***In vivo* DNase I-mediated footprinting analysis along the human bradykinin B1 receptor (*BDKRB1*) gene promoter: evidence for cell-specific regulation**

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By applying *in vivo* dimethyl sulphate and UV light type C-footprinting analysis, we previously showed that specific DNA sequences in the –1349/+42 core promoter region of the inducible human *BDKRB1* (bradykinin B1 receptor) gene correlated with its transcriptional activity. In the present study we used the highly sensitive DNase I *in vivo* footprinting approach to delineate more precisely the functional domains of the *BDKRB1* gene promoter in human SMCs (smooth muscle cells). Human lymphocytes that do not express a functional *BDKRB1* were also studied as a reference using dimethyl sulphate, UV light type C and DNase I treatments. An obvious difference was found in the DNase I-footprinting patterns between cellular systems that express a functional *BDKRB1* (SMCs) in comparison with human lymphocytes, where randomly distributed nucleosome-like footprinting patterns were found in the bulk of the core promoter region stud-

ied. Gel-shift assays and expression studies pointed to the implication of the YY1 and a TBP/TFIIB (TATA-box-binding protein/transcription factor IIB) transcription factor in the regulation of *BDKRB1* gene expression in SMCs and possible YY1 involvement in the mechanisms of nuclear factor κ B-mediated regulation of the receptor expression. No significant changes in the promoter foot-printing pattern were found after treatment with interleukin-1 β or serum (known *BDKRB1* gene inducers), indicating that definite regulatory motifs could exist outside the *BDKRB1* gene core promoter region studied.

Key words: bradykinin B1 receptor, DNase I *in vivo* genomic footprinting, G-protein-coupled receptor, human smooth muscle cell, ligation-mediated PCR, transcription factor-binding motif.

INTRODUCTION

BDKRB1 (bradykinin B1 receptor) is generally non-functional under normal physiological conditions, but is rapidly induced after different types of tissue injury and/or inflammation, under the effect of cytokines and growth factors [1,2]. The recent creation of the *BDKRB1* knockout mice confirmed the understanding that *BDKRB1*s are commonly inducible in pathological states, and their induction is controlled by IL-1 β (interleukin-1 β) together with the induction of other factors (cyclo-oxygenase 2) and receptors such as natural killer-1, in the framework of a generalized tissue response to injury [3]. The 5'-flanking regulatory region of the human *BDKRB1* gene has been studied in several laboratories, including ours [4–8]. These studies have indicated the presence of a functional TATA box and some positive and negative control regions in the human *BDKRB1* gene promoter [5,6], as well as the implication of NF- κ B (nuclear factor κ B) in the IL-1 β -mediated up-regulation of *BDKRB1* gene expression [4,7,8]. In an attempt to define the regulatory elements that account for the control of *BDKRB1* gene expression, we previously conducted *in vivo* DMS (dimethyl sulphate) and UVC (UV light type C)-footprinting analyses along the –1349/+42 *BDKRB1* gene core promoter in three human cell types: embryonic lung fibroblast cells (IMR-90), embryonic kidney cells [HEK-293 cells (human embryonic kidney 293 cells)] and

primary cultures of vascular umbilical SMCs (smooth muscle cells) [5]. Our initial *in vivo* footprinting analyses revealed that analogous complex protein–DNA interactions exist at the *BDKRB1* gene promoter in the three cell types studied before induction [5]. However, no additional changes in protein–DNA complexes were observed on treatment with IL-1 β or LPS (bacterial lipopolysaccharide), both known as inducers of *BDKRB1* gene expression [1–3].

In the present study, we further delineated the –1349/+42 promoter region of the *BDKRB1* gene using the more sensitive and informative DNase I *in vivo* footprinting approach. Additionally, we evaluated possible differences in the footprinting patterns of this promoter region between cells that express a functional *BDKRB1* (primary cultures of human SMCs) and cells that do not express a functional receptor (human peripheral blood lymphocytes). On the basis of the *in vivo* footprinting data obtained, we also examined the involvement of some specific transcription factors in the regulation of human *BDKRB1* gene expression.

MATERIALS AND METHODS

Materials

DNase I and lyssolecithin were obtained from Roche Molecular Biochemicals (Laval, Canada) and the oligonucleotide primers

Abbreviations used: *BDKRB1*, bradykinin B1 receptor; DMS, dimethyl sulphate; DMEM, Dulbecco's minimum essential medium; ds oligo, double-stranded oligonucleotide; EMSA, electrophoretic mobility-shift assay; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IL-1 β , interleukin-1 β ; LMPCR, ligation-mediated PCR; LPS, bacterial lipopolysaccharide; NE, nuclear extract; NF- κ B, nuclear factor κ B; PBL, peripheral blood lymphocyte; RT, reverse transcriptase; SMC, smooth muscle cell; TBP, TATA-box-binding protein; TFIIB, transcription factor IIB; UVC, UV light type C.

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were obtained from Life Technologies (Burlington, ON, Canada). *Taq* DNA polymerase and T_4 DNA ligase were purchased from Roche Molecular Biochemicals, whereas *Pfu* exo^- DNA polymerase was from Stratagene (La Jolla, CA, U.S.A.). [α - 32 P]-dCTP was supplied by New England Nuclear (Boston, MA, U.S.A.) and DMS, piperidine, K_2PdCl_4 and hydrazine were from Sigma-Aldrich Canada (Guelph, ON, Canada). The NF- κ B inhibitor MG-132 (Cbz-Leu-Leu-leucinal, where Cbz stands for carbobenzoxy) was purchased from BioMol Research Laboratories (Plymouth Meeting, PA, U.S.A.). All primer extensions and PCR amplifications were performed on a thermocycler PTC-100 obtained from MJ Research (Waltham, MA, U.S.A.) or a T-gradient thermocycler from Biometra (Montreal Biotech Kirkland, QC, Canada).

Cell cultures and drug incubations

Primary cultures of human SMCs were isolated from arteries of human umbilical cords obtained at normal deliveries, as described previously [5]. Cells were used only at passages 1–3 when the expression of smooth-muscle actin was clearly demonstrable (results not shown). The SMCs were grown to 80% confluence in DMEM (Dulbecco's minimum essential medium) containing 10% (v/v) fetal bovine serum. Under some experimental conditions, the culture medium was removed and replaced by non-supplemented DMEM for 24 h. Some serum-free SMC cultures were additionally treated with IL-1 β (0.5 ng/ml of culture medium) for 30 min at 37°C. After the incubations, the culture medium was removed and cells were washed with solution I (150 mM sucrose, 80 mM KCl, 35 mM Hepes, pH 7.4, 5 mM $MgCl_2$ and 0.5 mM $CaCl_2$) [9].

PBLs (peripheral blood lymphocytes) were isolated from a healthy human male as described in [9] and plated in supplemented DMEM. The culture medium was removed 24 h later, and cells were washed with solution I.

