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Prostaglandin E2 regulates B cell proliferation through a candidate tumor suppressor, *Ptger4*

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B cell receptor (BCR) signaling contributes to the pathogenesis of B cell malignancies, and most B cell lymphomas depend on BCR signals for survival. Identification of genes that restrain BCR-mediated proliferation is therefore an important goal toward improving the therapy of B cell lymphoma. Here, we identify *Ptger4* as a negative feedback regulator of proliferation in response to BCR signals and show that its encoded EP4 receptor is a principal molecule conveying the growth-suppressive effect of prostaglandin E2 (PGE2). Stable knockdown of *Ptger4* in B cell lymphoma markedly accelerated tumor spread in mice, whereas *Ptger4* overexpression yielded significant protection. Mechanistically, we show that the intrinsic activity of *Ptger4* and PGE2–EP4 signaling target a similar set of activating genes, and find *Ptger4* to be significantly down-regulated in human B cell lymphoma. We postulate that *Ptger4* functions in B cells as a candidate tumor suppressor whose activity is regulated by PGE2 in the microenvironment. These findings suggest that targeting EP4 receptor for prostaglandin may present a novel strategy for treatment of B cell malignancies.

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Abbreviations used: BCR, B cell receptor; DLBCL, diffuse large B-cell lymphoma; GC, germinal center; miRNA, micro RNA; PGE2, prostaglandin E2; qPCR, quantitative real-time PCR; Tet, tetracycline.

Recognition of foreign antigens by BCRs expressed on the surface of mature naive B cells triggers their massive proliferation, which is critically important for the effective defense of the organism against invading pathogens (1). Antigen-activated B cells undergo clonal expansion in dynamic structures called germinal centers (GCs) where much of the diversity of the Ig genes is generated by somatic hypermutation and class-switch recombination (2). These molecular processes require frequent DNA strand breaks that can, when deregulated, provide a rich source for the genesis of B cell lymphomas (3). For instance, the hallmark of Burkitt's lymphoma and some cases of diffuse large B cell lymphoma (DLBCL) is a reciprocal chromosomal translocation where the *MYC* protooncogene comes under the control of an active Ig locus, resulting in constitutive expression of *MYC* (4). However, although this and other translocations are thought to cause many types of B cell lymphoma, additional transforming events that override the normal mechanisms controlling B cell proliferation are

needed for malignant transformation. Indeed, mutations affecting the expression level or the activity of tumor suppressor genes, as well as genomic amplifications and hypermutations of multiple protooncogenes have also been implicated in the pathogenesis of B cell lymphomas (for review see reference [3]).

Despite the aggressive behavior of several types of B cell lymphoma, data gathered over the past few years have demonstrated that BCR signaling is essential for the survival of neoplastic B lymphoma cells, which also holds true for their nonmalignant counterparts (5–8). Observations that the majority of non-Hodgkin's lymphomas persistently express BCR and that IgH translocations almost never affect the functionality of Ig alleles, as well as the discovery of autoreactive BCR in certain neoplasms, indirectly suggests the need for BCR-derived survival and proliferation signals (3). Correspondingly, BCR

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signaling was found to promote growth of DLBCL and chronic lymphocytic leukemia B cells (5, 7), whereas follicular lymphoma B cells displayed potentiated BCR signaling versus tumor-infiltrating normal B cells (6). Most recently, a direct link between BCR signaling and B lymphomagenesis was established by demonstrating that *PAX5* promotes neoplastic growth by activating BCR signaling (8). These findings, together with the recognition that the survival of many B cell tumors depends on signals provided by their microenvironment, might lead to novel treatment options for B cell lymphoma.

More than three decades have passed since the first description of macrophage-derived PGE₂ as a potent suppressor of splenic B cell colony formation (9), and it was later demonstrated that the inhibitory effect of PGE₂ was caused by its direct influence on B cell proliferation (10). Further studies identified tingibile body macrophages as a major source of prostaglandins in the GC microenvironment, and proposed that these scavengers of apoptotic lymphocytes may use prostaglandin to down-regulate the GC reaction (11). However, since these seminal observations, no further insight into the mechanism or relevance of the attenuating effect of PGE₂ on B cell proliferation has been provided. In this study, we investigated how, mechanistically, PGE₂ and its receptors may regulate B cell proliferation triggered by BCR signaling, and evaluated the potential contribution to B cell lymphoma growth in mice and humans.

RESULTS

Ptger4 is a delayed-early gene that inhibits B cell proliferation

BCR-triggered activation of mature B cells leads to transcriptional reprogramming, which can be observed by a strong induction of immediate-early genes within minutes after mitogenic stimulation, preparing a cell to quickly respond with proliferation (Fig. 1 A; see also Fig. 2 C) (12). In an attempt to identify negative regulators of B cell proliferation, we performed transcriptome analysis at 2 h after BCR stimulation and looked for putative delayed-early genes, which are generally known to function as negative feedback regulators of growth factor signaling (GEO database accession no. GSE9215) (13). Among the most strongly induced genes, we found *Ptger4*, whose expression kinetics appeared similar to a wave of delayed-early genes (Fig. 1 B). In addition to its high induction upon BCR cross-linking, *Ptger4* also seemed interesting as a candidate regulator of B cell activation because it encodes an EP4 subtype of receptor for prostaglandin E₂ (PGE₂) (14), and PGE₂ is known for its potent yet poorly understood immunosuppressive role (15). Notably, none of the other genes coding for PGE₂ receptors showed altered expression after BCR triggering (Fig. 1 B). We found the induction of *Ptger4* to be conserved in mouse A20 and human P493-6 B cell lines, and confirmed that it resulted in protein expression by Western blotting and immunofluorescence (Fig. 1, B–D and Fig. S1, available at <http://www.jem.org/cgi/content/full/jem.20081163/DC1>; see also Fig. S2).

To investigate the role of *Ptger4* in peripheral B cell activation, we first examined the gross phenotypes of B cells extracted from spleen of *Ptger4*^{-/-} mice (16). We observed that knockout mice consistently harbored significantly increased numbers of B cells that showed greater resistance to spontaneous cell death in vitro compared with wild-type controls (Fig. 1, E and F). In addition, cell cycle analysis and ATP content measurements revealed an augmented mitogenic response of *Ptger4*-deficient B cells upon BCR ligation, indicating that the early induction of *Ptger4* may serve to counterbalance antigen-induced proliferation in mouse B cells (Fig. 1, G and H). To check whether the endogenously produced PGE₂ may have influenced the growth of BCR-activated B cells, we examined the levels of PGE₂ in culture supernatants and saw that these remained below the detection limit of 15 pg/ml during the course of the experiment (unpublished data). In addition, cotreatment with COX inhibitors indomethacin or NS-398 had no significant effect on proliferation or viability of BCR-stimulated cultures, suggesting that de novo-produced PGE₂ may not be a major factor in suppressing the growth of activated wild-type compared with *Ptger4*-deficient B cells in culture (Fig. S3, available at <http://www.jem.org/cgi/content/full/jem.20081163/DC1>). In summary, these observations suggested that *Ptger4* functions as a delayed-early gene upon BCR-triggering, and prompted us to further investigate the mechanism used by *Ptger4* to attenuate BCR-signaled proliferation.

Ptger4 acts as a negative feedback regulator of BCR-signaling

First, to understand how lack of *Ptger4* may have enhanced BCR-driven proliferation, we examined the early transcriptional response of both *Ptger4*-deficient and wild-type mouse B cells to stimulation with anti-IgM F(ab')₂ in culture. We were surprised by the regulation of genes known to affect lymphocyte proliferation, which showed two functionally coherent groups of genes defined by the presence or absence of *Ptger4* (Fig. 2 A). In line with the aforementioned observations, 11 out of 13 genes (85%) that had higher expression levels in knockout relative to wild-type B cells were found to promote proliferation, whereas all (5 out of 5) genes overexpressed in wild-types had an inhibitory role. Surprisingly, signaling through the BCR in wild-type cells resulted in a profound down-regulation of most genes encoding components of the proximal BCR-signaling cascade itself, suggesting the existence of a potent negative feedback mechanism (Fig. 2 B). Although triggering of *Ptger4*-deficient B cells also repressed the majority of these genes, several crucial genes of the upper BCR pathway, including *Ptprc* coding for CD45 (also known as B220) (17) and *Ighm* coding for the immunoglobulin heavy chain of the B cell receptor itself, were induced nearly twofold in *Ptger4*^{-/-} cells compared with wild-type controls (Fig. 2 B). This indicates that by maintaining the relatively high expression of certain signaling components, the lack of *Ptger4* may prolong the duration of signaling via the BCR, cumulatively delivering a stronger proliferative stimulus.

