A Hematopoietic Perspective on the Promiscuity and Specificity of $G\alpha_{16}$ Signaling

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Key Words

G-protein-coupled receptor • $G\alpha_{16}$ • Hematopoietic cells • Kinase • Signaling • Transcription

Abstract

$G\alpha_{16}$, a member of G_q subfamily, is expressed exclusively in hematopoietic cells, and its expression is highly modulated during lineage differentiation. Although functional redundancy within G_q subclass members has been observed in many established models, $G\alpha_{16}$ possesses unique structural and biochemical properties not shared by other family members. Its broad receptor-coupling capacity and unique downstream binding partners and effectors allow the occurrence of both inositol lipid-dependent and -independent signals. Apart from its recognized biological functions in hematopoietic cell responses, the enlistment of complicated signaling pathways further signifies the importance of $G\alpha_{16}$ in signal integration. This review aims to provide an updated appreciation and rational discussion of $G\alpha_{16}$ signaling with regard to its promiscuity and specificity.

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Introduction

Heterotrimeric G proteins are divided into four subfamilies (G_s , G_i , G_q , and G_{12}) based on the sequences and functional similarities of their $G\alpha$ subunits. $G\alpha_{16}$ is a

member of the G_q subfamily with molecular weight of 43.5 kDa [1]. Protein expression of $G\alpha_{16}$ is confined exclusively to normal and malignant hematopoietic cells [1, 2]. The murine homolog $G\alpha_{15}$ can also be detected in tissues such as spleen, thymus, lung and bone marrow where hematopoietic cells reside. $G\alpha_{16}$ shows 40% amino acid identity with $G\alpha_s$, 45% with $G\alpha_i$, and 57% with $G\alpha_{11}$ and $G\alpha_q$ [1, 3]. Like the other members in the G_q subfamily, $G\alpha_{16}$ lacks a cysteine residue four amino acids from the carboxyl terminus and is not a substrate for pertussis toxin (PTX)-mediated ADP-ribosylation site [1]. Instead of alanine or threonine, it contains a proline at position 50 in region A that is important for GTP binding and endogenous GTPase activity [3]. Based on sequence alignment, $G\alpha_{16}$ is predicted to have an extraordinarily long $\alpha 4/\beta 6$ loop which is an important region for both receptor and effector coupling [1, 4, 5]. Furthermore, the $G\alpha_{16}$ sequence includes a motif with a distinct homology to a hemoregulatory peptide pEEDCK associated with hematopoietic growth control [6]. pEEDCK induces reversible arrest of hematopoietic cells in G_0/G_1 phase without inducing differentiation, supporting the notion that $G\alpha_{16}$ may be involved in the maintenance of the immature state of the cells [7].

All heterotrimeric G proteins are composed of three subunits $G\alpha$, $G\beta$ and $G\gamma$. Ligand binding to a G-protein-coupled receptor (GPCR) induces the exchange of GDP bound on $G\alpha$ to GTP, and the resulting conformational changes allow both $G\alpha$ and $G\beta\gamma$ to regulate a wide range

Fig. 1. Covalent modifications and receptor-interacting regions of $G\alpha_{16}$. A schematic diagram shows the linear arrangement of the secondary structures of $G\alpha_{16}$. α -Helices and β -strands are colored in orange and blue, respectively. The two green linker regions (L1 and L2) connect the two major tertiary structural domains – helical and GTPase domains – of $G\alpha_{16}$. The amino acid sequences corresponding to the N-terminus and part of the $\alpha 4/\beta 6$ loop are shown, with the palmitoylated cysteines and the PKC phosphorylation site indicated in blue and green, respectively. Numbers in superscripts within the sequences correspond to the positions of the modified residues. Four known receptor-interacting regions on $G\alpha_{16}$ are marked below the schematic structural diagram, and are illustrated in a hypothetical $G\alpha_{16}$ structure. The model was generated by homologous modeling using Swiss-Model [87] and the template structures of $G\alpha_{11}$ and $G\alpha_{t1}$ in their corresponding trimeric G protein structures [88, 89]. The view shows the putative receptor-interacting surface on the GTPase domain of $G\alpha_{16}$, with the four receptor-interacting regions highlighted in yellow. The PKC phosphorylation site on $\alpha 4/\beta 6$ loop is highlighted by space-filled representation.

of downstream effectors. Following the activation of G_{16} , both $G\alpha$ and $G\beta\gamma$ dimer can stimulate at least 3 subtypes of phospholipase C β (PLC $\beta 1$ –3) and release inositol triphosphate (IP $_3$) and diacylglycerol as revealed in an exogenous expression system [8]. These second messengers in turn trigger intracellular Ca^{2+} mobilization and stimulation of protein kinase C (PKC), and eventually lead to the activation of several transcriptional factors. Emerging data suggest that $G\alpha_{16}$ participates in the activation of transcriptional factors such as signal transducer and activator of transcription 3 (STAT3) and nuclear factor κB (NF- κB). It has been demonstrated that C5a-induced phosphoinositide accumulation and Ca^{2+} release are im-

paired in macrophages from $G\alpha_{15}^{-/-}$ mice, despite of the normal hematopoiesis in these mice as well as in $G\alpha_{15}^{-/-}$ $G\alpha_q^{-/-}$ double-knockout and $G\alpha_{11}^{-/-}$ mice, suggesting that there is functional redundancy in G_q class signaling [9].

