Development of a Pan-Cancer 15 Gene Expression Signature to Detect a Subgroup Driven by MAPK Signalling

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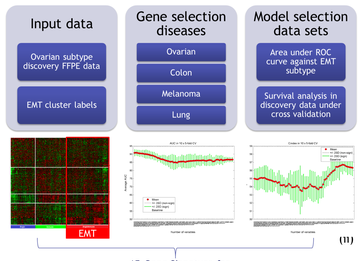
Background

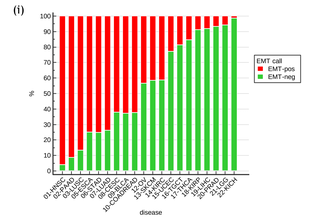
- Unsupervised hierarchical clustering of gene expression data from 265 high grade serous ovarian cancer (HGSOC) tumors identified 3 molecular subgroups.
- These were characterised by upregulation of angiogenesis (Angio subgroup), immune (Immune subgroup) and both angiogenesis and immune (Angioimmune subgroup) genes, respectively.
- Further characterisation of the AngioImmune subgroup reveals it to be driven by MAPK pathway activation and is associated with an EMT (Epithelial to Mesenchymal Transition) like phenotype.
- The aim of this study was to develop a gene signature which could detect the MAPK/EMT subgroup across multiple cancer types.
- Using our datasets and publically available gene expression datasets we have demonstrated that the MAPK/EMT subgroup (as defined by 'EMT assay') exists in other cancer types and is associated with poor prognosis.
- The EMT assay was validated in preclinical model systems.
- A positive result for the EMT assay is associated with higher sensitivity to drugs which target components of the MAPK and EMT pathways.

Development of Pan-Cancer gene expression Signature for MAPK/EMT That Is Predictive of Poor Prognosis

A. Development of the EMT Assay

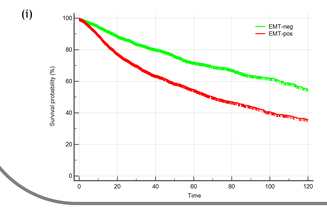


B. Proportion of MAPK/EMT subgroup Across Diseases



tcnc	head and neck
paad	pancreatic
lusc	lung squamous
brca	breast
esca	oesophageal
stad	stomach
luad	lung adeno
cesc	cervical
blca	bladder
coadread	colorectal
gbm	glioblastoma
ov	ovarian
skcm	melanoma
kirc	renal clear cell
ucec	UC endometrial
tgct	testicular
thca	thyroid
kirp	renal papillary
lihc	liver
prad	prostate
lgg	lower grade glioma
kich	renal chromophobe

C. EMT Assay Predicts Poor Prognosis Across Diseases

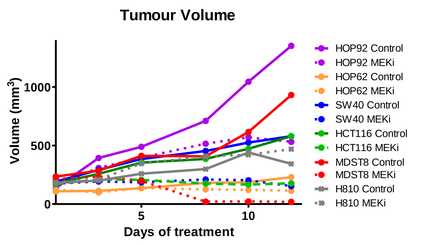


	Hazard ratio	P-value
All diseases	1.78 [1.65-1.92]	<0.0001
Bladder**	1.63 [1.22-2.18]	0.0011
Breast	1.02 [0.66-1.60]	0.92
Cervical**	2.16 [1.16-4.04]	0.016
Colorectal**	1.83 [1.04-3.25]	0.039
Oesophageal	0.90 [0.53-1.54]	0.71
Glioblastoma**	2.12 [1.17-3.83]	0.01
Head and Neck**	1.54 [1.02-2.30]	0.039
Renal clear cell**	4.12 [2.58-6.57]	<0.0001
Renal papillary**	2.98 [1.12-7.93]	0.029
Liver	1.02 [0.63-1.65]	0.94
Lower grade glioma**	2.42 [1.22-4.81]	0.012
Lung adeno**	1.77 [1.21-2.60]	0.003
Lung squamous	1.04 [0.71-1.51]	0.86
Pancreatic**	2.58 [1.52-4.38]	0.0005
Prostate	0.59 [0.05-6.68]	0.67
Melanoma**	1.42 [1.07-1.88]	0.015
Ovarian**	1.36 [0.99-1.86]	0.050
Stomach**	1.73 [1.09-2.75]	0.020
Testicular GCT	33.8 [0.47-2443.5]	0.11
Thyroid	1.96 [0.62-6.18]	0.25
UC endometrial	2.34 [0.93-5.90]	0.07

