פתוגנזה מולקולרית של אדרנוקורטיקל קרצינומה

ענת יפה

אנדוקרינולוגיה

הלל יפה

Weiss, MacFralane

- Histopathology combined with immuno-histochemistry (IHC) of ACTs is often adequate to provide highly informed diagnosis

molecular methods

- Diagnosis post/pre-op. -benign Vs malignant ACTs.
- Prognosis
- Pathophysiology

- Reliably? Accurate?

CLONAL ANALYSIS OF ACTs

Knudson hypothesis:

•Multistep process with an initiating event followed by events that result in tumor progression.

•The initiating event - mutation in a single cell that confers a growth advantage

•Resulting - monoclonal proliferation of that cell and cancer formation

Three clonal composition studies:

- 60%– 100% of ACCs are monoclonal
- 77.4%–100% of adrenal hyperplasias are polyclonal
- 12.5%– 43% of ACAs are polyclonal

Immunohistochemical Approaches

Detecting unique protein

- mib1/Ki-67 labeling index, topoisomerase 2 alpha labeling index, IGF2 protein, murine
- double minute 2, p21, p27, cyclin D1, Bcl-2,

- IGF2 IHC, 21/22 ACAs [-] vs 13/17 ACCs [+] specificity-95.5%, sensitivity-76.5%
- MIB1, 21 of 22 ACAs [-] vs 14 of 16 ACCs [+] specificity-95.5%, sensitivity-87.5%
- **Combining IGF2 + MIB1 IHC:** sensitivity-100%, specificity-95.5% in differentiating ACCs from ACAs

Diagnosis

MMP2-matrix metalloproteinase type 2 =gelatinase A

- IHC; MMP2 50 ACCs vs. 50 ACAs
  1/50 (2%) ACAs vs. 37/50 (74%) ACCs [+]
  focal vs. diffuse –associated [-] DFS

- MMP2; mRNA in situ hybridization 16 ACCs vs 14 ACAs
  [+] 13/16 (81%) ACCs 1/14 (7%) ACAs
  But, MMP2 mRNA was in surrounding stromal tissue and not in the neoplastic cell itself

- Serum MMP2 not useful in predicting either ACC or ACA

Mod Pathol 2006; 19:1563–1569,
World J Surg 1999;23:237–242,
Endocr Regul 2001;35:9 –16

SF1- Steroidogenic factor 1

- SF-1 maps to 9q33.3
- SF1 knockout mice -died on postnatal day 8 , adrenal- insufficiency, no adrenal
- SF1 heterozygous mice, adrenal=insufficiency
- In CGH studies 10/11 pediatric ACTs, 9q34 amplification
- Fluorescence in situ hybridization, 8/10 pediatric ACTs confirm higher # SF1 copy
- SF1 protein levels, however, were noted to be higher in all ACTs than in the normal
- **IHC with SF1 can not differentiate ACCs & ACAs**
- **Useful in distinguishing between primary ACC and metastasis from other sites**

VEGF-Vascular endothelial growth factor

- VEGF- increased in the majority of cancers & corr. poorer outcome

- EILSA; [+] corr, 18 ACAs, 12 transitional tumors, 13 ACCs
  !no corr. to tumor weight.
  !VEGF [+] corr. recurrence, in transitional or ACCs

- Serum VEGF levels, no corr. ACCs vs. ACAs

- **VEGF, not integrated into clinical practice as a molecular marker**

JCEM 2000;85:4734 – 4741

Genotyping Methods

Detecting DNA

- Chromosomal abnormalities, rearrangements, point mutation

- To understand the pathogenesis of ACTs
- To differentiate benign ACA & malignant tumors ACC

Genotyping Methods

Comparative Genomic Hybridization

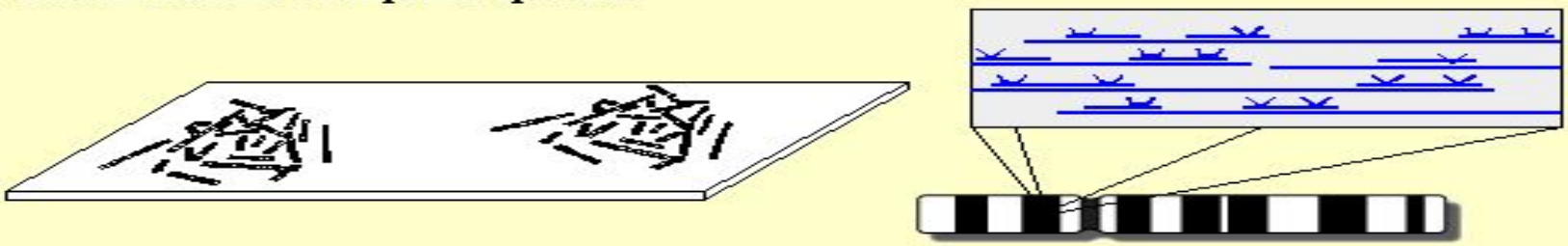
- CGH-a powerful tool for detecting genetic aberrations in tumors
- CGH potentially identifies regions that contain either oncogenes (regions of copy number gain) or tumor suppressor genes (regions of copy number loss)
- DNA copy losses identified on CGH corr. with LOH studies on the sub-chromosomal level

