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

Nuala McCabe,[1,2] Laura A. Knight, [2] Andrena McCavigan,[2] Aya El-Helali,[1] Caroline O. Michie,[3] Bethanie Price,[2] Niamh McGivern,[1] Michael Churchman,[1] Jaine K. Blayney [2], Kienan I. Savage, [2] Stuart McIntosh, [2] Alistair Williams,[4] W. Glenn McCluggage,[1,5] Charlie Gourley,[3] Denis P. Harkin[1,2,] and Richard D. Kennedy[1,2].

[1]Centre for Cancer Research and Cell Biology, Queens University Belfast, Northern Ireland, UK; [2]Almac Diagnostics, 19 Seagoe Industrial Estate, Craigavon, UK; [3]University of Edinburgh Cancer Research UK Centre, UK; [4]Division of Pathology, University of Edinburgh, UK; [5]Department of Pathology, Royal Group of Hospitals Trust, Belfast, UK.

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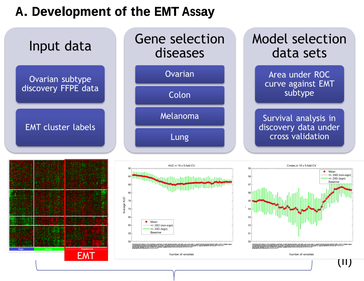
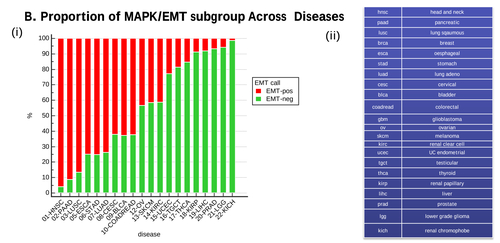
Background

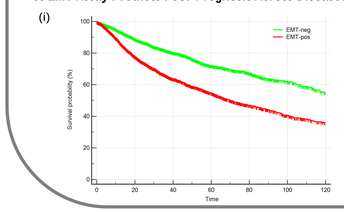
- Unsupervised hierarchical clustering of gene expression data from 265 high grade serous ovarian cancer (HGSOC) tumors identified 3 molecular subgroups.
- These were characterised by upregulation of angiogenesis (Angio subgroup), immune (Immune subgroup) and both angiogenesis and immune (Angioimmune 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.
- 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





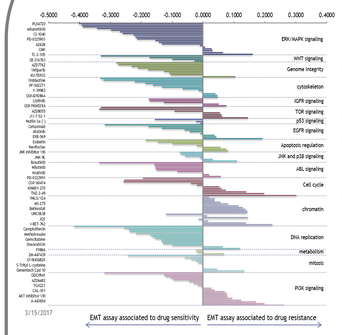
hnsc	head and neck
panc	pancreatic
lusc	lung squamous
brca	breast
esca	oesphageal
stad	stomach
luad	lung adeno
cesc	cervical
blca	bladder
coadread	colorectal
gbm	glioblastoma
ov	ovarian
skcm	melanoma
kich	renal clear cell
ucec	UC endometrial
tgct	testicular
thca	thyroid
kirp	renal papillary
lihc	liver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

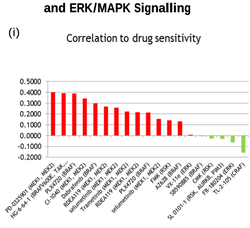


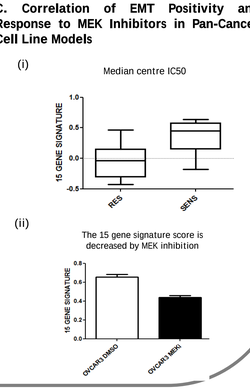
	Hazard ratio	P-value
All diseases	**1.78 [1.65-1.92]**	**<0.0001**
Bladder**	**1.63 [1.22-2.18]**	**0.0011**
Breast	1.02 [0.66-1.60]	0.92
Cervical**	**2.16 [1.16-4.04]**	**0.016**
Colorectal**	**1.83 [1.04-3.25]**	**0.039**
Oesophageal	0.90 [0.53-1.54]	0.71
Glioblastoma**	**2.12 [1.17-3.83]**	**0.01**
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).

Correlation of EMT Assay with Drug Response Data

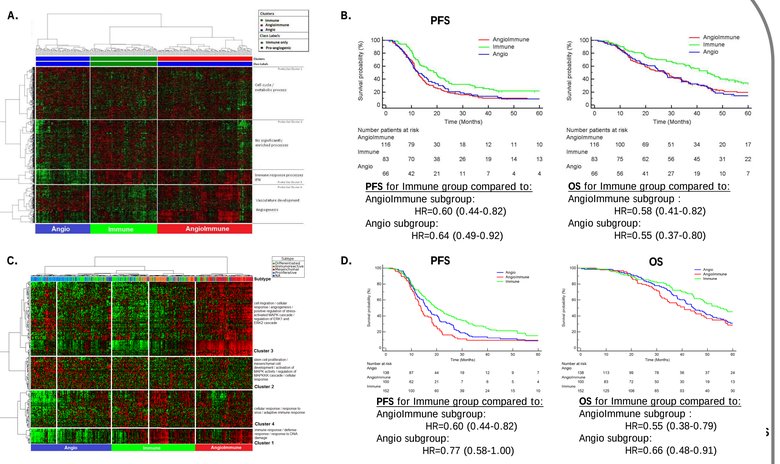






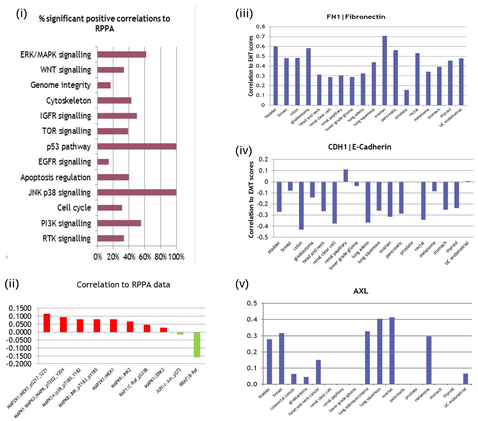
A. The ability of the EMT assay to predict sensitivity to a range of inhibitors was assessed using solid tumour cell lines accessed from the Sanger database. EMT positivity was shown to correlate with a low IC50 for many agents that target the ERK/MAPK pathway, indicating higher sensitivity of EMT positive cell lines to drugs that target MAPK/EMT. **B.** The association between EMT assay positivity and activation of the ERK/MAPK pathway is summarised based on its correlation to drug sensitivity (i). **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 (i). The EMT assay score was decreased upon treatment of sensitive cell lines with trametinib (ii).

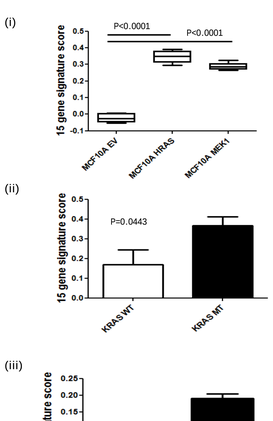
Identification of Molecular Subgroups of High Grade Serous Ovarian Cancer



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.

Correlation of EMT Assay with Protein Expression Data





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 (i-ii). A number of known EMT/MAPK-associated proteins and phosphoproteins were correlated with a high EMT signature score (iii-v). **B.** The EMT assay scores were significantly elevated in response to overexpression of HRAS and MEK1 (i); human KRAS mutant cell lines (ii) and in cells overexpressing SNAIL (iii).

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 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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Contact: nuala.mccabe@almacgroup.com