Hibernoma Development in Transgenic Mice Identifies Brown Adipose Tissue as a Novel Target of Aldosterone Action

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Abstract

Aldosterone is a major regulator of salt balance and blood pressure, exerting its effects via the mineralocorticoid receptor (MR). To analyze the regulatory mechanisms controlling tissue-specific expression of the human MR (hMR) in vivo, we have developed transgenic mouse models expressing the SV40 large T antigen (TAg) under the control of each of the two promoters of the hMR gene (P1 or P2). Unexpectedly, all five P1-TAg founder animals died prematurely from voluminous malignant liposarcomas originating from brown adipose tissue, as evidenced by the expression of the mitochondrial uncoupling protein ucp1, indicating that the proximal P1 promoter was transcriptionally active in brown adipocytes. No such hibernoma occurred in P2-TAg transgenic mice. Appropriate tissue-specific usage of P1 promoter sequences was confirmed by demonstrating the presence of endogenous MR in both neoplastic and normal brown adipose tissue. Several cell lines were derived from hibernomas; among them, the T37i cells can undergo terminal differentiation into brown adipocytes, which remain capable of expressing ucp1 upon adrenergic or retinoic acid stimulation. These cells possess endogenous functional MR, thus providing a new model to explore molecular mechanisms of mineralocorticoid action. Our data broaden the known functions of aldosterone and suggest a potential role for MR in adipocyte differentiation and regulation of thermogenesis. (*J. Clin. Invest.* 1998. 101:1254–1260.) Key words: mineralocorticoid receptor • alternative promoters • thermogenesis • ucp1 • steroid receptors

Introduction

Expression of the mineralocorticoid receptor (MR)[1] is restricted to specific tissues, including sodium reabsorbing epithelia (1), some areas of the brain (2, 3), and the cardiovascular system (4–6). Although physiological effects of aldosterone on sodium and potassium homeostasis are well established, the mechanism of MR action in both epithelial and nonepithelial target tissues is still poorly understood. In particular, the molecular mechanisms underlying mineralocorticoid signaling and the spectrum of primary mineralocorticoid-regulated target genes remain unclear.

Both in humans and rodents, multiple MR mRNA isoforms arising from alternative promoter utilization have been identified (7, 8). Two human MR (hMR) transcripts, hMRα and hMRβ, are expressed at approximately the same level in kidney, colon, heart, epidermis, and sweat glands; however, their relative abundance varies among these tissues, and there is strong evidence for existence of other hMR mRNA variants, most notably in the heart (9). Recently, we have shown that hMR gene transcription is under the control of two promoter regions, P1 and P2, located upstream of the two first untranslated exons 1α and 1β, respectively. Transient transfection studies have demonstrated that these promoters differ both by their basal as well as their corticosteroid-regulated transcriptional activity, in that only the distal P2 promoter is activated by aldosterone, whereas both regions are responsive to glucocorticoids (10).

In an attempt to study the regulatory mechanisms controlling MR expression in vivo, and to establish cellular models suitable for precise analysis of mineralocorticoid signaling both at the cellular and molecular level, we have developed a targeted oncogenesis strategy using different regulatory regions of the hMR gene fused to the large tumor antigen (TAg) of simian virus 40 (SV40) to direct oncogene expression in normally MR-expressing cells. Unexpectedly, all transgenic founder mice carrying 1.2 kb of the proximal P1 promoter region linked to TAg precociously developed voluminous malignant liposarcomas originating from brown adipose tissue (BAT). From these tumors we derived several cell lines harboring both immortalized features and differentiated characteristics of brown adipocytes. In both tumors and cell lines, as well as in normal BAT, we were able to detect endogenous MR. We have thus identified a novel aldosterone target tissue and established cellular models suitable for further studies on MR function as well as identification and characterization of aldosterone-induced genes.

Methods

Generation of P1-TAg constructs and transgenesis. The P1-TAg transgene was constructed using the transgenesis vector H31 kindly provided by Dr. L.M. Houdebine (INRA, Jouy en Josas, France) (Fig.

