探索·发现

荧光互补与光能量共振转移检测B类G蛋白偶联受体PAC1二聚化

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摘要 G蛋白偶联受体(G-protein couple receptors, GPCRs)是最大的超家族膜受体, 其中它的B家族成员垂体腺苷酸环化酶激活肽1(PAC1)是垂体腺苷酸环化酶激动多肽(PACAP)的特异受体, 介导PACAP神经保护等功能, 是神经系统疾病药物开发的重要靶点之一。二聚化或寡聚化是GPCRs普遍存在的现象, 但是目前尚没有关于PAC1形成同源二聚体或寡聚体的报道。为了验证PAC1也能进行同源二聚化, 该文采用生物发光能量转移(bioluminescence resonance energy transfer, BRET)方法进行检测, 结果显示不同浓度梯度共转染中国仓鼠卵巢细胞(Chinese hamster ovary, CHO)的PAC1-Rluc与PAC1-EYFP重组载体, 在底物腔肠素h(coelenterazine h)作用下呈现明显的BRET信号。双分子荧光互补(BiFC)检测显示, 带有EYFP N端基因标记的PAC1与带有EYFP C端基因标记的PAC1共转染CHO细胞, 能呈现完整的EYFP荧光信号。同时, Western blot检测也显示, 高表达PAC1的细胞中可检测到PAC1二聚体的大分子。因此, PAC1是能够进行正常同源二聚化的, 这个发现将为后续神经损伤药物的开发奠定全新的理论基础, 同时也为其他GPCRs同源二聚化的研究起到启发和借鉴作用。

关键词 GPCRs; PAC1; 同源二聚化

G蛋白偶联受体(G-protein couple receptors, GPCRs)是迄今为止发现的最大的超家族膜受体[1], 其成员有上千种, 被分为5个亚家族: A族——视紫红质样受体家族, B族——分泌素样受体家族, C族——谷氨酸盐样受体家族, D族——adhesion家族, E族——frizzkd/Taste2家族。GPCRs对大量的刺激包括生物胺、多肽、神经递质、气体、离子、脂类、核酸、光, 甚至蛋白酶都能产生应激反应, 当受到相应刺激时, 信号通过质膜被传到与它偶联的G蛋白酶, 从而启动相应的信号通路[2-3]。GPCRs具有共同的结构特征, 由七个α螺旋(含25-35个氨基酸)跨膜结构域、N端胞外域、C端胞内域三部分组成[4]。尽管它们有相似的结构, 但是它们却各自参与不同的生理和病理过程的调控[5]。它们的重要作用体现在, 在临床实践中大约40%~50%的药物是通过直接或间接对GPCRs进行修饰, 从而改善其活性来发挥药理学作用的[6-8], 但是很多药物都是针对单个G蛋白偶联受体作用而设计的, 很少是针对它的多聚化、寡聚化或二聚化来设计给药的[9]。已经有很多研究通过不同的生物物理、生物化学来探索该类受体的寡聚化或多聚化, 但大多数是针对GPCRs的A家族成员的, 很少是针对B家族成员的。PAC1主要分布在中枢神经系统、周围神经系统及神经内分泌系统(如脑、丘脑、耳蜗、视网膜、肾上腺及胰岛等), 主要介导了PACAP的神经递质、神经调质、神经保护、抗神经损伤及调控神经再生的功能[10-11], 是开发治疗帕金森病、老年痴呆症、手足舞蹈症等神经损伤药物的首选靶受体。PAC1同源二聚化机理的阐明

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将为神经药物的开发提供一个全新的设计切入点。

1 材料与方法

1.1 材料

1.1.1 细胞株 中国仓鼠卵巢细胞(Chinese hamster ovary, CHO)、人神经母细胞瘤细胞SH-SY5Y、人前列腺癌细胞22RV1以及经过G418筛选得到稳定表达PAC1受体的PAC1-CHO均由本实验室保存。细胞培养于含10%小牛血清的DMEM培养基(Gibco公司)中。菌株DH5α为本实验室保存。

1.1.2 酶及其他试剂 限制性内切酶*Eco*R I、*Sac* II、*Xho* I、*Not* I和 *Hin*d III购自美国New England Biolabs(NEB)公司, Taq酶和T4连接酶购自TaKaRa公司, PCR Purification Kit、Gel Extraction Kit、Endotoxin Plasmid Extractio Kit和Mini Plasmid Purification Kit购自OMEGA公司, 腔肠素h(coelenterazine h)购自Merck-Millipore公司, Lipofectamine LTX购自Invitrogen公司。

1.1.3 实验设备 Confocal Microscope(LSM 510 META-IEESS, Germany); Inverted Flurescence Microscopy(OLYMPUS-IX71, Japan); Victor3 1420(PerkinElmer, Wellesley, MA)。