$G\alpha_{16}$ is coupled to a wide variety of receptors that are not linked to $G\alpha_{q/11}$. The receptor promiscuity of $G\alpha_{16}$ and its exclusive expression in the hematopoietic system indicate that it has unique functions in hematopoietic cells not performed by other G_q subfamily members. This review summarizes the divergent biochemical features of $G\alpha_{16}$, and provides newly appreciated and logical speculations of the biological functions of this G protein.

Covalent Modifications of $G\alpha_{16}$

Like other members of the G_q subfamily, $G\alpha_{16}$ is predicted to be palmitoylated at its N-terminus. Three cysteine residues are located at the N-terminus of $G\alpha_{16}$, and recent data suggest that Cys 9 and Cys 10 but not Cys 13 are palmitoylated (fig. 1) [10]. Together with the polybasic residues found on the N-terminus of $G\alpha_{16}$, such modifications are critical for plasma membrane attachment and PLC β activation [10]. So far there is no other type of covalent modification detected on the N-terminus of $G\alpha_{16}$, and it is not known if the palmitoylation of $G\alpha_{16}$ is dynamically regulated [11] or whether there is any heterogeneity of the attached lipid moiety as that in $G\alpha_z$ [12].

$G\alpha_{16}$ is subjected to regulation by protein phosphorylation. The coupling of thyrotropin-releasing hormone receptor to $G\alpha_{16}$ in *Xenopus* oocytes is impaired by the activation of PKC [13]. Such regulation is unique among the members of the G_q subfamily. In vitro phosphorylation study shows that $G\alpha_{16}$ is a direct substrate of PKC, and the phosphorylation site has eventually been identified as Ser 336 on the unique long insert of the $\alpha 4/\beta 6$ loop of $G\alpha_{16}$ (fig. 1) [14], which is a critical receptor-interacting region as identified in another study [15]. Similar PKC-mediated phosphorylation has been noted with the murine homolog $G\alpha_{15}$ and the phosphorylation site falls in the same region [14]. The constitutive activity of the GTPase-deficient (Q 212 L) mutant of $G\alpha_{16}$ in PLC β activation is only weakly affected by the phosphorylation. Residual phosphorylation is observed in the $G\alpha_{16}$ Ser 336 mutant but not the quadruple mutant made in an earlier study [13], indicating that PKC might also phosphorylate the other three putative target sites (Ser 4 , Thr 6 and Ser 53). Since PKC is a major downstream effector of PLC β , the action of PKC on $G\alpha_{16}$ may serve as a negative feedback mechanism to limit the activity of $G\alpha_{16}$. To date, no oth-

er report shows the interaction of $G\alpha_{16}$ with other serine/threonine or tyrosine kinases.

A recent study shows that $G\alpha_{16}$ (and $G\alpha_q$) is degraded through proteasome pathway [16]. Proteasome inhibitors like MG132 and lactacystin block the $G\alpha_{16}$ degradation, whereas other non-proteasomal inhibitors show no obvious protection. The rate of degradation increases in the case of a palmitoylation-deficient mutant of $G\alpha_{16}$, indicating that receptor-induced cytosolic translocation of $G\alpha_{16}$ may facilitate its proteasomal degradation. Although there is no evidence for direct ubiquitination of $G\alpha_{16}$ in that study, two potential non-consensus ubiquitination sites (Arg³⁷ and Arg³⁰²) can be found [17]. It would be interesting to examine if ubiquitination of $G\alpha_{16}$ occurs in vivo.

