European Journal of Endocrinology (2005) **153** 629–636 ISSN 0804-4643

CASE REPORT

Shift from Conn's syndrome to Cushing's syndrome in a recurrent adrenocortical carcinoma

L Barzon, G Masi, K Fincati, M Pacenti, V Pezzi[1], G Altavilla[2], F Fallo[3] and G Palù

Department of Histology, Microbiology and Medical Biotechnologies, University of Padova,Via A. Gabelli 63, I-35121 Padova, Italy [1]Department of Pharmaco-Biology, University of Calabria, Arcavacata di Rende, Cosenza, Italy and Departments of [2]Pathology and [3]Medical and Surgical Sciences, University of Padova,Via A. Gabelli 63, I-35121 Padova, Italy

(Correspondence should be addressed to L Barzon; Email: luisa.barzon@unipd.it)

Abstract

Objective: Adrenocortical tumors may originate from the zona glomerulosa, zona fasciculata, or zona reticularis and be associated with syndromes due to overproduction of mineralocorticoids, glucocorticoids, or androgens respectively. We report an unusual case of recurrent adrenocortical carcinoma (ACC), which seems to contradict the paradigm of functional adrenal zonation.
Case report: A male patient presented with severe primary aldosteronism due to an ACC, which relapsed after adrenalectomy and adjuvant mitotane therapy. After removal of the tumor recurrence and eight cycles of chemotherapy with etoposide, doxorubicin and cisplatin, the patient presented again with ACC masses, but in association with overt Cushing's syndrome and normal aldosterone levels.
Methods and results: Extensive pathologic examination showed that this shift in steroid hormone production was paralleled by an attenuation of tumor cell atypia and polymorphism, whereas gene expression profile analysis demonstrated a change in expression of adrenal steroidogenic enzymes. Moreover, cancer progression was associated with overexpression of the inhibin-α subunit, which could have contributed to the phenotypic changes.
Conclusions: This case of recurrent ACC demonstrates that adrenocortical cells can reverse their differentiation program during neoplastic progression and change their specific hormone synthesis, as a consequence of modifications in the expression profile of steroidogenic enzymes and cofactors. We hypothesize that this shift in steroid hormone secretion is a consequence of chromosome amplification induced by chemotherapy. These findings, besides opening new perspectives to study adrenocortical cell plasticity and potential, demonstrate how conventional clinical and pathologic evaluation can be combined with genomic analysis in order to dissect thoroughly the biology of cancer.

European Journal of Endocrinology **153** 629–636

Case report

A 42-year-old man presented with severe hypertension (blood pressure, 200/120 mmHg) and hypokalemia (serum K^+, 2.2 mmol/l). Endocrine evaluation showed elevated plasma and urinary aldosterone levels (upright plasma aldosterone, 1371 pmol/l; normal values, 140–830 pmol/l) with suppressed plasma renin activity (PRA) (upright PRA, 0.1 ng/ml per h; normal values, 1.5–6 ng/ml per h), whereas cortisol, adrenal androgens and testosterone were within the normal range (Fig. 1). In particular, 24 h urinary free cortisol was 135 nmol/24 h (normal values, 82–330 nmol/l); plasma cortisol at 0800 h was 415 nmol/l (normal values, 138–550 nmol/l); at 1800 h, plasma cortisol was 287 nmol/l; and in the morning, 1 mg dexamethsone suppression test was 115 nmol/l (normal response, $<$138 nmol/l). Abdominal computed tomography (CT) scan demonstrated a 5 cm right adrenal mass, which was diagnosed as adrenocortical carcinoma (ACC) at histologic examination. After adrenalectomy, the patient received adjuvant mitotane therapy at doses up to 10 g/day, but after 9 months the patient presented again with isolated severe aldosteronism associated with a 3 × 5 cm tumor relapse in the right adrenal region and lymph-node and skin metastasis. The patient underwent complete removal of the tumor masses, followed by eight cycles of chemotherapy with etoposide, doxorubicin and cisplatin for residual disease. Notwithstanding initial control of the disease, tumor recurrence was identified by CT scan after about 8 months. Clinical examination demonstrated weight gain with central obesity (body-mass index change from 26 kg/m^2 at diagnosis to 31 kg/m^2), whereas endocrine investigations

