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# Gq突变在葡萄膜黑色素瘤中的作用及其抑制剂研究进展

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**摘要**: 葡萄膜黑色素瘤 (uveal melanoma, UM) 是成人眼部最常见的恶性肿瘤, 恶性程度极高, 且目前尚无有效治疗手段, 一旦发生转移生存期仅 2～7 个月。研究发现 83% 以上 UM 存在编码异源三聚体 G 蛋白的 Gαq 亚基 (GNAQ) 或编码异源三聚体 G 蛋白的 Gαq11 亚基 (GNA11) 互斥突变, 其中 95% 以上的 GNAQ/GNA11 突变是大鼠肉瘤 (rat sarcoma, RAS) 样结构域 209 位谷氨酰胺 (Q) 定点突变为亮氨酸 (L) 或脯氨酸 (P)。突变导致三磷酸鸟苷水解酶 (guanine triphosphatase, GTPase) 活性丧失并引起 G 蛋白持续活化。持续活化的 G 蛋白激活丝裂原活化蛋白激酶 (mitogen-activated protein kinase, MAPK)、磷脂酰肌醇 3-激酶 (phosphoinositide 3-kinase, PI3K)/蛋白激酶 B (protein kinase B, AKT)、Rho 激酶 (Ras homologue, Rho)/Rho 相关激酶 (Rho associated kinase, Rock)/Yes 相关蛋白 (Yes-associated protein, YAP) 等信号通路是诱发 UM 的重要原因, 因此靶向 GNAQ 与 GNA11 突变可能是治疗 UM 的全新策略。本文拟从 G 蛋白结构与功能、G 蛋白突变与 UM 发生、GNAQ/GNA11 小分子抑制剂的发现及其在 UM 中的抗癌活性等角度展开, 以期为相关临床及基础研究提供参考。

**关键词**: 葡萄膜黑色素瘤; G 蛋白; GNAQ/GNA11; G 蛋白偶联受体; 小分子

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# Study of Gq mutations and their inhibitors in uveal melanoma

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**Abstract**: Uveal melanoma (UM) is one of most common ocular cancers and is extremely malignant; so far there is no effective treatment. Moreover, the survival period is only 2−7 months after metastasis. It has been proven that more than 83% of uveal melanomas harbor mutations in G protein subunit α q (GNAQ) or G protein subunit α 11 (GNA11), among which 95% are a Q209P/L single-site mutation. Q209P/L mutations lead to dysfunction of guanine triphosphatase (GTPase) in the G protein and result in constitutive activation of downstream pathways including mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT), Ras homologue (Rho)/ Rho-associated kinase (Rock)/Yes-associated protein (YAP) and others. Therefore, targeting GNAQ/GNA11 mutations are potential strategies for UM treatment. This review will focus on roles of G protein mutations in UM progression, and the potential therapeutic effects of GNAQ/GNA11 inhibitors, and will provide insights into basic and clinical research on UM treatment.

**Key words**: uveal melanoma; G protein; GNAQ/GNA11; G protein-coupled receptor; small molecule

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葡萄膜黑色素瘤 (uveal melanoma, UM) 是成人眼部最常见的恶性肿瘤, 年发病率为1.3/$10^6$～8.6/$10^6$[1], 约占眼部黑色素瘤的83%、占所有黑色素瘤的3%～5%[2]。UM好发于脉络膜 (90%)、睫状体 (6%) 和虹膜 (4%)。针对UM的原位治疗主要包括保留眼球治疗 (放疗和激光治疗等) 和眼球摘除手术。经原位治疗后, 90% 的UM响应良好, 但仍有50% 患者在原位治疗后的1～15年内发生血行转移, 80% 以上转移至肝脏并导致死亡, 一旦发生转移生存期仅2～7个月[3]。针对转移性的UM可采用的有效治疗手段不足1%[4], 包括化疗和靶向治疗在内的临床常用手段均未显著改善转移性UM患者生存期[1,2,5]。临床常将皮肤性黑色素瘤 (cutaneous melanoma, CM) 治疗手段用于转移性UM探索性治疗[4], 虽然以程序性细胞死亡1 (programmed cell death-1, PD-1) 与程序性死亡配体1 (programmed cell death 1 ligand 1, PD-L1) 相互作用为免疫检查点的阻断疗法在CM上疗效明显, 但在转移性的UM中, PD-1抑制剂纳武单抗 (nivolumab, 商品名OPDIVO) 和派姆单抗 (MK-3475, 商品名Keytruda) 在Ⅱ期临床中均无显著疗效[6], 这可能与UM低肿瘤突变负荷 (tumor mutation burden, TMB) 和免疫抑制因子表达上调有关[2]。有数据显示, 83% 以上UM患者存在编码异源三聚体G蛋白的G$\alpha$q亚基 (G protein subunit $\alpha$ q, GNAQ) 或编码异源三聚体G蛋白的G$\alpha$q11亚基 (G protein subunit $\alpha$ 11, GNA11) 激活突变, 该突变是UM发生的致癌因素[7,8]。

