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Temporal regulation of Stat5 activity in determination of cell differentiation program

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Abstract

Although Stat5 is activated by various cytokines, only erythropoietin (Epo) and a small number of cytokines induce Stat5-dependent erythroid differentiation. Here, by using a reporter gene system to monitor transcriptional activity of Stat5, we showed that Epo but not interleukin (IL)-3 supports sustained activation of Stat5, which induces globin gene expression. IL-3 or IL-2 stimulation inhibits Epo-induced globin gene expression. The acidic region of the IL-2 receptor β-chain was essential for this inhibition. These results underscore the importance of temporal regulation of Stat activity for regulation of cytokine-specific cell differentiation.

Keywords: Erythropoietin; IL-3; IL-2; Globin genes; Transcription; Erythroid differentiation

Epo plays essential roles in erythropoiesis [1], in which Stat5 activation by Epo [2], is important [3–8]. Although Stat5 is activated by a variety of cytokines [2], only Epo and small number of other cytokines induce erythrocyte differentiation *in vivo* and *in vitro* model systems [1,9]. It is not well understood how specificity of Stat5-mediated globin gene expression by Epo is ensured.

Here we showed that LG-Stat5, an active form of Stat5, induces globin gene expression in response to epidermal growth factor (EGF) in EGF receptor (EGFR)-reconstituted Ba/F3 cells. LG-Stat5-induced globin gene expression is inhibited by IL-3, which activates Stat5 but does not induce globin gene expression. By using a reporter gene system, we showed that EGF and Epo stimulation induce sustained reporter gene expression by LG-Stat5, whereas IL-3-induced reporter gene expression is down-regulated gradually. These results indicate that sustained activation of Stat5 mediates erythroid differentiation and globin gene expression. Furthermore, we showed that IL-2 also mediates inhibition of Epo-induced globin gene expression, for which the acidic region (the A-region) of the IL-2 receptor (IL-2) β-chain (IL-2Rβ) is essential for this inhibition. These results underscore the importance of temporal regulation of Stat activity for regulation of cytokine-specific gene expression and may provide insight into mechanism of cross-talk in cytokine signaling.

Materials and methods

Plasmids and gene transfer. Plasmid construction and gene transfer are described in Supplemental methods.

Immunoblot analysis. Preparation of nuclear extracts and immunoblot analysis were described previously [10].

Inducible translocation trap (ITT) assay and flowcytometry. For ITT assay, BBLG or BLG transduced with pLG-Stat5 was analyzed for green fluorescent protein (GFP) with FACS Calibur as described previously [10].

DNA microarray analysis. BBLG expressing LG-Stat5 was cultured in the presence of mouse IL-3 (1 ng/ml) or human EGF (10 ng/ml). Gene expression was analyzed using Affymetrix expression array with GeneChip Murine Genome set U74Av2 according to the manufacturer's instruction with some modification.

Northern blot analysis. Northern blot analysis was performed as previously described [11]. cDNA probes of mouse α- and β-globin genes are described in Supplemental methods.

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Results and discussion

EGF induces sustained reporter gene expression by an active form of Stat5

To analyze nuclear translocation and target gene expression by Stat5 using ITT [10], we transduced a retroviral vector encoding a fusion protein consisting of LexA DNA-binding domain (DB), transactivation domain of Gal4 (Gal4 TA), and Stat5 (LG-Stat5) (Fig. 1A) into BBLG cell line containing LexA-d1EGFP reporter gene in the genome [10]. BBLG was derived from BB13, which was derived from murine IL-3-dependent hematopoietic Ba/F3 and ectopically expresses EGFR and Bcl-2 [12]. EGF supports proliferation of BB13 and BBLG but not parental Ba/F3. When LG-Stat5 is activated and translocates into the nucleus, it binds to LexA operator sites of the GFP reporter gene as well as Stat-binding elements of its target genes (Fig. 1B). In addition, since LG-Stat5 has a strong TA of Gal4, LG-Stat5 can function as an active form (Fig. 1B, see below). After Transduction of LG-Stat5 into BBLG cells, GFP (+) cells were sorted and cultured in the presence of EGF and/or IL-3. GFP expression levels were very high just after sorting of GFP (+) cells (day 0, Fig. 1C, left panel). Expression levels of GFP in cells cultured in the presence of IL-3 markedly decreased by day 8 (Fig. 1C, upper right panel). In