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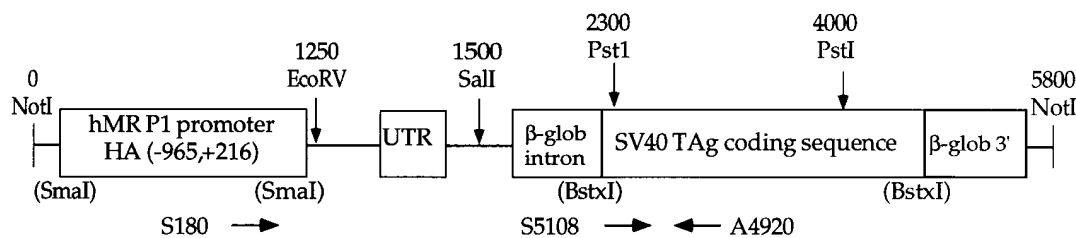
Figure 1. Schematic representation of the P1-TAg transgene. 1.2 kb of the proximal hMR promoter region was inserted upstream of the SV40 TAg coding sequence into the optimized vector H31, which contains stabilizing sequences for mRNA (*UTR,* untranslated region) and the terminal block of the human β-globin, including the last intron and the polyadenylation site. Restriction sites used for cloning are indicated in parentheses. Oligonucleotides S180, S5108, and A4920 used for PCR amplification are positioned on the transgene.

1). A HindIII-AvaII fragment (−965, +216) containing the hMR proximal promoter region (P1) and the beginning of the first untranslated exon 1α was blunt-ended using Klenow DNA polymerase (GIBCO BRL, Gaithersburg, MD) and ligated into the SmaI site of the H31 plasmid. The SV40 TAg coding region (2.3-kb StuI-BamHI fragment) was inserted into the BstXI site of the P1-H31 plasmid after fill-in of recessive ends with Klenow enzyme. The transgene was separated from plasmid vector sequences by NotI digestion and purified after agarose gel electrophoresis with Elutip-d columns (Schleicher & Schuell, Dassel, Germany) and ethanol precipitation. Microinjections into fertilized oocytes derived from B6D2 mice were realized at the Service d'Expérimentation Animale et Transgenèse (SEAT, CNRS, Villejuif, France).

Founders were identified by PCR analyses of tail DNAs from 2-wk-old mice using oligonucleotides specific to the SV40 TAg (sense primer S5108: 5′-TTGAAAGGAGTGCCTGGGGGAAT-3′; antisense primer A4920: 5′-CAGTTGCATCCCAGAAGCCTCCA) and to the P1 region (sense primer S180: 5′-TGCAACAGGTAGGCGAGAGA-3′); PCR conditions are available upon request. Number of integrated copies of transgenes was determined by Southern blot analysis.

Histology. Organs were fixed in 4% paraformaldehyde in PBS, dehydrated, and paraplast-embedded. 7-μm sections were cut and stained with hematoxylin and eosin.

Ribonuclease protection assays and Northern blot analyses. Total RNA was isolated from mouse tissues with Trizol (GIBCO BRL) according to the manufacturer's recommendations. For ribonuclease protection assays, Bluescript-KS plasmids (Stratagene, La Jolla, CA) containing a PstI fragment of exon 2 of the mouse MR (mMR, kindly provided by Drs. S. Berger and G. Schütz, Heidelberg, Germany) or a BamHI-StuI fragment of SV40 TAg were linearized by StuI and HinfI, respectively, and labeled antisense riboprobes synthesized with T3 and T7 RNA polymerases. The rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) probe was synthesized with T7 RNA polymerase, after digestion of a Bluescript-SK-GAPDH plasmid by PvuII-StyI. RNase protection assays were performed as described previously (11). Briefly, 50 μg of total RNA was hybridized overnight at 55°C with 4×10^5 cpm probe. Digestion with RNase A and T was performed at 30°C for 1 h followed by digestion by proteinase K at 37°C for 30 min. After phenol extraction and ethanol precipitation, protected fragments were electrophoresed on 6% polyacrylamide/urea gel. Unprotected mMR, TAg, and GAPDH riboprobes migrated at 452, 365, and 184 bp, with protected fragments migrating at 380, 291, and 164 bp, respectively.

Northern blot analysis was performed by standard techniques (12), using an [α-³²P]-labeled PCR fragment encompassing the coding region of the rat uncoupling protein 1 (ucp1) gene as probe (Megaprime DNA labeling system; Amersham, Arlington Heights, IL).

