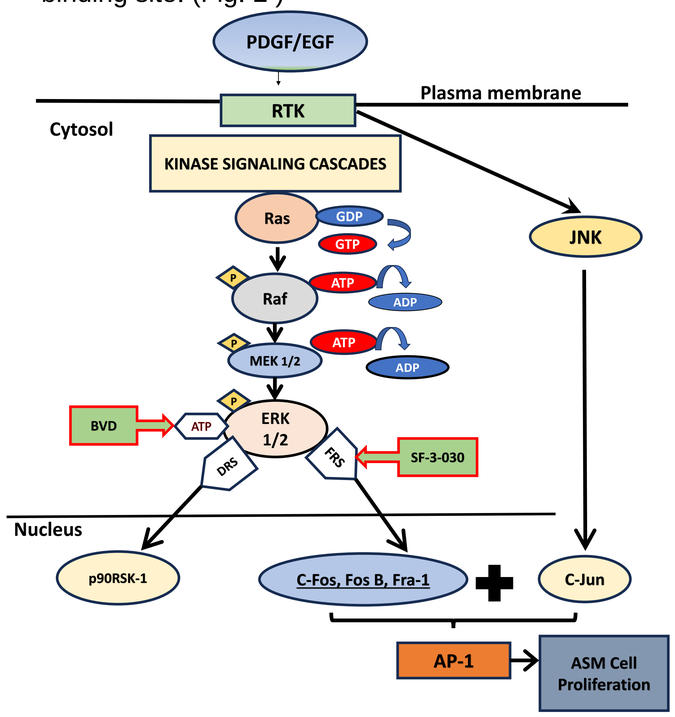

Figure 2. The extracellular signal-regulated kinase(ERK1/2) signaling pathway is a major mediator HASM cell hyperplasia. BVD 523 is ATP competitive inhibitor, SF-3030 is a function selective targeting FRS site. DRS is the D-recruitment site, FRS is the F-recruitment site.

Objective of Study

- Evaluating novel compounds targeting ERK1/2 and effects on AP-1 proteins in HASM cells.

Methodology

- The HASM cells are obtained from three healthy patients.
- HASM cells are treated with compounds targeting the ERK1/2 at the FRS site.
- The cells are stimulated with the growth factors such are EGF (epidermal growth factor) or PDGF (platelet-derived growth factor).
- Cells are harvested and analyzed for ERK1/2 pathway activation and AP-1 proteins by immunoblotting.

Figure 3. Basic flow of generating immunoblotting by running SDS-page

Results

Time course of ERK1/2 and AP-1 Protein levels in HASM cells treated with PDGF or EGF

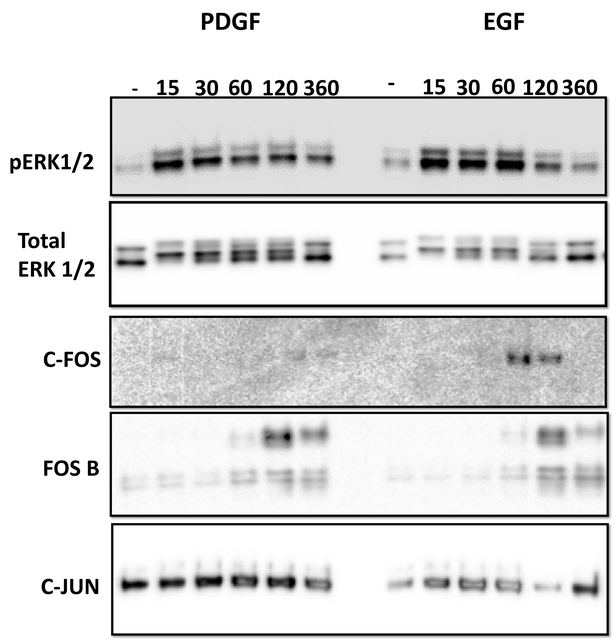

Figure 4. Cells are treated in a time dependent manner. The kinetics of ERK1/2 activation was analyzed. In addition, AP-1 protein induction C-FOS, FOSB, C-JUN response to PDGF or EGF was analyzed.

Results

A-7022 B-7027 C-7028
D- 3030 E-BVD

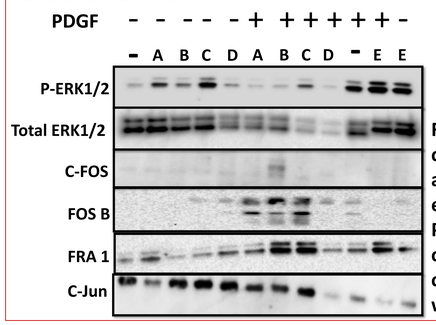

Figure 5. Immunoblot comparing ERK1/2 signaling and expression of immediate early genes (IEGs)in Activator Protein 1 (AP-1) using HASM cells after treatment with compounds and stimulation with PDGF for 2hr.

Conclusions

- Established the kinetics of ERK1/2 activation and AP-1 protein expression in response to EGF or PDGF treatment
- Data needs to be reproduced with other samples.
- Investigate more proteins in the AP-1 family
- Preliminary data shows that some of the compounds have different expression effects on Fos B and Fra1

Future Directions

- Investigate the changes in AP-1 proteins in the presence of compounds effects AP-1 activity.
- Investigate the additional compounds in HASM cells and other lung cells for example (Fibroblast)

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References

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2. Martinez, R., et al. *J Pharmacol Exp Ther* **2021**, *376* (1), 84-97. DOI: 10.1124/jpet.120.000266

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