Molecular mechanism of *CRLF2* overexpression in acute lymphoblastic leukaemia

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BACKGROUND

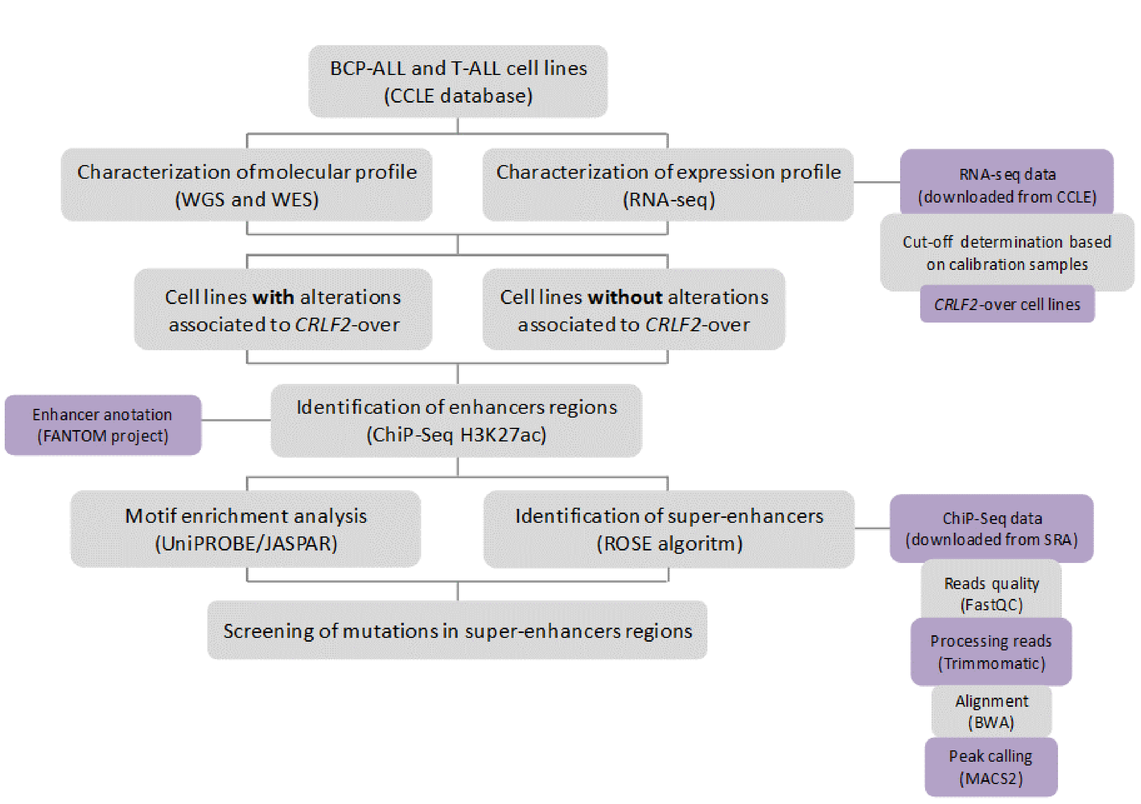
CRLF2 overexpression (*CRLF2*-over) has been associated with unfavourable prognosis in acute lymphoblastic leukaemia (ALL). In B-cell precursor ALL (BCP-ALL), the presence of *CRLF2* rearrangements, copy number gain of *CRLF2* and *CRLF2* mutations can explain this overexpression. Nonetheless, the mechanism is still unknown in ~50% of the remaining BCP-ALL cases. With respect to T-cell ALL (T-ALL), there is no evidence of somatic alterations accounting for *CRLF2*-over.

Recent discoveries have shown that somatically acquired mutations in non-coding regulatory regions can create new transcription factor binding sites, i.e. neomorphic super-enhancers, leading to an aberrant overexpression of critical oncogenes in human cancers. In this scenario, we hypothesise that the occurrence of these neomorphic enhancer mutations might be the mechanism behind oncogene overexpression in patients who lack other somatic abnormalities.

AIM

To identify the mechanism responsible for *CRLF2*-over in ALL by screening cases for the occurrence of super-enhancers.

