CD27 expression and its association with clinical outcome in children and adults with pro-B acute lymphoblastic leukemia

B-cell precursor acute lymphoblastic leukemia (BCP-ALL) is the most common type of cancer in children, representing up to 80% of pediatric, and ~20% of adult leukemias.1 BCP-ALL is divided into several genetic subtypes according to acquired chromosomal aberrations with varying prognosis.2 In children and young adults, BCP-ALL patients have an overall survival (OS) at 5 years around 90%. In contrast, OS in adults is much lower (30–40%), in part due to a higher frequency of ALL subtypes with poor prognosis.3,4 Hence, identifying new biomarkers for patients with poor prognosis is important as it might allow the development of new approaches to treat high-risk BCP-ALL.

CD27 is a member of the tumor necrosis factor receptor superfamily that regulates lymphocyte function.5 Expression of CD27 protein and mRNA has been reported in B-cell leukemia6 and adult T-cell leukemia/lymphoma.7 In acute myeloid leukemia CD27 has been shown to be a prognostic biomarker.8 We previously described a pro-B-cell molecular signature enriched in the ETV6-RUNX1 subtype, and found that one of these genes is CD27.9 Consistent with this, previous studies have shown high mRNA and surface levels of CD27 in ETV6-RUNX1 B-CP ALL.10,11 However, expression of CD27 in BCP-ALL of other subtypes and its potential clinical relevance is still unclear. Here, we determine the protein and mRNA expression pattern of CD27 during early B-cell development and in BCP-ALL, and investigate the prognostic relevance of CD27 mRNA expression in pediatric and adult patients with BCP-ALL.

To confirm and extend previous observations,10–12 we first analyzed CD27 expression during bone marrow (BM) B-cell development using flow cytometry (gating strategy, Supplementary Figure S1a). Pro-B cells (CD19+CD34−IgM−) from all five donors expressed CD27, whereas common lymphoid progenitor (CLP, CD19+CD34+IgM−), pre-B (CD19+CD34+IgM+), and immature B (CD19+CD34−IgM+ ) cells did not (Figures 1a and b, Supplementary Figure S1b). We also noted that only a fraction, on average 25%, of the pro-B cells were positive (Figure 1b). Thereafter, we analyzed six patient samples, of which two expressed CD27 at the time of diagnosis (Figure 1c and Supplementary Figure S1c). The CD27 levels in these two cases, classified as B-other, were much higher than those in the healthy pro-B cells, with the vast majority of the CD19+ leukemic cells being strongly positive. Consistent with the CD27 protein expression pattern, we found that CD27 mRNA levels were high in pro-B cells as compared to CLP, pre-B and immature B cells (Figure 1d). To determine which BCP-ALL subtypes express CD27, we analyzed its mRNA levels in public data sets (Supplementary Table S1). In pediatric leukemia samples, for example, data set GSE26281, CD27 was as expected highly expressed in over 90% of ETV6-RUNX1, compared to the mean level of the total samples (M0) in each data set (Figure 1f). Thus, CD27 is not only highly expressed in the ETV6-RUNX1, but also in BCR-ABL1, CRLF2-rearranged and B-other BCP-ALL. The CRLF2-rearranged subtype was only recently defined13,14 and, therefore, these patient samples are found within B-other in most public data sets. To determine the CRLF2-rearranged subtype in data sets where this has not been defined, the CRLF2 expression levels in already defined CRLF2-rearranged samples (Supplementary Table S1, GSE26281 and GSE11877) were analyzed. We found that the expression levels of CRLF2 in all CRLF2-rearranged samples were at least 10-fold higher than the median levels of total samples (Supplementary Figure S2a). Based on this, samples expressing 10-fold higher level of CRLF2 than the median are referred to as CRLF2-high in other data sets (Supplementary Figure S2b) with the assumption that most of these are CRLF2-rearranged. Thereafter, we queried which subtypes were enriched in samples with high CD27 expression levels, by performing meta-analyses based on eight data sets with over 1500 pediatric patient samples in total and two data sets with over 250 adult patient samples (Supplementary Table S1). Samples in each data set were first divided into four clusters (CD27++, CD27+, CD27− and CD27−) according to CD27 mRNA levels (Supplementary Figure S2c) and thereafter pooled. Subtype distribution analysis in pediatric BCP-ALL showed that ETV6-RUNX1, BCR-ABL1 and CRLF2-rearranged/high were proportionally enriched in the CD27++ and CD27− clusters, whereas the opposite was observed for, for example, KMT2A-rearranged (Figure 1g). Also in adult samples, the BCR-ABL1 subtype was enriched in the CD27++ and CD27− clusters, and the opposite was observed for, for example, KMT2A-rearranged (Figure 1h). Thus, pediatric and adult BCP-ALL showed similar expression patterns of CD27.

Because CD27 mRNA is highly expressed in the ETV6-RUNX1 subtype that display a pro-B-cell molecular signature,9 we hypothesized that BCP-ALL samples with high CD27 mRNA levels would also display a pro-B signature (Figure 1i). To test this hypothesis, we performed gene set enrichment analyses (GSEA) in leukemia data sets after excluding the ETV6-RUNX1 subtype. Independent of genetic subtype, pediatric BCP-ALL expressing high CD27 mRNA levels (CD27++ ) showed a molecular signature similar to pro-B cells (Figure 1j) and Supplementary Figure S3). However, we were unable to find a pro-B signature in adult BCP-ALL, which indicates that the molecular signature is different in children and adults.