Among the genes most significantly up-regulated in the absence of *Ptger4*, we found three potent cell cycle-regulating genes, *Cdk4*, *Plagl2*, and *Nfkb1*, which are all known to promote G1 to S phase transition (18–20), as well as *Cd74* and *H2-DMa*, which are both members of the MHC class II family (Table S1, available at <http://www.jem.org/cgi/content/full/jem.20081163/DC1>). Notably, CD74 has been shown to promote B cell lymphoma progression, and an anti-CD74 antibody is currently being clinically evaluated for the therapy of human B cell malignancies (21, 22).

Because B cells use antigen-presenting MHC class II molecules to attract activating help from helper T cells (23), endogenous control of the MHC-encoding genes by *Ptger4* may thus provide another means to keep B cell activation in check. Indeed, as we demonstrate in this study, signaling via the EP4 receptor potentially repressed the expression of multiple MHC components essential for effective antigen presentation by B cells (see also Fig. 5 B). Interestingly, it has been reported that PGE2 and EP2/EP4 agonists significantly inhibit the expression of MHC class II molecules in

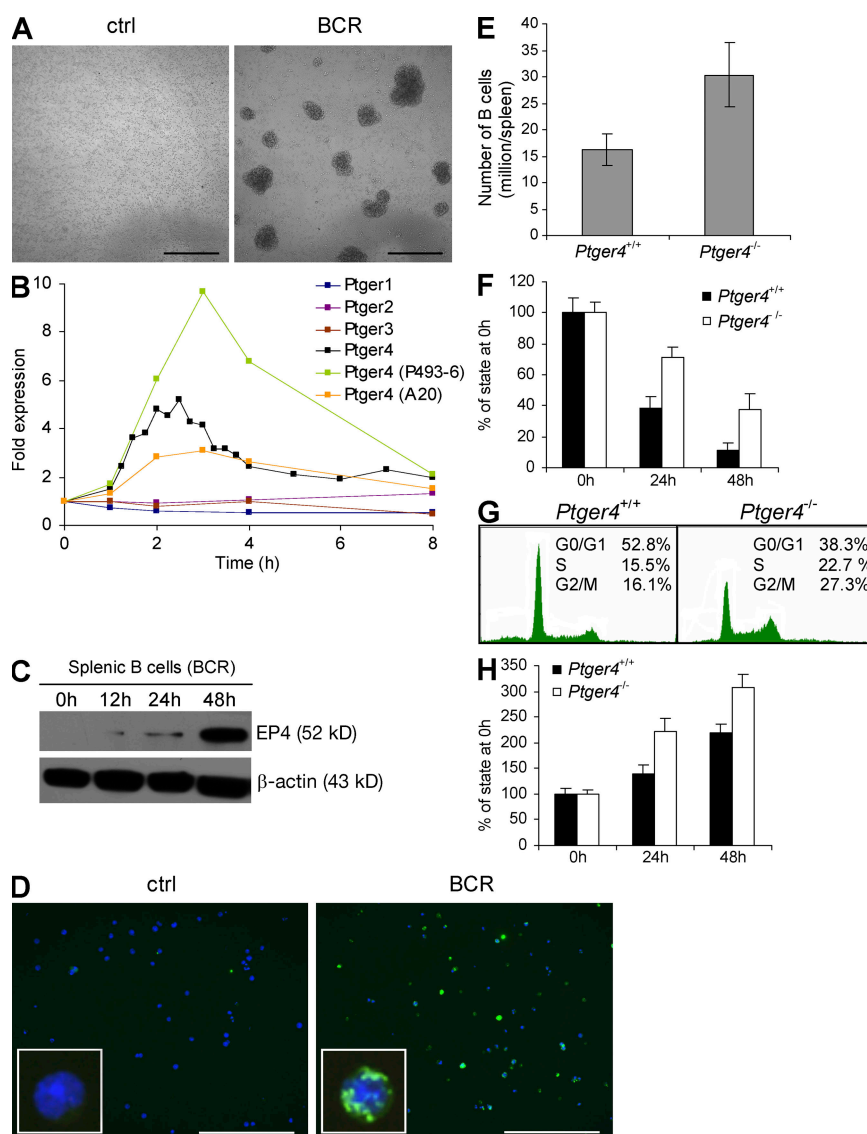


Figure 1. *Ptger4* is a delayed-early gene that inhibits B cell proliferation. (A) Mouse splenic B cells stimulated with (BCR) or without (ctrl) anti-IgM F(ab')₂ for 72 h in culture. (B) BCR-induced expression of *Ptger4* in mouse and human B cells as measured by qPCR at the indicated times. The relative abundances of each *Ptger* mRNA compared with *Hprt* transcripts in primary mouse B cells at time 0 h were as follows: *Ptger4* (0.0068; 100%), *Ptger2* (0.0013; 19.1%), *Ptger1* (0.0009; 13.2%), and *Ptger3* (2.2×10^{-6} ; 0.0003%). Western blotting (C) and immunofluorescence (D; at 48 h) of EP4 in BCR-stimulated mouse B cells. See Fig. S1 for higher protein loading and longer exposure immunoblot analysis, and Fig. S6 for higher quality immunofluorescence images. (E) Numbers of B cells isolated from spleen of *Ptger4*^{+/+} and *Ptger4*^{-/-} mice. (F) ATP content of unstimulated and (H) BCR-stimulated mouse B cells in culture. (G) Cell cycle analysis at 60 h after BCR-triggering of mouse B cells of the indicated genotypes. Error bars are the SD of six to eight independent experiments. Bars, (A) 200 μ m; (D) 100 μ m. Figs. S1 and S2 are available at <http://www.jem.org/cgi/content/full/jem.20081163/DC1>.

B lymphocytes stimulated with IL-4 and/or lipopolysaccharide (24, 25), suggesting that the inhibition of MHC class II by EP4 may occur independently of the stimulus used to activate B cells.

To verify the regulatory function of *Ptger4* in BCR-driven gene transcription, we focused on a set of immediate-early genes induced by antigen-receptor signaling (Fig. 2, C and D). Transient micro RNA (miRNA)-mediated knock-down of *Ptger4* in the A20 B cell lymphoma line resulted in increased transcriptional activity of NF-κB, in line with the observation from primary *Ptger4*^{-/-} B cells, where genes coding for essential components of the NF-κB signaling pathway, including *Traf5*, *Nfkb1*, *Nfkbie*, and *Fbi1*, have been found significantly induced over controls (GEO accession no. GSE9847) (20, 26). In contrast, ectopic expression of the

Ptger4 ORF region repressed BCR-driven transcription through NF-κB, complementing the results from loss-of-function studies. *Ptger4* exerted a similar inhibitory effect on transcriptional activity of the AP-1 components FOS and JUN, and potentially decreased expression from IL-2 promoter known to contain critically important AP-1 and NF-κB sites (Fig. 2 D) (27). On the other hand, neither silencing nor overexpression of *Ptger4* influenced transcriptional activity of EGR1 or transcription through cAMP-response element (CRE), both of which greatly increase upon BCR ligation (Fig. 2 D and Fig. S4, available at <http://www.jem.org/cgi/content/full/jem.20081163/DC1>) (28, 29). Together, these data indicate that *Ptger4* negatively regulates BCR-mediated gene expression and B cell proliferation, primarily by inhibiting NF-κB and AP-1 transcriptional activity.

