OPEN

Citation: Blood Cancer Journal (2014) **4**, e224; doi:10.1038/bcj.2014.44

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LETTER TO THE EDITOR

FAT1 expression and mutations in adult acute lymphoblastic leukemia

Blood Cancer Journal (2014) **4,** e224; doi:10.1038/bcj.2014.44; published online 27 June 2014

The cadherin gene *FAT1*, located on chromosome 4q34-35 (ref. 1) within a region frequently deleted in human cancers,[2] encodes a large protein with 34 extracellular cadherin repeats.[3] In solid tumors, aberrant expression of *FAT1* was found to be associated with disease progression.[4] Although the gene was originally cloned from a human T-cell acute lymphoblastic leukemia (T-ALL) cell line,[4] *FAT1* just recently gained interest owing to its altered gene expression levels and the detection of somatic mutations identified by next-generation sequencing (NGS) in acute leukemia.[2,5–7] *FAT1* was shown to be aberrantly expressed in pediatric patients with acute leukemia, whereas hematopoietic progenitors from healthy donors lacked *FAT1* expression.[5,8] In addition, a recent report correlated high *FAT1* expression with a higher probability of relapse in a small cohort of pediatric patients with B-cell precursor acute lymphoblastic leukemia (BCP-ALL) based on an *in silico* analysis comprising two BCP-ALL data sets including 32 and 27 patients.[5]

With the emergence of NGS, it has become obvious that *FAT1* is not only aberrantly expressed in various tumors, but also frequently mutated in solid tumors.[7,9] Morris *et al.*[2] were able to link the mutational inactivation of *FAT1* to the loss of its tumor suppressor capacity and the activation of the WNT pathway. In summary, these data make *FAT1* an interesting candidate for disease monitoring, risk stratification and the development of targeted therapies. Herein, we investigated *FAT1* expression in a large, homogenously treated cohort of adult acute leukemia patients, and explored the mutation status of *FAT1* and its clinical significance.

We analyzed *FAT1* expression by real-time PCR in different cell populations of healthy donors, various leukemia cell lines, a small cohort of acute myeloid leukemia (AML; $n = 13$), in 112 adult T-ALL samples and in 129 adult BCP-ALL samples (Supplementary Methods). We examined the clinical and molecular characteristics with respect to *FAT1* expression in this large cohort of adult ALL patients using specimens sent to the reference laboratory of the German Study Group for adult ALL (GMALL; $n = 231$). Of these, 180 patients were enrolled into the trials GMALL 06/99 and 07/03 with available clinical follow-up. The treatment strategy of the GMALL trials has been described previously (Supplementary Methods). We were able to confirm the reported expression pattern for *FAT1* in different cell lines (Supplementary Figure S1).[5] The cell line BE13 showed the lowest, nearly absent, expression of *FAT1* and was used as a cutoff to define samples with a high expression (*FAT1*pos) compared with a lower/absent expression (*FAT1*neg). We also investigated the expression of *FAT1* in different cell populations from healthy donors. Unselected bone marrow (BM), $CD34^+$ progenitors, peripheral blood and $CD3^+$ T cells from healthy donors lacked *FAT1* expression (Supplementary Figure S2), whereas *FAT1* expression was highly expressed in BM-derived mesenchymal stromal cells (BMSC) from healthy donors (Supplementary Figure S2). In contrast, *FAT1* was aberrantly expressed in adult leukemia: 23% of AML and 32% of BCP-ALL patients expressed *FAT1* and were defined as *FAT1*pos. The highest percentage of *FAT1*pos patients was found within the T-ALL cohort (54%, Supplementary Figure S2).

FAT1 expression was correlated with a more mature leukemic immunophenotype. In BCP-ALL, patients with a preB-ALL or a common ALL immunophenotype were in 57% and 26% classified as *FAT1*pos compared with only 9% of pro-B-ALL patients (see Supplementary Table S2). In T-ALL, a genotype–phenotype association was even more striking: 74% of patients with thymic T-ALL were *FAT1*pos compared with 45% of patients with mature T-ALL and only 4% of early T-ALL patients (see Supplementary Table S1). In accordance with the predominance of *FAT1* expression in more mature T-ALL, *FAT1*pos patients had a higher rate of clonal T-cell receptor rearrangement and a lower expression of the stem cell-associated genes *IGFBP7*, *BAALC* and *MN1* (Supplementary Table S1). Likewise, *FAT1*pos T-ALL patients showed higher white blood cell counts (WBC; $\geq 30\,000/\mu l$ at diagnosis) compared with *FAT1*neg T-ALL patients (78% vs 42%, $P < 0.01$, Supplementary Table S1). No significant differences were observed between the *FAT1*pos versus *FAT1*neg groups in age or sex among T-ALL patients.