Since CD27 mRNA was highly expressed in BCR-ABL1 and CRLF2-rearranged/high BCP-ALL that are associated with poor prognosis, we asked whether CD27 has prognostic value. To determine this, we first analyzed clinical data available from 207 high-risk pediatric BCP-ALL patients (Supplementary Table S1, GSE11877). Dividing the patient samples into four clusters according to CD27 expression levels, we found that the CD27++ cluster was associated with poor OS compared with the other clusters (Figure 2a). Moreover, approximately 60% of patients in the CD27++ cluster had relapse compared to 25–30% in the remaining clusters (Figure 2b). Thus, CD27 expression levels correlate with clinical outcome in this cohort with high-risk pediatric BCP-ALL patients. To further confirm this observation, we analyzed the clinical data available from an additional pediatric
cohort including 75 patients (Supplementary Table S1, GSE47051). Because the ETv6–RUNX1 subtype is associated with good prognosis in pediatric BCP-ALL, we excluded this subtype in the analyses. We did not detect a significant difference in OS between the clusters (Figure 2c), whereas a significantly lower OS was observed in the combined CD27++ compared to the CD27−/−.

**Figure 1.** Comparison of CD27 expression and molecular signature between pro-B cells and BCP-ALL. (a) Representative histogram shows surface CD27 expression in pro-B cells. (b) Scatter plot shows percentages of CD27+ cells in indicated subsets of BM samples from five healthy donors. (c) Representative histograms show CD27 surface expression in two BCP-ALL samples (CD27+ and CD27−). (d, e, f) Heat maps and scatter plots show CD27 mRNA expression levels in (d) healthy (GSE45460) BM; (e) pediatric (GSE26281) and (f) adult (GSE34861) BCP-ALL samples. (g and h) Pie charts show the proportions of BCP-ALL subtypes within each CD27 cluster based on meta-analyses of (g) eight pediatric data sets (GSE26281, GSE33315, GSE13576, GSE13425, GSE12995, Blood 2003, GSE11877, GSE47051) and (h) two adult data sets (GSE34861 and CCR 2005), after classifying BCP-ALL samples in each data set into four groups according to CD27 expression levels: CD27++ (> M0), CD27+ (< M0 to > M1), CD27− (< M1 to > M−1), CD27− (< M−1). Numbers in the center of pie charts represent number of patient samples, and those in segments the proportions of the indicated subtype. M0: mean expression of CD27 in all samples; M1: mean expression of CD27 in samples with levels above M0; M−1: mean expression of CD27 in samples with levels below M0. (i) Heat map shows genes highly expressed in pro-B cells (pro-B signature). Genes are selected according to the criteria: P-value (< 0.05), q-value (< 0.1) and fold change (> 1.5). (j) Heat map (left) and GSEA enrichment plots (right) reveals a pro-B molecular signature in CD27++ high-risk pediatric BCP-ALL data set (GSE11877). CLP, common lymphoid progenitor; IB, immature B; CRLF2-rearranged (previously defined) and CRLF2-high (defined herein) were pooled. Statistical analysis: (b and d) one-way ANOVA and (g and h) χ² analysis: **P < 0.01; ***P < 0.001; ****P < 0.0001.
**Figure 1.** Continued.

**Figure 2.** Clinical relevance of CD27 mRNA levels in patients with BCP-ALL. (a, c, e, f) Kaplan–Meier Log-rank survival analysis was used to compare survival of patients within the indicated CD27 clusters. (b, d, g) Percentages of patients with relapse within indicated CD27 clusters using Fisher’s exact test. (a, b) High-risk pediatric cohort GSE118877, (c, d) pediatric cohort GSE47051 (excluding ETV6-RUNX1); CD27++/+, CD27++ and CD27++; CD27++, CD27+ and CD27++, (e) adult cohort GSE34861 and (f, g) B-other patients within high-risk pediatric cohort GSE118877.
CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This work has been supported by grants from Barnancancerfonden (TJD2014-0083, TJD2016-0007, PR2016-0144), Cancerfonden (CAN2014/886, CAN2016/0668), AG Fond (FB 15-57, FB 16-15), Lions Cancerfond (2014-2015), ALF, IngaBritt och Arne Lundbergs Forsknings Stiftelse, Stiftelsen Wilhelm och Martina Lundgrens Vetenskap, Adlerbartska forskningsstiftelsena, KVS.

REFERENCES

6 Dong HY, Shahsafi A, Dorfman DM. CD148 and CD27 are expressed in B cell lymphomas derived from both memory and naive B cells. Leuk Lymphoma 2002; 43: 1855–1858.

© The Author(s) 2017

This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivs 4.0 International License. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in the credit line; if the material is not included under the Creative Commons license, users will need to obtain permission from the licensor holder to reproduce the material. To view a copy of this license, visit http://creativecommons.org/licenses/by-nc-nd/4.0/

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