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Deletion of 17p in cancers: Guilt by (p53) association

INVESTIGATING 17p DELETION IN CLL: A study of tumour-suppressive microRNAs

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BACKGROUND

The monoallelic deletion of Chromosome 17p (del17p) is the most common aneuploidy observed in cancer which associated with particularly poor prognosis across multiple malignancies[1], including CLL. The paradigm of the literature is that the aggressive tumour behaviour and resistance to therapy associated with del17p is due to the dysfunction or loss of *TP53*. However, clinical evidence suggests del17p incurs significantly shorter overall survival following therapy independent of *TP53*-status[2,3], although current understanding of this remains limited.

The dysregulation of microRNAs (miRNAs) is commonly observed in multiple cancer types[4]. Chromosome 17p harbours 25 miRNAs which bioinformatic analysis has revealed are likely to regulate essential cellular processes. Thus, we hypothesise that the loss of these miRNAs via the deletion of chromosome 17p may play a role in the pathology of del17p CLL.

17p HARBOURS 25 miRNAs

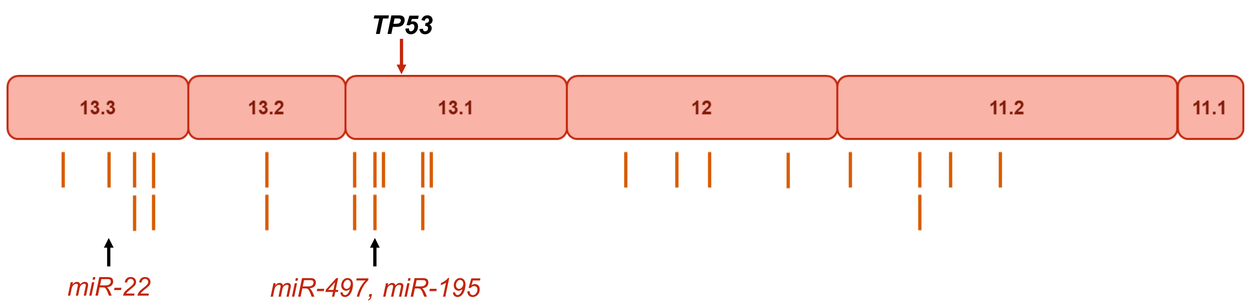


Figure 1: Schematic illustrating the loci of each miRNA along chromosome 17p, as well as *TP53* at 17p13.1. miRNAs of interest are situated in bands 13.3 and 13.1 and are marked with arrows.

miR-497, *-195*, and *-22* EXHIBIT STRAND BIAS AND HAPLOINSUFFICIENCY IN CLL CELL LINES

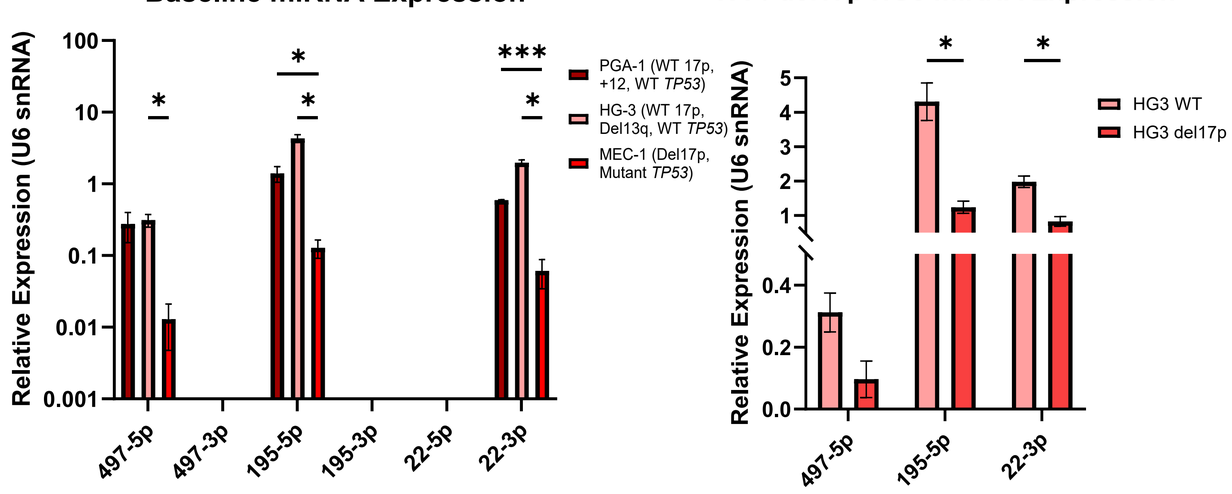


Figure 2: RT-qPCR data showing expression of mature miRNAs relative to U6 snRNA in **(a)** CLL cell lines with different 17p-status **(b)** WT HG3 and an isogenic del17p HG3 model. Error bars demonstrate standard error from the mean. Multiple unpaired T-tests used for statistical analysis.

