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B Cell Receptor (BCR) Cross-Talk: IL-4 Creates an Alternate Pathway for BCR-Induced ERK Activation That Is Phosphatidylinositol 3-Kinase Independent

Benchang Guo* and Thomas L. Rothstein2*†

IL-4 has pleiotropic effects on B cells. These effects include alteration of subsequent BCR-triggered responses. To identify a molecular basis for this receptor cross-talk, we examined ERK activation and NF-κB induction. We found that treatment with IL-4, but not other cytokines, affected subsequent BCR signaling by creating a new pathway in which the need for PI3K in ERK activation was eliminated. In contrast, the need for PI3K in NF-κB induction was not altered. The new pathway for ERK required time to develop, depended on STAT6, and was blocked by inhibition of macromolecular synthesis. As in the classical pathway, BCR-induced ERK activation in the new, PI3K-independent pathway required MEK and was reflected in c-Raf. Thus, IL-4 promotes an alternate pathway through which BCR is coupled to Raf/MEK/ERK that may function to heighten the responsiveness of B cells during times of immunological stress. The Journal of Immunology, 2005, 174: 5375–5381.

Engagement of the BCR is pivotal for the development, survival, and responsiveness of B lymphocytes (1–3). Among the recognized mediators triggered by BCR stimulation is the MAPK, ERK, which is responsible for activation of the AP-1 transcription factor complex component, Fos (4). Other transcription factors, such as NF-κB, are activated following BCR stimulation through a signaling pathway that includes signalosome elements such as Btk, B cell linker protein, PI3K, phospholipase C (PLC),3 and protein kinase C (PKC) (3, 5, 6). Recent evidence suggests that ERK activation and NF-κB induction may represent distal outcomes of two separate BCR-triggered pathways. Deficiency of Bam32 led to diminished activation of ERK in response to BCR cross-linking, whereas induction of NF-κB was unaffected (7). Conversely, deficiency of Carma1 led to diminished NF-κB induction following BCR engagement, whereas ERK activation proceeded normally (8–10). Thus, Bam32 and Carma1 appear to channel BCR-induced intracellular signaling toward distinct pathways (11). However, interconnections between these pathways certainly exist, and it has been shown that ERK activation is dependent on molecules such as PI3K and/or PKC that are key mediators of BCR-triggered NF-κB induction (12–17).

We have recently demonstrated that the need for signalosome elements such as Btk, PI3K, and PLC in BCR-triggered NF-κB induction is circumvented or bypassed by prior B cell treatment with CD40L. Although this alternate pathway for BCR signaling was initially delineated in *id B cells in which Btk is mutated (18), we have more recently shown that normal B cells initially exposed to CD40L and then stimulated with anti-Ig experience IκB kinase phosphorylation, IκBα degradation, and NF-κB activation in the presence of PI3K inhibitors such as LY294002 and wortmannin, and in the presence of the PLC inhibitor U731224 which normally block these outcomes. Further, CD40L treatment results in enhanced BCR-induced ERK activation that is resistant to inhibition of PI3K and PLCγ2 (54). Thus, the generally acknowledged need for several signalosome elements in BCR signaling for NF-κB induction and ERK activation may represent only an initial condition applicable to naive B cells, but not to experienced B cells.

CD40 triggering is not the only way in which B cell behavior is influenced by other immune cells. T cells and other cell types (including NK T cells, eosinophils, basophils, and mast cells) produce IL-4, which has pleiotropic effects on B cells (19). Among these effects is preparation for a more rapid response to subsequently added anti-Ig (20). The effects of IL-4 are mediated through the heterodimeric IL-4R, which consists of an IL-4Rα chain combined with a common γ-chain (reviewed in Ref. 21). Ligand binding induces receptor phosphorylation at specific residues that provide binding sites for Src homology 2- and phosphotyrosine binding domain-containing molecules (22). These interactions lead to the subsequent phosphorylation and activation of STAT6 and insulin receptor substrate-2 proteins (23, 24). STAT6 is a transcription factor that mediates many downstream effects of IL-4, as demonstrated by the phenotype of STAT6-deficient mice (25–27).

The capacity of IL-4 treatment to alter B cell responses to subsequently added anti-Ig raised the possibility that IL-4, like CD40L, might generate an alternate pathway for BCR signaling. The current study was designed to test this hypothesis and led to the demonstration that BCR-induced ERK activation, but not NF-κB induction, becomes PI3K-independent following IL-4 treatment.