Regarding response to a standard induction therapy, we found no differences between *FAT1*pos and *FAT1*neg BCP-ALL patients (Supplementary Table S2). In T-ALL, *FAT1*neg patients failed to achieve a complete remission more frequently (3/25) after induction therapy compared with *FAT1*pos patients (0/50, $P = 0.04$, Supplementary Table S1). In contrast to the *in silico* data of pediatric patients,[5] we found no differences in BCP-ALL or T-ALL between *FAT1*pos and *FAT1*neg patients regarding overall survival and remission duration (Figure 1). However, in the prognostic favorable subgroup of thymic T-ALL, we observed an inferior overall survival for *FAT1*pos patients, although not statistically significant.

On the basis of the high frequency of *FAT1* expression in T-ALL and recurrent *FAT1* mutations in early T-cell precursor (ETP)-ALL,[7] we examined 68 T-ALL samples for the presence of *FAT1* mutations by target enrichment and NGS (Supplementary Methods). Interestingly, *FAT1* mutations were detectable in a considerable number of adult T-ALL patients (8/68, 12%, Supplementary Table S3). One patient carried two point mutations within *FAT1*. All mutations were missense mutations, one leading to a frameshift and another encoding a stop codon (Supplementary Table S3). Mutations were predominantly located within the cadherin domains (Figure 2). T-ALL patients with *FAT1* mutations (*FAT1*mut) did not differ from T-ALL patients carrying a wild-type *FAT1* (*FAT1*wt) regarding sex, age, WBC and expression of specific cell surface antigens associated with an early differentiation stage. *FAT1* mutations were present in early T-ALL (3/12, 25%) and in thymic T-ALL (5/41, 12%), but absent in T-ALL with a mature immunophenotype (0/15, NS, Supplementary Table S4). Expression of *FAT1*, was more common in *FAT1*wt T-ALL patients than in *FAT1*mut patients (55% vs 25%, $P = 0.15$). No differences were observed in overall survival and remission duration between *FAT1*mut and *FAT1*wt patients (Supplementary Figure S3).

Although there are increasing data on the genetic characterization of ALL, only few molecular markers have been integrated into risk stratification for individualized therapies. The postulated correlation of high *FAT1* expression with inferior outcome in

