Fascin and Cdk2 are synthetic lethal partners with exceptional potential as joint therapeutic targets in malignant melanoma.

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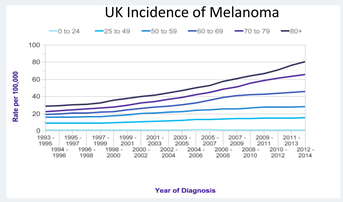
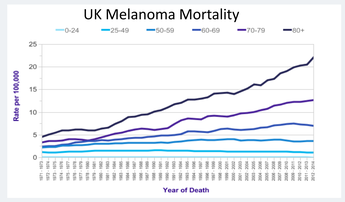
1. Introduction

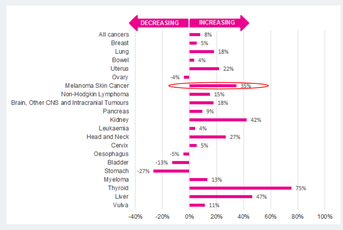
Objective was to identify **gene combinations** in malignant melanoma with therapeutic potential.

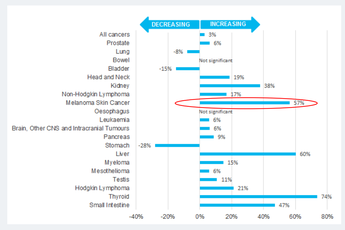
Motivation:
1. Malignant melanoma is deadly, with rising incidence and mortality[1].





2. Melanoma incidence is one of the fastest rising of all cancers in the UK is both women (pink) and men (blue).





3. Targeted therapies improve life expectancy but are expensive and only efficacious in a subset of patients[2].
4. Resistance to therapy is problematic – combination therapies may provide a route to avoiding this.

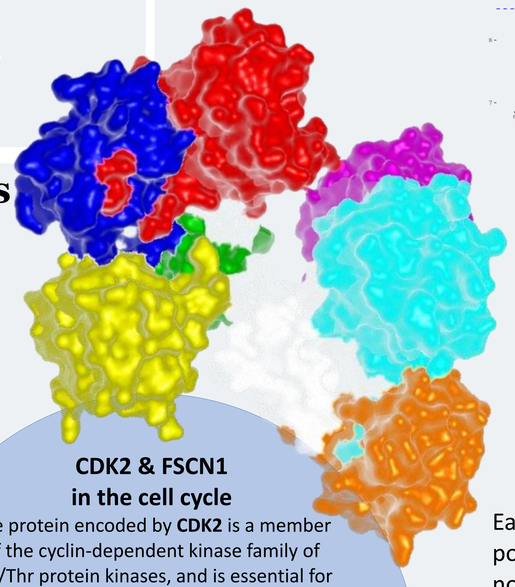
2. Materials and Methods

1. RNA-sequencing and clinical data from the Cancer Genome Atlas project (TCGA) were downloaded from gdac.broadinstitute.org (n=445 patients with melanoma)[3].

2. Gene expression combinations which showed functional redundancy and which were never expressed simultaneously at low levels were identified using the R programming language and the BISEP package.

3. Data from GTEx[4] (http://www.proteinatlas.org) and two microarray studies of melanoma gene expression[5-6] were obtained and analysed to verify that the effect seen in TCGA was replicated.

4. Initial screening of cancer-associated genes revealed a number of DNA-damage repair genes being associated with survival. GATAD2A is poorly characterised in melanoma, and has a plausible mechanism of action in carcinogenesis (see inset, right) so was chosen for further study.



CDK2 & FSCN1 in the cell cycle

The protein encoded by **CDK2** is a member of the cyclin-dependent kinase family of Ser/Thr protein kinases, and is essential for the G1/S transition. In melanocytic cell types CDK2 is tightly controlled by **MITF**[7]. Expression of the CDK2 protein regulates degradation of **beta-catenin**[8].
FSCN1 encodes an actin bundling protein which is critical for cell motility and is involved in regulation of cell cycle progression through interaction with **wnt/beta-catenin**[9].
A potential mechanism for the synthetic lethality seen here might be dependence of wnt/beta-catenin on CDK2/FSN1.

