Regulation of lysophosphatidic acid receptor-stimulated response by G-protein-coupled receptor kinase-2 and β -arrestin1 in FRTL-5 rat thyroid cells

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Abstract

Lysophosphatidic acid (LPA) is a naturally occurring phospholipid that activates a variety of biological activities including cell proliferation. Three mammalian LPA receptor (LPA_r) subtypes have been identified by molecular cloning, named lp_{A1}, lp_{A2} and lp_{A3}, that are coupled to heterotrimeric G-proteins for signal transduction. The LPA_r are endogenously expressed in the rat thyroid cell line FRTL-5 and we used the FRTL-5 cells permanently transfected to obtain moderate overexpression of G-protein-coupled receptor kinase-2 (GRK2) or β -arrestin1 to study whether GRK2 and β -arrestin1 desensitise LPA_r-mediated signalling and regulate LPA-stimulated functional effects.

Using RT-PCR we documented that lp_{A1}, lp_{A2} and lp_{A3} receptors are all expressed in FRTL-5 cells. We then analysed the signal transduction of the LPA_r in FRTL-5 cells. Exposure to LPA did not stimulate inositol phosphate formation nor cAMP accumulation but reduced forskolin-stimulated cAMP. LPA was also able to stimulate MAP kinase activation and this effect was abolished by pertussis toxin pretreatment. These results suggest that LPA_r are mainly coupled to a pertussis toxin-sensitive G-protein in FRTL-5 cells.

In order to investigate whether GRKs and arrestins are involved in the regulation of LPA_r-mediated signalling,

we used the FRTL-5 cell line permanently transfected to overexpress GRK2 (named L5GRK2 cells) or β -arrestin1 (L5 β arr1 cells). The ability of LPA to inhibit forskolin-stimulated cAMP accumulation was blunted in L5GRK2 and more markedly in L5 β arr1. The MAP kinase activation was also blunted in L5GRK2 and in L5 β arr1B cells. Exposure to 20 μ M LPA increased the phosphorylation of extracellular signal-regulated kinases ERK1/2 by ~ 3 -fold in L5pBJI cells (FRTL-5 cells transfected with the empty vector pBJI) while it induced a modest increase in L5 β arr1 and was ineffective in L5GRK2.

We measured [³H]thymidine uptake in L5 β arr1B and in L5 GRK2 cells to test whether GRK2 and β -arrestin1 could have a role in the regulation of LPA_r-mediated cell proliferation. The mitogenic response induced by 35 μ M LPA was substantially blunted in L5 β arr1 ($-69 \pm 6\%$) and in L5GRK2 ($-69.8 \pm 4.5\%$) cells as compared with L5pBJI. Our findings document that the receptor-mediated responses elicited by LPA are regulated by GRK2 and β -arrestin1 in FRTL-5 cells and indicate that this mechanism is potentially important for the control of the LPA-stimulated proliferative response.

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Introduction

Lysophosphatidic acid (LPA) is a naturally occurring phospholipid that activates a variety of biological activities in higher eukaryotes, promoting cell proliferation, smooth muscle contraction, platelet aggregation, reversal of neuroblastoma differentiation and changes in cell morphology and adhesion. LPA activates specific cell surface receptors that are members of the G-protein-coupled receptor (GPCR) superfamily (Contos *et al.* 2000, Kraneburg & Moolenaar 2001). Three mammalian LPA receptor (LPA_r) subtypes have been identified by molecular cloning,

named lp_{A1}, lp_{A2} and lp_{A3} (Contos *et al.* 2000), which can couple to different heterotrimeric G-proteins for signal transduction. Depending on the cell system, LPA_r stimulation could regulate the activity of different effectors, such as stimulation of phospholipase C and phospholipase A₂, activation of the MAP kinase cascade and inhibition of adenylyl cyclase.

