# An overview on G protein-coupled receptor-induced signal transduction in Acute Myeloid Leukemia

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**Abstract**

**Background:**

Acute myeloid leukemia (AML) is a genetically heterogeneous disease characterized by uncontrolled proliferation of precursor myeloid-lineage cells in the bone marrow. AML is also characterized with patients with poor long-term survival outcomes due to relapse. Many efforts have been made to understand the biological heterogeneity of AML and the challenges to develop new therapies are therefore enormous. G protein-coupled receptors (GPCRs) are a large attractive drug targeted family of transmembrane proteins, and aberrant GPCR expression and GPCR-mediated signaling have been implicated in leukemogenesis of AML. This review aims to identify the molecular players of GPCR signaling, focusing on the hematopoietic system, which are involved in AML to help developing novel drug targets and therapeutic strategies.

**Methods:** We undertook an exhaustive and structured search of bibliographic databases for research focusing in GPCR, GPCR signaling and expression in AML.

**Results and Conclusion:** Many scientific reports were found with compelling evidence for involvement of aberrant GPCR expression and perturbed GPCR-mediated signaling in the development of AML. The comprehensive analysis of GPCR in AML provides potential clinical biomarkers for prognostication, disease monitoring and therapeutic guidance. It will also help to provide marker panels for monitoring in AML. We conclude that GPCR-mediated signaling is contributing to leukemogenesis of AML, and postulate that mass spectrometry-based protein profiling of primary AML cells will accelerate the discovery of potential GPCR related biomarkers for AML.

Keywords: Leukemia; AML; G protein; GPCR; cell signaling; clinical biomarkers.

INTRODUCTION

Among the four main types of leukemia, acute myeloid leukemia (AML) is the deadliest with a 5-year survival rate of only 27% [1]. Leukemia is the broad term of hematopoietic cancers, thus affecting the immune cells in or from the bone marrow. Leukemia can develop slowly or very fast (i.e. chronic or acute), and can affect both the myeloid and lymphatic linage of the hematopoietic system. AML is characterized by uncontrolled proliferation of limited differentiated myeloid cells in the bone marrow [2-4]. Accumulation of these cells often results in reduced number of healthy and mature hematopoietic cells such as leukocytes, red blood cells and platelets in the blood and lymphoid organs. Patient heterogeneity is considerable in terms of the cellular phenotype (i.e. morphology, cytogenetic aberrations and mutations) and clinical outcome [3]. The prognosis is decided by cytogenetic and molecular abnormalities, and these factors are used to stratify AML patients into the following risk groups: favorable, intermediate and poor [4].

Advancing age, male gender and region of diagnosis are other risk factors [5], with age being the most pronounced. The prognostics will further guide the therapeutic decisions, such as who would benefit from an allogeneic hematopoietic stem cell transplantation (allo-HSCT) in their first complete remission, as even patients with a favorable risk have 35-40% chance of leukemic relapse (without allogeneic stem cell transplantation) [6].

To complicate the matter further, the leukemic cell population derived from a single patient can include different subpopulations of cells (i.e. cells with different genetic abnormalities and/or at different stages of maturation). Of particular importance is perhaps the leukemic stem cell (LSC) in the hierarchically organized cell population. This rare cell population (1 in 1.6 $x10^3$ to 1 in 1.1 x $10^6$ cells) can be defined by functional

assays, and their immunophenotype (i.e. surface molecule profile) differs between patients [7].

Important efforts have been put into understanding the biological heterogeneity of AML, aiming at improving the prognostication and therapy. This review will focus on involvement of G protein-coupled receptor (GPCR)-mediated signaling in cancer, especially in leukemogenesis of AML.

GPCRs are a large family of seven transmembrane receptors that binds a variety of endogenous ligands, including inflammatory mediators like chemokines and cytokines (reviewed in [8] and [9]). Proper GPCR signaling is essential in response to inflammation as well as critical for cell proliferation, survival and differentiation. Thus, receptor blocking with novel GPCR antagonists or inhibitors against their membrane-associated heterotrimeric G proteins or protein regulators are attractive cancer drug targets. The field of GPCR signaling is enormous and very well reviewed (as it will be acknowledged along this review). In this review we will just give a brief introduction of the main players in GPCR signaling to, then, focus in the recent achievements on GPCR signaling in AML. This information provides tools for researchers to explore future therapeutic targets.

## GPCR signaling

Many hormones, neurotransmitters, chemokines, odorants, ions and other stimuli act through the activation of different GPCRs that transmit the signal across the plasma membrane activating a number of signaling pathways [10]. Of those, the heterotrimeric G proteins, considered the canonical effectors, and arrestins are best characterized [11]. In the presence of ligand, GPCR undergoes an important rearrangement of internal helices 6 and 3 [12] that in turn triggers the exchange of GDP for GTP (acting as guanine nucleotide exchanges factors) on Gα, and their dissociation from Gβγ that results in the

activation of a plethora of downstream effectors. The cycle is terminated with the hydrolysis of GTP to GDP on Gα, a process accelerated by specialized proteins named Regulators of G protein Signaling (RGS) (acting as GTPase-activating proteins, i.e. GAPs)[13-14] and the re-formation of the heterotrimer. Further termination of the signal involves the phosphorylation of GPCR by specialized G protein receptor kinases (GRKs) and the binding of arrestin proteins [15] with the concomitant internalization of GPCRs. Internalization is also important for signaling since it has recently been proven that GPCRs can continue signaling once internalized in vesicles [16]. On the other hand, arrestin proteins can, in turn, act as adaptor proteins initiating alternative signaling cascades [17] (see figure 1). Furthermore, some ligands selectively activate certain pathways at the expense of others. This process has prompted the search of specialized ligands, named biased agonists, for selective drug development that can distinguish the activation through G proteins from the arrestin-based signal [18].

*GPCRs*

The GPCRs superfamily shares a common structure with seven transmembrane helices and can be grouped into several subfamilies [19-20]. Class A (rhodopsin-like), by far the largest and most studied in humans (more than 700 receptors), binding of the allosteric ligand produces the well-documented shift of the α-helices; Class B1 (secretin receptor-like) and Class B2 (adhesion receptors), with a large extracellular N-terminal domain which contains the high-affinity binding site for their peptide ligands; Class C (metabotropic glutamate receptor-like), work as homo- or hetero-dimers and also have a large N-terminal domain with a bilobal Venus flytrap domain (VFT); and class F (frizzled-like) subfamilies as well as the taste 2 sensory receptor subfamily. High conformational flexibility is a hallmark of GPCRs that together with other modifications

allow them to sense diverse stimuli. Those can regulate the receptor activity through conformational selection of distinct states that in turn selects the signaling response [21]. Among the downstream GPCR-interacting molecules are multiple adaptor and modulatory proteins, besides G proteins and arrestins, such as PDZ-containing scaffolds and non-PDZ-scaffolds. The latest revolution in cryo-electron microscopy and structural biology has allowed the comprehension of the conformational dynamics upon ligand binding and its control in the signaling output [18, 22-23].

