FBXW7/hCDC4 Is a General Tumor Suppressor in Human Cancer

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Abstract

The ubiquitin-proteasome system is a major regulatory pathway of protein degradation and plays an important role in cellular division. Fbxw7 (or hCdc4), a member of the F-box family of proteins, which are substrate recognition components of the multisubunit ubiquitin ligase SCF (Skp1-Cdc53/Cullin-F-box-protein), has been shown to mediate the ubiquitin-dependent proteolysis of several oncoproteins including cyclin E1, c-Myc, c-Jun, and Notch. The oncogenic potential of Fbxw7 substrates, frequent allelic loss in human cancers, and demonstration that mutation of *FBXW7* cooperates with p53 in mouse tumorigenesis have suggested that Fbxw7 could function as a tumor suppressor in human cancer. Here, we carry out an extensive genetic screen of primary tumors to evaluate the role of *FBXW7* as a tumor suppressor in human tumorigenesis. Our results indicate that *FBXW7* is inactivated by mutation in diverse human cancer types with an overall mutation frequency of ~6%. The highest mutation frequencies were found in tumors of the bile duct (cholangiocarcinomas, 35%), blood (T-cell acute lymphocytic leukemia, 31%), endometrium (9%), colon (9%), and stomach (6%). Approximately 43% of all mutations occur at two mutational "hotspots," which alter Arg residues (Arg^{465} and Arg^{479}) that are critical for substrate recognition. Furthermore, we show that $Fbxw7^{Arg465}$ hotspot mutant can abrogate wild-type Fbxw7 function through a dominant negative mechanism. Our study is the first comprehensive screen of *FBXW7* mutations in various human malignancies and shows that *FBXW7* is a general tumor suppressor in human cancer. [Cancer Res 2007;67(19):9006–12]

Introduction

The ubiquitin-proteasome system regulates important cellular processes including differentiation and development, apoptosis, protein trafficking, the immune and inflammatory responses, cell cycle progression, and cellular division (1). The latter processes are primarily regulated by two ubiquitin ligases known as the anaphase-promoting complex (APC) and SCF. SCF ubiquitin ligases are composed of Cul1, Rbx1 (also called Roc1 or Hrt1), and Skp1 bound to a member of the F-box protein family, which provide substrate specificity. Approximately 65 F-box proteins exist in humans, each characterized by an ~40-amino-acid F-box motif that associates with the SCF complex through Skp1 (1).

In *S. cerevisiae*, the F-box protein Cdc4 plays a critical role in cell cycle control by mediating the ubiquitin-dependent proteolysis of DNA replication protein Cdc6 and cyclin-dependent kinase (Cdk) inhibitors Far1 and Sic1 (2, 3). Similarly, the orthologue of Cdc4 in humans, designated Fbxw7 (also known as hCdc4, Fbw7, or SEL-10), mediates the ubiquitin-dependent proteolysis of several key regulatory proteins involved in cell division and cell fate determination, including cyclin E1, c-Myc, c-Jun, Notch, and Aurora-A (2). Furthermore, *FBXW7* deficiency in mice has been shown to result in early embryonic lethality (day ~10.5) with embryos exhibiting abnormalities in hematopoietic and vascular development (2).

Several lines of evidence suggest that Fbxw7 is a putative tumor suppressor in human tumorigenesis, including (*a*) the well-defined oncogenic potential of its putative substrates (described above); (*b*) localization of *FBXW7* to chromosome 4q31.3, which is deleted in ~30% of human cancers; (*c*) the finding that allelic loss of *FBXW7* cooperates with p53 in tumorigenesis in mice (4); and (*d*) demonstration that targeted disruption of *FBXW7* in cultured cells leads to an increase in genetic instability (5), a hallmark of human cancers.

In this study, we carry out an extensive genetic analysis of *FBXW7* in primary human tumors of diverse tissue origin to determine its role as a putative tumor suppressor. We find that *FBXW7* is mutated in a variety of human tumor types with an overall mutation frequency of ~6%. Furthermore, we show that expression of an Fbxw7 mutant corresponding to one of the major mutational hotspots in primary tumors interferes with wild-type Fbxw7 function, suggesting a potential dominant negative mechanism of Fbxw7 inactivation. Our results show that *FBXW7* is a general tumor suppressor in human tumorigenesis and provide insight into how Fbxw7 function is inactivated during tumorigenesis.