All procedures were approved by a local ethical committee.

DNase I treatment

SMCs, PBLs or purified DNA from PBLs were treated with DNase I. The plasma membrane of the SMCs and PBLs was permeabilized by lysolecithin for 1 min at room temperature (21°C) [9]. The permeabilized cells were then treated with DNase I for 10 min at room temperature and were then recuperated (referred to in the present study as *in vivo*) [9]. DNase I-cleaved genomic DNA was isolated from the *in vivo*-treated cells as described in [9]. Purified DNA was obtained from PBLs and digested with DNase I (referred to as *in vitro*) [9]. The single-strand DNA break frequencies were estimated using alkaline gel electrophoresis [10]. In some *in vivo* footprinting experiments, PBLs were treated with DMS or UVC as described previously [5].

LMPCR (ligation-mediated PCR)

The LMPCR procedure used herein has been already published in detail [9]. The primer extension step was performed using *Pfu* exo^- DNA polymerase as reported already [11] with the following modifications: DNA was denatured for 3 min at 98°C and the first primer was annealed for 5 min at its T_m (melting temperature, the temperature at which 50% of the oligonucleotide and its perfect complement are in duplex) as calculated using the GeneJockey software. With the exception of primer set B1, the same LMPCR primer sets designed for our initial *in vivo* footprinting analysis of 1.4 kb of the human *BDKRB1* gene core promoter region [5] were used again for the present study. Primer set B1 comprised oligonucleotides B1.1 (5'-TGACCAAATTC-AAAAGAG-3'; T_m = 48.9°C) and B1.2 (5'-GTGGCTCCACA-

AAAGCTGCTGCA-3'; T_m = 65.3°C) located on the bottom (transcribed) DNA strand of the *BDKRB1* gene promoter at -411/-393 and -382/-358 respectively (positions given are relative to the transcription initiation site [12]). Except for the primer set 48, all exponential PCR amplifications were performed using *Taq* DNA polymerase [10]. The *Pfu* exo^- DNA polymerase was used in the exponential PCR amplification by primer set 48, as described in [11].

Purified genomic DNA from human PBLs was chemically cleaved as reported previously [13,14]. The chemically cleaved G, A, T + C and C samples were included along with the other samples in the LMPCR assays as sequence markers. Only clear band intensity differences between *in vivo* and *in vitro* samples were considered as valuable footprints (*in vivo* band intensities being at least half lower or twice higher than *in vitro* band intensities). All LMPCR experiments were performed in duplicate and all DNA samples were double-analysed for each LMPCR method.

Sequence analysis

To identify potential transcription regulatory sites in the human *BDKRB1* gene promoter, we referred to our previously published data [5] and used both the Transcription Element Search Software (TESS; <http://www.cbil.upenn.edu/tess>) and the MatInspector software (<http://www.genomatrix.de/products/MatInspector/index.html>).

EMSAs (electrophoretic mobility-shift assays)

NEs (nuclear extracts) from primary cultures of human SMCs were isolated as described in [15]. The following oligos have been used to produce double-stranded DNA fragments: oligos 5'-TAGGAAAAATGTATCTGGTC-3' and 5'-TAGGAAAAATGTATCTGGTC-3' were used for the gel-shift analyses of the -529/-509 *BDKRB1* gene promoter region; oligos 5'-GCA-AAGTGAAATGAGAGTGG-3' and 5'-CCACTCTCATTTCACCTTTGC-3' were used for the gel-shift analyses of the -138/-119 *BDKRB1* gene promoter region; oligos 5'-GCAAGTGAAATGAGAGTGG-3' and 5'-CCACTCTCATTTCACCTTTGC-3' were used for the gel-shift analyses of the -138/-119 *BDKRB1* gene promoter region; oligos 5'-GGAGGTTATATAA-TTTAAAGCAACC-3' and 5'-GGTTGCTTTAAATTA-TATAA-CCTCC-3' were used for the gel-shift analyses of the -39/-15 *BDKRB1* gene promoter region. Finally, oligos 5'-CTCCTACGTTAGGGGGTGGCCTGGAA-3' and 5'-TTCCAGGCCA-CCCCCTACACGTAGGAG-3', containing the wild-type G allele of the -699G/C promoter polymorphism (underlined), and oligos 5'-CTCCTACGTTAGG-GCGGTGGCCTGGAA-3' and 5'-CTCCTACGTTAGGCGGTGGCCTGGAA-3', containing the mutant C allele of the -699G/C promoter polymorphism (underlined), were used for the gel-shift analyses of the -713/-687 *BDKRB1* gene promoter region.

The EMSA reactions were assembled as described previously [16], since those containing the NE received 70 μ g of protein. The reactions with recombinant transcription factors received 0.05 nM YY1 or 0.08 nM TBP (TATA-box-binding protein) and 0.15 nM TFIIB (transcription factor IIB; as indicated in the legend to Figure 6). All reactions contained 0.2 mg of BSA and 0.1 mg of poly(dG-dC) (polydeoxyguanylic-deoxycytidylic acid sodium salt; Amersham Biosciences, Baie d'Urfé, QC, Canada) as non-specific carriers. The EMSA autoradiograms were developed using a phosphorimager (Applied Biosystems, Foster City, CA, U.S.A.).

Analyses of YY1-mediated *BDKRB1* mRNA expression

Total RNA was isolated from primary SMCs, as described previously [5]. Before RNA isolation, some SMC cultures were

starved in serum-free medium for 72 h and consecutively treated with either control adenovirus or adenovirus expressing the YY1 transcription factor without or in combination with MG-132 (a proteasome and NF- κ B inhibitor; see the legend to Figure 7 for details). Our strategy for the construction of a recombinant adenovirus containing the human YY1 gene was based on the bacteriophage P1 Cre-lox site-specific recombination system [17]. The recombinant virus was constructed by co-transfection of recombinant Ad5 viral DNA and the shuttle plasmid pAdlox-YY1 vector, which contains the haemagglutinin-tagged YY1 cDNA fragment, into the cell line CRE8 (where CRE stands for cAMP-response element) expressing CRE recombinase [17]. Viral stocks were prepared and purified from plaque-purified viruses. A control virus was constructed with LacZ insert instead of YY1.

The *BDKRB1* gene expression levels in both control and treated cells were determined by semi-quantitative duplex RT (reverse transcriptase)-PCR as described in [18]. Briefly, 2 μ g of DNase I-treated RNA was reverse-transcribed into first-strand cDNA in a 20 μ l reaction using the MMLV (Moloney-murine-leukaemia virus) RT (Life Technologies, Burlington, ON, Canada). cDNA templates (2 μ l) were amplified in a 50 μ l reaction containing 2.5 pmol of each primer, 200 μ M dNTPs and 1.5 units of *Taq* polymerase (Life Technologies). The samples were initially denatured for 3 min at 94 °C and then submitted to 21 cycles of PCR (45 s at 94 °C, 45 s at 64 °C and 75 s at 72 °C) followed by a 10 min final elongation step at 72 °C. The number of cycles was chosen to keep the PCR-amplified DNA in the exponential phase of amplification. One-fifth of each PCR was run on a 10 % (w/v) polyacrylamide gel in 1 $\times$ TBE buffer (45 mM Tris/borate/1 mM EDTA) and the gel was documented and scanned using ScanJet 6 (HewlettPackard) and analysed with the NIH image 1.59 program.

