Role of Gas6 in erythropoiesis and anemia in mice

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Abstract

Many patients with anemia fail to respond to treatment with erythropoietin (Epo), a commonly used hormone that stimulates erythroid progenitor production and maturation by human BM or by murine spleen. The protein product of growth arrest-specific gene 6 (Gas6) is important for cell survival across several cell types, but its precise physiological role remains largely enigmatic. Here, we report that murine erythroblasts released Gas6 in response to Epo and that Gas6 enhanced Epo receptor signaling by activating the serine-threonine kinase Akt in these cells. In the absence of Gas6, erythroid progenitors and erythroblasts were hyporesponsive to the survival activity of Epo and failed to restore hematocrit levels in response to anemia. In addition, Gas6 may influence erythropoiesis via paracrine erythroblast-independent mechanisms involving macrophages. When mice with acute anemia were treated with Gas6, the protein normalized hematocrit levels without causing undesired erythrocytosis. In a transgenic mouse model of chronic anemia caused by insufficient Epo production, Gas6 synergized with Epo in restoring hematocrit […]

Reference


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SUPPLEMENTARY INFORMATION

SUPPLEMENTARY NOTE 1: Reticulocyte maturation, measured by the intensity of fluorescence (56), was normal in Gas6−/− mice. The proportion of immature reticulocytes with a high fluorescence versus mature reticulocytes with an intermediate or low fluorescence were, respectively, 12 ± 0.4% and 89 ± 1.9% in WT mice versus 11 ± 1.1% and 88 ± 0.6% in Gas6−/− mice (n = 3, P = NS).

SUPPLEMENTARY NOTE 2: RBCs were biotinylated in vivo. At 5 and 8 days after injection of the 34-3C-antibody, 62 ± 2% (day 5) and 39 ± 1% (day 8) biotinylated rbcs were detected in WT mice versus 66 ± 2% (day 5) and 43 ± 2% (day 8) in Gas6−/− mice, respectively (n = 6, P = NS).

SUPPLEMENTARY NOTE 3: Since iron reserves are necessary for a sufficient erythropoietic response and iron depletion is a common cause of resistance to Epo, we assessed the mice for iron stores. Perl’s staining of BM cytopsins revealed adequate iron stores in both WT and Gas6−/− mice (data not shown). Serum iron levels were also within the normal range (data not shown). In addition, BM histology revealed normal stainable iron stores in both WT and Gas6−/− mice (not shown). Thus, the impaired erythropoietic response in anemic Gas6−/− mice, in spite of elevated Epo levels (see Supplementary Note 4), was not attributable to iron deficiency.

SUPPLEMENTARY NOTE 4: Since maturation and proliferation of erythroid progenitors, as well as the generation of early erythroblasts are largely dependent on Epo (2), we considered whether the impaired erythropoietic response of Gas6−/− mice might be due to insufficient Epo production. In baseline conditions, serum Epo protein levels were undetectable in both genotypes (< 20 mIU/ml). Remarkably, after PHZ-induced hemolysis, serum Epo levels were higher in Gas6−/− than WT mice (mIU/ml: 53 ± 12 and 25 ± 0.5 in WT mice versus 440 ± 120 and 165 ± 50 in Gas6−/− mice at day 3 and 6 after PHZ, respectively; n = 4-7, P < 0.05). Thus, Gas6−/− mice displayed higher erythropoietin levels at day 3 and 6 after acute hemolysis than WT mice because they were more anemic at these time points (Figure 2a).
SUPPLEMENTARY NOTE 5: RBCs were biotinylated in vivo in WT and Gas6⁻/⁻ mice. rGas6 was administered from day 0 to day 8 (2 µg/day, i.p.). On day 0, autoimmune hemolytic anemia was induced by intraperitoneal injection of 200 µg purified anti-RBC 34-3C IgG2a (29). At 5 and 8 days after administration of rGas6 and anti-RBC antibodies, respectively, the percentage of biotinylated RBCs was 53 ± 3% and 39 ± 2% in WT mice versus 55 ± 7% and 37 ± 2% in Gas6⁻/⁻ mice (n = 6, P = NS), indicating that the clearance rate of biotinylated RBCs during acute hemolytic anemia was comparable in Gas6⁻/⁻ than WT mice in presence of rGas6 (see also Supplementary note 2).

SUPPLEMENTARY FIGURES

SUPPLEMENTARY FIGURE 1: Impaired erythropoiesis in Gas6<sup>−/−</sup> mice (red lines) as compared to WT mice (black lines) on a 100% C57BL/6 background in response to phenylhydrazine induced anemia (injection on day 0 and 1).

SUPPLEMENTARY FIGURE 2: Therapeutic potential of recombinant Gas6 in acute anemia induced by blood loss.
After bleeding (500 µl on day 0 and 500 µl on day 1), WT mice were treated with saline (control), recombinant human Gas6 (rGas6, 2 µg daily intraperitoneally) or recombinant human erythropoietin (Epo, 10 IU every second day intraperitoneally). The erythropoietic response was monitored by determining the hematocrit levels. Hematocrit levels are expressed as mean ± SEM (n = 6 mice) in all panels. *, P < 0.001.
Supplementary Fig. 1

A graph showing changes in hematocrit (%) over days. The graph includes data points connected by lines, with significance indicated by asterisks (*) and p-values less than 0.001.
Supplementary Fig. 2