The role of the Chemokine-like MARVEL trans membrane-type proteins CMTM6-8 in survival of B cell precursor Acute Lymphoblastic Leukaemia

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Aims

1. To write a critical review of the current literature concerning the CMTM6-8 gene cluster and formulate a testable research hypothesis;
2. To analyse Single Nucleotide Polymorphism (SNP) data available at the leukaemia research cytogenetic group (LRCG) for presence of focal deletions in CMTM6-8 and BLNK genes;
3. To analyse relevant gene expression data available from the internet database Gene Expression Omnibus (GEO);
4. To discuss whether the CMTM6-8 genes and associated downstream signalling pathways have potential as therapeutic targets in B cell precursor acute lymphoblastic leukaemia (BCP-ALL).

Hypothesis

- Loss of CMTM expression may provide a survival advantage for leukemic cells in patients or in a hostile xeno-environment.

Introduction

ALL is the most common cancer in children, accounting for around 25% of all childhood cancers in those less than 15 years of age [1]. This type of cancer can be rapidly fatal: early diagnosis and treatment is therefore essential.

Although up to 90% of children with ALL can now be cured, a significant number of patients do not respond to treatment and ultimately relapse with poor prognosis [2]. 25% of precursor B-ALL cases are genetically unclassified and have intermediate prognosis [3].

Developing novel therapeutics as well as identifying genetic markers of relapse is essential for improving treatment outcome in BCP-ALL.

Preliminary data shows potential use for CMTM7 and -8 as novel therapeutic targets and indicators of early relapse in BCP-ALL.

Background

B-Cell Precursor Acute Lymphoblastic Leukaemia

Leukemic cells in children with ALL are mostly pre-B cells, the precursor to the B cell [4]. The pre-B cell receptor (pre-BCR) is similarly the precursor to the B-cell receptor (BCR) and has a vital role in regulating B cell differentiation.

The pre-BCR checkpoint is known to regulate cell survival and proliferation in B cell ALL, with BCP-ALL usually being a consequence of pro-B or pre-B cells arrested in their development. Mutations affecting the pre-BCR checkpoint are known to cause pre-B cell ALL development [5, 6].

Acquired chromosomal and other genetic abnormalities are common in ALL [2]. Focal deletions and point mutations of crucial genes have also been observed, including those involved in lymphocyte development and signalling, transcription regulation, and tumour suppression [7].

The Human Chemokine-like factor -like MARVEL Trans Membrane (CMTM) Family

The CMTM6-8 genes are a gene cluster located on chromosome 3p22. CMTM8 is known to be involved in controlling EGF-R signalling whereas CMTM7 associates with the pre-B cell receptor and the signalling molecule BLNK (SLP-65) [8, 9].

Methods

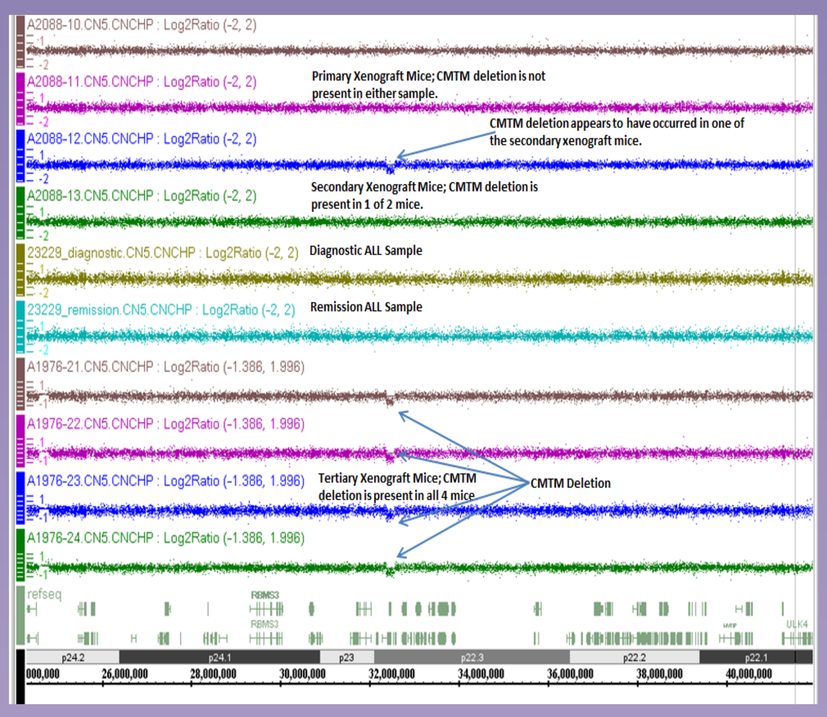
- Information for my literature review was obtained from research articles published on the PubMed website and referenced using Endnote.
- Affymetrix SNP6.0 array data, analysed using the bioinformatics software Genotyping Console, was used to look for the presence of focal deletions in the BLNK and CMTM6-8 genes across patient data sets and xenografts, available from the LRCG in Newcastle and St Judes Hospital, Memphis [10].
- Gene expression data from two ALL data sets were analysed using the online microarray platform r2. One data set, published by *Carroll et al.* [11], consisted of bone marrow samples obtained from 98 pre-B cell ALL patients; whereas another, published by *Murphy et al.* [12], contained 207 bone marrow samples from both B and T ALL patients.
- Microarray expression data uploaded from published papers onto GEO were also investigated [13, 14]. Relevant data was analysed and significance calculated using a paired T-test ($p<0.05$). Relapse patients were categorised into two groups: early relapse (defined as <36 weeks) and late relapse (defined as >36 weeks).