The following oligonucleotides were utilized as PCR primers: 5'-TGTCATGGCATCATCTGGC-3' and 5'-GGCAACCACGAGCGTGAGGAT-3' were used as sense and antisense primers, respectively, for the amplification of a specific human *BDKRB1* fragment; the primer sequences were selected from the published human cDNA sequence [19]. 5'-CACCATCTTCCAGGAGCGAGATCC-3' and 5'-GTCTTCTGGGTGGCAGTGATGGC-3' were used as sense and antisense primers respectively for the amplification of a specific human *GAPDH* (glyceraldehyde-3-phosphate dehydrogenase) fragment (primer sequences selected from [20]).

RESULTS

Experimental strategy

In an effort to understand better relevant transcriptional regulation of the inducible *BDKRB1* gene, we have mapped the DNA sequences involved in DNA-protein interactions along the *BDKRB1* gene promoter using the highly sensitive *in vivo* DNase I-mediated footprinting approach. The *in vivo* footprinting LMPCR analysis was performed on genomic DNA from primary cultures of human SMCs grown in serum-free media or stimulated with either IL-1 β or serum, since both IL-1 β and serum components can induce the *BDKRB1* gene expression in this cell type [1,5,6,12]. Cells were treated with DNase I and their DNA was analysed by LMPCR with primers specific for the region of the *BDKRB1* promoter extending from -1349/+42 relative to the transcription start site [12]. To assess better the functionality of the *BDKRB1* promoter, we also compared the *in vivo* DNA footprints for the same promoter region in a cell type (PBLs) that does not express a functional *BDKRB1* gene [21]. PBLs were treated with DNase I as well as with DMS or UVC to facilitate comparison with present and previously gathered *in vivo* footprinting data [5]. All

Figure 1 *In vivo* genomic DNA footprinting of the -1349/-734 distal negative regulatory module of the human *BDKRB1* gene promoter

Primer set 56 (primers 56.1 and 56.2) was used for the analysis of the -1350/-1165 upper strand sequences (A), and primer set 57 (primers 57.1 and 57.2) was used for the analysis of the -902/-1185 lower strand sequences (B). Lane 1, LMPCR of naked genomic DNA purified from PBLs and treated *in vitro* (t) with DMS. Lane 2, LMPCR of genomic DNA from PBLs treated *in vivo* (v) with DMS before DNA purification. Lanes 3-6, LMPCR of DNA treated with standard Maxam-Gilbert cleavage reaction. Lane 7, LMPCR of genomic DNA from PBLs treated *in vivo* (v) with UVC before DNA purification. Lane 8, LMPCR of naked genomic DNA purified from PBLs and treated *in vitro* (t) with UVC. Lanes 9-12, DNase I-mediated LMPCR of genomic DNA from SMCs stimulated with IL-1 β for 0 min (U, lane 9) or 30 min (T, lane 10) from SMCs stimulated with serum (S, lane 11) or from PBLs (P, lane 12); here, the cells were treated with DNase I *in vivo* (v) before DNA purification. Lane 13, LMPCR of naked genomic DNA purified from SMCs and treated *in vitro* (t) with DNase I. Previously reported DNA sequences, footprinted *in vivo* with DMS and UVC in SMCs, are indicated on the left. Open rectangles indicate difference in break frequencies between DNA from SMCs treated with DNase I *in vivo* (lanes 9-11) and *in vitro* (lane 13). Closed rectangles indicate difference in break frequencies between DNA from SMCs (lanes 9-11) and PBLs (lane 12), both treated *in vivo* with DNase I. The circle shows the location of UVC-footprinted pyrimidines from PBLs.

analyses were performed on both strands of the DNA (sense and antisense) using 16 different sets of primers: 44-57 [5] and B1. Due to limited space, we are presenting approximately half of the LMPCR experiments performed (Figures 1-3), and all *in vivo* footprinting data obtained are summarized in Figure 4.

The distal negative regulatory module (-1349/-734) of the *BDKRB1* promoter displays putative nucleosomal organization

We and others have shown previously that the 1.4 kb *BDKRB1* promoter comprises two composite regulatory regions: a distal negative regulatory module (-1349/-734) and a proximal module (-733/-1) containing both positive and negative regulatory elements and a functional TATA box [5,6]. Consecutive DMS- and UVC-mediated LMPCR in SMCs showed virtually a lack of *in vivo* footprinting activity in the distal negative promoter module (only one UVC-protected pyrimidine dimer found at position -1186/-1185 [5]; see also Figure 4A). Similar results were now obtained in PBLs for this promoter region, since with the exception of three short stretches of UVC-hyperactive pyrimidine

Figure 2 *In vivo* genomic DNA footprinting of the $-733/-1$ proximal promoter module of the human *BDKRB1* gene promoter

The regions shown were analysed as follows: primer set 46 (primers 46.1 and 46.2) was used for the analysis of the $-548/-308$ upper strand region (A); primer set 48 (primers 48.1 and 48.2) was used for the analysis of the $-615/-525$ upper strand region (B); primer set B (primers B.1 and B.2) was used for the analysis of the $-296/-70$ lower strand region (C); primer set 45 (primers 45.1 and 45.2) was used for the analysis of the $+42/-113$ lower strand region (D); primer set 44 (primers 44.1 and 44.2) was used for the analysis of the $-96/+2$ upper strand region (E). Lane indications and symbols used are the same as described in the legend to Figure 1.

dimers ($-1004/-1001$, $-982/-981$ and $-963/-962$), no other footprints were detected on DMS and UVC treatment (Figures 1A and 1B, lanes 2 and 7). Furthermore, the DNase I-mediated LMPCR analysis of the distal *BDKRB1* promoter module displayed, both in SMCs and PBLs, long successions of single DNase I-sensitive sites at approx. 10 bp intervals, covering almost the entire $-1352/-734$ region (Figures 1A and 1B, lanes 9 and 12), with the exception of a stretch of protected footprints ($-799/-778$) found in PBLs (LMPCR results not shown, see Figure 4B). The observed DNase I footprinting patterns suggest a nucleosomal organization of this promoter region in both cell types and thus could explain its role as a negative regulatory element.