DOI: 10.1530/eje.1.02011
Online version via www.eje-online.org

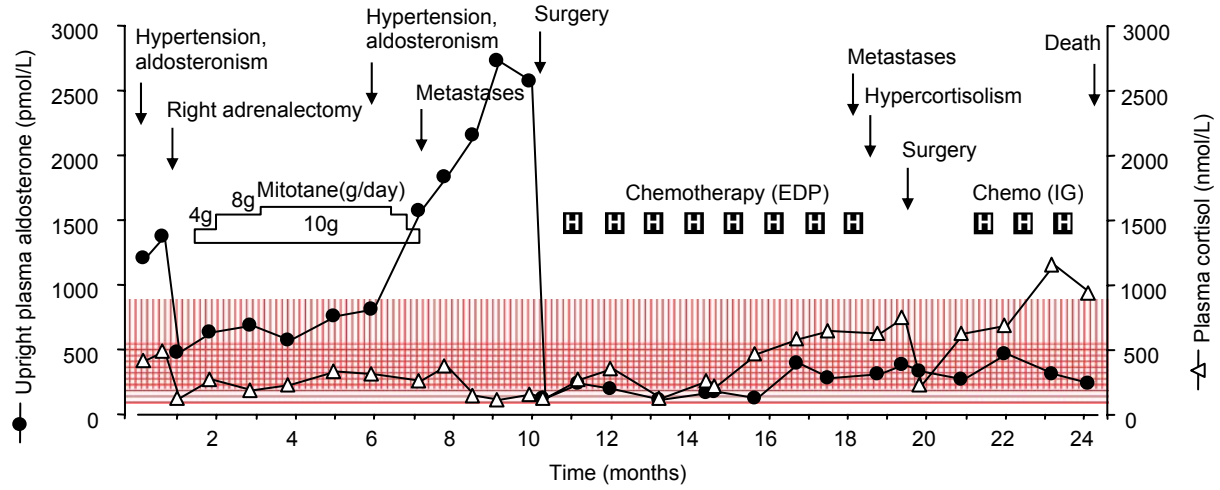


Figure 1 Plasma aldosterone and cortisol levels in a patient with ACC during the course of his disease. The red hatching indicates normal values; vertical hatching, upright plasma aldosterone, 140–830 pmol/l; horizontal hatching, plasma cortisol at 0800 h, 138–550 nmol/l. Mitotane doses are reported in grams per day; EDP: etoposide, doxorubicin, cisplatin; IG: irinotecan, gemcitabine.

revealed elevated 24 h urinary free cortisol (528 nmol/24 h), plasma cortisol (plasma cortisol at 0800 h, 615 nmol/l), plasma dehydroepiandrosterone sulfate (DHEA-S) (32.7 μmol/l; normal values, 0.5–9.0 μmol/l), and androstenedione (26.4 nmol/l; normal values, 2.0–9.2 nmol/l) values; undetectable plasma adrenocorticotropic hormone (ACTH); and normal levels of plasma aldosterone and PRA. Plasma and urinary cortisol were unresponsive to the high-dose dexamethasone suppression test. The patient was operated again to remove three abdominal masses of 7, 5.5 and 5 cm in maximum diameter, and subsequently treated with second-line chemotherapy with irinotecan and gemcitabine. Eventually, the patient died from persistent hypercortisolism and metastatic disease at 24 months after diagnosis (Fig. 1).

Results and discussion

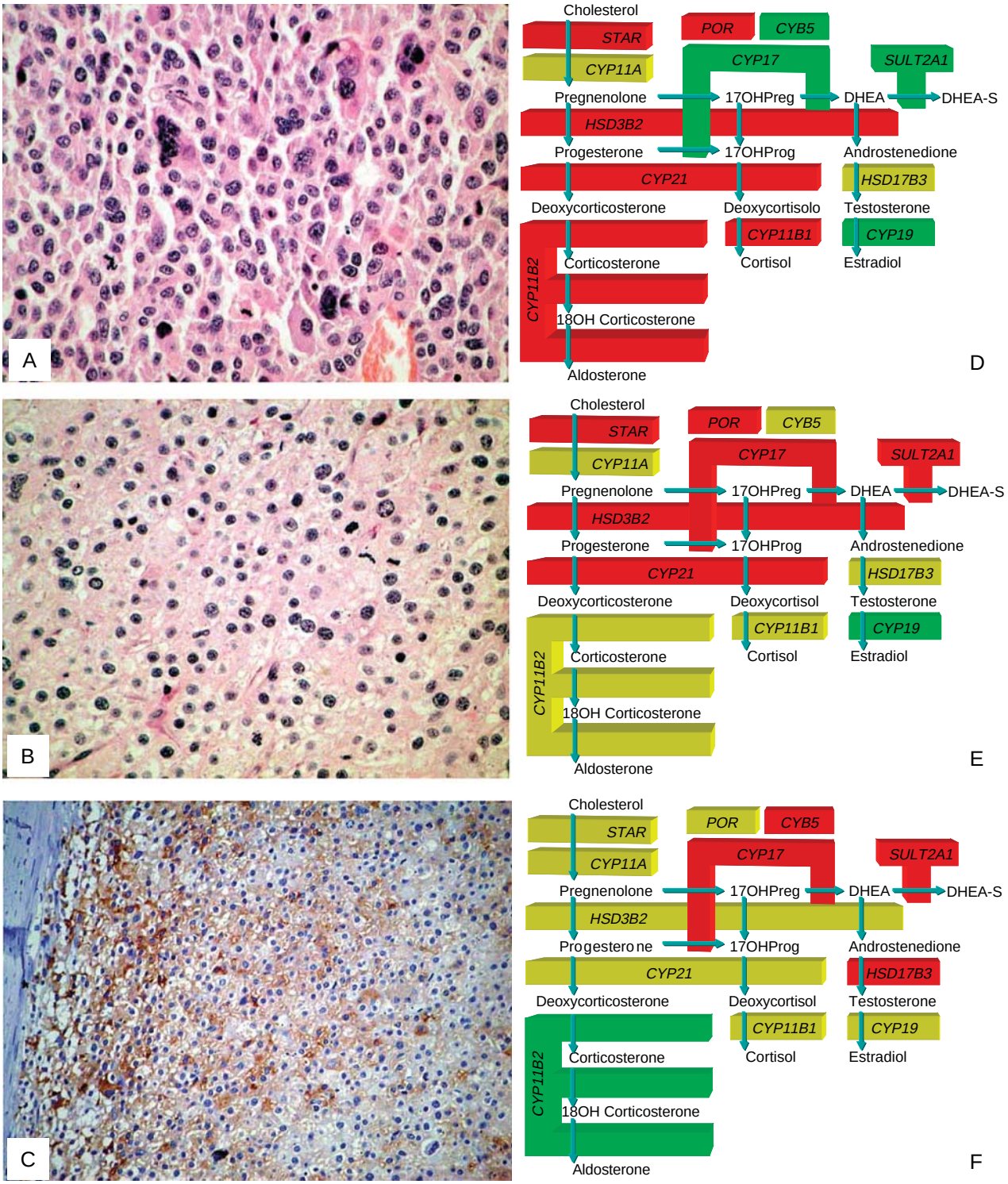
ACC is a rare and very aggressive cancer with poor prognosis. About half of cases are hormonally active and associated with clinical features of hypercortisolism (Cushing's syndrome), virilization, feminilization, or, rarely, primary aldosteronism (Conn's syndrome). Mixed syndromes due to overproduction of different steroid hormones and steroid precursors are also frequently observed.