UM致瘤信号通路具有复杂性, 在UM的临床治疗中, 常以G蛋白下游丝裂原活化蛋白激酶 (mitogen-activated protein kinase, MAPK)、磷脂酰肌醇3-激酶 (phosphoinositide 3-kinase, PI3K)/蛋白激酶B (protein kinase B, AKT)、Rho激酶 (Ras homologue, Rho)/Rho相关激酶 (Rho associated kinase, Rock)/Yes相关蛋白 (Yes-associated protein, YAP) 等级联信号通路以及受体酪氨酸激酶 (receptor protein tyrosine kinase, RTK) 通路激活为依据开展靶向治疗[9-14] (图1)。据统计, 45%～86% 的原发性UM肿瘤存在MAPK通路过活化[15,16], G蛋白作为该通路上游调节分子通过经典的第二信使途径触发MAPK通路激活[10]。当G蛋白发生突变活化时可直接与Rho鸟嘌呤核苷酸交换因子 (Rho family guanine nucleotide exchange factor, RhoGEF) 结合, 激活Rho/Rock信号通路, 促进F-肌动蛋白 (F-actin) 积聚、黏着斑激酶 (focal adhesion kinase, FAK) 磷酸化激活。F-actin通过竞争性结合促进YAP从无活性的胞质相关蛋白复合物中游离出来; FAK抑制YAP 127位丝氨酸 (S) 磷酸化, 促进YAP 357位酪氨酸 (Y) 磷酸化 (YAP 127位丝氨酸磷酸化导致YAP失活, 357位酪氨酸磷酸化提高YAP稳定性和活性[11]), 促进YAP转运至细胞核与转录因子TEAD (transcriptional enhanced associate domain)、SMAD (Smad protein) 发生转录激活[17]。经证实在多种癌细胞内, YAP激活可以诱导肿瘤干细胞特性, 促进肿瘤生长、转移、增强耐药性等[18]。此外, G蛋白突变激活下游PI3K/AKT信号通路, 研究统计50% 的UM存在PI3K/AKT信号通路过活化, 但在UM中该通路激活主要是由于RTK自分泌激活[9]和紧张素同源蛋白 (phosphatase and tensin homolog deleted on chromosome ten, PTEN) 功能缺失[12], 抑癌因子PTEN促进PIP3去磷酸化抑制PI3K/AKT通路激活[12]。目前, 临床上已经评估了丝裂原活化的细胞外信号调节激酶 (mitogen-activated extracellular signal regulated kinase, MEK) 抑制剂 (司美替尼、曲美替尼)、蛋白激酶C (protein kinase C, PKC) 抑制剂 (AEB071)、PI3K抑制剂 (BYL719)、RTK抑制剂 (c-Kit抑制剂舒尼替尼、c-Met抑制剂卡博替尼) 等对转移性UM的治疗效果[19-21], 不幸的是, Khoja等[22]回溯性分析近20年开展的29例针对转移性UM的Ⅱ期临床试验研究表明, 治疗组应答率普遍低于10%。因此, 针对转移性UM急需更为有效的治疗策略。G蛋白突变激活作为UM发生的关键, 其选择性抑制剂的发现提高了科研工作者将G蛋白作为药物研发靶点的信心[23]。临床前研究证实, 选择性G蛋白抑制剂FR900359在G蛋白突变的UM细胞中具有良好的抗肿瘤效果[24,25]。因此, 靶向G蛋白突变的抑制剂开发可能是治疗UM极具前景的发展方向。

## 1　G蛋白的生理功能与突变激活

G蛋白偶联受体 (G protein-coupled receptor, GPCR) 家族是一个庞大的细胞表面跨膜受体家族, 已经发现有800多个成员。GPCR参与内分泌和代谢等多种生理功能, 同时与肿瘤、免疫和心脏等重多疾病发生有密切联系, 是最重要的药物靶点之一, 目前靶向GPCR的药物占市售药物的20%～30%[26,27]。作为GPCR下游关键的分子开关, 鸟核苷酸结合蛋白 (G蛋白) 是一类对二磷酸鸟苷 (guanine dinucleotide phosphate, GDP) 和三磷酸鸟苷 (guanine trinucleotide phosphate, GTP) 有高度亲和力的膜内蛋白, 具有水解GTP为GDP的GTPase活性。G蛋白分为单体G蛋白 (小G蛋白) 和异源三聚体G蛋白复合物 (大G蛋白)。大G蛋白由G$\alpha$ (39～52 kDa)、G$\beta$ (37 kDa)、G$\gamma$ (6～9 kDa) 3个亚基组成[28,29] (图2)。

