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The Influence of Hepatocyte Growth Factor During Outer Segment Phagocytosis by Retinal Pigment Epithelium

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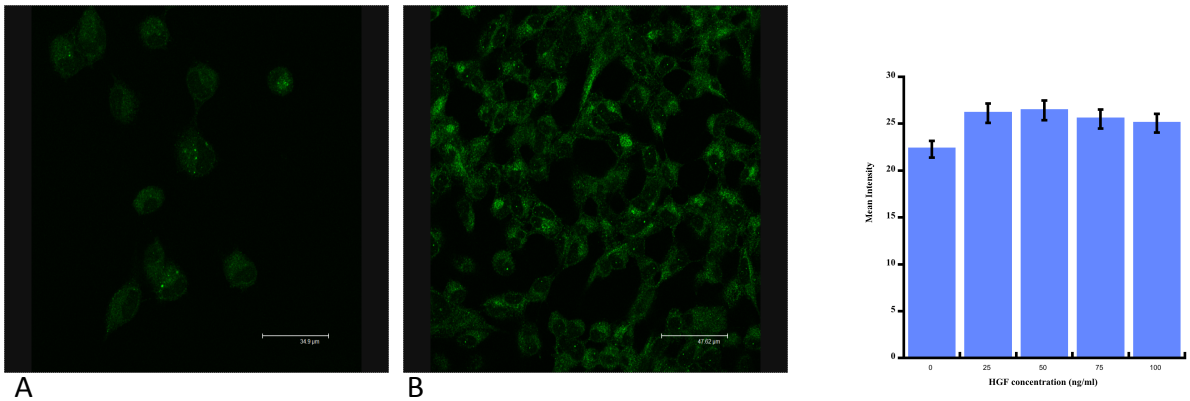
Inhibition of outer segment (OS) processing by retinal pigment epithelium (RPE) has been linked to photoreceptor injury and retinopathy onset. Sub-retinal clearance by RPE is facilitated by specialized phagocytosis featuring both RPE-specific and traditional FCγR mediated signaling cascades. As a result of this combinatory approach, RPE are capable of internalizing both specific and non-specific external targets alike. The discovery that lack of c-Met signaling results in impairment of phagocytosis in alveolar and hepatocyte macrophages [1] suggests c-Met's role as modulator of this activity in post-mitotic cells secreting HGF. Since activated PI3K has been identified as an activator of Rac1during FCγR mediated phagocytosis, we hypothesize that c-Met activation by HGF and subsequent PI3K activation is capable of mediating OS clearance by RPE.

To test our hypotheses, cultured ARPE-19 cells were grown to 70% confluence, then serum starved for 24 hr. Post starvation, cells were exposed to various concentrations of HGF for 24hr before fixation with 2.5% paraformaldehyde and .5% glutaraldehyde. Cells were then prepared for immunohistochemistry for receptor expression (non-phosphorylated and phosphorylated forms), focal adhesion kinase (FAK) and binding of fluorescently-labeled *E. coli*. Intensity values suggest that ARPE-19 respond maximally to concentrations of 25 ng/ml of HGF when compared to controls (Fig 1). While phosphorylated c-Met was not significantly altered (Fig 2) at 24 hr, this may be attributed to the transient expression of phospho-c-Met following activation by HGF. Our findings suggest that RPE respond to increases of exogenous HGF concentrations by up-regulating its receptor and subsequent second messengers systems. In addition, our data show a significant increase of fluorescently labeled *E. coli* (Fig 3). Taken together, these findings suggest that RTK cross-talk initiated by c-Met activation may be sufficient in mediating general uptake of external debris by RPE. Future studies including RPE challenge with fluorescently labeled OS during peak c-Met phosphorylation evoked by increased HGF exposure will provide evidence for HGF's role as a mediator of specialized phagocytosis of OS.