METHODS AND RESULTS



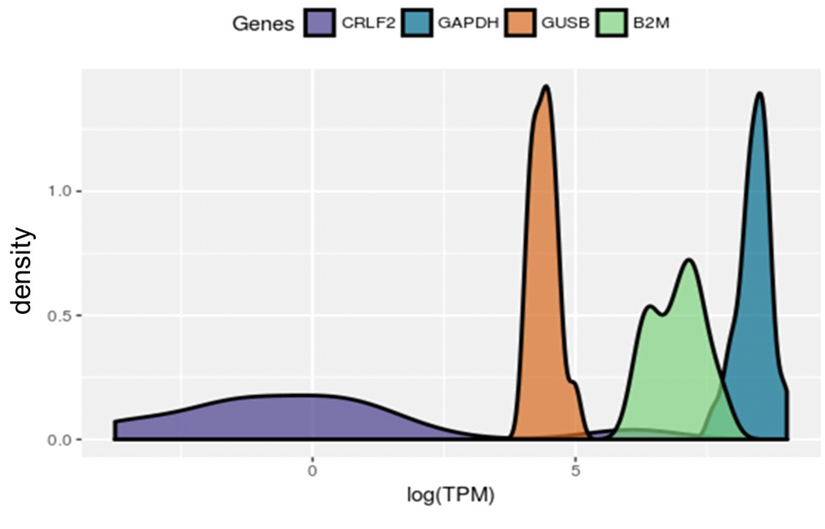


Figure 1. Distribution of *CRLF2, B2M, GAPDH* and *GUSB* expression in BCP-ALL and T-ALL cell lines.
B2M, GAPDH and *GUSB* expression showed a normal distribution while *CRLF2* expression had a more dispersed and non-normal distribution.

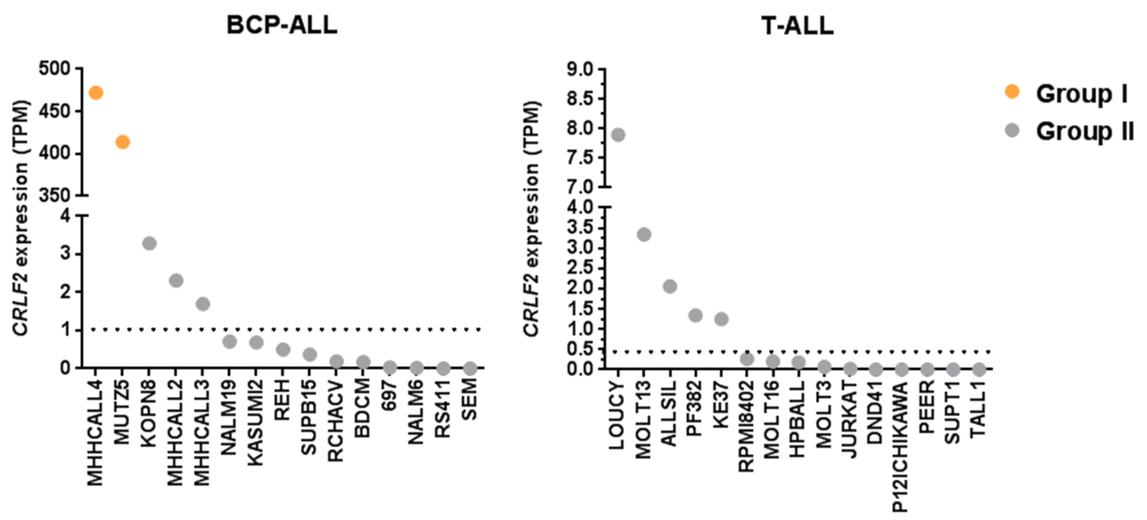


Figure 2. *CRLF2* expression in BCP-ALL and T-ALL cell lines according to the presence or absence of alterations that could explain *CRLF2*-over.
Group I: cell lines with alterations that could explain *CRLF2*-over; Group II: cell lines without alterations that could explain *CRLF2*-over. Ten of thirty cell lines presented *CRLF2*-over: MHH-CALL-4, MUTZ5, KOPN8, MHH-CALL-2 and MHH-CALL-3 (BCP-ALL); and LOUCY, MOLT13, ALL-SIL, PF-382 and KE37 (T-ALL). Of these, only MHH-CALL-4 and MUTZ5 harbour an alteration associated to *CRLF2*-over (*IGH-CRLF2)*.