It has been shown for several GPCRs that the process of signal transduction must be properly regulated in order to prevent overstimulation, achieve signal termination and render the receptor responsive to subsequent stimuli. Prolonged or repeated exposure of GPCRs to their

agonists induces diminished cellular response to following agonist stimulation. This process, named homologous desensitisation, involves the activity of proteins belonging to two distinct families: the G-protein-coupled receptor kinases (GRKs) and arrestins (Miller & Lefkowitz 2001, Pierce *et al.* 2001). The agonist-occupied receptor is phosphorylated by the GRK at serine/threonine residues, thus allowing the binding of arrestin and consequent uncoupling from the G-protein. The GRK family is composed of six cloned members named GRK1–6. GRK2 (previously known as β -adrenergic receptor kinase) is the prototype GRK. Molecular cloning has so far identified four arrestins. Two subtypes are selectively expressed in the retina, where they regulate phototransduction, while β -arrestin1 and 2 are expressed in many cell types. The two human splice variants of β -arrestin1 are named β -arrestin1A and B (Parruti *et al.* 1993).

Similar to many other GPCRs, agonist exposure induces the desensitisation of LPAr (Fischer *et al.* 1998, Kakizawa *et al.* 1998, Gueguen *et al.* 1999), but the mechanism involved in this process has not been elucidated. The present investigation was aimed at addressing this point. We used the rat thyroid cell line FRTL-5 permanently transfected to obtain moderate overexpression of GRK2 or β -arrestin1, which, as shown by our previous work, provides a useful tool to study the homologous desensitisation of GPCRs naturally expressed in FRTL-5 cells (Iacovelli *et al.* 1996, 1999). The LPAr is endogenously expressed in FRTL-5 cells and we document that in these cells GRK2 and β -arrestin1 desensitise LPAr-mediated signalling and regulate LPA-stimulated functional effects.

Materials and Methods

Cell culture and transfection

FRTL-5 cells are a continuous line of Fisher rat thyroid cells that maintain *in vitro* the proliferation and differentiation properties of the rat thyroid. FRTL-5 cells were cultured as previously described (Iacovelli *et al.* 1996). Briefly, FRTL-5 were maintained in Coon's modified F-12 medium supplemented with 5% calf serum, 20 mM glutamine and a mixture of six hormones (thyrotrophin (TSH), insulin, transferrin, somatostatin, cortisol and glycyl-L-histidyl-L-lysine acetate) and grown at 37 °C in a humidified atmosphere of 5% CO₂.

FRTL-5 cells overexpressing GRK2 (named L5 GRK2) or β -arrestin1 (named L5 β arr1) were derived from FRTL-5 cells stably transfected with GRK2 cDNA and β -arrestin1 (splicing 1B) cDNA. The transfection protocol and selection of transfectant clones have been already described (Iacovelli *et al.* 1996). Clones of FRTL-5 cells transfected with the empty vector pBJ1 were also produced to be used as control.

RT-PCR analysis of LPAr

RT-PCR was performed as previously described (Parruti *et al.* 1993). Five micrograms of FRTL-5 total RNA were reverse transcribed using random examers and PCR was performed for 30 s at 90 °C, 45 s at 60 °C and 1 min at 72 °C for 35 or 45 cycles, as indicated. The primers, derived from mouse sequences (Contos *et al.* 2000), were as follows: for the lp_{A1} CTAGCCACAGAATGGAACAC (forward primer) and TGCTCATTCGTGTATGGAGC (reverse primer); for the lp_{A2} ATGGGCCAGTGCTAC TACAA (forward primer) and AACATGAGGAAGAG GTAGGC (reverse primer); for the lp_{A3} ATGAATGAG TGTCATATGACAA (forward primer) and ACCGG GCCAGTGTAAACAT (reverse primer). Rat testis total RNA was used as positive control.

cAMP assay and inositol phosphate (IP) formation analysis

The intracellular content of cAMP in transfected cells was determined by a method previously described (Iacovelli *et al.* 1996). Briefly, cells were seeded in 96-well plates at a density of 5×10^4 cells/well and grown to confluency, then starved of hormones and serum for 48 h. Cells were washed twice with pre-warmed Hanks' balanced salt solution (HBSS) and added with HBSS containing 0.4% BSA, 10 mM Hepes and 0.5 mM 3-isobutyl-1-methylxanthine. Incubations were continued at 37 °C for 30 min with appropriate stimuli. The reaction was stopped by aspirating the incubation medium and adding ice-cold ethanol. The intracellular cAMP content was measured using a commercial RIA. The data shown are means $\pm$ S.E.M. of three or four independent experiments each assayed in triplicate.

