Convergence of hepatocyte growth factor and interleukin-6 signaling pathways in A549 adenocarcinoma growth

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INTRODUCTION

Non-small cell lung cancer (NSCLC) accounts for 85% of all lung cancer cases. It is associated with high mortality rates with the majority of patients diagnosed with advanced metastatic cancer. Within the pro-inflammatory tumour microenvironment of NSCLC, pleiotropic cytokines such as hepatocyte growth factor (HGF) and interleukin-6 (IL-6) and their receptors (c-Met and gp-130 respectively) are mutationally overexpressed in non-small cell lung cancer (NSCLC) [1, 2]. HGF and IL-6 share similar biological effects in cancer, influencing growth, migration and invasion in NSCLC [1, 3]. HGF and IL-6 have been shown to upregulate each others receptors to facilitate invasion in A549 cells [4]. Although extensive research has established the deregulated activity of HGF and IL-6 independently in cancer proliferation and invasion, the cooperation between HGF and IL-6 NSCLC in inducing proliferation remains unknown.

AIM: To investigate the potential additive or synergistic proliferation induced by HGF and IL-6 in A549 lung adenocarcinoma cells

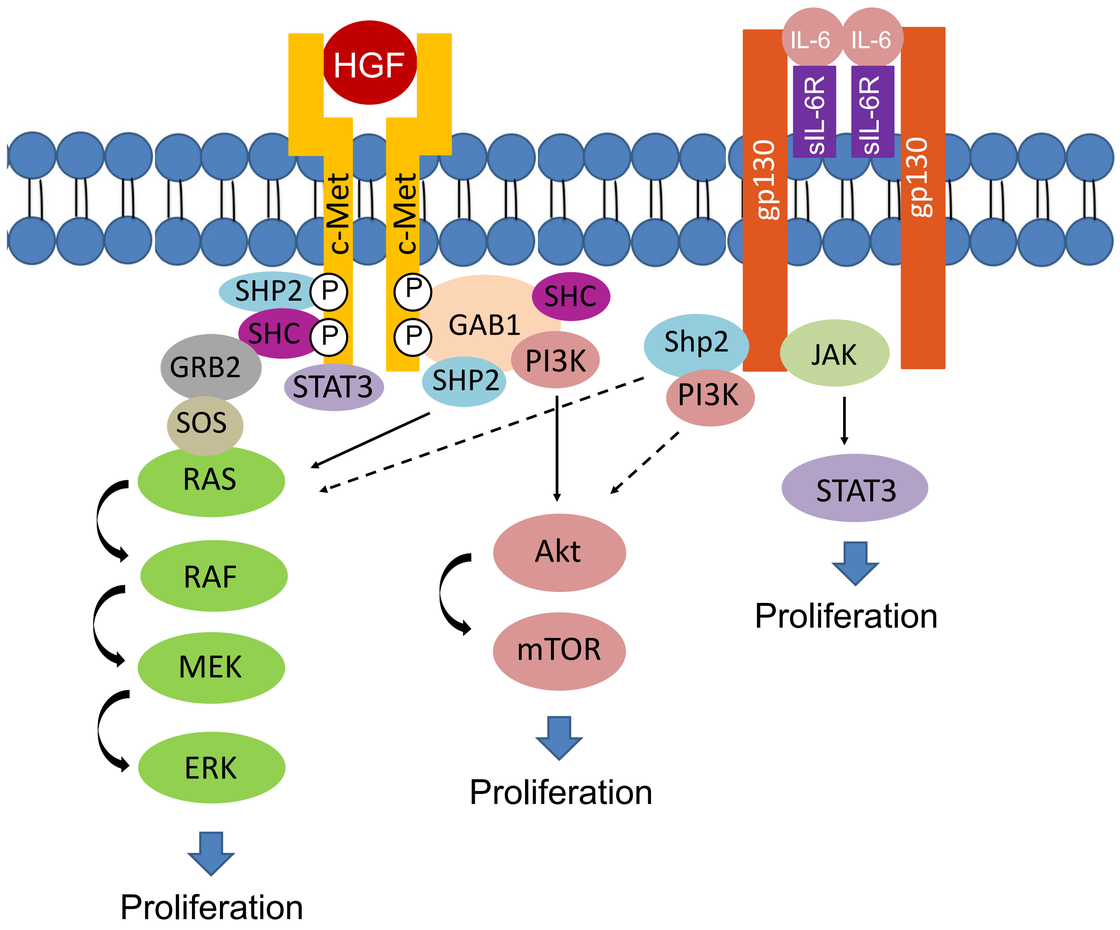

Figure 1: Downstream convergence of the MAPK, PI3K and STAT3 signalling pathways associated with HGF and IL-6 induced proliferation in A549 lung adenocarcinoma cells. HGF/c-Met signalling also leads to the recruitment of FAK which induces migration and cytoskeletal changes (not illustrated above).