Materials and Methods

Animals

Male BALB/cByJ mice and STAT6-deficient mice at 6–8 wk of age were obtained from The Jackson Laboratory (Bar Harbor, ME). Breeding pairs

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3 Abbreviations used in this paper: PLC, phospholipase C; HEL, hen egg lysozyme; PKC, protein kinase C.
of anti-hen egg lysozyme (anti-HEL) transgenic mice were kindly provided by Dr. C. C. Goodnow (John Curtin School of Medical Research, Australian National University, Canberra, Australia) and were bred at Boston University Medical Center. Mice were cared for and handled in accordance with National Institutes of Health and institutional guidelines.

B cell isolation

B cells were prepared from spleen cell suspensions by negative selection as previously described (28). Briefly, splenocytes were depleted of T cells by treatment with anti-Thy 1.2 Ab, followed by complement lysis; the resultant cells were then subjected to density separation using Lymphocyte M (Cedarlane Laboratories) to remove dead cells and RBC. B cells were cultured at 2 × 10^6/ml in RPMI 1640 medium (BioWhittaker) supplemented with 5% heat-inactivated FBS (Sigma-Aldrich), 10 mM HEPES (pH 7.25), 50 μM 2-ME, 2 mM l-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin.

B cell stimulation

B cells were stimulated by F(ab\(^{-}\))\(_2\) goat anti-mouse IgM (anti-Ig) after incubation in medium or treatment with IL-4 for 24 h in most experiments. In some experiments (Fig. 1C), B cells obtained from anti-HEL transgenic mice were stimulated with soluble HEL. Before anti-Ig (or HEL) stimulation, viable cells were obtained by density gradient separation with Lymphol, washed, and incubated for 3 h in medium. Inhibitors were added 1 h before stimulation with anti-Ig (or HEL).

Western immunoblot analysis

Proteins were extracted from B cell pellets with RIPA lysis buffer. Nuclear and cytoplasmic fractions were separately extracted from B cells with the Nuclear Extraction kit obtained from Active Motif according to the manufacturer’s instructions. In each experiment, equal amounts of protein for each condition (15–30 μg) were subjected to SDS-PAGE followed by immunoblotting as previously described (29). Immunoreactive proteins were detected by ECL (Amersham Biosciences). Immunoblots were stripped and reprobed with control Ab to verify that equal amounts of protein were loaded in each lane.

EMSA

Nuclear extracts were prepared as described previously (30). Briefly, nuclei were collected following hypotonic lysis of stimulated cells and extracted for 60 min on ice in a high salt buffer containing 430 mM NaCl and protease inhibitors. Binding reactions containing 1.0–1.2 μg of nuclear protein, 0.5 μg of poly(dI-dC) (Amersham Biosciences), and radiolabeled κB site-containing oligonucleotide probe (31) were incubated for 30 min at room temperature. NF-κB binding (Santa Cruz Biotechnology) was used to verify that equal amounts of nuclear extract protein were present in each sample (32).

Results

BCR-induced ERK phosphorylation becomes substantially PI3K independent after B cell treatment with IL-4

To evaluate the possibility that IL-4 cross-talk influences subsequent BCR signaling for MAPK activation, we stimulated purified primary splenic B cells from BALB/c mice with anti-Ig with or without prior IL-4 treatment (10 ng/ml) and then examined whole cell extracts by Western blotting using ERK- and phospho-ERK-specific Abs. We determined the PI3K-dependence of BCR-induced ERK phosphorylation by treating B cells with the specific inhibitor, LY294002, for 1 h before addition of anti-Ig; the dose of LY294002 used (20 μM) completely blocked BCR-induced phosphorylation of the PI3K substrate, Akt (33), in both medium-cultured and IL-4-treated B cells (Fig. 1A), and modestly exceeded the minimal Akt-inhibitory dose of 10 μM as determined in separate titration experiments (not shown).