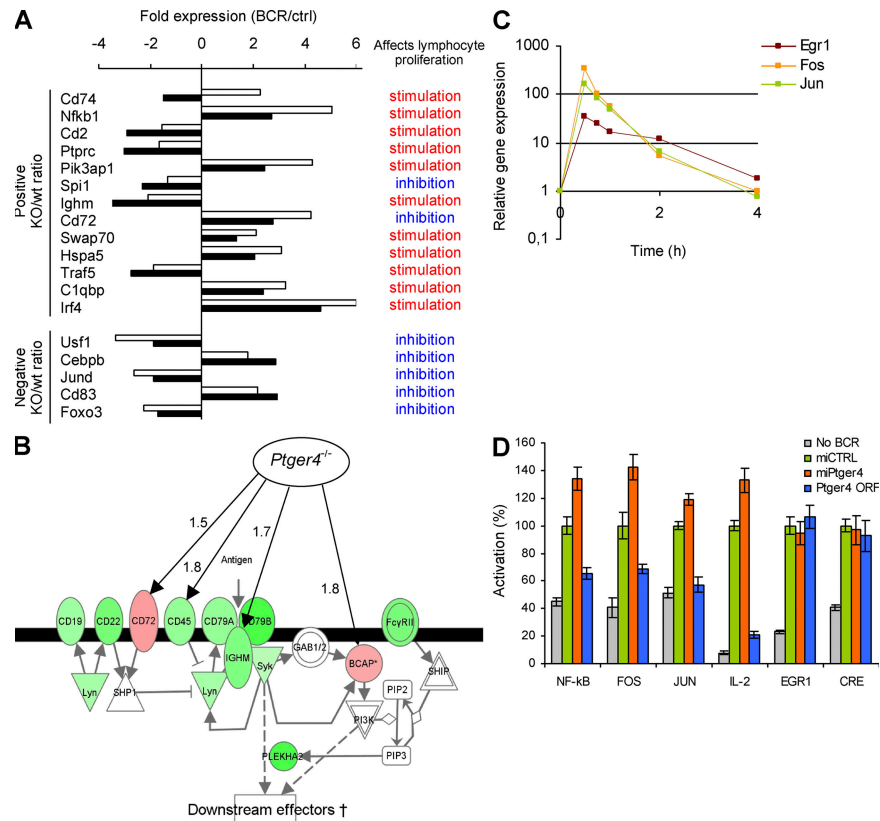


Figure 2. *Ptger4* is a negative feedback regulator of BCR-triggered proliferation. (A) Relative expression values and functions of genes known to affect lymphocyte proliferation, according to Ingenuity Pathway Analysis database (IPA; see URLs in Materials and methods). For each gene, the expression ratio in BCR-stimulated (BCR) versus control (ctrl) sample at 2 h is shown (filled bars, wild-type [wt] B cells; open bars, *Ptger4*^{-/-} [KO] B cells). Genes were ranked according to their absolute ratios in descending order. (B) Proximal part of the BCR-signaling canonical pathway. The molecules whose encoding genes showed at least a twofold change in expression upon BCR-triggering of wild-type B cells are colored (green, down-regulation; red, up-regulation). Black arrows indicate genes that showed at least a 50% difference in expression between *Ptger4*^{-/-} and wild-type B cells. Numbers indicate fold induction caused by the lack of *Ptger4*. (C) BCR-induced expression of the immediate-early genes *Egr1*, *Fos*, and *Jun* in A20 cell line. (D) A20 cells were cotransfected with plasmids encoding GFP cocistronic with nontargeting miRNA (miCTRL) or miRNA targeting *Ptger4* (miPtger4b) or the *Ptger4* ORF, together with the indicated luciferase reporter plasmid. The knockdown efficiency was similar to that achieved in stable cell lines (Fig. 3 A). Luminescence was determined after BCR stimulation as detailed in Materials and methods. Error bars are the SD of triplicates. To exclude the possibility of saturation, the assay for EGR1 and CRE activity was also performed at a lower concentration (5 μg/ml) of anti-IgG F(ab')₂ (Fig. S4). Fig. S4 is available at <http://www.jem.org/cgi/content/full/jem.20081163/DC1>.

Ptger4 is a candidate tumor suppressor in mouse B cell lymphoma

As primary *Ptger4*^{-/-} B cells resisted rapid cell death in vitro in the absence of activating stimuli, we next set out to determine how altering the *Ptger4* gene dosage would affect B cell growth and survival. To this end, we designed lentiviral vectors containing either GFP cocistronic with miRNA targeting *Ptger4* (miPtger4) or the cloned *Ptger4* ORF region to generate stable A20 B cell lines. Quantitative PCR (qPCR) and immunoblotting confirmed the efficiency of the stably integrated *Ptger4* knockdown and overexpressing cassettes (Fig. 3 A). Interestingly, although the introduced changes did not seem to influence the in vitro growth of A20 cells, which have a doubling time of <12 h, the slower growing P493-6 human B cell line was significantly impacted by a silencing construct that targeted both mouse and human *Ptger4* and achieved comparable gene knockdown levels in both cell lines (Fig. 3 B and unpublished data). The modified human B cells showed a similar rate of proliferation at low cell concentrations compared with controls, but later resisted overgrowth and reached about twice the density of viable control cells in the medium. This is in agreement with increased ex vivo survival of *Ptger4*^{-/-} cells and indicates that reducing the levels of *Ptger4* enhances survival of mouse and human B cells.

We then asked whether vigorous in vitro proliferation of A20 cells could have obscured the effect of over- or underexpressed *Ptger4* by setting up a more sensitive in vivo system. Because A20 cells are known to induce tumor formation when introduced into syngeneic mice, we injected 200,000 viable cells from each of the generated A20 lines in the BALB/c strain and monitored tumor development over time (Fig. 3 C). Stable co-expression of GFP in the injected cells allowed us to easily track tumor origin by fluorescence microscopy of the dissected or intact tumor tissue. Stunningly, although most of the control mice injected with nontargeting miRNA (miCTRL)-carrying cells succumbed to disseminated B-lymphoma development over the first two-month period, as expected, overexpression of *Ptger4* yielded significant protection against lymphoma spread, and the majority (70%) of mice survived beyond the monitoring period with no adverse symptoms (Fig. 3 C). In contrast, A20 cells with stably silenced *Ptger4* potently aggravated tumor burden, which frequently manifested as liver and neck region metastasis, as well as hind limb paralysis, and the mice quickly became terminally ill. Additionally, the more efficient miRNA, miPtger4b, triggered a more severe phenotype than the less efficient miPtger4a, as was also evident from a separate experiment in which a higher number of injected cells induced tumor formation at the site of injection in the eye, with the size of the tumor corresponding to the *Ptger4* knockdown efficiency (Fig. 3 D). Collectively, these data demonstrate that *Ptger4* negatively regulates B cell survival

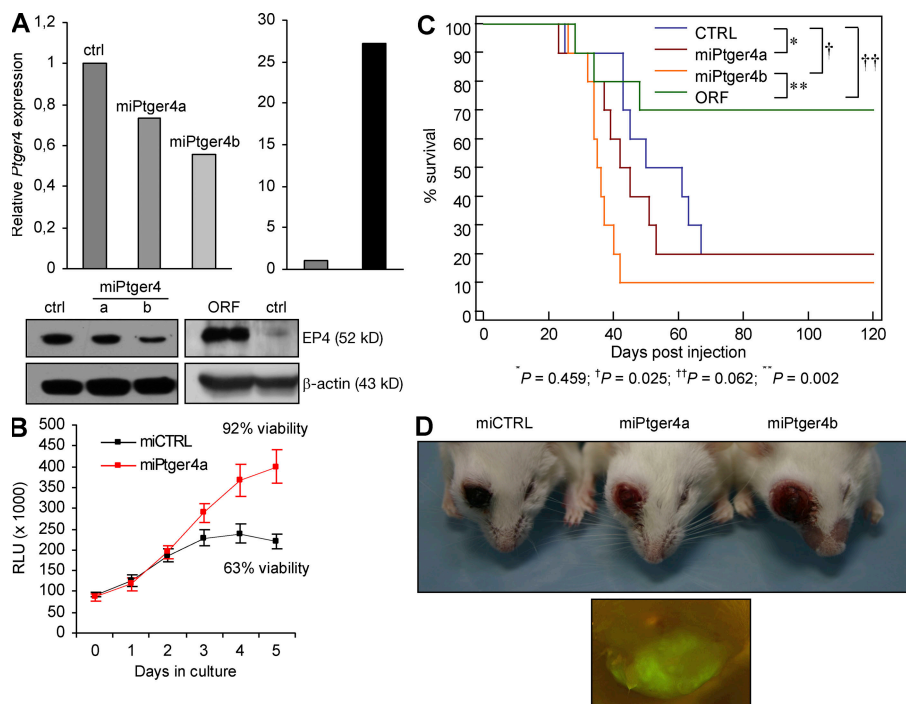


Figure 3. *Ptger4* is a candidate tumor suppressor in B cells. (A) Stable A20 cell lines were generated by lentiviral infection as described in Materials and methods. *Ptger4* expression was determined by qPCR and confirmed by Western blotting. (B) ATP content and viability of stable P493-6 cell lines in culture. RLU, relative luminescence units. Error bars are the SD of triplicates. (C) Survival of BALB/c mice injected with 2×10^5 A20 cells stably expressing the indicated constructs. 10 mice were used to derive each survival curve. Logrank test comparing two survival curves was used to derive P values, as indicated. (D) Tumor formation at the site of injection of the indicated stable A20 cell line at 20 d after injection (5×10^5 cells were injected per mouse; top), and a fluorescence micrograph showing GFP-expressing tumor tissue in the eye region (bottom).

and proliferation, and suggest that *Ptger4* may function as a tumor suppressor in B cells.

