Adrenocortical development and cancer: focus on SF-1

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Abstract
Steroidogenic factor-1 (SF-1/Ad4-binding protein; NR5A1) is an essential regulator of tissue-specific gene expression in steroidogenic cells and of adrenogonadal development. Here, I discuss recent data in the literature showing the implication of SF-1 and the importance of its dosage not only during development but also for adrenal cortex tumorigenesis in humans and mice.

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Introduction
The story of steroidogenic factor-1 (SF-1, also termed Ad4-binding protein, Ad4BP; NR5A1 in nuclear receptors’ official nomenclature) began in 1992 with the cloning by the Parker and Morohashi groups of a transcription factor that binds to and activates transcription from multiple P450 steroidogenic enzyme promoters (Lala et al. 1992, Morohashi et al. 1992). This factor turned out to be the homolog of Drosophila melanogaster FTZ-F1, a member of the nuclear receptor family which regulates the expression of the pair rule homeodomain transcription factor fushi tarazu. SF-1 binds as a monomer to nuclear receptor half sites on DNA (Wilson et al. 1993). Its transcriptional activity can be regulated by putative phospholipid ligands that bind inside its hydrophobic pocket (Krylova et al. 2005, Li et al. 2005, Wang et al. 2005), and by post-translational modifications, namely phosphorylation by different kinases at Ser203 (Hammer et al. 1999, Lewis et al. 2008) and sumoylation, which may affect subnuclear localization of SF-1 and its DNA-binding activity (Chen et al. 2004, Komatsu et al. 2004, Lee et al. 2005, Campbell et al. 2008).

An essential actor in adrenogonadal development
The demonstration of the pivotal role of SF-1 in adrenogonadal development came from Sf-1-null mice, which have no adrenal glands nor gonads at birth (Luo et al. 1994, Sadowsky et al. 1995). Interestingly, adrenal and gonadal development initiates normally in Sf-1-null mice, but then their primordia regress by apoptosis starting from embryonic day 12 (E12.0; Luo et al. 1994).

During development, gonads and adrenals are derived from a common precursor structure, the adrenogonadal primordium, located between the coelomic epithelium of the urogenital ridge and the dorsal aorta. This primordium is evidenced as early as E9 in mice. Afterwards, the adrenal and gonadal anlage progressively become distinct, which is well recognizable by E13. Primordial germ cells colonize the (at the time still sexually undifferentiated) gonadal anlage by E10. The bipotent gonad differentiates into a testis or into an ovary after E11.5–E12, when the Sry testis-determining gene starts to be expressed. The adrenal primordium is progressively colonized by cells originating from the neural crest that will later form the adrenal medulla. In mice, the adrenal cortex and medulla become distinct by E16.

SF-1 expression pattern during development is restricted to tissues involved in steroidogenesis (adrenal cortex, testis, and ovary) and reproductive function (pituitary gonadotropes and hypothalamic ventromedial nucleus; Ikeda et al. 1994), plus the spleen (Morohashi et al. 1999). This pattern is strikingly similar to the pattern of expression of Dax-1 (Nr0b1), another nuclear receptor that works as a negative regulator of SF-1 (Ikeda et al. 1996). SF-1 starts to be detected at E9 in the adrenogonadal primordium (being a useful marker for it; see Hatano et al. 1996), and continues
thereafter to be expressed in both the embryonal adrenal and gonad after their individualization. When the adrenal cortex and medulla separate, SF-1 expression localizes to the cortical region and remains expressed in this portion of the gland until adulthood. One study reported a transient down-regulation of SF-1 expression in the mouse adrenal after E18.5 and until postnatal day 6 (Martinez et al. 2003).