*G-proteins*

Although the amount of GPCR family members comprises over 800 members in the human genome, there are a relatively small number of G proteins that trigger a high number of intracellular signaling cascades [24]. A comprehensive analysis of the determinants of GPCR-G protein binding for the entire GPCR-G-protein signaling system has been recently released [25]. Thirty-five different genes encoding for G proteins can be found in the human genome, of which 16 correspond to Gα-subunits, 14 to Gβ and 5 to Gγ [26]. The four major Gα families Gs, Gi, Gq, and G12 [27] regulate different key effectors (for example, adenyl cyclase by the Gi/Gs, phospholipase C (PLC) by the Gq subfamily, Rho by G12 subfamilies) that generate secondary messengers, which trigger different signaling cascades. The Gi subfamily is blocked by pertussis toxin and thereby many different signaling pathways are inhibited, among them chemotaxis triggered by chemokines. The $G\alpha_q$ family embraces four members: $G\alpha_q$ and $G\alpha_{11}$ (ubiquitously expressed and with close protein sequence similarity), $G\alpha_{14}$ (found in kidney, liver and lung) and $G\alpha_{15/16}$ (mouse/human orthologous respectively, expressed only in hematopoietic and epithelial cells) [28]. To date functional redundancy has been assumed for Gq and G11, with few exceptions [29-30]. Besides, both are present in most cells

except for platelets [31] and purkinje cells [32]. Phospholipase C β is considered to be the canonical effector of the $G\alpha_q$ family, although an extensive variety of cellular proteins have been described to interact with $G\alpha_q$ that can either function as effectors, regulators or be considered as accessory proteins [24]. Different non-canonical functions and locations of Gq proteins have also been shown, as the control of mitochondria physiology [33]. The role of $G\alpha_q/G\alpha_{11}$ in regulating multiple cellular and physiological functions is well established: controlling cardiovascular physiology; smooth muscle tone and nervous system [34].

On the other hand, another member of the Gq family, G15, is quite unique in sequence and properties [35]. $G\alpha_{15/16}$ can couple with a variety of GPCRs for PLCβ activation and subsequent $Ca^{2+}$ mobilization and downstream signaling in the cells [28] [36] [37]. Moreover, G15-coupled signaling is quite resistant to GPCR-internalization by arrestins [35, 38] and is phosphorylated by protein kinase C (PKC) [39]. Although all these properties confer the protein's functional differences, its specific function has remained quite elusive. But on the other hand, these properties have been used to make $G\alpha_{15}$ as a laboratory tool for functional studies of ligand binding to orphan GPCRs [37, 40-41], reviewed in [38].

**Regulators of GPCR signaling: RGSs, GRKs, and Arrestins**

*RGS Proteins*

The prototype role of RGS proteins is the acceleration of GTP hydrolysis by Gα, promoting the re-association of Gα and Gβγ subunits with the receptor. RGS stabilize the transition state conformation lowering the free energy required for the hydrolysis reaction [42-43]. Hence, they regulate the magnitude and duration of the cellular response by GPCRs [42, 44]. There are 20 canonical members of the RGS family in mammals grouped

in four subfamilies. Almost all containing a core domain of 120 amino acid, i.e. the RGS-domain, which mediates interaction to Gα subunits. Additionally, they contain non-RGS domains or modulatory regions that either gives G-protein specificity or additional roles [45]. Multiple RGS proteins are expressed in a given cell and tissue making the study of their physiological function very challenging. Nevertheless, along the years many studies have contributed to understand their involvement in the control of many physiological processes including cardiovascular biology, metabolism, inflammation and neurophysiology [46-47]. On the other hand, RGS are implicated in multiple pathologies such as cardiovascular (hypertension and atherosclerosis) and neurodegenerative disorders (schizophrenia, depression, addiction, anxiety and many others) [13, 48], and references herein. RGS proteins are key modulators of many physiological systems and they are tightly regulated by different mechanisms ranging from protein subcellular localization, protein stability, transcriptional control or epigenetic regulation.

*GRKs*

G protein receptor kinases (known as GRK) are members (7 in mammals) of the AGG kinase family that specifically recognize and phosphorylate agonist-bound GPCRs in the C-terminal tail and/or cytoplasmic loop [49-51] and, together with arrestins, are part of the mechanism for desensitization of the response. The GRK family members are multidomain proteins with a central catalytic domain necessary for the phosphorylation of serine/threonine residues at the C-terminal and internal loops of the agonist-stimulated GPCRs. GRKs can be subdivided into three main groups: visual GRK (GRK1 and GRK7), the β-adrenergic receptor kinase (GRK2 and GRK3) and the GRK4 subfamily (GRK4, GRK5 and GRK6). The non-catalytic domain of the GRKs houses the regions (like RH and PH domains) involved in the interaction to other cellular partners and

regions for modulation of their activity that coordinate the recruitment and activation of the different isoforms [51]. GRK2 is the most abundant and studied isoform and is the isoform that provided the finding that GRK-mediated phosphorylation promoted GPCR endocytosis [52]. Further it was proven that GRK2 interacts with several proteins that are involved in or regulates the endocytosis process, like clathrin, GRK-interacting protein 1 (GIT1), phosphoinositide-3-kinase (PI3K) and ezrin. More recently, it has been shown that GRK2 displays a complex interactome, for instance it interacts with Gαq, mitogen-activated protein kinase kinase (MEK), serine-threonine protein kinase (AKT) and Raf kinase inhibitor protein (RKIP) [53-54]. This led to the suggestion that GRKs, as arrestins, can act as scaffold proteins to form signaling platforms on the receptor [17, 55-56]. GRK2 participates in basic cellular processes such as migration, cell-cycle progression, among others.