1. Labeling of genomic tumor DNA and normal genomic control DNA by Nick translation

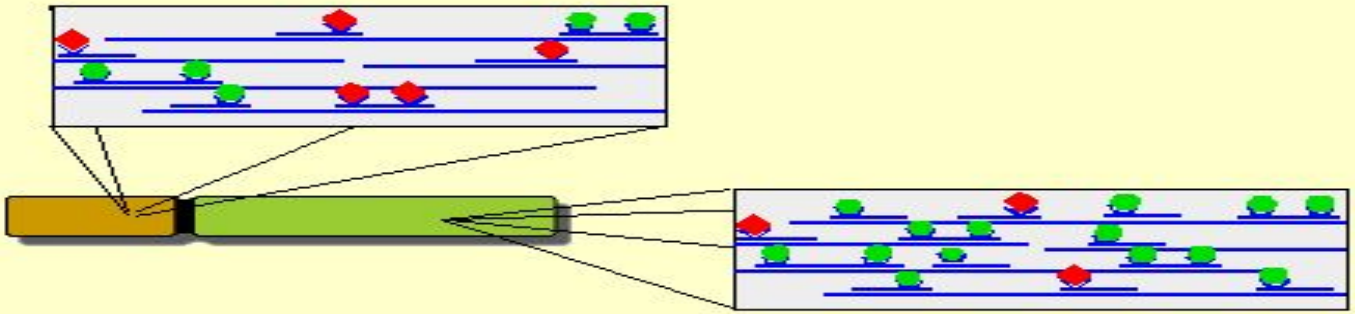
Biotin-labeled tumor DNA

Digoxigenin-labeled control DNA

2. Simultaneous hybridization of differentially labeled tumor and control DNAs to normal human metaphase spreads



3. Fluorescence detection of the hybridized DNAs



4. Result

balanced DNA content

overrepresentation of the whole chromosome within the tumor DNA

underrepresentation of the long arm within the tumor DNA

high level amplification

Genotyping Methods

Comparative Genomic Hybridization

- To date, 4 CHG studies performed on adult ACTs
- There were more copy # changes in ACCs than in ACAs,
- 7.6 –14 changes in ACCs vs 1.1–2 changes in ACAs
- Smaller ACAs were less likely to have genetic abnormalities, and chromosomal changes # corr. tumor size.
- In ACCs, # genetic changes corr. tumor size
- **The CGH data support the theory of an adenoma-to-carcinoma progression because there are more chromosomal changes in ACCs than in ACAs and the number of these changes increases tumor size**

- CGH studies have not yet yielded a reliable and accurate genotype that can be clinically exploited to assist in the diagnosis of these tumors.

Robinson BG, JCEM 2002 Jul;87(7):3467-74

Results of CGH Studies of Adrenal Cortical Carcinoma

Table 1. Results of CGH Studies of Adrenal Cortical Carcinoma

Locus of gain or loss	Sidhu [14] (n = 13)	Kjellman [13] (n = 8)	Zhao [15] (n = 12)	Dohna [12] (n = 14)
1p loss	62%	13%	67%	14%
2q loss	31%	38%	42%	0%
4p gain	31%	38%	25%	21%
4q gain	31%	50%	0%	7%
5p gain	46%	50%	25%	57%
5q gain	38%	50%	33%	50%
11q loss	31%	50%	42%	0%
12p gain	38%	13%	17%	43%
12q gain	38%	13%	50%	86%
17p loss	54%	50%	8%	0%
19 gain	31%	38%	0%	43%
19 loss	23%	0%	0%	0%
22 loss	38%	38%	0%	7%

Giordano, Endocrine pathology 2006 17: 14

Genotyping Methods

Single Marker Genotyping

- identification of cancer-related genes; genes responsible for familial cancer syndromes

Two syndromes -ACC is a common

- Beckwith-Wiedemann syndrome (BWS)
- Li–Fraumeni syndrome

Beckwith-Wiedemann syndrome

- An overgrowth disorder associated several tumor types

- linked to the chromosomal region 11p15.5,
- imprinted region that contains several genes including H19, KIP2, and **IGF2**.
- I**GF2** -rearrangements, mostly paternal isodisomy

- **IGF2** – familial & sporadic ACC , increased **IGF2** expression
- I**GF2** – rearrangements, restricted to ACCs

Li–Fraumeni syndrome

- High occurrences of breast cancer, brain tumors, acute leukemia, soft tissue, sarcomas, bone sarcomas & ACC
- Linked to mutations of the TP53 gene, 17p13 locus
- TP53 mutations were found in 20–67% of ACCs, rare in ACAs

Table 1. Summary of hereditary tumor syndromes associated with ACTs

Hereditary tumor syndrome	Gene (chromosomal locus)	Manifestation of tumor syndrome	Prevalence of ACTs
Li-Fraumeni syndrome	*TP53* (17p13), *hCHK2* (22q12.1), 1q23	Soft tissue sarcoma, osteosarcoma, breast cancer, brain tumor, leukemia, ACC	ACC, 3%–4%
Beckwith-Wiedemann syndrome	*IGF2, H19, CDKN1C, KCNQ1* (11p15)	Exomphalos, macroglossia, gigantism, ACC, nephroblastoma, hepatoblastoma, rhabdomyosarcoma	ACC, 5%
Carney complex	*PRKAR1A* (17q23-q24) 2p16	Cardiac, endocrine, cutaneous, and neural myxomatous tumors, and pigmented lesions of the skin and mucosa	PPNAD, 90%–100%
Multiple endocrine neoplasia 1	*MEN1* (11q13)	Parathyroid, pancreatic islet cell, anterior pituitary and ACTs	ACT, 55%; ACC, rare
Congenital adrenal hyperplasia	*CYP21B* (6p21.3)—most common, *CYP11B*, *CYP17A*, *HSD3B2*	Adrenal hyperplasia, virilization, salt-wasting	Adrenal tumors, 82%; hyperplasia, 100%

Abbreviations: ACA, adrenocortical adenoma; ACC, adrenocortical carcinoma; ACT, adrenocortical tumor; PPNAD, primary pigmented nodular adrenocortical disease.