Tissue culture. Cell lines were derived from 2-wk-old transgenic mice (founders 34 and 37, Table I). Interscapular tumors were removed under sterile conditions, cut in small pieces, rinsed twice with

defined medium (DME-Ham's F12 [GIBCO BRL] supplemented with 10% FCS, 2 mM glutamine, 100 IU/ml penicillin, 100 μg/ml streptomycin, 20 mM Hepes) and teased with two forceps in a Petri dish containing 10 ml of defined medium. After centrifugation, pelleted cells were rinsed twice and grown at 37°C in a humidified atmosphere with 5% CO_2. Three cell lines were established, T34, T37s, and T37i, and the latter was further analyzed. Differentiation into adipocytes was achieved by treatment with 2 nM triiodothyronine and 20 nM insulin (GIBCO BRL) for at least 3 d; ucp1 expression was induced by adding 1 μM retinoic acid or isoproterenol for 6 h.

*Immunohistochemical and cytochemical analyses.* Immunodetection of SV40 TAg was performed using a monoclonal anti-SV40 TAg antibody (Ab-2; Calbiochem, La Jolla, CA). Cells were fixed with 100% ethanol, rinsed with PBS, and incubated with a 1:100 dilution of the primary antibody for 45 min at 37°C. After washing, samples were incubated with biotinylated horse anti–mouse IgG and streptavidin-fluorescein isothiocyanate (1:100). To confirm the TAg nuclear labeling, cells were treated for 5 min with propidium iodide. Cellular lipid accumulation was detected on the basis of red oil coloration (13).

Binding assays. Cells were grown 24 h before harvesting in the presence of 10% charcoal stripped FCS. After being rinsed twice with cold PBS, pelleted cells were frozen in liquid nitrogen. One volume of cells was homogenized in one volume of TEGW buffer (20 mM Tris-HCl, 1 mM EDTA, 20 mM sodium tungstate 10% [vol/vol] glycerol, pH 7.4, at 20°C) in a Teflon-glass Potter-Elvehjem (Poly Labo, Strasbourg, France) apparatus. Cytosols obtained after centrifugation at 4°C at 15,000 *g* for 15 min were incubated for 4 h at 4°C with 10 nM [³H]aldosterone (Amersham) in the absence or presence of a 100-fold excess unlabeled aldosterone or RU38486 (a generous gift of Roussel-Uclaf, Romainville, France). Bound and unbound steroids were separated by the dextran-charcoal technique. Values of binding parameters were determined at equilibrium by Scatchard analysis using a computerized method as described previously (6).

Table I. Characteristics of P1-TAg Transgenic Mice

Founder	Sex	Age of death	Copy number	Localization of liposarcomas
		wk		
16	F	17	15	Interscapular and mediastinal
17	M	5	5	Perirachidian
20	F	15	2	Interscapular and mediastinal
34	F	2	10	Dorsal subcutaneous
37	F	2	1	Dorsal subcutaneous

Number of integrated transgene copies was determined by Southern blot analysis after digestion of 10 μg genomic DNA with SalI and hybridization with the HA-TAg transgene following standard techniques.
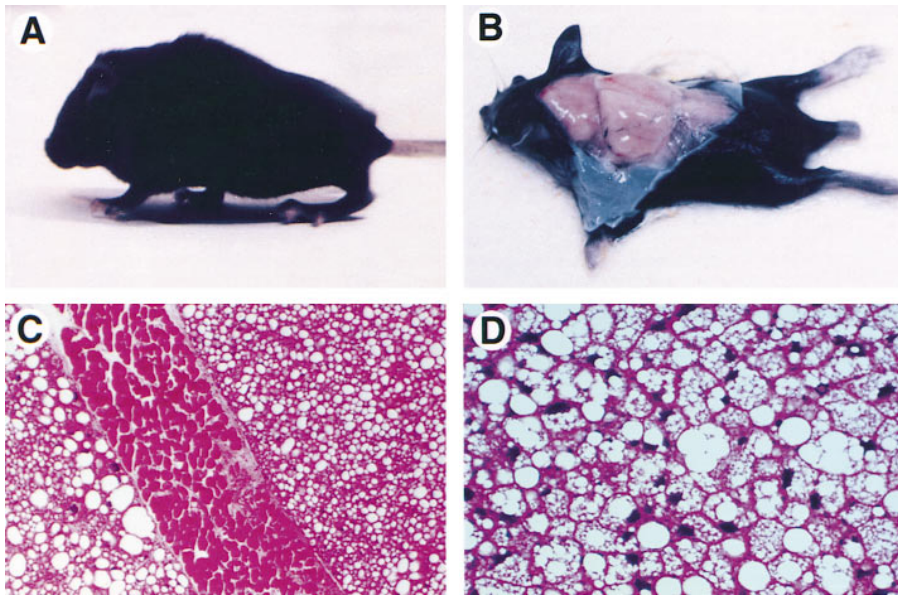
Figure 2. Liposarcoma development in P1-TAg transgenic mice. (*A*) Founder 37 presented with a voluminous dorsal mass. (*B*) Postmortem macroscopic examination revealed the presence of two 3 × 2 cm large tumors (inferior and superior) in the interscapular region of founder 37, which were removed and used to derive the T37i and T37s cell lines, respectively. (*C* and *D*) Hematoxylin and eosin–stained sections of a paravertebral liposarcoma from founder 37, infiltrating striated muscle tissue (*C*, ×250). Note the multivacuolated cells with lipid droplets and atypical nuclei (*D*, ×400).