The proximal promoter module ($-733/-1$) controls *BDKRB1* gene expression in a cell-type-specific manner

Contrary to the distal negative regulatory module, the proximal *BDKRB1* promoter module ($-733/-1$) displayed quite different *in vivo* footprinting patterns in SMCs compared with PBLs. The DNase I footprints obtained in SMCs correlated well with the positions of the diverse control elements previously delineated in the proximal promoter module [5,6]. Thus the $-639/-436$ positive control region exhibited pockets of DNase I footprints on both strands including sequences previously shown to be DMS- and UVC-footprinted [5]. Two regions spanning $-629/-576$ on

Figure 3 *In vivo* genomic DNA footprinting of the $-615/-525$ region of the human *BDKRB1* gene promoter

The upper strand of the $-615/-525$ region was analysed with primer set 53 (primers 53.1 and 53.2). Lanes 1–13 are described in the legend to Figure 1. All footprinted nucleotides are indicated by arrowheads. Pyrimidines protected from UVC and pyrimidines hyper-reactive to UVC in PBLs are indicated by $\square$ and $\blacksquare$ respectively. DNase I-hyperactive nucleotides (+) and DNase I-protected nucleotides (–) are indicated. The nucleotides bracketed with (1) represent serum-induced footprints.

the lower strand (LMPCR results not shown, see Figure 4A) and $-473/-436$ on the upper strand (Figure 2A, lane 9) showed uninterrupted stretches of hyper-reactive footprinted nucleotides, since the $-473/-436$ region displayed the strongest signals that were observed along this promoter. The later region also showed a stretch of hypersensitivity on the lower strand ($-471/-462$; see also Figure 4A). In PBLs, DMS and UVC footprints were notably absent for the $-639/-436$ positive control domain (Figures 2A and 2B, lanes 2 and 8), and the DNase I footprints found there were scarcely distributed, suggesting the presence of nucleosome-like structures (Figures 2A and 2B, lane 12). Otherwise, the two previously delineated $-684/-639$ and $-435/-375$ negative control regions [5,6] displayed, both in SMCs and PBLs, similar footprinting patterns: either nucleosome-like footprinting periodicity ($-684/-639$; LMPCR results not shown, see Figure 4) or lack of *in vivo* footprinting activity ($-435/-375$; Figure 2A, lanes 2, 7, 9 and 12).

Further downstream, occurrence of distinct DNase I footprints was monitored in SMCs on both strands of the $-333/-270$ promoter region that has previously shown evident DMS and UVC footprinting activities (Figure 2A, lane 9, and Figure 2C, lane 9; see also Figure 4A). These footprints corresponded or were in the vicinity of myogenin, musculo-aponeurotic fibrosarcoma and Oct-1 DNA control elements formerly identified with DMS and UVC (Figure 4A). In contrast, the $-333/-270$ region displayed no footprinting activity in PBLs (Figure 2A, lanes 2, 7 and 12, and Figure 2C, lanes 2, 7 and 12).

Finally, no significant DNase I footprints were observed in SMCs for the $-296/11$ promoter segment (Figures 2C, 2D and 2E, lane 12). Scarce DNase I footprints were also found in the vicinity of the TATA box ($-54/-26$), a region that has previously demonstrated distinct DMS and UVC footprints ([5]; see also Figure 4A). The $-269/-1$ promoter region was more heavily footprinted in PBLs, especially in the $-221/-182$ segment, where a long stretch of DNase I footprints (containing both hyper-

sensitive and protected nucleotides) and UVC-protected pyrimidine dimers was observed (Figure 2C, lanes 7 and 12).

No significant alterations of the DNA–protein interactions along the ($-1349/-1$) *BDKRB1* gene promoter after IL-1 β or serum treatment

Since both IL-1 β and serum components are known inducers of the *BDKRB1* gene expression in SMCs [1–5,12,19], we also applied the more sensitive DNase I treatment approach in search of IL-1 β - and/or serum-mediated changes in the *in vivo* footprinting pattern of the ($-1349/-1$) *BDKRB1* gene promoter region. However, both treatments did not produce significant alterations in the DNase I footprints along the promoter region studied (lanes 10 and 11 in all footprints shown in Figures 1 and 2). The only exception was a stretch of DNase I-protected footprints ($-713/-699$) that were observed on serum treatment (Figure 3, lane 11). Interestingly, this serum-induced $-713/-699$ protection footprint is located in one of the previously delineated positive control regions of the proximal promoter module [5] that includes the (-699 G/C) polymorphic nucleotide. The -699 G/C polymorphism was first discovered in our laboratory [22], and we and others have consecutively reported its clinical relevance to renal, cardiovascular and gastrointestinal pathology [23–25]. In gel-shift experiments, the $-713/-687$ region supported specific protein–DNA interactions (Figure 5); however, no differences were observed when using NEs isolated from serum-treated or serum-deprived (growth-arrested) SMCs (Figure 5). Additionally, ds oligos (double-stranded oligonucleotides) containing the wild-type (G) or the polymorphic (C) allele displayed an identical gel-retardation pattern (results not shown).

Possible implication of the transcription factors YY1 and TBP/TFIIB in the control of *BDKRB1* gene expression

Sequence analysis of the core *BDKRB1* gene promoter region has indicated the presence of different transcription binding sites including two putative consensus binding sites for the transcription factor YY1 [26]. One of these sites is located in the heavily footprinted positive control region ($-529/-509$), whereas the other is located in a region displaying relatively weaker footprinting activity ($-138/-119$). In gel-shift experiments, only the $-529/-509$ region supported definite YY1–DNA interactions as a specific gel-shift signal was observed with SMCs-derived NEs (Figure 6A, panel a, lane 3), similar to that obtained for highly purified recombinant YY1 protein (Figure 6A, panel a, lane 2). Additional experiments using monoclonal mouse anti-YY1 antibodies displayed identical supershifts in both the positive control reaction assembled with the recombinant YY1 protein (Figure 6A, panel b, lane 3) and in the reaction containing a NE from SMCs (Figure 6A, panel b, lane 5). No supershifted bands were detected when using non-specific antibodies (Figure 6A, panel b, lanes 2 and 4). The above results suggest that YY1 probably interacts with the $-529/-509$ promoter sequence. Similar experiments performed with an oligonucleotide corresponding to a putative consensus YY1-binding site located at $-138/-119$ of the *BDKRB1* gene promoter region indicated that this sequence would support YY1 binding with a very low affinity. As expected, we did not observe any specific interaction with both recombinant YY1 and SMC NEs at this site (Figure 6B). Most probably, the protein complexes that form in the $-138/-119$ promoter region are YY1-independent. Implication of the YY1 transcription factor in the transcriptional regulation of the human *BDKRB1* gene was further supported by additional experiments, including a semi-quantitative RT–PCR analysis of *BDKRB1* gene expression in SMCs that were infected with adenovirus expressing the human

Figure 4 For legend see facing page

Figure 5 Gel shift of the $-713/-687$ polymorphic *BDKRB1* promoter region

NEs were isolated from primary cultures of growth-arrested and serum-stimulated human SMCs. Band-shift reactions received NE ds oligos (0.15 nM) with different promoter sequences. **(a)** Band-shift reactions received NE from growth-arrested cells. Lane 1 received 32 P-labelled ds $-713/-687$; lane 2, labelled ds oligo and NE; lanes 3 and 4 received the unlabelled competitor ds $-713/-687$ oligo at different concentrations as indicated above the lanes; lanes 5 and 6 received mutant (G698C) ds competitor at different concentrations as indicated above the lanes; lanes 7 and 8, non-related oligo was added as a competitor. **(b)** Band-shift reactions received NE from serum-stimulated cells. Reactions were assembled as in **(a)** with different competitors as indicated above the lanes. The position of free 32 P-labelled ds oligo is indicated on the right of the (F); NS indicates the position of a non-specifically shifted band.