Total IP production was determined as described (Iacovelli *et al.* 1999). Briefly, cells plated for IP assay were overnight loaded with [³H]D-myo-inositol (5 μ Ci/ml) in M199 serum-free medium. Cells were washed twice with pre-warmed HBSS, incubated for 15 min at 37 °C in HBSS buffer containing 10 mM Hepes and 10 mM LiCl, pH 7.3 and then stimulated with the indicated agonists for 30 min. Total [³H]IP was extracted with perchloric acid, neutralised and analysed by ion exchange chromatography on a Dowex column.

Western blot for phosphorylated extracellular signal-regulated kinases (ERK1/2)

The Western blot for phosphorylated ERK1/2 was performed as previously described with minor modifications (Brondello *et al.* 1997, Della Rocca *et al.* 1997). Briefly cells were grown to 50% confluency in six-well plates and starved of hormones and serum for 48 h. Cells were stimulated in the same buffer as for cAMP assay at 37 °C for 30 min, then washed twice with ice-cold PBS and lysed in Triton X-100 lysis buffer for 15 min at 4 °C.

Figure 1 RT-PCR analysis of LPAr subtypes in FRTL-5 cells. Five micrograms of FRTL-5 total RNA were reverse transcribed using random examers and PCR was performed for 45 cycles. Rat testis RNA was analysed in parallel as a positive control. Molecular mass markers (M) are shown.

Samples were clarified by centrifugation at 12 000 *g* for 10 min at 4 °C. Equal amounts of proteins from supernatants (50 µg) were separated by SDS-PAGE (12.5% acrylamide, 0.0625% bisacrylamide). Proteins were transferred and probed using a commercial anti-phospho specific antibody against phosphorylated ERK1/2.

Confocal analysis and immunofluorescence

Confocal analysis was performed as previously described (Iacovelli *et al.* 2001). FRTL-5 cells were seeded on glass chamber slides. After agonist stimulation, cells were fixed with 4% paraformaldehyde in PBS (0.1 M pH 7.4) for 15 min at room temperature. The autofluorescence was quenched by incubation for 30 min in 50 mM NH₄Cl, 50 mM glycine in PBS and non-specific interactions were blocked by treatment with blocking solution (0.05% saponin, 0.5% BSA in PBS) for 30 min at room temperature. Cells were incubated overnight at 4 °C with anti-arrestin monoclonal antibody F4C1 (kindly provided by L A Donoso, Wills Eye Hospital, Philadelphia, PA, USA) in blocking solution. The chamber slides were then incubated with blocking solution containing Alexa-488 (Molecular Probes, Eugene, OR, USA) (1:400) anti-mouse IgGs for 1 h at room temperature. From the incubation with the secondary antibody, each incubation step was carried out in the dark and followed by careful washes with PBS (six times/3 min each). After immunostaining the coverslips were mounted on slides with

Mowiol 4-88, and analysed by the Confocal Imaging System Ultraview (Perkin Elmer, Cambridge, UK).

[³H]thymidine uptake

This experiment was performed essentially as previously described (Iacovelli *et al.* 1996), with minor modifications. Briefly, cells were plated in 96-well plates (10⁴ cells/well) and allowed to grow for 4 days. After 48 h of starvation in Coon's modified F-12 medium plus 0.3% BSA and 20 mM glutamine, the cells were stimulated as appropriate for 30 h and a pulse of [³H]thymidine was given in the last 6–8 h.

Statistical analysis

All the experiments are presented as the average of duplicate or triplicate determinations repeated at least three times. Statistical analysis was carried out by either Student's *t*-test or Dunnett ANOVA.

Materials

5-Bromo-4-chloro-3-indolyl phosphate and nitro blue tetrazolium were from Gibco BRL; forskolin (FSK) from Calbiochem (Darmstadt, Germany); glutamine, penicillin/streptomycin and calf serum from Gibco; Tris, SDS, bromophenol blue and G418 from Fluka (Sigma-Aldrich); acrylamide and bisacrylamide from Promega; cAMP assay

kit and [³H]thymidine from Amersham; alkaline phosphatase-conjugate goat anti-mouse IgG from Pierce (Rockford, IL, USA); anti-phospho ERK1/2 antibody from Promega. All the other materials were from Sigma (Milan, Italy).