This case of recurrent ACC attracted our attention because of the atypical clinical presentation, characterized by a shift from primary aldosteronism to Cushing's syndrome during tumor progression. This shift in adrenal steroid synthesis, which has been very rarely reported in the literature (1–3), seems to contradict the paradigm of functional adrenal zonation, according to which the three zones of the adult adrenal, that is, zona glomerulosa, zona fasciculata and zona reticularis, have specialized steroidogenetic activity, being committed to produce mineralocorticoids, glucocorticoids and androgens respectively.

In order to investigate the mechanism at the basis of this endocrine shift, we performed a thorough pathologic, genetic and gene expression profile analysis of the primary lesion and its recurrences. The patient gave written, informed consent for the scientific evaluation of the tumor samples.

Pathologic examination revealed quite variable findings, since cells of the primary tumor and the first recurrence showed great polymorphism and atypia, frequent and atypical mitoses, and no evident organoid growth pattern (Fig. 2A). In contrast, metastases of the second relapse showed monomorphism of the tumor

Figure 2 Pathologic examination of ACC specimens (A–C): A) hematoxylin and eosin (HE) staining of abdominal lymph-node metastasis from the primary ACC, showing polymorphic cells with wide eosinophil dense cytoplasm, atypical central nucleus of variable size and with chromatin, and no evident organoid growth pattern. Mitoses were very frequent and often atypical (mitotic index $> 20 \times 10$ high-power field); necrotic areas and vascular tumor cell invasion were also frequent. B) HE staining of a skin metastasis from the second ACC recurrence, showing monomorphism of the tumor cell population with a nodular growth pattern simulating an organoid structure. The neoplastic cells had large eosinophilic cytoplasm, but this was more regular than in the primary ACC and first recurrence, and nuclei were less variable in size and chromatin distribution. Mitosis was found, but necrosis was absent. Analysis of α-inhibin expression by the use of anti-α-inhibin antibody (Serotec Ltd, Oxford, UK; 1:50) demonstrated positive staining in these second



metastatic cells (C), but not in the primary ACC and first recurrence. Representation of steroidogenic enzyme expression in ACC specimens as determined by real-time quantitative RT–PCR and DNA microarray analysis (D and E). Comparisons between (D) primary ACC and normal adrenal cortex, (E) second ACC recurrence and normal adrenal cortex, and (F) second recurrence and primary ACC. Genes showing at least a twofold difference in expression between samples were considered under- or overexpressed. Upregulated genes are represented as red boxes; downregulated genes as green boxes; no significant differential expression as yellow boxes. Results are consistent with the prevalence of the aldosterone biosynthetic pathway in the primary ACC and a shift to the androgen and gluocorticoid biosynthetic pathway in the second ACC relapse.

Table 1 Results of microarray analysis of expression of steroidogenic enzymes and transcription factors in the recurrent ACC vs normal adrenocortical tissues.