The oncologist 2008: 13:548-561

Table 2. Summary and evidence of genes involved in sporadic ACTs

Gene (chromosomal locus)	Evidence of involvement in sporadic ACTs
TP53 (17p13)	Mutation of *TP53* found in 20%–27% of ACCs and 0%–6% of ACAs [52, 53]; 17p13 LOH occurs in up to 87.5% of ACCs and up to 30% of ACAs [63, 105, 106]
IGF2 (11p15)	Overexpression of IGF2 mRNA in ACCs compared with ACAs [63, 67–69, 134]; 11p15 LOH occurs in up to 83% of ACCs and 34% of ACAs [62, 63]
PRKAR1A (17q23-q24)	LOH of 17q22–24 occurs in 53% of ACCs and 23% of ACAs; mutation of *PRKAR1A* occurs in 10% of ACAs and not in ACCs [76]
MEN1 (11q13)	LOH of 11q13 occurs in 100% of ACCs and 25% of ACAs [73, 74, 107]; *MEN1* mutation occurs in 7% of ACCs and ACAs [73, 74]
GNAS (20q13.2)	Mutation of *GNAS* occurs in ACAs and tumors of patients with AIMAH [80, 81]

Abbreviations: ACA, adrenocortical adenoma; ACC, adrenocortical carcinoma; ACT, adrenocortical tumor; AIMAH, adrenocorticotropic hormone–independent macronodular adrenocortical hyperplasia; LOH, loss of heterozygosity.

IGF2–transgenic mice :

- Weights of the adrenal glands higher, hyperplasia of the zona fasciculata.
- 18-month period, transgenic mice did not develop tumors in their adrenal glands
- Overexpression of IGF2 alone is insufficient to cause ACT formation, other factors are required for tumorigenesis

Genotyping Methods

Molecular Profiling Studies

Pitfalls

- Different types of mutations can inactivate /activate the same gene
- Point mutations can be distributed across large genes
- Translocations & amplifications

Gene expression in tissues

- Commercially available DNA microarrays

Diagnosis, prognosis, therapeutic targets

Genotyping Methods

Molecular Profiling Studies

Pitfalls

- low incidence of ACC, small # samples
- different microarray platforms
- different software and algorithms to analyze the data
- different significance level cutoffs
- **Contamination with normal adrenocortical, medullary, or stromal tissue**

Molecular Profiling Studies

- **A first microarray paper**:
Small cohort of normal adrenal cortex, ACAs, & ACCs, 2400 genes set
-robust list of differentially expressed genes

- **A second microarray paper:** larger set of tumors, but a 230 genes set

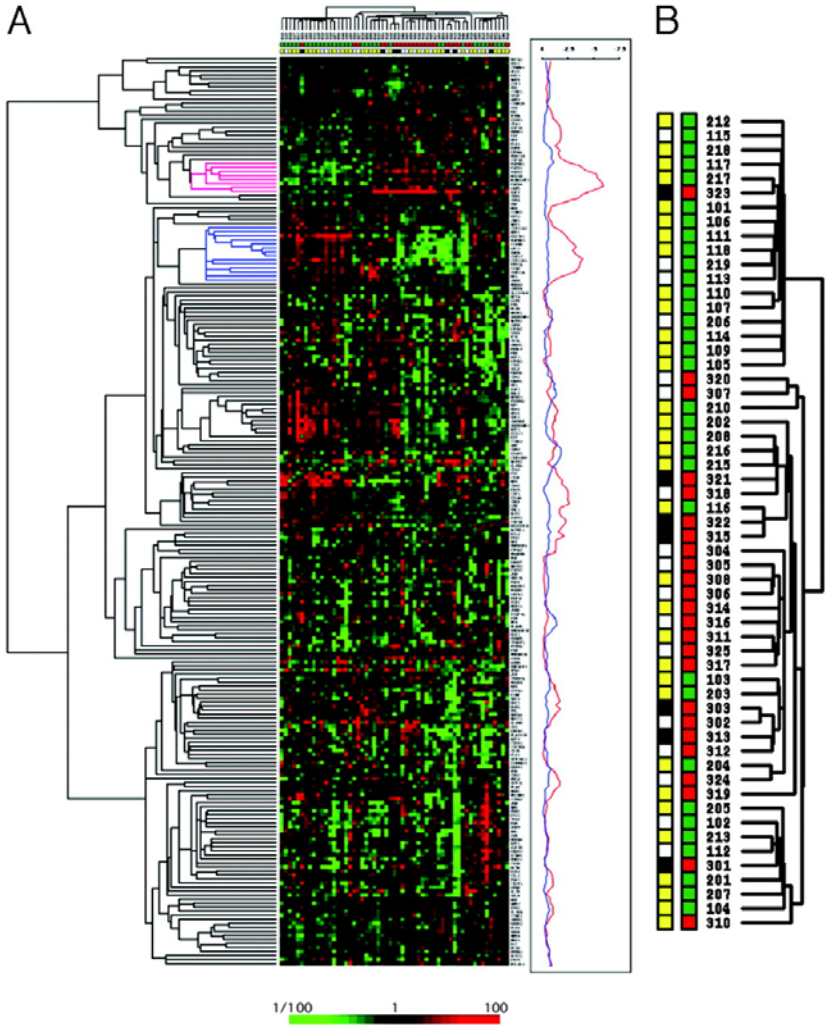
Giordano TJ, Am J Pathol 162:521–531, 2003
de Fraipont F, JCEM 2005;90:1819 –1829.