*Arrestins*

Arrestins are small globular proteins that bind specifically to the broad family of active phosphorylated GPCRs and numerous non-receptor partners [57]. The arrestin family has four members in mammals: arrestin-1 (known as visual or rod arrestin); arrestin-2 (also called β-arrestin-1); arrestin-3 (β-arrestin-2) and arrestin-4 (cone arrestin). Arrestins modulate GPCR activation by direct competition with G proteins [58]. Their recruitment to phosphorylated GPCRs arrest G protein binding through steric hindrance and induces receptor internalization from the cell surface through clathrin-coated vesicles [59-61]. It is well documented that arrestins can also serve as adaptor/scaffold proteins that connect and promote multiple independent signaling pathways [17, 62-63]. Their high flexibility ensures their ability to scaffold multiple proteins [64]. Arrestins, β-arr1 and β-arr2, present different properties in terms of their affinity for GPCRs, subcellular localization,

interacting partners and signaling [17, 52, 65-66]. Extracellular signal-regulated kinase 1/2 (ERK1/2) is one of the best characterized example of interaction partners, but proto-oncogene tyrosine kinase Src [67], small GTPases, transcription factors, PI3K/AKT proteins, proteins from the wingless-type MMTV integration site family (Wnt)/β-catenin pathway [68-69] and cytoskeletal proteins are also included (see [57] for extended list). Surprisingly, two recent studies [70-71] have suggested that arrestins control the amplitude and kinetics of ERK, as other multiple studies have shown, but only in the presence of G proteins. What it is clear is that arrestins acting as scaffolds, bind key pathway intermediates that influence the tonic level of pathway activity in cells and, in some cases, serve as ligand-regulated scaffolds for GPCR-mediated signaling [72-73]. Therefore, arrestins play important roles in embryological development, perhaps reflecting their interaction with non-GPCR elements of the Sonic hedgehog (Shh)-Smoothened, Wnt, and Notch signaling pathways [68].

## GPCR signaling in hematopoietic cells

### *GPCRs in hematopoietic cells*

G protein–coupled receptors (GPCRs) are expressed in hematopoietic cells and their function is only partially understood (see model in Figure 1). Hematopoietic cells change location during development and circulate in mammals throughout life, moving in and out of the bloodstream to engage different niches. The interaction with the surrounding environment is very important for the regulation of the hematopoietic cell fate. The migration and circulation of various types of blood cells is regulated by chemokines in particular, membrane proteins, its GPCRs, and other GPCRs expressed in hematopoietic and lymphoid tissues [74]. CXCL12 (also called SDF-1) is one of the most abundant and important chemokine that regulates HSC (hematopoietic stem cells) quiescence and

differentiation [75-76]. CXCR4, the receptor for CXCL12, is expressed by more than 95% of hematopoietic cells in bone marrow including HSCs and hematopoietic progenitors. CXCL12 is expressed by heterogeneous populations of cells: mesenchymal stem and progenitor cells (MSPCs) and sinusoidal endothelial cells, which express the highest amounts, as well as osteoblasts besides certain hematopoietic cells. In addition, other cytokines such as granulocyte colony-stimulating factor (G-CSF) act partially through the modulation of CXCL12/CXCR4 signaling to induce hematopoietic stem cell mobilization in the bone marrow [77-78]. CXCL12 can also cross-talk with other GPCR signaling pathways, including sphingosine-1-phosphate (S1P) and Lipoprotein (A) (LPA) [79]. Both S1P and LPA synergistically enhance the chemotactic migratory response of the hematopoietic stem cells to CXCL12 [80-82].

The complement receptor C3aR is another inflammatory GPCR [83]. Similar to CXCR4 antagonists, blocking of C3aR augmented G-CSF mobilization of hematopoietic cells [84]. Interestingly, expression of endocannabinoids by stromal cells modulate G-CSF mobilization via the endocannabinoid receptor CB2 [85], but in contrast to antagonists of CXCR4 and C3aR, which promote circulation of hematopoietic cells, CB2 antagonists reduced G-CSF–induced stem cell mobilization [86]. Other GPCRs expressed in hematopoietic stem cells are the cysteinyl leukotriene D4 receptor (cysLT1) [87] and lysophospholipid receptors such as $S1PR_1$ that differentially regulate chemotaxis, adhesion, and proliferation [88]. The co-activation of both Gq and Gi by cysLT1 results in stronger proliferation of hematopoietic stem cells than stimulation of Gi by CXCL12 or S1P alone [88].

On the other hand, about one-third of the 33 human adhesion GPCRs are expressed in hematopoietic stem, progenitor, or mature cells, where they define distinct cellular populations (see [89]for an extensive study of adhesion GPCRs in immune system). In

particular, G protein-coupled receptor 56 (GPR56) is a versatile marker for all human cytotoxic lymphocytes, including natural killer (NK) cells and $CD4^+$ and $CD8^+$ T cells [90]. Interestingly, in a whole-blood gene expression meta-analysis in 14,983 individuals of European ancestry, GPR56 was the second most highly upregulated gene associated with age [91]. CD97 is expressed in both immature hematopoietic stem and progenitor cells (HSPCs), as well as more differentiated peripheral blood cells. Adhesion G Protein-coupled receptor L1 (LPHN1), GPR124, GPR125, Cadherin EGF LAG seven-pass G-type receptor 3 (CELSR3), GPR113, GPR114, and GPR126 are equally expressed in hematopoietic progenitor cells and granulocytes. GPR125 is expressed in noncommitted HSPCs ($CD34^+$ $CD45RA^-$) and also in B cells and erythroid precursors. In contrast, expression of CD97, Egf-Like Module-Containing Mucin-Like Hormone Receptor-Like 2 (EMR2), and EMR3 is low in HSPCs, but gradually increases upon differentiation, reaching maximum expression levels in terminally differentiated mature peripheral blood granulocytes.

*Gq subfamily in hematopoietic cells*

Although the different members of the Gq subfamily of G proteins are present in the hematopoietic cells where they couple to different membrane receptors, their role in hematopoiesis has remind controversial. On one hand, Gq/11 has been shown to couple to some chemokine receptors (CCR2, CXCL12 and CXCR4) [92-95], but it doesn't seem to be required for leukocyte chemotaxis that it is mainly driven by Gi proteins [96]. Other GPCR receptors present in hematopoietic cells also couple to both Gi and Gq/11. On the other hand, it was shown that Gq-deficient (GNAQ−/−) monocytes are unable to migrate to inflammatory sites and lymph nodes *in vivo*, demonstrating that Gq-coupled chemokine receptor signaling pathway may be needed for the initiation of the immune

responses [93]. Notably, Gi and Gq/11 proteins are involved in dissemination of myeloid leukemia cells to liver and spleen, whereas bone marrow colonization involves only Gq/11 [97]. Therefore, $G\alpha_q$ activation could contribute to determining the commitment and threshold of hematopoietic cells either to migration or activation [98] (see [8] for a recent review).

