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Review Article

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Evaluation of Signaling Pathways Involved in γ-Globin Gene Induction Using Fetal Hemoglobin Inducer Drugs

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ABSTRACT

Potent induction of fetal hemoglobin (HbF) production results in alleviating the complications of β-thalassemia and sickle cell disease (SCD). HbF inducer agents can trigger several molecular signaling pathways critical for erythropoiesis. Janus kinase/Signal transducer and activator of transcription (JAK/STAT), mitogen activated protein kinas (MAPK) and Phosphoinositide 3-kinase (PI3K) are considered as main signaling pathways, which may play a significant role in HbF induction. All these signaling pathways are triggered by erythropoietin (EPO) as the main growth factor inducing erythroid differentiation, when it binds to its cell surface receptor, erythropoietin receptor (EPO-R) HbF inducer agents have been shown to upregulate HbF production level by triggering certain signaling pathways. As a result, understanding the pivotal signaling pathways influencing HbF induction leads to effective upregulation of HbF. In this mini review article, we try to consider the correlation between HbF inducer agents and their molecular mechanisms of γ-globin upregulation. Several studies suggest that activating P38 MAPK, RAS and STAT5 signaling pathways result in efficient HbF induction. Nevertheless, the role of other erythroid signaling pathways in HbF induction seems to be indispensible and should be emphasized.

KEY WORDS: β-thalassemia, Sickle cell disease, Fetal hemoglobin

INTRODUCTION

Fetal hemoglobin inducer drugs can improve symptoms of α-globin chain precipitation and ineffective erythropoiesis in β-thalassemia, and are able to reduce hemoglobin S production in sickle cell disease (SCD).[1] Effective expression induction of fetal hemoglobin (HbF) is directly associated with improved clinical status of patients, and targeting the signaling pathways active in inducing the expression of fetal hemoglobin can result in increased level of HbF production up to effective therapeutic level, reducing the complications of disease.[1-3]

Signaling pathways in Erythroid Differentiation

HSC differentiation to erythroid series is associated with activation of multiple signaling pathways. It has been shown that activation of these signaling pathways during erythropoiesis is mediated by interaction between erythropoietin (EPO) and erythropoietin receptor (EPO-R).[4] Mitogen-Activated Protein Kinase (MAPK),

Phosphatidyl Inositol 3 Kinase (PI3K) and JAK-STAT are three major signaling pathways involved in erythroid differentiation.[5-7] MAPK signaling pathway includes three separate signaling pathways of SAPK/JNK, Extracellular-Regulated Kinase (ERK1/2) and P38 MAPK (SAP kinase/Jun kinase).[8] ERK1 and ERK2 play a role in proliferation of early erythroid precursors and also in differentiation of final erythroid precursors.[9] This is while activation of SAPK and P38 signaling pathways through their binding to EPO receptor is more important in erythroid differentiation.[10, 11] Activation of MAPK signaling pathway and associated molecules of Ras and Raf-1 is done by binding of Grb2 adapter molecule to EPO receptor.[12, 13] PI3-Kinase signaling pathway has been shown to play an important role in proliferation, differentiation and maturation of erythroid precursors. Direct binding of P85 regulatory subunit of PI3Kproteinor, indirect binding of it via an adapter protein to EPO-R and activation of Akt results in activation of PI3-Kinase/Akt signaling pathway.[14-16] This signaling pathway results in increased phosphorylation and activation of transcription factors involved in normal erythropoiesis including GATA-1 and Foxo3a.[17, 18] Activation of JAK/STAT signaling pathway mediated by EPO binding to EPO-R plays an important role in proliferation and differentiation of erythroid precursors.[19, 20] In this signaling pathway, dimerization of Janus Kinase 2 (JAK2) and EPO-R autophosphorylates JAK2 and creates the binding site for signaling protein. Several STAT (Signal tranducer and activator of transcription) proteins play a role in erythroid differentiation by binding to dimerized JAK2 and are phosphorylated and thereby activated by JAK2.[21] In this signaling pathway, STAT1, STAT3 and STAT5a/b are dissociated from EPO-R following phosphorylation, and are transferred to the nucleus following homodimerization or heterodimerization, increasing expression of transcription factors containing Interferon γ Activated Sequence (GAS).[22-25]