Description	Gene	Normal adrenal[a]	Ratio ACC1/normal[b]	Ratio ACC2/normal[b]	Ratio ACC2/ACC1[b]
Cytochrome P450, subfamily I, polypeptide 2	*CYP1A2*	8.59	1.92	1.99	1.49
P450 (cytochrome) oxidoreductase	*POR*	5.90	**2.16**	**2.39**	1.11
Cytochrome P450, subfamily XVII	*CYP17*	5.44	*0.42*	**4.86**	**6.67**
Steroidogenic acute regulatory protein	*STAR*	5.40	**2.16**	**2.53**	1.50
24-dehydrocholesterol reductase	*DHCR24*	5.02	0.94	1.35	1.91
Nuclear receptor subfamily 4, A1	*NR4A1*	4.98	1.95	**2.28**	1.16
Cytochrome P450, subfamily XXIA, polypeptide	*CYP21A2*	4.66	**2.40**	**2.70**	1.31
Cytochrome P450, subfamily IIB, polypeptide 6	*CYP2B6*	3.91	1.27	**2.31**	**3.14**
Cytochrome P450, subfamily IIS, polypeptide 1	*CYP2S1*	3.53	1.42	**2.79**	**3.93**
Hydroxysteroid (17-beta) dehydrogenase 1	*HSD17B1*	3.30	**2.93**	**2.49**	1.66
Cytochrome P450, subfamily XIB, polypeptide 1	*CYP11B1*	2.50	**2.13**	1.11	0.81
Cytochrome P450, subfamily IIA, polypeptide 7	*CYP2A7*	2.45	1.67	**3.09**	**2.98**
Hydroxyacyl-coenzyme A dehydrogenase, type II	*HADH2*	2.20	1.56	1.93	1.78
Hydroxysteroid (3-beta) dehydrogenase 2	*HSD3B2*	2.17	**2.22**	**2.09**	1.29
Cytochrome P450, subfamily XIA (cholesterol sec)	*CYP11A*	2.17	1.28	1.60	1.76
Hydroxysteroid dehydrogenase, 3 beta 1	*HSD3B1*	2.12	**4.51**	**5.71**	**2.53**
7-Dehydrocholesterol reductase	*DHCR7*	2.12	1.98	**3.73**	**3.14**
Steroid sulfotransferase 2A1, DHEA-preferring	*SULT2A1*	2.01	***0.42***	**2.43**	**4.47**
Hydroxysteroid (17-beta) dehydrogenase 7	*HSD17B7*	1.97	1.16	**3.08**	**2.40**
Aldo-keto reductase, 7A2	*AKR7A2*	1.95	1.42	1.29	1.28
Steroid sulfotransferase 1C2	*SULT1C2*	1.74	**2.58**	**2.68**	1.22
Steroid sulfatase (microsomal), isozyme S	*STS*	1.60	0.74	0.89	0.88
Ferrodoxin reductase	*FDXR*	1.58	**3.19**	**2.85**	1.21
Cytochrome P450, subfamily IIC9	*CYP2C9*	1.56	1.40	0.86	0.69
Steroidogenic factor-1 (SF-1)	*NR5A1*	1.51	1.38	1.53	1.15
Nuclear receptor subfamily 4, A2	*NR4A2*	1.48	***0.37***	***0.22***	0.61
Cytochrome P450, subfamily XIB, polypeptide 2	*CYP11B2*	1.43	**5.10**	1.19	***0.40***
Aldo-keto reductase, 1B1	*AKR1B1*	1.36	1.26	1.09	**2.47**
Cytochrome b5	*CYB5*	1.17	***0.49***	0.79	**2.67**
Hydroxysteroid (17-beta) dehydrogenase 4	*HSD17B4*	1.12	1.26	1.23	1.26
Aldo-keto reductase, 1A1	*AKR1A1*	1.09	0.89	0.59	0.65
Steroid sulfotransferase 1C1	*SULT1C1*	0.98	0.86	1.21	0.71
Steroid reductase, alpha polypeptide 1	*SRD5A1*	0.98	0.84	0.81	***0.45***
Aldo-keto reductase, 1B10	*AKR1B10*	0.96	***0.22***	***0.39***	***0.17***
Aldo-keto reductase, 1D1	*AKR1D1*	0.89	0.62	***0.35***	***0.25***
Steroid sulfotransferase, 2B1	*SULT2B1*	0.88	0.80	0.54	***0.34***
Liver receptor homolog 1 (LRH-1)	*NR5A2*	0.83	0.90	0.60	***0.24***
Cytochrome P450, subfamily XIX	*CYP19*	0.81	***0.19***	***0.39***	1.31
Aldo-keto reductase, 1C1	*AKR1C1*	0.69	1.01	***0.36***	0.84
Hydroxysteroid (17-beta) dehydrogenase 2	*HSD17B2*	0.60	0.72	***0.48***	***0.19***
Steroid sulfotransferase, 1B1	*SULT1B1*	0.59	0.55	0.66	1.21
Hydroxysteroid (11-beta) dehydrogenase 2	*HSD11B2*	0.55	***0.31***	0.73	1.88