Table 3. Genes that were significantly differentially expressed in ACCs compared with ACAs in five microarray gene-profiling studies

Study	Samples	Upregulated genes	Downregulated genes
Giordano et al. [67]	11 ACCs, 4 ACAs, 1 macronodular hyperplasia, 3 normal adrenal cortices	*IGF2*; ubiquitin carrier protein E2-C (*UBCH10*); *KIAA0101*; secreted phosphoprotein 1 (*SPP1*); chromosome 20 open reading frame 1 (*C20ORF1*)	Alcohol dehydrogenase 1 (*ADH1*); *ADH2*; tropomodulin (*TMOD*); stromal cell–derived factor 1 (*SDF1*); *KIAA1024*
de Fraipont et al. [68]	24 ACCs (Weiss score ≥4); 33 ACAs (Weiss score <4)	*IGF2*; *TGFβ2*; *FGFR1*; *FGFR4*; macrophage stimulating 1 receptor (*MST1R*); *TGFBR1*; KCNQ1 overlapping transcript 1 (*KCNQ1OT1*); glyceraldehyde-3-phosphate dehydrogenase (*GAPD*)	Steroidogenic acute regulatory protein (StAR) Cytochrome *CYP11A*; hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 1 (*HSD3B1*); *CYP11B1*; *CYP21A2*; *CYP17*; protein phosphatase 1, catalytic subunit, alpha isoform (*PP1A*); S100 calcium binding protein B (*S100B*); glypican 3 (*GPC3*); inhibin α-chain (*INHA*); cAMP response element modulator (*CREM*); retinoblastoma 1 (*RB1*); nonmetastatic protein 23 (*NM23H5*); *TGFB3*

adult ACCs versus ACAs,

FIG. 1. Expression patterns of 230 genes in 57 human sporadic adrenocortical tumors



de Fraipont, F. et al. J Clin Endocrinol Metab 2005;90:1819-1829

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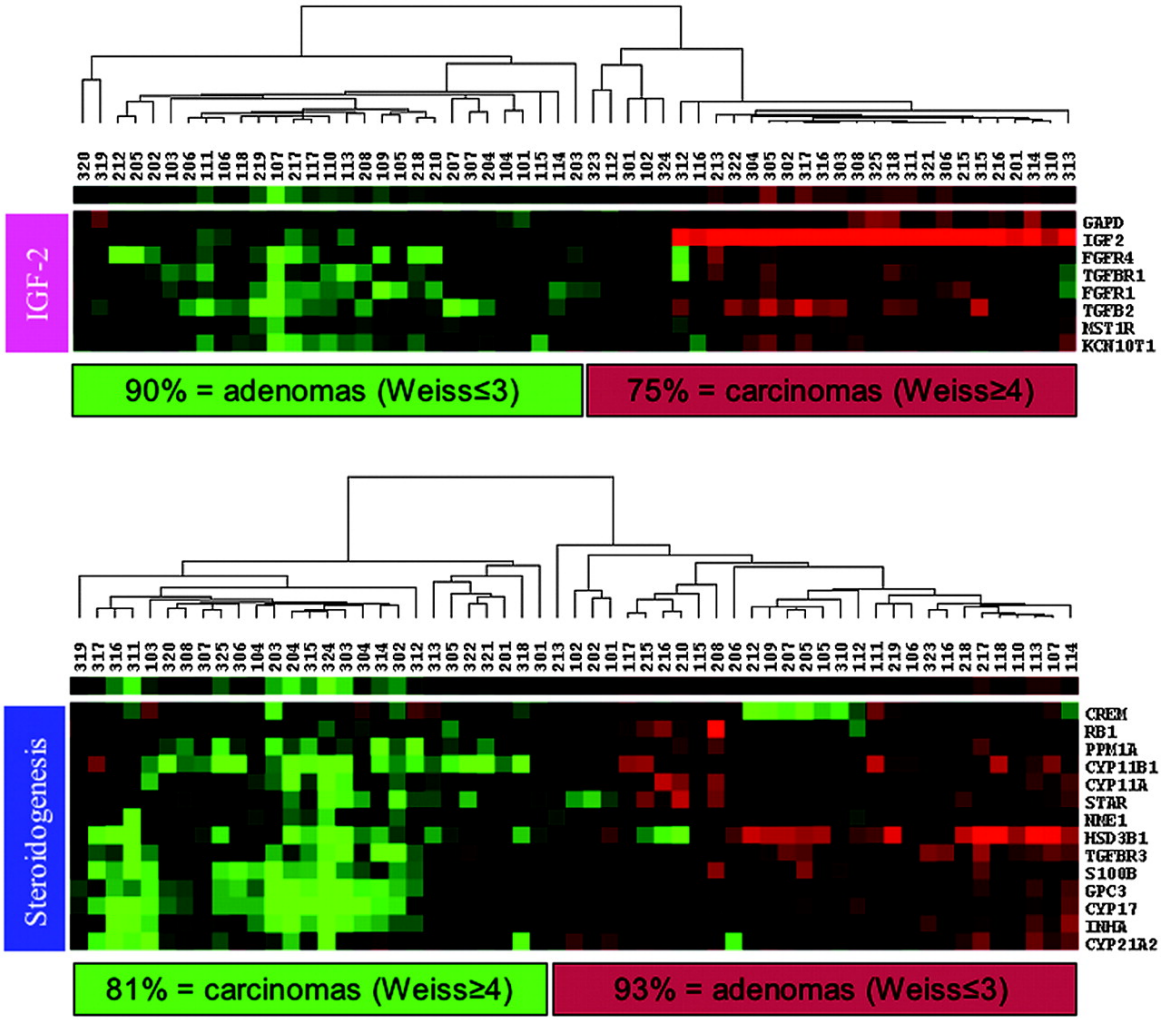
FIG. 2. Classification of adrenocortical tumors using specific gene clusters