**1.1　G蛋白的活化周期与生理功能**　G蛋白的活化呈周期样循环, 在非活化状态下G$\alpha$亚基与GDP结合, 维

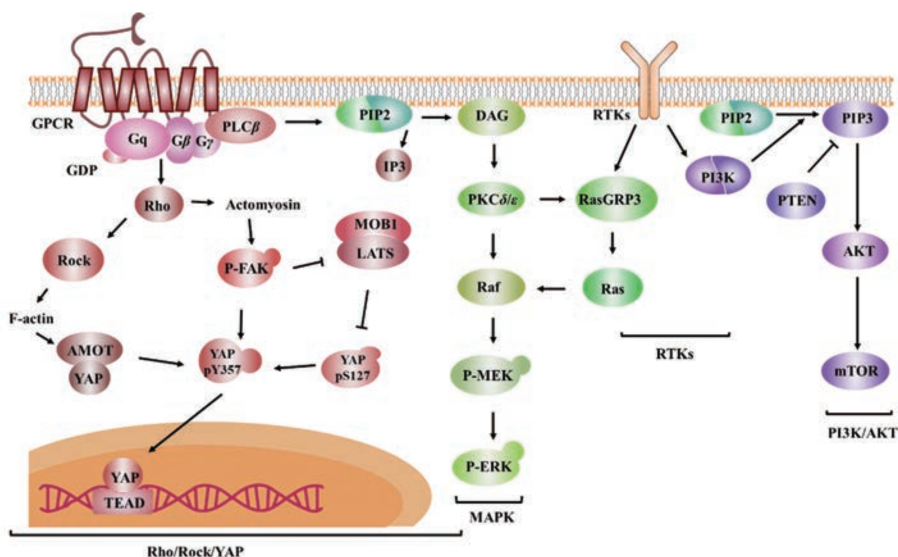


**Figure 1**　Tumorigenic signaling pathway in UM. MAPK signal pathway: phosphorylation level of MEK/ERK be promoted by G protein activation, at the same time, the selective overexpression of RasGRP3 in G protein mutation UM synergistic promotes the activation of MAPK pathway. Rho/Rock/YAP signal pathway: G protein activates F-actin accumulation and FAK phosphorylation by Rho activation. F-actin accumulation causes the dissociation of AMOT-YAP complexes, thereby contributing to YAP nuclear translocation; FAK phosphorylation can directly inhibit the phosphorylation of YAP S127 by MOB1/LATS (YAP S127 phosphorylation leads to YAP inactivation), while increasing the phosphorylation of YAP Y357 (YAP Y357 phosphorylation promotes the stability and activity of YAP). PI3K/AKT and RTK signal pathways: the decrease or loss of PTEN function or RTK autocrine activation often occurs in UM. PTEN can dephosphorylate PIP3 to antagonize PI3K activation. In addition, RTK can promote the MAPK signal pathway through Ras/Raf. UM: Uveal melanoma; MAPK: Mitogen-activated protein kinase; MEK: Mitogen-activated extracellular signal regulated kinase; ERK: Extracellular regulated protein kinases; RasGRP3: Ras guanyl releasing protein 3; Rho: Ras homologue; Rock: Rho associated kinase; YAP: Yes-associated protein; FAK: Focal adhesion kinase; AMOT: Angiomotin; MOB1: MOB kinase activator 1; LATS: Large tumor suppressor kinase; RTK: Receptor protein tyrosine kinase; PTEN: Phosphatase and tensin homolog deleted on chromosome ten; PIP3: Phosphatidylinositol 3,4,5-trisphosphate; Ras: Rat sarcoma; Raf: Rapidly accelerated fibrosarcoma

持G蛋白以异源三聚体 (G$\alpha\beta\gamma$) 形式与GPCR形成复合体 (图2a)。当上游GPCR受到外界刺激并通过构象变化将信号传递给G蛋白后, GTP取代GDP (图2b)[30,31]。随后G$\alpha$与G$\beta$G$\gamma$二聚体解聚, 并各自传递信号。G$\alpha$亚基在与效应蛋白结合的同时或之后, 将结合的GTP水解为GDP (图2c)[32], 并再次与G$\beta$G$\gamma$形成三聚体回到非活化状态 (图2d), 等待下一次信号转导。

基于G$\alpha$亚基序列和功能的相似性, 可以将G蛋白分为4种亚类家族: Gi、Gs、G12/13和Gq[23,33] (图3)。Gs家族包含G$\alpha_s$和G$\alpha_{olf}$[34], 活化后促进腺苷酸环化酶 (adenylate cyclase, AC) 产生第二信使环磷酸腺苷 (cyclic adenosine monophosphate, cAMP), 继而激活cAMP依赖的蛋白激酶。Gi是G蛋白中最大且最多样的家族, 主要包括G$\alpha_{i1}$、G$\alpha_{i2}$、G$\alpha_{i3}$、G$\alpha_{oA}$、G$\alpha_{oB}$、G$\alpha_s$、G$\alpha_{t1}$、G$\alpha_{t2}$、G$\alpha_{gust}$和G$\alpha_z$[34], Gi活化后产生与Gs相反的生物学功能。G12/13家族包含G$\alpha_{12}$和G$\alpha_{13}$, 其活化后主要负责调控RhoGEFs[28]。人源性Gq家族主要包括G$\alpha_q$、G$\alpha_{11}$、G$\alpha_{14}$、G$\alpha_{16}$。G$\alpha_q$和G$\alpha_{11}$在体内广泛分布, 且二者氨基酸序列相似性高达88%[33]。G$\alpha_{14}$主要分布在肾、肺和肝等器官, G$\alpha_{16}$只在特定的造血细胞内表达[34]。Gq活化后促进磷脂酶C$\beta$ (phospholipase C$\beta$, PLC$\beta$) 水解4,5-二磷酸脂酰醇 (phosphatidylinositol 4,5-biphosphate, PIP2) 产生第二信使三磷酸肌醇 (inositol 1,4,5-triphosphate, IP3) 和二酰甘油 (diacylglycerol, DAG), IP3可进一步导致胞质$Ca^{2+}$浓度增高并引发一系列生理反应。

**1.2　G蛋白与GPCR信号传导的选择性**　GPCR家族包含800多个成员, 可以被上千种配体激活, 而G蛋白数量却极其有限, GPCR与G蛋白间偶联模式错综复杂。研究表明, GPCR与G蛋白间的偶联存在选择性[35], 如$\beta$1肾上腺素受体 ($\beta$1-adrenoceptor, $\beta$1AR) 和五羟色胺受体6 (5-hydroxytryptamine receptor 6, 5-HT6) 均偶联Gs (图4a); 又如某些GPCR可以偶联多种G蛋白, 如$\beta$2肾上腺素受体 ($\beta$2-adrenoceptor, $\beta$2AR) 既可以偶联Gs又可以偶联Gi[35,36] (图4b); 某些GPCR只偶联某一种G蛋白, 如乙酰胆碱受体1 (muscarinic

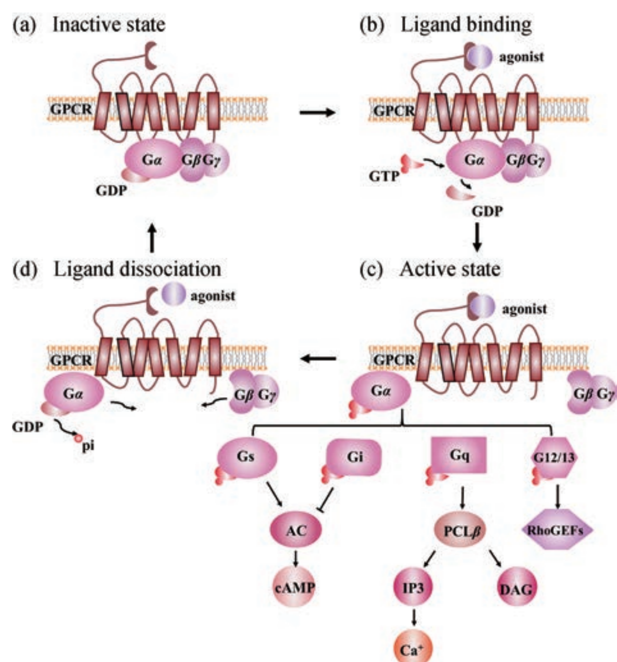


**Figure 2** G protein cycle and mechanism of action. GDP: Guanine dinucleotide phosphate; GTP: Guanine trinucleotide phosphate; AC: Adenylate cyclase; cAMP: Cyclic adenosine monophosphate; PLC*β*: Phospholipase C*β*; IP3: Inositol 1,4,5-trisphosphate; DAG: Diacylglycerol; RhoGEFs: Rho guanine nucleotide-exchange factors