Table 1 *Continued*

Description	Gene	Normal adrenal[a]	Ratio ACC1/normal[b]	Ratio ACC2/normal[b]	Ratio ACC2/ACC1[b]
Steroid sulfotransferase, 1A2	*SULT1A2*	0.55	1.66	1.03	**2.03**
Steroid sulfotransferase, 1A3	*SULT1A3*	0.49	1.37	1.17	1.54
Hydroxysteroid (11-beta) dehydrogenase 1	*HSD11B1*	0.43	0.80	1.70	1.29
Hydroxysteroid (17-beta) dehydrogenase 3	*HSD17B3*	0.42	0.62	1.79	**2.12**
Steroid sulfotransferase, 1A1	*SULT1A1*	0.31	0.68	0.90	1.26

[a]Signal intensity values in normal adrenocortical tissues; [b]ratios >2 (i.e., overexpressed genes) are in boldface, ratios <0.5 (i.e., underexpressed genes) are in boldface and italic; ACC1: primary ACC; ACC2; second ACC recurrence.

cell population with a nodular growth pattern simulating an organoid structure. The neoplastic cells had large eosinophilic cytoplasm, but this was more regular than in the primary ACC and first recurrence, and nuclei were less variable in size and chromatin distribution (Fig. 2B).

Sequence analysis of candidate genes (i.e., *TP53*, *PTEN*, *GNAS1*, *GNAI2*, *CDKN1C*, *MEN1*, *PRKAR1A*, *INHA* and *APC*) typically involved in adrenal tumorigenesis failed to demonstrate pathologic mutations either in the primary tumor or in recurrences, whereas measurement of mRNA levels of ACC marker genes (i.e., *IGF2*, *H19*, *CDKN1C*, *EGFR* and *TOP2A*) by quantitative real-time RT–PCR demonstrated very high *IGF2* and *TOP2A* mRNA levels and underexpression of *H19* and *CDKN1C* in both primary primary tumor and metastases, as typically observed in ACC (4).

DNA microarray analysis and quantitative real-time RT–PCR demonstrated that the expression pattern of steroidogenic enzymes was concordant with endocrine activity of the ACC masses. In fact, mRNA levels of *CYP11B2* (aldosterone synthase) were extremely high in the aldosterone-producing tumors but very low in the second relapse, which, in contrast, had high mRNA levels of genes encoding enzymes involved in the production of cortisol and adrenal androgens, such as *CYP17*, *CYP21* and *SULT2A1* (Table 1 and Fig. 2D–F) (5). Of interest, microarray analysis (performed with microarray glass slides containing 70 mer oligonucleotide sequences of 21 329 human genes, produced by CRIBI Core Facility, University of Padova, Italy) also showed that the most overexpressed genes in the second cortisol-secreting ACC recurrence, as compared with the aldosterone-producing primary ACC and first relapse, included a large number of genes mapping to the 19q13.3–4 chromosomal region. Among these genes, there were a large cluster of cytochrome P450 genes involved in the metabolism of steroids and xenobiotics (6) (e.g., *CYP2B6, CYP2S1 CYP2A7*) and the *INHA* gene, encoding the inhibin α-subunit (Table 2). Overexpression of cytochrome P450 genes leading to increased inactivation of anticancer drugs has been linked to chemotherapy resistance (7). Chromosomal gains and amplifications in 19q13 are often found in ACC (8), and, in our case, they could indeed have occurred during chemotherapy, causing gene overexpression. The product of the *SULT2A1* gene, DHEA sulfotranspherase, also located in 19q13.3–4, normally sulfates DHEA to DHEA-S, as well as pregnenolone and 17α-hydroxypregenolone to their sulfated metabolites, removing these substrates from mineralocorticoid and glucocorticoid pathways respectively (9). *SULT2A1* overexpression, in the presence of high levels of CYP17 and CYP21, might have shifted aldosterone biosynthesis to both cortisol and androgen biosynthesis. These findings are in accordance with the clinical shift from Conn's syndrome to Cushing's syndrome seen in our patient.

Table 2 Transcripts expressed at >3 threefold higher or lower in the second ACC recurrence vs the primary ACC.

