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Wilms Tumor Chromatin Profiles Highlight Stem Cell Properties and a Renal Developmental Network

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SUMMARY

Wilms tumor is the most common pediatric kidney cancer. To identify transcriptional and epigenetic mechanisms that drive this disease, we compared genome-wide chromatin profiles of Wilms tumors, embryonic stem cells (ESCs), and normal kidney. Wilms tumors prominently exhibit large active chromatin domains previously observed in ESCs. In the cancer, these domains frequently correspond to genes that are critical for kidney development and expressed in the renal stem cell compartment. Wilms cells also express "embryonic" chromatin regulators and maintain stem cell-like *p16* silencing. Finally, Wilms and ESCs both exhibit "bivalent" chromatin modifications at silent promoters that may be poised for activation. In Wilms tumor, bivalent promoters correlate to genes expressed in specific kidney compartments and point to a kidney-specific differentiation program arrested at an early-progenitor stage. We suggest that Wilms cells share a transcriptional and epigenetic landscape with a normal renal stem cell, which is inherently susceptible to transformation and may represent a cell of origin for this disease.

INTRODUCTION

The fundamental role of genetic changes in cancer progression is now unquestioned. These aberrations are being cataloged at an unprecedented pace through the application of high-throughput genomic tools (Stratton et al., 2009). In contrast, the extent to which epigenetic events and chromatin environments contribute to cellular transformation remains controversial. The genomic instability present in many cancers complicates the study of DNA methylation and chromatin. Moreover, the most relevant self-renewing cell populations are frequently obscured by tumor heterogeneity. Nonetheless, there is increasing evidence that aberrant DNA methylation and chromatin regulation profoundly contribute to specific types of cancer (Feinberg et al., 2006; Jones and Baylin, 2007). The advent of new epigenomic tools provides an opportunity to investigate their contributions broadly (Barski et al., 2007; Lister et al., 2009; Mikkelsen et al., 2007). Pediatric cancers represent attractive models to study because their relatively normal genomic background facilitates epigenomic characterization and suggests that epigenetic factors may play particularly critical roles in pathogenesis.

Wilms tumor is characterized by a multipotent "triphasic" histology that includes an undifferentiated "blastemal" component and varying amounts of epithelial and stromal elements (Rivera and Haber, 2005). These tumors can also be associated with developmental abnormalities, including persistent embryonic tissue known as nephrogenic rests, and are thus believed to be intimately connected to kidney organogenesis.

Genetic abnormalities described in Wilms tumor involve genes that regulate the metanephric mesenchyme, a kidney-specific stem cell population that resembles blastemal tumor cells and gives rise to most epithelia in adult kidneys. The tumor suppressor *WT1* has been linked to survival and differentiation of these cells (Call et al., 1990; Gessler et al., 1990; Kreidberg et al., 1993; Moore et al., 1999), and the activation of *β-catenin* is a crucial step in epithelialization (Koesters et al., 1999). Similarly, the recently identified tumor suppressor *WTX* is expressed in kidney stem cells and has been linked to *Wnt* signaling and *WT1* transcriptional control (Major et al., 2007; Rivera et al., 2007; Rivera et al., 2009). Yet known mutations account for less than 50% of Wilms tumors, leaving a majority without any known causal genetic alteration.

Two aspects of Wilms tumor suggest that epigenetic alterations also play critical roles in pathogenesis. First, the classical

imprinted gene *IGF2*, normally expressed only from the paternal allele, frequently exhibits biallelic expression in sporadic Wilms tumors (Ogawa et al., 1993). *IGF2* imprinting is also lost in Beckwith-Wiedeman, an overgrowth syndrome associated with an elevated risk of Wilms tumors (Weksberg et al., 1993). Second, similarities in gene expression between Wilms tumor and fetal kidney raise the possibility that pathways active in organ-specific stem cells may be shared by the tumor (Li et al., 2002). This relationship is further supported by the occasional spontaneous regression of nephrogenic rests, which may reflect the reactivation of kidney differentiation pathways (Beckwith et al., 1990).

Whole-genome analysis of chromatin state is now feasible by combining chromatin immunoprecipitation (ChIP) with sequencing (ChIP-Seq) (Barski et al., 2007; Mikkelsen et al., 2007). Of particular interest are specific histone modifications that relate closely to transcriptional programs, cellular state, and epigenetic processes (Kouzarides, 2007). Maps of histone H3 trimethylated at lysine 4 (K4me3), lysine 36 (K36me3), or lysine 27 (K27me3) identify promoters, transcripts, or sites of Polycomb repression, respectively (Barski et al., 2007; Li et al., 2007; Mikkelsen et al., 2007). In embryonic stem cells (ESCs), "bivalent domains" with overlapping K27me3 and K4me3 are associated with developmental genes that are presently silent, but poised for activation upon differentiation (Azuara et al., 2006; Bernstein et al., 2006). Bivalent domains have been proposed to predispose gene promoters to DNA methylation in cancer (Ohm et al., 2007). However, the global role of such marks in cancer has not been explored. Such an analysis could provide insight into the developmental state of tumor cells and how they relate to nonmalignant counterparts.