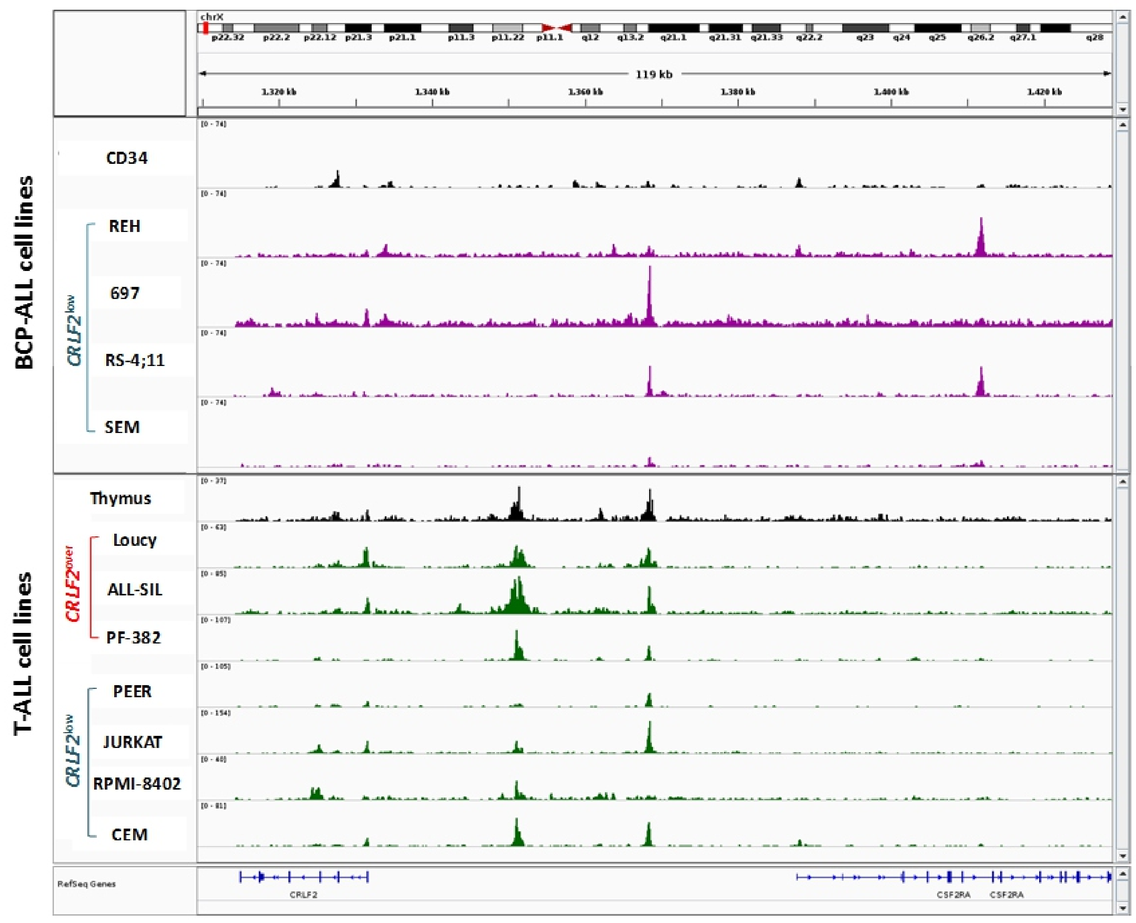


Figure 3. ChiP-Seq analyses of cell lines based on H3K27ac marks.
ChiP-Seq data showed that LOUCY, ALL-SIL, PF-382, REH and 697 presented aberrant H3K27ac marks located ±2kb upstream of the *CRLF2* transcription start site (TSS).

CONCLUSIONS

Our results have so far shown that LOUCY, ALL-SIL and PF-382 are potential carriers of neomorphic super-enhancers, but these evidences need to be further explored. These partial results are part of an ongoing investigation, therefore due to the preliminary nature of the current data we are still unable to draw any definitive conclusions.

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MINISTÉRIO DA SAÚDE