YY1 gene. As shown in Figure 7, *BDKRB1* gene expression was strongly up-regulated after infection with an YY1-expressing adenovirus. We also observed some up-regulation of *BDKRB1* gene expression with a control adenovirus that indicates that the viral infection could induce mechanisms of *BDKRB1* transcription activation similar to those found in inflammation. Interestingly, pretreatment of the SMCs with the NF- κ B/proteasome inhibitor MG-132 completely abolished the YY1/adenovirus-mediated increase in *BDKRB1* gene expression.

We also investigated TBP-containing complexes with the TATA-box-containing sequence located at $-39/-15$ of the *BDKRB1* gene promoter region (Figure 6C). Using this promoter motif, definite interactions were observed with highly purified TBP and TFIIB (Figure 6C, panel a, lane 2), as well as with NEs from SMCs (Figure 6C, panel a, lane 3). Monoclonal mouse anti-human TBP antibodies were further used to verify the specificity of TBP complexes with ds oligo $-39/-15$ (panel b). TBP-specific supershifted complexes were observed only in the positive control reaction assembled with recombinant human TBP and TFIIB (Figure 6C, panel b, lane 3) and in the reaction containing SMC NEs (Figure 6C, panel b, lane 5). Thus TBP and TFIIB probably participate in the formation of a specific complex with the TATA-box domain of the *BDKRB1* gene promoter in SMCs.

DISCUSSION

By applying *in vivo* DMS- and UVC-DNA footprinting analysis, we previously showed that specific DNA sequences in the human *BDKRB1* gene promoter are implicated in the transcriptional control of this receptor gene [5]. We have now used a more informative *in vivo* footprinting approach after treatment with DNase I to understand better the molecular basis for the regulation of *BDKRB1* gene expression. DNase I treatment has been routinely applied for *in vitro* footprinting experiments aiming to reveal DNA–protein interactions using total cellular extracts and purified DNA [27]. Compared with DMS or UVC, DNase I cleavage is more efficient at detecting minor groove DNA–protein contacts, provides more information on chromatin structure, displays larger and clearer footprints, better delimits the boundaries of DNA–protein interactions and can also position nucleosomes and reveal specific DNA secondary structures [9,27–34]. Therefore DNase I is the preferred footprinting agent for studying DNA–protein interactions in living cells.

The DNase I-mediated *in vivo* footprinting analyses confirmed previous *in vivo* and *in vitro* promoter studies by our laboratory and other authors [5,6] concerning the functional importance of the $-733/-375$ *BDKRB1* gene promoter domain in the regulation of the receptor gene expression in SMCs. Moreover, we were now able to demonstrate *in vivo* footprinting activity at the $-733/-685$ positive control region (this region did not display any DMS- or UVC-mediated *in vivo* footprinting activity [5]). Additionally, nucleosome spacing-like footprinting patterns [35] were found in the $-1349/-734$ distal promoter module, shown previously to play a role in the negative control of the human *BDKRB1* gene expression [5,6]. Again, this region did not display any footprints in our previous *in vivo* footprinting analyses [5]. The fact that no DMS- and UVC-footprinted DNA sequences were identified combined with possible multiple nucleosomes positioned along this negative control promoter module suggests that particular DNA sequences seem to be inaccessible to eventual transcription modulators in this promoter region.

In our previous DMS and UVC *in vivo* footprinting analyses, we have obtained identical footprints for the core promoter of the *BDKRB1* gene in three human cell lines [5]; amongst them, HEK-293 cells were considered to be cells not expressing a functional *BDKRB1* (D. Bachvarov, unpublished work). However, on recent verification by semi-quantitative RT–PCR, we have found significant *BDKRB1* gene expression in this cell line (results not shown). Thus, to interpret better the DNase I footprints obtained for the *BDKRB1* promoter in SMCs, we also proceeded to an extensive *in vivo* DNA analysis of the *BDKRB1* promoter in a cell type (PBLs) that does not express a functional *BDKRB1* [23]. Indeed, we were not able to register any *BDKRB1* gene expression (by semi-quantitative RT–PCR) or *BDKRB1* functional activity (by performing *BDKRB1* binding assays) in primary human PBL cell cultures (results not shown). The $-1349/+42$ promoter region was investigated in PBLs using DMS, UVC and DNase I treatments to facilitate comparison with the footprinting data gathered before [5]. Our present LMPCR data show an obvious difference in the footprinting pattern between cellular systems that express a functional *BDKRB1* (SMCs) in comparison with

Figure 4 Summary of DMS, UVC and DNase I *in vivo* footprinting data of the human *BDKRB1* gene promoter in SMCs and PBLs

Guanines protected from DMS in living cells and hyper-reactive to DMS are indicated by '○' and '●' respectively. Pyrimidines protected from UVC in living cells and hyperreactive to UVC are indicated by '□' and '■' respectively. Nucleotides protected from DNase I and hyper-reactive to DNase I are indicated by arrows pointing down and up, respectively. Putative transcription factor binding sites, as identified by the program, are listed along the footprinted sequences. **(A)** Summary of the *in vivo* footprinting data obtained in SMCs. **(B)** Summary of the *in vivo* footprinting data obtained in PBLs.

