Life Sciences 83 (2008) 192–197

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Differential effects of diabetes induced by streptozotocin and that develops spontaneously on prostate growth in Bio Breeding (BB) rats

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ARTICLE INFO

Article history:
Received 11 March 2008
Accepted 5 June 2008

Keywords:
BB rats
Spontaneous diabetes
STZ-induced diabetes
Prostate

ABSTRACT

We investigated molecular changes in the response to insulin in prostates of spontaneously developed (Bio Breeding) and streptozotocin (STZ)-induced diabetic rats that received sufficient amounts (euglycemic group), or suboptimal doses (hyperglycemic group) of insulin for 32 weeks, using Affymetrix GeneChip analysis of gene expression. Alterations in gene expression levels identified by microarray analysis, having potential biological relevance to prostate growth, were verified by real-time reverse transcription polymerase chain reaction (RT-PCR). A significant decrease in the weight of ventral prostate was observed in the hyperglycemic STZ-induced but not spontaneously developed diabetic group. Microarray analysis revealed that gene expression profiles were distinctly different in each region of the prostate, and that hyperglycemic diabetes in spontaneously developed and STZ-diabetic rats was associated with differential changes in the prostatic expression levels of 856 genes, of which 35 were related to cell growth, proliferation and death. RT-PCR data verified significant differences in the mRNA expression levels of Igfbp6, Tieg, and Clu between euglycemic and hyperglycemic groups, whereas expression levels of these genes in control and euglycemic diabetic groups were not significantly different. In ventral prostate, the mRNA expression levels of Igfbp6 and Tieg were significantly higher in the hyperglycemic STZ-induced diabetic than in the hyperglycemic spontaneously diabetic BBDP/Wor rats. Our data demonstrate that the diabetes induced by STZ in the BBDR/Wor rats affects prostate growth and the molecular response to insulin differently than that observed in BBDP/Wor rats that develop diabetes spontaneously.

Introduction

Testosterone regulates the growth, development and differentiation of the prostate partly via growth factors (Desai and Kondaiah, 2000; Gnanapragasam et al., 2000). We previously showed that streptozotocin (STZ)-induced diabetes in rats causes a significant reduction in prostatic weight and in serum testosterone levels and that the prostatic involution is accompanied by up-regulation of transforming growth factor (TGF)-β, a potent inhibitor of prostatic cell growth, at protein and gene transcript levels (Ikeda et al., 2000). Insulin treatment of diabetic rats normalized the changes in prostatic weight and serum testosterone levels, and reversed the alterations in TGF-β expression at protein and gene transcript levels to control levels (Ikeda et al., 2000). The STZ diabetes-induced changes in the growth factors are similar to those seen in castrated rats (Nishi et al., 1996; Desai and Kondaiah, 2000), suggesting that decreases in testosterone may contribute to the changes observed in diabetic rat prostate.

In our recent study, diabetes prone bio breeding (BBDP/Wor) rats, in which diabetes develops spontaneously, and diabetes resistant bio breeding (BBDR/Wor) rats, in which diabetes is induced by a single injection of STZ, received two different doses of insulin in order to maintain the rats in either a hyperglycemic or euglycemic state (Yono et al., 2005b). BBDP/Wor rats that received insulin treatment had normal prostatic weights, whereas hyperglycemic STZ-induced diabetic rats had a smaller ventral prostate. Insulin treatment resulted in normal and/or increased serum testosterone levels in both groups (Yono et al., 2005b). The differences between spontaneously developed and STZ-induced diabetes in BB rats can reflect biological and functional variations between these two diabetic animal models.

In this communication, we report several molecular changes that occur in the BBDP/Wor and STZ-induced diabetic rat prostate using Affymetrix GeneChip analysis of gene expression. The differential expression of genes with known involvement in prostatic growth processes is then validated in euglycemic and hyperglycemic rats, representing two different states of insulin treated diabetes, by real-time reverse transcription polymerase chain reaction (RT-PCR). These results provide important information regarding gene expression changes in the prostate, specifically those related to the glycemic control with insulin in BBDP/Wor and STZ-induced diabetic rats, and

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doi:10.1016/j.lfs.2008.06.006

Table 1
Sequences of oligonucleotides used as primers

mRNA		Sequence (5′→ 3′)	Length[a]	Accession no.[b]
Igfbp6	Sense	GCCAGAGGGCCGTCGGAAG	149	NM_013104
	Antisense	CAGGGGCCCATTTCACCATC		
Tieg	Sense	GCCAACCATGCTTAACTTCG	108	NM_031135
	Antisense	TCAGCTTTGTCCCAGGGATGTGCT		
Clu	Sense	CTGACCCAGCAGTACAACGA	196	NM_053021
	Antisense	TGACACGAGAGGGGACTTCT		
β-actin	Sense	CTATGAGCTGCCTGACGGTC	115	NM_031144
	Antisense	AGTTTCATGGATGCCACAGG		
GAPDH	Sense	TGACTCTACCCACGGCAAGT	124	NM_017008
	Antisense	AGCATCACCCCATTTGATGT		

[a]Amplicon length in base pairs.
[b]Genbank accession number of cDNA and corresponding gene, available at http://www.ncbi.nlm.nih.gov/.

improve our understanding of the differences between spontaneously developed and STZ-induced diabetes.