1.1.4 引物设计 由上海生工合成(表1)。

1.2 方法

1.2.1 coelenterazine h配制 将coelenterazine h按5 μmol/L浓度溶于pH7的PBS溶液后, 分装成200 μL/管, 储存于–20 ºC备用。

1.2.2 重组载体的构建 利用PAC1-EYFP-F和PAC1-EYFP-R互补引物成功构建重组载体PAC1-EYFP; 利用PAC1-Rluc-F和PAC1-Rluc-R互补引物成功构建重组载体PAC1-Rluc; 利用EYFP/N-F和EYFP/N-R互补引物成功构建重组载体PAC1-EYFP/N; 利用EYFP/C-F和EYFP/C-R互补引物成功构建重组载体PAC1-EYFP/C。

1.2.3 共聚焦显微镜观察带有EYFP荧光标签基因的PAC1重组载体在CHO细胞表达 取处于对数生长期的CHO细胞, 消化后计数, 调整细胞浓度为2×10^5/mL, 接种于35 mm激光共聚焦培养皿(直径20 mm), 待细胞完全贴壁后按转染试剂说明书单转染PAC1-EYFP。转染换液后24~36 h在共聚焦显微镜(激发光波长488 nm, 吸收光波长545 nm)下观察重组载PAC1-EYFP是否在细胞膜部位正常表达。

1.2.4 细胞培养与转染 取处于对数生长期的CHO细胞, 消化后计数, 调整细胞浓度为2×10^4/mL, 接种于96孔板, 待细胞贴壁后按转染试剂说明书进行共转染PAC1-EYFP与PAC1-Rluc(EYFP与Rluc)。设计8个浓度梯度, PAC1-EYFP:PAC1-Rluc(ng)=0:1、1:1、2:1、4:1、6:1、8:1、10:1、12:1, 对照组EYFP/Rluc同此, 其中控制PAC1-Rluc(Rluc)载体的量为恒定1 000 ng, 只单一改变PAC1-EYFP载体量。

1.2.5 生物荧光能量共振转移检测PAC1二聚化 待共转染换液24~36 h后, 将上述96孔板CHO细胞吸取上清, PBS洗三遍, 然后每孔加入 50 μL浓度为5 μmol/L的coelenterazine h, 上机读取525 nm、488 nm

表1 寡核苷酸引物设计

Table 1 Oligonucleotide primers design

引物 Primer	序列(5′→3′) Sequence (5′→3′)	目的 Purpose
PAC1-EYFP-F	GAA TTC ATG GCC AGA ACC CTG CAG CTC TCC CTG ACT GCT CTC CTC CTG CTG	Generation of gene PAC1 tagged C-terminally with complete EYFP gene (restriction sites, *Eco*R I and *Sac* II)
PAC1-EYFP-R	CCG CGG GGT GGC CAA GTT GTC GGC CGG GAG GCT	
PAC1-Rlu-F	CTC GAG ATG GCC AGA ACC CTG CAG CTC TCC CTG ACT GCT CTC	Generation of gene PAC1 tagged C-terminally with Rlu gene(restriction sites, *Xho* I and *Hin*d III)
PAC1-Rlu-R	AAG CTT GGG TGG CCA AGT TGT CGG CCG GGA GGC T	
EYFP/N-F	CCG CGG ATG GTG AGC AAG GGC GAG GAG CTG TTC	Amplification of the EYFP N-terminal 172AA gene and tagging gene PAC1 C-terminally (restriction sites, *Sac* II and *Not* I)
EYFP/N-R	GCG GCC GCT TAC TCG ATG TTG TGG CGG ATC TTG AAG TT	
EYFP/C-F	CCG CGG GAC GGC AGC GTG CAG CTC GCC GAC CAC	Amplification of the EYFP C-terminal 67AA gene and tagging gene PAC1 C-terminally (restriction sites, *Sac* II and *Not* I)
EYFP/C-R	GCG GCC GCT TAC TTG TAC AGC TCG TCC ATG CCG AG	

下划线为酶切位点。

Underline for enzyme site.

