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Regulation of Acute Myeloid Leukaemia Maintenance by Wilms Tumour 1 in an Isoform Specific Manner

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INTRODUCTION

The gene encoding Wilms Tumour 1 (*WT1*) is recurrently upregulated in Acute Myeloid Leukaemia (AML) and encodes a Zinc Finger Transcription Factor. Increased *WT1* transcript levels are associated with primary refractory disease and with relapse. In an shRNA depletion screen against transcription factors in vitro and in murine xenotransplantation experiments, we have previously shown that *WT1* was essential for leukemic maintenance (Martinez-Soria et al., Cancer Cell, 2018).

WT1 produces at least 8 distinct isoforms in haematopoietic cells depending upon which start site is employed and whether or not alternative splicing at the exon 5 and exon 9 sites occur. In particular, alternative splicing of a 3 amino acid sequence, Lysine-Serine-Threonine 'KTS' in the Zinc Finger encoded by exon 9 alters the DNA binding of WT1.

Here, we investigate the role of individual WT1 isoforms in leukaemia and relate the distinct DNA binding sites of the WT1 +KTS and WT1 –KTS isoforms to the phenotypic behaviour of leukaemic cells. We also investigate how WT1 may co-operate with other Transcription factors, with a particular focus on other Zinc Finger Transcription Factors.

DISCUSSION

We have shown that WT1 is critical for leukaemic maintenance in AML but is not required in healthy stem cells. In particular the WT1 +KTS isoform is responsible for leukaemic growth and has distinct binding sites to the WT1 –KTS isoforms. Both isoforms compete or cooperate with other Zinc Finger transcription factors for their binding sites.

Several vaccine studies and more recently a T cell receptor study targeted against WT1 have been undertaken but they have shown mixed efficacy in AML (Chapuis et al, Nature Medicine, 2019). Since we find that the different isoforms of *WT1* have antagonistic effects, we hypothesise that a more effective therapeutic strategy would be to selectively target only WT1 +KTS isoforms.

Whilst direct inhibition of the WT1 transcription factor is not currently possible, we show that with pharmacological or genetic perturbation of upstream parts of the Transcription Factor or signalling networks (CBFβ-RUNX1 inhibitor, dominant negative FOS or FLT3 inhibitor), *WT1* can be knocked down (Figure 7).

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RESULTS

WT1 is overexpressed in all subtypes of AML

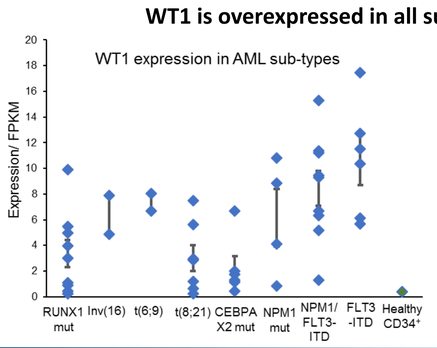

Figure 1: Expression of *WT1* mRNA in purified primary AML leukemic blasts with different mutational subtypes. Error bars show standard error of the mean.

Knockdown of *WT1* reduces leukaemic cell growth and colony formation ability

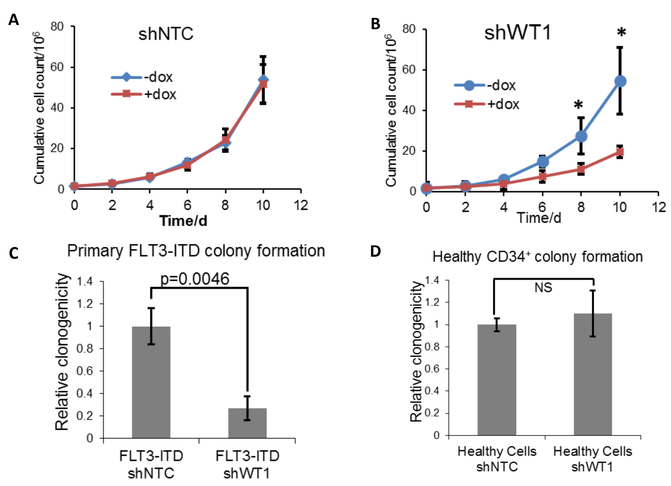

Figure 2: (A-B) Growth curves of Kasumi-1 cells transduced with doxycycline-inducible shRNA against (A) Non-targeting control (NTC) or (B) *WT1* with or without induction with doxycycline. (C-D) Colony formation ability of (C) primary FLT3-ITD AML cells or (D) primary healthy CD34+ cells transduced and induced with doxycycline-inducible shRNA against Non-targeting control (NTC) or *WT1*.