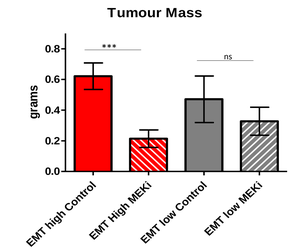
A. Gene selection for the MAPK/EMT subgroup was performed in different cancer types using publicly available and our own datasets. Genes were identified based on variance and intensity between replicates and across all diseases. These genes were used to develop a 15-gene assay which could detect the MAPK/EMT subgroup. **B.** The TCGA database was used to assess prevalence of the MAPK/EMT subgroup in various cancer types (i). TCGA disease codes are provided in the corresponding table (ii) **C.** EMT positivity is associated with a poorer overall survival across different diseases (i). Hazard ratios, which reflect the association between EMT positivity and survival probability for individual diseases, are shown in the corresponding table (ii).

EMT Assay predicts response to MEK inhibitors in mouse xenograft models

A. EMT Positive xenografts are Sensitive To treatment with a MEK Inhibitor

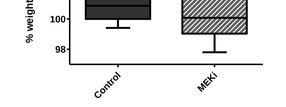


B. MEK inhibition reduces tumour mass in EMT positive xenograft models

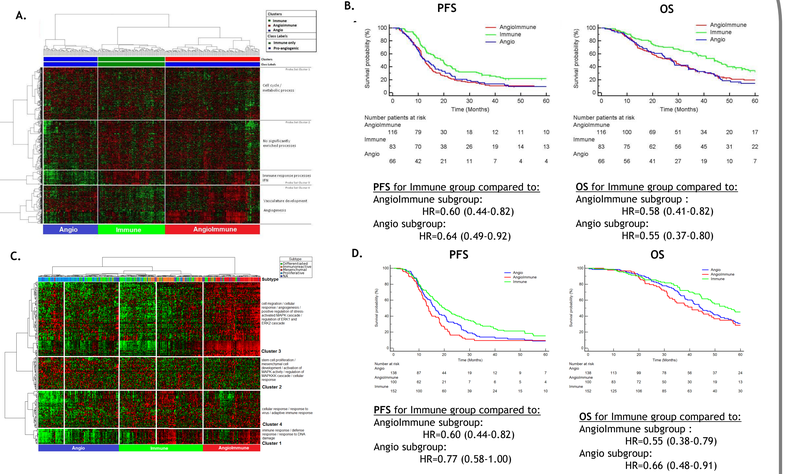


A. EMT assay predicts sensitivity to trametinib *in vivo*. Athymic nude mice were engrafted with solid tumour cell lines, EMT assay high cell lines and EMT assay low cell line. When tumours reached 150mm3 mice were treated with vehicle or trametinib (1 mg/kg) once daily by oral gavage for 14 days. Tumour volume variation of all the cell lines during treatment shows that EMT positive cell lines are highly responsive to trametinib treatment. **B.** Average of the mass of the tumours from all cell lines collected after treatment. Trametinib treated tumours from the EMT High cell lines had reduced their size significantly ($p<0.01$). **C.** Body weight percentage variation of vehicle or Trametinib treated mice is shown (not significant).