Until recently, only factors regulating SF-1 expression in extra-adrenal sites were known. An E-box-binding site within the basal promoter of the gene is critical for SF-1 expression (Nomura et al. 1995), and the transcription factor POD1/capsulin was shown to suppress SF-1 expression in the gonad through binding to the E-box (Tamura et al. 2001, Cui et al. 2004), while WT1 has a positive role in SF-1 expression in the developing gonad (Wilhelm & Englert 2002), similarly to the LIM homeobox gene (Birk et al. 2000). In addition, a conserved distal enhancer in intron 6 of the Sf-1 gene is important for its VMH-specific expression (Shima et al. 2005). More recently, elegant studies from K Morohashi’s group have elucidated the mechanisms leading to SF-1 expression in the developing mouse adrenal. By the use of transgenic mice, the existence of a fetal adrenal enhancer (FAdE) has been defined in intron 4 of the Sf-1 gene locus, which drives SF-1 expression in the fetal, but not in the adult, adrenal. Importantly, binding sites for the homeodomain factors Pbx/Prep and Pbx/Hox in the FAdE are required to initiate the establishment of SF-1 expression in the mouse fetal adrenal, while thereafter SF-1 maintains its own expression through an autoregulatory loop binding to sites inside the FAdE (Zubair et al. 2006). Further lineage-tracing studies demonstrated that the adult adrenal cortex in mice derives from precursor cells in the fetal cortex in which the FAdE was activated during early development. However, the ability of precursor cells that activate the FAdE to contribute to the adult adrenal cortex largely disappears by E14.5 (Zubair et al. 2008). After birth, fetal zone cells where the FAdE is active are restricted to the X-zone, a transient zone of the mouse adrenal cortex which is located in an innermost position and regresses at puberty in the males and after the first pregnancy in the females (Zubair et al. 2006). Interestingly, during adrenal development, the expression of the SF-1 repressor DAX-1 localizes in the outer part of the adrenal primordium (the zone that will later give rise to the adult adrenal cortex), while FadE-dependent expression of SF-1 progressively restricts to the inner part of the adrenal cortex (Zubair et al. 2008). This suggests that DAX-1 represses expression of SF-1 driven by its FAdE during the transition from the fetal to the adult adrenal, and that a subtle balance between SF-1 and DAX-1 is required for execution of the full genetic program needed for adrenal development.

DAX-1 mutations in humans in fact cause adrenal hypoplasia congenita, an adrenocortical hormone deficiency syndrome caused by a defect of adrenal development (Zanaria et al. 1994).

Dosage at the core of SF-1 biological activity

A critical factor to be considered when examining the role of SF-1 during development is its dosage. The first clue about the importance of SF-1 dosage for its biological activity came from the description of a patient with adrenal failure and complete 46,XY sex reversal bearing a heterozygous loss-of-function SF-1 mutation (G35E; Achermann et al. 1999). Afterwards, several other patients have been described where SF-1 haploinsufficiency causes variable degrees of gonadal and adrenal dysgenesis (Jameson 2004). To date, only one homozygous SF-1 mutation has been described (R92Q) in a patient with adrenal hypoplasia and 46,XY sex reversal. This mutation reduces SF-1 transcriptional activity only partially, consistent with its phenotypic expression only when transmitted as a homozygous trait (Achermann et al. 2002). Notably, in humans, gonadal development appears to be more sensitive to SF-1 haploinsufficiency than adrenal development (Lin & Achermann 2008). Conversely, SF-1 heterozygote mice have smaller adrenal glands and higher evening ACTH levels than wild-type mice, displaying a condition of latent adrenal insufficiency that becomes overt under stressful stimulations (Bland et al. 2000; Fig. 1). While gonads are also smaller in SF-1 heterozygote mice (Bland et al. 2000), these animals are fertile (Luo et al. 1994). Furthermore, the presence of two SF-1 copies is critical for producing adrenal hypertrophy and hyperplasia in a model of postnatal adrenal growth following unilateral adrenalectomy (Beuschlein et al. 2002). Modulation of SF-1 dosage is also relevant for the mechanism of adrenal development impairment induced by the lack of CITED2, a transcriptional cofactor supposedly interacting with WT1 (Val et al. 2007). On the other hand, transgenic overexpression of SF-1 in mice under the control of its own FAdE led to increased adrenal size and to the formation of ectopic adrenal tissue in the thorax (Zubair et al. 2009). All together, these data evidence the importance of SF-1 dosage for both adrenal and gonadal development.