Promiscuous Receptor Coupling of G_{16}

One of the interesting features of G_{16} is its promiscuous receptor-coupling property, which channels a wide variety of GPCRs to activate PLC β and induce rapid intracellular Ca^{2+} mobilization (table 1) [18]. Such a discovery has facilitated the deorphanization of novel GPCRs and a number of successful cases have been reported [reviewed in 19–21]. However, $G\alpha_{16}$ is far from being the ideal ‘universal adaptor’ for all kinds of GPCRs. Significant and increasing numbers of GPCRs are found to be incapable of recognizing G_{16} [discussed in 22, 23] and most of them belong to the G_i -coupled receptors. The quest of a ‘better coupler’ has evolved based on the early finding that the extreme C-terminal tails of $G\alpha$ subunits determine the receptor-coupling specificity [24, 25]. Substitutions of the C-terminal residues in the $\alpha 5$ helix of $G\alpha_{16}$ with those of either $G\alpha_{i2}$, $G\alpha_{o1}$ or $G\alpha_z$ can greatly enhance the coupling with G_i -linked receptors that normally do not interact with $G\alpha_{16}$ [23, 26]. The same strategy worked equally well for G_s -coupled receptors [27]. Chimeric $G\alpha_{16}$ subunits with broadened receptor-coupling capability have been successfully employed in high throughput screening platforms [23, 28–30]. Further efforts have been made to decipher the minimal requirements for switching the receptor-coupling specificity. Individual residue lying on the exposed surface of the $\alpha 5$ helix of $G\alpha_{16}$, as well as a stretch of six amino acids between the $\alpha 5$ helix and the extreme C-terminal tail, plays an important role in determining the receptor-coupling specificity [30]. Other potential receptor-interacting regions on $G\alpha_{16}$ have also been explored, such as the $\beta 2/\beta 3$ loop [30], $\alpha N/\beta 1$ loop [31], as well as the uniquely long

$\alpha 4/\beta 6$ loop (fig. 1) [14]. Preliminary data from our laboratory indicate that reduction of the long $\alpha 4/\beta 6$ loop of $G\alpha_{16}$ can greatly diminish the receptor-coupling profile [M.K.C. Ho and Y.H. Wong, unpubl. data].

Ligand selectivity may reflect the distinct conformations of a GPCR in association with different G proteins [reviewed in 32]. Since G_{16} couples to a great variety of GPCRs, the coexistence of $G\alpha_{16}$ with other $G\alpha$ subunits in the same cellular milieu may modify the ligand selectivity of a G_{16} -coupled receptor. Emerging experimental evidence speaks for the hypothesis. Binding of specific agonist U50,488 to the κ -opioid receptor (OR) activates G_{16} in COS-7 cells [33]. Yet, co-expression of κ -OR with δ -OR or OR-like receptor 1, but not μ -OR, attenuates the U50,488-elicited G_{16} -mediated PLC β activation [34]. Conformational changes of the 6th and 7th membrane-spanning helices of κ -OR have been studied by substituting cysteine residues with serines and assessing their accessibilities by sulfhydryl reagent (2-aminoethyl)methanethiosulfonate. Interestingly, non-overlapping cysteine residues are found to be sensitive to the sulfhydryl reagent when κ -OR is co-expressed with either $G\alpha_{16}$ or $G\alpha_{i2}$ [35]. Furthermore, the affinity of salvinorin A and U69593, but not dynorphin A (1–13), towards κ -OR is significantly enhanced when $G\alpha_{16}$ is co-expressed [35]. These results suggest that G_{16} can functionally alter the ligand selectivity of the coupled receptor, thereby affecting the pharmacological outcome through the confinement of a subpopulation of the receptors at a particular active state. The concept of receptor-based biased functional selectivity caused by the presence of a defined set of G proteins [36, 37] has been reported [38, 39]. This phenomenon of biased ligand may profoundly affect the functions of GPCRs that are coupled to G_{16} .

Transcriptional Activation by $G\alpha_{16}$

It is believed that $G\alpha_{16}$ has highly organized mechanisms for sorting and amplifying transmembrane signals due to its finely tuned expression regulation. With its unique expression in the hematopoietic lineages, it is plausible that $G\alpha_{16}$ may play a role in hematopoietic cell differentiation and function. Although the underlying mechanisms as to how $G\alpha_{16}$ is involved in the regulation of lineage exclusion and maturation remain elusive, there is evidence suggesting that $G\alpha_{16}$ mediates the activation of several important transcription factors such as STATs and NF- κ B. In combination of the importance of the