Figure 6 EMSA of putative YY1- and TBP/TFIIB-binding motifs in the human *BDKRB1* gene promoter

NEs were isolated from primary cultures of human SMCs. Band-shift reactions received 32 P-labelled, ds oligos (0.15 nM) with different promoter sequences. **(A)** EMSA using ds oligos covering the -529/-509 domain of the *BDKRB1* gene promoter. **(a)** Lane 1, ds oligo only; lane 2 represents EMSA using recombinant YY1 (rYY1) protein (0.05 nM); the reactions in lanes 3-6 received NE alone (lane 3) or different competitors (lanes 4-6), as indicated at the top of the lanes. Competitors used: DNA oligonucleotide (8 nM) (lane 4); the initiator element from the adeno-associated virus P5 promoter, to which YY1 binds (lane 5); Sp1-binding ds oligo (lane 6). **(b)** Supershifts using anti-YY1 antibodies (α YY1): lane 1, gel-shift reaction in the presence of 1 μ g of anti-YY1, radioactively labelled oligo and reaction buffer (20 mM Hepes, pH 7.4, 150 mM NaCl, 0.5 mM dithiothreitol, 5% glycerol and 50 mg/ml BSA); lane 2, reaction with 1 μ g of non-specific antibodies (α NS), recombinant-YY1 protein and radioactively labelled oligo; lane 3, reaction with 1 μ g of anti-YY1 and recombinant-YY1 protein; lane 4, NE from SMCs (NE SMC) was mixed with 1 μ g of anti-NS; lane 5, gel shift with NE from SMC and 1 μ g of anti-YY1. Migration of the YY1 antibody-supershifted complexes is indicated on the left of the panel. The identity of both antibodies and proteins (rYY1, NE SMC) in the individual reactions is indicated at the top of the lanes. **(B)** EMSA using ds oligo covering the -138/-119 domain of the *BDKRB1* gene promoter. The reaction in lane 2 received only recombinant-YY1 protein (0.05 nM). The reactions in lanes 3, 4 and 5 received NE. Unlabelled competitor oligonucleotides were used for the reactions in lanes 4 and 5 (as indicated at the top of the lanes). **(C)** EMSA using ds oligos covering the -39/-15 domain of the *BDKRB1* gene promoter. **(a)** The reaction in lane 2 received only highly purified recombinant TBP protein (0.08 nM) together with 0.12 nM recombinant TFIIB [36]. Reactions in lanes 3-6 received NE and no competitor DNA (lane 3), homologous DNA competitor (lane 4), AdMLP TATA-box oligo as a competitor [36] (lane 5) or P5 heterologous DNA (lane 6). The absence (none) or presence of competitor unlabelled oligonucleotides is indicated at the top of the lanes. The positions of

Figure 7 Duplex RT-PCR analysis of *BDKRB1* mRNA expression in human SMCs

Total RNA was isolated from different SMC cultures and used for the synthesis of cDNA by RT and an oligo-dT primer. The analogous cDNA was used as a template for simultaneous amplification of two PCR fragments: one corresponding to the *BDKRB1* and the other corresponding to a *GAPDH* fragment, serving as an internal standard. One-fifth of each PCR was run on a 10% polyacrylamide gel. **(A)** Representative RT-PCR PAGE of *BDKRB1* (BIR) versus *GAPDH* gene expressions in control (lane 1) and treated cells (lanes 2-4). SMC cultures were treated as follows: serum-free culture medium for 72 h (lane 2); serum-free culture medium for 72 h, then adding control adenovirus (Ad: 1 μ l/ml medium) for an additional 48 h (lane 3); serum-free culture medium for 72 h, and then adding YY1-expressing adenovirus (1 μ l/ml of medium) for an additional 48 h (lane 4); serum-free culture medium for 72 h, and then adding 1 μ M MG-132 (a proteasome and NF- κ B inhibitor) and, 3 h later, a YY1-expressing adenovirus (1 μ l/ml of medium) was added for an additional 48 h (lane 5). The positions of the *BDKRB1* and *GAPDH* PCR fragments are indicated. Lane M contains a molecular-mass standard. The treatments of the SMCs before cell harvesting and RNA isolation are displayed above each lane. **(B)** Quantification of the *BDKRB1* gene expression. Results are shown after densitometry and after normalization to *GAPDH* mRNA levels.

cells not expressing a functional *BDKRB1* (PBLs), especially in the -733/-1 proximal promoter module. In general, the UVC and DMS footprinting patterns obtained in PBLs were very scarce, whereas the DNase I footprinting patterns were indicative of randomly distributed nucleosome-like structures along most of the promoter region studied. Such a footprinting pattern of the core

free DNA (F) and complexes (YY1 and TBP-TFIIB) are indicated on the left of the panels. **(b)** Supershifts using anti-TBP antibodies (α TBP): lane 1, control gel-shift reaction in the presence of 0.72 μ g of anti-TBP, radioactively labelled oligo and reaction buffer; lane 2, reaction with 0.72 μ g of non-specific antibodies (α NS), recombinant TBP and TFIIB proteins together with radioactively labelled oligo; lane 3, reaction with 0.72 μ g of anti-TBP, recombinant TBP and TFIIB proteins and radioactively labelled oligo; lane 4, NE from SMCs was mixed with 0.72 μ g of anti-NS; lane 5, gel shift with NE from SMCs and 0.72 μ g of anti-TBP. The migration of the TBP antibody-supershifted complexes is indicated on the left of the panel. The identity of both antibodies and proteins (TBP, TFIIB, NE SMC) in the individual reactions is indicated at the top of the lanes.

BDKRB1 gene promoter could be associated with a transcriptionally inactive receptor gene.

Sequence analysis of the *BDKRB1* gene promoter region has revealed the presence of consensus binding sites for different transcription factors, including YY1 and TBP/TFIIB [35,36]. Indeed, competition gel-shift reactions using SMC NEs with both specific and non-specific oligonucleotides (Figure 6, panel A, lanes 3–6) suggested that definite sequences of the promoter could be specifically occupied by protein complexes with the participation of YY1 and a TBP/TFIIB transcription factor. These results were indirectly supported by our previous (DMS, UVC) and present (DNase I) *in vivo* footprinting experiments, since the *BDKRB1* gene promoter regions, comprising –539/–439 (the YY1-binding motif) and –39/–15 (the TBP/TFIIB-binding motif), were among the most consistently and heavily footprinted domains of the promoter sequence studied in SMCs (Figure 4A). YY1 is a Zn-finger DNA-binding transcription factor that regulates the expression of genes with important functions in DNA replication, protein synthesis and cellular response to external stimuli during cell growth and differentiation [26,36,37]. YY1 could provide the retinoblastoma protein [15] as well as chromatin-modifying enzymes' [37] dependence on the promoter activity, and can function as a repressor or as an activator, depending on the promoter region [26,37]. How YY1 accomplishes such a variety of functions is unknown. Our results indicated that YY1 might represent a positive regulator of the human *BDKRB1* gene expression in SMCs. We also showed that YY1 is possibly implicated in the mechanisms of NF- κ B-mediated regulation of *BDKRB1* gene expression, since pretreatment of human SMCs with the NF- κ B inhibitor MG-132 completely abolishes the up-regulation of the *BDKRB1* gene, mediated by YY1. Numerous data are indicative of the involvement of the transcription factor NF- κ B in the induction of the *BDKRB1* gene expression [4,7,38,39]. Yet, the mechanisms and/or sites of interaction of activated NF- κ B with the *BDKRB1* gene expression are currently not known. Earlier reports using *in vitro* gene promoter analyses with a reporter gene suggested the putative implication of two different potential NF- κ B-binding sites, one located at –1172/–1162 of the human *BDKRB1* gene promoter [4], and the other located at –67/–57 of the rat *BDKRB1* gene promoter [37]. However, our previous (DMS and UVC) [5] and present (DNase I) *in vivo* footprinting analyses have not found any significant footprinting activity in any of the putative consensus NF- κ B-binding domains along the human *BDKRB1* gene core promoter sequence studied so far. Now, we demonstrate for the first time that NF- κ B could indirectly modulate the *BDKRB1* gene expression through possible interactions with the YY1 transcription factor. Furthermore, the specific binding of the TBP/TFIIB transcription factors to the –39/–15 TATA-box-containing sequence confirms the functional importance of this *BDKRB1* gene promoter domain.