PGE2 suppresses B cell proliferation by targeting the EP4 receptor

We next examined the potential physiological relevance of the inhibitory role of *Ptger4* on B cell growth by focusing on the effects of the only known but extremely potent endogenous ligand for EP4 receptor, prostaglandin E2 (PGE2). Several reports have documented a repressive role of PGE2 on immune responses and B cells in particular (10, 30–32), but no study to date has identified the exact mechanism by which this inhibition occurs. Because we identified *Ptger4* as a delayed-early gene and the only BCR-induced gene of the *Ptger* series (Fig. 1 B), we speculated that the EP4 receptor may be important for conveying the growth-regulatory

actions of PGE2 in B cells. We first checked the influence of PGE2 on BCR-triggered proliferation of wild-type cells in vitro and saw that PGE2 at a concentration as low as 1 nM effectively prevented B cell cluster formation and proliferation (Fig. 4 A). In contrast, EP4-null B cells were completely insensitive to the inhibition by PGE2, forming massive clusters of proliferating cells similar to the vehicle-treated controls, even at high doses of PGE2 (Fig. 4 B). This strongly suggested that EP4 was the major, if not the only receptor transmitting the PGE2-delivered signal to block B cell growth. In addition, treatment with the EP4-specific agonist PGE1-OH faithfully mimicked the inhibitory activity of PGE2 in wild-type, but not EP4-null, cells, as opposed to stimulating the closest EP4-related receptor, EP2, which did not influence proliferation of either cell type (Fig. 4 C).

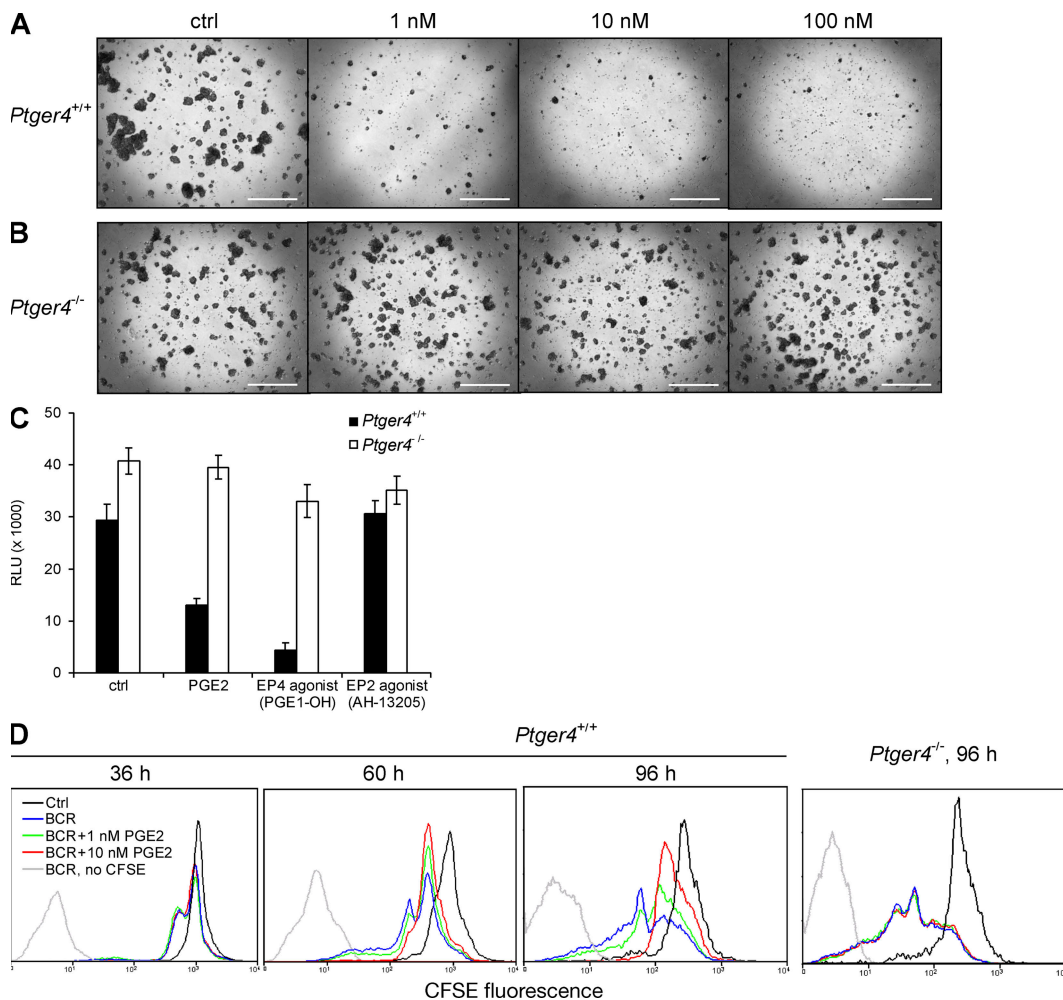


Figure 4. The suppressive effect of PGE2 on B cell proliferation is mediated by the EP4 receptor. *Ptger4*^{+/+} (A) and *Ptger4*^{-/-} (B) splenic B cells were incubated in vitro for 60 h with anti-IgM F(ab')₂ and cotreated with PGE2 at the concentrations as indicated (ctrl, vehicle-treated control). Bar, 200 μm. (C) BCR-stimulated splenic B cells were cotreated as indicated for 60 h, followed by determination of ATP content. Final concentrations of PGE1-OH and AH-13205 were adjusted to compensate for the differences between inhibitory constants for the mouse EP4 receptor (56). RLU, relative luminescence units. Error bars are the SD of triplicates. (D) The CFSE staining method was used to follow proliferation of *Ptger4*^{+/+} and *Ptger4*^{-/-} mouse B cells. Flow cytometry analysis of viable cells is shown (see Materials and methods).

To more closely examine how triggering of EP4 by PGE2-affected B cell activation, we used time-lapse microscopy to follow cell growth and cluster development of BCR-triggered *Ptger4*^{+/+} and *Ptger4*^{-/-} B cells in the presence of PGE2 over several days. Although the two cell populations did not differ during the initial phase (the first 18–24 h) of cluster formation, the effect of PGE2 became progressively more apparent, as the wild-type cells were less efficient at forming bigger groups of cells. Once *Ptger4*^{-/-} cells entered a cluster, they stayed associated with it until the activation reaction was terminated, as opposed to wild-type cells, which dynamically shuttled in and out of clusters, resulting in a smaller net size of the clusters and in more cells found dispersed throughout the culture plate (Videos S1 and S2, available at <http://www.jem.org/cgi/content/full/jem.20081163/DC1>). Notably, this time-dependent activity of PGE2 was in line with the kinetics of BCR-induced EP4 expression, as revealed by immunoblotting (Fig. 1 C). To discern the impact of signaling through EP4 on cell proliferation, we used the CFSE method to follow cell division of BCR-activated B cells (Fig. 4 D). In agreement with a previous study (10), PGE2 allowed wild-type B cells to undergo only one round of cell division, but significantly inhibited further proliferation of daughter B cells. In contrast, *Ptger4*-deficient cells displayed complete protection against the proliferation-limiting effects of PGE2, confirming the suggestion that EP4 is the primary molecule mediating the negative influence of PGE2 on B cell activation. The addition of PGE2 diminished the viability of

activated wild-type B cells in a time- and dose-dependent manner (Fig. S5), suggesting that PGE2 signaling via the EP4 receptor promoted the apoptosis of replicating progeny. The greater proportion of undivided cells at 96 h compared with 60 h in cultures supplemented with 10 nM PGE2 (Fig. 2 D) is thus a likely consequence of a preferential death of the divided population at the later time point.

