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c-Met signalling and endocytosis in clear cell ovarian cancer

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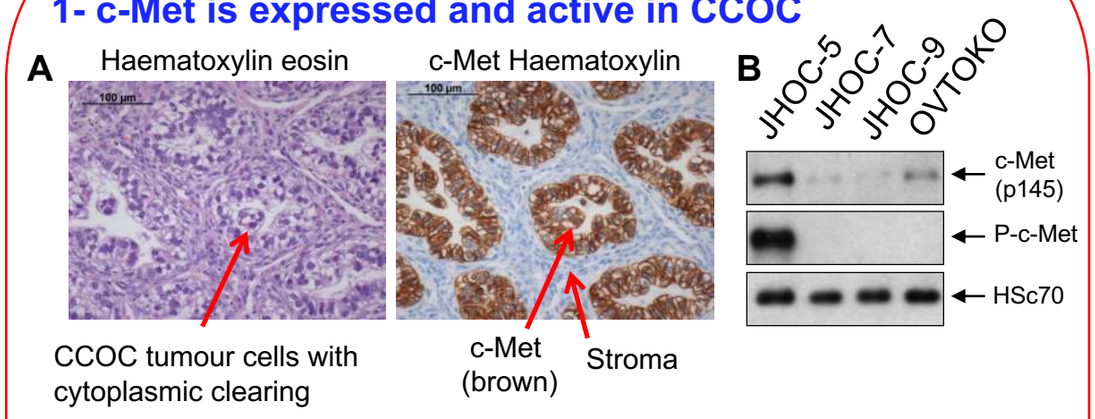
Introduction Clear cell ovarian cancer (CCOC) is a rare subtype of ovarian cancer. Response rate to first line chemotherapy is only 20%. As a result, survival in advanced CCOC is poor and new therapies are urgently required. Upon binding its ligand, Hepatocyte Growth Factor (HGF), the receptor tyrosine kinase c-Met promotes proliferation, survival and motility. c-Met amplification has been demonstrated in 37% of CCOC, compared to 2.6% of epithelial cancers. Recent clinical trials with c-Met inhibitors in ovarian cancer showed response in only 20% of patients. More comprehensive understanding of c-Met signalling in CCOC is necessary to establish optimal therapeutic strategies. We have previously shown that c-Met transmits oncogenic signalling post-endocytosis and endocytosis inhibition reduces c-Met driven tumourigenesis (Joffre et al., Nat Cell Biol 2011). Consequently, manipulating c-Met endosomal signalling may provide a novel therapeutic approach. c-Met endocytic trafficking has never previously been analysed in CCOC.