BDKRB1 gene expression was shown to considerably increase after exposure to IL-1 β , LPS or serum components [1,5,11,19], since the accumulation of *BDKRB1* transcripts was partially attributed to mRNA stabilization, but mostly to increased gene transcription levels [4,7]. Using the more informative DNase I LMPCR approach, we were again not able to find any significant changes in the footprinting activity upon IL-1 β or serum treatment in the core human *BDKRB1* gene promoter sequence. A serum-induced stretch of DNase I protected footprints (–713/–699) was observed in SMCs; however, these results were not confirmed by consecutive gel-shift analyses. *In vitro* promoter studies performed by us and others [5,6] have also failed to reveal any domain in the human *BDKRB1* gene core promoter region that could be involved in the induction of expression by IL-1 β , LPS and TNF- α

(tumour necrosis factor α). Recent evidence based on a reporter gene coupled with different 5'-upstream elements of the human *BDKRB1* gene suggests that, at least the LPS-mediated induction could involve motif(s) located outside the core promoter [40]. We are currently trying to confirm these results, and if the results of these experiments are positive, we intend to further target the indicated regions by *in vivo* footprinting analyses.

In summary, by using the more informative and sensitive DNase I-based *in vivo* footprinting approach, we were able to delineate more precisely the active control regions in the core promoter domain of the human *BDKRB1* gene. We also demonstrated that cells expressing a functional *BDKRB1* gene (SMCs) display quite different DMS-, UVC- or DNase I-mediated *in vivo* footprinting patterns compared with cells not expressing a functional receptor (PBLs), the latter exhibiting mostly nucleosome-like spacing footprints. We additionally showed that YY1 and TBP/TFIIB transcription factors are probably involved in the control of the human *BDKRB1* gene expression, as YY1 might be implicated in the mechanisms of NF- κ B-mediated control of *BDKRB1* gene transcription. Similar to our previous DMS- and UVC-mediated *in vivo* footprinting analyses, the more sensitive DNase I LMPCR approach did not reveal any significant change in footprinting activity in the core promoter sequence of the human *BDKRB1* gene after IL-1 β and/or serum treatments. Further analysis of some flanking regions outside the *BDKRB1* core promoter sequence might be necessary to discover the control domains responsible for IL-1 β -, serum- and LPS-mediated induction of the *BDKRB1* gene expression.

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REFERENCES

- 1 Marceau, F., Larrivee, J. F., Saint-Jacques, E. and Bachvarov, D. R. (1997) The bradykinin B1 receptor: an inducible G protein coupled receptor. *Can. J. Physiol. Pharmacol.* **75**, 725–730
- 2 Marceau, F., Hess, J. F. and Bachvarov, D. R. (1998) The B1 receptors for bradykinins. *Pharmacol. Rev.* **50**, 357–386
- 3 Pesquero, J. B., Araujo, R. C., Heppenstall, P. A., Stucky, C. L., Silva, Jr, J. A., Walther, T., Oliveira, S. M., Pesquero, J. L., Paiva, A. C., Calixto, J. B. et al. (2000) Hypoalgesia and altered inflammatory responses in mice lacking bradykinin B1 receptors. *Proc. Natl. Acad. Sci. U.S.A.* **97**, 8140–8145
- 4 Schanstra, J. P., Bataille, E., Marin Castano, M. E., Barascud, Y., Hirtz, C., Pesquero, J. B., Pecher, C., Gauthier, F., Girolami, J. P. and Bascands, J. L. (1998) The B1-agonist [des-Arg10]-kallidin activates transcription factor NF-kappaB and induces homologous upregulation of the bradykinin B1-receptor in cultured human lung fibroblasts. *J. Clin. Invest.* **101**, 2080–2091
- 5 Angers, M., Drouin, R., Bachvarova, M., Paradis, I., Marceau, F. and Bachvarov, D. R. (2000) *In vivo* protein-DNA interactions at the bradykinin B(1) receptor gene promoter: no modification on interleukin-1 beta or lipopolysaccharide induction. *J. Cell. Biochem.* **78**, 278–296
- 6 Yang, X., Taylor, L. and Polgar, P. (1998) Mechanisms in the transcriptional regulation of bradykinin B1 receptor gene expression. Identification of a minimum cell-type specific enhancer. *J. Biol. Chem.* **273**, 10763–10770
- 7 Ni, A., Chao, L. and Chao, J. (1998) Transcription factor nuclear factor kappaB regulates the inducible expression of the human B1 receptor gene in inflammation. *J. Biol. Chem.* **273**, 2784–2791
- 8 Zhou, X., Polgar, P. and Taylor, L. (1998) Roles for interleukin-1beta, phorbol ester and a post-transcriptional regulator in the control of bradykinin B1 receptor gene expression. *Biochem. J.* **330**, 361–366
- 9 Drouin, R., Therrien, J. P., Angers, M. and Ouellet, S. (2001) *In vivo* DNA analysis. *Methods Mol. Biol.* **148**, 175–219
- 10 Drouin, R., Gao, S. and Holmquist, G. P. (1996) Agarose gel electrophoresis for DNA damage analysis. In *Technologies for Detection of DNA Damage and Mutations* (Pfeifer, G. P. ed.), pp. 37–43, Plenum Press, New York