波长处的发光值Em525、Em488, 每组4个重复。按[Em525–(Em488×Cf)]/Em488(Cf=Em525/Em488)计算BRET Ratio。

1.2.6 双分子荧光互补检测PAC1同源二聚化 取处于对数生长期的CHO细胞, 消化后计数, 调整细胞浓度为5×10^5/mL, 接种于6孔板, 待细胞贴壁后按转染试剂说明书进行单转染PAC1-EYFP或PAC1-Rluc以及共转染PAC1-EYFP和PAC1-Rluc(1:1)。换液后24~36 h在倒置荧光显微镜下观察, 变换明场和荧光场, 在同一视野下拍照。

1.2.7 免疫荧光检测PAC1-EYFP/N和PAC1-EYFP/C能否在细胞膜上正常表达 完成双分子互补实验的上述三种细胞在室温下用含4%(W/V)多聚甲醛的PBS溶液处理5 min, 然后吸取溶液, PBS洗两遍。接着用含3%(W/V) BSA的PBS溶液室温下处理1 h。为了增强细胞通透性, 以便抗体能顺利进入细胞与特定PAC1表位集合, 改用含0.05%(V/V) Triton X-100的PBS溶液室温下处理5 min。然后吸取溶液, PBS洗两遍, 加入能特异性集合在鼠PAC1 N端跨膜区61-115位的rabbit polyclonal antibody(1:100; Santa Cruz Biotechnology, USA), 室温下孵育1 h。PBS洗两遍, 接着加入Alexa594-conjugated anti-rabbit antiserum (1:400), 室温孵育1 h。然后细胞PBS洗两遍, 倒置荧光显微镜下[excitation (520±30) nm和emission (595±30) nm]观察, PAC1-EYFP/N和PAC1-EYFP/C是否成功表达并转运到细胞膜部位。

1.2.8 Western blot确证PAC1二聚化 分别取处于对数生长期的CHO、SH-SY5Y、22RV1和PAC1-CHO细胞, 消化后计数, 调整细胞浓度为5×10^5/mL, 接种于6孔板, 待长满后收集细胞制样, 采用抗PAC1的N端61-115位氨基酸的一抗、小鼠二抗进行分子量鉴定。

1.2.9 Victor3 1420多标记检测仪检测PAC1同源二聚化 带有完整EYFP荧光标记基因的PAC1在仪器给定488 nm激发光刺激下, 会发出525 nm的绿色荧光。从而可以通过检测525 nm的绿色荧光有无及强弱, 来间接判断是否有同源二聚化及同源二聚化的水平。

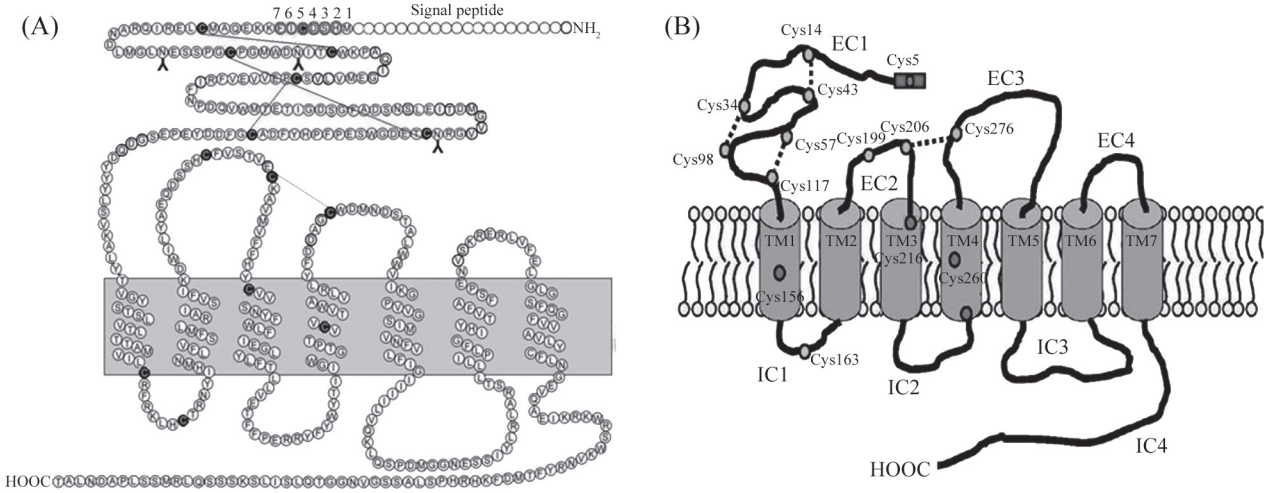
1.3 统计学处理

实验数据用($\bar{x}\pm s$)表示, 多组数据比较采用单因素方差分析, 两组均数比较采用t检验, 所有数据均由GraphPad Prism 5软件进行处理, P<0.01为统计学差异显著。

2 结果

2.1 *PAC1*基因的分析

PAC1 N端胞外域含有7个半胱氨酸, 其中Cys14和Cys43、Cys34和Cys98、Cys57和Cys117分别都



2-7连续五环是受体内部激动域HSDCIF, 线(实线和虚线)代表保守的二硫键。A: PAC1的氨基酸序列图; B: PAC1的分子结构图。

2-7 continuous rings for receptor endogenous excited domain HSDCIF, linear representative conservative disulfide bonds. A: the amino acids sequence draft of PAC1; B: the molecular structure draft of PAC1.