WT1 exhibits isoform specific effects based upon inclusion or exclusion of a 3 amino acid 'KTS' splice site

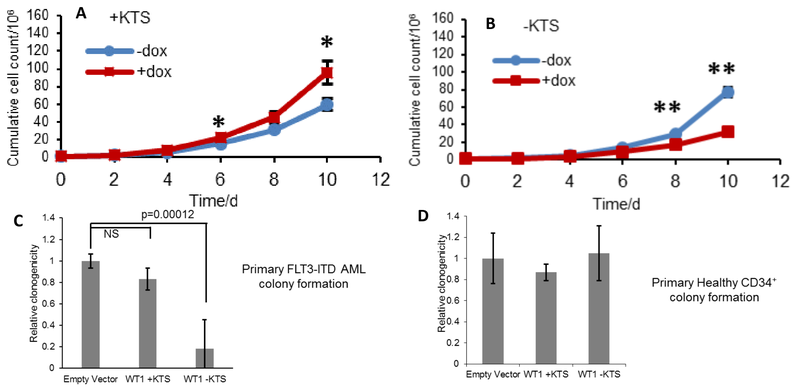

Figure 3: (A-B) Growth curves of Kasumi-1 cells transduced with doxycycline-inducible WT1 isoforms (A) with or (B) without inclusion of the KTS splice site, with or without doxycycline. (C-D) Colony formation ability of (C) primary FLT3-ITD AML cells or (D) primary healthy CD34+ cells transduced and induced with doxycycline-inducible WT1 isoforms (C) with or (D) without the KTS splice site.

WT1 –KTS has distinct and more binding sites compared with WT1 +KTS

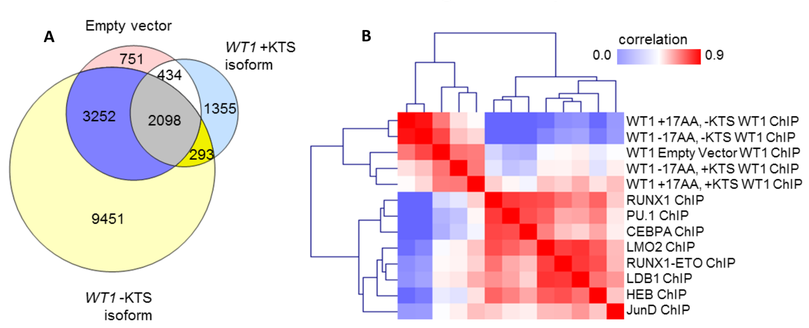

Figure 4: (A) Venn diagram showing the overlap between WT1 ChIP-seq binding sites in Kasumi-1 cells transduced with doxycycline-inducible Empty Vector (endogenous WT1), WT1 –KTS or WT1 +KTS. (B) Heatmap showing hierarchical clustering of Pearson Correlation Coefficients of WT1 isoform ChIP-seq sequence profiles and that of the indicated transcription factor ChIP-seq profiles. Some ChIP-seq data from (Ptasinska et al., 2014).

Zinc finger transcription factors EGR1, WT1 and Sp1 bind to overlapping site and compete or cooperate for binding

