Molecular and Cellular Endocrinology 399 (2015) 122–130

Contents lists available at ScienceDirect

Molecular and Cellular Endocrinology

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

Novel markers of gonadectomy-induced adrenocortical neoplasia in the mouse and ferret

Maximiliaan Schillebeeckx [a,1], Marjut Pihlajoki [b,c,1], Elisabeth Gretzinger [b,d], Wei Yang [a], Franziska Thol [b,d], Theresa Hiller [b,d], Ann-Kathrin Löbs [b,d], Theresa Röhrig [b,d], Anja Schrade [b,c], Rebecca Cochran [b], Patrick Y. Jay [a,b], Markku Heikinheimo [b,c], Robi D. Mitra [a], David B. Wilson [b,e,*]

[a] Department of Genetics, Washington University School of Medicine, St. Louis Children's Hospital, St. Louis, MO 63110, USA
[b] Department of Pediatrics, Washington University School of Medicine, St. Louis Children's Hospital, St. Louis, MO 63110, USA
[c] Children's Hospital, University of Helsinki and Helsinki University Central Hospital, Helsinki 00290, Finland
[d] Hochschule Mannheim, University of Applied Sciences, Mannheim 68163, Germany
[e] Department of Developmental Biology, Washington University School of Medicine, St. Louis Children's Hospital, St. Louis, MO 63110, USA

ARTICLE INFO

Article history:
Received 21 January 2014
Received in revised form 18 July 2014
Accepted 29 September 2014
Available online 5 October 2014

Keywords:
Adrenal cortex
Endocrine tumor
Gonadotropin
Ovariectomy
Steroidogenesis

ABSTRACT

Gonadectomy (GDX) induces sex steroid-producing adrenocortical tumors in certain mouse strains and in the domestic ferret. Transcriptome analysis and DNA methylation mapping were used to identify novel genetic and epigenetic markers of GDX-induced adrenocortical neoplasia in female DBA/2J mice. Markers were validated using a combination of laser capture microdissection, quantitative RT-PCR, *in situ* hybridization, and immunohistochemistry. Microarray expression profiling of whole adrenal mRNA from ovariectomized *vs*. intact mice demonstrated selective upregulation of gonadal-like genes including *Spinlw1* and *Insl3* in GDX-induced adrenocortical tumors of the mouse. A complementary candidate gene approach identified *Foxl2* as another gonadal-like marker expressed in GDX-induced neoplasms of the mouse and ferret. That both "male-specific" (*Spinlw1*) and "female-specific" (*Foxl2*) markers were identified is noteworthy and implies that the neoplasms exhibit mixed characteristics of male and female gonadal somatic cells. Genome-wide methylation analysis showed that two genes with hypomethylated promoters, *Igfbp6* and *Foxs1*, are upregulated in GDX-induced adrenocortical neoplasms. These new genetic and epigenetic markers may prove useful for studies of steroidogenic cell development and for diagnostic testing.

© 2014 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Steroidogenic cells in the adrenal cortex and gonads arise from a common pool of progenitors in the adrenogonadal primordia, but the mechanisms that determine whether a given precursor cell adopts an adrenocortical or gonadal phenotype are not fully understood (Bandiera et al., 2013; Hu et al., 2007; Laufer et al., 2012; Morohashi and Zubair, 2011; Pihlajoki et al., 2013b; Shima et al., 2012; Simon and Hammer, 2012; Val et al., 2006; Wood and Hammer, 2011; Wood et al., 2013). One experimentally tractable model for the study of steroidogenic cell fate determination is gonadectomy (GDX)-induced adrenocortical neoplasia. In response to GDX and the ensuing changes in serum hormone levels [↑ luteinizing hormone (LH), ↓ inhibins, etc.], sex steroid-producing tumors arise in the adrenal glands of certain mouse strains and ferrets (Bernichtein et al., 2007, 2008, 2009; Beuschlein et al., 2012; Bielinska et al., 2005, 2006; Doghman and Lalli, 2009; Johnsen et al., 2006; Miller et al., 2013). This phenomenon is thought to reflect gonadotropin-induced metaplasia of stem/progenitor cells in the adrenal capsule or cortex, although the term "neoplasia" is used more often than "metaplasia" to describe the process (Bielinska et al., 2006). The neoplastic tissue resembles luteinized ovarian stroma and expresses gonadal-like differentiation markers, including LH receptor (*Lhcgr*), anti-Müllerian hormone (*Amh*) and its receptor (*Amhr2*), inhibin-α (*Inha*), transcription factors *Gata4* and *Wt1*, and enzymes required for sex steroid biosynthesis (*Cyp17a1, Cyp19a1*) (Bandiera et al., 2013; Bielinska et al., 2003, 2005, 2006; Johnsen et al., 2006; Krachulec et al., 2012). Prototypical markers of adrenocortical cell differentiation, such as the ACTH receptor (*Mc2r*) or corticoid biosynthetic enzymes (*Cyp21a1, Cyp11b1, Cyp11b2*), are not expressed in the neoplastic tissue (Bielinska et al., 2003, 2005, 2006; Johnsen et al., 2006).