图1 PAC1序列和结构模式图

Fig.1 PAC1 amino acids sequence and structure mode

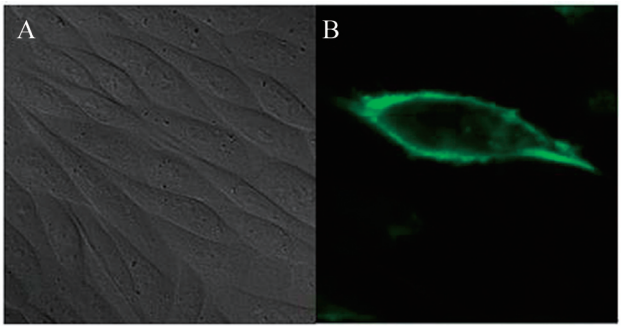
形成了分子内二硫键, 只有Cys5单独存在, 因此推测该半胱氨酸可能参与形成PAC1同源二聚化分子间二硫键(图1)。

2.2 重组载体PAC1-EYFP表达情况

未处理的CHO细胞, 转染PAC1-EYFP重组载体后, 在共聚焦显微镜荧光场下发出绿色荧光, 并且分布在细胞膜部位。说明重组后的*PAC1*基因仍然能够正常表达, 并且不影响它向细胞膜部位转运(图2)。

2.3 Western blot确证PAC1同源二聚体的存在

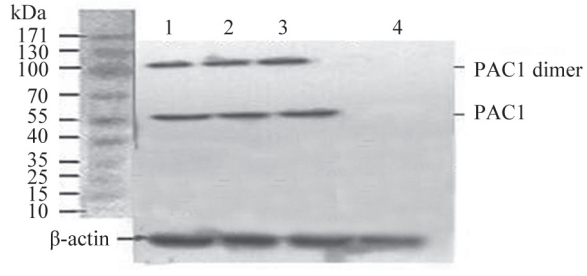
多株高表达PAC1的肿瘤细胞(人前列腺癌细胞22RV1、人神经母细胞瘤细胞SH-SY5Y)及重组细胞株PAC1-CHO中均检测到110 kDa左右与PAC1二聚体大小相吻合的大分子(图3)。PAC1由468 aa组成, 单体分子量为55 kDa, 二聚体分子为110 kDa, 而PAC1的最大剪切变体只有523 aa, 单体大小不会超过70 kDa，表达PAC1的细胞中可检测出PAC1二聚体的大分子, 这是PAC1二聚化的直接证据。



A: 从形态学上证明荧光标记受体的正常表达, 在共聚焦显微镜明场下观察被EYFP标记的PAC1-EYFP在CHO细胞中的表达; B: 在荧光场下观察的EYFP标记的PAC1-EYFP在CHO细胞中的表达。
Morphologic demonstration of normal expressing of fluorescently tagged recptors. Shown was confocal fluorescence micrographs(single optical section) of formaldehyde-fixed CHO cells expressing EYFP-tagged versions of PAC1-EYFP(A) at the lighted field and EYFP-tagged versions of PAC1-EYFP(B) at fluorescent field.

图2 共聚焦显微镜观察重组载体在CHO细胞中的正常表达

Fig.2 Recombinant plasmid normally expressing in CHO cells by confocal microscope



1: PAC1-CHO细胞; 2: SH-SY5Y细胞; 3: 22RV1细胞; 4: CHO细胞.
1: PAC1-CHO cells; 2: SH-SY5Y cells; 3: 22RV1 cells; 4: CHO cells.

图3 Western blot检测PAC1的表达

Fig.3 Western blot testing PAC1 expressing

2.4 生物发光能量转移检测PAC1受体之间发生同源二聚化

BRET是目前应用于鉴定GPCRs二聚化或寡聚化的主要方法之一, 当受体二聚化时(距离<100 A°), 一个受体所偶联的海肾荧光素酶(renila luciferase, Rluc)作用底物coelenterazine h所产生的生物发光luminescence(488 nm)能量被“靠得足够近”的另一受体所携带的改良黄色荧光蛋白(enhanced yellow fluorescent protein, EYFP)接受而激发出525 nm荧光, 通过检测488 nm(代表Rluc发出的luminescence)和525 nm(代表EYFP发出的fluorescence)的信号强度, 计算BRET Ratio(525 nm/488 nm), 从而评价A与B的相互作用(图4)。BRET饱和曲线, 固定能量供体PAC1-Rluc质粒浓度(1 000 ng DNA/10^4 cells), 逐步增加能量受体PAC1-EYFP质粒浓度[(600~12 000) ng DNA/10^4 cells], 共转染细胞后48 h后, 测定并计算BRET Ratio (525 nm/488 nm), 发现BRET Ratio随EYFP/Rluc (浓度比)增加而增加, 表明发生典型的BRET反应, 作为受体二聚化的表征(图5)。