Triglyceride measurements. Undifferentiated T37i cells were plated in 12-well plates at a density of 5×10^4 cells per well and grown for 7 d in the presence of 10^{-8} M aldosterone or 2 nM triiodothyronine and 20 nM insulin. Cells were rinsed twice with cold PBS, harvested in 250 µl PBS with a rubber policeman, and counted. Cells were subsequently disrupted by sonication (10 s × 2) and homogenates centrifuged for 10 min at 12,000 rpm at 4°C. The protein concentration was measured in the supernatant by the Bradford technique; triglyceride content was determined using a colorimetric determination kit (procedure 336; Sigma Chemical Co., St. Louis, MO).

Results

We have generated transgenic mice carrying the SV40 TAg under the control of the hMR proximal P1 promoter. Five transgenic founders were obtained, which all died within a few weeks after birth (Table I). Only female mouse 20 produced offspring, although none of the six pups inherited the transgene, preventing establishment of a lineage. Macroscopic examination of transgenic animals revealed the presence of large dorsal subcutaneous tumors, resulting in a characteristic buffalo appearance (Fig. 2, *A* and *B*). In some transgenic mice, other neoplastic localizations were detected in the mediastinum and paravertebral region. All tumors were histologically identified as malignant liposarcomas displaying a lobular pattern and some of them were infiltrating striated muscle tissue (Fig. 2 *C*). The cells showed varying degrees of differentiation, but most were granular and multivacuolated with lipid droplets and major nuclear abnormalities (Fig. 2 *D*). Systematic histopathological examination disclosed liposarcomatous foci in the pleura, the pericardium, and in the perirenal region. No other tumors were observed in other organs of these animals (kidney, lung, brain, heart, liver, muscle, spleen, colon, salivary glands, skin); however, some cardiomyocytes exhibited nuclear abnormalities (data not shown).