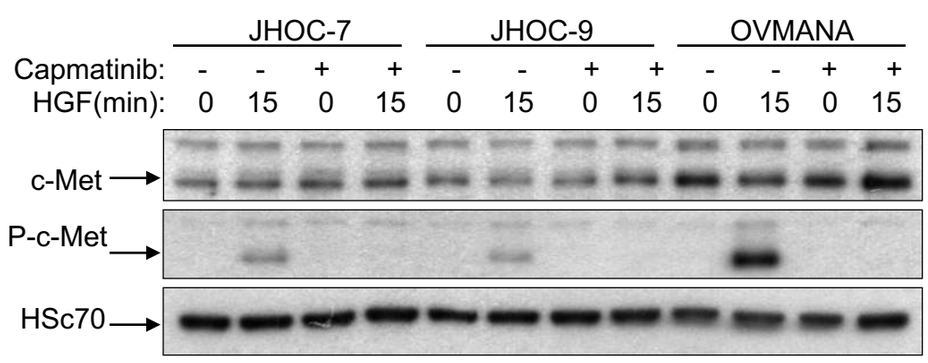
c-Met is a target in CCOC

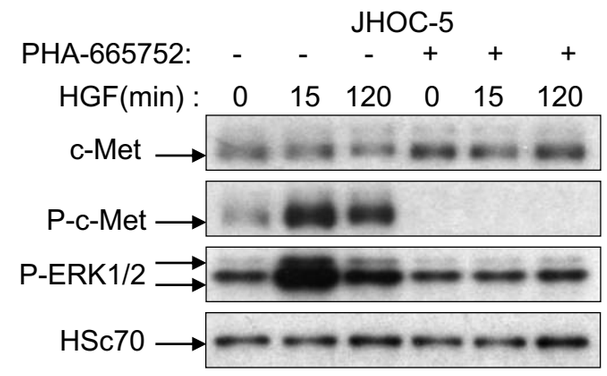
1- c-Met is expressed and active in CCOC



A: Intense c-Met immunostaining (brown) in the malignant epithelial cells of a human CCOC tumour. **B:** Western blots showing c-Met expression in CCOC cell lines. JHOC-5 cells express constitutively phosphorylated c-Met.

2- c-Met inhibitors reduce c-Met activation and signalling

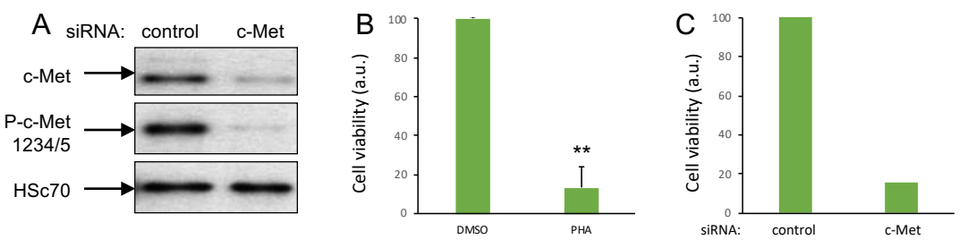




Western blots showing that HGF stimulates c-Met and downstream ERK1/2 phosphorylation.

These responses are inhibited by the c-Met inhibitors Capmatinib (10nM) or PHA-665752 (1µM).

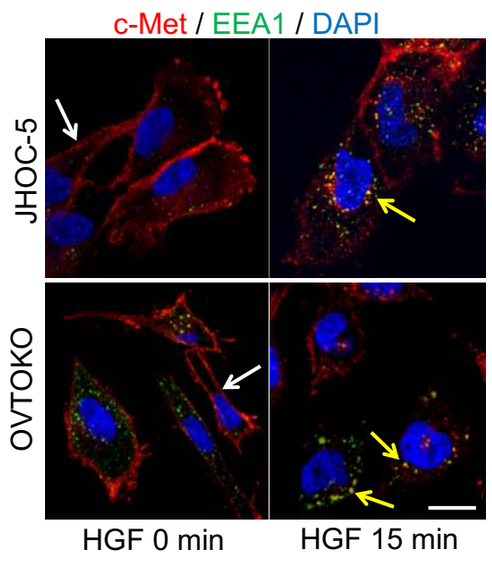
3- Cell survival depends on c-Met expression and activity



A: Western blot demonstrating effective knockdown of c-Met with siRNA in JHOC-5. **B:** the c-Met inhibitor PHA-665752 reduced survival (n=3; ** p<0.001, student t-test). **C:** silencing c-Met reduced survival (n=2).