As mentioned before one feature of $G\alpha_{15}$ is its high degree of promiscuity,

its presence in immature bone marrow cells and, that its expression decrease upon cell maturation [99]. In particular it has been shown that $G\alpha_{15}$ expression is mainly present with the CD34 marker for stem and progenitor cell population [100-101]. Consistent with this several chemokine receptors like CCR1, CCR2, CXCR1 and CXCR2 have been shown to couple to $G\alpha_{15}$. These receptors utilize $G\alpha_{15}$ to activate nuclear factor NF-kappa-B (NF-$\kappa$B) [38, 102-103]. Likewise, chemoattractant receptors such as CCR8, C3a and C5a have been reported to activate NF-$\kappa$B via $G\alpha_{15}$ [102, 104]. Moreover, CCR1-induced STAT3 (signal transducer and activator of transcription 3) tyrosine phosphorylation and subsequent production and release of the chemokine CXCL8 in THP-1 macrophage like cells can also be mediated through $G\alpha_{14/15}$ signaling [105]. Therefore, it was quite puzzling the results that show Gα15 knockout mice display normal maturation of all cell lineages and a normal response to the immune challenges [106]. Another feature of $G\alpha_{15}$ is its resistance to arrestin-dependent desensitization. Taking this into account, it has been proposed that $G\alpha_{15}$ could be relevant in exceptional conditions, as could be the case of intense GPCR activation in high proliferation states, in certain immune responses or in cases like cancer [35].

*RGS in hematopoietic cells*

Importantly, RGS proteins are highly enriched in the hematopoietic compartment and their function have been associated with the immune system and with hematopoiesis and platelet formation [107]. For example, RGS1, RGS2, RGS10, RGS13 and RGS18 are expressed in lymphocytes (see [108] and [109] for details). Perhaps the most important G-protein-coupled receptors in lymphocytes regulated by RGS proteins are the chemokine receptors. Of interest is the fact that RGS1, RGS3, RGS4, and RGS13 in B lymphocytes and RGS16 in T cells impair chemokine-induced signaling [110-114]. In particular, RGS1-deficient mice are hyper-responsive to the chemokines CXCL12 and CXCL13, which results in abnormal architecture of the spleen [115]. RGS2 targeted mutation in mice cause a reduction in T-cell proliferation [116]. On the other hand, hypertension in humans is associated with reduced expression of RGS2 or mutations in its gene [117-119]. RGS13 is expressed in B and T lymphocytes and mast cells and its function has been associated to B and T-cell migration and/or differentiation besides controlling mast cell allergic inflammation [120].  RGS13 function is also associated to CXCR4-mediated migration of T cells [121]. On the other hand, other reports have demonstrated the involvement of RGS16 and RGS18 in megakaryopoiesis and/or platelet function (see [107] for more details). RGS18 was actually present in progenitor and mature myeloerythroid and lymphoid lineage blood cells [122].

*GRKs in hematopoietic cells*

GRKs are critically involved in immune response through regulation of cytokine receptors in mature leukocytes, but their role in hematopoiesis is largely unknown. GRK2 phosphorylates and regulates several chemokine receptors such as CCR5, CCR2b, CXCR4 [49, 123]. Altered GRK2 levels are observed in several cardiovascular and inflammatory pathologies. Interestingly, GRK2 is highly expressed in different cellular

types of the immune system [124]. In T cells and monocytes decreased GRK2 levels correlate with enhanced ERK activation and cell migration in response to chemokines [125-126] while in some other cellular models GRK2 down-regulation impairs migration [127]. Moreover, GRK6 is highly expressed in vertebrate immune organs and peripheral blood cells [128-129]. Interestingly, GRK6 knockout mice show increased severity of acute inflammatory arthritis [130] and colitis [131] because of enhanced granulocyte chemotaxis, and develop autoimmune diseases due to impaired macrophage engulfment [132]. GRK6 regulates chemotaxis through SDF/CXCLs-CXCR4 [126, 133-134] leukotriene B4-induced CGRP receptor [135] and BLT receptor [136] activation. Also, it has been reported that the expression and activity of GRK6 change during differentiation of the promyelocytic cell line HL-60 [137] suggesting the potential involvement of GRK6 in earlier leukocyte development. Recently, it has been shown that GRK6 knockout mice exhibit lymphocytopenia, loss of the hematopoietic stem cell (HSC) and multiple progenitor populations, demonstrating the importance of GRK6 in regulation of hematopoietic stem cell self-renewal [138].

*Arrestin proteins in hematopoietic cells*

In the immune system, *β*-arrestin scaffolds perform key roles through the negative regulation of G protein–mediated responses, promotion of chemotaxis, regulation of exocytosis and degranulation, and signal dampening through sequestration of pathway components [139]. β-arrestins regulate macrophage chemotaxis both by desensitizing chemokine CCL2-induced $Ca^{2+}$ signaling and by scaffolding ERK1/2-dependent assembly of the actin cytoskeleton in pseudopodia [49, 140]. In polymorphonuclear leukocytes, β-arrestin1–bound hematopoietic cell kinase (c-Hck) and proto-oncogene c-Fgr regulate IL-8 CXCR1 receptor–stimulated granule exocytosis [141], similar to the

reported role of a β-arrestin1–c-Yes complex in the control of endothelin-1–stimulated translocation of exocytic granules containing the glucose transporter GLUT4 [142]. Isolated polymorphonuclear leukocytes lacking β-arrestin2 exhibit increased basal and lipopolysaccharide-stimulated release of the inflammatory cytokine TNF-*α* and IL-6 [143], perhaps due to the loss of tonic inhibition of NF-*κ*B transcriptional pathways by β-arrestin2–dependent sequestration of I*κ*B*α* and I*κ*B kinases [144]. β-Arrestin2 also negatively regulates the activity of natural killer cells by recruiting the protein-tyrosine phosphatases SHP-1 and SHP-2 to the inhibitory killer cell immunoglobulin-like receptor 2DL1 (KIR2DL1) [145]. Zebrafish embryos lacking *β*-arrestin1 fail to undergo hematopoiesis and exhibit severe posterior defects resulting from downregulation of *cdx4*, a homeobox transcription factor that specifies the hematopoietic lineage by modulating *hox* gene expression [146].