PGE2–EP4 signaling results in general repression of activating genes

We next asked how, mechanistically, PGE2–EP4 signaling antagonizes the proliferative burst of B cells triggered by antigen receptor cross-linking. Therefore, we inspected the transcriptional status of activated wild-type B cells incubated with or without PGE2 in comparison to EP4-null cells at 24 h after BCR triggering, a time point at which the inhibited phenotype started to become apparent and when EP4 protein was first readily detected by immunoblotting. Interestingly, of the 263 genes whose expression was altered by more than twofold in wild-type B cells because of the presence of PGE2, 91% (240 genes) were down-regulated, indicating a general repressive effect of PGE2 on gene transcription in activated B cells (Fig. 5 A). To the contrary, only 10 of the ~22,000 genes examined were either induced or repressed in knockout cells, suggesting that EP4 was indeed the primary receptor through which PGE2 exerted its attenuation of the BCR-induced response. This finding was confirmed by an independent direct comparison of activated and

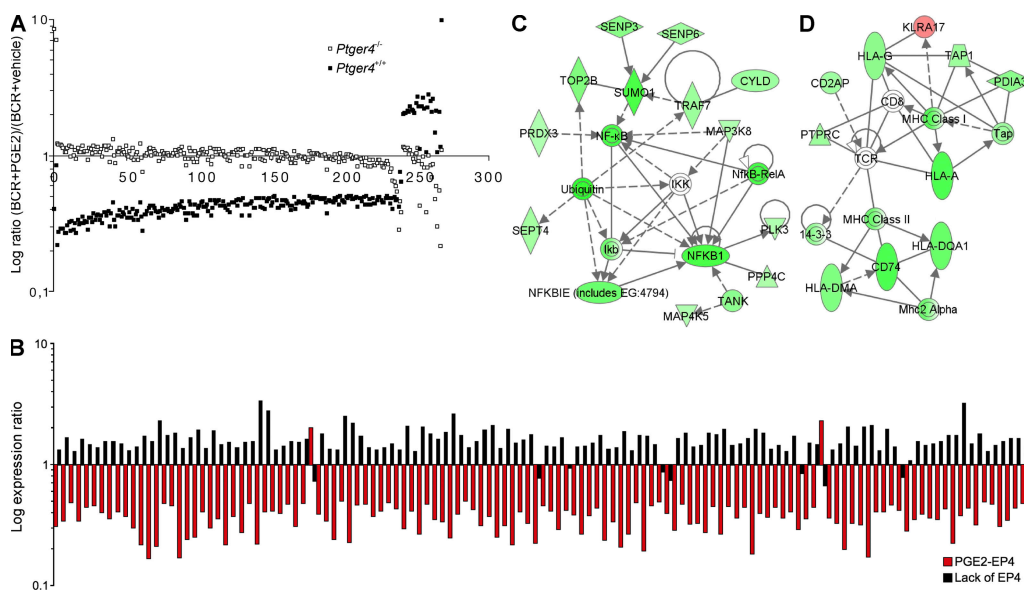


Figure 5. PGE2–EP4 signaling results in general repression of activating genes. (A) Genes regulated at least twofold in BCR-triggered *Ptger4*^{+/+} and/or *Ptger4*^{-/-} B cells by 1 nM PGE2 at 24 h. For each genetic background, log expression ratios of PGE2-treated (BCR+PGE2) versus vehicle-treated (BCR+vehicle) activated B cells are shown for each gene. The x axis denotes number of genes. (B) Genes that are induced when EP4 is absent are repressed when EP4 is abundant and activated. Shown are the log expression ratios of 128 genes commonly regulated in conditions designated as “lack of EP4” (*Ptger4*^{-/-} versus *Ptger4*^{+/+} BCR-triggered B cells at 2 h) and “PGE2–EP4” (*Ptger4*^{+/+} versus *Ptger4*^{-/-} BCR-triggered and 1 nM PGE2 cotreated B cells at 24 h). The identity of genes and their values are listed in Table S2. (C, D) Genes from the “PGE2–EP4” condition in (B) were connected using the IPA knowledge database. Two most significant and coherent networks consisted of genes associated with the NF- κ B pathway (C) and MHC class I and II molecules (D). Green, down-regulated genes; red, up-regulated genes.

PGE2-treated wild-type and *Ptger4*^{-/-} B cells, which revealed EP4-mediated 85% (527 out of 623 genes affected) attenuation of gene transcription (unpublished data).

We then examined the identity of the genes regulated by the PGE2–EP4 pathway, and observed that a significant proportion (~21%) of these genes were also impacted in *Ptger4*^{-/-} B cells early after triggering of the BCR (Fig. 5 B and Table S2, available at <http://www.jem.org/cgi/content/full/jem.20081163/DC1>). However, genes that changed expression in both models had a reciprocal pattern of change; genes that were up-regulated because of the lack of EP4 were down-regulated in conditions where EP4 was abundant and stimulated by PGE2, confirming that this set of genes was specifically controlled by the EP4 receptor. PGE2–EP4 signaling most profoundly attenuated the expression of several genes coding

for molecules of the NF-κB pathway, ribosomal proteins, translation-initiation factors, and ATP-producing molecules, as well as components of the MHC and (immuno)proteasome, most of which are known to be mandatory for efficient B cell activation (Fig. 5, C and D, and Table S2). These data validate and complement the observations from *Ptger4*-deficient B cells, indicating that EP4 functions to prime the inhibitory activity of its endogenous ligand PGE2 by repressing transcription of a functionally coherent set of activating genes, eventually leading to a systemic attenuation of B cell proliferation.

***Ptger4* antagonizes GC B cell development**

To verify the implications of our findings beyond the model of in vitro BCR-stimulated cells, we first screened the published data for potential differences in *Ptger4* expression levels

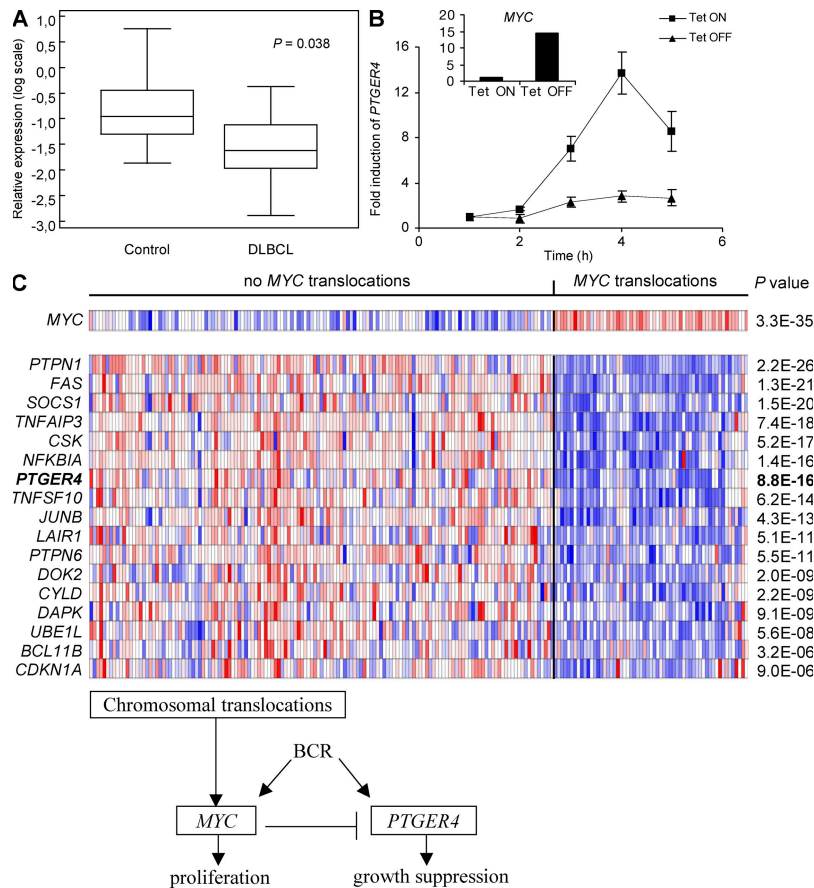


Figure 6. MYC down-regulates the expression of PTGER4 in human B cell lymphoma. (A) *PTGER4* is significantly down-regulated in human DLBCL cells relative to normal B cells. Data were analyzed from reference 30. The P value from Wilcoxon rank sum test is shown. (B) *MYC* inhibits BCR-triggered induction of *PTGER4* in the human P493–6 B cell line. Cells were incubated with or without Tet (Tet ON) or without Tet (Tet OFF) for 3 d, and the expression of *PTGER4* after BCR-triggering was determined by qPCR. Tet-dependent *MYC* expression is shown in the insert. Error bars are s.d. of triplicates. (C) *PTGER4* and several known tumor suppressors are coordinately down-regulated in multiple human B cell lymphomas with *MYC* translocation (38). Data were analyzed from a comprehensive study, including samples of 220 mature aggressive B cell lymphomas using the algorithm of the human cancer-profiling database OncoPrint (see URLs in Materials and methods) (57). Shown is the heat map comprising the microarray results, where red indicates induction of genes and blue indicates repression of genes. Samples were classified according to the presence (or absence) of *MYC* translocations, as indicated. Genes showing the lowest P values were selected and ranked in ascending order. The selected genes, except *PTGER4*, have been described as tumor suppressors in B cell lymphoma according to Pubmed (see URLs in Materials and methods). The scheme on the bottom presents the interaction between *MYC* and *PTGER4* in a lymphoma-relevant context.