HbF Inducers Act via Multiple Signaling Pathways

In several studies, drugs such as thalidomide, pomalidomide, hydroxyurea (HU), decitabine and sodium butyrate have been cited as high potential drugs in induction of HbF expression. As an immunomodulator drug, thalidomide has a high inductive effect in increasing HbF level compared with sodium butyrate. It has a higher potential than sodium butyrate in increasing proliferation of erythroid precursors.[26] In fact, thalidomide performs its inductive effect in increasing HbF levels through activation of P38MAPK signaling pathway along with Reactive oxygen Species (ROS).[1, 27] Butyrate as a histone deacetylase (HDAC) inhibitorcan activate P38 MAPK signaling pathway and guanylatecyclase in γ-globin gene induction in addition to causing epigenetic changes.[28-30] In addition to increasing global acetylation in H4 histone, butyrate can launch signaling pathways associated with activation of C-myc, C-myb and STAT-5 in human K562 and burst-forming units-erythroid (BFU-E) cells, resulting in increased proliferation of erythroid precursors.[31] Thricostatin, as a HDAC inhibitor, is capable of inducing γ-globin gene expression by phosphorylating P38MAPK.[32] As a HDAC inhibitor, apicidin may increase H3 and H4 acetylation in LCR region and in the region adjacent to promoter of γ-globin gene by activating MAPK signaling pathway in K562 cells.[33] Moreover, treatment with butyrate and trichostatin activates γ-globin expression by activating transcription factor-2 (ATF2) and CRE binding protein 1 (CREB1) via p38 MAPK signaling.[34] Decitabine [35] and 5-azacitidine [30,36] are known inhibitors of the DNA methylation. They cause demethylation of γ-globin promoter CPGs, leading to activation of this gene to differentiate adult erythroid cells. As a cytotoxic drug, HU can increase the level of HbF and produce fetal like cells in β-thalassemia and sickle cell patients following erythroid differentiation induction. HU and butyrate have been found to increase γ-globin expression in vivo by activating cGMP/soluble guanylate cyclase (sGC)/PKG (cGMP-dependent protein kinase) pathway.[37, 38] This drug can also induce HbF production by inducing signaling pathways associated with increased production of cAMP, small GTP-binding protein and secretion associated and RAS related protein (SAR).[39, 40] Butyrate and 5–azacitidine can induce HbF production by cAMP pathway.[39] In addition, Decitabin, 5-azacitidin,[41] thalidomide , sodium

butyrate[30] as hypomethylating agents induce HbF production (Figure1).

Thalidomide, butyrate and hydroxyurea (HU) modulate γ-globin gene expression via an upstream γ-globin cAMP response element (G-CRE), in addition to c-jun induction of HbF via similar pathway. Butyrate, thricostatin and thalidomide activate γ-globin expression via reactive oxygen species (ROS) and p38 mitogen activated protein kinase (MAPK) signaling. In addition, HU acts via NO/P38 MAPK. Alternatively, butyrate and HU induce HbF by increasing cGMP. For other details refer to text.