Table 1. Receptor-coupling specificity of G₁₆

Receptor category	Subtype	G _{15/16} -coupled?	References
<i>G_i-coupled receptors</i>			
Adenosine	A ₁	G _{15/16}	23, 90
Adrenergic	α ₂	G ₁₆	23
Cannabinoid	CB ₁	G ₁₆	91
Chemoattractant	C3a, C5a, fMLP	G _{15/16}	18, 23, 92–94
Chemokine	CCR1, CCR2b, CCR3, CXCR1, CXCR2	G ₁₆	95–97
	CCR2a, CCR5, CCR7, CXCR4	No	95, 96, AUD
Dopamine	D ₂	G ₁₆	23
γ-Aminobutyric acid, metabotropic	GABA _{B1a} + GABA _{B2} , GABA _{B1b} + GABA _{B2}	G ₁₆	28
Glutamate, metabotropic	<i>Drosophila</i> , mGlu ₂	G _{15/16}	98
	mGlu _{4a} , mGlu _{7a} , mGlu _{8a}	G ₁₅ only	98
Melatonin	MT ₁ , MT ₂	G ₁₆	23
	<i>Xenopus</i>	No	23
Muscarinic acetylcholine	M ₂	G _{15/16}	18, 99
	M ₄	G ₁₅ only	90
Neuropeptide	AF/FF	G ₁₆	100
Opioid	μ	G _{15/16}	18, 33
	δ, κ, nociceptin	G ₁₆	33
Purinergic	UDP-glucose	G ₁₆	101
Serotonin	5-HT _{1A}	G _{15/16}	18
Somatostatin	SST ₁ , SST ₂	G _{15/16}	23, 90
Tastant/L-amino acids	mouse T2R ₅ , human T2R ₁₆	No	102
	T1R ₁ + T1R ₃ , T1R ₂ + T1R ₃	G ₁₅ only	103, 104
Smoothed	–	G ₁₅ only	105, 106
<i>G_s-coupled receptors</i>			
Adenosine	A _{2A}	G _{15/16}	18, 23
Adrenergic	β ₁	G ₁₆	99
	β ₂	G _{15/16}	18, 23, 99
Dopamine	D ₁	G _{15/16}	18, 23
	D ₅	G ₁₆	27
Glucagon		No	27
Histamine	H ₂	G ₁₆	27
Luteinizing hormone		G ₁₆	23, 99
Parathyroid hormone		G ₁₆	27
Prostaglandin	IP	G ₁₆	AUD
Secretin		G ₁₆	27
Thyrotropin-releasing hormone		G ₁₆	27
Vasoactive intestinal peptide		G ₁₅ only	90
Vasopressin	V ₂	G _{15/16}	18, 23, 99
<i>G_q-coupled receptors</i>			
Adrenergic	α _{1A} , α _{1C}	No	23
	α _{1B}	G ₁₆	107
Bombesin		G ₁₆	23
Metabotropic glutamine	mGlu _{1a}	No	98
Muscarinic acetylcholine	M ₁ , M ₃ , M ₅	G ₁₆	23, AUD
Serotonin	5-HT _{1C/2C}	G _{15/16}	18
Thrombin		G _{15/16}	18
Thromboxane	TXA ₂	G _{15/16}	18
Vasopressin	V _{1A}	G _{15/16}	18

AUD = Authors' unpublished data.

transcription factors regulated by $G\alpha_{16}$ and its promiscuity in the coupling of the receptors, many GPCRs would possess the ability to modulate the activities of the transcription factors in response to signals from the extracellular surroundings. Vasoconstrictors such as arginine vasopressin or angiotensin II induce hypertrophy of the vascular smooth muscle cells (VSMC) [40, 41]. Stable expression of constitutively active $G\alpha_{16}QL$ in VSMC stimulated c-Jun N-terminal kinase (JNK) activity, causing hypertrophy of the cells [42]. These results indicate that arginine vasopressin or angiotensin II signals through $G\alpha_{16}$ -coupled receptor, causing the VSMC hypertrophy in a JNK-dependent manner.

In human erythroleukemia (HEL) cells where $G\alpha_{16}$ and $G\alpha_{14}$ are endogenously expressed, stimulation of the prostacyclin receptor by its specific agonist cicaprost induces STAT1 and STAT3 phosphorylation in a PTX-insensitive manner. In addition, phosphorylations of extracellular signal-regulated kinase (ERK) and JNK, but not p38 mitogen-activated protein kinase, can also be induced by cicaprost [43]. Another study indicated that in human embryonic kidney 293 (HEK293) cells expressing a STAT3-responsive luciferase reporter, the $G\alpha_{16}$ -coupled opioid receptor-like (ORL_1) and formyl peptide (fMLP) receptors stimulated luciferase activity upon stimulation by specific agonists. Co-expression of $G\alpha_{16}$ was required and the GPCR-mediated STAT3 activation was dependent on JAK and Raf-1 signaling [44]. More detailed illustration for the requirements of PLC β /PKC/CaMKII, Ras/Raf-1/ERK and Rac1 in the activation of STAT3 is accomplished by the application of constitutively active $G\alpha_{16}$ ($G\alpha_{16}QL$) in heterologous expression systems using HEK293 cells. The non-receptor tyrosine kinases, c-Src, JAK2 and JAK3 are also involved as demonstrated by the use of specific inhibitors and dominant-negative mutants (fig. 2) [45]. $G\alpha_{16}QL$ induces STAT1-dependent c-fos transcriptional activation via PLC β , c-Src/JAK and ERK pathways [46]. The differential involvements of STAT1 and STAT3 in $G\alpha_{16}$ -induced signaling pathways reveals that there are functional differences between these two STAT proteins, although they can always be activated by the same stimuli and form homo- and heterodimers [47, 48]. The biological effects of these two proteins can be antagonistic, as revealed by their actions on cell growth and survival. Given that $G\alpha_{16}$ can activate STAT1 and STAT3 simultaneously, the ultimate response of a particular cell type will depend on the availability of the two STAT proteins as well as the delicate control of the balanced levels between the homo- and/or heterodimer of STAT1 and STAT3.