Results and Discussion

The presence of LPAr on the rat thyroid FRTL-5 cells was suggested by previous evidence mostly based on pharmacological approaches (Okajima *et al.* 1997, Falasca *et al.* 1998). More recently, three LPAr subtypes have been identified by molecular cloning making the human and mouse sequences available (Contos *et al.* 2000, Pierce *et al.* 2001). We used RT-PCR to document the expression of LPAr in FRTL-5 cells and to identify the LPAr subtypes (Contos *et al.* 2000) which are expressed on these cells. Rat testis, in which the three LPAr subtypes are expressed, was used as a positive control. RT-PCR analysis of the FRTL-5 RNA showed one band of the expected size for each of the LPAr subtypes considered, indicating that FRTL-5 express lp_{A1}, lp_{A2} and lp_{A3} receptors (Fig. 1). Based on these experiments, the lp_{A3} appeared to be less abundant, as compared with the testis.

Since LPAr can couple to different heterotrimeric G-proteins for signalling, and the signalling pathways are cell-type dependent (Bandoh *et al.* 1999, Young *et al.* 1999, Contos *et al.* 2000, Hernandez *et al.* 2000, Pierce *et al.* 2001), in initial experiments we characterised the LPA-mediated signalling in FRTL-5 cells. Exposure of FRTL-5 cells to LPA reduced FSK-stimulated intracellular cAMP accumulation in a dose-dependent manner (IC₅₀ = 10 µM). Maximal reduction of FSK-stimulated cAMP levels was observed at doses between 20 and 70 µM. At 20 µM LPA, FSK-stimulated cAMP was decreased by 45–50% (Fig. 2A). At this concentration LPA alone did not stimulate cAMP production in FRTL-5 cells. In these cells, the stimulation of LPAr activated MAP kinases and this effect was likely mediated by a pertussis toxin (PT)-sensitive G-protein, since the pretreatment with PT totally prevented the phosphorylation and hence the activation of ERK1/2 (Fig. 2C). The possible coupling to Gq was assessed by measuring total IP production after LPA exposure. We found that exposure to LPA (up to 50 µM) did not induce any detected IP accumulation, while in parallel experiments the activation of the α1 adrenergic receptors by 1 µM norepinephrine (NE) increased IP production by 2- to 3-fold (Fig. 2B). Taken together this set of experiments indicates that in FRTL-5 cells the LPAr are mainly coupled to a PT-sensitive

Figure 2 Lysophosphatidic acid (LPA)-stimulated signalling in FRTL-5. (A) FRTL-5 cells were stimulated for 30 min with 20 µM LPA or with 10 µM forskolin (FSK) or both and cAMP was measured. Data are the means ± S.E.M. of three separate experiments performed in triplicate. (B) FRTL-5 cells were stimulated for 30 min with 50 µM LPA or 1 µM norepinephrine (NE) and the inositol phosphate (IP) formation was measured. Data are the means ± S.E.M. of three separate experiments performed in duplicate. (C) Immunoblot representing phospho-ERK1/2 in L5pBJ untreated or treated with 20 µM LPA and 1 nM pertussis toxin (PT) (which was preincubated for 16 h). The experiment is representative of two similar.

Figure 3 RT-PCR analysis of LPA subtypes in L5pBJI, L5βarr1 and L5GRK2. Five micrograms of total RNA were reverse transcribed using random examers and PCR was performed for 35 cycles (exponential phase of the reaction). Lanes are: 1: molecular mass markers, 2: rat testis, 3: L5pBJI; 4: L5βarr1; 5: L5GRK2.

G-protein. We hypothesise that the stimulation of LPA induces the dissociation of the PT-sensitive G-protein heterotrimer and the release of free $G\alpha$ inhibits adenylyl cyclase activity and reduces intracellular cAMP levels, while the MAP kinase cascade could be stimulated by free $G\beta\gamma$.

The main goal of this study was to investigate whether the LPA are regulated by the GRK/arrestin-dependent mechanism for homologous desensitisation. In order to investigate whether GRKs and arrestins are involved in the regulation of LPA-mediated signalling, we used the FRTL-5 cell line permanently transfected to overexpress GRK2 (named L5GRK2 cells) or β -arrestin1 (L5βarr1 cells). We transfected GRK2 and β -arrestin1 since these subtypes are expressed in FRTL-5 cells and, as documented by our previous studies, represent the physiologically relevant subtypes in these cells (Iacovelli *et al.* 1996, 1999). FRTL-5 cells transfected with the empty vector (L5pBJI cells) were used as control cells in this study. All the cell clones were used for limited cell passages (15–20 passages), in order to avoid changes in cellular responses due to ageing. In all experiments the L5pBJI,