GENERATION OF miRNA KNOCKOUTS USING CRISPR/CAS9 IN PGA-1 CELLS

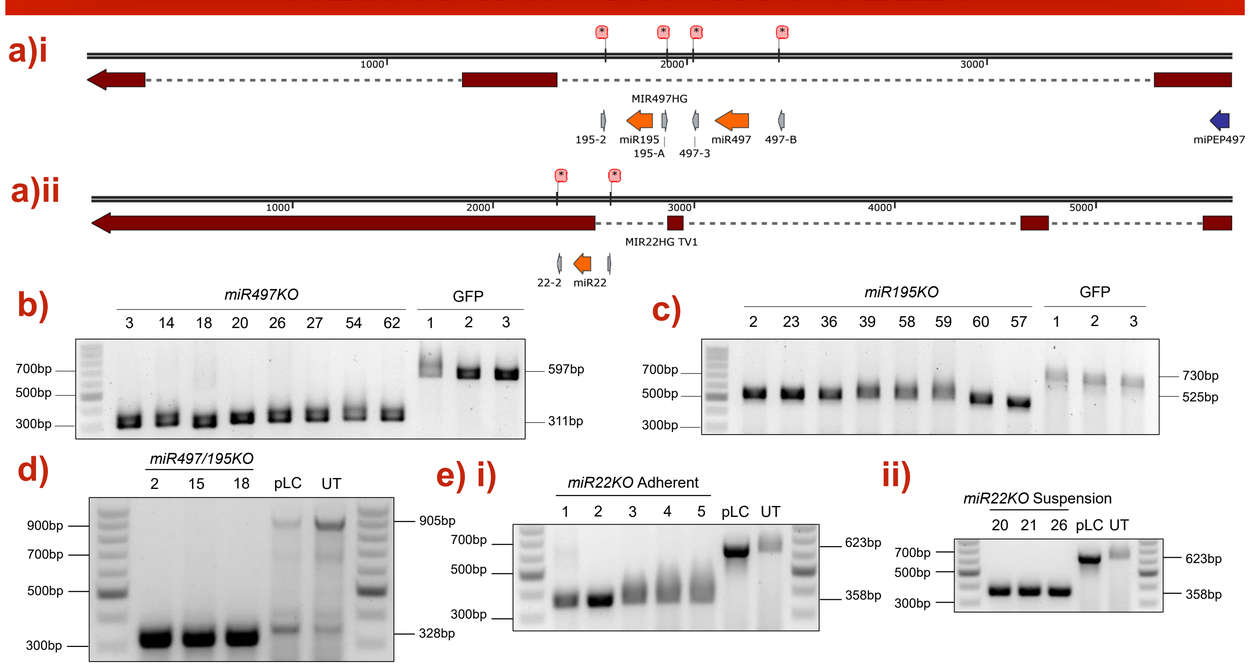


Figure 3: a) Schematic illustrating the placement of Cas9 guide RNAs for the deletion of their respective pre-miRNAs. Genotype PCR screening of puromycin-selected PGA-1 clones for the knockout of **b)** *miR-497* **c)** *miR-195* **d)** *miR-497/195* dual knockout **e)** *miR-22* in adherent**(i)** and suspension**(ii)** clones. Deletions were validated using qRT-PCR (data not shown).