between naive follicular B cells and activated GC B cells in mice and humans (33, 34). In accord with the expectations, GC B cells exhibited profoundly reduced levels of *Ptger4* compared with naive controls, confirming the in vitro observation that low *Ptger4* expression favors B cell proliferation (Fig. S6, A and B, available at <http://www.jem.org/cgi/content/full/jem.20081163/DC1>). We then examined the expression of EP4 protein and saw that GC B cells expressed it at a much lower level compared with naive B cells, supporting the data from the aforementioned transcriptional analyses (Fig. S6 C). Furthermore, the analysis of GC B cell content in *Ptger4*^{-/-} mice immunized with a T cell–dependent antigen found a significant increase compared with wild-type controls, indicating that the lack of *Ptger4* was causative for the enhanced activation of B cells in vivo (Fig. S6 D). The importance of these findings is underscored by the fact that many B cell malignancies in humans show expression patterns of differentiation markers and global gene-expression profiles characteristic of GC B cells (35), thus most types of B cell lymphoma are thought to originate from GC B cells. It should be noted, however, that the higher proportion of GC B cells in *Ptger4*^{-/-} mice may have resulted, at least in part, as a consequence of altered functioning of other EP4-expressing cell types, such as T cells (31) and dendritic cells (36), which also participate in T cell–dependent immune responses.

The protooncogene *MYC* down-regulates *PTGER4* in human B cell lymphomas

To investigate the relevance of *PTGER4* to human disease, we first looked for altered expression of *PTGER4* in normal versus malignant B cell populations by mining the GEO expression database (37). Interestingly, the analysis showed that *PTGER4* is markedly down-regulated in the most commonly occurring, aggressive form of B cell malignancy, DLBCL, indicating that the reduction of proliferation-attenuating function of *PTGER4* may indeed contribute to human B cell lymphoma growth (Fig. 6 A). We then asked about the possible mechanism leading to the observed decrease in *PTGER4* expression in human neoplasia by focusing on the *MYC* gene. Because of chromosomal breakpoints at the *MYC* locus, increased transcription of *MYC* is a frequent cause of B lymphomagenesis, and has been associated with an adverse clinical outcome in several cases of aggressive B cell lymphoma (38). To that end, we used a human B cell line (P493-6) carrying a conditional tetracycline (Tet)-regulatable *MYC* allele that allows for abundant expression of *MYC* in the absence of Tet, but only weak *MYC* expression when Tet is present (39). We saw that the presence of Tet markedly influenced BCR-induced expression of *PTGER4*; in the high-*MYC* condition, the induction of *PTGER4* was moderate, whereas low amounts of *MYC* transcripts greatly facilitated *PTGER4* up-regulation, suggesting that *MYC* represses *PTGER4* expression (Fig. 6 B). To examine whether the association between *MYC* and *PTGER4* is relevant in vivo, we selected *PTGER4* and several known tumor suppressors with established roles in human B cell lymphoma, and examined their expression in

clinical cases of Burkitt's lymphoma, DLBCL, and some other types of mature aggressive B cell lymphomas with or without *MYC* translocations (38). We observed that this set of tumor-suppressor genes, including *PTGER4*, was coordinately down-regulated in cases with *MYC* translocations compared with cases without *MYC* translocations, irrespective of morphological, clinical, or immunohistochemical characteristics of the investigated B cell lymphomas (Fig. 6 C). These findings are consistent with a tumor-suppressive role of *PTGER4* in human B cell cancer, and implicate the *MYC* oncogene as a potent negative regulator of *PTGER4* expression. Although our data allow for a causative correlation, the extent to which the reduced *PTGER4* expression contributes to the origin of these malignancies remains speculative, and further studies will be required to evaluate the potential of *PTGER4* and its encoded EP4 receptor as a target for the therapy of human B cell lymphoma.

DISCUSSION

Ptger series of genes have traditionally been viewed and studied in light of the interactions of their encoded receptors with PGE2 as the natural ligand, whereas kinetics of gene expression or the intrinsic functioning of EP receptor subtypes has received little attention. In this study, we identify *Ptger4* as a typical delayed-early gene negatively regulating the robust proliferative response of B cells to antigen receptor triggering and use transcriptome analysis to delineate the attenuating mechanism used by *Ptger4*.

The observation that *Ptger4* knockout B cells display a more activated genotype compared with wild-type controls early after BCR-stimulation in vitro suggests an inherent growth-suppressing activity of *Ptger4* that is independent of PGE2–EP4 interactions. This is supported by the finding that BCR-stimulated mouse B cells do not secrete any substantial amounts of PGE2 in culture and that incubation of wild-type B cells with COX inhibitors to block the potential PGE2 biosynthesis does not augment proliferation of activated B cells, excluding the possible influence of de novo-produced PGE2. In addition, stable knockdown of *Ptger4* promoted growth of a human B cell line in culture, suggesting that the intrinsic growth-limiting role of *Ptger4* is conserved between mouse and human B cells. Although there is evidence for the secretion of relatively high amounts of PGE2 by quiescent and activated human B cells (40, 41), we are not aware of any studies supporting the production of PGE2 by mouse B lymphocytes, with the exception of a study by Graf et al. (42) showing that a biphenotypic B/macrophage population of cells produces substantial amounts of PGE2 upon stimulation with lipopolysaccharide, CD40, or BCR ligand. In contrast, it has been noted that the expression of COX-1 and -2 is not observed in mouse B cells (41, 42), and the lack of induction of COX-2 is confirmed by our microarray analysis of mouse B cells after BCR engagement (GSE9215 and GSE9847). Interestingly, although controversy exists regarding the net effect of PGE2 on proliferative responses of activated human B cells (10, 40, 41, 43–45), there appears to be a consensus on

the inhibitory function of PGE2 on mouse B cell proliferation (9, 24, 46, 47). Depending on the mitogenic stimulus studied, reports have documented inhibition (10), no influence (43), stimulation (45), or concentration-dependent effect (41) of PGE2 on proliferation of human B cells. It is possible that the various stimuli differently regulate the expression of or signaling through each of the four distinct PGE2 receptors, some of them with opposing functions, thus critically influencing the net result of PGE2 action on humans B cells. Further studies will be required to show whether PGE2–EP4 signaling is inhibitory in each of these B cell-activating conditions.

Two main observations lead us to assume that *Ptger4* acts as a feedback regulator to attenuate BCR–signaling pathway. First, *Ptger4* counteracted the transcriptional activity of early-induced NF- κ B and AP-1 complexes, which are both essential for propagating B cell growth and proliferation, and, second, it facilitated down-regulation of several proximal BCR–signaling components. Given the “natural” tendency of the BCR to undergo autoinactivation upon signal transmission, the negative feedback regulation by *Ptger4* may thus shorten the activation period of the BCR, ultimately resulting in a weaker proliferative response of wild-type compared with *Ptger4*-deficient B cells.