Figure 4 Regulation of LPA signalling by β -arrestin1 and GRK2. L5pBJI ($\square$), L5βarr1 ($\blacksquare$) and L5GRK2 ($\bullet$) were stimulated for 30 min with 10 μ M FSK plus the indicated concentrations of LPA and cAMP accumulation was measured. Data are the means $\pm$ S.E.M. of three separate experiments performed in triplicate. All the values in L5βarr1B were statistically significant vs L5pBJI at $P < 0.01$, except for LPA 1, 40 and 70 μ M in L5GRK2 which were significant at $P < 0.05$.

L5βarr1 and L5GRK2 cells were age-matched. These cell lines represent a useful experimental model for investigating the regulation of GPCR signalling, as described in previous studies. We selected FRTL-5 clones in which the level of GRK2 or β -arrestin1 overexpression did not exceed 3- to 4-fold that of the endogenous proteins, since the moderate overexpression of GRK2 and β -arrestin1 resulted in a selective regulation of different GPCRs endogenously expressed in FRTL-5 cells (Iacovelli *et al.* 1999). For example we could demonstrate that TSH receptors, which in these cells mediate fundamental responses such as proliferation and differentiation, are strictly controlled by GRK/ β -arrestin regulatory mechanisms, while the $\alpha 1$ -adrenergic receptors were unaffected by GRK2 overexpression (Iacovelli *et al.* 1999).

The expression of the three LPA subtypes was determined in L5pBJI, L5βarr1 and L5GRK2 by RT-PCR. The PCR was performed for 35 cycles, when the reaction is at the exponential phase (not shown). The expression of the IpA₁, IpA₂ and IpA₃ was similar in all the FRTL-5 clones analysed, indicating that the overexpression of β -arrestin1 and GRK2 did not alter the levels of LPA (Fig. 3). The ability of LPA to inhibit FSK-stimulated cAMP accumulation was blunted in L5GRK2 and more markedly in L5βarr1. LPA (35 μ M) decreased FSK-stimulated intracellular cAMP by $46.2 \pm 7\%$ in L5pBJI and only by $19.2 \pm 6\%$ ($P < 0.01$ vs control) and $3.3 \pm 8.7\%$ ($P < 0.01$ vs control) in L5GRK2 and in L5βarr1 respectively (Fig. 4). This indicates that the LPA-stimulated signalling is desensitised by GRK2 and β -arrestin1 overexpression. Consistently, the MAP kinase

Figure 5 Regulation of LPA-stimulated ERK1/2 phosphorylation by β -arrestin1 and GRK2. On the left: representative immunoblot showing phospho-ERK1/2 in L5pBJI, L5 β arr1 and L5GRK2 after exposure to the indicated concentrations of LPA. On the right: the quantitative analysis of three separate immunoblots of cells untreated (first bars in each group) or treated with 20 μ M (second bars) or 40 μ M (third bars) LPA. Data are expressed as fold of basal of ERK1/2 phosphorylation in which ERK1/2 phosphorylation in L5pBJI was defined as 1. Values represent means $\pm$ S.E.M. Statistically significant differences vs L5pBJI were observed at 20 μ M LPA in L5 β arr1 and in L5GRK2 ($P < 0.01$) and at 40 μ M LPA in L5GRK2 ($P < 0.05$).

activation, which is the other signalling cascade activated by the LPAr (presumably through $G\beta\gamma$) was blunted in L5 GRK2 and in L5 β arr1 cells. Exposure to 20 μ M LPA increased the phosphorylation of ERK1/2 by ~ 3 -fold in L5pBJI while it induced a modest increase in L5 β arr1 ($P < 0.01$ vs control) and was ineffective in L5 GRK2 ($P < 0.01$ vs control). At 40 μ M LPA, ERK1/2 phosphorylation was increased by ~ 6 -fold in L5pBJI and by ~ 5 -fold and ~ 2.5 -fold in L5 β arr1 (difference from control not statistically significant) and L5GRK2 ($P < 0.05$ vs control) respectively (Fig. 5).