de Fraipont, F. et al. J Clin Endocrinol Metab 2005;90:1819-1829

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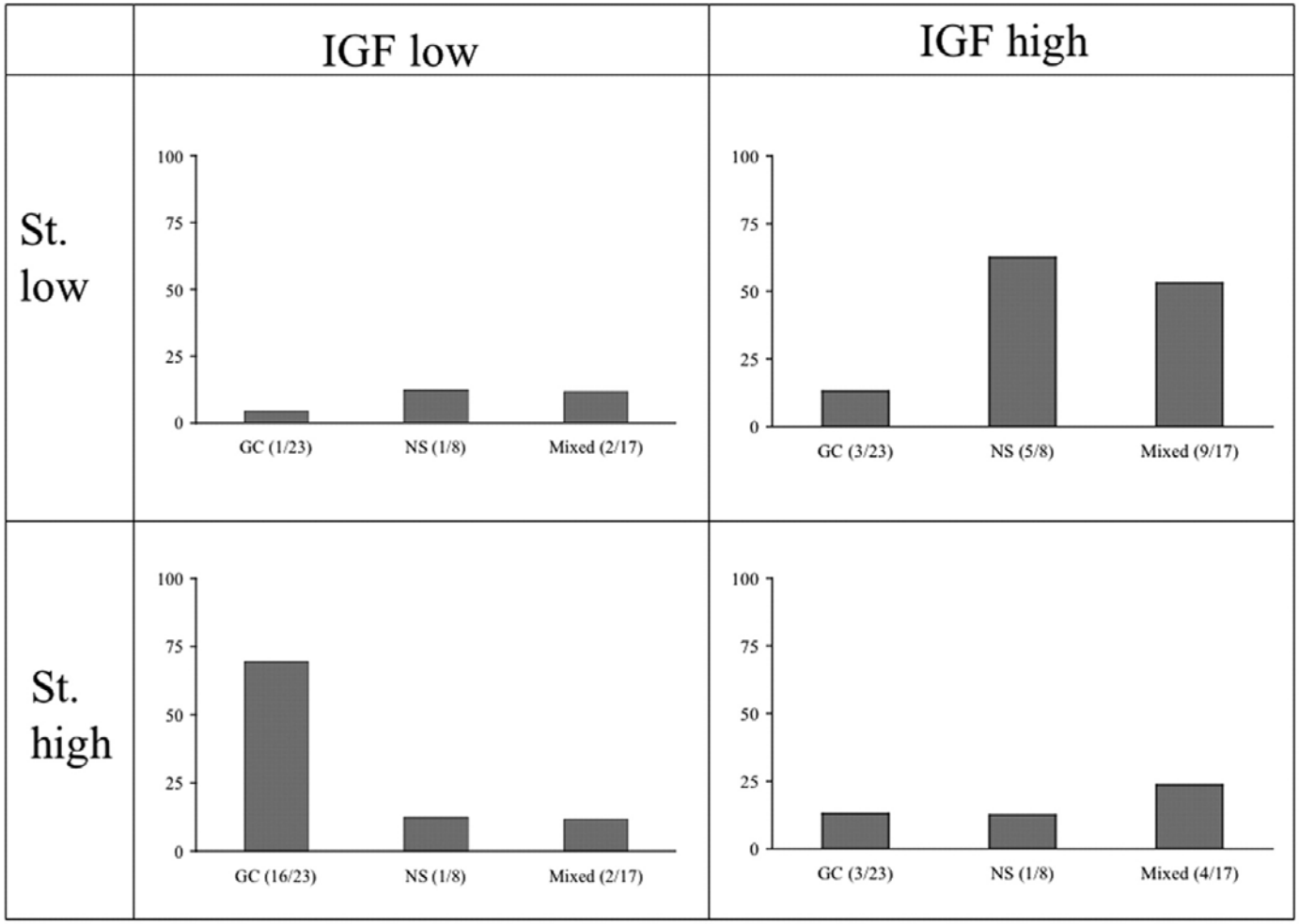
FIG. 5. Correlation between the steroid secretion profiles and the gene expression profiles of adrenocortical tumors



de Fraipont, F. et al. J Clin Endocrinol Metab 2005;90:1819-1829

The Journal of Clinical Endocrinology & Metabolism

Molecular Profiling Studies

- **A second microarray paper:** larger set of tumors, but a 230 genes set

- IGF2 gene–related cluster -8 genes-
- 90%, low expression of the IGF2 gene–related cluster were ACAs
- 75% of tumors with high expression of these genes were ACCs
- Steroidogenesis cluster -14-gene- could identify ACAs with high accuracy

IGF2 gene–related cluster , select the subgroup of ACCs who were at a high risk for recurrence and who would therefore benefit from adjuvant therapy