B cell stimulation with anti-Ig produced substantial phosphorylation of ERK1 and ERK2 within 5 min, consistent with earlier work (16). Further, inhibition of PI3K with LY294002 before addition of anti-Ig eliminated BCR-induced ERK phosphorylation, as reported (17). However, the situation was very different for B cells previously treated with IL-4. Following exposure of B cells to IL-4 for 24 h, anti-Ig produced ERK phosphorylation that was not blocked by LY294002 and was thus PI3K-independent (Fig. 1B). Similar results were obtained with the structurally unrelated PI3K inhibitor, wortmannin (data not shown).

To determine whether the effect of IL-4 on subsequent BCR signaling depended on the particular kind of cross-linking produced by anti-Ig, we examined B cells obtained from anti-HEL transgenic mice that were then stimulated in vitro with specific Ag (Fig. 1C). B cell stimulation with soluble HEL produced substantial phosphorylation of ERK1 and ERK2 within 5 min, and inhibition of PI3K with LY294002 before addition of HEL eliminated BCR-induced ERK phosphorylation. Most importantly, following
exposure of B cells to IL-4 for 24 h, HEL produced ERK phosphorylation that was not blocked by LY294002 and was thus PI3K-independent, much like the results obtained with anti-Ig, above.

To determine whether the effect of IL-4 on subsequent BCR signaling depended on a shift of phospho-ERK between intracellular locations (combined with inefficient nuclear extraction), we prepared and tested separate cytoplasmic and nuclear fractions from previously untreated and IL-4-treated BALB/c B cells (Fig. 1D). These fractions were >97% pure as determined by Western blotting for anti-tubulin and anti-lamin A/C (data not shown). Stimulation of previously unmanipulated B cells with anti-Ig produced substantial levels of phospho-ERK in both cytoplasmic and nuclear fractions; BCR-induced phospho-ERK in both locations was inhibited by LY294002. However, following exposure of B cells to IL-4 for 24 h, anti-Ig induced LY294002-resistant phospho-ERK in both cytoplasmic and nuclear fractions. Importantly, the ratio of N/C phospho-ERK induced by anti-Ig in IL-4/-IL-4002 B cells was little different than the ratio induced by anti-Ig in unmanipulated B cells.

Notably, IL-4 treatment did not induce ERK phosphorylation on its own (Fig. 2A), nor did IL-4 treatment alter the protein level of ERK, in previously unmanipulated B cells (Fig. 2B). Taken together, these results indicate that IL-4 treatment eliminates the need for a specific intracellular mediator, PI3K, that is otherwise required for propagation of BCR signaling to the endpoint of ERK phosphorylation.

**Figure 2.** IL-4 does not induce ERK phosphorylation nor alter the resting level of ERK protein. A, IL-4 does not induce ERK phosphorylation; B cells were untreated (“0” time) or were treated with IL-4 at 10 ng/ml for 1–24 h, or were stimulated with anti-Ig at 15 μg/ml for 15 min as a positive control. Whole cell extracts were Western blotted with anti-phospho-ERK Ab, after which blots were stripped and reprobed with anti-ERK Ab. B, IL-4 does not alter the resting level of ERK protein. B cells were cultured in medium alone (M) or were treated with IL-4 at 10 ng/ml for 24 h. Whole cell extracts were Western blotted with anti-ERK Ab, after which blots were stripped and reprobed with anti-actin Ab. One of three comparable experiments is shown.

**IL-4-mediated reprogramming of BCR signaling for ERK phosphorylation requires time to develop and depends on protein synthesis**

To determine the speed with which IL-4 affects subsequent BCR signaling, we treated B cells with IL-4 for various periods of time before stimulation with anti-Ig, which was in turn conducted in the presence or absence of PI3K inhibition. B cell treatment with IL-4 for as little as 6 h led to the appearance of some LY294002-resistant, BCR-induced ERK phosphorylation (Fig. 3A and data not shown) in comparison to B cells cultured in medium alone. Longer periods of B cell treatment with IL-4 resulted in increased levels of PI3K-independent phospho-ERK1/ERK2 after subsequent stimulation with anti-Ig. The level of BCR-induced, PI3K-independent ERK phosphorylation reached a peak after 24 h of IL-4 treatment, at which point the bulk of anti-Ig-triggered phospho-ERK was unaffected by LY294002. Thus, IL-4-mediated acquisition of PI3K independence in BCR signaling for ERK phosphorylation is a time-dependent process.