SF-1 dosage and adrenocortical tumorigenesis in humans

Adrenocortical tumors (ACTs) in children are in many cases diagnosed in the context of multiorgan cancer syndromes, but they can also occur sporadically (Koch et al. 2002). Their incidence is highest during
the first 3 years of life. Epidemiologically, it is remarkable that these tumors are much more frequent in Southern Brazil than in the rest of the world. Overall, their response to therapy is still poor, with 5-year survival rates that range at 50% (Michalkiewicz et al. 2004). In Southern Brazil, childhood ACTs are found to be associated with a specific low-penetrance germline substitution of arginine with histidine at codon 337 (Ribeiro et al. 2001, Figueiredo et al. 2006). Remarkably, only certain inbred (e.g. C3H and DBA/2J) or transgenic (inhibin a-null mice/inhibin a promoter–SV40 Tantigen transgenic mice) mouse strains are susceptible to gonadectomy-induced ACT formation (Matzuk et al. 1994, Kananen et al. 1996, Rilianawati et al. 1998, Bielinska et al. 2003, 2005, Johnsen et al. 2006). One major genomic locus implicated in gonadectomy-induced adrenocortical tumorigenesis has been mapped on chromosome 8, which is modulated by epistasis by another quantitative trait locus on chromosome 18 (Bernichtein et al. 1999). This chromosomal region harbors the human SF-1 gene, which is indeed amplified and overexpressed in childhood ACTs (Figueiredo et al. 2005, Pianovski et al. 2006). Considering the pivotal function of SF-1 in adrenal gland development, we hypothesized that its increased dosage might play an important role in ACT tumorigenesis. Using the H295R human adrenocortical cell model, we showed that SF-1 overexpression significantly increases proliferation through combined effects on cell cycle progression and apoptosis (Doghman et al. 2007b). Importantly, this effect is dependent on the transcriptional activity of the factor, since overexpression of an activation function-2 (AF-2) mutant does not trigger an increase in cell proliferation.

Increased SF-1 levels selectively modulate steroidogenic enzyme expression and the pattern of steroids secreted by H295R cells, with reduction of cortisol and aldosterone and maintenance of dehydroepiandrosterone-sulfate (DHEA-S) production. SF-1 overexpression in human ACT cells has a significant impact on the expression of genes involved in steroid metabolism, cell cycle, apoptosis, and cell adhesion (Doghman et al. 2007a). An increased SF-1 dosage in ACT cells can reproduce several molecular features of childhood ACTs, where some enzymes implicated in steroid metabolism (HSD3B2 and CYP21A2) are also down-regulated (West et al. 2007). NOV/CCN3 is one of the most significantly repressed transcripts in childhood ACTs compared with normal adrenal cortex. This is a secreted multimodular protein whose expression was described to be mostly restricted to the definitive zone of the fetal adrenal cortex (Ratcliffe et al. 2003). NOV/CCN3 is down-regulated in childhood ACTs, independently from their degree of malignancy, and in human adrenocortical cells in a manner dependent on SF-1 dosage. Moreover, NOV/CCN3 is a selective proapoptotic factor for human adrenocortical cells (Doghman et al. 2007a). These properties suggest that this factor may have an important role during adrenal development and oncogenesis.

