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Short communication

SF-1 overexpression in childhood adrenocortical tumours

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ABSTRACT

The steroidogenic factor 1 (SF-1) gene encodes a transcription factor playing a pivotal role in the regulation of adrenogenital development. We have recently shown that *SF-1* is amplified in childhood adrenocortical tumours (ACT). This study was aimed to assess if an increase in *SF-1* gene copy number was associated with increased protein levels and to study the correlation between *SF-1* expression and ACT clinical parameters. An increased *SF-1* copy number was detected in eight of the 10 ACT cases studied. Conversely, the SF-1 protein was found to be overexpressed in all cases, compared to normal age-matched adrenal glands. No significant correlation was found between SF-1 protein levels and its gene copy number. Furthermore, no significant correlation existed with histological grade or with the clinical manifestation or evolution of disease. This data show that *SF-1* overexpression is widespread in childhood ACT and is likely to play a role in its pathogenesis.

1. Introduction

Tumours of the adrenal cortex are rare in children, with the highest worldwide incidence in southern Brazil, where adrenocortical tumours occur at a rate of 3.4–4.2 cases per million children under the age of 15, 12–18 times higher than worldwide estimates.[1,2]

In 2001, we identified a *TP53* R337H germline mutation that is consistently present in children from Southern Brazil bearing adrenocortical tumours (ACT).[3] This mutation is associated with loss of heterozygosity (LOH) at the *TP53* locus, with elimination of the wild-type *TP53* gene in the tumours. The *TP53* R337H mutation is the main genetic alteration involved in the initial formation of ACT in these children.[3]

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Among sporadic adult ACT, genetic alterations such as LOH at 11p, 13q and 7p, *ACTH* receptor gene mutations and 17q amplifications were identified.[4] In childhood ACT, using comparative genomic hybridization (CGH), we have shown that the most consistent genetic abnormality is the amplification of 9q34, which was detected in 8/9 cases of pediatric ACT from Southern Brazil.[5] A similar study conducted in Britain showed similar 9q34 amplification in 10/11 patients.[6] These findings led us to propose that the 9q34 amplification is an event intrinsically related to the biology of this type of cancer and is not related to environmental factors.[7] Several genes related to tumour development are located in the 9q34 region, such as the *ABL1* oncogene, which was found not to be amplified in ACT (our unpublished data). It is noteworthy that the steroidogenic factor 1 (*SF-1*) gene maps to 9q33.3,[8] a region in close proximity to the common 9q34 amplicon in ACT. We have recently shown an increase in copy number of the *SF-1* gene by fluorescence in situ hybridization (FISH) in ACT samples that were also evaluated by CGH.[9]

SF-1 (also known as *Ad4BP*) encodes a member of the nuclear hormone receptor superfamily (NR5A1 according to the standard nomenclature), which plays a key role in the regulation of adrenal gland development and in the expression of steroidogenic enzymes (see[10] for review). The purpose of the present study was to ascertain whether increased *SF-1* copy number in pediatric ACT translates to an increase in SF-1 protein levels in the tumours. In this study, we analyzed 10 childhood tumours from six girls and four boys, aged between 11 months and 11 years of age. All subjects carried the *TP53* R337H mutation, inherited from one of the parents, and had LOH at the *TP53* locus in the tumours. In children, ACT are associated with symptoms related to the production of androgens (virilizing form, >85% of cases), glucocorticoids (Cushing's syndrome) and less frequently to the production of mineralocorticoids (Conn's syndrome) or estrogens (feminizing syndrome).[1,2] On the other hand, SF-1 is considered as a regulator of steroidogenic gene expression.[10] For this reason, the correlation of SF-1 protein levels and hormonal production by the tumours was also analyzed.

2. Patients and methods

2.1. Patients

The study included 10 children with ACT (six boys and four girls), of age ranging from 11 months to 11 years. Histologically, three tumours were classified as adenomas and seven as carcinomas. Eleven normal adrenal glands resected from age-matched children undergoing surgery for Wilms' tumour were used as controls. All patients and control subjects were included in the study after one of the parents or legal guardians signed an informed consent form approved by the Ethics Committee of the Hospital de Clínicas of the Federal University of Paraná.

Seven patients presented with virilization and three with virilization and Cushing's syndrome. Of the 10 patients analyzed in this study, four died of disease progression, while six are still alive (Table 1). All patients included in this study carried the germline *TP53* R337H mutation previously described[3] and had *TP53* LOH.[3,11]

2.2. Fluorescence in situ hybridization (FISH)

To evaluate copy number changes of the *SF-1* gene in ACT, we used fluorescence in situ hybridization (FISH) using a probe for the *SF-1* gene, as previously reported.[9] In brief, the FISH probe consisted of a bacterial artificial chromosome (BAC) clone containing sequences of the *SF-1* gene: RP11-91G7 (BACPAC Resources, Oakland, CA). BAC clone DNA preparation, labelling and FISH conditions were all previously described in detail.[9] Considering artifact and loss of genomic contents in partially cut nuclei, FISH signals in 50 cells for each specimen were counted. The presence of 2 FISH signals per cell in at least 50% of the nuclei was considered as normal diploid. Three fluorescence signals in at least 30% of the nuclei with detectable signals were considered as increased copy number/gain. Amplification was scored in those cases where 30% or more of the cells showed 4 or more copies of the *SF-1* gene.

Table 1 – Clinical and molecular data of ACT patients

ID	Age (months)	Gender	Clinical manifestation	Histology	Clinical stage	Tumour volume (cm^3)	Outcome	Cortisol (μg/dL)	DHEA-S (times above normal)	Virilization signs P(1–5)/ Acne	*SF-1* copy number
1	139	F	V	Ad	I	90	A	19.6	9	5/Yes	Amplified[a]
2	43	F	V	Ad	I	21	A	10.8	17	4/Yes	Normal
3	11	F	C	Ad	I	24	A	10.9	14	3/No	Increased[b]
4	52	F	V	Ca	II	1800	D	25.4	>3	3/Yes	Amplified[a]
5	110	M	V + C	Ca	II	968	D	51.5	10	5/No	Amplified[a]
6	21	M	C	Ca	I	61	A	10.1	2	3/Yes	Amplified[a]
7	25	M	V + C	Ca	I	108	A	23.5	ND	3/Yes	Amplified[a]
8	39	F	V	Ca	III	1450	D	13.5	ND	3/Yes	Amplified[a]
9	72	M	V	Ca	III	196	D	23.9	ND	1/No	Increased[b]
10	130	F	V + C	Ca	II	924	A	21.5	2	3/Yes	Normal

Clinical manifestations were virilization (V) and/or Cushing's syndrome (C). Outcome: alive (A) or deceased (D). Among the virilization signs examined were the degree of pubic hair growth according to Tanner stages (P1–P5)[19,20] and the presence of facial acne.
a Indicates that 4 or more copies of the *SF-1* gene were detected in over 30% of the cells by FISH.
b Indicates that 3 copies of the *SF-1* gene were detected in over 30% of the cells by FISH.