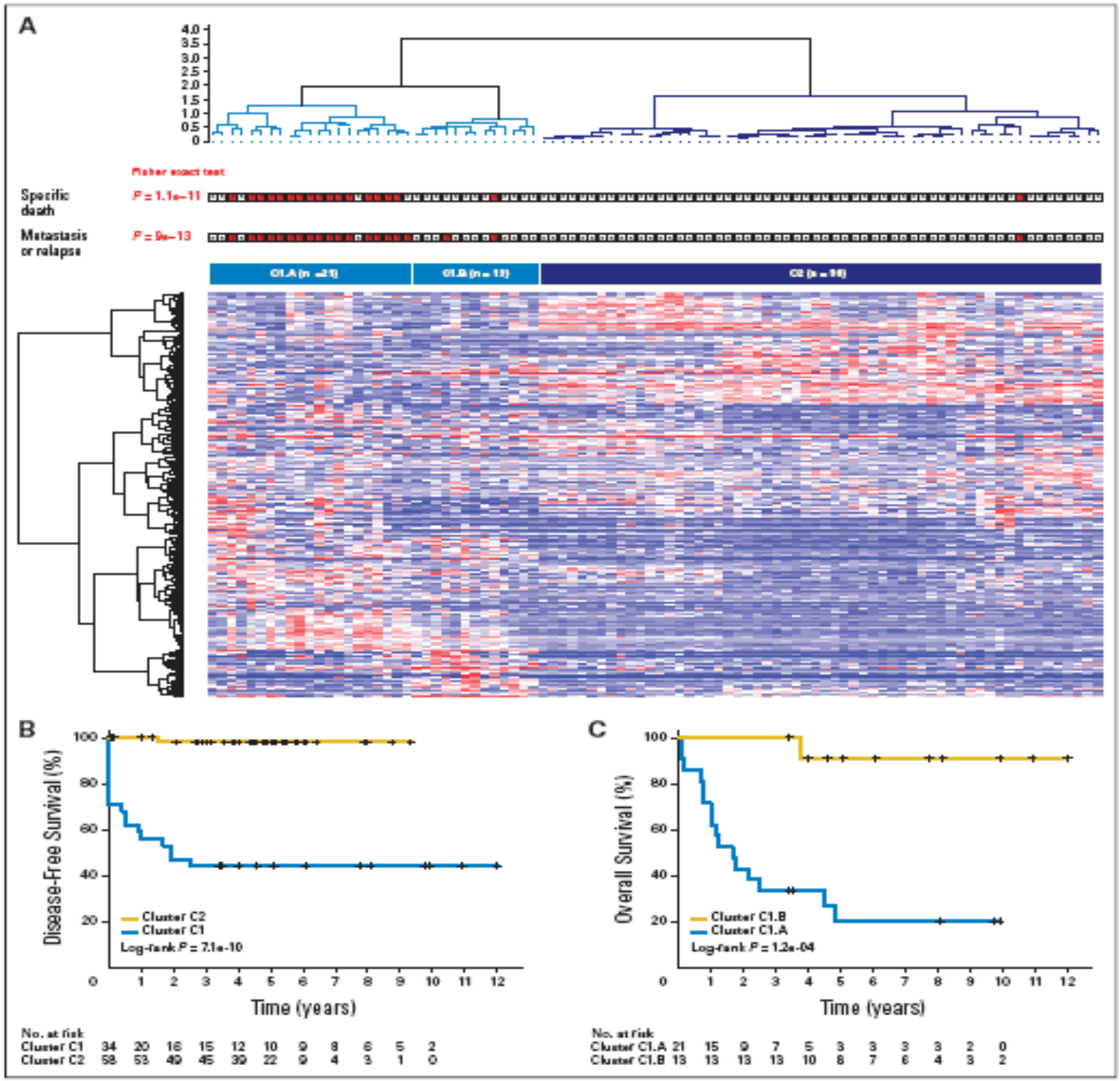
- Analyzing a sub-group of 40 tumors with follow-up data showed that either the IGF2 or steroidogenesis cluster of genes alone was not as effective as the Weiss score in terms of predicting malignancy and postoperative recurrence

Giordano TJ, Am J Pathol 162:521–531, 2003
de Fraipont F, JCEM 2005;90:1819 –1829.

Table 3. Genes that were significantly differentially expressed in ACCs compared with ACAs in five microarray gene-profiling studies

Velázquez et al. [135] adult ACCs versus ACAs,	7 ACCs, 13 ACAs	Ubiquitin specific peptidase 4 (*USP4*); ubiquitin fusion degradation 1 like (*UFD1L*); inositol polyphosphate phosphatase-like 1 (*INPPL1*); aquaporin 3 (*AQP3*); H3 histone, family 3B (*H3F3B*)	Chemokine (CXC motif) ligand 10 (*CXCL10*); retinoic acid receptor responder 2 (*RARRES2*); aldehyde dehydrogenase 1 family, member A1 (*ALDH1A1*); cytochrome b reductase 1 (*CYBRD1*); glutathione S-transferase A4 (*GSTA4*)
Slater et al. [69] adult ACCs versus ACAs,	10 ACCs, 10 ACAs	Cathepsin H (*CTSH*); mucolipin 3 (*MCOLN3*); *FGFR1*; aldo-keto reductase family 1, member C1 (*AKR1C1*); fibronectin 1 (*FN1*)	*MGC5306*; cytoplasmic FMR1 interacting protein 2 (*CYFIP2*); Purkinje cell protein 4 (*PCP4*); glutaminyl-peptide cyclotransferase (*QPCT*); paralemmin (*PALM*)
West et al. [134] pediatric ACCs versus ACAs.	18 ACCs, 5 ACAs, 1 indeterminate ACT	Thyroid hormone receptor interactor (*TRIP*); delta-like 3 (*DLL3*); *FLJ22814*; dual oxidase 2 (*DUOX2*); *FLJ10458*	Phenylalanine hydroxylase (*PAH*); major histocompatibility complex, class II, DR alpha (*HLA-DRA*); pleiomorphic adenoma gene-like 1 (*PLAGL1*); *CYP11B1*; *HLA-DPA1*

Velázquez-Fernandez D, Surgery 2005;138:1087–1094,
Slater EP, Eur J Endocrinol 2006;154:587–598,
West AN, Cancer Res 2007;67:600 – 608



153 ACT
microarray (n= 92)
RT-qPCR (n=148)

1993-2005
746 probe sets

ACA vs. ACC

Malignant ACC vs. Less Malignant

C1A-[21]
C1B-[13]
C2- [58]

de Reynies A, Bertherat J
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Molecular Profiling Studies

153 ACT microarray (n = 92) or RT-qPCR(n = 148) ; 1993-2005
746 probe sets
ACA vs. ACC

A two-gene predictor of malignancy was built using DFS as the end point in a training cohort (n = 47), then validated in an independent validation cohort (n = 104)

ACC

A two-gene predictor of survival was built using the OS as the end point in a training cohort (n = 23), then tested in an independent validation cohort (n = 35).