2.5 双分子荧光互补和免疫荧光检测PAC1受体之间发生同源二聚化

单独转染PAC1-EYFP/N或PAC1-EYFP/C的CHO细胞在倒置荧光显微镜荧光场下并没有发出绿色荧光信号, 而共转染PAC1-EYFP/N和PAC1-EYFP/C (1:1)的CHO细胞有很强的绿色荧光信号, 说明共转

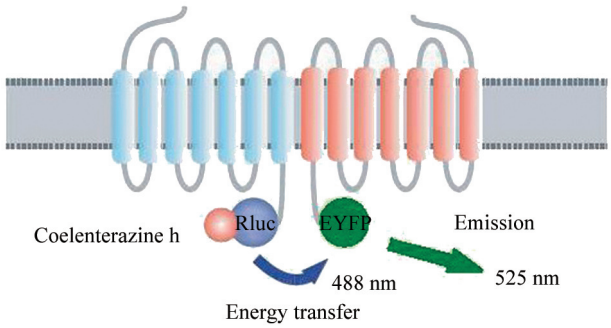
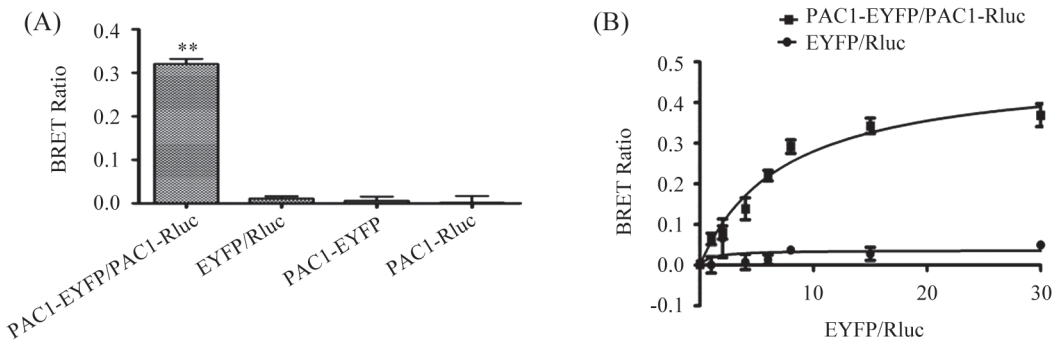