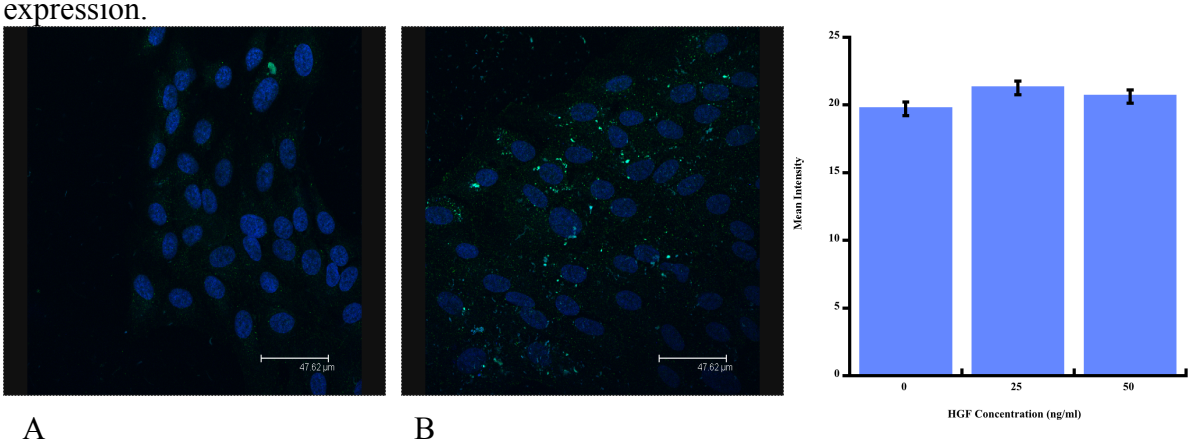
References

[1] C.G. Huh et al., *Proceedings of the National Academy of Science in the United States of America*. 13 (2004) 101.
[2] Support for this project comes from PSC-CUNY and the LS-AMP program of CUNY.



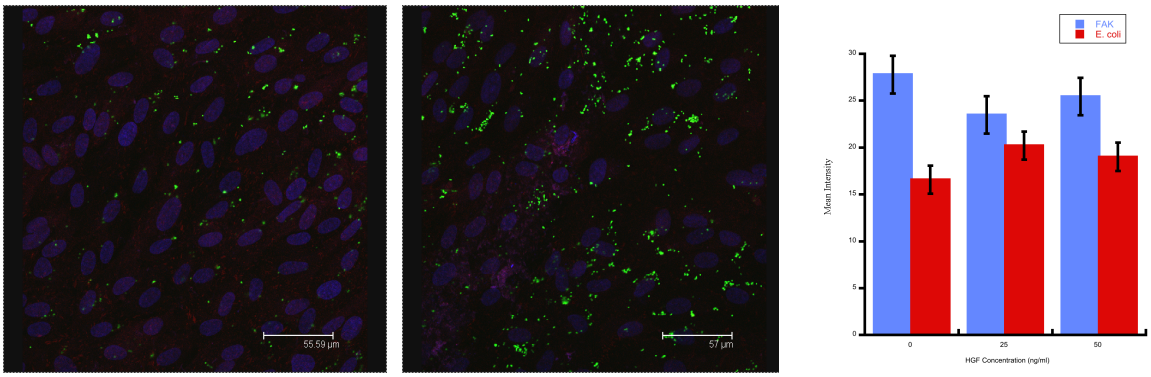
A B

Figure 1 A & B illustrate the up-regulation of c-Met in ARPE-19 when exposed to increasing concentrations of HGF. Statistical analyses reveal that all concentrations $\geq$ 25 ng/ml are significantly higher than controls, but concentrations higher than 25 ng/ml do not increase c-Met expression.



A B

Figure 2 A & B demonstrate that while cMet is up-regulated with HGF treatment, the phosphorylated cMet is not at 24 hr. This is likely due to both the constitutive expression of the protein and lack of HGF activation at 24 hr.



A B

Figure 3 A & B show an increase in extracellular target binding of fluorescently labeled *E. coli* by ARPE-19 treated with 25ng/ml HGF. Interestingly, total expression of FAK, a key mediator of specialized phagocytosis, is reduced after HGF treatment.

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