Molecular and Cellular Endocrinology 408 (2015) 145–155

Contents lists available at ScienceDirect

Molecular and Cellular Endocrinology

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

Review

Steroidogenic organ development and homeostasis: A WT1-centric view

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ARTICLE INFO

Article history:
Received 3 November 2014
Received in revised form 6 January 2015
Accepted 6 January 2015
Available online 14 January 2015

Keywords:
Adrenal development
Gonadal development
WT1
β-Catenin
Progenitor cells
Steroidogenic differentiation

ABSTRACT

Adrenal and gonads are the main steroidogenic organs and are central to regulate body homeostasis in the vertebrate organism. Although adrenals and gonads are physically separated in the adult organism, both organs share a common developmental origin, the adrenogonadal primordium. One of the key genes involved in the development of both organs is the Wilms' tumor suppressor *WT1*, which encodes a zinc finger protein that has fascinated the scientific community for more than two decades. This review will provide an overview of the processes leading to the development of these unique organs with a particular focus on the multiple functions WT1 serves during adrenogonadal development. In addition, we will highlight some recent findings and open questions on how maintenance of steroidogenic organs is achieved in the adult organism.

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1. Introduction

Steroid hormones are a class of lipid-based molecules that in the vertebrate system act as signaling molecules during sexual development, body homeostasis and in the response to inflammation. Due to their peculiar chemical structure, a unique set of enzymes is

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http://dx.doi.org/10.1016/j.mce.2015.01.009
0303-7207/

required for their biosynthesis. Although several tissues (pituitary, placenta, brain, gut, heart and skin) contain cells that are capable of producing steroids, vertebrates have evolved dedicated steroidogenic organs: the adrenals and gonads. During embryonic development these two steroidogenic organs share a common primordium: the adrenogonadal primordium (AGP) (Bland et al., 2003). A set of transcription factors orchestrates events leading to the development of the AGP, its separation into two organ-specific primordia and, ultimately, differentiation of progenitors into terminally differentiated cell types (Morohashi, 1997). These include sexually dimorphic development of gonads into testes and ovaries in males and females, respectively (Carre and Greenfield, 2014; DeFalco and Capel, 2009; Quinn and Koopman, 2012). Research over the past 20 years has identified the Wilms' tumor suppressor *WT1* as a central gene involved in several steps during development and homeostasis of steroidogenic organs (Barbaux et al., 1997; Hammes et al., 2001; Kikuchi et al., 1998; Klamt et al., 1998; Kreidberg et al., 1993; Pelletier et al., 1991; Riccardi et al., 1978; Wilhelm and Englert, 2002). In this review we will give an overview of the known functions of WT1 at each stage of adrenogonadal development and homeostasis.

2. WT1 structure–function relationships

WT1 has been cloned in 1990 as a gene mutated in children suffering from Wilms' tumor, a pediatric kidney tumor affecting approximately 1 out of 10,000 newborns (Gessler et al., 1990; Haber et al., 1990). The *WT1* gene encodes a zinc finger protein that can bind to DNA and regulate downstream target genes both as a transcriptional activator or suppressor (Madden et al., 1991). WT1 action seems to be highly cell type dependent, with for example *Wnt4* being repressed in the heart, but activated within the developing kidney (Essafi et al., 2011). This differential action appears to be achieved by recruitment of co-activators such as CBP or p300 (Essafi et al., 2011) or co-repressors such as BASP1 (Carpenter et al., 2004) or WTIP (Srichai et al., 2004). WT1 binding not only affects the transcription of neighboring genes, but induces changes in the epigenetic landscape that can spread over large distances (Essafi et al., 2011). Whether these changes are transmitted to their progeny even when *WT1* becomes switched off remains to be determined.

Understanding WT1 function is further complicated by the fact that as many as 36 different isoforms are produced by a combination of alternative translation start sites, alternative splicing, and RNA editing (Fig. 1; for details see Hohenstein and Hastie, 2006). How these variants affect the function of the resulting WT1 protein has only been partly addressed: The alternatively spliced exon 5 seems to mediate interaction with the prostate apoptosis response factor PRKC (PAR4) (Richard et al., 2001), but deletion of this exon in mice did not affect development (Natoli et al., 2002). Similarly, removal of an alternative translation start site that leads to an additional 68 amino acids at the N-terminus had no overt effect on development, and mutant mice survived normally (Miles et al., 2003).