mRNA-SEQ REVEALS POTENTIAL DIRECT TARGETS

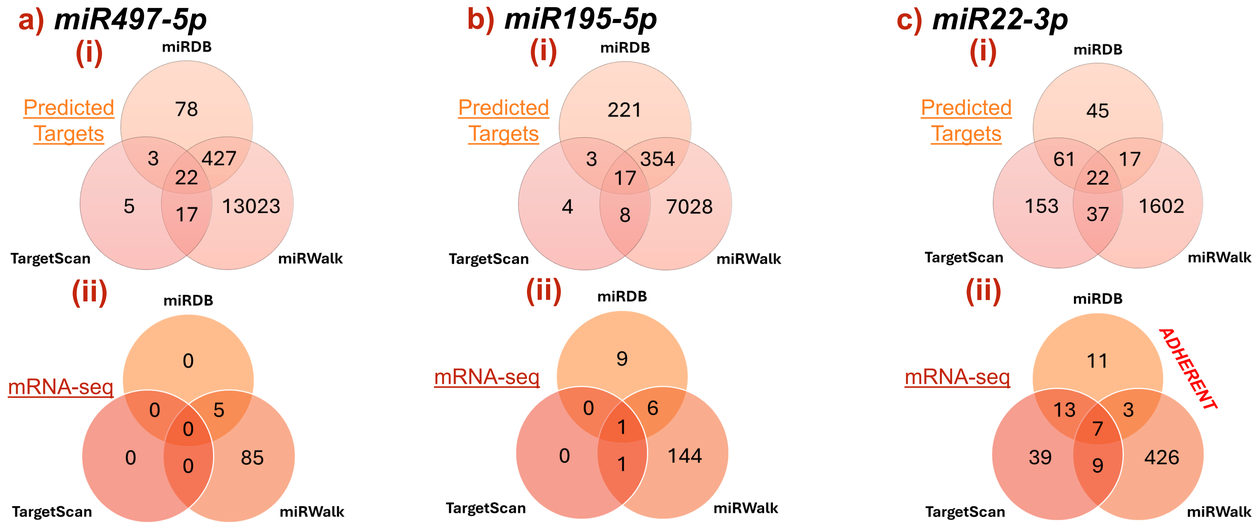
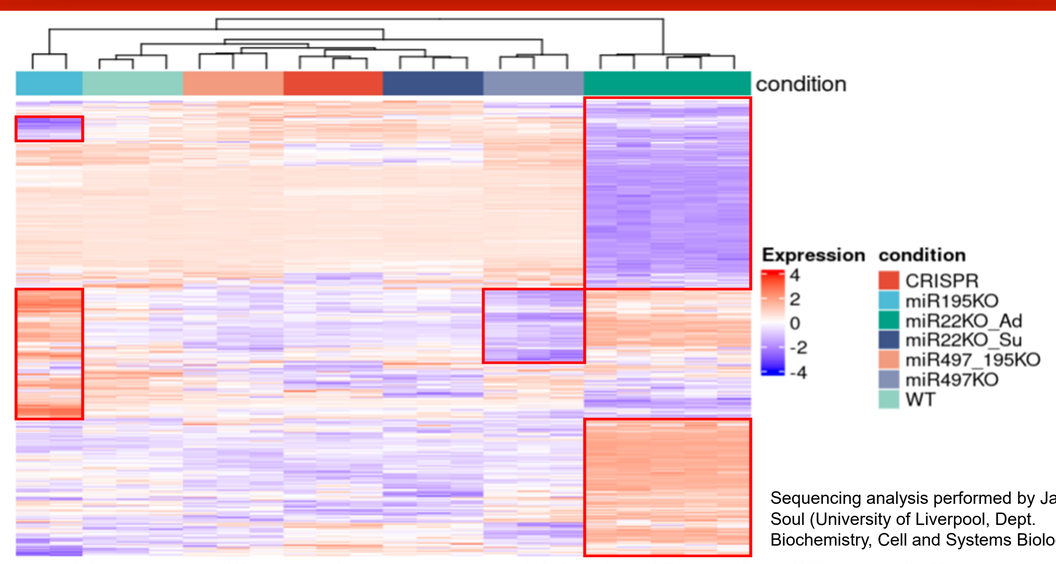


Figure 4: Venn diagrams for **(a)** *miR-497-5p* **(b)** *miR-195-5p* **(c)** *miR-22-3p* showing **(i)** predicted targets from three different algorithms (miRDB, miRWalk, TargetScan), and **(ii)** predictions cross-referenced with mRNA-seq data (*miR22-3p* suspension data not shown). Data was filtered for significantly upregulated genes compared to CRISPR controls (Padj<0.05, log2FC>+1).

miRNA KOs INDUCE TRANSCRIPTOMIC CHANGES



Sequencing analysis performed by Jamie Soul (University of Liverpool, Dept. Biochemistry, Cell and Systems Biology)

Figure 5: Heatmap illustrating the top 100 significantly differentially expressed genes between each condition and its WT and CRISPR control.