SV40 TAg mRNA expression was analyzed in various tissues of founders 34 and 37 by ribonuclease protection assay.
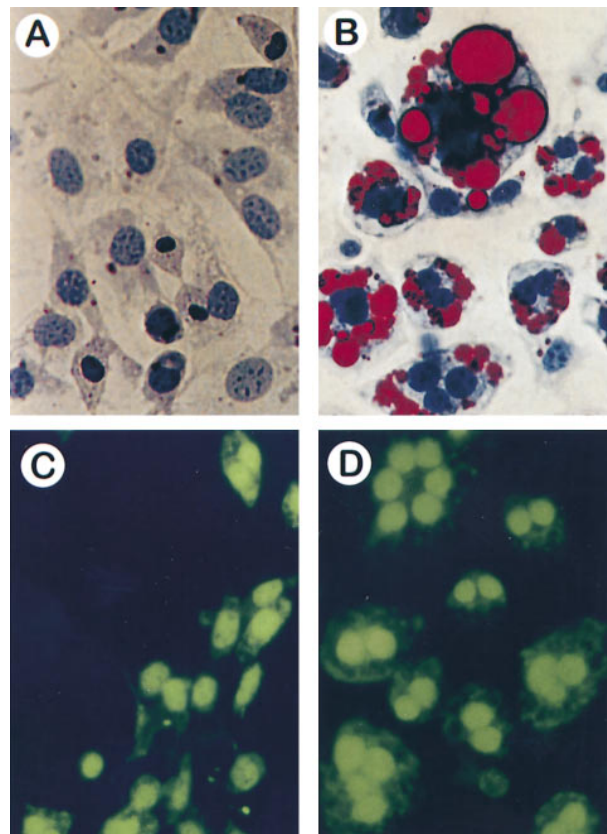
Figure 4. Establishment of a new brown adipocyte cell line. The T37i cell line was derived from the dorsal inferior liposarcoma from founder 37. Treatment of undifferentiated cells (*A*) with insulin and triiodothyronine induced differentiation into brown adipocytes with multivacuolar lipid accumulation, as evidenced by red oil staining (*B*). Characteristic nuclear staining with a monoclonal anti-TAg antibody was seen in both undifferentiated (*C*) and differentiated (*D*) T37i cells. ×800.
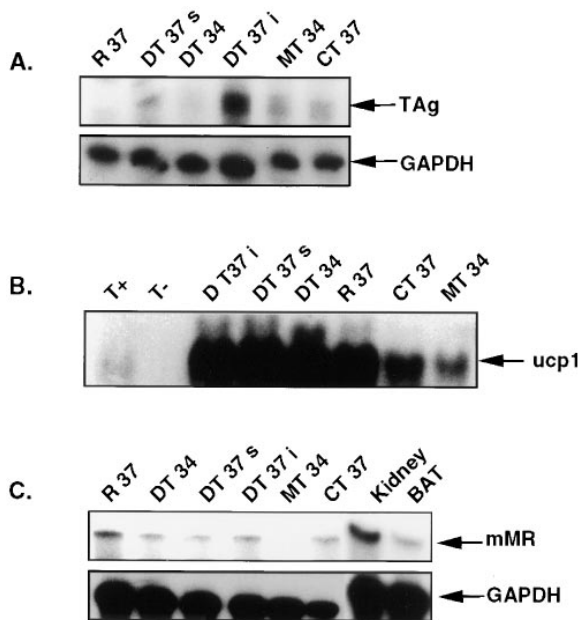
Figure 3. Expression of SV40 TAg, ucp1, and mMR mRNA in various hibernomas of founders 34 and 37. (*A* and *C*) Ribonuclease protection assay was used to detect TAg (*A*) and mMR (*C*) expression in RNAs originating from a paravertebral, a dorsal superior, a dorsal inferior, and a cervical tumor of mouse 37 (*R 37, DT 37 s, DT 37 i,* and *CT 37,* respectively), as well as from a dorsal and a mediastinal tumor of mouse 34 (*DT 34* and *MT 34*). Endogenous mMR mRNA was detected in normal BAT. Total mouse kidney RNA was used as a positive control for MR expression, whereas a rat GAPDH probe was used as internal control in ribonuclease protection assays. (*B*) ucp1 expression in neoplastic tissues was detected by Northern blot analysis. *T+,* differentiated 1B8 cells treated with 1 μM norepinephrine, positive control for ucp1 expression; *T−,* differentiated 1B8 cells, nontreated, negative control. The presence of two 1.6- or 1.9-kb ucp1 transcripts corresponds to different polyadenylation sites.

All liposarcomas were shown to express significant amounts of TAg, with highest levels in the inferior dorsal tumor of founder 37 (Fig. 3 *A*); TAg expression was largely independent of the integrated transgene copy number (see Table I). The interscapular localization of liposarcomas together with their histomorphology were highly suggestive of malignant BAT tumors (hibernomas). This origin was confirmed by demonstrating expression of the mitochondrial uncoupling protein ucp1, a BAT-specific gene product, in liposarcomas of mice 34 and 37 (Fig. 3 *B*).

The occurrence of hibernomas in all transgenic mice carrying the SV40 TAg under the control of the hMR P1 proximal promoter was totally unexpected and prompted us to investigate whether BAT could be a site of MR gene expression. Ribonuclease protection assays were performed on total RNA from tumors as well as from BAT of nontransgenic animals. Endogenous mMR transcripts were detected in both normal and neoplastic BAT, providing conclusive evidence for MR gene transcription in BAT, although to a lesser extent than in the kidney (Fig. 3 *C*). These results further indicate that SV40 TAg expression and hibernoma formation in transgenic mice are the consequence of appropriate tissue-specific expression of the transgene.