Description	Function	Map	UniGene	Gene	Ratio
Overexpressed genes					
Ubiquitin carboxyl-terminal esterase L1	Ubiquitin hydrolysis; neuroendocrine tissues	4p14	76 118	*UCHL1*	11.40
Synuclein, gamma	Oncogene	10q23	349470	*SNCG*	10.06
Clusterin	Downregulated in prostate cancer	8p21	75 106	*CLU*	7.79
Chymotrypsinogen B1	Serine protease	16q23	74 502	*CTRB1*	7.05
Cytochrome P450, subfamily XVII	Steroid 17-alpha-hydroxylase	10q24.3	1363	*CYP17*	6.67
Paternally expressed 3	Growth-promoting functions, but also tumor suppressor	19q13.4	139033	*PEG3*	6.25
K562 cell-derived leucine-zipper-like protein 1	Transcription factor	19q13.43	31 854	*LOC57106*	5.91
Adlican	VEGF receptor	Xp22.33	72 157	*DKFZp56411922*	5.78
Zinc finger protein 573	Regulation of transcription	19q13.13	278871	*ZNF573*	5.59
K1AA1198 protein	Zink-finger protein 490	19p13.2	175475	*KIAA1198*	5.38
Synaptophysin	Adrenocortical neoplasm marker, cholesterol binding	Xp11.23	75 667	*SYP*	5.19
Leukocyte receptor cluster member 5	tRNA splicing	19q13.4	15 580	*LENG5*	4.82
Retinol binding protein 1, cellular	Retinol transport, antitumor activity	3q23	101850	*RBP1*	4.76
Solute carrier family 4, anion exchanger, member 3	Anion transport	2q36	1176	*SLC4A3*	4.74
Protein phosphatase 1, regulatory subunit 14A	Inhibitor of smooth muscle myosin phosphatase	19q13.1	348037	*PPP1R14A*	4.73
HMT1 bnRNP methyltransferase-like 1	Signal transduction	21q22.3	235887	*HRMT1L1*	4.71
Solute carrier family 25	Mitochondrial carrier with calcium-binding domains	19q13.3	32 246	*SLC25A23*	4.66
Sulfotransferase 2A, DHEA-preferring, member 1	Steroid metabolism	19q13.3	81 884	*SULT2A1*	4.47
K1AA1415 protein	Guanine nucleotide exchange factor	20q13.13	109315	*KIAA1415*	4.43
Inhibin, alpha	Activin inhibitor activity	2q33–36	1734	*INHA*	4.41
Filamin B, beta	Actin cytoskeleton organization	3p14.3	81 008	*FLNB*	4.32
Cargo selection protein	Similar to mannose-6-P receptor binding protein 1	19p13.3	140452	*TIP47*	4.26
Retinol dehydrogenase 13 (all-*trans* and 9-*cis*)	Oxidoreductase activity	19q13.42	178617	*RDH13*	4.19
Fatty acid desaturase 1	Fatty acid biosynthesis	11q12.2-q13.1	132898	*FADS1*	4.09
PRP31 pre-mRNA processing factor 31	Retinitis pigmentosa, snRNP formation	19q13.42	183438	*PRPF31*	4.08
Ubiquitin-conjugating enzyme E2C	Cell growth and malignant transformation	20q13.12	93 002	*UBE2C*	3.98
Stem-loop (histone) binding protein	Histone processing	4p16.3	75 257	*SLBP*	3.95
Cytochrome P450, subfamily IIS, polypeptide 1	Drug metabolism and cholesterol synthesis	19q13.1	98 370	*CYP2S1*	3.93
Neuronal pentraxin I	Central nervous system development	17q25.1-q25.2	84 154	*NPTX1*	3.92
Ectodermal-neural cortex (with BTB-like domain)	Actin-binding protein	5q12-q13.3	104925	*ENC1*	3.78
Niemann-pick disease, type C1	Intracellular transport of cholesterol	18q11	76 918	*NCP1*	3.72
Thioredoxin-like 4A	Electron transport	18q23	5074	*TXNL4A*	3.72
Actinin, alpha 4	Actin-binding protein; tumorigenicity	19q13	182485	*ACTN4*	3.70
Reticulon 4	Neuroendocrine secretion	2p16.3	65 450	*RTN4*	3.70
Zinc-finger protein 83 (HPF1)	Transcription factor	19q13.3	305953	*ZNF83*	3.68
Protein tyrosine phosphatase, receptor type, H	Tumor suppressor	19q13.4	179770	*PTPRH*	3.65
Host cell factor C1 regulator 1 (XPO1 dependent)	HCF-1 beta-propeller interacting protein	16p13.3	279581	*HCFC1R1*	3.61
Anti-müllerian hormone	Gonadal development	19p13.3	112432	*AMH*	3.60
Thioredoxin reductase 1	Protection against oxidative stress	18q23	13 046	*TXNRD1*	3.48
Pituitary tumor-transforming 1 interacting protein	Facilitates the nuclear translocation of PTTG1	21q22.3	111126	*PTTG11P*	3.46
Thy-1 cell surface antigen	Tumor suppressor gene	11q22.3-q23	125359	*THY1*	3.45
Porcupine	Processing of Wnt proteins	Xp11.23	5326	*MG61*	3.45
KRAB zinc-finger protein KR18	Regulation of transcription	19q13.41	206882	*KR18*	3.43
Tropomyosin 2 (beta)	Structural constituent of muscle	9p13.2-p13.1	300772	*TPM2*	3.40
Similar to zinc-finger protein 268		19q13.42	209430	*LOC91664*	3.34
Thromboxane A2 receptor	Thromboxane A2 receptor activity	19p13.3	89 887	*TBXA2R*	3.34
Serine carboxypeptidase 1	Serine carboxypeptidase activity	17q23.2	106747	*SCPEP1*	3.31
Growth hormone receptor	Growth factor	5p13-p12	125180	*GHR*	3.27