A striking finding of our study is the reduced in vivo tumorigenic potential of the aggressive A20 B-lymphoma cells overexpressing *Ptger4*. Regardless of whether the diminished tumorigenicity resulted from the enhanced cell-intrinsic effects of *Ptger4*, increased PGE2–EP4 signaling, or both, the over-present *Ptger4* was clearly causative for protection of mice against lymphoma spread. Conversely, the aggravated symptoms caused by tumor development and the increased mortality imposed by the ~40–45% silenced expression of *Ptger4* demonstrate the inhibitory potential of *Ptger4* and indicate that its deficit was, indeed, the primary genetic lesion leading to the elevated rate of tumor invasion. We were therefore not surprised to find *PTGER4* significantly down-regulated in DLBCL, the most common subtype of non-Hodgkin’s lymphoma that accounts for 30–40% of all lymphoma cases and that frequently shows a poor prognosis in humans (3, 38). Together, these data further establish *Ptger4* as a negative regulator of BCR–signaling and identify it as a candidate tumor suppressor in mouse and human B cells.

Consistent with previous reports, PGE2 potently inhibited B cell activation, and we for the first time provide definitive proof that the EP4 receptor is the principal molecule responsible for the repressive activity of PGE2 in B cells. This finding indicates that PGE2 potentiates the existing intrinsic inhibition by EP4, which is supported by the progressively greater negative influence of PGE2 on B cell proliferation and viability correlating with the expression level of EP4, and, importantly, by the reciprocal EP4-related gene regulation between activated *Ptger4*^{-/-} and PGE2-treated wild-type B cells. Transcriptional profiling of PGE2–EP4 signaling uncovered a systemic down-regulation of numerous pro-survival genes that likely accounted for the impacted pheno-

type. Several of these genes mapped to the most significantly affected NF- κ B pathway, indicating that the latter could play a key role in the EP4–phenotype correlation. Importantly, genetic studies in mice have confirmed the essential role of NF- κ B in promoting B cell activation (18), and activated B cell–like DLBCL cells depend on constitutive NF- κ B activity for survival (48). The EP4-attenuated expression of cell-cycling genes, as well as genes coding for components of the energy-producing and protein-translation machinery might have, at least in part, come as a consequence of the aberrant upstream NF- κ B activity, together leading to a proliferation shutdown.

Another functionally coherent network consisted of MHC class I and II members, which B cells use to present antigens to and thus stimulate T cells, thereby ensuring appropriate immune response to foreign antigens. This suggests that EP4 not only functions to negatively regulate the activation of the B cell in which it is expressed, but may also limit the responses of other immune cells in its vicinity. Nevertheless, one of the genes most significantly up-regulated by the lack of *Ptger4* and repressed by PGE2–EP4 signaling, the MHC class II chaperone CD74, was found to directly stimulate B cell survival and promote malignant B cell proliferation (21). The expression of *PTGER4* may thus be relevant to the efficiency of the anti-CD74 monoclonal antibody LL1, which is currently under evaluation as a novel therapeutic agent to treat B cell lymphoma (22).

Activation of the *MYC* protooncogene by chromosomal translocations or hypermutation is a key event in the pathogenesis of many B cell lymphomas, and *MYC* breakpoints in lymphomas with a high chromosomal complexity score strongly correlate with a poor survival rate (38). Given that the critical mechanisms by which *MYC* contributes to malignant transformation are not clear, it is tempting to speculate that the coordinate down-regulation of multiple tumor suppressor genes, including *PTGER4*, plays an important role in *MYC*-induced lymphomagenesis. It is noteworthy that *PTGER4* has also been identified as one of a few genes targeted by the transcription factor and candidate tumor suppressor ETV6, deletions of which are observed in up to 47% of childhood pre-B acute lymphoblastic leukemia (49), implying a different mechanism of *PTGER4* down-regulation in B cell malignancy. Regardless of the primary genetic lesion leading to reduced expression of *PTGER4*, our findings support the possibility that regulation of B cell proliferation is a contributing mechanism by which *PTGER4* could hold human B cell lymphoma formation in check.

Collectively, our data postulate *Ptger4* as a delayed-early gene and a negative feedback regulator of BCR–signaled activation of B cells. This gene functions via its encoded EP4 receptor, which is also the principal molecule transmitting the inhibitory message of PGE2 to proliferating B cells. Regulation of *Ptger4* inversely correlates with the expression of many genes that promote growth and survival of B cell lymphoma, such as *MYC*, members of the NF- κ B pathway, and *Cd74*, but appears to be coordinated with several tumor suppressors. Varying the *Ptger4* expression level significantly affects B cell

lymphoma spread in mice and we find it down-regulated in human B cell malignancy, indicating that *Ptger4* is a tumor suppressor in B cells. Finally, because EP4 is expressed as an easily accessible membrane receptor, interfering with the PGE2–EP4 signaling pathway may present a clinically useful alternative or supplement to chemotherapy in the treatment of B cell lymphoma.

MATERIALS AND METHODS

Mice and reagents. We obtained female C57BL/6 and BALB/c mice from The Jackson Laboratory. Female *Ptger4*^{-/-} mice and *Ptger4*^{+/+} littermate controls were generated as previously described (16). All mice were housed in the Exploration and Experimental Functional Research Center (CERFE; Evry, France), and all experiments and protocols using animals were approved by the CERFE direction committee and the Institut de Radiobiologie Cellulaire et Moléculaire (CEA) animal research committee. Mice used for experiments were 8–12 wk old. PGE2, AH-13205, indomethacin, and tetracycline were purchased from Sigma-Aldrich. Prostaglandin E1 alcohol (PGE1-OH) and NS-398 were obtained from Cayman Chemical. FITC-, PE-, APC-, or biotin-conjugated antibodies to CD19, CD23, CD38, CD93, CD95, or B220 were obtained from eBioscience. F(ab')₂ anti-human IgM was purchased from SouthernBiotech, F(ab')₂ anti-mouse IgM was purchased from Jackson ImmunoResearch Laboratories, and F(ab')₂ anti-mouse IgG was purchased from Caltag Laboratories.

Cells, cell sorting, and culture. Single-cell suspensions were prepared from spleen of mice according to standard protocols. Negatively depleted B cells were recovered using B cell Isolation kit (Miltenyi Biotec) and mature B cells were further sorted as CD93⁺CD23⁺ by FACS using a MoFlo cell sorter (Dako) (50). Sorted cells were ≥95% pure. B cells were cultured at a density of 2 × 10⁶ cells/ml in IMDM supplemented with 5% heat-inactivated FCS, 100 U/ml of penicillin, 100 µg/ml of streptomycin, 2 mM L-glutamine, and 50 µM β-mercaptoethanol. To trigger the BCR, cells were stimulated with F(ab')₂ anti-mouse IgM at 20 µg/ml. A20, a mature B lymphoma cell line of BALB/c origin (American Type Culture Collection [ATCC]), and P493-6, a human B lymphoma cell line containing a Tet-regulatable *MYC* gene (provided by G.W. Bornkamm, GSF Research Centre, Neuherberg, Germany) were cultivated in RPMI-1640 supplemented with 10% heat-inactivated FCS, 100 U/ml of penicillin, 100 µg/ml of streptomycin, 2 mM L-glutamine and 50 µM β-mercaptoethanol. To inhibit the expression of *MYC*, P493-6 cells were incubated with 0.1 µg/ml Tet for 3 d. For BCR triggering, A20 cells were incubated with F(ab')₂ anti-mouse IgG and P493-6 cells with F(ab')₂ anti-human IgM, both at 20 µg/ml. NIH3T3 cells were cultivated as recommended by the ATCC. All cells were maintained at 37°C in a humidified atmosphere containing 5% CO₂.

Proliferation and viability assays. The ATP content of B cells was measured using a bioluminescence assay (ViaLight Plus; Cambrex). Tests were performed in 96-well plates at 10⁵ cells/well according to the manufacturer's instructions. Cell cycle was analyzed using flow cytometry according to standard protocols. The CFSE method to follow B cell proliferation over time was performed according to a previously published protocol (51). Cell viability was monitored by the exclusion of 0.4% trypan blue solution visualized by light microscopy.