Results

SNP Data:

At the LRCG, we identified CMTM6-8 focal deletions in mouse xenografts of a case of BCP-ALL with intrachromosomal amplification of chromosome 21 (iAMP21), as shown in figure 1.

Figure 1: SNP Data from the LRCG, Newcastle. CMTM6-8 focal deletion is present in 1 of 2 secondary xenografts as well as all 4 tertiary xenografts. From this data the CMTM6-8 deletion does not appear to be present in the primary xenografts, diagnostic or remission samples.



The CMTM6-8 gene region was found to be deleted in a primary ALL (cytogenetically normal subgroup) patient sample (n=107), as shown in figure 2.

Figure 2: SNP Data from a Cytogenetically Normal pre-B ALL Patient referred to Newcastle. An 11 year old male was found to have a focal deletion of the genes CMTM7 and -8.

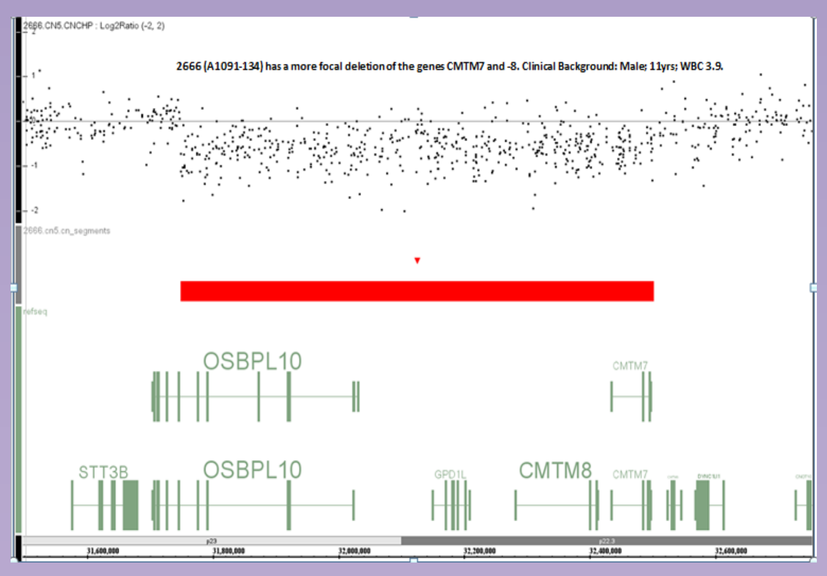
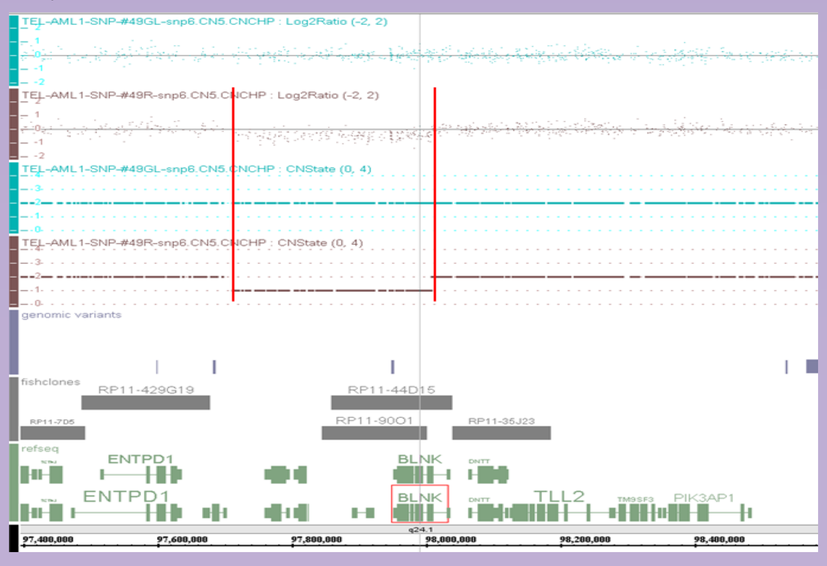


Figure 3: SNP Data from BCP-ALL Patients referred to St Judes, Memphis USA [10]. SNP data from St Judes hospital (n=242) included a patient that carried the TEL-AML1 translocation and a focal deletion of the BLNK gene located on chromosome 10q24.



