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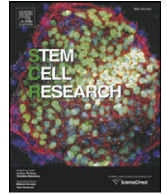

Short report

GABP is necessary for stem/progenitor cell maintenance and myeloid differentiation in human hematopoiesis and chronic myeloid leukemia

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ABSTRACT

Maintenance of hematopoietic stem cells and their potential to give rise to progenitors of differentiated lymphoid and myeloid cells are accomplished by a network of regulatory processes. As a part of this network, the heteromeric transcription factor GA-binding protein (GABP) plays a crucial role in self-renewal of murine hematopoietic and leukemic stem cells. Here, we report the consequences of functional impairment of GABP in human hematopoietic and in leukemic stem/progenitor cells. Ectopic overexpression of a dominant-negative acting GABP mutant led to impaired myeloid differentiation of CD34$^+$ hematopoietic stem/progenitor cells obtained from healthy donors. Moreover, drastically reduced clonogenic capacity of leukemic stem/progenitor cells isolated from bone marrow aspirates of chronic myeloid leukemia (CML) patients underlines the importance of GABP on stem/progenitor cell maintenance and confirms the relevance of GABP for human myelopoiesis in healthy and diseased states.

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1. Introduction

Transcriptional regulation is of particular relevance for self-renewal of hematopoietic stem cells and accurate hematopoietic differentiation. The potential to give rise to mature hematopoietic cells as well as to preserve stem cell properties is regulated by a set of transcription factors, of which only few are well-characterized (Orkin and Zon, 2008). The ETS transcription factor GA-binding protein (GABP) was recently shown to play a crucial role for myeloid differentiation in mice and humans (Yang et al., 2011; Yu et al., 2011; Ripperger et al., 2015). Moreover, GABP was reported to directly impact propagation of leukemic clones in mice transplanted with BCR-ABL1$^+$ murine leukemic stem cells, which resemble human chronic myeloid leukemia (CML) (Yu et al., 2012; Yang et al., 2013). Our recent work supported these observations by showing that GABP affects viability and imatinib sensitivity in human CML cell lines (Manukjan et al., 2015).

Here, we focus on the effects of GABP in human normal and leukemic hematopoietic stem/progenitor cells and demonstrate that GABP is required for proper myeloid differentiation in human primary cells. GABP functions as an obligate heterodimer, in which the alpha-subunit (GABPα) binds targeted DNA-motifs and the beta-subunit (GABPβ1) contributes to transcriptional regulation via its transactivation capacity (Rosmarin et al., 2004). Hence, ectopic overexpression of GABPβ1 lacking the transcriptional activation domain (TAD) results in a dominant-negative GABP, as we show here and have already demonstrated in the preliminary work (Manukjan et al., 2015). In this context, we further show that impaired GABP decreases leukemic stem/progenitor cell capacity of human CD34$^+$ cells derived from CML patients.

2. Material & methods

Mobilized CD34$^+$ cells were purified from peripheral blood leukapheresis material from three individuals in hematological remission after treatment for acute myeloid leukemia (AML). Bone marrow CD34$^+$ cells were obtained from healthy donors. Bone marrow CD34$^+$ cells from five CML patients (three females; two males; age range at diagnosis: 44–87 years) in chronic phase were obtained at the time of diagnosis. Patients did not receive tyrosine kinase inhibitor therapy prior to sampling. Diagnosis of CML was confirmed by standard cytogenetic and molecular genetic analyses to detect translocation t(9;22)(q34;q11) and detection and quantification of p210 BCR-ABL1 fusion transcripts as described previously (Emig et al., 1999; Hughes et al., 2006). The investigation was approved by the Hannover Medical

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School Ethics Committee (No. 2899) and written consent was obtained from each patient in accordance with the Declaration of Helsinki. In all cases, $CD34^+$ cells were purified by magnetic separation according to the manufacturer's recommendations (Miltenyi Biotec, Bergisch Gladbach, Germany).

Cultivation, transduction and chemical treatment (DMSO and imatinib) of K-562 cells (ACC-10, DSMZ, Braunschweig, Germany) were performed as described earlier (Manukjan et al., 2015).

The pRSF91.IRES.dTomato.pre* vector was used to ectopically overexpress *GABPB1*.ΔTAD, following published protocols (Ripperger et al., 2015). $CD34^+$ cells were transduced with VSV-G pseudotyped retroviral particles using a RetroNectin protocol (Takara Bio Europe/Clontech, Saint-Germain-en-Laye, France) and a multiplicity of infection (MOI) of 50. Quantitative RT-PCR was performed using the QuantiTect SYBR Green RT-PCR Master Mix (Qiagen, Hilden, Germany) under standard conditions. Relative expression was calculated by the $\Delta\Delta C_t$ method in correlation to *SDHA*. Primer sequences are as follows: *GABPB1* (forward: 5′-GGT CAA GAT GAT GAA GTT CG-3′; reverse: 5′-CTG GCA TCT CTG CTC ACA C-3′); *SDHA* (forward: 5′-GCC ATC CAC TAC ATG ACG-3′; reverse: 5′-TCC ATA TAA GGT GTG CAA TAG C-3′).