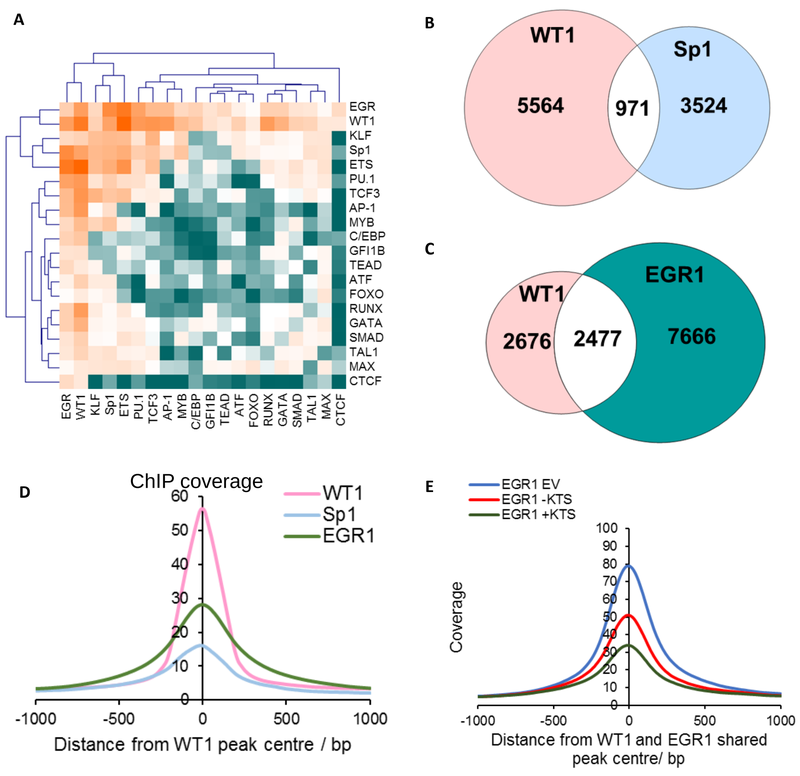

Figure 5: (A) Bootstrapping analysis clustering the significance of co-localisation of the indicated Transcription Factor binding motifs within 50 bp in WT1 ChIP-seq peaks in Kasumi-1 cells. (B) Venn diagram showing the overlap between WT1 and Sp1 ChIP-seq binding sites in Kasumi-1 cells. (C) Venn diagram showing the overlap between WT1 and EGR1 ChIP-seq binding sites in Kasumi-1 cells. (D) Average profile plot of ChIP coverage relative to distance from the WT1 peak centre in Kasumi-1 cells. (E) Average profile plots of EGR1 ChIP coverage in Kasumi-1 cells transduced with Empty Vector, *WT1* –KTS or *WT1* +KTS.

The FLT3-ITD AML Transcription Factor Network

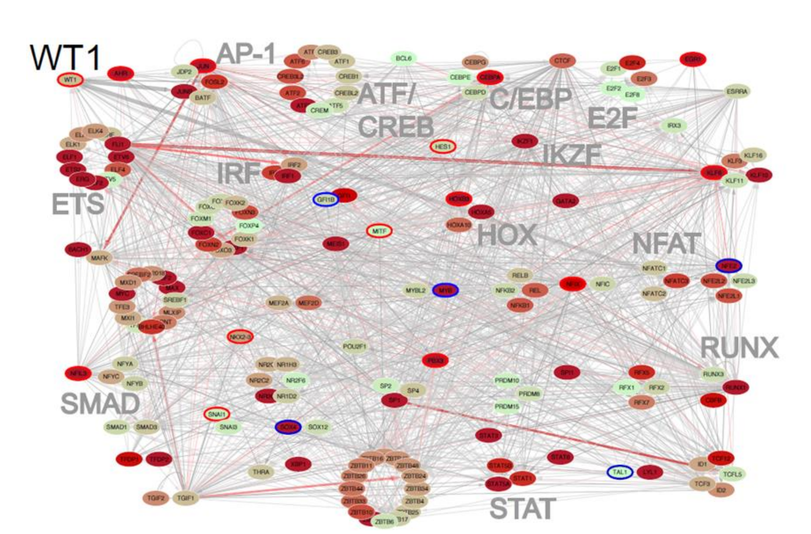

Figure 6: The transcription factor network in FLT3-ITD AML. Utilising ChIP-seq data of transcription factor binding sites and motif enrichment in open chromatin sites, regulation of one factor by another may be deduced. Upregulated transcription factors compared with healthy CD34+ cells are shown and the arrows are dependent of the number ChIP binding sites or motif enrichment in cis-regulatory elements of the target gene. The key below explains the colour code.

Pharmacological or genetic perturbation of the FLT3-ITD transcription factor network demonstrate mechanisms by which *WT1* may be (indirectly) inhibited

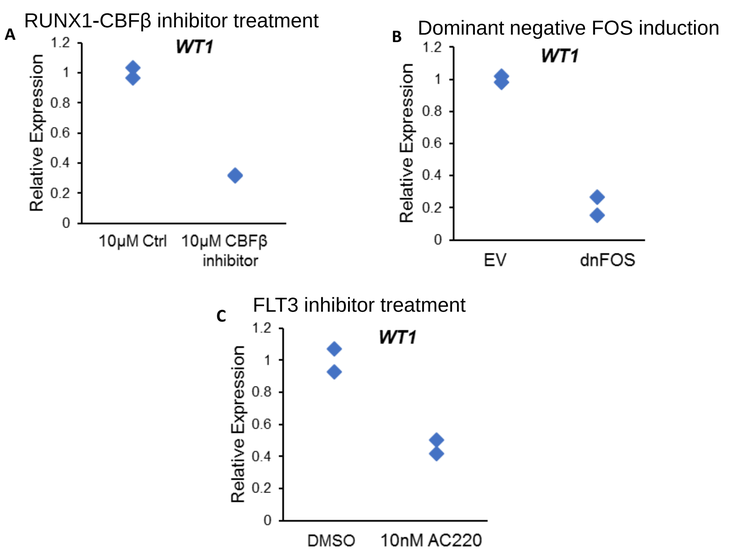

Figure 7: Dot plots of relative gene expression as determined by RNA-seq of *WT1* in primary FLT3-ITD cells with (A) treatment with DMSO or the FLT3-ITD inhibitor Quizartinib (AC220), (B) treatment with a control compound or the RUNX1-CBFβ inhibitor (Illendula et al., 2016), (C) transduction with doxycycline-inducible Empty Vector or Dominant Negative FOS (dnFOS) (Olive et al., 1997).

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