Expression Microarray Data:

Figure 4: Expression Data for BLNK and CMTM7 and -8 Genes [14]. Expression data from normal cells during B cell development showed that BLNK and CMTM7 and -8 had up regulated gene expression during their transition from common lymphoid progenitors to pre-BI cells. Key: S1 – Lymphoid progenitors; S2 - Pre-B-I cells; S3 - Pre-B-II cells; S4 - Immature B cells.

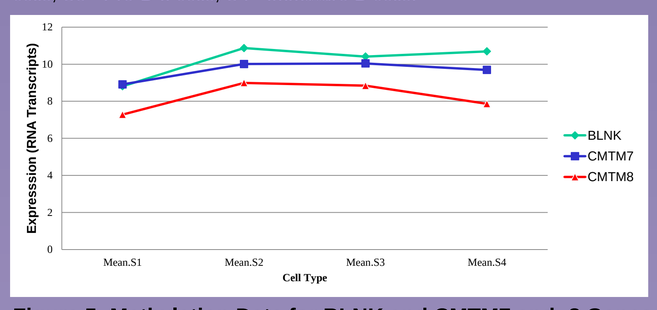


Figure 5: Methylation Data for BLNK and CMTM7 and -8 Genes [14]. Average methylation beta values ranging from 0 (unmethylated) to 1 (fully methylated). Increased BLNK and CMTM7 & -8 gene expression correlated with demethylation of the promoter regions of these genes. Key: S1 – Lymphoid progenitors; S2 - Pre-B-I cells; S3 - Pre-B-II cells; S4 - Immature B cells.

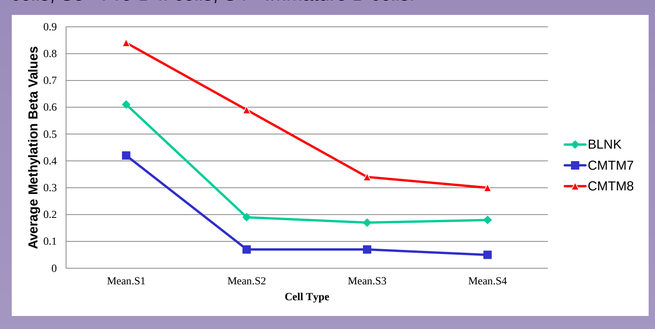


Figure 6: CMTM7 and -8 Gene Expression (log values) across Patient Samples from the Carroll Data Set [11]. CMTM7 and -8 expression largely vary across patient samples.

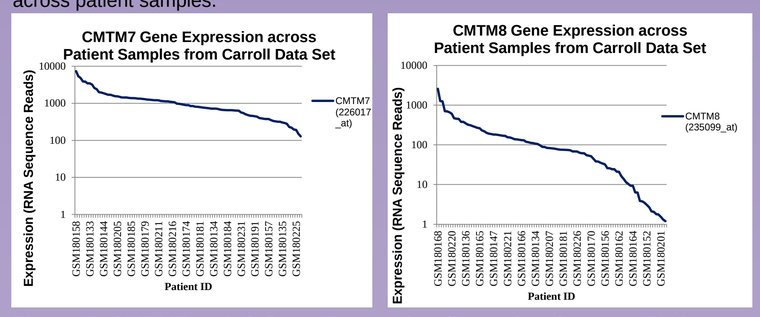


Figure 7: CMTM7 and -8 Gene Expression (log values) across Patient Samples from the Murphy Data Set [12]. CMTM7 and -8 expression largely vary across patient samples.