Transfection and luciferase reporter assays. A20 cells were transfected by electroporation using Nucleofector II (Amaxa Biosystems) according to the manufacturer's instructions. NF-κB, FOS, JUN, IL-2, EGR1, and CRE activities were measured after electroporation of cells with plasmids (2 µg) encoding the indicated response element or promoter fused to a firefly luciferase reporter gene together with the indicated *Ptger4*-expression vectors (2 µg) and pRL-TK as an internal control (200 ng; Promega). 48 h after transfection, cells were incubated with 20 µg/ml F(ab')₂ anti-mouse IgG for 8 h,

and luminescence was determined using Dual-Luciferase Reporter Assay System (Promega) according to the manufacturer's instructions. The luciferase reporter plasmids were gifts, as follows: NF-κB reporter plasmid from K.A. Reedquist (University of Amsterdam, Amsterdam, Netherlands), FOS and JUN reporter plasmids from H. van Dam (Leiden University, Leiden, Netherlands), IL-2 reporter plasmid from G.R. Crabtree (Stanford University, Stanford, CA), EGR1 reporter plasmid from J. Milbrandt (Washington University School of Medicine, St. Louis, MO), and CRE reporter plasmid from V.C. Manganiello (National Institutes of Health, Bethesda, MD).

Expression vectors, lentiviruses, and stable cell lines. Expression vectors to silence or overexpress mouse *Ptger4* were constructed using Gateway technology and Block-iT Lentiviral Pol II miR RNAi Expression System (Invitrogen). For the silencing vectors, seven different premiRNAs targeting *Ptger4* were designed using the manufacturer's RNAi Designer program, cloned into pcDNA 6.2-GW/EmGFP-miR vectors, and tested by qPCR for *Ptger4* knock-down efficiency upon transfection into NIH3T3 cells with Lipofectamine 2000 (Invitrogen). The most efficient premiRNA expression cassette (miPtger4b) a less efficient cassette targeting both mouse and human *Ptger4* (miPtger4a), and a nontargeting control cassette (miCTRL) were selected and recombined into pLenti6/V5-DEST destination vector. For the overexpression construct, the ORF region of mouse *Ptger4* was amplified by PCR from total cDNA of A20 cells and transferred into pLenti6/V5-DEST vector. Lentiviruses were generated by cotransfecting each recombined destination vector with ViraPower packaging mix (Invitrogen) into 293FT cells as recommended by the manufacturer. A20 and P493-6 cell lines stably expressing silencing or control cassettes were generated by in vitro transduction with the appropriate lentiviral particles, followed by selection of GFP⁺ cells by FACS at 12 d after transduction. Similarly, A20 cells with stably integrated overexpressing cassette were generated using the blasticidin-selection method. The expression of *Ptger4* in stable cell lines was verified by qPCR and immunoblotting. All synthetic oligonucleotide sequences used are listed in Table S3 (available at <http://www.jem.org/cgi/content/full/jem.20081163/DC1>).

Lymphoma injection and immunization of mice. Cells of each generated A20 stable cell line were intravenously injected into BALB/c mice, and tumor development was followed over a period of 120 d. The mice were killed when they showed excessive tumor growth. GFP expression analysis by fluorescence microscopy was used to validate the mutated A20-origin of tumors. The results are presented as Kaplan-Meier survival curves. To analyze GC B cell development in response to a T cell-dependent antigen, *Ptger4*^{-/-} mice and wild-type controls were injected intraperitoneally with 100 µg of 4-hydroxy-3-nitrophenylacetyl chicken γ-globulin (NP-CGG; Biosearch Technologies) mixed with Imject Alum (Thermo Fisher Scientific) as an adjuvant. At day 14 after immunization, mice were killed and GC B cell content in splenic single-cell suspensions was determined by flow cytometry as the percentage of CD19⁺CD38^{low}CD95^{high} cells (52). This strategy was also used to sort wild-type GC and naive B cells for the immunoblot analysis.

Microarray analysis. For the initial screen to identify negative regulators of B cell proliferation, we used microarrays containing 15,247 cDNA clones for the analysis of ~11,000 mouse genes (53). RNA was isolated from BCR-stimulated or vehicle-treated mature B cells from pooled spleens of 10 C57BL/6 mice using Trizol reagent (Invitrogen), followed by one round of amplification with MessageAmp kit (Ambion). The amplified RNA was reverse-transcribed, labeled, and hybridized to the arrays as previously described (54). Each sample was hybridized to the arrays in 6 replicates using a dye-swap strategy. Slides were scanned with GenePix 4000B array scanner and feature extraction was performed with GenePix Pro 6.0 software (Axon Instruments). For transcriptome analysis of *Ptger4*^{-/-} and *Ptger4*^{+/+} control B cells, microarrays with 24,109 spotted mouse oligonucleotides were used (55). RNA was isolated using RNeasy Mini kit (QIAGEN), amplified, labeled, and hybridized following a published protocol (55). Oligonucleotide arrays were scanned with Agilent G2565AA Microarray Scanner (Agilent Technologies). Data analysis, including intensity-dependent Lowess normalization of raw data, was performed

with GeneSpring 7.0 software (Silicon Genetics). Ingenuity Pathway Analysis (Ingenuity Systems) was used for gene ontology and network analysis.

Real-time quantitative PCR. Total RNA was isolated using RNeasy Mini kit, and reverse-transcribed by Superscript II Reverse transcription (Invitrogen) according to the manufacturers' protocols. Samples were processed on ABI PRISM 7700 Sequence Detection System (Applied Biosystems) using SYBR Green JumpStart Taq ReadyMix (Sigma-Aldrich). All experiments were performed in triplicate, and results were normalized to *Hprt1* expression levels. Primer sequences are listed in Table S3.

Immunoblot analysis and immunofluorescence. Total cell protein extracts were prepared from B cells by lysis in RIPA Buffer supplemented with Protease Inhibitor Cocktail (both from Sigma-Aldrich). Proteins were separated by SDS-PAGE and immunoblots were performed using anti-EP4 (C-term; Cayman Chemical) and anti-actin (Sigma-Aldrich) antibodies. Immunoblots were developed with SuperSignal ECL reagent (Thermo Fisher Scientific). Immunofluorescence of BCR-stimulated mouse B cells was done using anti-EP4 antibody (C-18; Santa Cruz Biotechnology) following the manufacturer's protocol. To assure specificity of staining, anti-EP4 antibody was preincubated with fivefold (by weight) excess of the specific EP4 blocking peptide (Santa Cruz Biotechnology) for 1 h at room temperature. Indirect detection of EP4 was performed using secondary FITC-conjugated antibody.

Measurement of PGE2 production. Mouse B lymphocyte culture supernatants were harvested at 0, 2, and 4 d of cocubation with anti-IgM F(ab')₂ with or without indomethacin (20 μM) or NS-398 (10 μM), and analyzed by a highly specific competitive enzyme immunoassay (Prostaglandin E2 EIA kit; Cayman Chemical) to determine the concentration of PGE2. The levels of PGE2 in all samples of culture supernatants were below the detection limit of the assay (15 pg/ml). Three independent experiments were performed.

Statistical analysis. Results are mean values ± SD. Statistical analyses were performed using the logrank test, Wilcoxon rank sum test, or Student's *t* test (OncoPrint), as indicated. P values of 0.05 or less were considered significant, and exact P values are shown in the figures.

Accession nos. The complete microarray datasets are available from the Gene Expression Omnibus (GSE9215 and GSE9847).

URLs. Ingenuity Pathway Analysis: <http://www.ingenuity.com/>; OncoPrint database: <http://www.oncoPrint.org/>; Pubmed database: <http://www.ncbi.nlm.nih.gov/pubmed/>.

Online supplemental material. Fig. S1 shows that EP4 protein is expressed at low levels in quiescent B cells and is induced upon BCR triggering. Fig. S2 shows that BCR-triggering induces expression of the EP4 receptor on the plasma membrane of B cells. Fig. S3 shows that COX inhibition has no effect on proliferation or viability of BCR-stimulated mouse B cells. Fig. S4 shows that *Ptger4* expression does not affect the BCR-induced transcriptional activity of EGR1 or transcription through CRE. Fig. S5 shows that PGE2 diminishes the viability of BCR-activated B cells in culture. Fig. S6 shows that *Ptger4* antagonizes GC B cell development in mice. Table S1 shows the lack of *Ptger4* facilitates BCR-triggered induction of activating genes. Table S2 shows genes that are induced when EP4 is absent are repressed when EP4 is abundant and activated. Table S3 shows synthetic oligonucleotide sequences used for qPCR, amplification of *Ptger4* ORF region, and knockdown of *Ptger4* expression. The videos show freshly isolated *Ptger4*^{-/-} (Video S1) and *Ptger4*^{+/+} (Video S2) splenic B cells cocultured with 20 μg/ml F(ab')₂ anti-IgM and 10 nM PGE2 in culture. Online supplemental material is available at <http://www.jem.org/cgi/content/full/jem.20081163/DC1>.

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