REVIEW

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, Sadovsky *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

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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 *Lhx9* (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

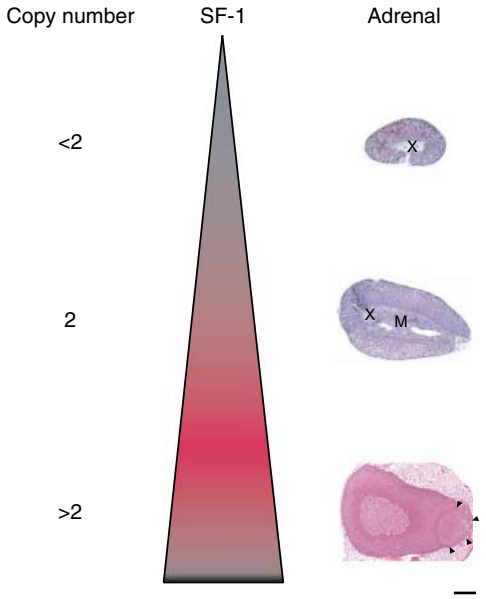

Figure 1 Effect of SF-1 dosage on mouse adrenal gland development and tumorigenesis. SF-1 haploinsufficiency causes adrenal hypoplasia and latent insufficiency, while normal adrenal development ensues in the presence of two normal *Sf-1* alleles. Increased SF-1 dosage by transgenic overexpression causes tumor formation (arrowheads) in the adrenal cortex. X, X-zone; M, medulla. Scale bar, 200 μm. Images are reproduced with permission from Bland *et al.* (2000) (Copyright 2000, National Academy of Sciences, USA) and Doghman *et al.* (2007*a*) (Copyright 2007, The Endocrine Society).

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 mutation of tumor protein p53 (*TP53*), leading to the substitution of arginine with histidine at codon 337 (Ribeiro *et al.* 2001, Figueiredo *et al.* 2006). The diagnosis of childhood ACTs is most frequently made because of symptoms associated with hormone excess. Virilization is the most common sign, and in several cases, it is associated with Cushing's syndrome. These tumors are believed to be derived from the fetal adrenal because of their early age of onset, their pattern of hormone secretion, and their very high insulin-like growth factor 2 expression (Wilkin *et al.* 2000). Using comparative genomic hybridization (CGH), it has been shown that childhood ACTs are characterized by a high frequency of chromosomal aberrations. It is remarkable that the gain/amplification of 9q33–q34 emerged as the most consistent finding in a great majority of cases of childhood ACTs (Figueiredo *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.* 2007*b*). 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.* 2007*a*). 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.* 2007*a*). 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 α-null mice/inhibin α promoter–SV40 T-antigen 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.* 2008).

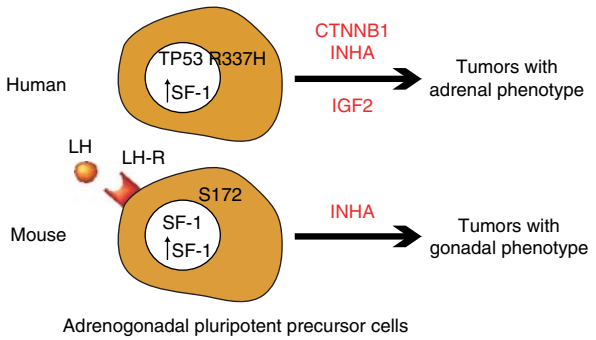

Figure 2 A model for the implication of an increased SF-1 dosage in adrenocortical tumorigenesis in humans and mice. In childhood adrenocortical tumors (top), increased SF-1 dosage in the presence of a germline TP53 mutation with loss of heterozygosity in the tumor would trigger proliferation of adrenocortical cells around the period of physiological fetal adrenal regression. Other genetic lesions (e.g. inhibin-α 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-1*^{S172} allele, which may predispose to increased expression, or lack the tumor suppressor inhibin-α. 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, inhibin-α. 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.* 2007*b*; Fig. 1). Remarkably, tumors arising in *Sf-1* transgenic mice histologically resemble granulosa cell tumors and express gonadal markers such as *Amh* and *Gata-4*, while they do not express the steroidogenic enzyme *P450scc* (Doghman *et al.* 2007*b*). 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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