* Corresponding author. Box 8208, Washington University School of Medicine, 660 S. Euclid Ave., St. Louis, MO 63110, USA. Tel.: +1 314 286 2834; fax: +1 314 286 2892.
E-mail address: wilson_d@wustl.edu (D.B. Wilson).
[1] These two authors contributed equally.

http://dx.doi.org/10.1016/j.mce.2014.09.029
0303-7207/© 2014 Elsevier Ireland Ltd. All rights reserved.

The genetic basis of GDX-induced adrenocortical neoplasia has been investigated in the mouse. Hypophysectomy, parabiosis, and transplantation experiments have shown that the adrenal glands of susceptible strains of mice exhibit an inherent predisposition to develop tumors in response to gonadotropin stimulation (reviewed in Bielinska et al., 2005, 2006). Linkage analysis of crosses between susceptible (DBA/2J) and non-susceptible (C57Bl/6) inbred strains has established that post-GDX adrenocortical neoplasia is a complex trait influenced by multiple genetic loci (Bernichtein et al., 2007, 2008, 2009). Targeted mutagenesis of *Gata4*, a gene normally expressed in gonadal but not adrenal steroidogenic cells of the adult mouse, attenuates post-GDX adrenocortical tumor formation in susceptible strains (Krachulec et al., 2012), and transgenic expression of *Gata4* induces adrenocortical neoplasia in a non-susceptible strain (Chrusciel et al., 2013).

In addition to genetic factors, epigenetic changes such as DNA methylation may contribute to the pathogenesis of GDX-induced adrenocortical neoplasia. Altered methylation of cytosine residues in CpG dinucleotides has been shown to modulate gene expression and progenitor cell fate in various tissues, including endocrine organs (Aranda et al., 2009; Hoivik et al., 2011). For example, conditional mutagenesis of the mouse *Dnmt1* gene, which encodes the maintenance DNA methyl-transferase, causes reprogramming of pancreatic β-cells into α-cells (Akerman et al., 2011; Dhawan et al., 2011). It has been suggested that GDX-induced adrenocortical neoplasia may be another example of DNA methylation-regulated cell fate interconversion in an endocrine tissue (Bielinska et al., 2009; Schillebeeckx et al., 2013). According to this hypothesis, epigenetic alterations affect the phenotypic plasticity of adrenocortical stem/progenitor cells, allowing them to respond to the hormonal changes associated with GDX (Bielinska et al., 2009; Feinberg et al., 2006).

The current study was undertaken to identify novel genetic and epigenetic markers of GDX-induced adrenocortical neoplasia, so as to gain a better foothold for investigations into the mechanistic basis of tumorigenesis. Complementary approaches, including genome-wide DNA methylation mapping and microarray expression profiling, were used to screen for genes that are hypomethylated and/or overexpressed in post-GDX adrenocortical neoplasms of the mouse. Candidate genes were validated using a combination of laser capture microdissection (LCM), quantitative RT-PCR (qRT-PCR), and *in situ* hybridization or immunohistochemistry. One of the validated genes was found to be a marker of post-GDX adrenocortical neoplasia in not only mice but also ferrets.