The mechanism of GPCR regulation by β -arrestin has been, at least in part, elucidated (Miller & Lefkowitz 2001). It has been shown for many GPCRs that agonist stimulation induces the translocation of β -arrestin to plasma membranes, where it binds to the phosphorylated receptor and prevents the interaction between the receptor and the G-protein. This step is usually followed by the internalisation of the receptor and β -arrestin in intracellular vesicles. We used a confocal microscopy analysis to assess whether the exposure to LPA promotes the redistribution of β -arrestin in FRTL-5 cells (Fig. 6). Under basal conditions β -arrestin was diffused in the cytosol of FRTL-5 cells. LPA treatment (35 μ M) induced a rapid (2 min) translocation of β -arrestin to plasma membranes which was followed (at 10 min treatment) by the internalisation of β -arrestin in intracellular vesicles. Similar results were obtained with L5 β arr1 (Fig. 6) and with L5pBJI (not shown) cells. These findings document that in FRTL-5 β -arrestin is redistributed in an LPA-dependent manner and suggest that the LPAr are regulated by β -arrestin by a mechanism similar to that documented for the majority of GPCRs.

Previous studies documented that LPA stimulates FRTL-5 cell proliferation (Falasca *et al.* 1998) and we measured [3 H]thymidine uptake in L5 β arr1 and in L5 GRK2 cells to test whether GRK2 and β -arrestin1 could have a role in the regulation of LPAr-mediated cell proliferation. The mitogenic response induced by 35 μ M LPA was substantially blunted in L5 β arr1 and in L5GRK2 cells as compared with L5pBJI. [3 H]thymidine uptake was inhibited by $69 \pm 6\%$ in L5 β arr1 and $69.8 \pm 4.5\%$ in L5GRK2 cells as compared with L5 pBJI cells (Fig. 7). This finding demonstrates that the regulation of LPAr by GRK2 and β -arrestin1 is functionally relevant. The observation that the overexpression of GRK2 and β -arrestin1 reduced both TSH-stimulated (Iacovelli *et al.* 1996) and LPA-stimulated (present work) [3 H]thymidine uptake in FRTL-5 further supports our previous hypothesis that GRK2 and β -arrestin1 act as negative regulators of GPCR-stimulated proliferative responses in thyroid cells.

In summary, we have shown that FRTL-5 thyroid cells express three subtypes of LPAr which are functionally coupled to a PT-sensitive G-protein. Agonist-stimulated receptor activation inhibited the adenylyl cyclase and stimulated ERK1/2 phosphorylation. This pathway is involved in the proliferative response stimulated by LPA, as indicated by the analysis of [3 H]thymidine uptake. All these responses elicited by LPA were substantially blunted in cells transfected to overexpress GRK2 and β -arrestin1, indicating that, similar to many other GPCRs, the LPAr is desensitised by a GRK2/ β -arrestin1-mediated mechanism. It should also be noted that the moderate levels of GRK2 and β -arrestin1 overexpression achieved by transfection in FRTL5 cells are similar to those obtained in cells after different treatments (Iacovelli *et al.* 1999). This

Figure 6 Translocation of β -arrestin by LPA in FRTL-5. The fluorescence imaging of β -arrestin using confocal microscopy is shown. L5 β arr1 cells were untreated (Basal) or treated with LPA (35 μ M) for the indicated times. The experiment shown was repeated four times with similar results.

Figure 7 LPA-induced DNA synthesis is blunted in FRTL5 cells overexpressing β -arrestin1 and GRK2. Quiescent L5 β arr1 and L5GRK2 cells were stimulated with 35 μ M LPA and [³H]thymidine uptake was measured. Data are means $\pm$ S.E.M. of four separate experiments each performed in triplicate. The [³H]thymidine uptake reduction in L5 β arr1 and L5GRK2 vs L5 pBJI cells was statistically significant ($P < 0.01$).

indicates that such levels can be reached in cells that naturally express these regulatory proteins and that the modulation of the levels of GRK2/ β -arrestin1 represents one factor that could play a role in controlling the LPA-mediated effects, including the stimulation of proliferative responses in thyroid cells. In this regard it should be emphasised that a recent study has documented the overexpression of the high-affinity receptor edg-4 (lp_{A2}) in differentiated thyroid cancer (Schulte *et al.* 2001). A knowledge of the mechanisms that regulate receptor-mediated proliferative responses in thyroid cells may provide a novel therapeutic target to control pathological cell proliferation.

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