图4 BRET检测受体二聚化的原理示意图

Fig.4 BRET detecting dimerization of receptor principle diagram

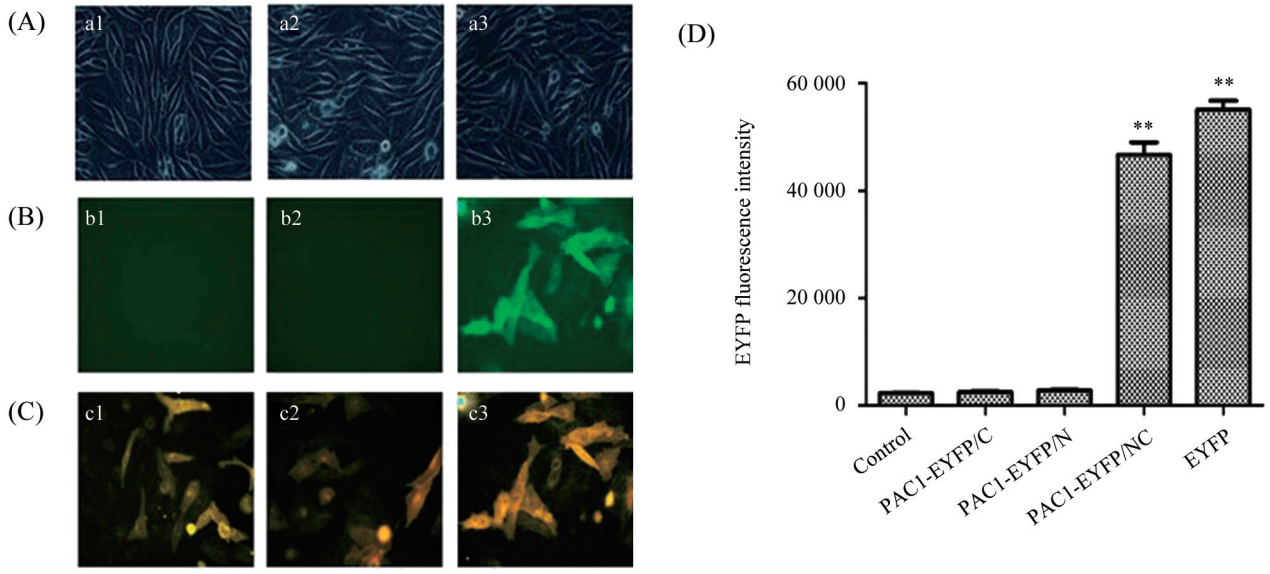


A: 固定供体Rluc和受体EYFP总量为3 μg共转染CHO细胞, 以单转染PAC1-Rluc做为对照, 进行BRET检测, 定量分析, 其中阴影部分代表供体PAC1-Rluc和受体PAC1-EYFP之间发生二聚化而产生的BRET信号, 每组数据都是经过6次独立的重复试验后取平均值, **P<0.01, 与对照组相比; B: 固定供体Rluc的量, 改变受体EYFP的量共转染CHO细胞, 进行BRET检测, 得到BRET饱和曲线。随着受体浓度的不断增加, BRET Ratio呈现不断增加趋势, 并最终达到饱和状态, 符合PAC1发生同源二聚化, 每组数据都是经过6次独立实验后取平均值。

Shown were BRET Ratios generated from CHO cells expressing Rlu-tagged and EYFP-tagged receptorconstructs. For static BRET(A) a total of 3 μg of DNA was utilized, divided equally among the noted constructs in each condition. Cells tranfected with PAC1-Rluc alone was used as negative control. The shaded area represents the specific BRET signal generated between PAC1-Rlu and PAC1-EYFP protein, with BRET signals above this area considered to be significant. Data were presented as mean±S.E. of six independent experiments. **P<0.01, significantly above the background and significantly higher than negative control(PAC1-Rluc). For saturation BRET analysis(B), shown were the BRET saturation curves plotted as ratios of EYFP fluorescence to Rlu luminescence that were observed for tagged receptor constructs studied with a fixed amount of donor and increasing amounts of acceptor. PAC1-Rluc/PAC1-EYEP receptor constructs yielded exponential curves that reached asymptotes indicating significant homo-dimerization of PAC1, while EYFP/Rluc yielded curves is a straight line. Data were presented as mean±S.E. of six independent experiments.

图5 BRET表征PAC1二聚化

Fig.5 BRET characterizing dimerization of PAC1



a1、b1、c1单转转染PAC1-EYFP/N的CHO细胞, a2、b2、c2单转染PAC1-EYFP/C的CHO细胞, a3、b3、c3共转染PAC1-EYFP/N和PAC1-EYFP/C的CHO细胞; A:在倒置荧光显微镜明场下观察结果; B: 荧光场下观察结果; C: 免疫荧光场下观察结果; D: 对荧光信号通过Victor3 1420多标记检测仪定量分析结果, **P<0.01, 与对照相比。

CHO cells were alone-transfected PAC1-EYFP/N, observed at lighted and different fluorescent field of converted fluorescence microscope(a1,b1); CHO cells were alone-transfected PAC1-EYFP/C, observed at lighted and different fluorescent fields of converted fluorescence microscope(a2,b2); CHO cells were co-transfected PAC1-EYFP/N and PAC1-EYFP/C, observed at lighted and fluorescent field of converted fluorescence microscope(a3,b3); C: immunocytochemistry fluorescence was proceeded at last row; D: the statistical analysis of the EYFP fluorescence intensity showed that only cells were co-transfected PAC1-EYFP/N and PAC1-EYFP/C or EYFP(positive control) had significant higher EYFP fluorescence intensity than the negative control. **P<0.01 vs control.

图6 Victor3 1420多标记检测仪和双分子荧光互补检测PAC1同源二聚化, 免疫学荧光检测重组载体在CHO细胞膜上正常表达

Fig.6 Multi-label detector Victor3 1420 and BiFC detecting dimerization of PAC1, immunofluorescence assaying recombinant plasmid normally expressing on the membrane of CHO cells

染CHO细胞, 实现两个蛋白二聚化形式的共表达。如果PAC1同源二聚化, EYFP/N172与EYFP/C67靠近, 恢复EYFP完整荧光功能。无论是单独转染的PAC1-EYFP/N或PAC1-EYFP/C的CHO, 还是共转染PAC1-EYFP/N和PAC1-EYFP/C(1:1)的CHO细胞, 经过免疫荧光检测, 都可以观察到PAC1在细胞膜上的正常表达(图6 A-图6C)。