Materials and methods

Animals

We maintained male 60 to 70 days old BBDP/Wor, STZ (65 mg/kg)-injected BBDR/Wor and their age matched control (BBDR/Wor) rats for a period of 32 weeks as previously described (Yono et al., 2005b). Each diabetic group received either sufficient amounts of insulin to maintain serum blood glucose levels close to normal levels, approximately 5.5–11 mM (euglycemic groups) or a suboptimal dose of insulin that resulted in serum blood glucose levels of approximately 16.5–22 mM (hyperglycemic groups). Insulin therapy was provided throughout the maintained period via a single daily subcutaneous injection of protamine zinc insulin, a long-acting insulin. The dose of insulin was carefully adjusted by monitoring the serum glucose level. After the completion of the maintained periods, non-fasted rats that had not received insulin for the last 24 h were killed and blood samples were taken for measurement of serum glucose, HbA1c, insulin and testosterone levels. The prostate was dissected and separated into ventral and dorsolateral regions. The number of rats in control, euglycemic BBDP/Wor, hyperglycemic BBDP/Wor, STZ-injected euglycemic BBDR/Wor, and STZ-injected hyperglycemic BBDR/Wor groups were 22, 14, 13, 8 and 7, respectively.

Glucose concentration was measured by the hexokinase method (Kunst et al., 1984). Serum insulin and total testosterone levels were determined by radioimmunoassay kits (Diagnostic Systems Laboratories, Webster, TX).

Microarray experiments

Affymetrix GeneChip expression analysis experiments were performed as previously described (Yono et al., 2005a). Total RNA was extracted using TRIzol reagent and further purified using RNeasy kit (Qiagen, Valencia, CA). The quality of total RNA was evaluated by A_{260}/A_{280} ratio, which was at least 1.9, and by gel electrophoresis pattern, which revealed two major bands of 28S and 18S RNA. The RNA quality was further assessed by Agilent Bioanalyzer (Agilent Technologies, Palo Alto, CA). Labeled cRNA was prepared and the subsequent hybridization to rat 230 2.0 arrays was performed by the Affymetrix Resource facility at Yale University (http://keck.med.yale.edu/affymetrix). The Affymetrix 230 2.0 array represents 31,000 transcripts and provides comprehensive coverage of the entire transcribed rat genome. The hybridized arrays were scanned using a confocal laser Affymetrix scanner.

Real-time RT-PCR

The alterations in gene expression levels of candidate genes identified by microarray analysis with potential biological relevance were verified by real-time RT-PCR using SYBR Green I as previously described (Yono et al., 2005a). Sequences of oligonucleotides used as primers for Insulin-like growth factor binding protein-6 (Igfbp6), TGF-β inducible early gene (Tieg), clusterin (Clu), β-actin and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) are summarized in Table 1. Immediately after the amplification, melt curve protocols were performed to ensure that primer-dimers and other non-specific products had been minimized or eliminated.

Data analyses

Microarray data were scaled to an average intensity of 500 and analyzed independently using GeneChip Operating software (Affymetrix, Santa Clara, CA) as previously described (Yono et al., 2005a). The hybridization intensity data were converted into presence/absence calls for each gene, and changes in gene expression between experiments were detected by comparison analysis. NetAffx website (Affymetrix, Santa Clara, CA) and Gene Spring software (Silicon Genetics, Redwood City, CA) were used for data annotations, and further downstream analysis including cluster analysis (Goldsmith and Dhanasekaran, 2004; Liu et al., 2004). Cut off of fold change >1.5 and FDR (false discovery rate) <0.05 were used to identify differentially expressed transcripts.

RT-PCR data were analyzed with the iCycler iQ Real-Time PCR Detection System (Bio-Rad, Hercules, CA) as previously described (Yono et al., 2005a). In brief, an initial copy number of the target gene was calculated from its standard curve and normalized against an initial copy number of two housekeeping genes, i.e., β-actin and GAPDH, which also were calculated from their standard curves.

Results

The general features of experimental animals are shown in Table 2. The rate of increase in body weight for both BBDP/Wor and STZ-

Table 2
General features of experimental animals

	BBDR/Wor	BBDP/Wor		STZ-injected BBDR/Wor	
	Control (n=22)	Euglycemic (n=14)	Hyperglycemic (n=13)	Euglycemic (n=8)	Hyperglycemic (n=7)
Initial body wt, g	314±7	284±9*	269±10*	231±12*	247±7*
Final body wt, g	478±7	442±8*	378±6*,**	440±16*	365±9*,**
Ventral prostate, mg/body wt, g	1.2±0.1	1.3±0.1	1.5±0.1	0.9±0.1	0.8±0.1*
Dorsolateral prostate, mg/body wt, g	0.8±0.02	0.9±0.03	0.9±0.04	0.7±0.03	0.7±0.04
Serum glucose, mM	7.2±0.2	16.3±1.4*	16.9±0.8*	15.6±0.4*	18.4±1.2*
HbA1c, %	4.3±0.1	4.1±0.2	6.3±0.4*,**	5.6±0.5	9.8±0.9*,**
Serum insulin, pmol/L	20.5±2.2	22.6±4.6	7.7±1.4*,**	24.7±8.3	4.7±0.9*,**
Serum testosterone, ng/mL	1.2±0.2	1.8±0.3	1.7±0.3	2.5±0.4	2.5±0.6

Each value represents the mean±S.E.M. n the number of rats in each group.
*Significantly different from comparable values for control rats.
**Significantly different from comparable values for euglycemic rats within the same diabetic group.