acetylcholine receptor 1, M1) 激活后仅偶联Gq; Flock等[35,37]认为GPCR与G蛋白选择性偶联取决于Gα上一段特定的保守氨基酸序列, 该序列可以被GPCR的不同区域所识别, 不同的GPCR通过不同的残基与Gα上保守氨基酸序列结合, 而GPCR长期进化导致了不同配体使用不同识别残基来识别相同Gα蛋白, 以此提高信号转导效率。GPCR在基因分化期间主要积累两种突变类型: 一种维持Gα蛋白偶联选择性, 但改变配体结合特异性; 另一种保持配体结合特异性, 但改变Gα蛋白偶联选择性。偶联相同Gα蛋白的GPCR如果其Gα蛋白偶联选择性是从同一祖先继承而来, 则共享相同的结合残基 (GPCR-R1与GPCR-R2, 图4c), 但如果GPCR通过改变原始祖先Gα蛋白选择性而实现偶联相同Gα蛋白, 则享有不同的结合残基[35] (GPCR-R3与GPCR-R2', 图4c)。GPCR与G蛋白间偶联选择性为G蛋白小分子抑制剂的开发和功能验证提供了基础理论依据。

**1.3 G蛋白的激活突变与UM** UM是一种以突变为典型特征的肿瘤, 如GNAQ、GNA11、半胱氨酰白三烯受体2 (cysteinyl leukotriene receptor 2, CYSLTR2) 间互斥突变, 以及真核翻译起始因子1X (eukaryotic translation initiation factor 1A, X-linked, EIF1AX)、剪切因子3B1亚基 (splicing factor 3b, subunit 1, SF3B1)、BRCA1相关蛋白1 (BRCA1 associated protein 1, BAP1) 间互斥突变等 (表1[9,13,38])[38,39], G蛋白突变激活与UM的发生和转移密切相关[7,8]。测序结果表明, 95%以上的GNAQ、GNA11突变集中在209位[40], 以209位谷氨酰胺 (Q) 突变为亮氨酸 (L) 或脯氨酸 (P) 为主。209位谷氨酰胺位于GNAQ和GNA11的RAS结构域, 该结构域对Gq的GTPase活性至关重要[17]。209位突变可能导致Gα的GTPase活性丧失, 从而使Gα处于持续活化状态[7,8]。Q209L和Q209P突变提高Gα亚基对效应器的亲和力, 促进Gα与GβGγ亚基解聚。此外, Q209P突变还可抑制G蛋白信号转导调节蛋白

