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Role of G proteins and ERK activation in hemin-induced erythroid differentiation of K562 cells

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Abstract

Heterotrimeric G proteins which couple extracellular signals to intracellular effectors play a central role in cell growth and differentiation. The pluripotent erythroleukemic cell line K562 that acquires the capability to synthesize hemoglobin in response to a variety of agents can be used as a model system for erythroid differentiation. Using Western blot analysis and RT-PCR, we studied alterations in G protein expression accompanying hemin-induced differentiation of K562 cells. We demonstrated the presence of $G_{\alpha s}$, $G_{\alpha i2}$ and $G_{\alpha q}$ and the absence of $G_{\alpha i1}$, $G_{\alpha o}$ and $G_{\alpha 16}$ in K562 cells. We observed the short form of $G_{\alpha s}$ to be expressed predominantly in these cells. Treatment of K562 cells with hemin resulted in an increase in the levels of $G_{\alpha s}$ and $G_{\alpha q}$. On the other hand, the level of $G_{\alpha i2}$ was found to increase on the third day after induction with hemin, followed by a decrease to levels lower of those of uninduced cells. The mitogen-activated protein kinase ERK1/2 pathway is crucial in the control of cell proliferation and differentiation. Both G_i- and G_q-coupled receptors stimulate MAPK activation. We therefore examined the phosphorylation of ERK1/2 during hemin-induced differentiation of K562 cells. Using anti-ERK1/2 antibodies, we observed that ERK2 was primarily phosphorylated in K562 cells. ERK2 phosphorylation increased gradually until 48 h and returned to basal values by 96 h following hemin treatment. Our results suggest that changes in G protein expression and ERK2 activity are involved in hemin-induced differentiation of K562 cells.
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Keywords: Erythroid differentiation; Hemin; G proteins; ERK1/2 phosphorylation; K562

Introduction

Heterotrimeric guanine nucleotide binding proteins (G proteins) which couple many cell surface receptors to effectors on the plasma membrane mediate a series of events which ultimately lead to diverse cellular responses. G proteins are composed of an α-subunit that binds to and hydrolyzes GTP, and a βγ-subunit complex. To date, 20 different Gα, 6 Gβ and 11 Gγ subunits have been described. Gα subunits have been divided into four families (G_s, $G_{i/o}$, $G_{q/11}$, $G_{12/13}$) based on the homology at the amino acid level. Their mass is between 39 and 52 kDa (Hepler and Gilman, 1992; Offermans and Simon, 1996). G proteins regulate critical processes such as cell growth, differentiation and development (Malbon, 1997). Studies in human pheochromocytoma PC12 cells which were treated with nerve growth factor (Strittmatter et al., 1990) and in the neuroblastoma × glioma hybrid NG 108-15 cell line, cultured with agents that elevate intracellular levels of cAMP (Mullaney and Milligan, 1989) suggested a role of $G_{\alpha o}$ in development and differentiation of nervous tissue. Changes in the levels of G protein α-subunits such as $G_{\alpha s}$, $G_{\alpha o}$ and $G_{\alpha i}$ were associated with differentiation of 3T3-L1 cells from fibroblasts into adipocytes. As these cells were induced to differentiate to adipocytes, a decline in the level of $G_{\alpha s}$ (Wang et al., 1992), a decline (Gierschik et al., 1986) or an increase (Watkins et al., 1987) in the expression of $G_{\alpha i2}$ was observed. Constitutive activation of $G_{\alpha s}$ by cholera toxin blocked and oligonucleotide(s) antisense to $G_{\alpha s}$ accelerated the differentiation process (Wang et al., 1992). Interestingly, the modulating effect of $G_{\alpha s}$ on differentiation was independent of adenylyl cyclase activation and cAMP levels (Wang and Malbon, 1996). A subsequent study demonstrated differential expression of $G_{\alpha q/11}$ at three different stages of adipogenesis: in confluent preadipocytes, differentiated preadipocytes and mature adipocytes (Denis-Henriot et al.,

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1996). In F9 teratocarcinoma stem cells induced to differentiate with retinoic acid, the inhibitory G protein, $G_{\alpha i}$ repressed (Galvin-Parton et al., 1990), whereas expression of $G_{\alpha s}$ induced differentiation (Gao and Malbon, 1996). G proteins are also known to modulate differentiation of hematopoietic cells. Increase in expression of $G_{\alpha i2}$ and decrease in expression of $G_{\alpha 16}$, a G protein specifically expressed in hematopoietic cells, were shown in a promyelocytic cell line, HL-60, in the course of differentiation along the neutrophil pathway (Amatruda et al., 1991). When HL-60 cells were induced to differentiate to mature granulocyte-like cells with retinoic acid a decrease in the level of $G_{\alpha s}$ was observed (Meissner et al., 1996). The content of $G_{\alpha i2}$ remained unchanged in a human erythroleukemia cell line, HEL, induced to differentiate to megakaryocytes (Ashby et al., 1991); however, the levels of $G_{\alpha i2}$ and $G_{\alpha i3}$ increased in a human megakaryoblastic leukemia cell line, MEG-01, induced to differentiate with TPA (Nagata et al., 1995). Terminal differentiation of an erythropoietin-sensitive murine erythroleukemia cell line, RED-1, was associated with a loss of $G_{\alpha i3}$ and an increase in the cytosolic form of $G_{\alpha i2}$ (Kesselring et al., 1994). In human myeloid progenitors and mature blood cells $G_{\alpha s}$, $G_{\alpha i2}$ and $G_{\alpha q/11}$ proteins were expressed at high levels during every stage of granulomonocytic and erythroid differentiation, whereas $G_{\alpha 12}$ and $G_{\alpha 16}$ proteins were expressed in a lineage-specific manner in normal myeloid cells (Tenailleau et al., 1997). Taken together, these studies reveal that G protein alpha subunits can regulate cell differentiation in distinct ways, depending on the cell type or tissue under investigation as well as the type of inducer.