C. MEK inhibition has little effect on body weight



Identification of Molecular Subgroups of High Grade Serous Ovarian Cancer



PFS for Immune group compared to:
AngioImmune subgroup:
HR=0.60 (0.44-0.82)
Angio subgroup:
HR=0.64 (0.49-0.92)

OS for Immune group compared to:
AngioImmune subgroup :
HR=0.58 (0.41-0.82)
Angio subgroup:
HR=0.55 (0.37-0.80)

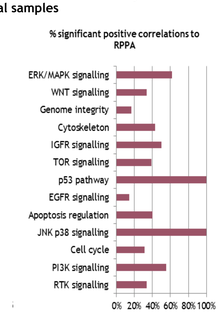
PFS for Immune group compared to:
AngioImmune subgroup:
HR=0.60 (0.44-0.82)
Angio subgroup:
HR=0.77 (0.58-1.00)

OS for Immune group compared to:
AngioImmune subgroup :
HR=0.55 (0.38-0.79)
Angio subgroup:
HR=0.66 (0.48-0.91)

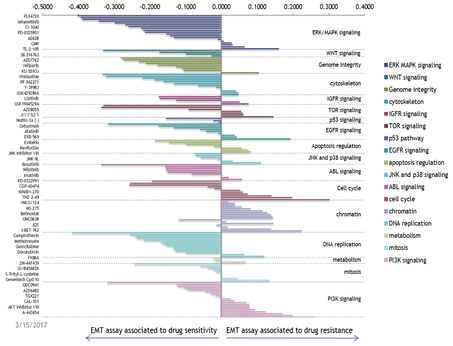
A. Hierarchical clustering analysis of 265 FFPE treatment naive HGSOC who were subsequently treated with platinum-based SoC chemotherapy (carboplatin +/- paclitaxel). 3 subgroups were identified: Immune, Angio and AngioImmune. **B.** Survival analysis of newly identified molecular subgroup. Immune subgroup is a good prognosis subgroup and the Angio and AngioImmune subgroups are poor prognostic subgroups in the context of SoC. **C.** Recapitulation of molecular subgroups in TCGA dataset. The AngioImmune subgroup is associated with the TCGA mesenchymal subgroup. **D.** Survival analysis of molecular subgroups in TCGA dataset.

EMT Assay predicts response to MEK inhibitors *in vitro*

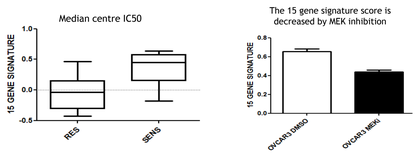
A. Correlation Between EMT assay and Protein expression in TCGA clinical samples



B. Correlation Between EMT Assay Sensitivity To MEK Inhibitors



C. Correlation of EMT Positivity and Response To MEK Inhibitors in Pan-Cancer Cell Line Models



A. EMT signature scores were correlated with protein expression across multiple disease types (RPPA data, TCGA database). The ERK/MAPK pathway is strongly associated with a high EMT signature score. A number of known EMT/MAPK-associated proteins and phosphoproteins were correlated with a high EMT signature score **B.** The ability of the EMT assay to predict sensitivity to a range of inhibitors was assessed using solid tumour cell lines accessed from the Sanger database. EMT positivity was shown to correlate with a low IC50 for agents that target the ERK/MAPK pathway, **C.** Colony formation assays were performed to measure response of cancer cell lines to the MEK inhibitor (trametinib). Cells that were sensitive to treatment with trametinib had a higher 15 gene EMT assay score. The EMT assay score was decreased upon treatment of sensitive cell lines with trametinib.

Conclusions

- A 15 gene signature has been developed from formalin fixed paraffin embedded samples to detect a molecular subgroup driven by MAPK/EMT signalling across multiple diseases .
- This assay predicts sensitivity to MEK inhibitors in pre-clinical cell line and mouse model systems.
- Further work aims to validate the EMT assay in clinical samples from patients treated with a MEK or EMT inhibitor.
- This assay may be helpful for clinical trial enrichment to select patients that are likely to benefit from MAPK or EMT targeted therapies.

Acknowledgements

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