**GPCR signaling and cancer**

The balance between activation and de-activation in GPCR signaling is crucial for cell homeostasis and loss of it may recur in pathologies. Several studies indicate that GPCRs and their signaling pathways control different aspects of cancer progression. As stated before there are many current drugs that target GPCRs [147] but conversely there are no current drugs for treatment of cancer of specialized GPCRs. GPCR activity can be altered in cancer through changes in their expression levels or in increased production and/or secretion of their ligands, also through gain-of-function activating or inactivating mutations (estimated to be present in 20% of human cancer), by both tumor cells and surrounding stromal cells (see reviews [148-153]). The Catalogue of Somatic Mutations in Cancer [154] reveals the presence of mutations in multiple GPCRs. Among those GPCRs: CXCR5, GPR183, GPR153, GPM8, DRD2, LPHN3, P2RY2, P2RY8, FZD1,

F2RL2, NPSR1 and GPRC6A are found in hematopoietic and lymphoid tissues [153]. A more recent study has approached the expression of GPCR in cancer cells using TaqMan qPCR [155]. The analysis revealed that certain cancer cell types may possess a "GPCR signature" with no mutations but altered expression of several GPCRs. The authors found that in patients with B-cell Chronic Lymphocytic leukemia (CLL), cells express 106 common GPCRs but some of them, e.g. GPR92, GABBR1, CNR2, CELSR1 are overexpressed compared to normal B cells [155]. Additionally, certain GPCRs (e.g., CD97 and GPR56) are found highly expressed in multiple types of cancer, including AML [156-157]. Moreover, high expression of CXCR4 has been associated as prognostic predictor associated with poorer clinical outcome [158-160]. Malignant transformation and oncogenesis can also be obtained by expression of G proteins in constitutively active state (GTP-bound) either by blocking the ability to hydrolyze GTP (i.e., GTPase-deficient mutants) or by reducing its sensitivity to the action of GAPs (i.e., GAP-insensitive). GNAS is the most frequently mutated G proteins in human cancer ([161], see references herein). Active mutations in GNAS have been found in pituitary tumors, thyroid adenomas, colon cancer, pancreatic tumors, hepatocellular carcinoma, parathyroid cancer and a few others. The most frequent gain of function mutation of GNAQ or GNA11 is found in around 60% of ocular melanomas, in meninges (59%), in most blue nevus of the skin (83%), and in a subset of cutaneous melanomas linked to chronic sun-induced damage (around 6%). GNA11 and amphiregulin (AREG) are also downregulated in B-lineage acute lymphoblastic leukemia (B-ALL) [162] and GNA15 is significantly mutated in skin melanoma. Of interest, mutations in GNA13 are found in a significant amount of cancers derived from hematopoietic and lymphoid tissues obtained from a whole genome study data from COSMIC v62, in addition to GNAI1 and GNAI2 [153]. In fact, suppressive mutations in Gα13 and its downstream effector Rho were found in

Burkitt's lymphoma and diffuse large B- cell lymphoma (DLBCL), which led to suggest that in fact these proteins act as tumor suppressors. On the other hand, RGSs can function as both inhibitors and promoters of cancer progression in breast, ovarian, lung and prostate cancer as they can function as GAP or GAP-independent mechanisms for G proteins and GPCR signaling pathways. For example, RGS1 expression in DLBCL was associated with poor prognosis [163]. RGS13 is increased in adult T cell leukemia/lymphoma [164-165]. Adding another layer of complexity, changes in GRKs expression or activity will have an impact on the amplitude of GPCR signaling and in turn mediate tumorigenesis. Recently reviewed in Nogués et al. [166], GRKs have been suggested to be relevant regulators of cancer progression, in particular due to their role as main modulator of chemokines. Specifically, changes in GRK2 levels or functionality have been reported to affect mitogen-activated protein kinase (MAPK)/ERK activation and cell proliferation in different ways, depending on the cell type and mitogen stimuli involved. The mechanisms underlying such effects can be varied, including “canonical” desensitization of G-protein–dependent MAPK stimulation by GPCR, modulation of GPCR-*β*-arrestin-MAPK cascades or of GPCR crosstalk with epidermal growth factor receptor (EGFR) or other growth factor receptors or by directly interacting and/or phosphorylating non-GPCR cellular partners [167]. Moreover, GRKs appear to play a central role in tumor endothelium functionality and in the homing of immune cells to the tumor microenvironment. Thus, it is tempting to suggest that concurrent changes in the dosage of different GRKs in vascular endothelial cells and in circulating monocytes and other immune cell types might cooperate in fueling tumor progression [166].

Finally, acting as a scaffold protein, β-arrestin1 and β-arrestin2 are also important for both initiation and progression of tumors. In particular, β-arrestin2 is influential in chronic myeloid leukemia (CML) by inhibiting the Wnt/β catenin pathway [168-169]. On

the other hand, β-arrestin1 has been shown to mediate nicotine-induced metastasis through e2f1 [170], ovarian cancer cell invasion though β-catenin [171] and breast cancer through hypoxia-inducible factor 1 (HIF-1)-dependent vascular endothelial growth factor (VEGF) expression [172].

Besides the presence of different mutations in GPCRs and their signaling proteins, changes in expression levels or activity of these proteins can also regulate important cellular functions necessary for cancer, such as proliferation, apoptosis and migration. Of particular interest is the fact that many different types of cancer that involves inappropriate GPCR signaling pathways described before have an altered Wnt/β-catenin pathway in common [173]. Wnt signaling plays critical roles in development and diseases. In fact, Wnt/β-catenin signaling contributes to the transformation of hematopoietic stem cells (HSC) into LSCs [174]. It is clear that a comprehensive picture of the complex of signaling pathways by GPCR is needed to design signaling-biased proteins with scientific and therapeutic potential.

**GPCR signaling in AML**

As mentioned before, AML is a heterogeneous disease with multiple molecular pathways driving its progression and the impact of GPCR signaling proteins is just starting to be investigated (Table 1).