Table 2 *Continued*

Description	Function	Map	UniGene	Gene	Ratio
Liver-specific bHLH-Zip transcription factor	Receptor activity	19q13.12	95 697	*LISCH7*	3.26
Brain abundant, membrane attached signal protein 1	Membrane bound protein	5p15.1-p14	79 516	*BASP1*	3.26
CCR4-NOT transcription complex, subunit 3	Transcription regulator activity	19q13.4	343571	*CNOT3*	3.24
Dynein, cytoplasmic, light polypeptide	Inhibition of NOS activity	12q24.23	5120	*PIN*	3.23
Plexin A1	Semaphorin receptor activity	3q21.3	334666	*PLXNA1*	3.23
Oxysterol binding protein-like 10	Intracellular lipid receptor	3p22.3	285123	*OSBPL10*	3.22
WD repeat domain 18		19p13.3	325321	*WDR18*	3.22
Leukocyte receptor cluster member 8	Receptor	19q13.42	348571	*LENG8*	3.21
Clone MGC:9381 IMAGE:3865583		19q13.3	76 277		3.19
Ovary-specific acidic protein		4q31.1	154140	*OSAP*	3.18
Stearoyl-CoA desaturase	Fatty acid biosynthesis	10q23-q24	119597	*SCD*	3.16
Cytochrome P450, subfamily IIB, polypeptide 6	Drug metabolism and cholesterol synthesis	19q13.2	1360	*CYP2B6*	3.14
7-dehydrocholesterol reductase	Endogenous cholesterol synthesis	11q13	11 806	*DHCR7*	3.14
3-hydroxy-3-methylglutaryl-coenzyme A reductase	Rate-limiting enzyme for cholesterol synthesis	5q13.3-q14	11 899	*HMGCR*	3.10
CNDP dipeptidase 2 (metallopeptidase M20 family)	Metallopeptidase activity	18q22.3	273230	*CNDP2*	3.09
Pro-oncosis receptor inducing membrane injury gene	Oncosis-like cell death	11q22.1	172089	*PORIMIN*	3.08
Aquaporin 2 (collecting duct)	Water channel	12q12	37 025	*AQP2*	3.07
Glutathione S-transferase A4	Cellular defense against toxic compounds	6p12.1	169907	*GSTA4*	3.07
Breast cancer anti-estogen resistance 1	Signal transduction	16q22-q23	273219	*BCAR1*	3.06
Nucleobindin 2	Calcium-binding EF-hand protein	11p15.1-p14	3164	*NUCB2*	3.05
Cytochrome P450, subfamily IIA, polypeptide 7	Drug metabolism and cholesterol synthesis	19q13.2	250615	*CYP2A7*	3.05
Mitogen-activated protein kinase 4	MAP kinase activity	18q12-q21	269222	*MAPK4*	3.04
Underexpressed genes					
Fatty acid binding protein 4, adipocyte	Fatty acid uptake, transport, and metabolism	8q21	83 213	*FABP4*	0.05
Cell adhesion molecule with homology to LICAM	Neural cell adhesion molecule	3p26.1	210863	*CHL1*	0.08
Urotensin 2	Vasoconstrictor	1p36	162200	*UTS2*	0.10
37 kDA leucine-rich repeat protein		7q22.1	155545	*P37NB*	0.11
Plasticity related gene 3	Lipid phosphate phosphatase activity	9q31.1	106825	*PRG-3*	0.14
Hypothetical protein MGC10981		4p16.1	115912	*MGC10981*	0.15
Estrogen-related receptor gamma	Orphan nuclear receptor	1q41	151017	*ESRRG*	0.16
Chemokine (C-X3-C motif) ligand 1	Chemokine activity	16q13	80 420	*CX3CL1*	0.19
Chromosome 20 open reading frame 103		20p12	22 920	*C20orf103*	0.20
Solute carrier family 25 member 15	L-ornithine transporter activity	13q14	78 457	*SLC25A15*	0.23
Growth differentiation factor 10	Cell growth and differentiation	10q11.22	2171	*GDF10*	0.25
Fatty-acid-coenzyme a ligase, long-chain 5	Long-chain-fatty-acid-CoA ligase activity	10q25	11 638	*FACL5*	0.26
Putative nuclear protein	Acidic repeat containing	Xq13.1	135167	*NAAR1*	0.29
Cathepsin Z	Tumorigenesis	20q13	252549	*CTSZ*	0.29
Interferon, gamma-inducible protein 30	Lysosomal thiol reductase	19p13.1	14 623	*IFI30*	0.30
Cytochrome P450, subfamily XIB, polypeptide 2	Aldosterone synthesis	8q21-q22	184927	*CYP11B2*	0.30
Secretin	Intestinal hormone	11p15.5	302005	*SCT*	0.31
Maternally expressed 3	Tumor suppressor	14q32	112844	*MEG3*	0.32
Neurotrimin	Cell adhesion, neuronal cell regonition	11q25	288433	*HNT*	0.32
Glutathione S-transferase M3 (brain)	Detoxification of electrophilic compounds	1p13.3	2006	*GSTM3*	0.33