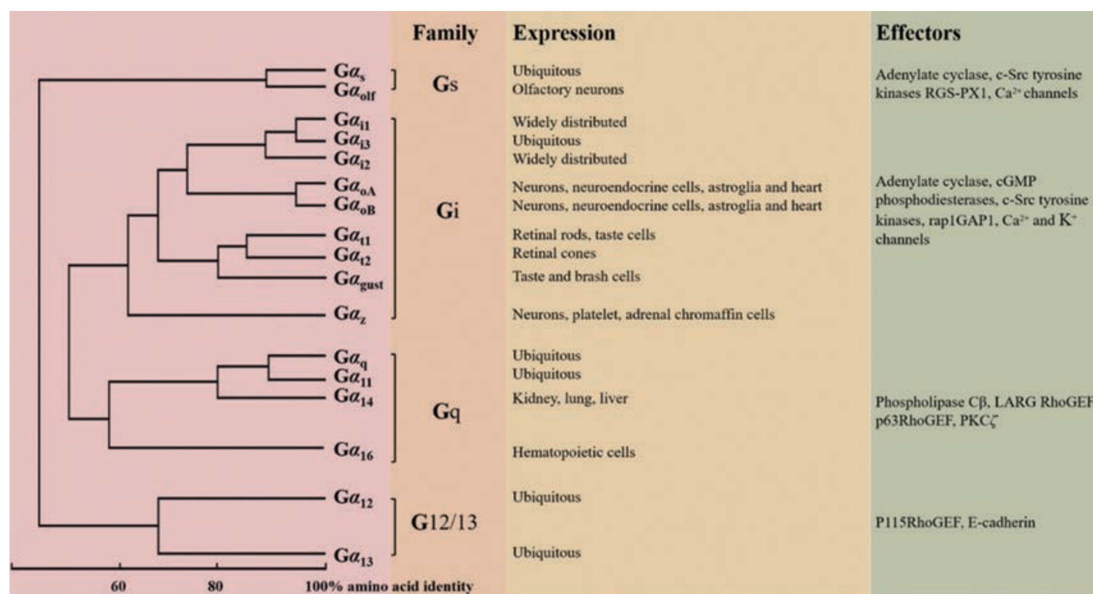


**Figure 3** Phylogenetic relationship of human G*α* subunits and their expression and effectors

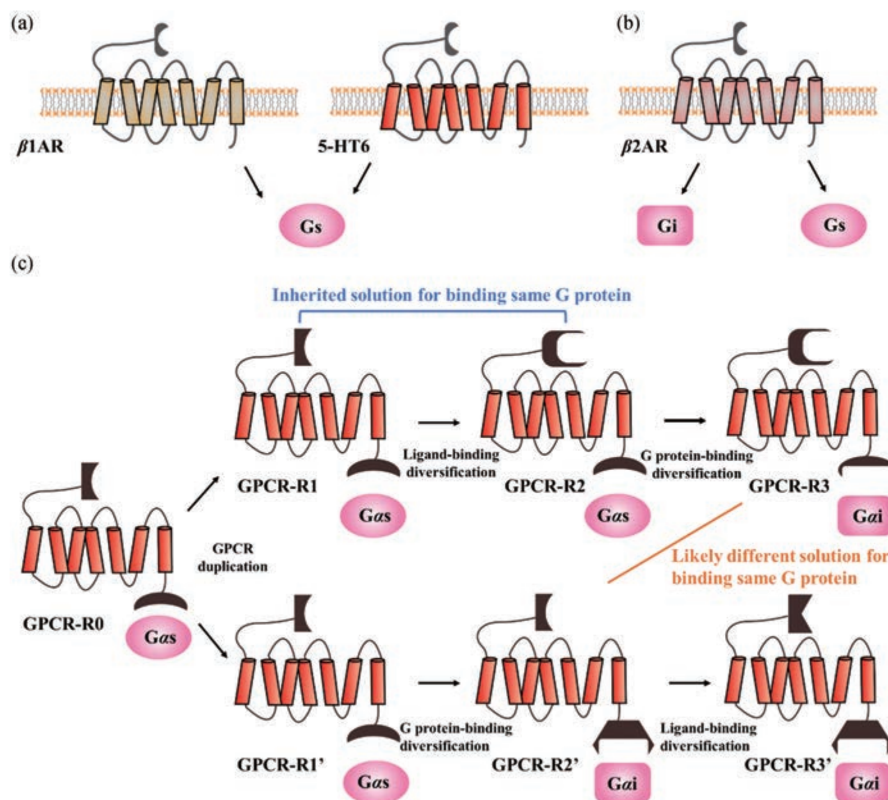


**Figure 4** Selective conjugation of G protein-coupled receptor (GPCR) with G protein. a: Several distinct receptors couple to the same G$\alpha$ protein [$\beta$1-adrenergic receptor ($\beta$1AR) and 5-hydroxytryptamine 6 receptor (5-HT6)]; b: Receptors couple to more than one G$\alpha$ protein [$\beta$2-adrenergic receptor ($\beta$2AR)]; c: Evolution model for ligand and G protein selectivity of GPCRs. GPCR-R1 and GPCR-R2 conjugate the same G protein by changing ligand selectivity and preserving G protein selectivity, and share the same G protein binding residues. GPCR-R3 and GPCR-R2' conjugate the same G protein by changing G protein selection, but share different binding residues

**Table 1** Mutations in UM

| Gene | Description | Percentage of gene alterations | Mutation type |
|---|---|---|---|
| GNAQ | G protein subunit $\alpha$ q | 50% | Missense mutation: activating hot spot mutations on Gln209 and Arg183[9,13] |
| GNA11 | G protein subunit $\alpha$ 11 | 45% | Missense mutation: activating hot spot mutations on Gln209 and Arg183[9,13] |
| CYSLTR2 | Cysteinyl leukotriene receptor 2 | 4% | Missense mutation[9,38] |
| EIF1AX | Eukaryotic translation initation factor 1A, X-linked | 13% | Missense mutation and inframe mutation[38] |
| SF3B1 | Splicing factor 3b subunit 1 | 23% | Missense mutation[38] |
| BAP1 | BRCA1-associated protein-1 | 33% | Truncating mutation[38] |

(regulator of G protein, RGS) 对G$\alpha$负性调控[40] (图5), RGS是一类GTPase激活蛋白 (GTPase-accelerating protein, GAP), 通过直接与激活的G$\alpha$结合加速GTP水解 (>1 000倍), 促进G蛋白信号通路失活[32,41]。部分突变也发生于183位, 以R (精氨酸) 183C (半胱氨酸) 突变为主, 但该突变的发生频率和对G蛋白激动效果都远不及209位突变[8]。因此, 靶向G$\alpha$亚基突变, 尤其是Q209位突变研发新型UM治疗药物具有重大意义。

## 2 靶向Gq抑制剂的研发进展

基于GNAQ/GNA11在UM的高频突变, 靶向GNAQ/GNA11小分子抑制剂开发可能是UM治疗极具前景的策略。然而, 靶向Gq突变的研究仍处于初始阶段。目前经确证的选择性Gq抑制剂仅有YM-254809和FR900359及其衍生物, 泛G蛋白抑制剂包括BIM-46714及其二聚体BIM-46187和多肽类G蛋白拮抗剂-2A、27残基肽 (I860A) 等。

### 2.1 选择性Gq抑制剂FR900359和YM-254890

FR900359又名UBO-QIC, 于1988年从植物朱砂根*Ardisia crenata sims*中提取分离得到, 具有抑制血小板聚集和降血压等作用[42]。YM-254890于2003从色素

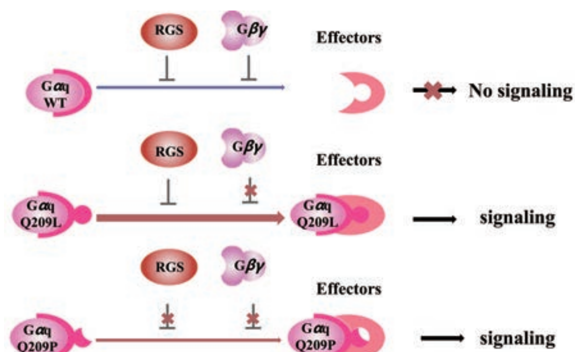


**Figure 5**　Gα mutant activation. When GPCRs are not activated by ligands, wild-type Gαq maintains a low activation state, mainly due to low affinity for effectors (purple arrow) and inhibition of regulator of G protein (RGS) and GβGγ. When Gαq 209 mutated, its affinity for effectors increases (the thicker the red line, the stronger the affinity), and its sensitivity to negative regulation of GβGγ decrease. In addition, the Q209P mutation reduces the sensitivity to negative regulation of RGS