The mitogen-activated protein kinase ERK1/2 pathway is essential in the control of growth, differentiation and survival in many cellular systems. The nature of the response to ERK activation in different cells depends on the agent used to induce cells to growth or differentiation and the duration of ERK activation (Pouyssegur and Lenormand, 2003). Several hematopoietic growth factors and cytokines such as erythropoietin (EPO) stem cell factor (SCF) and interleukin-3 (IL-3) by acting on receptor tyrosine kinases activate members of the ERK or MAPK family (Platanias, 2003). It is documented that G_q- and G_i-coupled receptors also activate the ERK1/2 pathway (Gutkind, 2000). A role for the ERK/MAPK pathway in erythroid differentiation has been proposed in a number of studies. Shelly et al. (1998) established that inhibition of the basal activity of the ERK/MAPK pathway enhanced the erythroid phenotype of K562 cells. Likewise, Kang et al. (1999) demonstrated that inactivation of ERK by MAPK inhibitors resulted in growth arrest and erythroid differentiation of K562 cells. Treatment of K562 cells with sodium butyrate resulted in a rapid but transient activation of ERK1/2 signaling in one study (Rivero and Adunyah, 1996) but caused inhibition of ERK and activation of p38 MAPK pathway in another (Witt et al., 2000). Recently, the involvement of ERK phosphorylation in both growth and hemin-induced erythroid differentiation of K562 cells has been shown (Woessmann and Mivechi, 2001). The signaling pathways mediating hemin-induced differentiation of K562 cells have not been well characterized. In this study, we demonstrate alterations in the level of $G_{\alpha s}$, $G_{\alpha i2}$ and $G_{\alpha q}$ proteins and ERK1/2 phosphorylation during erythroid differentiation of K562 cells induced by hemin.

Materials and methods

Hemin, RPMI 1640 and penicillin/streptomycin solutions, alkaline phosphatase-conjugated goat anti-rabbit IgG and other electrophoresis reagents were purchased from Sigma (St Louis, MO, USA). Fetal calf serum (FCS) was from Harlan Sera-Lab (Leicestershire, UK). G protein antisera RM/1 (recognizing C-terminal sequence RMHLRQYELL of $G_{\alpha s}$), GC/2 (recognizing C-terminal peptide GCTLSAEERAALERS of $G_{\alpha o}$), and AS/7-Transducin (recognizing C-terminal peptide KENLKDCGLF of $G_{\alpha i12}$) were purchased from Dupont NEN (Lachine, Quebec, Canada). $G_{\alpha q}$ antibody (internal 115–133) and antibody reacting with the C terminus (360–373) of the $G_{\alpha 16}$ subunit were obtained from Calbiochem (San Diego, CA, USA). Antisera against the $G_{\beta\gamma}$ (S217) and $G_{\alpha 16}$ (AS339) subunits were kindly provided by Dr. Alfred Gilman, University of Texas and Dr. Bernd Nürnberg, Heinrich-Heine University, Dusseldorf, respectively. Phosphorylated ERK1/2 antibody was from Santa Cruz Biotechnology (San Diego, CA, USA). Anti-ERK1/2 antibody was from Promega (Madison, WI, USA). Affinity purified peroxidase-conjugated anti-rabbit IgG was purchased from Jackson ImmunoResearch Europe Ltd. (Cambridgeshire, UK). All other chemicals and enzymes were obtained from commercial sources.

Cell preparations

K562 and HL-60 cells obtained from ATCC (MD, USA) were cultured in RPMI medium supplemented with 10% fetal calf serum (FCS), 100 IU/ml penicillin, 100 μg/ml streptomycin, 25 μg/ml amphotericin B and 2 mM L-glutamine at 37 °C in humidified air containing 5% CO_2. Peripheral blood mononuclear cells (PBMC) were obtained from the fresh heparinized blood of healthy human volunteers. AML cells were prepared from the fresh heparinized blood of patient diagnosed with acute myelocytic leukemia AML. PBM and AML cells were isolated by density gradient centrifugation over Ficoll-Hypaque.

Induction of hemoglobin synthesis

Cells seeded at 1×10^5 cells/ml were induced to differentiate with 20 μM hemin (day 0) and were harvested at days 1–6 after induction. At this time, benzidine staining was carried out to assess the extent of differentiation.

Measurement of erythroid differentiation of K562 cells by benzidine staining

Erythroid differentiation was scored by staining the cells for hemoglobin synthesis with benzidine reagent. A benzidine dihydrochloride stock solution, 0.2% (w/v), was prepared in

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