2.6 Victor3 1420多标记检测仪检测荧光互补信号

单独转染EYFP、PAC1-EYFP/N、PAC1-EYFP/C的CHO细胞以及共转染PAC1-EYFP/N和PAC1-EYFP/C(1:1)的CHO细胞, 在Victor3 1420给出488 nm激发光前提下, 分别检测525 nm波长(图6D)。

3 讨论

GPCRs常常通过N端胞外域、C端胞内域或七次跨膜域形成分子间二硫键而发生二聚化或寡聚化[12-13], 从而被激活, 进而启动下游偶联信号通路发挥功能。PAC1和VPACs都属于G蛋白偶联受体B家族, 该家族成员普遍都能被多肽激素激活, 例如分泌素、胰高血糖素、生长激素、促肾上腺皮质激素、甲状旁腺素、降钙素等[14-16], 并且此类受体的显著特点是具有较长的N端胞外域, 该区域主要用于配体的结合[17], 可能介导自身同源二聚化而被激活。

二聚化或寡聚化是GPCRs普遍存在的现象[18]。目前尚没有PAC1形成二聚体或寡聚体的报道, 但属于B族GPCRs的VPAC1、VPAC2及SR均可形成同源或异源二聚体或寡聚体。VPAC1和VPAC2的同源或异源二聚化并不影响配体VIP的结合与激活: 但配体的结合能够导致同源或异源二聚体的解聚。而SR可形成组成型同源二聚体, 配体的结合不会使SR同源二聚体及其和VPAC1或VPAC2形成的异源二聚体解聚。SR是通过第4跨膜区(transmembrane domain 4, TM4)形成二聚体[18], 而且TM4被认为是介导B族GPCRs二聚化的关键区域[19], PAC1的TM4与SR的TM4同源性极高, 因此探索PAC1的同源二聚化已经具备很充分的理论基础。

GPCRs的固有活性, 尤其是神经肽类激素受体的固有活性被认为与神经系统基础活性有关, 目前对PAC1非配体依赖激活的固有活性的研究只限于人为的氨基酸突变的个例研究, 不能有效诠释PAC1所担负的生理角色。PAC1的同源二聚化所介导的固有活性机制符合PAC1介导调控神经生理节律[20]、学习记忆[21]、社会行为及精神状态[22]等神经活动的生物学角色与功能, 有助于我们深入诠释PAC1的生理学角色与作用。

总之, PAC1同源二聚化的确证不仅有助于诠释高表达PAC1的生理学与病理学的作用和意义, 而且为以PAC1为靶点的药物开发提供了新的理论依据和研究基础。例如, 可以用提高PAC1固有活性的方法替代原有的配体激活的方法来开启PAC1所介导的信号通路, 实现以PAC1为靶点的相关神经系统疾病的治疗: 这样可以避免PACAP等多肽配体激活非特异受体而造成的副作用。对于和肿瘤或某些病理过程密切相关的PAC1高表达, 可以通过抑制其固有活性[抑制其二聚化或筛选有效反向激动剂(inverse agonist)]实现对某些肿瘤(神经内分泌肿瘤)或某些病理过程(如创伤后应激障碍, 压力性应激障碍等)的干预和治疗。

参考文献 (References)

1 Fredriksson R, Schiöth HB. The repertoire of G-protein-coupled receptors in fully sequenced genomes. Mol Pharmacol 2005; 67(5): 1414-25.

2 Wei H, Ahn S, Shenoy SK, Karnik SS, Hunyady L, Luttrell LM, *et al*. Independent beta-arrestin 2 and G protein-mediated pathways forangiotensin II activation of extracellular signal-regulated kinases 1 and 2. Proc Natl Acad Sci USA 2003; 100(19): 10782-7.

3 Luttrell LM. Transmembrane signaling by G protein-coupled receptors. Methods Mol Biol 2006; 332: 3-49.

4 Szidonya L, Cserzo M, Hunyady L. Dimerization and oligomerization of G-protein-coupled receptors: Debated structures with established and emerging functions. J Endocrinol 2008; 196(3): 435-53.

5 Xin Cai, Jing Chen, Bo Bai. Exploring the specific role of GPCRs dimerization in drug discovery. Journal of Chinese Pharmaceutical Sciences 2011; 20(6): 535-41.

6 Tyndall JD, Sandilya R. GPCR agonists and antagonists in the clinic. Med Chem 2005; 1(4): 405-21.

7 Terrillon S, Bouvier M. Roles of G-protein-coupled receptor dimerization. EMBO Rep 2004; 5(1): 30-4.

8 Jacoby E, Bouhelal R, Gerspacher M, Seuwen K. The 7 TM G-protein-coupled receptor target family. ChemMedChem 2006; 1(8): 761-82.

9 Milligan G. G protein-coupled receptor hetero-dimerization: Contribution to pharmacology and function. Br J Pharmacol 2009; 158(1): 5-14.

