A matter of dosage: SF-1 in adrenocortical development and cancer

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Steroidogenic factor-1 (SF-1), a nuclear receptor transcription factor, has a pivotal role in adrenal and gonadal development in humans and mice. It was previously shown that SF-1 dosage critically regulates adrenal size and function in mice, while in humans mutation of a single SF-1 allele leads to adrenal and/or gonadal dysgenesis. Recent studies have elucidated the role of an increased SF-1 dosage as an important pathogenetic factor in childhood adrenocortical cancer and in experimental adrenocortical cancer in mice and identified genes that are regulated by SF-1 in a dosage-dependent fashion. Drugs targeting SF-1 transcriptional activity may then represent potential therapeutic tools to be associated to current chemotherapeutic regimens for the treatment of adrenocortical cancer in children.

The adrenal cortex is an essential component of the hypothalamic-pituitary-adrenal axis, synthesizing essential steroid hormones (mineralocorticoids, glucocorticoids and androgens) and regulating diverse biological processes, such as stress response, blood pressure, glucose metabolism and sexual development.

The structure of the fetal adrenal cortex is unique in human and primates compared to other species. In fact, during intrauterine life, most of the inner part of the adrenal cortex (fetal zone) is occupied by large, steroid-secreting cells, which synthesize large amounts of dehydroepiandrosterone and its sulfate derivative (reviewed in [1]). Conversely, a few layers of cells lying in an outer, subcapsular position (definitive zone) are nonsteroidal and constitute a proliferative compartment in which most probably the precursors of fetal zone cells are generated. An additional zone (transitional zone) develops between the definitive and the fetal zone late in pregnancy and synthesizes cortisol. Steroid hormones produced by the fetal zone regulate intrauterine homeostasis, the maturation of the fetal organs necessary for survival after birth and, in some species, the timing of parturition. After birth, the fetal adrenal undergoes a process of rapid involution and remodeling. The fetal zone regresses by apoptosis whereas cells of the definitive zone proliferate and differentiate to form the glomerulosa, fasciculata and reticularis zones of the adult adrenal cortex.

Adrenal glands and gonads are derived from the same embryological primordium. Several transcriptional regulators play an important role for the genesis of both organs. An essential function is played by the nuclear receptor SF-1 (SF-1/Ad4BP/NR5A1) (reviewed in [2]). In addition to SF-1, other factors such as Wilm’s tumor-1 (WT-1), Pbx-1, DAX-1 and Cited-2 (reviewed in [3]) have been shown to be essential for differentiation of the urogenital ridge and the adrenal primordium. SF-1 gene dosage has been associated with different adrenal and gonadal phenotypes in mice and humans and we have recently shown that it also plays an important role in adrenocortical tumorigenesis [4].

1. SF-1, an essential transcription factor in adrenal development

SF-1 is a key regulator of endocrine function and modulates the transcription of many genes involved in gonadal and adrenal development, sexual differentiation, steroidogenesis and reproduction (reviewed in [2]). It has a restricted and specific expression pattern in the gonads, adrenal cortex, anterior pituitary, hypothalamus and spleen. SF-1 belongs to the nuclear receptor superfamily and functions as a constitutive transcriptional activator. The C-terminal portion of SF-1 harbors a ligand-binding domain (LBD), which contains a conserved transcriptional activation domain (AF-2). The crystal structure of the SF-1 LBD revealed a large binding pocket filled with phospholipids, which could play an important role in the factor’s transcriptional properties [5–7]. In addition, posttranslational...
modifications may regulate SF-1 activity. SF-1 is the target of phosphorylation by MAPK and cyclin dependent kinase (Cdk7) at Ser203 [8,9]. Other posttranslational modifications, including acetylation, ubiquitination and sumoylation, regulate SF-1 transcriptional activity, degradation and DNA binding [10–15]. Many studies led to the identification of several SF-1-target genes, its regulation and expression pattern. However, the mechanisms that direct SF-1 expression in the adrenal gland are poorly understood. Recently, an enhancer that selectively directs the expression of Ad4BP/SF-1 to the mouse fetal adrenal (fetal adrenal specific enhancer [FADE]), has been identified, whose expression is autoregulated by Ad4BP/SF-1 [16].

SF-1 was initially identified as a key regulator of adrenal and gonadal development, as SF-1 null mice present adrenal and gonadal agenesis, XY sex reversal and impaired gonadotropin secretion, resulting in postnatal death due to severe adrenal insufficiency [17,18]. SF-1 inactivation also causes abnormalities of the ventromedial hypothalamic nucleus, revealing its important role in feeding and anxiety behavior [19,20]. Heterozygous animals have a milder phenotype with an impaired corticosterone production in response to stress, associated with an impaired compensatory adrenal growth after unilateral adrenalectomy [21,22]. In humans, SF-1 heterozygote mutations have been described in patients with different degrees of adrenal insufficiency and gonadal dysgenesis (reviewed in [23]). The effects of SF-1 haploinsufficiency suggested that its gene dosage may be critical for optimal adrenal development and also play an important role in adrenocortical tumorigenesis.