2. Materials and methods

2.1. Experimental animals

Procedures involving mice were approved by an institutional committee for laboratory animal care and were conducted in accordance with NIH guidelines for the care and use of experimental animals. DBA/2J mice were purchased from Jackson Laboratories (Bar Harbor, ME). Female mice were anesthetized and gonadectomized at 3–4 weeks of age (Bielinska et al., 2005). We limited our analysis to females because they develop post-GDX adrenocortical neoplasms more readily than their male counterparts (Beuschlein et al., 2012; Bielinska et al., 2006). Adrenal tissue was harvested for analysis 3 months later.

2.2. Isolation of neoplastic and normal tissue using LCM

Cryosections (10 μm) of adrenal glands from ovariectomized or intact mice were collected on membrane slides (PEN-Membrane 2.0 μm; Leica), fixed in acetone (for collecting DNA) or ethanol (for collecting RNA) at –20° C, stained with hematoxylin and eosin (H&E) or crystal violet, and then dehydrated by passage through successively higher concentrations of ethanol followed by xylene (Pihlajoki et al., 2013a; Schillebeeckx et al., 2013). LCM was used to isolate samples from GDX-induced adrenocortical neoplasms, adjacent normal adrenocortical tissue [zona glomerulosa (zG) + zona fasciculata (zF) cells], and the adrenal X-zone. Dissectates were collected in SDS/proteinase K for genomic DNA isolation or in RNA extraction buffer (RNeasy Mini Kit, Qiagen, Valencia, CA).

2.3. DNA methylation analysis

DNA from neoplastic or normal adrenocortical tissue was subjected to genome-wide methylation analysis using LCM-reduced representation bisulfite sequencing (LCM-RRBS) (Schillebeeckx et al., 2013).

2.4. Microarray expression profiling

RNA was isolated from whole adrenal glands of intact, virgin ($n = 3$) or ovariectomized ($n = 3$) female DBA/2J mice using RNeasy® Mini Kit (Qiagen), amplified using the TotalPrep RNA amplification kit (Illumina, San Diego, CA), and hybridized on an Illumina Mouse6v2 oligonucleotide array. Array hybridization was performed by the GTAC Microarray Core facility at Washington University according to standard protocols.

Expression values were normalized using Partek Genomics Suite (Partek Inc.). More specifically, gene expression intensities were treated by background adjustment, quintile normalization, and log-transformation before statistical analyses. There were 45,281 probes and 6 samples. Features were filtered first by "Detection Pvalue"; if all 6 samples had detection P value > 0.05, the probe was rejected. A total of 19,620 probes were retained after the filtering. The R package genefilter was used to further filter on the basis of interquartile range (IQR), a measure of statistical dispersion. Applying the default value of 50% suggested by genefilter reduced the number of probes to 9810. Permutation-based analysis was performed using the significance analysis of microarrays (SAM) test with the R statistical package siggenes. After adjusting for multiple testing, there were 120 significant probes (corresponding to 95 genes) with permutation FDR < 0.1 (Supplementary Table S1). Unsupervised hierarchical clustering was performed with R function hclust, using a Euclidean distance metric and complete linkage.

2.5. Enrichment analysis

Genes that were differentially expressed in adrenal glands from gonadectomized *vs*. intact mice were compared with pooled Gene Expression Omnibus (GEO) data for different mouse tissues, using a recently developed method (Chen et al., 2013). Genes with a statistically significant expression specificity (P value < 0.01; Bonferroni corrected) for adrenal, brain, ovary, or testis tissues were considered to be tissue-specific (Supplementary Table S2). Enrichment for upregulated or downregulated genes within the tissue-specific gene dataset was determined using the Fisher's Exact Test.

2.6. qRT-PCR

Total RNA was isolated and subjected to qRT-PCR analysis as described (Slott et al., 1993). Expression was normalized to the housekeeping genes *Actb* and *Gapdh*. Primer pairs are listed in Supplementary Table S3.

2.7. In situ *hybridization*

Nonradioactive *in situ* hybridization was performed (Val et al., 2006) using paraformaldehyde-fixed, paraffin-embedded adrenal sections (5 μm). To prepare riboprobes, cDNA fragments of *Igfbp6,*

Download English Version:

https://daneshyari.com/en/article/2195956

Download Persian Version:

https://daneshyari.com/article/2195956

Daneshyari.com