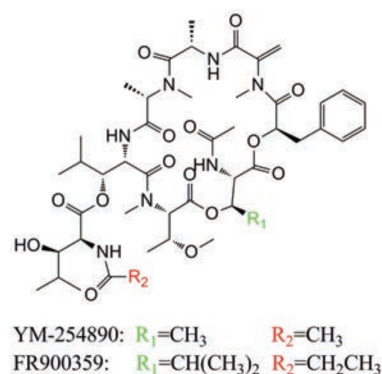


**Figure 6**　Chemical structures of YM-254890 and FR900359

杆菌*sp.QS3666*发酵液中分离得到, 起初作为一种新型血小板凝集抑制剂[43], 用于抗血栓和溶血栓研究[44]。YM-254890和FR900359提取来源不同, 但结构却极其相似[45] (图6)。

YM-254890相对FR900359发现较晚, 但却是第一个被证实可选择性靶向Gq的抑制剂[46]。YM-254890的发现及作为Gq选择性抑制剂对研究Gq激活及Gq偶联的信号通路具有重要意义。2010年Nishimura等[47]成功得到了YM-254890与Gq蛋白的结晶, 首次提供了G蛋白与小分子结合的结构信息。基于结构相似性, 2015年Inamdar等[48]证实FR900359也是一种选择性Gq抑制剂, 对G12/13、Gs和Gi均不具有抑制作用。YM-254890和FR900359作为核苷酸解离抑制剂 (GDP dissociation inhibitor, GDI) 均可结合到靠近核苷酸结合口袋的Gq铰链1 (linker 1) 和开关Ⅰ (switch Ⅰ) 结构域之间。Switch Ⅰ构象变动是启动G蛋白的关键[49], 当化合物结合后阻碍了switch Ⅰ运动, 导致GDP被束缚于核苷酸结合口袋内, 通过阻滞GDP/GTP交换致使Gq处于失活状态[25,47] (图7)。由于YM-254890提取工艺复杂、产率低、合成困难, 因此长期以来仅作为Gq研究工具使用, 而未开展相关临床试验。2016年Xiong等[45]报道了YM-254890和FR900359及其衍生物的全合成路线, 并首次测定出YM-254890和FR900359抑制Gq的$IC_{50}$分别为0.095和0.033 μmol·L$^{-1}$, 证实FR900359是目前对Gq抑制效果最强的化合物, 但复杂的合成过程使得YM-254890和FR900359的大规模制备仍然不可能实现。经测定YM-254890对Gαq R183C突变激活抑制效果明显好于Gαq Q209L[46], 而FR900359无论对野生型、183突变和209突变都有明显的抑制作用[25]。目前常以FR900359作为UM研究的工具化合物。

在体外实验中, Feng等[13,24,50,51]证明FR900359在GNAQ/GNA11突变的MEL270、OMM1.3、92.1和UM002B细胞中剂量依赖地抑制细胞存活, 而对BRAF

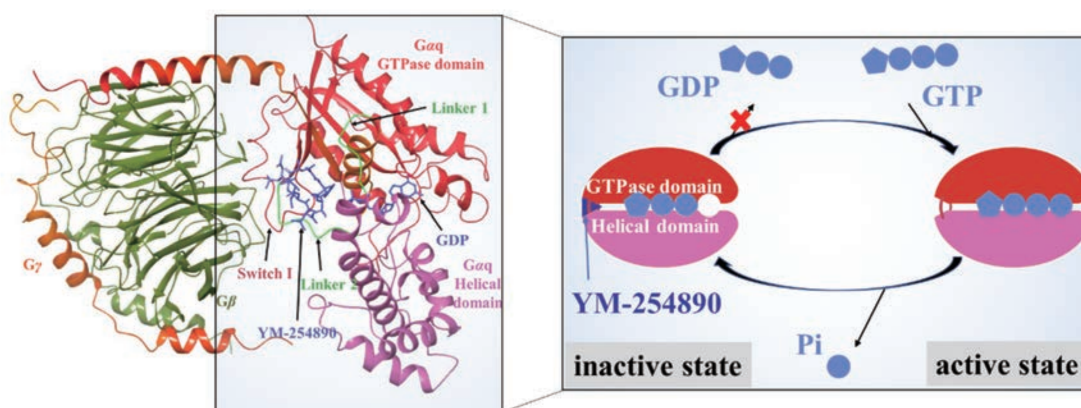


**Figure 7**　Crystal structure of Gα with YM-254890 (PDB: 3AH8) and schematic for YM-254890 inhibition of Gq GTPase. YM-254890 bounds to the hydrophobic activity near the GTPase domain, thereby allosterically stabilizing the GDP-bound fraction by inhibiting the release of GDP from Gq

突变的SK-MEK-28和OCM3细胞, 在同剂量下几乎不具有杀伤细胞作用。FR900359可在3D培养中剂量依赖抑制OMM1.3细胞克隆形成, 促进UM细胞G1期阻滞、细胞凋亡和抑制细胞迁移等。FR900359的作用效果与G蛋白突变具有强烈的相关性, 提示G蛋白突变激活作为UM驱动因素在UM恶性增殖中承担重任。在UM复杂的致瘤信号通路中, FR900359可抑制Rho/Rock/YAP通路关键蛋白FAK磷酸化, 并以剂量依赖方式促进YAP S127磷酸化, 抑制YAP转录入核[13,24]。在MAPK信号通路中, FR900359对G蛋白突变株可剂量依赖抑制下游ERK/MEK磷酸化水平, 而对多数非G蛋白突变株抑制效果不显著[24,25]。此外, FR900359可介导起始复合物2 (polycomb repressive complex 2, PRC2)沉默[51], 促进黑色素细胞再分化, 经证实PRC2在包括UM在内的多种癌症中具有维持肿瘤干细胞特性和促进自我更新等作用[52]。体内实验表明, 相较于BRAF突变的UM, FR900359对Gq突变的UM异种移植瘤有明显效果[50]。以上研究提示, 直接靶向G蛋白突变作为抗肿瘤药物研发靶点是可行且有效的。