2. Role of SF-1 in childhood adrenocortical tumorigenesis

Tumor formation in the adrenal cortex results from a multistep process that combines genetic alterations, signaling pathways deregulation and possibly environmental factors. Genetic alterations present in adrenocortical tumors (ACT) include TP53 mutations, loss of heterozygosity at 11p15 causing IGF2 overexpression, mutations of CTNNB1 (β- catenin) and other genes [24,25].

2.1. Adrenocortical tumors in children

ACT in children are found isolated or associated with other types of cancers in the context of genetically determined syndromes [24]. The incidence of ACT in children follows a bimodal distribution, being highest during the first 3 years of life and presenting a second peak during adolescence. These tumors are 10–15 times more frequent in southern Brazil than in the rest of the world. Therapeutic results are still unsatisfactory, with a survival rate at 5 years of only about 50%. Tumor size, weight and histology are the most useful factors to discriminate between adenomas and carcinomas. Favorable prognostic factors are stage I at diagnosis, tumor weight of 200 g or less, age younger than 4 years, and presence of virilization alone (reviewed in [26]).

In southern Brazil, childhood ACT is found associated with a specific germline tumor protein p53 (TP53) mutation (R337H) [27], but its penetrance is low (less than 10% of mutations carriers develop ACT; [28]). Functional analysis has shown that the mutant R337H TP53 retains its transactivation capacity. However, this mutation is predicted to impair p53 function by inducing a pH-dependent destabilization of protein tetramers [29]. The degree and the type of endocrine disturbance appear to be related to patient’s age. In fact, childhood ACT are most commonly functional, causing virilization due to production of androgenic steroids by the tumor, which can be associated with signs of excess glucocorticoid production. Childhood ACT are thought to be derived from the fetal adrenal because of their early age of onset, their pattern of hormone secretion and their molecular phenotype. It has been shown that an apoptotic process is responsible for fetal zone regression after birth [30] and childhood ACT are thought to be caused by defective apoptosis.

The molecular basis of childhood adrenocortical diseases remains poorly understood, despite several studies investigating the role of specific genes in adrenal tumorigenesis. Using comparative genomic hybridization (CGH), it has been shown that ACT are characterized by a high frequency of chromosomal amplification or loss. Chromosomal segments showing gain/amplification by CGH are generally sites of amplified genes, and gene amplification is one of the ways how oncogenes are activated. Clearly the identification of such genes and the characterization of their mechanism of action are critical to better understand the process of tumorigenesis for a given tumor. Interestingly the gain/amplification of the most telomeric segment of the long arm of chromosome 9 chromosomal region emerged as the most consistent finding in a great majority of cases of childhood ACT [31]. We have shown that the SF-1 gene, mapping to 9q33.3 region, is amplified and overexpressed in childhood ACT and hypothesized so that it may play an important role in the genesis and progression of those tumors [32,33].

2.2. SF-1 dosage effects on adrenocortical tumorigenesis

By using human ACT cell cultures, we have recently defined a critical role of SF-1 dosage in regulating the proliferation of human adrenocortical cells [4]. These data show that SF-1 dosage is a critical factor for the control of adrenocortical cell growth during development but also for the process of adrenocortical tumorigenesis. SF-1 overexpression effect on adrenocortical cell proliferation can be explained by an activation of cell proliferation and a decrease in apoptosis and requires the factor to be transcriptionally active through its AF-2 domain [4].

Interestingly, increased levels of SF-1 result in the selective modulation of steroidogenic enzyme expression and pattern of steroids secretion, with reduction of cortisol and aldosterone and maintenance of DHEA-S production. We suggested that overexpression of SF-1 in H295R cells reinforces their differentiation towards the fetal adrenal phenotype, characterized by the production of high levels of DHEA-S, while it inhibits differentiation towards an adult adrenal cell that produces glucocorticoids and mineralocorticoids [4]. Moreover, SF-1 overexpression in adrenocortical cells is able to phenocopy several features of
Fig. 1. Model showing the role of increased SF-1 dosage in human and mouse adrenocortical tumorigenesis. In the human adrenal gland, in the presence of a germline TP53 mutation and loss of heterozygosity, increased SF-1 dosage may increase proliferation and inhibit apoptosis of adrenocortical cells around the period of physiological fetal adrenal regression. Concomitantly with other genetic lesions (e.g. inhibin-α mutations, IGF2 overexpression due to imprinting defects in the 11p15 region), adrenocortical tumors with a fetal adrenal phenotype (androgen producing) may be generated. In mice, pluripotent adrenogonadal progenitor cells are present in the subcapsular region of the adrenal cortex. Susceptible mouse strains harboring the S172 Sf-1 allele, which may predispose to increased Sf-1 expression, or lacking inhibin-α develop gonadal type cell tumors in the adrenal cortex under the control of pituitary LH. The same effect is produced by increased Sf-1 levels in the C57B6 background in the absence of elevated gonadotropin levels. INHA: inhibin-α; IGF2: insulin-like growth factor 2; LH: luteinizing hormone; LH-R: LH receptor.