Given our interest in establishing a mineralocorticoid-sensitive cellular model, we derived three cell lines from hibernomas of mice 34 and 37; one of them, cell line T37i, originating from the inferior dorsal tumor of transgenic mouse 37, was further characterized. This cell line was subcultured for more than 10 passages over 3 mo, and subcloned by limiting dilution. Under normal culture conditions, T37i cells displayed a fibroblast-like appearance (Fig. 4 *A*). After reaching confluency and additional treatment for 3–5 d with 20 nM insulin and 2 nM triiodothyronine, two known positive regulators of adipogenic differentiation, a striking morphological modification was observed with cells becoming larger and the appearance of numerous intracytoplasmic vacuoles consisting of small lipid droplets, demonstrated by red staining with oil red O (Fig. 4 *B*). The majority of the cells presented with multiple nuclei and underwent terminal adipocyte differentiation in a reproducible manner. It should be noted that the two other cell lines T37s and T34, derived from a superior dorsal tumor of transgenic mouse 37 and a dorsal hibernoma of mouse 34, respectively, did not fully differentiate, even though cultured under the same conditions. Immunofluorescence studies showed that both undifferentiated and differentiated T37i cells expressed TAg, as demonstrated by specific nuclear labeling (Fig. 4, *C* and *D*).

ucp1 expression was also studied in T37i cells to establish whether these cells maintained differentiated features of BAT. Whereas undifferentiated T37i cells did not express the ucp1 gene even upon stimulation, isoproterenol (1 μM) and retinoic acid (1 μM) significantly activated ucp1 gene expression in terminally differentiated cells (Fig. 5 *A*). These results indicate that the tumor-derived T37i clone behaves as a preadipocyte
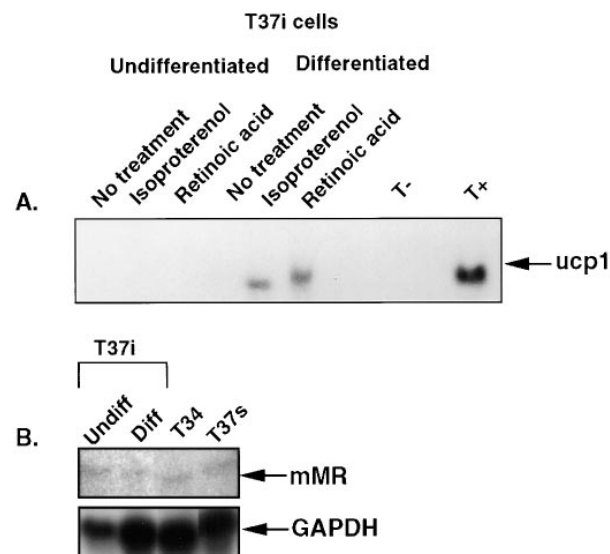
Figure 5. Expression of ucp1 and mMR in T37i cells. (*A*) ucp1 transcripts were detected by Northern blot analysis only in differentiated T37i cells treated with 1 μM isoproterenol or retinoic acid. No ucp1 mRNA is detected in either undifferentiated or untreated differentiated cells. (*B*) Both undifferentiated and differentiated T37i cells express mMR mRNA, as detected by ribonuclease protection assay. mMR transcripts are also detected in the cell line T34 derived from a dorsal hibernoma of founder 34, as well as in cell line T37s, derived from the superior dorsal tumor of founder 37.
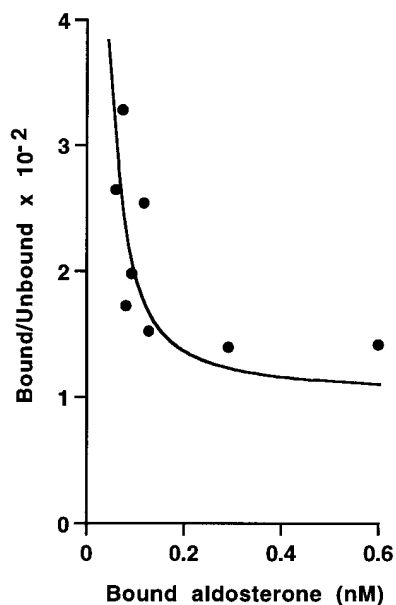
Figure 6. Scatchard plot of aldosterone binding to T37i cells. Cytosolic fractions of undifferentiated T37i cells (passage 11) were incubated with increasing concentrations of [³H]aldosterone (1–50 nM) for 4 h at 4°C. Bound (B) and unbound (U) hormone were separated by the charcoal dextran technique. U was calculated as the difference between the total and the bound hormone concentration. The curve was simulated from the best model of interaction ($B = nU/(K_d + U) + \beta U$), where n is the maximum number of specific binding sites ($n = 56 \pm 7$ pM), K_d is the dissociation constant ($K_d = 0.81 \pm 0.49$ nM), and β is the constant of nonspecific binding ($\beta = 0.01$). Mean values are given with the corresponding intraexperimental SD. Experimental points are means of triplicate determinations.