**2.2 泛G蛋白抑制剂BIM-46174和BIM-46187** BIM-46174是一类咪唑并哌嗪类小分子化合物 (图8)。2006年Prévost等[53]报道BIM-46174为泛G蛋白抑制剂, 可选择性抑制霍乱毒素 (cholera toxin, CTX) 介导Gs激活引起的cAMP水平升高, 而对毛喉素介导AC激活引起cAMP积累无抑制效果, 并以可逆方式抑制Gs偶联GPCR介导的cAMP积累。此外, BIM-46174在体内外实验中均显示出抑制肿瘤细胞增殖、存活和侵袭等作用, 其在细胞水平抑制细胞增殖的$IC_{50}$为0.6～25 μmol·L$^{-1}$ [53]。对G蛋白其他亚型, BIM-46174抑制Gq介导的IP1 (IP3水解产物[54]) 产生, 并对偶联Gi/o的Wnt-2卷曲受体和偶联Gq的高亲和性神经降压素受体介导的癌细胞侵袭表现出抑制效果。初步说明, BIM-46174对泛G蛋白均有一定抑制作用[53]。

BIM-46187是BIM-46174的氧化二聚体形式 (图8), 起初被发现可引起强烈的痛觉过敏反应, 并与吗啡有很强协同作用[55]。2009年Ayoub等[56]发现BIM-46174可抑制多种GPCR (抗利尿激素受体V2、β2肾上腺素受体、五羟色胺受体、蛋白酶激活受体1、溶血磷脂酸受体和γ氨基丁酸受体) 介导的IP1、cAMP的产生和SRE-Luc荧光素酶报告基因表达, 表明BIM-46187对Gs、Gi、Gq和G12/13具有普遍抑制作用。使用生物发光 (BRET[57]) 和荧光共振能量转移 (FRET[54]) 发现, 在重组的GPCR、G蛋白细胞株中BIM-46174可通过直接与Gα亚基结合, 阻碍G蛋白与GPCR复合物间相互作用, 实现GDP/GTP交换抑制[56]。2014年Schmitz

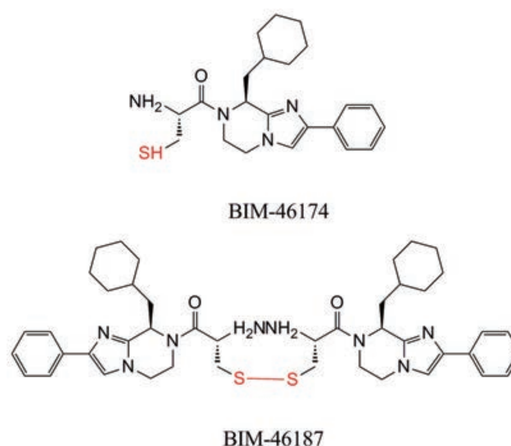


**Figure 8** Chemical structures of BIM-46174 and BIM-46187

等[58]通过分子动力学模拟 (molecular dynamics, MD) 对BIM (BIM-46174和BIM-46187) 的作用模式展开深入研究, 推测BIM (BIM-46174和BIM-46187) 绑定到Gα亚基α4环 (α4 loop) 和α4螺旋 (α4 helix) 之间, 以允许GDP从核苷酸结合口袋中退出但阻止GTP进入方式将Gα困在空腔结构中, 有别于YM-254890和FR900359将GDP束缚在核苷酸结合口袋内[58] (图9)。该研究结果与Ayoub等[56,58]报道的BIM-46187作用于$G\alpha_{i2o}$偶联的白三烯受体时显示BIM-46187允许GDP解离但阻止GTP结合一致。虽然BIM-46187作为一种泛G蛋白抑制剂, 但呈细胞环境依赖方式干扰G蛋白信号。在特定细胞内BIM-46187只抑制Gq信号, 当胞内Gα蛋白表达丰度存在差异也会影响该化合物的抑制效果[58]。在结构上BIM-46187比单体BIM-46174仅多了1个二硫键, 但Schmitz等[58]报道在CHO细胞和HEK293细胞中, BIM-46187比BIM-46174显示更强的Gq抑制作用。此外, 在胞外特定条件下小单体BIM-46174可完全转化为二聚体形式, 表明二硫键的存在似乎不影响化合物的细胞膜透过性, 甚至改善了反应动力学和化学计量学效应, 致使二聚体比单体显示更高活性[59]。

**2.3 G蛋白拮抗剂-2A** G蛋白拮抗剂-2A (G protein antagonist-2A, GP-2A) 是一类十一氨基酸神经肽 (substance P, SP) 类似物, 氨基酸序列为Arg-Pro-Lys-Pro-Gln-*D*-Trp-Phe-*D*-Trp-Met-$NH_2$ (图10), 但二级结构一直未见报道。起初作为一种SP拮抗剂有刺激组胺释放作用[60], 后被发现可抑制Gq偶联的M1受体介导的GTP水解, 而对Gi偶联的M2受体介导的GTP水解无明显抑制[61]。有文献[60]报道, GP-2A截短型肽GPAnt-2 (pGlu-Gln-*D*-Trp-Phe-*D*-Trp-*D*-Trp-Met-$NH_2$) 可抑制Gi偶联的M2受体和Gs偶联的β2肾上腺素受体介导的GTP水解, GPAnt-2以可逆方式与Gi/Gs竞争性结合