The slowly progressive induction of PI3K independence produced by IL-4 in the BCR-ERK pathway suggested a role for protein synthesis. To evaluate this, we exposed B cells to the protein synthesis inhibitor, cycloheximide (10 μM), during IL-4 treatment for 12 h; we chose this period of time because the toxic effects of cycloheximide on primary B cells are minimal within 12 h, even though IL-4 induction of PI3K independence in BCR signaling is suboptimal. As noted above, IL-4 treatment for 12 h produced partial LY294002 resistance of subsequent BCR signaling for ERK activation; however, this was completely reversed when cycloheximide was present along with IL-4, such that LY294002 sensitivity was reinstated (Fig. 3B). Thus, IL-4-mediated acquisition of PI3K independence in BCR signaling for ERK phosphorylation depends on protein synthesis.

**IL-4-mediated reprogramming of BCR signaling for ERK phosphorylation requires STAT6**

It is well known that many of the effects of IL-4 are mediated via activation of STAT6 (25–27), and involvement of this transcription factor would be consistent with the requirement of new macromolecular synthesis for alteration of BCR signaling. To evaluate the role of STAT6 in establishing a PI3K-independent pathway for BCR-induced ERK activation, we compared B cells obtained from STAT6-deficient mice with B cells obtained from normal control mice. In untreated B cells, anti-Ig stimulated phosphorylation of
ERK1/2 that was blocked by LY294002, regardless of whether B cells were obtained from normal or STAT6-deficient mice. As noted above, prior treatment of normal B cells with IL-4 reprogrammed intracellular signaling such that BCR-induced ERK phosphorylation became, for the most part, resistant to inhibition by LY294002. In the absence of STAT6, however, IL-4 failed to alter BCR signaling—even after IL-4 treatment, anti-Ig-induced ERK phosphorylation was blocked by LY294002 in B cells obtained from STAT6-deficient mice (Fig. 4A). Thus, the ability of IL-4 to influence subsequent BCR signaling depends, like many other outcomes of IL-4R engagement, on STAT6.

Other cytokines fail to alter the PI3K dependence of BCR-induced ERK activation

To evaluate the possibility that other STAT-activating cytokines provide cross-talk that influences BCR signaling for ERK activation, we treated primary B cells with IL-2 or IL-6, in place of IL-4, and then stimulated these B cells with anti-Ig in the presence or absence of LY294002. We then determined levels of ERK and phospho-ERK by Western blotting. Whereas IL-4 treatment largely reversed the inhibition of anti-Ig-induced ERK phosphorylation imposed by LY294002, IL-2 treatment and IL-6 treatment failed to do so (Fig. 4B). Further, IL-13 treatment had no effect on subsequent BCR signaling, in keeping with its reported lack of effect on murine B cells (34) (data not shown). Although only a few cytokines were tested, these results suggest that reprogramming of BCR signaling for ERK phosphorylation is not a universal property of STAT-activating cytokines and is a specific effect of IL-4.

**FIGURE 4.** IL-4-mediated reprogramming of BCR signaling for ERK phosphorylation requires STAT6 and is not reproduced by some other cytokines. A, IL-4-mediated reprogramming requires STAT6. B cells from wild-type control (WT) or STAT6-deficient (KO) mice were cultured in medium alone (MED, top panels) or with IL-4 at 10 ng/ml for 24 h (IL-4, bottom panels), as indicated. Viable B cells were then stimulated with F(ab')
2 of goat anti-mouse IgM Ab at 15 μg/ml (odg) for the indicated times. B cells were exposed to the PI3K inhibitor, LY294002 at 20 μM (LY), or diluent control DMSO (DM) starting 60 min before addition of anti-Ig. Whole cell extracts were prepared and Western blotted with anti-phospho-ERK Ab. Blots were stripped and reprobed with anti-ERK Ab. One of three comparable experiments is shown. B, IL-4-mediated reprogramming is not reproduced by other cytokines. B cells were cultured in medium alone (MED) or with IL-4 at 10 ng/ml (IL-4), IL-2 at 10 ng/ml (IL-2), or IL-6 at 10 ng/ml (IL-6) for 24 h, after which viable B cells were stimulated with F(ab')
2 of goat anti-mouse IgM Ab at 15 μg/ml (odg) for the indicated times. B cells were exposed to the PI3K inhibitor, LY294002 at 20 μM (LY), or diluent control DMSO (DM) starting 60 min before addition of anti-Ig. Whole cell extracts were prepared and Western blotted, as described in Materials and Methods with anti-phospho-ERK Ab. Blots were stripped and reprobed with anti-ERK Ab. One of three comparable experiments is shown.