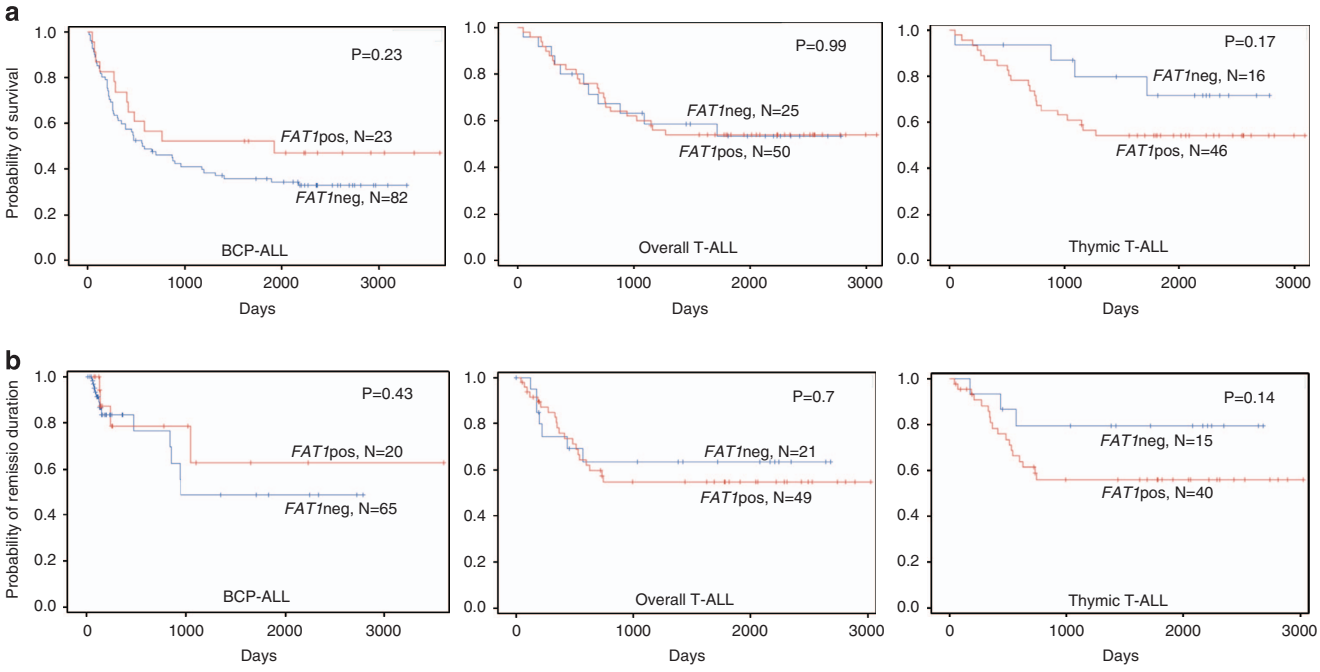

Figure 1. Overall survival (**a**) and duration of remission (**b**) for patients with BCP-ALL, overall T-ALL and the standard-risk subgroup of thymic T-ALL enrolled into GMALL trials.

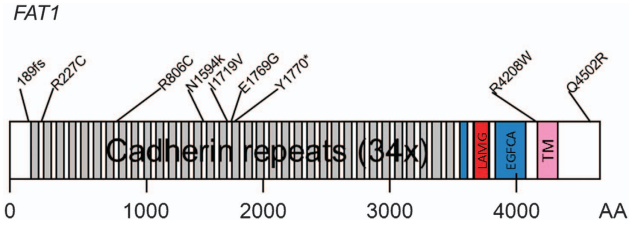

Figure 2. Protein domain plot of FAT1 with mutations ($n = 9$) found in 8 of 68 T-ALL patients. One patient carried two mutations. Changes are annotated in Supplementary Table S3.

pediatric BCP-ALL[5] could not be confirmed in our cohort of adult BCP-ALL. The most obvious reasons for these conflicting results might be different therapeutic approaches and large age differences between pediatric and adult patients as shown for other prognostic markers.[10] Also limitations of *in silico* analyses of cohorts including low number of patients might at least in part explain these conflicting findings. Although *FAT1* might not have a prognostic value, its expression and mutation profile make it an interesting candidate for minimal residual disease monitoring, the development of targeted therapies, and improved understanding of leukemogenesis in different ALL subgroups.

In addition to its potential role in leukemogenesis, it is tempting to speculate about the role of *FAT1* in the interaction of leukemic cells with the microenvironment. It is known that, *FAT1* is associated with cell migration, polarity and cell–cell adhesion and direct interaction with β-catenin.[2,4,11] As we found a high *FAT1* expression in BMSC, *FAT1* might have a role in the stabilization of the interaction of leukemic cells with the bone marrow niche and/or thymic homing. This might also explain the significantly higher expression of *FAT1* in the more differentiated subgroups of T-ALL and BCP-ALL. On the other hand, inactivating mutations of *FAT1* in different human cancers have been linked to the inability to bind β-catenin and deregulated activation of the WNT pathway.[2] These mechanisms might have a role in solid cancer leading to higher treatment sensitivity and evasion of tumor metastasis.[2,12] Interestingly, in gingiva-buccal oral squamous cell cancer, *FAT1* mutations occur in addition to mutations in *NOTCH1* and *MLL2*, a spectrum very similar to the one observed in ETP-ALL.[7,12] Deregulation of the WNT pathway has been linked to leukemogenesis in T-ALL.[13,14] The previously unreported *FAT1* mutation rate of 15% in adult T-ALL stresses the importance of the WNT pathway in T-ALL.

In conclusion, we explored the pattern of *FAT1* expression and its mutation status in a large, homogenously treated cohort of adult ALL patients. Our analysis revealed an aberrant expression predominantly in mature BCP-ALL and thymic T-ALL and a high rate of *FAT1* mutations in T-ALL. Further studies should explore a link to WNT pathway activation and potential therapeutic implications.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We thank Liliana H Mochmann for critical reading of the manuscript. This work was supported by grants from the Deutsche Krebshilfe (Mildred Scheel Professur) and Gutermuth-Stiftung to CDB, Deutsche Krebshilfe grant 70-2657-Ho2 to DH and partly BMBF 01GI 9971 to DH and NG, and Deutsche Krebshilfe grant 109031 to PAG.

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Supplementary Information accompanies this paper on Blood Cancer Journal website (http://www.nature.com/bcj)