**SF-1 dosage and ACTs in mice**

ACTs can occur after gonadectomy in certain rodent species, including mice (reviewed by Bielinska et al. 2006). Remarkably, only certain inbred (e.g. C3H and DBA/2J) or transgenic (inhibin a-null mice/inhibin a promoter–SV40 Tantigen transgenic mice) mouse strains are susceptible to gonadectomy-induced ACT formation (Matzuk et al. 1994, Kananen et al. 1996, Rilianawati et al. 1998, Bielinska et al. 2003, 2005, Johnsen et al. 2006).
Figure 2 A model for the implication of an increased SF-1 dosage in adrenocortical tumorigenesis in humans and mice. In childhood adrenocortical tumors (top), increased SF-1 dosage in the presence of a germline TPS3 mutation with loss of heterozygosity in the tumor would trigger proliferation of adrenocortical cells around the period of physiological fetal adrenal regression. Other genetic lesions (e.g. inhibitin-a and β-catenin mutations, and IGF2 overexpression) may participate in the tumorigenic process. In mice (bottom), adrenocortical tumors may arise after gonadectomy in certain susceptible strains, which develop gonadal-type tumors. These tumors arise under the control of pituitary LH from adrenogonadal precursor cells residing in the subcapsular region of the adrenal cortex. Notably, susceptible strains harbor the SF-1S172 allele, which may predispose to increased expression, or lack the tumor suppressor inhibitin-a. The same effect is produced by increased SF-1 levels in the C57/B6 background in the absence of elevated gonadotropin levels. CTNNB1, β-catenin; INHA, inhibitin-a. Adapted with the permission of Doghman & Lalli (2009); Elsevier Masson, Editor.

Neoplastic cells in these tumors express gonadal markers, and their growth is dependent upon the high levels of circulating gonadotropins present after gonadectomy. Recent studies have demonstrated the origin of these tumors from pluripotent adrenogonadal precursor cells situated in a subcapsular position in the adrenal cortex, which retain the potential to differentiate into cells harboring features of gonadal stroma (Looyenga & Hammer 2006).

The spatiotemporal expression of the endogenous SF-1 gene can be recapitulated in YAC transgenic mice carrying the rat SF-1 gene locus (Karpova et al. 2005). Several transgenic lines were generated, each one of which carried a different transgene copy number. In each case, SF-1 overexpression triggered adrenocortical tumorigenesis (Doghman et al. 2007b, Fig. 1). Remarkably, tumors arising in SF-1 transgenic mice historically resemble granulosa cell tumors and express gonadal markers such as Amh and Gata-4, while they do not express the steroidogenic enzyme P450sc (Doghman et al. 2007b). As such, this closely matches the phenotype of ACTs occurring in some strains of mice after gonadectomy. These data show that in both humans and mice, SF-1 acts as an important regulator of ACT cell proliferation, even if profound differences exist in the phenotype of ACTs in the two species (Fig. 2). For this reason and also because of its restricted pattern of expression, SF-1 represents an appealing therapeutic target in childhood ACTs. Compounds able to block SF-1 transcriptional activity have been identified (Del Tredici et al. 2008, Madoux et al. 2008), and we have recently shown that SF-1 inverse agonists of the isoquinolinone class are able to reverse the effect of increased SF-1 dosage on proliferation of the H295R ACT cell line (Doghman et al. 2009). These data suggest the potential clinical utility of molecules targeting SF-1 in the therapy of advanced childhood ACTs.

In adult ACTs, some CGH studies also showed frequent amplification of the region harboring the SF-1 gene (Dohna et al. 2000). Different authors have described variable expression of SF-1 mRNA or protein. While some studies reported similar SF-1 levels in normal adrenal compared to cortisol-producing adenomas (Sasano et al. 1995, Shibata et al. 2001) and in adrenocortical adenomas and carcinomas (Kiiveri et al. 2005), others described increased SF-1 mRNA expression in aldosterone- and cortisol-producing adenomas (Bassett et al. 2005), and in adenomas relative to carcinomas (Lefrançois-Martinez et al. 2004). Possible explanations for these discrepancies are differences between the methods used to measure SF-1 expression at the mRNA or protein level. Furthermore, one has to consider that SF-1 activity in adrenocortical cells also depends on the relative abundance of its repressors (e.g. DAX-1 and COUP-TF), and on its post-translational modifications and availability of putative activating ligands. For these reasons, further studies are required to assess whether an increased SF-1 activity may be involved in the pathogenesis of ACTs in adults.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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