- Unsupervised clustering analysis discriminated robustly the malignant and benign tumors, and identified two groups of malignant tumors with very different outcome.
- The combined expression of DLG7 and PINK1 was the best predictor of DFS, overcome the uncertainties of intermediate pathological Weiss scores, and remained significant after adjustment to the Weiss score
- Among the malignant tumors, the combined expression of BUB1B and PINK1 was the best predictor of OS and remained significant after adjusting for MacFarlane staging
- Gene expression analysis unravels two distinct groups of adrenocortical carcinomas.

Molecular predictors of malignancy and of survival are reliable and provide valuable

Therapeutic ?

Preclinical Targeting of the Type I Insulin-Like Growth Factor Receptor in Adrenocortical Carcinoma

Objective: The objective of the study was to profile human adrenal tumors and ACC cell lines to assess activated IGF signaling and determine the efficacy of two IGF receptor (IGF-1R) antagonists alone and in combination with mitotane.

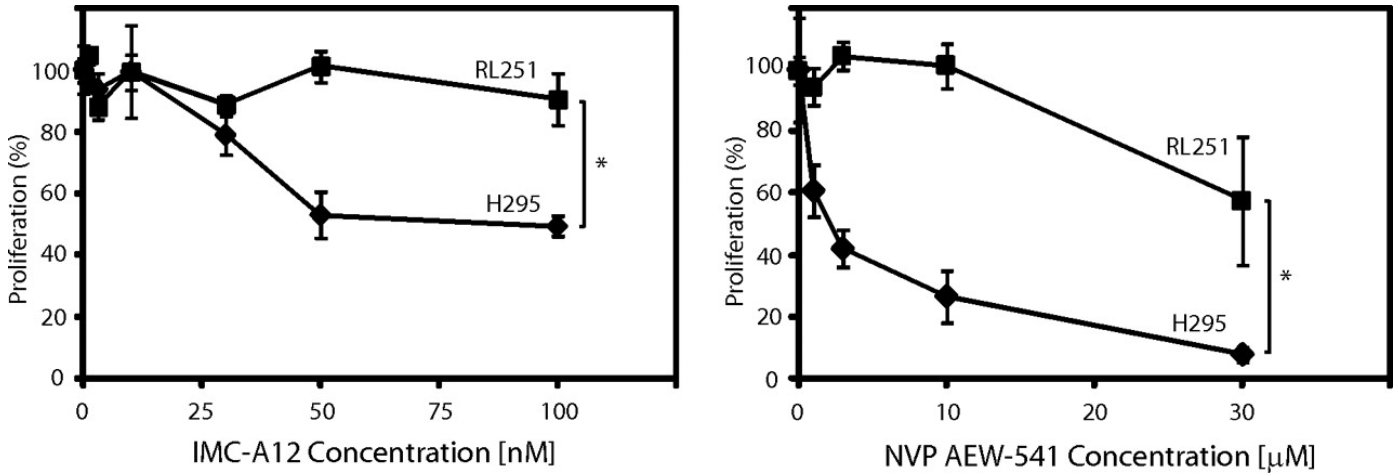
Experimental Design: ACC cell lines that display or lack activated IGF signaling are used to assess the effects of two IGF-1R antagonists in cultured cells and ACC xenograft tumors

•The majority of ACC cell lines tested display constitutive IGF ligand production and activation of downstream effector pathways

•Both IGF-1R antagonists cause significant dose-dependent growth inhibition in ACC cell lines

•Furthermore, we observe that mitotane, the first-line adrenolytic drug used in patients with ACC, results in enhanced growth inhibition when used in combination with the IGF-1R antagonists

FIG. 4. Antiproliferative effects of IGF-1R antagonist treatments in vitro



Barlaskar, F. M. et al. J Clin Endocrinol Metab 2009;94:204-212

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FIG. 5. Targeted inhibition of tumor growth in vivo