progenitor cell, which could be fully differentiated into a brown adipocyte phenotype under appropriate conditions.

To investigate whether MR expression was maintained in T37i cells, the presence of mMR at the mRNA and protein level was sought. Both undifferentiated and differentiated T37i cells expressed low but detectable amounts of mMR transcripts, as evidenced by ribonuclease protection assay (Fig. 5 *B*); note that the two other cell lines T37s and T34 also contained significant levels of mMR mRNA. Finally, specific aldosterone binding sites were detected in the cytosolic fractions of undifferentiated T37i cells (34 ± 4 fmol/mg protein, $n = 3$); nonspecific binding was < 45%. These sites were identified as MRs since aldosterone binding was totally unaffected by the addition of a 100-fold excess of RU38486, a glucocorticoid receptor (GR) ligand (data not shown). Scatchard plot representation of aldosterone binding in the cytosol of T37i cells is presented in Fig. 6. Computerized analysis of the experimental data revealed the presence of one class of high-affinity binding sites with a K_d value estimated at 0.81 ± 0.49 nM and a maximum number of sites $n = 56 \pm 7$ pM (corresponding to 16 ± 2 fmol/mg protein). In contrast, MR concentration was much lower in differentiated cells (8 fmol/mg protein), suggesting that in this cell line MR expression might be repressed during the differentiation process.

In an attempt to assess a biological role for mineralocorticoids in BAT, we have studied the influence of aldosterone treatment on T37i cell differentiation. A significant, twofold increase in triglyceride accumulation of T37i cells was observed after a 7-d treatment with 10^{-8} nM aldosterone, as compared with nontreated cells (118.9 ± 19.2 vs. 56.4 ± 5.8 mg triglycerides/g of protein, $n = 3$, $P < 0.01$), corresponding to ~ 50% of triglyceride content observed after insulin–thyroid

hormone treatment (215.2 ± 23.9 mg triglycerides/g of protein). These results were highly suggestive of an involvement of aldosterone in promoting brown adipocyte differentiation.

Discussion

We have shown recently that expression of multiple hMR mRNA isoforms is under the control of alternative promoters, which are submitted to differential hormonal control (10). To identify particular *cis*-acting elements involved in tissue-specific and developmental hMR expression, we have developed transgenic animal models, in which the SV40 TAg was placed under the control of the hMR P1 or P2 promoter regions. Targeted oncogenesis was chosen for the additional possibility of establishing immortalized cell lines, originating from various aldosterone target tissues, which could be used to further define cellular and molecular events underlying MR-mediated effects. During the course of these studies, unexpected findings were obtained with the transgenic mice harboring the P1-TAg hybrid gene. Whereas no tumors were apparent in classical aldosterone target tissues such as kidney or colon, all transgenic founders prematurely died from hibernomas. Subsequently, we demonstrated that MR is normally expressed in BAT. The hMR P1 promoter sequences from −965 to +216 thus clearly contain elements sufficient to direct tissue-specific expression of the transgene in BAT. Early development of hibernomas in P1-TAg mice indicates that the P1 region is transcriptionally active during early life, in agreement with recent studies reporting MR expression in the mouse fetus from E13.5 onwards (14). The fact that no other tumors were found in these animals, despite expression of TAg in several other tissues, including kidney, heart, and brain (Le Menuet, D., M.-C. Zennaro, S. Viengchareun, and M. Lombès, unpublished results), supports previous observations that BAT possesses a high tumor potential (15, 16).