Materials and Methods

Tumor specimens. Primary tumor specimens (534 in total) were obtained from (*a*) Department of Obstetrics and Gynecology, Innsbruck Medical University, Innsbruck, Austria (breast and ovarian tumor specimens);

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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***FBXW7* mutation screening.** Screening for *FBXW7* mutations was done as previously described (6).

Methylation analysis. Methylation status of mutational hotspot 1435-6 was determined by bisulfite sequencing as described (7). PCR amplifications were done with primers 5′-TGTGGAATGTAGAGATTGGAGAATGTATA-3′ and 5′-AAAAAATCCCAACCATAACAAAATTT-3′.

Antibodies. Antibodies used in this study include anti-Flag (Sigma), anti-Skp1 (Lab Vision), anti–cyclin E1 (HE12, Santa Cruz Biotechnology), anti–phospho-T380 cyclin E1 (Santa Cruz Biotechnology), anti-Myc (9E10, Santa Cruz Biotechnology), anti–glutathione *S*-transferase (GST; Santa Cruz Biotechnology), anti-actin (Santa Cruz Biotechnology), anti-p53 (DO-1, Santa Cruz Biotechnology), and anti-p21 (Ab-1, Calbiochem). Precipitation experiments were done using anti-Flag agarose (Sigma) or Glutathione-Sepharose (GE Healthcare). Immunohistochemical analysis of p21 and p53 was done as described (8). Analysis of ploidy was determined by Feulgen staining of histopathologic sections and image cytometry.

Plasmids and transfection. The full-length α-Fbxw7 cDNA was cloned into pFlag-CMV2 (Sigma) and GST expression vector pEBG. Expression plasmids for all *FBXW7* mutants were generated using the GeneTailor Site-Directed Mutagenesis System (Invitrogen). Expression plasmid for 3× Myc-cyclin E1 was generated by cloning the full-length cyclin E1 cDNA into pcDNA3.1 (Invitrogen). Transfections were done using Lipofectamine 2000 (Invitrogen).

Molecular modeling. The molecular structure of α-Fbxw7 was created using the SWISS-MODEL program and viewed using the DeepView-SwissPDB-Viewer.

Results

***FBXW7* is mutated in a variety of primary human tumor types.** To determine the role of *FBXW7* as a tumor suppressor in human tumorigenesis, we genetically analyzed the complete coding region, including sequences unique to the three alternatively spliced Fbxw7 isoforms (α, β, and γ), in a variety of human tumor types. The results of this analysis, combined with previously reported data (9–17) for *FBXW7* in primary tumors (1,556 specimens in total), are presented in Table 1. *FBXW7* mutations were detected in tumors of diverse tissue origin, including those of the blood, breast, bile duct, colon, endometrium, stomach, lung, bone, ovary, pancreas, and prostate. The highest frequencies of mutations were observed in tumors of the bile duct (cholangiocarcinomas, 35%), blood (T-cell acute lymphocytic leukemia, 31%), colon (9%), endometrium (9%), and stomach (6%). Overall, the frequency of *FBXW7* mutations in all primary human cancers analyzed was ∼6% (87 of 1,556). DNA sequencing of matching negative biopsies from patients with stomach cancers showed that the *FBXW7* mutations were exclusively in the tumor specimens, indicating that the mutations were somatic in nature.