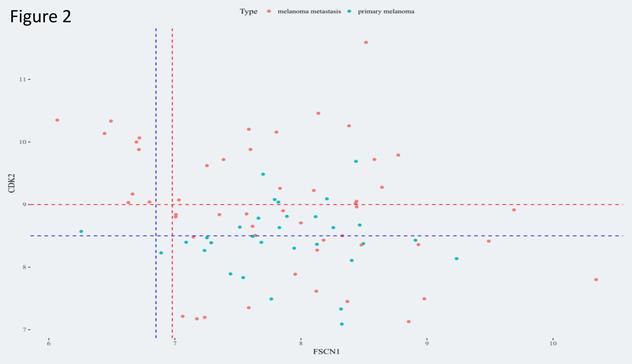
3. Results

Figure 1

TCGA expression data from melanoma patients (n=445) showed no simultaneous low expression of CDK2 and FSCN1, implying a synthetic gene dose lethality in melanoma (Figure 1).

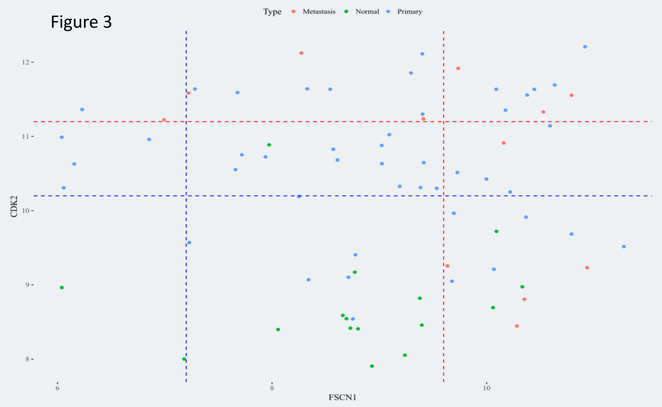
To assess reproducibility of the result a second data set[5] was analyzed which showed the same result (Figure 2), and also demonstrated that metastatic samples showed more sensitivity to the gene dose synthetic lethality than primary samples.

Figure 2



A third data set[6] which included normal skin samples reproduced the findings, and showed that normal skin was not susceptible to the gene dose synthetic lethality (Figure 3).

Figure 3



A large collection of normal skin (GTEx) compared with melanoma (TCGA), despite different analysis pathways demonstrated significantly higher sensitivity to gene dose synthetic lethality of CDK2 and FSCN1 in normal skin than melanoma (Figure 4).

Figure 4

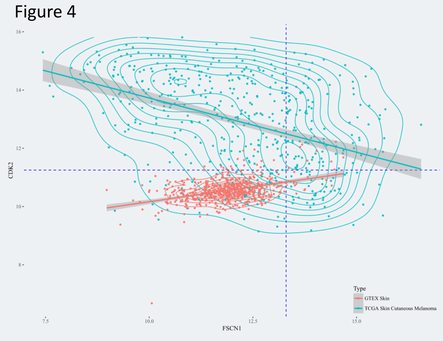
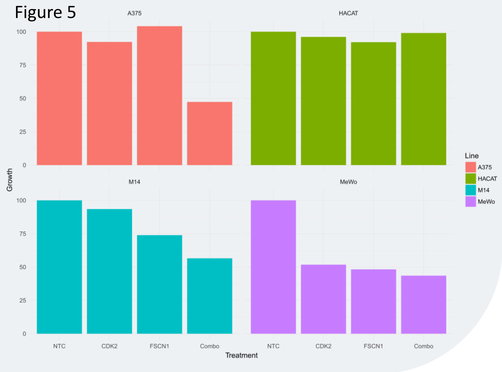


Figure 5



Early *in-vitro* data suggests that siRNA knock-down of both CDK2 and FSCN1 together powerfully inhibits growth in 3 melanoma cell lines (MeWo, A375 and M14), but leaves non-melanoma (HACAT cells) cell growth intact (Figure 5). (Data from J Gao & S Bailey).

4. Discussion and Conclusions

- *CDK2 and FSCN1* gene expression is show **synthetic dose lethality** in melanoma samples.
- Synthetic lethality is seen in three **independent** data sets.
- **Normal skin** does not show the gene dose lethality.
- Early data shows that interventional **knock-down** of the two genes in combination restricts melanoma growth more than either alone, and does not affect non-melanoma cells.
- Both genes have established **roles in cancer pathogenesis**, but have never been associated as joint therapeutic targets.
- The combination represents an attractive pair of genes for **novel therapeutic combinations**.
- There is a potential mechanistic basis for the synthetic lethality via the effects on **wnt/beta-catenin and cell cycle progression**.

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Author Biography

I am an MRC-funded Post-doctoral Clinical Research Fellow at the University of Cambridge and EMBL-European Bioinformatics Institute and honorary registrar in Dermatology and Clinical Pharmacology in Cambridge. My research is focused on applying bioinformatics techniques with a clinician's view of disease phenotypes. I am particularly interested in drug repositioning and in the role of alternative RNA splicing in skin cancers.