**BCR-induced MEK phosphorylation becomes substantially PI3K-independent after B cell treatment with IL-4 and MEK is required for IL-4-induced reprogramming of BCR signaling for ERK phosphorylation**

MEK is an upstream MAPK kinase that directly phosphorylates ERK (35). To elucidate the mechanism responsible for mediating IL-4-induced PI3K independence in the BCR-ERK pathway, we stimulated B cells with anti-Ig with or without prior IL-4 treatment, then evaluated MEK phosphorylation and LY294002 sensitivity. Anti-Ig stimulation produced phosphorylation of MEK within 5 min, in keeping with previous work (36). This BCR-induced MEK phosphorylation was highly sensitive to PI3K inhibition and was largely inhibited by B cell exposure to LY294002 concurrently with anti-Ig. However, much like the situation with ERK phosphorylation, IL-4 markedly altered the MEK response to BCR engagement. After IL-4 treatment, a substantial portion of anti-Ig-induced MEK phosphorylation became resistant to inhibition by LY294002 (Fig. 5A). Thus, IL-4 treatment alters the signaling requirements for BCR-induced MEK phosphorylation just as it does BCR-induced ERK phosphorylation.

To determine whether MEK is responsible for LY294002-resistant ERK phosphorylation induced by anti-Ig following IL-4 treatment, we blocked MEK activation with the specific inhibitor, U0126 (37). BCR-induced MEK phosphorylation was completely blocked by U0126, and when MEK activation was blocked with U0126, anti-Ig-induced ERK phosphorylation was inhibited in B cells, in keeping with previous work (38). Similarly, anti-Ig-induced ERK phosphorylation was inhibited by U0126 in IL-4-treated B cells (Fig. 5B). Thus, inhibition of MEK blocks BCR-induced ERK phosphorylation in the presence or absence of prior IL-4 treatment, strongly suggesting that BCR-induced MEK activation, which itself becomes LY294002-resistant after IL-4 treatment, is directly connected to BCR-induced, PI3K-independent ERK phosphorylation.

**IL-4-mediated reprogramming of BCR signaling for ERK phosphorylation is reflected in c-Raf**

c-Raf is an upstream MAPK kinase kinase that phosphorylates MEK (39). To determine whether IL-4-mediated reprogramming is reflected in the behavior of c-Raf, we stimulated B cells with anti-Ig with or without prior IL-4 treatment and then evaluated c-Raf phosphorylation and LY294002 sensitivity. Anti-Ig stimulation produced phosphorylation of c-Raf within 5 min, in keeping with previous work (40). This BCR-induced c-Raf phosphorylation was sensitive to PI3K inhibition and was largely inhibited by B cell exposure to LY294002 concurrently with anti-Ig. However, much like the situation with MEK/ERK phosphorylation, IL-4 markedly altered the c-Raf response to BCR engagement. After IL-4 treatment, a substantial portion of anti-Ig-induced c-Raf phosphorylation became resistant to inhibition by LY294002 (Fig. 5C). Thus, IL-4 treatment alters the signaling requirements for BCR-induced c-Raf phosphorylation just as it does BCR-induced MEK and ERK phosphorylation.

**BCR-induced ERK phosphorylation becomes substantially PLC-independent after B cell treatment with IL-4**

PLCγ2 is located downstream of PI3K and generates second messenger molecules that activate PKC and elevate Ca2++. To determine whether IL-4-induced reprogramming of BCR signaling for ERK activation occurs independently of additional signalosome elements, we stimulated B cells with anti-Ig in the presence or absence of the PLCγ2 inhibitor, U73122 (17), with or without prior IL-4 treatment, and then examined ERK phosphorylation.
BCR engagement produced substantial phosphorylation of ERK which was completely eliminated by B cell exposure to U73122 concurrently with anti-Ig. However, much like the situation with PI3K, IL-4 markedly altered the PLC dependence of BCR-induced ERK phosphorylation. After IL-4 treatment, a substantial portion of anti-Ig-induced ERK phosphorylation became resistant to PLC inhibition, which viable B cells were stimulated with F(ab')2 of goat anti-mouse IgM Ab at 15 μg/ml (αIg) for the indicated times. B cells were exposed to the PI3K inhibitor, LY294002 at 20 μM (LY), or to diluent control DMSO (DM) starting 60 min before addition of anti-Ig. Whole cell extracts were prepared and Western blotted with anti-phospho-MEK Ab. Blots were stripped and reprobed with anti-ERK Ab. One of three comparable experiments is shown.