Fig. 2. Transcriptional stimulation of NF- κ B and STAT3 by activated $G\alpha_{16}$. GTP-bound $G\alpha_{16}$ stimulates PLC β /PKC/CaMKII and Ras/Raf-1/MEK/ERK signaling cascades, leading to the activation of STAT3. Activated $G\alpha_{16}$ indirectly interacts with c-Src, which in turn activates JAK/STAT pathway. Rac1 is required for the full activation of STAT3. Additionally, through both $G\alpha_{16}$ and activation-released $G\beta\gamma$, Ras/Raf-1/MEK/ERK pathway and c-Src become activated, which lead to the stimulation of IKK, followed by the phosphorylation and degradation of I κ B α . The unsequestered p65/p50 heterodimer then translocates into the nucleus, and promotes NF- κ B-dependent transcription. The adaptor protein TPR1 bridges the interaction between activated $G\alpha_{16}$ and Ras, which contributes to the activation of STAT3 and NF- κ B. P stands for the phosphorylation site.

In addition to the regulation of STAT activity, $G\alpha_{16}$ can couple to chemoattractant receptors such as CCR8, C3a, C5a, and fMLP receptors to activate NF- κ B, but the detailed signaling pathways involved have not been delineated [49, 50]. In the resting state, the two subunits of NF- κ B, p65 and p50, are retained in the cytosol by anchoring to the inhibitor- κ B α (I κ B α). Upon cell activation, an upstream I κ B kinase (IKK) complex comprised of the catalytic subunits (IKK α and IKK β) and a linker subunit (IKK γ) becomes activated, and subsequently phosphorylates I κ B α , leading to the degradation of I κ B α . The released heterodimer then translocates into the nucleus and promotes NF- κ B-dependent transcription. Studies of other G_{16} -coupled receptors provide hints on how G_{16} may regulate NF- κ B. Adenosine A_1 receptor (A_1R) can couple to both $G\alpha_i$ [51] and $G\alpha_{16}$ proteins [23, 33]. Stimulation of Reh cells with A_1R agonist, N^6 -cyclohexyladenosine, results in PTX-insensitive phosphoryla-

tion of IKK. Interestingly, only Reh cells expressing $G\alpha_{16}$ supported the N^6 -cyclohexyladenosine-induced IKK phosphorylation and NF- κ B-driven luciferase activity. Further examination revealed the participation of c-Src, as well as signaling intermediates along ERK cascade including Ras, Raf-1 and MEK1/2, in $G\alpha_{16}$ -dependent NF- κ B activation in the Reh cells (fig. 2) [52].

The ability to integrate incoming signals is an important feature of biological systems. The final biological response is the combinational consequences of cell-type specificities, the strength and duration of the signals, and the balanced effects between the elicited signaling pathways. More than one transcription factor might be activated upon encountering a single extracellular stimulation. Recent studies from our laboratory have demonstrated that Lkn-1 stimulation of $G\alpha_{16}/G\alpha_{14}$ -coupled CCR1 leads to the activation of both STAT3 and NF- κ B in HEK293 cells expressing CCR1 together with $G\alpha_{14}$ or $G\alpha_{16}$ [unpubl. data]. $G\alpha_{16}$ may be involved in the modulation of growth factor receptor signaling. Exogenous expression of $G\alpha_{16}QL$ in Swiss 3T3 cells results in the growth inhibition of the cells, and downregulation of the signal pathway in response to platelet-derived growth factor [53]. Transfection of constitutively active $G\alpha_{16}QL$ markedly enhance tumor necrosis factor- α -induced NF- κ B reporter activity in HeLa cells [50]. It has also been revealed that preactivation of $G\alpha_q$ family members including $G\alpha_{16}$ permits subsequent stimulation of PLC β by G_i -coupled receptors through the release of $G\beta\gamma$ dimer. In transiently transfected COS-7 cells, agonists binding of the G_i -coupled δ - and κ -opioid receptors cannot stimulate PLC β unless they are co-expressed with $G\alpha_{16}$ [54]. Another study shows that leukotriene B_4 receptor can activate PLC β_2 -mediated IP accumulation via $G\alpha_{12}$ -derived $G\beta\gamma$, and a synergistic effect could be observed when these cells co-express $G\alpha_{16}$ [55]. Given that both $G\alpha_{12}$ and $G\alpha_{16}$ are expressed in the hematopoietic cells [1, 56], the physiological relevance of the synergistic actions mediated by these two $G\alpha$ subunits deserves further investigations. Altogether, these studies suggest that activation of G_{16} can synergize the effects of cytokines and other GPCRs in regulating transcriptional events.