*GPCRs in AML*

A recent analysis using next-generation sequencing (RNA-seq) has addressed the analysis of GPCRs in AML [157]. In a significate cohort of AML patients (n=772), Maiga et al. investigated the expression of GPCRs (transcriptome) in samples from bone marrow and peripheral blood and compared it with normal CD34-positive cells. They found as much as 30 different GPCRs upregulated and 19 GPCRs downregulated in the primary AML

cells. The upregulated GPCRs included the adhesion family (EMR1, EMR2, CD97 and GPR114), as well as members of the chemokine receptor family (CCR1, CCR2, CCR7, CCRL2, CXCR1 and CXCR4) and some members of the purinergic receptor family like P2RY2 and P2RY13. Among those, a key GPCR in AML seems to be CXCR4. In an independent work, Spoo et al. found that AML patients with low CXCR4 expression, as assessed by flowcytometry, had a significantly longer relapse-free survival and overall survival than patients with intermediate or high CXCR4 expression [159]. Moreover, receptor blocking with CXCR4 antagonists such as plerixafor increase remission rate for patients undergoing chemotherapy and have a positive effect on stem cell mobilization with G-CSF in transplanted patients [175-176]. As for the purinergic receptors, it was shown that higher expression of the purinergic receptor P2RY14 is linked to relatively poor survival compared to AML patients having lower expression [177]. Although this receptor has not been studied much in hematopoietic malignancies, it seems to have a role in the localization of hematopoietic stem cells (HSCs) and in promoting regenerative capabilities following injury. The authors' show that cells that displayed increased levels of expression of P2RY14 show resistance to PI3K/mTOR inhibition. The PI3K/mTOR pathway is the second most frequently deregulated pathway in a majority of cancers and it is one of the characteristics of the AML cells. They also show that the inhibitory effect of P2RY14 involved the activation of ERK pathway. On the other hand, the adhesion GPCR family member CD97 is a well-known LSC specific marker in AML [178]. It is frequently expressed in CD34+CD38- LSC and its expression correlates with poor chemotherapy effect and prognosis, and higher recurrence rate [156, 178]. Mutations in the *FLT3* gene, which is a land-mark of AML, is associated with AML samples with high levels of CD97 expression [156]. Moreover, CD97 was recently reported to be a critical regulator of AML stem cells [179], [178]. Martin et al. [179] verified that CD97 had 10-

fold higher expression on LSC-enriched (CD34+CD38-) blasts, as assessed by flowcytometry, compared to HSCs in all AML patients (n = 30) inspected. Another recent study to monitor minimal residual disease after AML treatment, also found increased gene expression levels of CD97 (and also CX3CR1/GPR13) among 22 markers aberrantly expressed in leukemic cells from 157 AML patients [180]. The authors suggest that these markers can help to discriminate between residual cells and normal cells. Another adhesion receptor found [157] to be correlated to AML is GPR56. GPR56 is under-expressed in AML patients' cells compared to normal CD34-positive cells, however high expression of GPR56 has previously been reported as an LSC-specific signature, as assessed by xenografting primary human AML cells into immunodeficient mice, for AML patient samples (n = 16) [7]. Moreover, high GPR56 expression level on AML LSC was recently reported to have high repopulating capacity and thus contributes to the development of AML in xenograft studies in mice [181-182]. The highly expressed GPR56 LSC signature was associated with various high-risk genetic lesions and poor outcome [182]. GPR56 has also been associated to the maintenance of HSCs [183]. In the case of the GPR84, increased levels of the receptor were found in AML LSCs compared to normal cells [178, 184]. The same study showed that GPR84 sustained aberrant β-catenin signaling and that GPR84 impaired stem cell leukocyte function and inhibited the development to an aggressive and drug resistant subtype of AML. Another upregulated receptor in AML is the proton sensing G protein coupled receptor 132 (GPR132), also termed G2A [157]. Interestingly, the GPR132 agonist ONC212 reduced cell viability in AML cells, thus GPR132 is a potential therapeutic GPCR target in leukemia [185-186]. The involvement of GPR132 in AML have in fact prompted M.D. Anderson and Oncoceutics [187] to declare their intention to bring to Phase I and Phase II clinical trials ONC212 for patients with refractory acute myeloid leukemia (AML).

Several downregulated GPCRs that were found in the study of Maiga et al., also belonged to the adhesion GPCR family (including GPR125, GPR126, LPHN1 and CELSR3). In addition, protease-activated and Frizzled family receptors were found lower expressed in AML patient cells compared to normal CD34-positive cells [157].

As mentioned before, it has been long assumed that relapse of AML arises from a population of leukemia stem cells (LSC) that were dormant and therefore likely protected from chemotherapy. But this concept has been challenged by a recent study [188] that provides evidence that, in fact, LSCs are not present after chemotherapy. On the contrary, they found a small population of cells that they named "leukemic regenerating cells" (LRCs) that were only present after chemotherapy and not in healthy regenerating cells in the bone marrow (HSC). These cells had a gene expression profile distinctly different from that of LSCs. One feature of these cells was the functional association and the production of several G-protein-coupled receptors (GPCRs), among them elevated levels of GPR1, GPR139, DRD2, GRM5 and GPR148. The authors found that antagonist treatment of one of these receptors, DRD2, had profound effects on regenerating LRCs in chemotherapy-treated mice. The possibility to use markers of LRC will discriminate between relapsing versus disease-free survival in human AML patients. It also highlights the importance of identifying specific markers to monitor resistance after chemotherapy. Opening also the chances for specific therapy directed towards the small population of regenerating cells, as stated by the editor [189].

*G proteins in AML*

As described above, CCR1, CCR2β and CXCR1 may interact with Gα15/16, which subsequently bring about phosphorylation and transcriptional activation of NF-κB [38, 102-103]. NF-κB is often found constitutively activated in AML patients' cells and is

associated with resistance to apoptosis and increased proliferative signaling (reviewed in[190]). Thus, receptor blocking of one or several of these chemokine receptors and/or selective inhibitor drug against the Gα15 protein may be a therapeutic option, which dampen downstream signaling and NF-κB activation. Interestingly, by performing gene expression profiling of the LSC-enriched CD34+ fraction from AML patients (n=46), de Jonge et al. found high transcription level of Gα15 (GNA15), as well as ankyrin repeat domain 28 (ANKRD28) and UDP-glucose pyrophosphorylase (UGP2), which was significantly linked to poorer overall survival in two other independent larger cohorts (n=163 and n=218) of AML patients with normal karyotype [191]. Approximately 40-50 % of all AML patients show no aberrant karyotype [191]. Thus, Gα15 transcription expression analysis may be useful for risk-benefit evaluation of potential allotransplanted patients with normal karyotype AML.