Spectra of *FBXW7* mutations reveal mutational "hotspots" and isoform-specific mutations. Of the mutations detected in *FBXW7*, the vast majority (94%) were single nucleotide changes, with the remainder (6%) being either small nucleotide deletions or insertions. Seventy-four percent of mutations introduced a single amino acid substitution (missense) in the Fbxw7 protein, whereas 26% induced a premature termination of translation (nonsense; Table 1). *FBXW7* mutations were found to occur throughout the coding region, including the F-box motif, WD40 repeats, and two of three unique 5′ exons that determine the Fbxw7 isoforms (Fig. 1). Interestingly, isoform-specific mutations were found to represent ∼6% of *FBXW7* mutations. Approximately 43% of all *FBXW7* mutations occurred at two mutational hotspots positioned at codons Arg^{465} (nucleotides 1,393–1,394; 29%) and Arg^{479} (nucleotides 1,435–1,436; 14%). Additional minor mutational hotspots were detected at nucleotides 670 (Arg^{224}; 3%), 832 (Arg^{278}; 4%), 1,177 (Arg^{393}; 3%), and 1,745 (Ser^{582}; 4%). Molecular modeling revealed that codons Arg^{465}, Arg^{479}, and Ser^{582} are localized to the surface or lumen of the β-propeller structure of Fbxw7; Arg^{393} is at the rear of the β-propeller structure; and Arg^{278} is situated close to the isoform-specific NH_2 terminus of Fbxw7.

Mutation hotspots in *FBXW7* correspond to methylated CG dinucleotides with signatures of deaminations of 5′-methylcytosine. Investigation of the mutational hotspots of *FBXW7* revealed that all seven are centered on CG dinucleotides, potential sites of DNA methylation in humans. Interestingly, the prominent mutation type that occurs at each mutational hotspot was either a C→T or G→A transition within the CG dinuclotide, consistent with the spontaneous deamination of 5′-methylcytosine→thymine in DNA (18). Because 5′-methylcytosine has been shown to act as an endogenous mutagen (18), we analyzed the methylation status of DNA isolated from several primary tumor types to determine whether the cytosine corresponding to the mutation hotspot at nucleotides 1,435–1,436 (Arg^{479}) was methylated *in vivo*. Indeed, bisulfite-based sequence analysis revealed that the cytosine was methylated in 18 of 18 primary tumors examined, including those of the ovary, breast, and endometrium (data not shown).

Functional analysis of Fbxw7 mutants reveals defects in localization and substrate binding. Substrate recognition by Fbxw7 is mediated through the interaction of the β-propeller surface formed by the WD40 repeats with the phosphodegron of the substrate protein (2, 3). To examine the consequence on substrate binding of the hotspot mutations, we expressed α-$Fbxw7^{Arg465}$ and α-$Fbxw7^{Arg479}$ in HEK 293T cells and assessed their ability to bind substrate cyclin E1. Whereas wild-type α-Fbxw7 readily bound cyclin E1 *in vivo*, mutants α-$Fbxw7^{Arg465}$ and α-$Fbxw7^{Arg479}$ did not (Fig. 2), despite their proper localization as assessed by immunofluorescence (data not shown). Interestingly, many *FBXW7* mutations fall outside of the WD40 repeats and the effect of these on Fbxw7 function is unclear. To analyze this in more detail, we functionally tested mutant $FBXW7^{+p16}$ identified in a prostate tumor specimen that contains a proline residue inserted at amino acid 16 of the α-isoform (Table 1). Expression of α-$Fbxw7^{+p16}$ in HEK 293T cells showed that it was incapable of binding cyclin E1 substrate *in vivo*, although it could readily associate with the SCF core components (Fig. 2). Because residue 16 is in close proximity to the proposed nuclear localization signal of the α-isoform (2), we next tested whether α-$Fbxw7^{+p16}$ is properly localized in cells. Immunofluorescence analysis showed that whereas wild-type α-Fbxw7 is almost exclusively localized to the nucleus in HEK 293T cells, α-$Fbxw7^{+p16}$ is restricted to the cytoplasm (Fig. 2). These results show that the insertion of proline at residue 16 abolished the nuclear localization signal, preventing interaction with cyclin E1 substrate in the nucleus.