Overexpression of the inhibin α-subunit in the cortisol-producing ACC recurrence (Fig. 2C) could have also contributed to the shift from mineralocorticoid to glucorticoid formation. The inhibin α-subunit is highly expressed in the fetal zone of the developing adrenal cortex and in the zona reticularis of adult adrenal cortex and tumors derived thereof. In this regard, the inhibin α-subunit has been found to stimulate cortisol and androgen secretion by antagonizing activin signaling through a dominant-negative effect (10).

In conclusion, this case of recurrent ACC, characterized by the sequential presentation of two endocrine syndromes, Conn's and Cushing's syndromes, demonstrates that adrenocortical cells can reverse their differentiation program during neoplastic progression and change their specialized hormone production, as a consequence of modifications in the expression profile of steroidogenic enzymes and cofactors. We hypothesize that this shift in steroid hormone secretion is a consequence of chromosome amplification induced by chemotherapy, even though we cannot exclude other molecular mechanisms, such as point mutations in steroidogenic enzymes or transcription factors, chromosomal translocations, and clonal progression of cells with different functional properties. Shift in endocrine activity has been also recently observed in a case of small-cell lung cancer treated by chemotherapy (11). Our findings, besides opening new perspectives to study adrenocortical cell plasticity and potential, demonstrate how conventional clinical and pathologic evaluation can be combined with genomic analysis to dissect thoroughly the biology of cancer.

Acknowledgements

This study was supported by grant no. RSF 168/04 from the Veneto Region and by funds from IOV (Istituto Oncologico Veneto) to G. Palù.

References

1 Barzon L, Fallo F, Sonino N, Daniele O & Boscaro M. Adrenocortical carcinoma: experience in 45 patients. *Oncology* 1997 **54** 490–496.
2 Arteaga E, Biglieri EG, Kater CE, Lopez JM & Schambelan M. Aldosterone-producing adrenocortical carcinoma. Preoperative recognition and course in three cases. *Annals of Internal Medicine* 1984 **101** 316–321.
3 Hisamatsu H, Sakai H, Irie J, Maeda K & Kanetake H. Adrenocortical carcinoma with primary aldosteronism associated with Cushing syndrome during recurrence. *BJU International* 2002 **90** 971–972.
4 Gicquel C, Bertagna X, Gaston V, Coste J, Louvel A, Baudin E, Bertherat J, Chapuis Y, Duclos JM, Schlumberger M, Plouin PF, Luton JP & Le Bouc Y. Molecular markers and long-term recurrences in a large cohort of patients with sporadic adrenocortical tumors. *Cancer Research* 2001 **61** 6762–6767.
5 Fallo F, Pezzi V, Barzon L, Mulatero P, Veglio F, Sonino N & Mathis JM. Quantitative assessment of *CYP11B1* and *CYP11B2* expression in aldosterone-producing adenomas. *European Journal of Endocrinology* 2002 **147** 795–802.
6 Hoffman SMG, Nelson DR & Keeney DS. Organization, structure and evolution of the *CYP2* gene cluster on human chromosome 19. *Pharmacogenetics* 2001 **11** 687–698.
7 McFadyen MC, Melvin WT & Murray GI. Cytochrome P450 enzymes: novel options for cancer therapeutics. *Molecular Cancer Therapy* 2004 **3** 363–371.
8 Dohna M, Reincke M, Mincheva A, Allolio B, Solinas-Toldo S & Lichter P. Adrenocortical carcinoma is characterized by a high frequency of chromosomal gains and high-level amplifications. *Genes, Chromosomes and Cancer* 2000 **28** 145–152.
9 Rainey WE, Carr BR, Sasano H, Suzuki T & Mason JI. Dissecting human adrenal androgen production. *Trends in Endocrinology and Metabolism* 2002 **13** 234–239.
10 Beuschlein F, Looyenga BD, Reincke M & Hammer GD. Role of the inhibin/activin system and luteinizing hormone in adrenocortical tumorigenesis. *Hormone and Metabolic Research* 2004 **36** 392–396.
11 Mayer S, Cypess AM, Kocher ON, Berman SM, Huberman MS, Hartzband PI & Halmos B. Uncommon presentations of some common malignancies. Case 1. Sequential paraneoplastic endocrine syndromes in small-cell lung cancer. *Journal of Clinical Oncology* 2005 **23** 1312–1314.

Received 16 May 2005
Accepted 26 July 2005