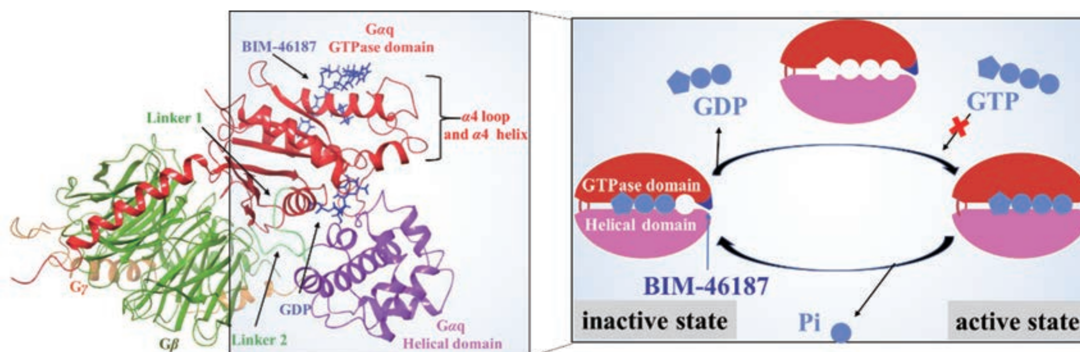


**Figure 9**　Proposed binding sites of Gα with BIM-46187 by molecular dynamics and schematic for BIM-46187 inhibition of Gq. BIM-46187 binding epitopes encompass amino acids 292-311 of both α4 loop and α4 helix. BIM-46187 traps Gq in the empty pocket conformational intermediate along the activation pathway by allowing GDP exit but preventing GTP entry

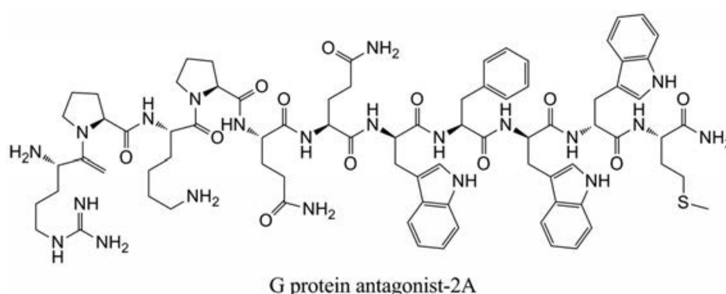


**Figure 10**　Chemical structure of G protein antagonist-2A (CAS number: 89430-38-6)

GPCR抑制G蛋白活化。而GP-2A对G蛋白抑制机制一直有待确证, 此外, 其细胞透过性、对G蛋白的选择性和疾病模型中的作用也需进一步考量。

**2.4　27残基肽 (I860A)**　27残基肽 (I860A) (27mer (I680A)) 是一类基于蛋白-蛋白相互作用衍生出的一类合成肽, 研究发现Gq下游效应器 (PLCβ和p63RhoGEF) 均以相似的卷曲螺旋结构 (helix-turn-helix, HTH) 结合到Gq的Switch Ⅱ和螺旋结构α3之间。27mer (I860A) 是PLCβ HTH结构域860位异亮氨酸 (I) 突变为丙氨酸 (A) 的27残基肽 (His-Gln-Asp-Tyr-Ala-Glu-Ala (860位)-Leu-Ala-Asn-Pro-Ile-Lys-His-Val-Ser-Leu-Met-Asp-Gln-Arg-Ala-Arg-Gln-Leu-Ala-Ala), 期望以残基取代全长效应器竞争性结合Gq而抑制G蛋白活化。体外实验表明27mer (I860A) 对活化的Gq有较高的亲和力 ($K_D$ = 400 nmol), 但不结合$G\alpha_{i1}$、$G\alpha_t$和$G\alpha_s$[62]。但目前27mer (I860A) 的构效关系、细胞透过性和G蛋白选择性等都需要进一步考究。

## 3　小结与展望

UM中Gq的高频突变促使更多研究者把视线聚焦到GPCR-G蛋白通路高频突变与癌症发生上, 随着深度测序技术的发展, 越来越多G蛋白突变在各种癌症中被揭示, 如在某些类型的胰腺癌中$G\alpha_s$突变率高达70%[63], 在上皮性T淋巴瘤中$G\alpha_{i2}$突变率在24%左右[64], G蛋白突变在癌症研究中不断彰显。目前转移性的UM仍然是难以治疗的癌种, 临床治疗手段极其有限。基于UM中Gq的高突变率, 靶向Gq突变的抑制剂开发可能是攻克UM极具前景的方向。但由于不同亚型G蛋白在一级序列和三维结构等方面具有高度相似性, 因此, 开发对不同G蛋白亚型有高度选择性的抑制剂极富挑战性。目前经证实, 可以选择性靶向Gq的抑制剂仅有YM-254890和FR900359及其类似物, 但该类化合物提取分离工艺复杂、产率低、合成困难而难以批量化生产, 此外其在突变型和野生型Gq间选择性不强, 若开发成药物需要制定策略将化合物精准地传递到靶细胞, 以避免干扰正常的Gq级联信号。因此, 急需开发新型成药性良好的G蛋白选择性抑制剂。

UM致瘤信号通路具有复杂性, 基于UM致瘤信号通路开展的各项临床研究表明, 如果仅靶向单一潜在靶点开展单独用药, 难以取得显著生存获益, 因此, 目前针对转移性的UM一般主张多通路多靶点联合用药。此外, 对UM患者需要有针对性地检测基因表达谱从而更精准地判断预后, 制定更加个体化和更有效的诊疗方案。如Gq突变的UM患者中, 基于Gq突变可直接触发Rho/Rock/YAP信号通路激活[13,17,65], 提示对Gq突变的UM患者在进行靶向治疗时, 可针对Rho/Rock/YAP通路结合MAPK、PI3K等通路开展联合用

药[9,10,15,66,67]。总体来说, 针对UM患者, 开发高活性、高选择性的G蛋白抑制剂结合个体化联合治疗将是未来治疗的发展方向。

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