Binding Partners of $G\alpha_{16}$

New insights on the functions of G_{16} can be gleaned from the discovery of novel binding partners of $G\alpha_{16}$. Recently, an adaptor protein called tetratricopeptide repeat 1 (TPR1) was found to bind to $G\alpha_{16}$ in yeast two-hybrid

screening [57]. TPR1 is a 292-amino-acid protein containing three TPR motifs that was originally identified in the yeast cell division cycle protein Cdc23p [58] and the nuclear protein nuc2 [59], and was subsequently found to be present in many other proteins [60]. It has been demonstrated that TPR1 interacts with Ha-Ras preferentially in its active form. Expression of $G\alpha_{16}$, especially its constitutively active form strongly enhances the interaction between TPR1 and Ras (fig. 1) [57]. In an attempt to define the structural requirement for $G\alpha_{16}$ to interact with TPR1, a series of $G\alpha$ chimeras have been constructed in our laboratory to test for their association with TPR1. Our preliminary data indicated that a stretch of six amino acids (201–206) in the $\beta 3$ region of $G\alpha_{16}$ is required for interaction with TPR1 without affecting the activation of PLC β . Further experiments confirmed that the identified region is devoid of PLC β -stimulating activity. It appears that there are two discrete functional regions of $G\alpha_{16}$ for the activation of PLC β and Ras, two signaling molecules important for the induction of downstream transcription factors such as STAT3 and NF- κ B [unpubl. data].

Regulators of G protein signaling (RGS) protein binds to the active $G\alpha$ subunits and stabilizes the transition state of $G\alpha$ -catalyzed GTP hydrolysis, resulting in the acceleration of GTP hydrolysis, and therefore, termination of G protein signaling [61]. Among the more than 30 RGS proteins identified to date, only RGS2 has been shown to interact with $G\alpha_{16}$ and block of PLC β signaling. RGS2 also inhibited IP production in HEK293 cells expressing the constitutively active $G\alpha_{16}QL$ [62]. Interestingly, GRK2 (a GPCR kinase bearing some resemblance to RGS proteins) selectively interacts with $G\alpha_q$ over $G\alpha_{16}$ [62]. GRK2 binds to the 'switch loops' of $G\alpha_q$ and inhibits its signaling [63]. The amino acid sequence of the switch loop region of $G\alpha_{16}$ is notably different from that in other $G\alpha_q$ subfamily members [3], which might account for the discrimination between $G\alpha_q$ and $G\alpha_{16}$ in their binding to GRK2. It is possible that $G\alpha_{16}$ may interact with a unique array of RGS and RGS-like proteins.

Another recently identified $G\alpha_{16}$ -binding partner, p63RhoGEF, belongs to the guanine nucleotide exchange factor (GEF) family. p63RhoGEF contains the Dbl and pleckstrin homology domains [64], and has been described as a novel mediator of $G\alpha_{q/11}$ signaling to enhance RhoA activation [65, 66]. Physical association between $G\alpha_{16}$ and p63RhoGEF has been recently demonstrated in our laboratory. Further investigation revealed that instead of the enhancement of RhoA signal, overexpression of p63RhoGEF and $G\alpha_{16}$ in HEK293 cells inhibits PLC $\beta 2$

activation and its downstream signaling such as the stimulation of transcription factors STAT3 and NF- κ B [unpubl. data]. These results suggest that p63RhoGEF may play differential roles in regulating the signaling of different G_q subfamily members.

$G\alpha_{16}$ Expression during Myeloid Differentiation

$G\alpha_{16}$ expression fluctuates significantly during lineage differentiation [1, 67]. The pluripotent hematopoietic stem cells in the bone marrow can be divided into two types of progenitors – a common lymphoid progenitor that gives rise to natural killer cells and T and B lymphocytes, and a common myeloid progenitor that gives rise to different types of leukocytes, erythrocytes and megakaryocytes that produce platelets. The leukocytes include monocytes, the myeloid dendritic cells, and neutrophils, eosinophils and basophils. The latter three are generally termed granulocytes. $G\alpha_{16}$ protein expression is high in normal $CD34^+$ cells ($CD34$ is the surface marker for the hematopoietic progenitor cells), then decreases sharply when differentiating into granulocytes and erythrocytes [67]. In contrast, expression of $G\alpha_{16}$ protein remains high during normal monocytic differentiation and in HL60 cells (committed myeloid precursor cell line) differentiating into monocytes when induced by phorbol 12-myristate 13-acetate (PMA) or interferon- γ [67]. Acute leukemias are clonal diseases in which the malignant cells lose their differentiation capacity. In primary myeloid leukemoblasts, as well as leukemic cell lines such as myeloid leukemia HL60, KG-1, HEL, and K562 cells, expression of $G\alpha_{16}$ protein is higher than that found in their normal $CD34^+$ counterparts [1, 67]. These results imply that $G\alpha_{16}$ -dependent signaling may modulate cell proliferation or differentiation, depending on the specific cellular environment. Therefore, elucidating the functions of $G\alpha_{16}$ in hematopoietic cells, and identifying the surface receptors to which they couple, should lead to a better understanding of the mechanisms regulating human hematopoiesis and leukemogenesis.