A much more important protein modification appears to be the insertion or omission of the three amino acids KTS between zinc fingers 3 and 4. WT1(+KTS) and WT1(–KTS) variants are produced by an alternative splice-donor site at the end of exon 9, a feature that has been conserved throughout evolution (Kent et al., 1995). Insertion of the KTS sequence changes the spacing between zinc fingers 3 and 4 and, not surprisingly, affects the biochemical properties of the protein. WT1(–KTS) isoforms have strong affinity to DNA and show a diffuse staining pattern in nuclei (Larsson et al., 1995). By contrast, WT1(+KTS) isoforms have lower affinity to DNA (Laity et al., 2000) and, since WT1 can interact with RNA (Caricasole et al., 1996; Kennedy et al., 1996; Ladomery et al., 2003) and splicing factors (Davies et al., 1998), it has been suggested that these isoforms may regulate splicing. A confirmation or refutation of this hypothesis is however still missing. Alternatively, WT1(+KTS) may be involved in splicing-independent posttranscriptional regulation and evidence that this isoform enhances interaction of certain RNAs with polyribosomes has been reported (Bor et al., 2006). Finally, WT1(+KTS) may interact with other DNA-binding proteins to regulate downstream targets, as seems to be the case for the activation of *Sry* gene expression (see discussion later).

Whatever its precise molecular role, it is clear that WT1(+KTS) is important for normal development. Indeed, heterozygous mutations abrogating production of this isoform from the affected allele lead to Frasier syndrome, a disease characterized by XY sex reversal and early onset glomerular disease (Barbaux et al., 1997; Klamt et al., 1998). Since the wildtype allele continues to produce both variants, it must be the change of ratio between +KTS and –KTS (Fig. 1B and C), a general reduction of +KTS, or an increase of –KTS variants that is causing the severe phenotype in this disorder. However, while WT1 isoforms seem to have specific roles, they must also share certain functions, as gene targeting experiments reveal a much less severe phenotype of splice specific knockouts when compared to the full deletion of the *Wt1* gene (Hammes et al., 2001).

3. The adrenogonadal primordium: WT1 as a survival factor

Adrenals and gonads are mesoderm derived and develop through proliferation of the coelomic epithelium, an initially single-layered epithelium overlying the nephrogenic cord that becomes stratified (Hatano et al., 1996). In mice, the AGP can first be detected at around E10. Its formation depends on GATA4 (Fig. 2A and B) (Hu et al., 2013), a transcription factor that is required for the activation of the steroidogenic factor *Sf1* (*Nr5a1*) and Lim homeobox protein 9 (*Lhx9*), but not *Wt1*. Expression of *Sf1* at this early time point also depends on the presence of WT1 (Wilhelm and Englert, 2002) and transcriptional activation appears to be enhanced via physical interaction with the CBP/p300-interacting transactivator CITED2 (Val et al., 2007). *Sf1* regulation is however more complex (Zubair et al., 2006) and also requires the transcription factors SIX1 and SIX4 (Fujimoto et al., 2013). Whether these transcription factors form a multiprotein complex or whether they act on independent enhancer elements is presently unknown. The upregulation/maintenance of *Sf1* expression is crucial and deletion leads to widespread apoptosis and, as a consequence, mutant animals lack both adrenals and gonads (Luo et al., 1994). Consistent with the lack of *Sf1* expression, *Wt1* and *Cited2* knockout mice also lack adrenals (Bamforth et al., 2001; Moore et al., 1999) and, in the case of *Wt1* mutants, also gonads (Kreidberg et al., 1993). How these factors suppress apoptosis in steroidogenic organs is not clear, but it maybe interesting to draw parallels to kidney development. In early kidney anlagen WT1 suppresses cell death by directly activating fibroblast growth factors (FGFs), such as *Fgf20*, and suppressing BMP-pSMAD signaling (Motamedi et al., 2014). Whether a similar mechanism is in place to suppress apoptosis in adrenogonadal development remains to be elucidated.

4. Wt1 in gonadal development: Lessons from mice and humans

While adrenals and gonads share a common origin on both the genetic and developmental level, by E10.5 the adrenogonadal primordium has split. The adrenal cells remain rostral moving to a slightly more medial position, whereas the coelomic epithelium continues to proliferate along the rostral–caudal axis contributing to further growth of the gonadal primordium (Fig. 2A and B). Primordial germ cells migrate along the hindgut with the first cells reaching the adreno-gonadal primordium by E10 (Molyneaux et al., 2001).