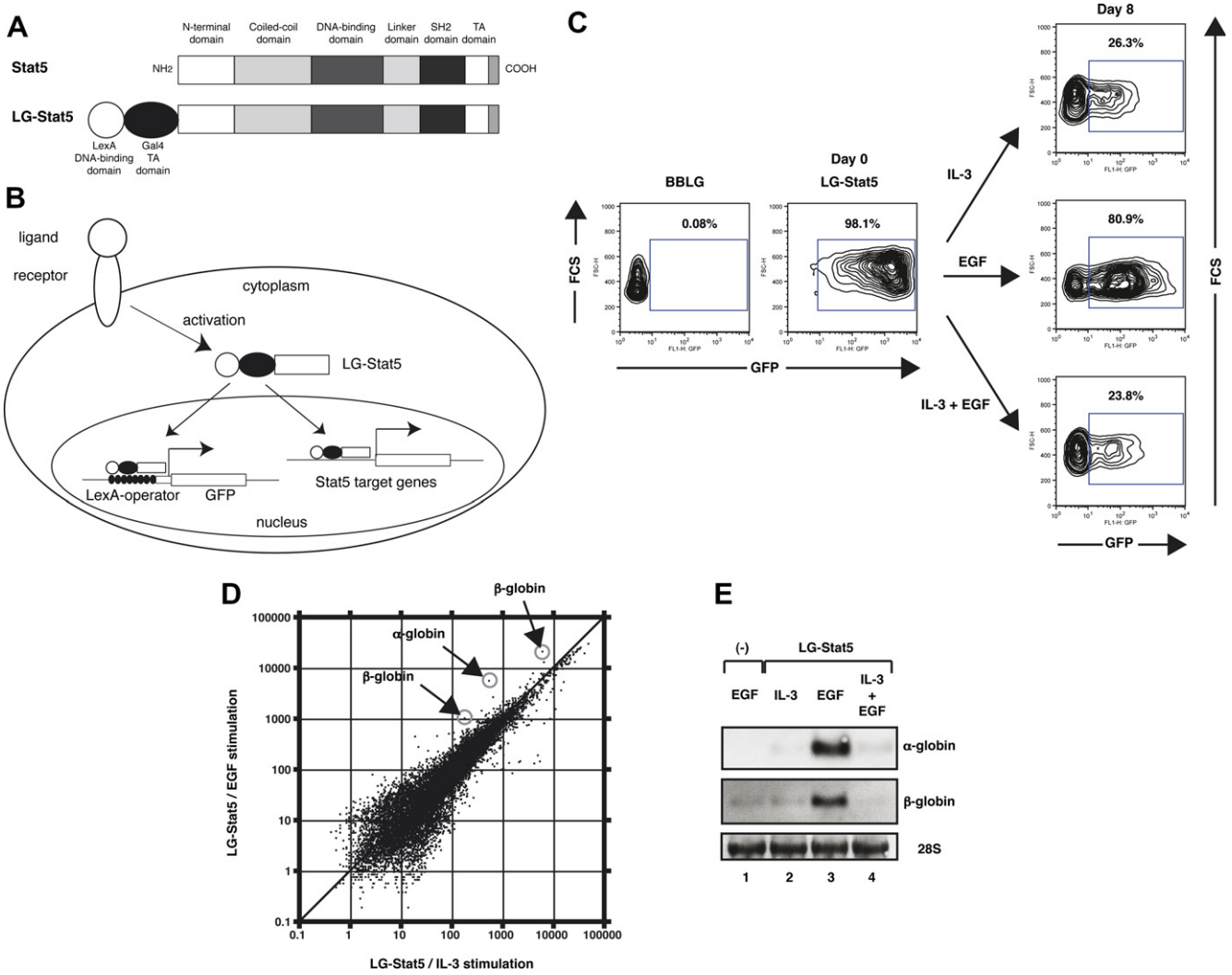

Fig. 1. Sustained activation of Stat5 induces globin gene expression. (A) LG-Stat5 consists of LexA DB (a.a. 1–87), Gal4 TA (a.a. 768–881), and human Stat5a. (B) When LG-Stat5 translocates into the nucleus by cytokine stimulation inducing Stat5 activation, it binds to the LexA operators with LexA DB and activates GFP expression by the action of Gal4 TA. Activated LG-Stat5 also binds to recognition sequences of Stat5 and activates expression of Stat5 target genes. (C) EGF stimulation induces sustained reporter expression by an active form of Stat5. After transduction of LG-Stat5 into BBLG cells, GFP (+) cells were sorted and cultured in the presence of EGF and/or IL-3. Expression of GFP was examined just after sorting (day 0) and 8 days after sorting (day 8). (D) Total RNA prepared from BBLG expressing LG-Stat5 cultured in the presence of IL-3 or EGF were subjected to microarray analysis with GeneChip Murine Genome set U74Av2. For all expressed genes, the changes relative to IL-3-stimulated cells (x axis) or EGF-stimulated cells (y axis) are plotted on $\log_{10}$ format. (E) Expression of globin genes in BBLG expressing LG-Stat5 cultured in the presence of IL-3, EGF, or IL-3 + EGF. BBLG was transduced with LG-Stat5, and sorted GFP (+) cells and BBLG were cultured with IL-3 (1 ng/ml), EGF (10 ng/ml), or IL-3 + EGF for 4 days.