Further information about the potential roles of $G\alpha_{16}$ accumulated from studies in which $G\alpha_{16}$ expression level or functional activity was altered. HEL cells endogenously express the $P2Y_2$ purinergic receptor that is coupled to $G\alpha_{16}$. $P2Y_2$ -mediated elevation of intracellular Ca^{2+} is suppressed in HEL cells expressing antisense $G\alpha_{16}$; the growth rate is also impaired in this cell line [68]. In MB-02 erythroleukemia cells containing a GFP-reporter sys-

tem that associates with erythroid differentiation, overexpression of wild type, constitutively active $G\alpha_{16}$, or transfection of a $G\alpha_{16}$ antisense plasmid led to significant growth inhibition and differentiation promotion [69]. This data indicate that an optimal functional level of $G\alpha_{16}$ is required for maintaining MB-02 cell proliferation, and any deviation from it will induce cell differentiation.

$G\alpha_{16}$ Expression during B-Cell Differentiation

In accordance with its expression in myeloid lineage, $G\alpha_{16}$ expression is restricted to progenitor B cells and poorly differentiated B-cell malignancies such as pre-B-acute lymphocytic leukemia, but not in its differentiated counterparts such as peripheral mature B cells and non-Hodgkin's lymphoma [1, 70]. When a pre-B-cell line BLIN-1 is stimulated to undergo maturation development, $G\alpha_{16}$ expression was found to disappear during the transition from pre-B to B-cell differentiation stages [70].

The $G\alpha_{16}$ -associated cell surface receptor of B-cell origin is largely unknown. However, there are several pieces of data indicating that GPCRs do exist on B cells and they might play important functional roles in modifying B-cell proliferation and differentiation. In blood cells, the most abundant known GPCRs are receptors for chemokines [71]. Other than the transduction of chemotactic signals, a number of chemokines are also believed to have functions in hematopoietic proliferation and differentiation. Although the definitive influence of chemoattractants on B cells remains elusive, recent studies have demonstrated that there is stage-specific microenvironment (niches) in the bone marrow for the development of B-cell progenitors [72]. A chemokine called stromal-derived factor-1 (SDF-1) plays a pivotal role in the migration and retention of B-cell progenitors in the bone marrow niches, which might indirectly affect their differentiation via cellular compartmentalization [73, 74].

A number of viruses make use of GPCR mimetics to hijack our immune system. Kaposi's sarcoma-associated herpesvirus (KSHV) infects B cells and expresses a viral IL-8 receptor-like GPCR (KSHV-GPCR) which is constitutively active and also capable of binding various CXC chemokines including IL-8, SDF-1, IP-10 (interferon- γ -inducible protein-10) [75, 76]. Exogenous expression of KSHV-GPCR in BJAB cells upregulates numerous cellular transcripts. The most abundantly in-

duced genes are the CC chemokines macrophage inflammatory protein (MIP)-1 α and -1 β [77]. Activation of KSHV-GPCR induces the tyrosine phosphorylation of RAFTK (related adhesion focal tyrosine kinase, also known as Pyk2 and CAK β), a kinase that can be activated when cross-linking the B-cell receptor and acts as an oncogene to stimulate JNK and p38 [78]. Consequently, there appears to be a functional cross-talk between signals generated by ligation of B-cell receptor and KSHV-GPCR, leading to modulation of B-cell proliferation [78, 79].

There is no evidence to indicate how G α_{16} might affect B-cell differentiation in the bone marrow. Strikingly, as revealed in an early report, PLC β was not expressed in murine B cells throughout all developmental stages [80]. Therefore, signals elicited by G α_{16} -coupled receptors may not go through the conventional PLC β -engaged pathways. Data from our laboratory suggests that activated G α_{16} may induce the activation of transcription factors in a PLC β -independent manner. Interaction between G α_{16} and TPR1 allows the coupling of diverse receptors to the stimulation of Ras, as well as the subsequent activation of STAT3 [57]. A recent study shows that the absence of STAT3 impaired bone marrow B-cell development. Mice selectively lacking STAT3 in the bone marrow progenitor cells display reduced numbers of mature B cells which is believed to result from the diminished numbers of B-cell precursors including pro-, pre-, and immature B cells [81]. Given the fact that TPR1 is universally expressed and the fact that G α_{16} expression is dramatically disrupted during pre-B- to B-cell transition, it is conceivable that G α_{16} may regulate B-cell differentiation by modulating gene transcription at particular developmental stages through its interaction with TPR1.