Wang et al. previously demonstrated that the Wnt/β-catenin signaling pathway is required for mixed-lineage leukemia (MLL)-AF9-induced AML in mice [192]. MLL-translocations and fusion genes are found in approximately 10 % of AML cases and are generally associated with poor prognosis (reviewed in [193]). Interestingly, a crucial role for Gαq and downstream β-catenin signaling in maintenance of AML-LSC were recently reported [174]. By using selective inhibition of Gαq, as assessed with the GP-antagonist 2A inhibitor peptide of Gαq or shRNA silencing, in pre-LSC and leukemic cells it was demonstrated that Gαq promotes proliferation and extends survival of leukemic cells both in vitro and in vivo [174]. Interestingly, Gαq-inhibition was linked to reduced expression of mitochondrial complex 1 subunits and impairment of the oxidative phosphorylation in the myeloid leukemic cells. The results agree with previous data that show regulation of mitochondria respiration capacity by Gq proteins [33]. Thus, leukeomogenesis of AML-

LSC may be associated to dysfunctional mitochondrial function via Gαq- and subsequent β-catenin- signaling. In another study, $G\alpha_{11}$ (GNA11) [162] was found downregulated in AML patients together with amphireguling (AREG), albeit the results were found in only two patients. On the other hand, functional platelet-activating factor (PAF) receptor and Gq protein were detected in AML and ALL patient cells [194]. In a recent study, utilizing parallel targeted next generation sequencing, changes in GNAQ expression were found in childhood AML (n=20) compared to adult AML [195]. In another study searching for GNAQ-Q209 mutation in different tumors including AML, breast, colorectal, lung, glioma, ovary, pancreas, thyroid and melanomas, the mutation was only found in blue nevus samples (n=13) [196]. Hence, it seems that the GNAQ activated mutation can only be found in specialized cells. Thus, the potential of GNAQ and GNA11 as a marker for AML is promising, but it will need further investigation.

*RGSs in AML*

Given the important role that RGSs have in controlling chemokine signaling and their pattern of expression it is plausible that they play an important role in tumor induction or proliferation of hematopoietic cells. In fact, it was detected decreased expression of RGS2 in AML patients that also presented fetal liver tyrosine kinase 3 internal tandem duplications in the juxtamembrane domain (Flt3-ITD) [197]. The authors demonstrated that forced expression of Flt3-ITD in myeloid cell lines downregulated RGS2, whereas overexpression of RGS2 inhibited Flt3-ITD-induced phosphorylation of Akt and clonal growth of myeloid cells. RGS2 mRNA expression in primary AML bone marrow samples was repressed in the majority of cases compared with controls from healthy donors, also in the absence of activating Flt3 mutations. They also presented a strong correlation of RGS2 expression and myeloid differentiation in several leukemia cell line models. On

the other hand, the authors demonstrated that the cytoplasmic RGS2 levels could regulate $G\alpha_q$ activity and thus serve as a modulator of GPCR signaling and receptor tyrosine kinase crosstalk in AML. Thus, RGS2 and other RGSs can emerge as new targets for AML therapies. Interestingly, Mosakhani and collaborators identified miR-363 as a miRNA in samples from patients that respond poorly to chemotherapy in AML [198]. The levels of miR-363 are known to increase in CD4+ cells from peripheral blood mononuclear cells [199], and one of the targets of miR-363 is RGS17. RGS17 is associated with chemoresistance, and its high expression leads to a reduced susceptibility to chemotherapeutic cytotoxicity [200]. As stated before, other RGSs are expressed specifically in hematopoiesis, for instance RGS18, thus it may be reasonable to think that future studies may uncover other RGS targets for AML.

*GRKs in AML*

Although there is not a clear link between the GRKs and AML, multiple small molecule kinase inhibitors are currently being developed for this disease [201]. It has been described that Akt, a critical substrate of PI3 kinase, is activated in AML blasts [202]. Moreover, aberrant PI3K/Akt/mTOR signaling has been implicated in many human cancers, including AML [203]. Efforts to exploit pharmacological inhibitors of the PI3K/Akt/mTOR cascade are currently under investigation. Interestingly, GRKs has been described to interact and modulate PI3K function [54, 204].

*Wnt signaling in AML*

As we have mentioned before, growing evidences from both preclinical and clinical investigations reveal the critical role of Wnt signaling for the development of many cancers and their response to chemotherapy [173]. Specifically, Wnt inhibitors reduce

proliferation and chemoresistance of AML cells in culture or co-culture with bone marrow stroma cells. Thus, active Wnt signaling appears to play an important role in the propagation/acceleration of AML and has been shown to be an important secondary oncogenic event to transform pre-LSCs into LSCs in mouse models of AML [205]. Interestingly, it has been described that Wnt-pathway inhibitors, which inhibit the interaction between β-catenin and LEF1, selectively induce cell death in AML cell lines and primary AML blasts [206].

**Protein biomarkers for AML**

The perturbed expression of GPCRs and signaling proteins described previously are examples of potential biomarkers that can be used in diagnostics or prognostic evaluation of individual cancer patients. In AML, new biomarkers are needed for better classification and hence further personalized treatment of AML patients. A biomarker or a biomarker panel that can predict the therapy response would help the hematological clinicians to better identify patients that will benefit from allo-HSCT early during the first remission after standard chemotherapy.

A biomarker can take many forms (e.g. gene mutations or altered abundances or presence of cells, transcripts, proteins or metabolites). Protein biomarkers for prognostication, disease monitoring and therapeutic guidance have great potential to improve clinical assessment of cancer, also in AML [207-208]. Over the past years, the major focus in biomarker studies have been genetic approaches. Likewise, most of the current potential biomarkers for aberrant GPCR and downstream signaling molecules in AML, as given in Table1, were found by genomic- and transcriptomic-based approaches. Hitherto, protein biomarkers in the clinical practice are often assessed with non-MS techniques (mainly immunoassay and immunohistochemistry) [209] [210]. An example is flowcytometric

drug monitoring of therapeutic surface levels of the GPCR-linked protein biomarker CXCR4 after plerixafor administration to AML patients [175-176]. However, the poor prognostic CD97 biomarker on LSC-enriched (CD34+CD38-) AML blasts was detected by LC-MS and validated by flow cytometry [179]' [178]. Many biomarkers candidates from proteomic studies have been suggested but very few have been implemented into the clinic [211]. While technical limitation previously could be used as explanation to why proteomics has not resulted in many new clinical biomarkers, it may currently rather be a result of poor study design. This can include the use of underpowered studies, insufficient understanding of the analytical evaluation criteria required to pass through the approval pipeline by the U.S. Food and Drug Administration (FDA), unreproducible pre-analytical sample processing and/or inappropriate statistical and experimental design [211-212]. Moreover, earlier AML discovery-based biomarker studies have to a large extent been performed with low performance 2D-PAGE based approaches combined with MALDI-TOF or LC-MS, which results in a low number of quantifiable proteins and potential biomarkers compared to what is achievable with liquid chromatography on modern mass spectrometers like Q Exactive HF Orbitrap LC-MS/MS system [213]. Interestingly, by using the state-of-the-art Q HF-X mass spectrometer it is possible to identify 5000 phosphopeptides and 55000 peptides (5900 proteins) with only short 15 min and 30 min LC-MS/MS run, respectively [213]. Mass spectrometry-based protein quantification may thus be applied as an efficient tool for improved clinical assessment of cancer, and step by step protocols for handling primary AML samples have recently been published [214-215]. The global phosphoproteome study by Schaab et al. demonstrated how a phosphosignature can predict the response to the tyrosine kinase inhibitor Quizartinib, as currently assessed in clinical trials [216]. For patients with non-small-cell lung cancer a proteomic signature was also found to have predictive and

therapeutic value in a phase-III trial [217]. Thus, this might be the era afore a big break-through in clinical application of proteomics-based biomarkers.