This work, which demonstrates that BAT is a novel mineralocorticoid target tissue, constitutes the first step of a detailed in vivo study of regulatory elements of the hMR gene. TAg expression in BAT of P1-TAg mice is promoter specific, since transgenic animals in which TAg was driven by the distal hMR promoter P2 do not develop hibernomas at 36 wk. To identify functional elements responsible for tissue-specific expression in BAT, the hMR P1 region was aligned with promoter sequences of the BAT specific gene ucp1. A strong enhancer located at −2.4 kb in the 5′-flanking region of the rat ucp1 gene has been shown recently to be essential for brown fat specificity as well as hormonal regulation (17, 18). Interestingly, some P1 promoter sequences showed high degrees of nucleotide identity with specific motifs located in the ucp1 enhancer, in particular the Ets1/NF1 binding sites, the TRE, RXR/TR regions, and the ucp gene-activating region UAR (18). Thus, these sequences might represent important *cis*-acting elements involved in tissue-specific expression of hMR. However, further studies are required to precisely determine the role played by these elements in directing mMR expression in BAT.

We then derived a new cell line, T37i, from a dorsal hibernoma of one transgenic founder. T37i cells exhibited differentiated features of BAT under appropriate conditions, as demonstrated by expression of ucp1 after adrenergic or retinoic acid stimulation. MR expression is maintained in these cells, constituting a unique model to unravel molecular mechanisms of aldosterone action. Indeed, despite many attempts to estab-

lish a mammalian mineralocorticoid-sensitive cell line (19–21), up until now no cellular model has been described which maintains adequate response to aldosterone in terms of ion transport or transactivation of a reporter gene. Besides its potential usefulness to study in detail expression and regulation of specific BAT genes, the T37i cell line appears to represent an attractive experimental system suitable to examine regulated MR expression and to identify new aldosterone target genes. Furthermore, this and other potential cellular models derived from our transgenic animals, in which the P1 or P2 promoters are stably integrated into the genome, will allow functional analysis of hMR regulatory elements in an appropriate chromatin structure.

Recent studies have clearly demonstrated that aldosterone action is not only confined to the control of sodium and potassium homeostasis in tight epithelia but rather appears to be pleiotropic, including specific mineralocorticoid effects in the central nervous and cardiovascular systems (22, 23). Recently, a distinction between epithelial and nonepithelial target tissues in terms of aldosterone action has been proposed. Indeed, mineralocorticoid specificity is closely related to the presence of the enzyme 11β-hydroxysteroid dehydrogenase 2 (11HSD2), which is present in sodium transporting epithelia; in unprotected tissues, MR appears to be predominantly occupied by glucocorticoids, which are not converted to inactive 11-dehydro-congeners, although specific aldosterone actions have also been described (23–25). BAT seems to be a bona fide nonepithelial target tissue, since we failed to detect 11HSD2 mRNA either in hibernomas or in the T37i cell line (data not shown).

The specific role of aldosterone in BAT remains to be elucidated. Our preliminary studies demonstrate that aldosterone is able to induce differentiation of T37i cells into brown adipocytes. This is in accord with recent data showing that aldosterone promotes differentiation of 3T3-L1 cells into adipocytes (26). In this context, an enlargement of brown fat depots in the perirenal and periadrenal regions has been reported recently in patients affected by primary hyperaldosteronism (27). Involvement of aldosterone and MR in the regulation of non-shivering thermogenesis should also be considered. Indeed, effects of glucocorticoids in BAT are well documented (28, 29). In vivo, corticosterone has been shown to decrease thermogenic activity, to increase lipid accumulation, and to be a potent inhibitor of ucp1 gene expression; further investigation should clarify whether these effects are mediated via GR and/or MR. Finally, recent data have demonstrated heterodimerization between MR and GR on different promoters (10, 30, 31). Colocalization of both receptors in several unprotected tissues suggests a role for MR as a widespread dimerization partner for GR in mediating glucocorticoid effects.

In conclusion, targeted oncogenesis appeared to be a powerful strategy for discovering new aldosterone-responsive tissues and for deriving differentiated cell lines constituting valuable models for the evaluation of MR function in vivo. Future studies of P1-TAg and P2-TAg transgenic animals should thus allow precise characterization of alternative promoter usage in directing tissue-specific and developmental MR gene expression.

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