10 Vaudry D, Falluel-Morel A, Bourgault S, Basille M, Burel D, Wurtz O, *et al*. Pituitary adenylate cyclase-activating polypeptide and its receptors: 20 years after the discovery. Pharmacol Rev 2009; 61(3): 283-357.

11 Bourgault S, Vaudry D, Dejda A, Doan ND, Vaudry H, Fournier A. Pituitary adenylate cyclase-activating polypeptide: Focus on structure-activity relationships of a neuroprotective peptide. Curr Med Chem 2009; 16(33): 4462-80.

12 Gao F, Harikumar KG, Dong M, Lam PC, Sexton PM, Christopoulos A, *et al*. Functional importance of a structurally distinct homodimeric complex of the family B G protein-coupled secretin receptor. Mol Pharmacol 2009; 76(2): 264-74.
13 Harikumar KG, Morfis MM, Lisenbee CS, Sexton PM, Miller LJ. Constitutive formation of oligomeric complexes between family B G protein-coupled vasoactive intestinal polypeptide and secretin receptors. Mol Pharmacol 2006; 69(1): 363-73.
14 Laburthe M, Couvineau A, Tan V. Class II G protein-coupled receptors for VIP and PACAP: Structure, models of activation and pharmacology. Peptides 2007; 28(9): 1631-9.
15 Laburthe M, Couvineau A, Gaudin P, Maoret JJ, Rouyer-Fessard C, Nicole P. Receptors for VIP, PACAP, secretin, GRF, glucagon, GLP-1, and other members of their new family of G protein-linked receptors: Structure-function relationship with special reference to the human VPAC1 receptor. Annu NY Acad Sci 1996; 805: 94-109.
16 Dickson L, Finlayson K. VPAC and PAC receptors: From ligands to function. Pharmacol Ther 2009; 121(3): 294-316.
17 Dautzenberg FM, Mevenkamp G, Wille S, Hauger RL. N-terminal splice variants of the type I PACAP receptor: Isolation, characterization and ligand binding/selectivity determinants. J Neuroendocrinol 1999; 11(12): 941-9.
18 张从芬, 彭代银, 李庆林, 池志强, 刘景根. G蛋白偶联受体二聚化研究进展. 中国药理学通报(Zhang Congfen, Peng Daiyin, Li Qinglin, Chi Zhiqiang, Liu Jinggen. Dimerization of G protein-coupled receptors and its possible role in signaling transduction. Chinese Pharmacological Bulletin) 2006; 22(7): 774-9.
19 Simpson LM, Taddese B, Wall ID, Reynolds CA. Bioinformatics and molecular modelling approaches to GPCR oligomerization. Curr Opin Pharmacol 2010; 10(1): 30-7.
20 Cao YJ, Gimpl G, Fahrenholz F. A mutation in the second intracellular loop of the pituitary adenylate cyclase activating polypeptide type I receptor confers constitutive receptor activation. FEBS Lett 2000; 469(2/3): 142-6.
21 Cao Y, Gimpl G. A constitutively active pituitary adenylate cyclase activating polypeptide(PACAP) type I receptor shows enhanced photoaffinity labeling of its highly glycosylated form. Biochim Biophys Acta 2001; 1548(1): 139-51.
22 Simpson LM, Taddese B, Wall ID, Reynolds CA. Bioinformatics and molecular modelling approaches to GPCR oligomerization. Curr Opin Pharmacol 2010; 10(1): 30-7.

Researching Homologous Dimerization of G Protein Coupling Receptor PAC1 through BRET and BiFC

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Abstract G-protein couple receptors (GPCRs) are the biggest super family membrane receptors. PAC1 belongs to the B family of GPCRs and is pituitary adenosine acid cyclization enzyme excited peptide (PACAP) specific receptors, mediating PACAP neural protection function, which is one of the important targets for drug development to diseases of the nervous system. Dimerization or oligomerization is a common phenomenon to GPCRs. But there is no report homologus dimerization or oligomerization for PAC1 at present. In order to verify PAC1 dimerization, we use BRET to test CHO cells which are co-transfected PAC1-Rluc and PAC1-EYFP with different density gradient. The result presents obviously BRET signal by adding coelenterazine h. While BiFC test shows that CHO cells which are co-transfecte PAC1-EYFP/N and PAC1-EYFP/C appear complete EYFP fluorescent signal. Western blot test also shows that cells which high expressing PAC1 contain macromolecules of PAC1 dimer. So PAC1 can normally form homologous dimerization. This discovery will lay the novel theoretical foundation for the subsequent drug development, and offer illumination and reference for researching other GPCRs.

Key words GPCRs; PAC1; homologous dimerization

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