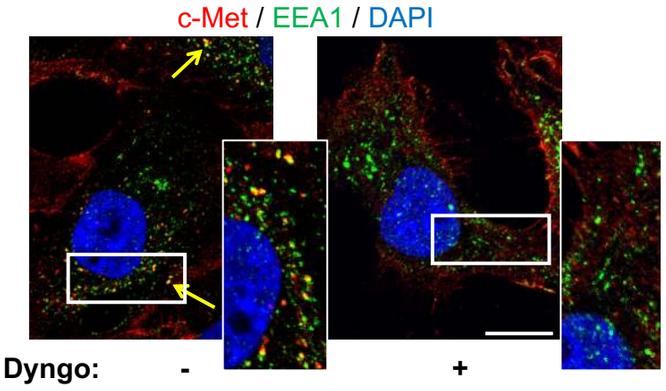
c-Met signals on endosomes in CCOC

1- HGF stimulation triggers rapid c-Met endocytosis



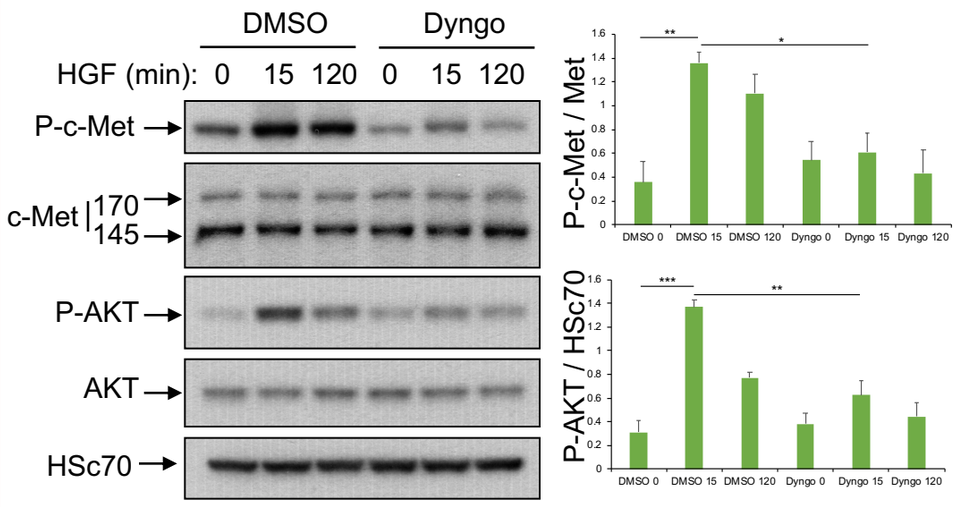
c-Met is localised at the plasma membrane (white arrow) at 0 min, it has endocytosed in vesicles at 15 min of HGF stimulation. Yellow arrows indicate colocalisation between c-Met and EEA1. Scale bar 10 µm.

2- c-Met endocytosis can be impaired pharmacologically



Endocytosis was impaired by pharmacological inhibitor Dyngo in JHOC-5. c-Met colocalisation with EEA1 (yellow arrows) was detected without but not with Dyngo. Scale bar 10 µm.

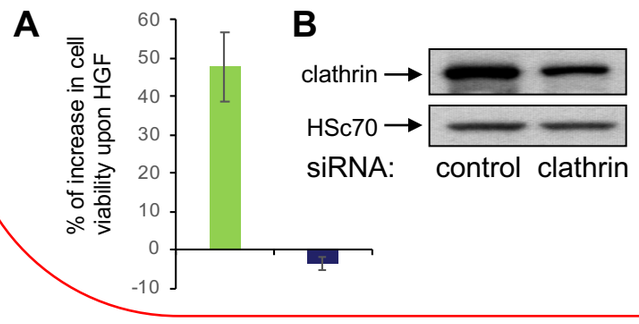
3- Endocytosis inhibition reduces c-Met activation and signalling



Western blots showing that HGF stimulates c-Met and AKT phosphorylation in JHOC-5. These decrease significantly upon treatment with Dyngo.

The graphs are densitometry results averaged from n=3 independent experiments, student t-test, * p<0.05, ** p<0.01, *** p<0.005.

4- Endocytosis inhibition reduces c-Met driven cell survival



A: Transfection with Clathrin Heavy Chain siRNA impairs endocytosis and reduces cell viability in JHOC-5. **B:** Western blots for heavy chain of clathrin and HSc70 are shown.

Conclusion This study confirms c-Met as a therapeutic target in CCOC and illustrates, for the first time, that c-Met is endocytosed in CCOC. The reduction of c-Met signalling following pharmacological blockade of endocytosis strongly indicates that c-Met needs to internalise to transmit its downstream signalling fully. Further work is ongoing to unravel these pathways and to identify novel treatment approaches.