We have previously reviewed the proteomics-based scientific contributions to AML research with main focus on AML patient material and biomarkers [218], but perhaps due to reasons mentioned above, only one protein (CLCX4) related to the GPCR signaling pathway was proposed as a potential biomarker. However, we envision that e.g. stimulation of different GPCR pathways in different AML subgroups, and subsequent targeted analysis of several downstream GPCR mediators will unveil how GPCRs and the mediators are differently activated or altered in AML subsets of this heterogeneous disease. Here, the sensitive and high throughput quantification technology named Parallel Reaction Monitoring (PRM) can be used to measure the abundance of tens to hundreds of targeted peptides from each AML patient in a short time. In brief, PRM involves isolation of the target precursor ion in the quadrupole (Q1), fragmentation of the selected ion in the collision cell (Q2) and detection of the generated product ions in the orbitrap [219]. The targeted PRM method can be used for quantification of aberrant GPCR-mediated protein and phosphorylation dynamics in signalling pathways in a sensitive and specific manner without the need of antibodies.

**Conclusion**

As described elsewhere herein, there is compelling evidence for involvement of aberrant GPCR expression and perturbed GPCR-mediated signaling in the development of cancer. Many of these measurable indicators of abnormal expression have large potential as predictive markers of therapy response, and thus can be used for therapeutic guidance of cancer patients. To our knowledge, no large proteome AML patient cohorts with focus on GPCR-signaling have been published. Regarding AML, testing of available approved

drugs as well as synthesis of new small molecule therapeutics against AML enriched GPCRs or GPCR signaling proteins are warranted [147]. We postulate that mass spectrometry-based protein profiling of primary AML cells will accelerate the discovery of potential GPCR related biomarkers for AML. Targeted quantitative proteomics approaches (e.g. PRM) can then be used to accurately and simultaneously measure the abundance of 10s of AML-disease related proteins (including phosphorylation status and other PTMs) in large patient cohorts. This will lead to new information regarding the significance and clinical potential of these AML-disease related proteins as markers for prognostication, disease monitoring and therapeutic guidance.

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**Figure legends**

**Figure. 1.** GPCRs are expressed in hematopoietic cells. Receptors stimulate heterotrimeric G proteins by promoting GDP to GTP exchange in the Gα subunit and dissociation from the βγ dimer. Both Gα and βγ initiate signaling through different effector proteins. Activated GPCRs are phosphorylated by GRKs on the internal loops creating recognition sites for β-arrestins that can in turn act as adaptor proteins initiating

alternative signaling cascades. Interestingly, a crosstalk between GPCRs and Wnt/β-catenin signaling pathways is also highlighted.

**Table 1**: Overview of GPCR signaling in the pathogenesis of AML.

| GPCR signaling | Experimental effect in AML | Reference |
|---|---|---|
| CXCR4 | Low expression correlated with longer relapse-free survival.<br>Upregulation in AML.<br>High CXCR4 expression is associated with poorer clinical outcome.<br>Pharmacological target for HSC mobilization from bone marrow.<br>*CXCR4 antagonist Plerixafor increase remission rate.* | [159]<br>[157]<br>[175],[176]<br> |
| CCR1, CCR2, CCR7, CCRL2, CXCR1 | High expression on primary AML cells. | [157] |
| GPR84 | High expression is linked to poor prognosis. Stimulates aberrant β-catenin signaling for maintenance of AML-LSC leukemogenesis. | [184],[178] |
| CD97 | Critical regulator of AML stem cell. High expression on LSC-enriched (CD34+CD38-) blasts. Upregulation in AML. | [156],[157], [178],[179], [180] |
| GPR56 | LSC-specific signature.<br>High repopulating capacity in xenograft studies in mice.<br>Under-expression in AML patients.<br>Maintenance of HSC. | [7]<br>[181],[182]<br>[157]<br>[183] |
| P2RY2, P2RY13 | Purine receptor family. Upregulated in AML. | [157] |
| P2RYY14 | High expression correlates with poor survival AML.<br>Resistance to PI3K/mTOR inhibition. | [177] |
| GPR125, GPR126, LPHN1, CELSR3 | Adhesion family. Downregulated in AML. | [157] |
| PAR and Gq | Downregulated in AML | [157] |
| EMR1, EMR2, GPR114,<br>GPR312 | Adhesion family receptors. Upregulated in AML.<br><br>Upregulated receptor in AML.<br>*GPR132 agonist ONC212 reduced cell viability.* | [157]<br><br><br>[185],[186] |
| GNA15 | High transcription level with ANKRD28 and UGP2, linked to poorer overall survival. | [191] |
| GNAQ | Stimulates proliferation and survival of AML-LSC.<br>Activate β-catenin signaling and increases expression of mitochondrial complex 1 subunits in AML-LSC.<br>Mitochondrial disfunction.<br>Changes in GNAQ expression were found in childhood AML compared to adult AML.<br>Gq protein and Platelet-activating factor (PAF) receptor proteins were detected in AML and ALL patient cells. | [174]<br><br>[33]<br>[195]<br>[194] |
| GNA11 | Downregulated in AML patients together with AREG. | [162] |
| RGS2 | Decreased expression in AML patients with Flt3-ITD. | [197] |
| RGS17 | Putative inhibition by miR-363 in patients that respond poorly to chemotherapy in AML. | [199] |
| Wnt signaling | Wnt inhibitors reduce proliferation and chemoresistance of AML cells.<br>Needed for MLL-AF9 induced AML in mice.<br>Wnt-pathway inhibitors induce cell death in AML cell lines and primary AML blasts. | [173], [205]<br>[192]<br>[206] |