ACT, which display a similar downregulation of HSD3B2 and CYP21A2 and whose general pattern of gene expression is highly correlated to the fetal adrenal pattern [34].

Our gene expression profiling study showed that an increased SF-1 dosage modulates the expression of transcripts involved in steroid metabolism, cell cycle, apoptosis, cell adhesion and allowed us to identify NOV/CCN3 as a downregulated gene in childhood ACT and to characterize its role in human adrenocortical cells [35]. NOV/CCN3 is a multidomain-secreted protein, member of the CCN family, whose expression was described to be mostly restricted to the definitive zone of the fetal adrenal cortex [36]. Its known functions are related to cell adhesion and angiogenesis, and its expression is negatively correlated to proliferation and associated with differentiation in many cellular systems (reviewed in [37]). We have shown that NOV/CCN3 is downregulated at the protein level in childhood ACT independently from their degree of malignancy and in human adrenocortical cells in a manner dependent on SF-1 dosage. Moreover, we characterized its selective proapoptotic activity for human adrenocortical cells [35]. We suggest that NOV/CCN3 is a factor involved in growth control in specific cell types and may have a relevant role during adrenal development and tumorigenesis. Given its proapoptotic activity on adrenocortical cells, the role of NOV/CCN3 in the physiological process of fetal zone regression remains open to investigation.

2.3. Adrenocortical tumors in mice

ACT occur in ferrets and in certain mouse strains after gonadectomy (reviewed in [38]). In mice, gonadectomy-induced ACT are found either in certain inbred (e.g. C3H and DBA/2J) or in specific laboratory-engineered strains (inhibin-α-null mice and inhibin-α promoter-SV40 T antigen transgenic mice) [39–43]. A common feature of all types of gonadectomy-induced ACT occurring in rodents is the gonadal phenotype of the neoplastic cells, which express gonadal markers (e.g. AMH, LHR, and Gata-4). Increased gonadotropin production in gonadectomized animals has been shown to play a fundamental role in triggering adrenocortical tumorigenesis in these susceptible strains. A recent study has identified one major significant locus for gonadectomy-induced adrenocortical tumorigenesis on mouse chromosome 8, which is modulated by epistasis by another quantitative trait locus on chromosome 18 [44].

Transgenic mice harboring YAC constructs containing the Sf-1 gene locus have been generated and recapitulate the spatiotemporal expression of the endogenous Sf-1 gene expression [45]. We observed that Sf-1 overexpression in those mice, due to the presence of the transgene, is sufficient to trigger adrenocortical tumorigenesis. Contrarily to childhood ACT, tumors arising in mice transgenic for Sf-1 have a gonadal phenotype resembling granulosa cell tumors, characterized by expression of gonadal markers such as Amh and Gata-4 and the absence of steroidogenic enzymes like P450scc [4]. The phenotype of ACT arising in Sf-1 transgenic mice closely resembles the phenotype of ACT occurring in gonadectomized animals as described before. Those tumors are thought to be derived from pluripotent adrenogonadal precursor cells lying beneath the outer adrenal connective capsule, which in the susceptible strains still have the potential to differentiate into cells of gonadal stroma phenotype [46].
While the difference in tumor phenotypes underlies the differences in adrenocortical development and physiology between humans and mice, in both species SF-1 appears to play a pivotal role in adrenocortical tumorigenesis (Fig. 1).

3. New therapeutical approaches in adrenocortical tumors and future perspectives

Although ACT are a rare type of cancer, they are very aggressive and highly resistant to chemo- and radiotherapy. Therapeutic results are still unsatisfactory, with an overall survival rate at 5 years of only about 50% [26]. Current therapy for pediatric ACT primarily consists in surgical resection of the tumor. Moreover, the use of the adrenolytic agent, mitotane (o,p-DDD), associated or not with DNA-damaging drugs, is the only medical therapy available up to date [47]. Thus, a better knowledge of the molecular mechanisms underlying tumor growth and progression is necessary in order to develop more selective and specific treatments.

The important role of increased SF-1 dosage in adrenocortical tumorigenesis suggests that modulation of SF-1 activity may represent an important therapeutic target in childhood ACT. Our cellular model recapitulates several molecular features of childhood ACT and constitutes a useful tool to understand the molecular mechanisms of ACT pathogenesis needed for the development and for the study of drugs targeting SF-1 transcriptional activity for ACT therapy.

Recently, studies based on high-throughput screening of chemical libraries led to the identification of two distinct classes of small molecules described as selective SF-1 inverse agonists [48,49]. These drugs may then represent interesting therapeutic tools to be associated to mitotane and newer drugs in ACT chemotherapy [50–52]. The characterization of the molecular mechanism of action of these compounds are crucial for the design of increasingly selective molecules targeting SF-1 in a cell specific fashion to limit their side effects.

References