Here, we present a whole-genome analysis of chromatin in primary Wilms tumors. We focused on Wilms as an initial model because (1) resected tumors provide a ready source of homogeneous, undifferentiated "blastemal" cells that resemble embryonic renal tissue and may represent a model of tumor stem cells (Rivera and Haber, 2005) and (2) Wilms cells exhibit relatively normal genetic backgrounds with few copy number alterations or known mutations, thus facilitating and highlighting the importance of epigenomic analysis (Rivera et al., 2007). We mapped K4me3, K27me3, and K36me3 in Wilms tumors, normal kidneys, and fetal kidneys, and we compared the maps to analogous data for human ESCs. These chromatin data were integrated with published transcript profiles and complemented by mutation and copy number analyses.

The data reveal an interconnected network of genes that appear to drive Wilms tumor phenotype and proliferation. Many of these genes correspond to known regulators of kidney development, but some may be novel master regulators of this process. The maps also point to critical roles for Polycomb repression in both fully silenced and bivalent patterns, an important feature of ESC biology that is recapitulated in Wilms tumor. For example, the *p16* tumor suppressor is repressed by Polycomb in a manner reminiscent of normal stem cells, but distinct from many adult tumors. Similarly, markers of epithelial differentiation are maintained in a bivalent, poised state that may signal a latent differentiation potential akin to a normal renal stem cell. In summary, in-depth analysis of Wilms tumor chromatin points to a transformed phenotype that is sustained through the precise control of developmental and proliferative mechanisms shared with early kidney precursors and ESCs.

RESULTS

Genome-wide Maps of Chromatin State in Wilms Tumor

Genome-wide chromatin maps were generated for three *WTX* mutant Wilms tumors, normal kidney, and fetal kidney via ChIP-Seq. We selected tumors with pronounced blastemal compartments and few large-scale copy number changes in order to enrich for homogeneous tumor cells with defined genetic abnormalities. These tumors did not contain alterations in *WT1* or *β-catenin*. After manually dissecting blastemal compartments, we performed ChIPs for K4me3, K27me3, or K36me3 (see Experimental Procedures). ChIP DNA was sequenced on an Illumina Genome Analyzer and reads were aligned to the human genome (hg18) to produce density maps and identify genomic intervals enriched for a given modification (Mikkelsen et al., 2007). These data were integrated with maps of ESC chromatin (Ku et al., 2008), with sequence-based annotations for promoters, transcripts, and other genomic features, and with transcript profiles for Wilms tumors and other renal tissues (Brunskill et al., 2008; Yusenko et al., 2009). Although we focused our analysis on these *WTX* mutant tumors, we also mapped K4me3 in a *WT1* mutant tumor for comparison. Chromatin data sets are summarized in Tables S1–S5 available online and are publicly available at http://www.broadinstitute.org/cgi-bin/seq_platform/chipseq/shared_portal/clone/Wilms.py and at Gene Expression Omnibus (GEO).

Shared Patterns of Active Chromatin in Wilms Tumor and ESCs

We began our analysis by comparing promoter states across the different samples, focusing on K4me3 as a marker of transcriptional initiation (Li et al., 2007). We annotated promoters based on the presence of K4me3 and performed unsupervised clustering of the various samples. As expected, all three *WTX* mutant Wilms tumors cluster closely (Figure 1A; Figure S1). Remarkably, K4me3 patterns in the tumors resemble those in ESCs to a significantly greater extent than those in normal kidney. This relationship does not extend to other precursor populations, as indicated by the fact that Wilms tumors more closely resembled ESCs than hematopoietic progenitors (Figure S1; Cui et al., 2009).

Wilms tumors also resembled ESCs with respect to the chromatin patterns seen at developmental loci. Both cell types exhibit an overabundance of broad K4me3 intervals or "domains" that contrast markedly with the punctate K4me3 peaks seen at typical promoters (Figures 2A and 2B).

Active Chromatin Domains Mark Kidney Development Genes in Wilms Tumor

In ESCs, the largest K4me3 domains coincide with master regulators of pluripotency, including *OCT4* and *SOX2* (Mikkelsen et al., 2007). Initial examination of our data suggested a similar effect in Wilms tumor. For instance, one of the largest K4me3 domains in Wilms tumor overlaps *SIX2*, which encodes a transcription factor (TF) with essential functions in maintaining kidney stem cells in an undifferentiated state (Figures 2B and 2C; Kobayashi et al., 2008; Self et al., 2006). *SIX2* is expressed