***FBXW7* mutants corresponding to a mutational hotspot in primary tumors act dominant negatively to inactivate Fbxw7 function.** Recently, it was shown that isoforms of Fbxw7 can form

Table 1. *FBXW7* mutations in primary human tumors

Tumor type	Current study, frequency (%)	Previous studies, frequency (%)	Nucleotide change*	Amino acid change
Breast	1/122 (1)		A791G	Q264R
Bladder	0/20 (0)			
Cholangiocarcinoma	7/20 (35)		C1393T (×4)	R465C
			G1394A	R465H
			C1513T	R505C
			A1556G	Y519C
Colon †	3/31 (10)		C1393T (×2)	R465C
			G1510A	V504I
		45 ‡ /492 (9)	C658T	Q220X
			C670T (×3)	R224X
			C832T (×4)	R278X
			C845A	S282X
			C907T	Q303X
			A936C	R312S
			C1099T	R367X
			C1177T (×3)	R393X
			G1190A	G397D
			G1268T	G423V
			C1313T	S438F
			G1338A	W446X
			C1393T (×7)	R465C
			G1394A (×2)	R465H
			G1436A (×7)	R479Q
			G1457A	W486X
			C1513T	R505C
			G1514C	R505P
			G1628A	R543K
			A1634G	Y545C
			C1745T (×4)	S582L
			C1787T	S596F
Endometrium§		9/102 (9)	β-G67F	V23I
			G370T	D124Y
			C1099T	R367X
			2bp ins. 1110	376X
			G1394A	R465H
			1bp ins. 1417	476X
			G1436A (×2)	R479Q
			C1972T	R658X
Esophagus‖		0/27 (0)		
Leukemia¶		AML, 0/35 (0)		
	B-ALL, 0/20 (0)			
	B-CLL, 0/20 (0)			
	HCL, 0/20 (0)			
	T-ALL**, 8/26 (31)		G1394A (×4)	R465H
			C1435G(×2)	R479G
			G1436T	R479L
			C2065T	R689W
Liver	0/12 (0)			
Lung ††	NSCLC, 1/38 (3)	0/50 (0)	A32G	K11R
Melanoma	0/20 (0)			
Bone ‡‡		1/47 (2)	3bp del., 424	
Ovarian§§	0/32 (0)	2/111 (2)	G734T	S245I
			G1411T	E471X
Prostate	1/83 (1)		3bp ins., 45	+CCT, +P
Pancreas‖‖		1/11 (9)	A1379G	H460R
Stomach¶¶	8***, ††† /52 (15)		C1393T (×4)	R465C
			G943A	A315T
			G1436T	R479L

(*Continued on the following page*)

Table 1. *FBXW7* mutations in primary human tumors (Cont'd)

Tumor type	Current study, frequency (%)	Previous studies, frequency (%)	Nucleotide change*	Amino acid change
			G1451C	R484T
			G1510A	V504I
		6/162 (4)	G1318A	D440N
			C1393T	R465C
			G1947A	W649X
			1bp del, 1996	706X
			G2021A	R674Q
			G2077A	E693K

Abbreviations: B-CLL, B-cell chronic lymphocytic leukemia; B-ALL, B-cell acute lymphocytic leukemia; T-ALL, T-cell acute lymphocytic leukemia; HCL, hairy cell leukemia; NSCLC, non–small cell lung carcinoma.
*Nucleotide and codon position based on α-isoform transcript unless indicated.
† Includes tumors analyzed in refs. 5, 9.
‡ Includes three tumors with multiple *FBXW7* mutations.
§Refs. 6, 10.
‖Ref. 11.
¶Ref. 12.
**Malyukova et al., submitted for publication.
†† Ref. 13.
‡‡ Ref. 14.
§§Ref. 15.
‖‖Ref. 16.
¶¶Ref. 17.
***All mutations were confirmed as somatic.
††† Restriction digest-based analysis showed five tumors with mutations had no LOH of the remaining *FBXW7/hCDC4* allele.