METHODS

Growth experiments: A549 cells, human lung adenocarcinoma epithelial cells modelling NSCLC, were seeded at a density of $1x10^5$cells/well in a 12-well plate with DMEM media containing 10% FBS, 2mM l-glutamine and 10 000 U/ml penicillin and streptomycin. The A549 cells were incubated at 37°C with CO_2 level of 5% in humidified atmosphere. After 24 hours, cells were treated with HGF (0.01-1000ngml) or IL-6 (0.01-300ng/ml) for a further 24 hours and the number of cells present in the wells were counted using a haemocytometer. To establish the synergistic or additive effects of HGF and IL-6 on A549 cell proliferation, cell counts were carried out as above utilising the EC_{50} and maximal concentrations of HGF and IL-6. To determine the signalling convergence between HGF and IL-6, cells were seeded at a density of $5x10^4$ cells/well. After 24 hours, the cells were pre-treated with 20μM of PD 98059 (MEK1 inhibitor) and DMSO as a vehicle control for 30 minutes before the addition of the maximal concentrations of HGF (30ng/ml) and IL-6 (10ng/ml). Cell counts were then carried out as above. Determination of significance in all growth data were assessed with one-way ANOVA and Tukey's multiple comparison test.

RESULTS

HGF and IL-6 induce proliferation in A549 cells

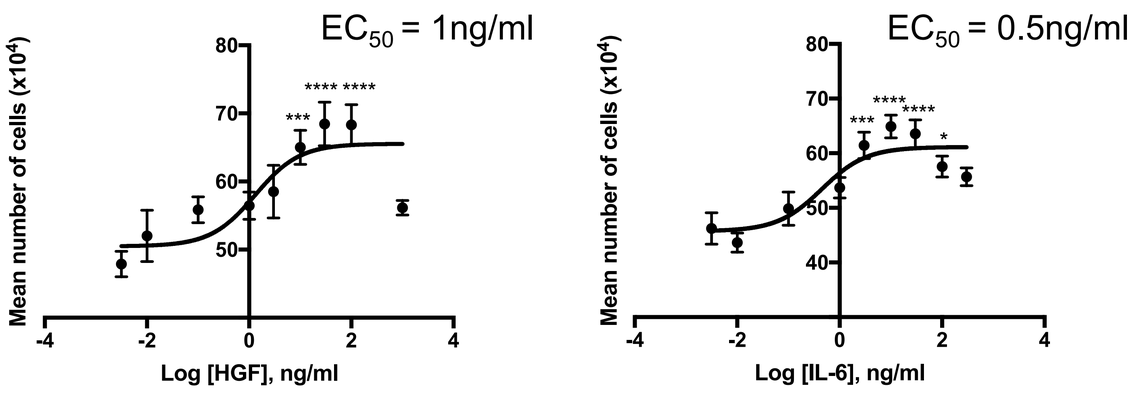

Figure 2: IL-6 and HGF log concentration response curve displays a significant increase in A549 cell proliferation in a concentration dependent manner. The EC_{50} values of IL-6 and HGF are 0.5ng/ml and 1ng/ml respectively. The maximal values of IL-6 and HGF are 10ng/ml and 30ng/ml respectively. *n=3,* **P*<0.05, ****P*<0.001, **** *P*<0.0001

No synergistic effect of IL-6 and HGF on A549 proliferation

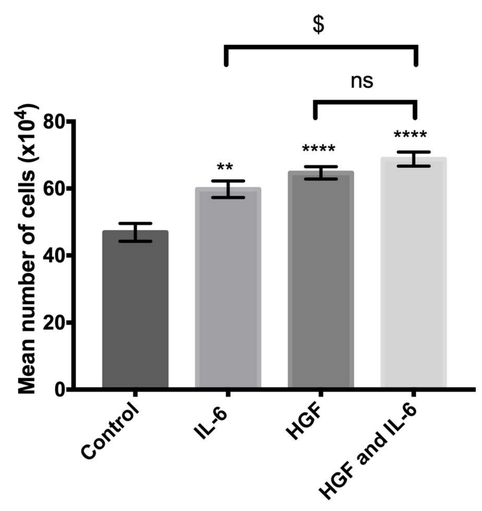

Figure 3: A549 cells were stimulated with its EC_{50} concentrations of HGF (1ng/ml) and IL-6 (0.5ng/ml) individually and in combination for 24 hours. There was a significant increase in the mean cells counts of IL-6 and HGF compared to control. A significant increase in cell counts was observed between IL-6 treated A549 cells and the HGF and IL-6 treated cells in combination. No significant increase was observed between the HGF treated A549 cells and the HGF and IL-6 treated cells in combination. *n=3, $*P<0.05, **P<0.01, ****P<0.0001.*