IL-4 treatment does not affect the PI3K dependence of BCR-induced NF-κB activation

To evaluate the specificity of IL-4 cross-talk for BCR-induced ERK activation, we questioned whether IL-4 influences other signaling pathways that lead to other downstream outcomes, and in so doing we focused on BCR-induced activation of NF-κB, which previously has been shown to be affected by CD40 cross-talk (18). We stimulated primary B cells with anti-Ig in the presence or absence of LY294002, with or without prior IL-4 treatment, and then examined nuclear extracts by EMSA. Anti-Ig induced NF-κB, and this was inhibited by LY294002, as expected. Importantly, this pattern of PI3K dependence was not altered by IL-4. Thus, in IL-4-treated B cells, inhibition of PI3K again blocked induction of nuclear NF-κB by anti-Ig (Fig. 7), in contrast to the situation with BCR-induced ERK phosphorylation which is not blocked by LY294002 after B cell treatment with IL-4 (Fig. 1). These results indicate that IL-4 reprogramming in intracellular signaling affects the pathway leading from BCR to ERK but not from BCR to NF-κB.

Discussion

The present study demonstrates that IL-4 treatment establishes a new, alternate pathway for BCR-induced ERK activation that operates independently of PI3K and PLCγ2, in direct contrast to the need for both mediators in naive B cell signaling, and thus, IL-4 reprograms the connection between the BCR and downstream ERK activation. This reprogramming is equally applicable to physiological BCR engagement by specific Ag as to BCR cross-linking by anti-Ig. Because the requirement for both PI3K and PLCγ2 is eliminated by IL-4, the possibility is raised that this novel pathway...
circumvents multiple signalosome elements. As with the traditional pathway for BCR-induced ERK activation, the new, IL-4-induced, PI3K-independent pathway depends on MEK.

Reprogramming the new, PI3K-independent, pathway for BCR signaling by IL-4 depends completely on activation of STAT6, the mediator for many outcomes of IL-4 signaling (25–27). Consistent with the role of STAT6 as a transcriptional activator, the new pathway requires hours to develop and is blocked by inhibition of macromolecular synthesis. The means by which BCR and MEK/ERK are coupled in the absence of PI3K likely involves c-Raf (Fig. 5C), but not MEKK1 (activation requirements of which for BCR triggering were not altered by IL-4 pretreatment; data not shown). The finding of BCR-induced c-Raf phosphorylation in the face of LY294002 after B cell treatment with IL-4 suggests the possibility of a role for PKCα. However, preliminary results on this point are conflicting. Whereas the relatively specific PKCα inhibitor Ro32–0432 completely blocked BCR-triggered ERK phosphorylation after B cell treatment with IL-4, two other PKC inhibitors (that inhibit PKCα as well as other PKC isofoms) failed to do so.

The new pathway for BCR signaling is specific, in that NF-κB induction did not become independent of PI3K following IL-4 treatment. The results for MEKK1 and NF-κB raise the possibility of a role for Bam32 or associated molecules (7). Notably, the mechanism by which only ERK activation, and not NF-κB induction, becomes PI3K independent after IL-4 treatment stands in contrast to the situation with CD40L, in which pretreatment establishes a novel pathway that bypasses PI3K for both outcomes. This strongly suggests that IL-4 does not simply activate the same pathway as that produced by CD40L.

Receptor cross-talk between IL-4R and BCR has been reported previously in that IL-4 treatment hastens the proliferative response of B cells to subsequently added anti-Ig, and this effect requires time to develop (20), paralleling the resistance of BCR-induced ERK activation to PI3K inhibition. The role of ERK in stimulating cyclin D2 expression (36) suggests a possible connection between ERK activation to PI3K inhibition. The role of ERK in stimulating prolifereation but not survival.

Discussion

The authors have no financial conflict of interest.

References