Following fluorescence activated cell sorting (FACS) for dTomato-reporter expression, HSPCs were maintained for ten days in liquid culture using StemSpan medium (StemCell Technologies, Grenoble, France) supplemented with 1% penicillin/streptomycin, 1% L-glutamine (Biochrom AG, Berlin, Germany) and the following recombinant human cytokines: 100 ng/ml M-CSF, 50 ng/ml FLT3-L, 20 ng/ml SCF, and 20 ng/ml IL-6 (all PeproTech, Hamburg, Germany). For flow cytometry, the following antibodies were used: anti-human CD11b APC (ICRF44) (eBioscience, Frankfurt am Main, Germany); anti-human CD14 APC (555399) (BD Pharmingen, Heidelberg, Germany). Colony formation assays were performed two days after transduction with 5000 FACS-purified reporter-positive cells per 35 mm^2 culture dish using MethoCult™ H4230 methylcellulose (StemCell Technologies) supplemented with 1% penicillin/streptomycin (Biochrom AG), as well as a recombinant human cytokine cocktail containing 100 ng/ml SCF, 20 ng/ml G-CSF, 20 ng/ml GM-CSF, 20 ng/ml IL-3, and 20 ng/ml IL-6 (PeproTech). Granulocyte/macrophage colony-forming units (CFU-GM) were determined after 14 days of cultivation under standard conditions.

3. Results & discussion

To study the impact of GABP during myeloid differentiation and self-renewal of primary human hematopoietic stem/progenitor cells (HSPCs), GABP function was impaired by ectopic overexpression of a GABPβ1 subunit lacking the TAD (*GABPB1*.ΔTAD, shown schematically in Fig. 1A) (Ripperger et al., 2015). *GABPB1*.ΔTAD ectopic overexpression was performed since knockdown efficiencies of shRNAs targeting GABP subunits were not sufficient in HSPCs (data not shown). Initially, effects of *GABPB1*.ΔTAD ectopic overexpression were studied in the BCR-ABL1$^+$ K-562 cell line in combination with imatinib treatment to confirm the dominant-negative action of the mutant protein (Fig. 1B). A similar proliferation behavior in terms of elevated sensitivity to imatinib upon *GABPB1*.ΔTAD ectopic overexpression was observed as compared to our previous investigations applying shRNA-mediated knockdown of *GABPA* in K-562 cells (Manukjan et al., 2015). Hence, the approach is feasible to be used in primary HSPCs particularly as overexpression efficiency could be proven by qPCR (Fig. 1C).

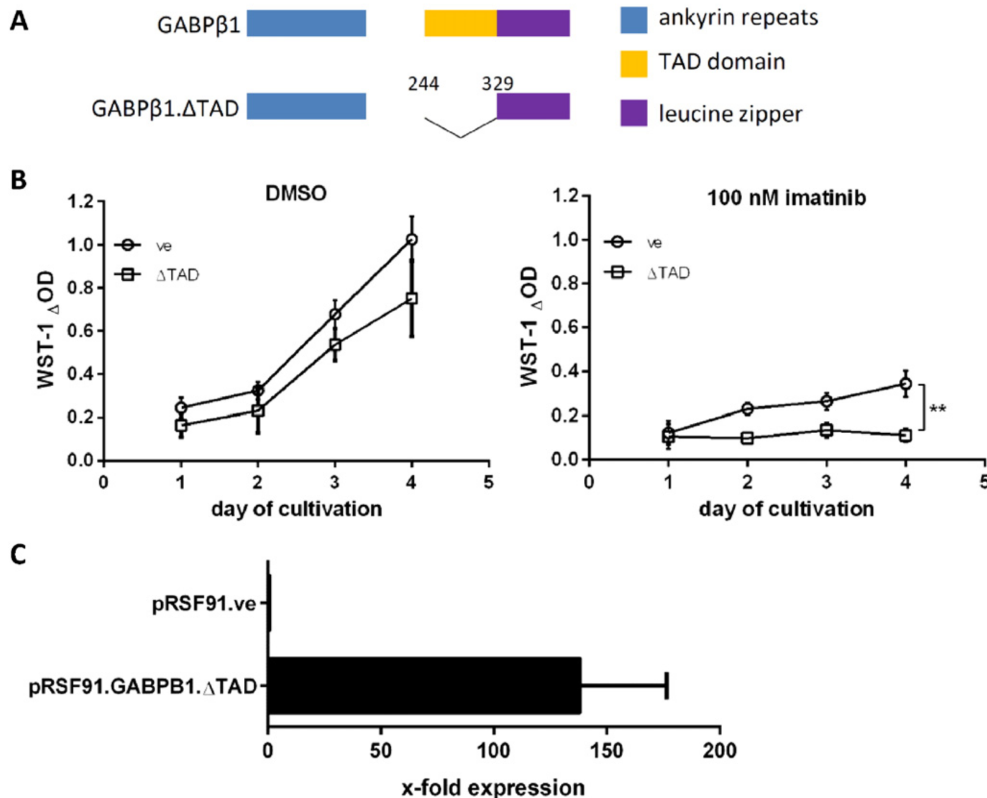

Fig. 1. *GABPB1*.ΔTAD acts as dominant-negative mutant. (A) Schematic representation of GABPβ1 subunit domains. The transactivation domain (TAD) of *GABPB1* subunit, transcript variant beta-2 (NM_016654.4) was deleted by restriction digestion to create the *GABPB1*.ΔTAD construct (bottom) used for ectopic overexpression. Numbers indicate amino acid residues. (B) WST-1 proliferation assay on K-562 cells. Cells were FACS-purified for dTomato expression upon transduction with the ectopic overexpression vector (pRSF91.IRES.dTomato.pre*) either as empty vector control (ve) or containing a *GABPB1*.ΔTAD (ΔTAD) cDNA cassette and subsequently treated with 100 nM imatinib or 0.1% DMSO as solvent control. The WST-1 assay was performed every 24 h. The background-corrected optical density (ΔOD) is plotted over time (mean ± s.d., $n = 3$; unpaired t-test; $^{**}P < 0.01$). (C) Quantitative PCR results of three independent experiments performed two days after transduction of HSPCs comparing *GABPB1*.ΔTAD with the empty vector control (pRSF91.ve) (mean + s.d.). Primers allow amplification of both, endogenous wild-type *GABPB1* and ectopically expressed *GABPB1*.ΔTAD.

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