No additive effect of IL-6 and HGF on A549 proliferation

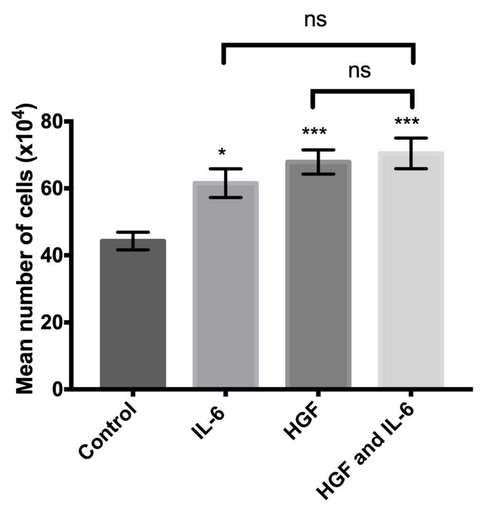

Figure 4: A549 cells were stimulated with its maximal concentrations of HGF (30ng/ml) and IL-6 (10ng/ml) individually and in combination for 24 hours. There was a significant increase in the mean cells counts of IL-6 and HGF, individually and in combination, compared to control. No significant increase in mean cell counts was observed between HGF and IL-6 treated A549 cells alone compared to A549 cells treated with HGF and IL-6 in combination. *n=3, *P<0.05, ***P<0.001.*

HGF and IL-6 converge on the MAP kinase pathway in A549 adenocarcinoma growth

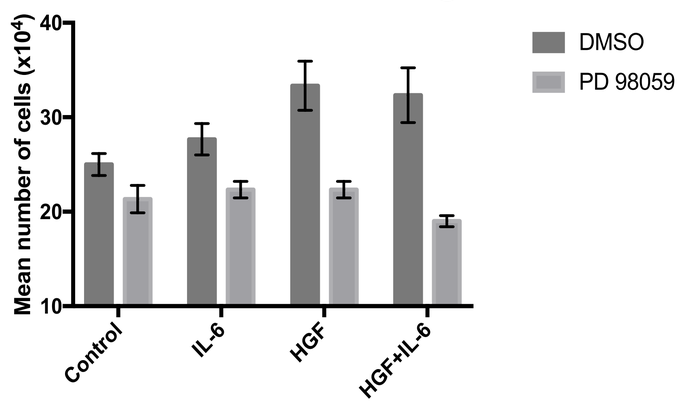

Figure 5: A549 cells were pre-treated for 30 minutes with 20μM of PD 98059 or DMSO, and HGF (30ng/ml) or IL-6(10ng/ml) added individually and in combination for 24 hours. *n=1.*

SUMMARY

- HGF, IL-6 significantly induced proliferation in A549 cells in a concentration dependent manner.
- No synergistic or additive proliferation was observed using co-treatment of HGF and IL-6 at either EC_{50}concentrations (1ng/ml and 0.5ng/ml respectively) or maximal concentrations (30ng/ml and 10ng/ml respectively) compared to either IL-6 or HGF alone.
- Preliminary results suggest HGF and IL-6 converge on the MAPK pathway to induce A549 proliferation.

Conclusion: These results indicate a convergence of the HGF and IL-6 signalling pathways at the MAPK pathway in inducing A549 cell proliferation

FUTURE WORK

- Use inhibitors of signalling pathways (e.g. MAPK, STAT3 and PI3K) to determine the signalling pathways involved in the HGF and IL-6 induced proliferation of A549 cells. Identifying the HGF and IL-6 induced signalling convergence will help to optimise the targeted inhibition of NSCLC growth

References: 1. Lee, S.O., et al. Oncotarget. 2016. 7(6):p.6626; 2. Bahcall et al. Cancer discovery. 2016. 6: 1334-41; 3. Naldini et al. Oncogene. 6: 501-04; 4. To et al. American journal of respiratory cell and molecular biology, 27: 220-26.