- 11 Angers, M., Cloutier, J. F., Castonguay, A. and Drouin, R. (2001) Optimal conditions to use Pfu exo(−) DNA polymerase for highly efficient ligation-mediated polymerase chain reaction protocols. *Nucleic Acids Res.* **29**, E83
- 12 Bachvarov, D. R., Hess, J. F., Menke, J. G., Larrivee, J. F. and Marceau, F. (1996) Structure and genomic organization of the human B1 receptor gene for bradykinins (BDKRB1). *Genomics* **33**, 374–338
- 13 Iverson, B. L. and Dervan, P. B. (1987) Adenine specific DNA chemical sequencing reaction. *Nucleic Acids Res.* **15**, 7823–7830
- 14 Maxam, A. M. and Gilbert, W. (1980) Sequencing end-labeled DNA with base-specific chemical cleavages. *Methods Enzymol.* **65**, 499–560
- 15 Petkova, V., Romanowski, M. J., Suljoadikusumo, I., Rohne, D., Kang, P., Shenk, T. and Usheva, A. (2001) Interaction between YY1 and the retinoblastoma protein. Regulation of cell cycle progression in differentiated cells. *J. Biol. Chem.* **276**, 7932–7936
- 16 Usheva, A. and Shenk, T. (1996) YY1 transcriptional initiator: protein interactions and association with a DNA site containing unpaired strands. *Proc. Natl. Acad. Sci. U.S.A.* **93**, 13571–13576
- 17 Hardy, S., Kitamura, M., Harris-Stansil, T., Dai, Y. and Phipps, M. L. (1997) Construction of adenovirus vectors through Cre-lox recombination. *J. Virol.* **71**, 1842–1849
- 18 Dukas, K., Sarfati, P., Vaysse, N. and Pradayrol, L. (1993) Quantitation of changes in the expression of multiple genes by simultaneous polymerase chain reaction. *Anal. Biochem.* **215**, 66–72
- 19 Menke, J. G., Borkowski, J. A., Bierilo, K. K., MacNeil, T., Derrick, A. W., Schneck, K. A., Ransom, R. W., Strader, C. D., Linemeyer, D. L. and Hess, J. F. (1994) Expression cloning of a human B1 bradykinin receptor. *J. Biol. Chem.* **269**, 21583–21586
- 20 Ercolani, L., Florence, B., Denaro, M. and Alexander, M. (1988) Isolation and complete sequence of a functional human glyceraldehyde-3-phosphate dehydrogenase gene. *J. Biol. Chem.* **263**, 15335–15341
- 21 Prat, A., Weinrib, L., Becher, B., Poirier, J., Duquette, P., Couture, R. and Antel, J. P. (1999) Bradykinin B1 receptor expression and function on T lymphocytes in active multiple sclerosis. *Neurology* **53**, 2087–2092
- 22 Bachvarov, D., Landry, M., Pelletier, I., Chevrette, M., Betard, C., Houde, I., Bergeron, J., Lebel, M. and Marceau, F. (1998) Characterization of two polymorphic sites in the human kinin B₁ receptor gene: altered frequency of an allele in patients with a history of end stage renal failure. *J. Am. Soc. Nephrol.* **9**, 598–604
- 23 Zychma, M. J., Gumprecht, J., Zukowska-Szczechowska, E. and Grzeszczak, W. (1999) Polymorphisms in the genes encoding for human kinin receptors and the risk of end-stage renal failure: results of transmission/disequilibrium test. The End-Stage Renal Disease Study Group. *J. Am. Soc. Nephrol.* **10**, 2120–2124
- 24 Dhamrait, S. S., Payne, J. R., Li, P., Jones, A., Toor, I. S., Cooper, J. A., Hawe, E., Palmen, J. M., Wootton, P. T., Miller, G. J. et al. (2003) Variation in bradykinin receptor genes increases the cardiovascular risk associated with hypertension. *Eur. Heart J.* **24**, 1672–1680
- 25 Bachvarov, D., Landry, M., Houle, S., Paré, P. and Marceau, F. (1998) Altered frequency of a promoter polymorphic allele of the kinin B₁ receptor gene in inflammatory bowel disease. *Gastroenterology* **115**, 1045–1048
- 26 Hiromura, M., Choi, C. H., Sabourin, N. A., Jones, H., Bachvarov, D. and Usheva, A. (2003) YY1 is regulated by O-GlcNAcylation (O-linked N-acetylglucosaminylation). *J. Biol. Chem.* **278**, 14046–14052
- 27 Leblanc, B. and Moss, T. (2001) DNase I footprinting. *Methods Mol. Biol.* **148**, 31–38
- 28 Pfeifer, G. P. and Riggs, A. D. (1991) Chromatin differences between active and inactive X chromosomes revealed by genomic footprinting of permeabilized cells using DNase I and ligation-mediated PCR. *Genes Dev.* **5**, 1102–1113
- 29 Pfeifer, G. P. and Riggs, A. D. (1993) Genomic sequencing. *Methods Mol. Biol.* **23**, 169–181
- 30 Rozek, D. and Pfeifer, G. P. (1993) *In vivo* protein-DNA interactions at the c-jun promoter: preformed complexes mediate the UV response. *Mol. Cell. Biol.* **13**, 5490–5499
- 31 Chin, P. L., Momand, J. and Pfeifer, G. P. (1997) *In vivo* evidence for binding of p53 to consensus binding sites in the p21 and GADD45 genes in response to ionizing radiation. *Oncogene* **15**, 87–99
- 32 Chen, C. and Yang, T. P. (2001) Nucleosomes are translationally positioned on the active allele and rotationally positioned on the inactive allele of the HPRT promoter. *Mol. Cell. Biol.* **21**, 7682–7695
- 33 Tanguay, R. L., Pfeifer, G. P. and Riggs, A. D. (1990) PCR-aided DNase I footprinting of single copy gene sequences in permeabilized cells. *Nucleic Acids Res.* **18**, 5902
- 34 Tormanen, V. T., Swiderski, P. M., Kaplan, B. E., Pfeifer, G. P. and Riggs, A. D. (1992) Extension product capture improves genomic sequencing and DNase I footprinting by ligation-mediated PCR. *Nucleic Acids Res.* **20**, 5487–5488
- 35 Tornaletti, S., Bates, S. and Pfeifer, G. P. (1996) A high-resolution analysis of chromatin structure along p53 sequences. *Mol. Carcinog.* **17**, 192–201
- 36 Usheva, A. and Shenk, T. (1994) TATA-binding protein-independent initiation: YY1, TFIIB, and RNA polymerase II direct basal transcription on supercoiled template DNA. *Cell (Cambridge, Mass.)* **76**, 1115–1121
- 37 Thomas, M. J. and Seto, E. (1999) Unlocking the mechanisms of transcription factor YY1: are chromatin modifying enzymes the key? *Gene* **236**, 197–208
- 38 Cabrini, D. A., Campos, M. M., Tratsk, K. S., Merino, V. F., Silva, Jr, J. A., Souza, G. E., Avellar, M. C., Pesquero, J. B. and Calixto, J. B. (2001) Molecular and pharmacological evidence for modulation of bradykinin B(1) receptor expression by endogenous glucocorticoids hormones in rats. *Br. J. Pharmacol.* **132**, 567–577
- 39 Sabourin, T., Morissette, G., Bouthillier, J., Levesque, L. and Marceau, F. (2002) Expression of bradykinin B(1) receptor in fresh or cultured rabbit aortic smooth muscle: role of NF-kappa B. *Am. J. Physiol. Heart Circ. Physiol.* **283**, H227–H237
- 40 Yang, X., Taylor, L., Yu, J., Fenton, M. J. and Polgar, P. (2001) Mediator caused induction of a human bradykinin B1 receptor minigene: participation of c-Jun in the process. *J. Cell. Biochem.* **82**, 163–170

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