G α_{16} Expression during T-Cell Activation

T cells originate from the hematopoietic stem cells in the bone marrow, and develop into mature naive T cells (CD4 $^{+}$ or CD8 $^{+}$) in the thymus. Many G α subunits including G $\alpha_{q/11}$ and G α_{16} have been identified in human thymocytes [82]. It has been suggested that a coordinated regulation of G α_{16} expression is required for optimal T-cell activation: G α_{16} protein expression reaches the highest level at day 4 in blood T cells after Leuco A stimulation and then decreases afterwards. Responses to Leuco A in Jurkat T cell are strongly inhibited when G α_{16} is overexpressed, and surprisingly, weak inhibition has also been

observed when increases in G α_{16} expression were prevented expressing an antisense G α_{16} construct [83]. Collectively, these data suggest that alterations of G α_{16} expression impair activation-induced T-cell responses.

The T-cell receptor (TCR) complex consists of the highly variable α : β heterodimer which recognizes and binds antigen, the invariant CD3 complex (composed of CD3 γ , CD3 δ , and CD3 ϵ) and ζ chains. Upon anti-CD3-mAb engagement, G $\alpha_{q/11}$ and G α_{16} are activated [84], and TCR/CD3-mediated signaling is inhibited in Jurkat T cells expressing a dominant negative mutant of G α_{16} [85]. A profound defect in the production of IL-2 and IL-10, as well as in the expression of CD69 was observed. Accordingly, significant reductions in anti- ϵ -induced tyrosine phosphorylation of ζ , ϵ , ZAP-70 (ζ -associated protein 70) and intracellular Ca $^{2+}$ mobilization are detected. Similarly, TCR/CD3-mediated activation of two Src-family tyrosine kinases, Fyn and Lck, are also reduced by the same G α_{16} mutant. However, the PMA-induced IL-10 production and CD69 expression, and the ionomycin plus PMA-induced IL-2 production are not affected in mutant G α_{16} -expressed Jurkat cells. Together, these results imply a crucial functional role of G α_{16} in TCR signaling. Recently, we have performed co-immunoprecipitation studies and confirmed that G α_{16} is physically associated with the TCR, and the observed interaction is dependent on the activation of G α_{16} but not the T cells. Constitutively active G α_i subunits are unable to co-immunoprecipitate with the TCR, suggesting the specificity of the interaction between G α_{16} and TCR [unpubl. data]. These studies provide further supportive evidence for the specific association between G α_{16} and the TCR complex, and raise the possibility that signaling through G α_{16} -coupled receptors may affect the activation of T cells. Similar results have been obtained regarding the co-stimulation of T cells by chemokine receptors coupled to G α_q and/or G α_{11} via their recruitment to the immunological synapse [86]. Altogether, these results increase our understanding of the molecular mechanisms responsible for the involvement of G α_{16} in T-cell activation.

Conclusion

We have outlined the evidence demonstrating the distinctive biochemical properties of G α_{16} , together with its proven and speculated biological functions. Identification of the covalent modifications of G α_{16} , which are important for its membrane targeting and signal transduction, is extremely helpful in our appreciation of its func-

tional activity. Sequence substitutions and mutations of $G\alpha_{16}$ help to illustrate the receptor binding promiscuity and advance our understanding of its receptor-coupling capability and specificity. Great achievements have been made in the delineation of the $G\alpha_{16}$ -associated signaling molecules and pathways involved in the activation of the transcription factors, which allow the appreciation to the complexity of the linked signaling network. $G\alpha_{16}$ is uniquely expressed in the hematopoietic cells and in tissues harboring these cells. Some progress about its functions in hematopoietic development and differentiation has been achieved since its discovery. However, due to technical limitations such as the low efficiency of hematopoietic cell transfection, and the complexity of lineage maturation network, questions as to how exactly $G\alpha_{16}$ fits into the hematopoietic signaling and development remain unaddressed. The signaling complexity, and the re-

ceptor binding promiscuity, together with its restrained cell/tissue distribution, raises the possibility that $G\alpha_{16}$ is able to elicit a unique profile of signaling events and cellular responses. It is of great interest in the future to investigate the biological function of $G\alpha_{16}$ and its involvement in the cross-talk with other signaling pathways such as BCR- and TCR-induced lymphocyte differentiation and activation.

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