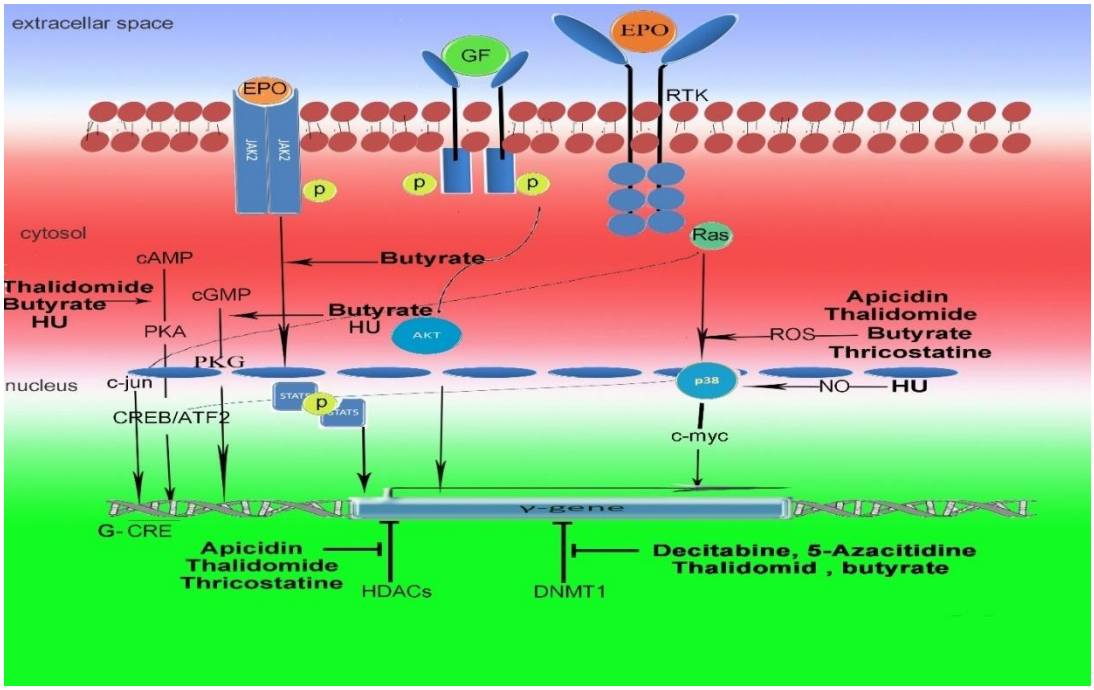

Figure1. Drugs and signaling pathways involved in γ-globin gene induction

Abbreviation: JAK2, janus kinase 2; STAT, Signal transducer and activator of transcription; EPO, erythropoietin; p, phosphate; GF, growth factor;PI3K, Phosphoinositide 3-kinase; AKT, proline-rich AKT substrate; HDACs, Histone deacetylases; cAMP, cyclic AMP; PKA, protein kinase A; CREB, Cyclic AMP-responsive element-binding protein; ATF2, activating transcription factor; cGMP, cyclic GMP; PKG, cGMP-dependent protein kinase; NO, nitric oxide.

Comparison of Drug Effects in HbF Induction

According to multiple studies, hemoglobin F inducers have been shown to increase total Hb level 1-5 g/dl above baseline. HU will increase it from 0.6 to 2.7 g/dl. Other pharmacological inducers of HbF production including EPO preparation, butyrate and 5-azacitidine have been shown to increase mean total Hb level by approximately 2-3 g/dl above baseline.[42] Decitabine increases mean Hb level by 1g/dl, and is tolerated at a dose with limited cytotoxicity.[43]

DISCUSSION

The use of drugs with a high potential to stimulate erythropoiesis appears to induce HbF expression by increasing proliferation of erythroid precursors. Thus, activation of signaling pathways involved in erythroid differentiation may play an important role in increased expression of γ-globin gene. Suppression of PI3K signaling pathway has been found to cause a 75% decrease in induction of erythroid precursors. This is while suppression P38 MAPK and mTOR (mammalian target of rapamycin) signaling pathways cause 40% and 60% reduction in erythroid precursors, respectively.[36] Since EPO as a major growth factor in erythroid differentiation could enhance the expression of genes such as KIT (CD117) and CDH1 (E-cadherin) through the activation of PI3-kinase,[44, 45] targeting this signaling pathway seems to be effective in inducing HbF expression. Treatment with EPO is limited because

it is associated with potential risk; erythroid expansion can increase iron absorption and extramedullary hematopoiesis.[46] Butyrate is an effective HbF inducer but its development has been limited because of suppressive effect on erythropoiesis.[47, 48] DNMT inhibitors used in SCD patients resistant to HU[49] might increase the risk of cancer in long time.[36] Therefore, their use is limited. Decitabine is not carcinogenic[36] but its effect on HbF induction is limited.[43] Among HbF inducers, the use of thalidomide is debatable because of its teratogenic effects.[50]

HU is a common drug used for therapeutic goals in hemoglobinopathies, but the effectiveness of this drug appears to decline with long term use.[51] Moreover, it only increases HbF in approximately half of SCD patients,[52] and is less effective in increasing HbF in β-thalassemia.[53] In addition, HU is a potent mammalian teratogen. Various other toxic effects have been attributed to it: interference with enzymes for DNA synthesis, granulocytopenia, gastrointestinal and cutaneous toxicity, etc.[54] It should be mentioned that HU can be toxic, and it can still be considered generally safe for therapeutic use by appropriate dosage and proper frequency of use.

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