miR-22 DELETION INDUCES AN ADHERENT PHENOTYPE

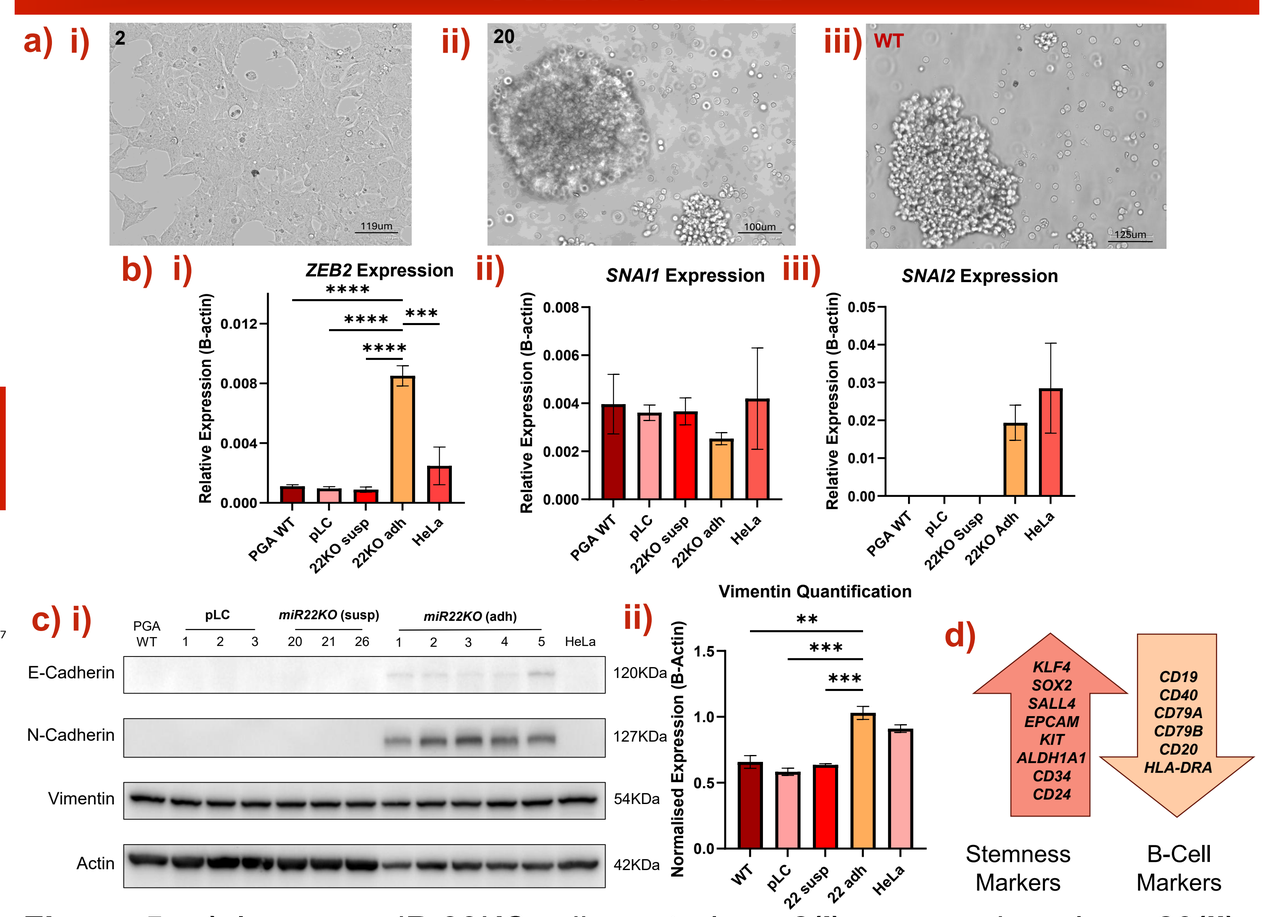


Figure 5: a) Images *miR-22KO* adherent clone 2**(i)**, suspension clone 20**(ii),** and untransduced Wild Type PGA-1 cells**(iii)**. **b)** RT-qPCR data for the expression of EMT transcription factors **(i)***ZEB2* **(ii)***SNAI2* **(iii)***SNAI1* relative to B-actin. **c) (i)**Western blot for the presence of EMT markers N/E-Cadherin and Vimentin **(ii)** densitometry quantification of Vimentin expression. **d)** Data from mRNA-seq showing significant downregulation of B-cell markers (Padj<0.05, Log2FC<-1), and upregulation of stemness markers (Padj<0.05, Log2FC>+1)

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