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The role of fibroblast growth factor receptor 4 overexpression and gene amplification as prognostic markers in pediatric and adult adrenocortical tumors

Dear Editor

In the last decade, relevant progresses in the molecular basis of adrenocortical tumors (ACTs) were achieved and abnormalities involving growth pathway deregulation were frequently associated with malignancy. Remarkably, upregulation of insulin-like growth factor 2 (*IGF2*) and its receptor (*IGF1R*) has been demonstrated in a significant proportion of ACTs, and the presence of these abnormalities has both prognostic and therapeutic implications (Almeida *et al*. 2008). In fact, clinical trials involving pharmacological blockade of IGF1R are currently under process. Besides the IGF system, other growth signaling pathways have been suggested to be important for ACC progression and are possible therapeutic targets. Among these, fibroblast growth factor receptor 4 (*FGFR4*) overexpression has been observed in both adult and pediatric ACT by genome-wide expression studies in the same extent as the IGF system (de Fraipont *et al*. 2005, Laurell *et al*. 2009). However, these data have not been validated in an independent cohort and the molecular mechanisms responsible for *FGFR4* upregulation have not been assessed. Therefore, we studied the expression levels of *FGFR4* and gene amplification in a cohort of ACT patients from our institution. This study was approved by the Ethics Committee of Hospital das Clinicas, Sao Paulo, Brazil, and an informed written consent was obtained from all patients and/or parents. Our cohort consisted of 57 patients – 32 adults (age $\geq$18 years, 18–66 years) and 25 pediatric (age $<$18 years, 0.9–17 years) with the diagnosis of ACT, with a mean follow-up period of 77.5 ± 53 months. *FGFR4* transcript levels were assessed through quantitative real-time RT-PCR in all 57 samples using TaqMan gene expression assays (Applied Biosystem, Foster City, CA, USA). The endogenous control gene used was *ACTB* for each sample and the reactions were carried out in triplicate. The relative expression levels of *FGFR4* were calculated using the $2^{-\Delta\Delta C_t}$ method as described previously (Livak & Schmittgen 2001). A commercial pool of 61 human adrenal glands of autopsy was the reference sample (Clontech). The criteria used for under- and overexpression was a twofold change in comparison with the reference sample. *FGFR4* amplification was determined using the SALSA MLPA kit P026-C1 Sotos (MRC-Holland, Amsterdam, The Netherlands), which contains two probes for *FGFR4* gene and 24 probes for *NSD1* gene at locus 5q35.1. *FGFR4* copy number was assessed in 45 ACTs. MLPA was performed as described previously (Schouten *et al*. 2002). The tumor sample-normalized peak height was divided by the average normalized peak height from normal adrenals. Dosage quotient areas outside the range 0.70–1.3 were considered abnormal. Control samples were included in each multiplex ligation-dependent probe amplification (MLPA) experiment. Each result was confirmed by two independent tests.

Overall, *FGFR4* overexpression was detected in 65% (37 out of 57) of the cases. Noteworthy, there was a positive correlation between *FGFR4* and *IGF2* expression levels (Pearson's $r = 0.84$, $P < 0.001$). This finding is in accordance with previous data (de Fraipont *et al*. 2005), which suggested that *FGFR4* and *IGF2* belong to a cluster of genes that are simultaneously overexpressed in adrenocortical carcinoma (ACC). Among the 25 pediatric ACTs, *FGFR4* overexpression was detected in all except three cases (88%). In the adult group, *FGFR4* overexpression was detected in 47% (15 out of 32) of ACTs, being significantly different between carcinomas and adenomas (9.35 (0.21–35.73) vs 1.44 (0.53–9.85); Mann–Whitney *U* test, $P = 0.004$). Survival analysis revealed that *FGFR4* overexpression was a predictor of poor outcome in adult patients (log rank test, $P = 0.049$; Fig. 1A). In addition, *FGFR4* amplification was detected in three out of 22 (13.5%) of the pediatric tumors and in seven out of 23 (30.4%) of adult tumors, suggesting that gene amplification may be the molecular mechanism underlying *FGFR4* overexpression in a subset of tumors. Furthermore, *FGFR4* locus amplification was associated with adverse outcome in adults (log rank test, $P = 0.008$; Fig. 1B).

We demonstrated *FGFR4* overexpression in a significant proportion of pediatric and adult ACTs,

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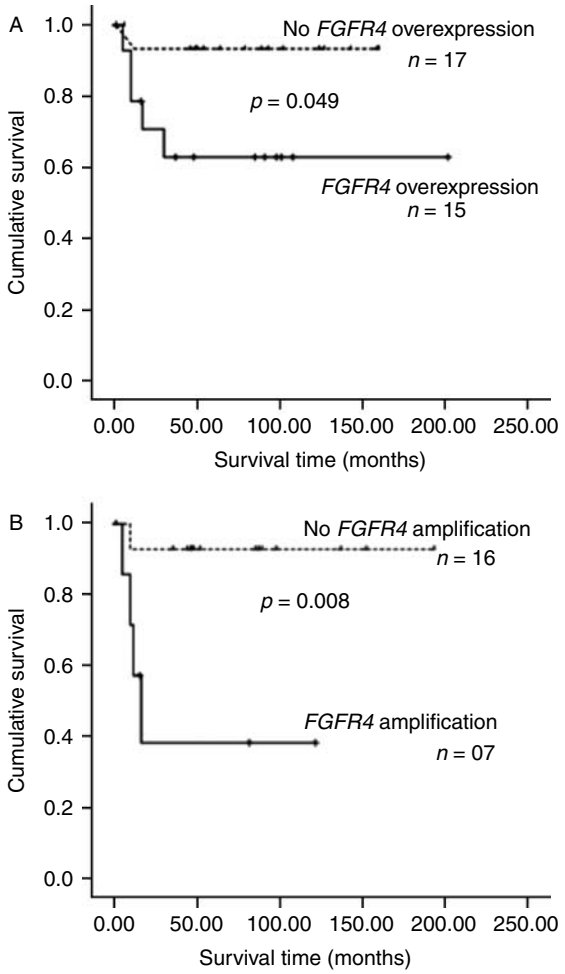

Figure 1 Kaplan–Meier survival analysis of adult patients with adrenocortical tumors. (A) Overall survival of 32 patients (17 ACA and 15 ACC) according to *FGFR4* expression level. (B) Overall survival of 23 patients (12 ACA and 11 ACC) according to the amplification status of *FGFR4* locus. Survival time is expressed in months.

confirming previous observations (de Fraipont *et al*. 2005, West *et al*. 2007, Laurell *et al*. 2009). According to our data, we consider that *FGFR4* overexpression and amplification may be important steps in adrenocortical tumorigenesis and *FGFR4* is possibly an interesting therapeutic target.

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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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