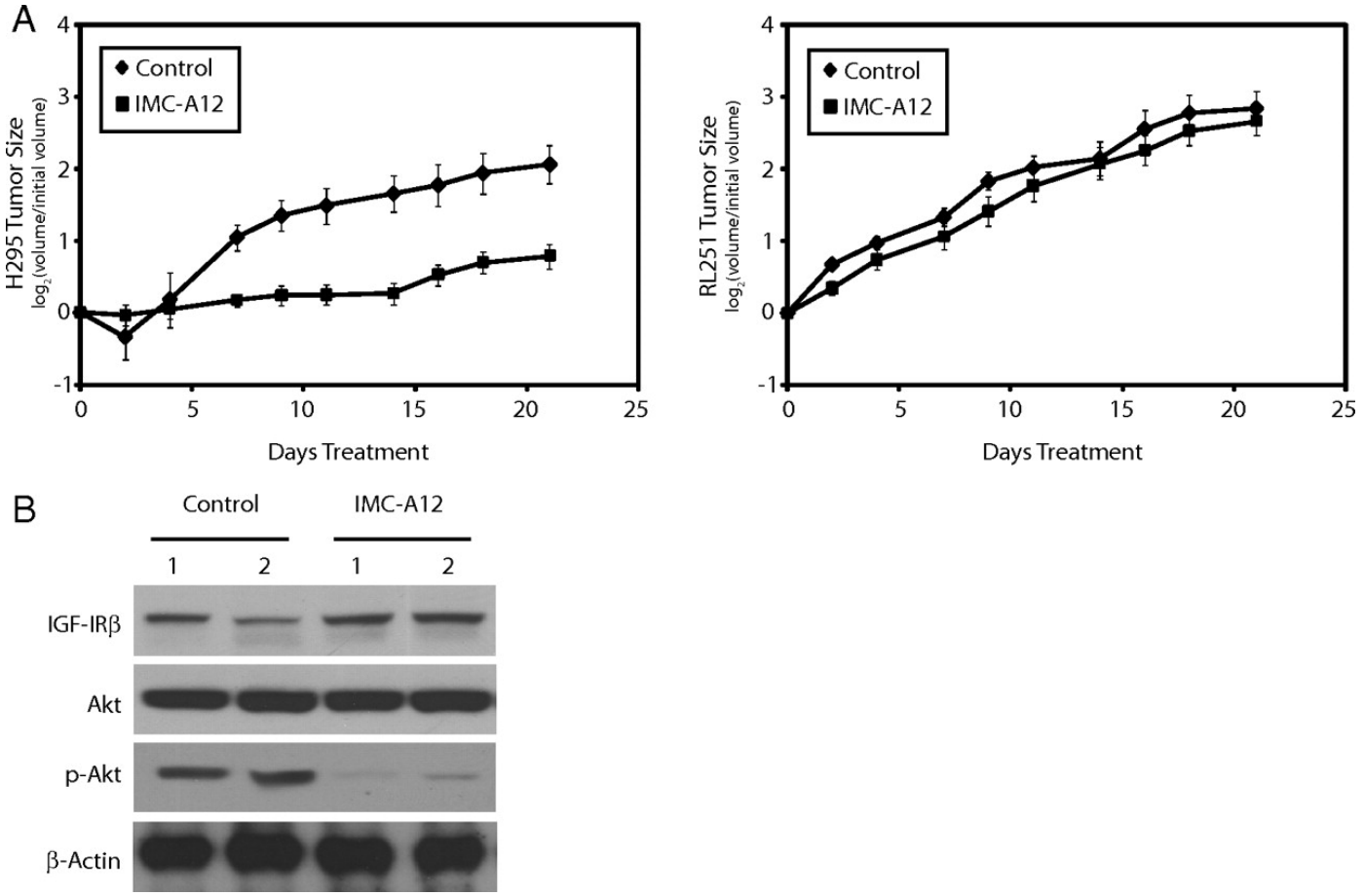

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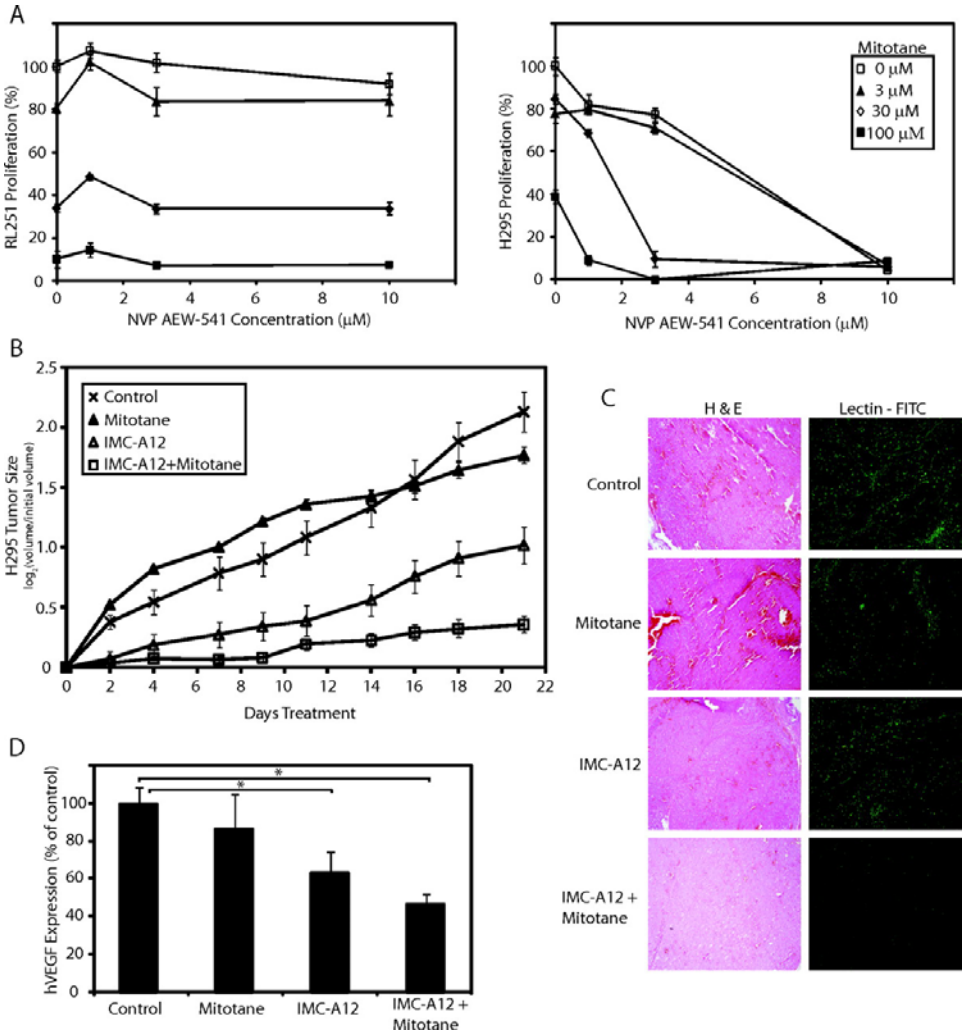
FIG. 6. IGF-1R antagonists enhance the inhibitory effects of mitotane



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