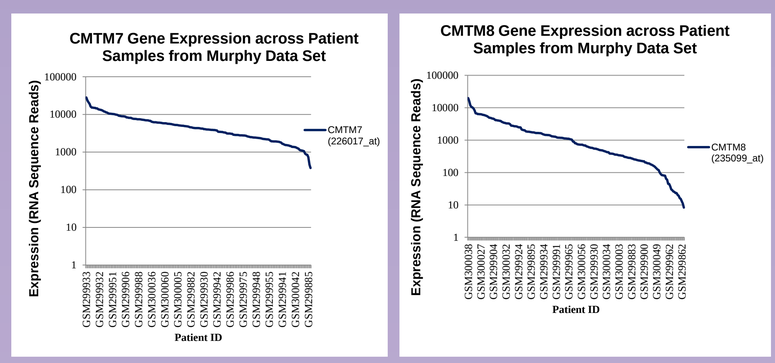


Figure 8: CMTM8 expression compared at diagnosis and early relapse in matched patient samples [13]. A paired T-test for these data showed that there was a significant difference (0.00622) between CMTM8 expression at diagnosis and early relapse in matched samples.

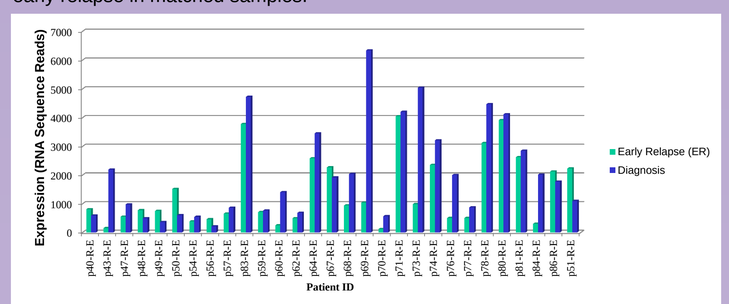
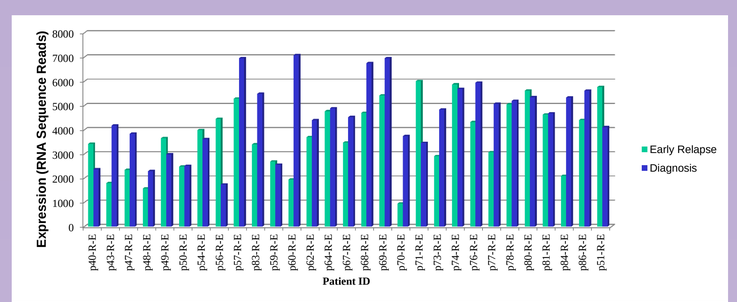


Figure 9: CMTM7 Expression compared at Diagnosis and Early Relapse in Matched Patient Samples [13]. A paired T-test for these data showed that there was a significant difference (0.0108) between CMTM7 expression at diagnosis and early relapse in matched patient samples.



Discussion

Evidence from SNP data suggests that the CMTM6-8 focal deletion was not initially present but instead arose in one of the secondary xenografts. However, I am unable to unequivocally determine at what point the CMTM focal deletion occurred. Interestingly, I found a cytogenetically normal patient with a very similar focal deletion of CMTM7 and -8 as well as a TEL-AML patient with a BLNK focal deletion.

Methylation and expression data in normal B cells suggests that BLNK and CMTM7 and -8 have important roles during B cell development. Data analysed from the Murphy and Carroll cohorts suggest that CMTM7 and -8 have a wide gene expression range.

From the current literature, it seems that CMTM7 and -8 have crucial roles in B cell development and function. CMTM7 may have a role as a trans membrane linker protein that interacts with the pre-BCR and its intracellular signalling machinery as well as exerting a tumour-suppressor function. CMTM8 might interact with the EGFR and also have an apoptotic function. These results agree with the data for normal B cell development. Overexpression of these genes may have potential roles as novel therapeutics.

CMTM7 and -8 were found to have significantly different expression profiles from diagnosis to early relapse (0.0108 and 0.00622, respectively). CMTM7 and-8 appears to have decreased expression at early relapse when compared to corresponding values at diagnosis, although not all patients showed this expression pattern. No significant difference was found between diagnosis and late relapse for CMTM7 (0.336) or CMTM8 (0.476). Interestingly, the prognosis for early relapse is worse that it is for late relapse patients [13]. Low CMTM7 and -8 expression may be possible markers of early relapse in certain subgroups of BCP-ALL patients. Multivariate analysis, for example by analysing white cell counts, would be necessary to conclude this however.

Overall, these results agree with my initial hypothesis that loss of CMTM6-8 expression may provide a survival advantage in ALL cells. Further research into the precise function of the membrane-localised CMTM6-8 will help to elucidate their involvement in BCP-ALL, their therapeutic value, and their significance in early relapse.

Conclusions

- Loss of CMTM6-8 expression may provide a survival advantage for leukemic cells in humans or a hostile xeno-environment.
- Low CMTM7 and -8 expression may be possible markers of early relapse in certain subgroups of BCP-ALL patients, although multivariate analysis is required to prove this.
- Preliminary data shows potential for CMTM7 and -8 to be used as novel therapeutic targets.

Acknowledgements

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