homodimers and heterodimers *in vivo* (19), raising the possibility that mutants of Fbxw7 could interfere with wild-type Fbxw7 function in a dominant negative fashion. To explore this possibility, we cotransfected HCT116$^{FBXW7-/-}$ cells that have both *FBXW7* alleles inactivated by targeted disruption (5) with plasmids that express Myc-cyclin E1, Cdk2, α-Fbxw7, and increasing amounts of α-Fbxw7^{R465C}. The results of this experiment showed that coexpression of α-Fbxw7^{R465C} with wild-type α-Fbxw7 resulted in a marked accumulation of cyclin E1 compared with expression of wild-type α-Fbxw7 alone (Fig. 3*A*). Similar results were observed for expression of α-Fbxw7^{R479Q}, corresponding to the other major mutational hotspot in primary tumors, in HCT116$^{FBXW7-/-}$ cells and for both mutants in HEK 293T cells that contain wild-type *FBXW7* alleles (data not shown). We next carried out cycloheximide-chase experiments to determine whether the increase in cyclin E1 level observed for α-Fbxw7^{R465C} expression was due to increased protein stability. As expected, expression of wild-type α-Fbxw7 resulted in a decrease in cyclin E1 stability (half-life of ~1.2 h) compared with expression of α-Fbxw7^{R465C} or vector alone (half-life >3 h). In contrast, coexpression of α-Fbxw7 and α-Fbxw7^{R465C} resulted in an increase in cyclin E1 half-life (>3 h), comparable to expression of α-Fbxw7^{R465C} or vector alone (Fig. 3).

Discussion

Our results show that *FBXW7* is a general tumor suppressor in human tumorigenesis. *FBXW7* mutations were detected in primary human tumors of the blood, breast, bile duct, bone, colon, endometrium, stomach, lung, ovary, prostate, and pancreas, with an overall mutation frequency of ~6%. Mutations were most frequent in tumors of the bile duct (35%), blood (T-cell acute lymphocytic leukemia, 31%), colon (9%), endometrium (9%), pancreas (9%), and stomach (6%), suggesting a potential tissue specificity of *FBXW7* inactivation in human tumorigenesis.

FBXW7 mutations were found localized throughout the coding region of the gene including the isoform-specific 5′-exons, nuclear localization signal, F-box motif, and WD40 repeats. These mutations are predicted to adversely affect isoform-specific functions, subunit dimerization, Pin-1 association, localization, SCF assembly, and substrate recognition, highlighting the plethora of ways Fbxw7 can be functionally inactivated in tumors. Interestingly, ~6% of *FBXW7* mutations are isoform specific, suggesting that inactivation of an individual isoform could play a causative role in human tumorigenesis. This finding could reflect the potential of a mutant Fbxw7 isoform to act dominant negatively (see below) or that individual Fbxw7 isoforms may target a unique set of substrates for ubiquitination. In support of the latter hypothesis, Fbxw7 isoforms are differentially localized in mammalian cells (α-nuclear, β-cytoplasmic, and γ-nucleolar; ref. 20). Furthermore, ubiquitination of c-Myc has been shown to be mediated by γ-Fbxw7, which selectively binds substrate in the nucleolus (2). Additionally, ubiquitination of cyclin E1 is mediated through a cooperative effort of α- and γ-Fbxw7, in which α-Fbxw7 binds cyclin E1 and stimulates prolyl-isomerization by Pin-1, which then promotes efficient ubiquitylation by SCF$^{γ\text{-}Fbxw7}$ (20). Indeed, one of the α-isoform–specific mutations detected in tumors (D124Y) was shown to be defective in supporting Pin-1–mediated isomerization of cyclin E1 but was not defective in ubiquitylation function (20). It is currently not understood whether other

SCF^{Fbxw7} substrates are ubiquitinated via an analogous cooperative mechanism of Fbxw7 isoforms.

Our analysis identified seven mutational hotspots in *FBXW7*, representing >60% of the total mutations. Two major hotspots located at nucleotides 1,393–1,394 (Arg^{465}) and 1,435–1,436 (Arg^{479}) account for ~43% of all mutations. The amino acid residues corresponding to six of seven mutational hotspots in primary human tumors are evolutionarily conserved in flies, worms, and yeast (2), showing their potential importance in Fbxw7 function. Interestingly, all seven *FBXW7* mutational hotspots involve CG dinucleotides, where the resultant mutation is primarily a C→T or G→A transition. Consistent with 5′-methylcytosine deamination as a mechanism for *FBXW7* mutation, the cytosine nucleotide at hotspot position 1,435–1,436 (Arg^{479}) was found to be methylated in all primary tumors analyzed. These data stress the potential importance of endogenous methylation in mutational inactivation of Fbxw7 in tumors, as has previously been postulated for the tumor suppressor p53 (18).

It is currently not understood what role, if any, other genetic and epigenetic mechanisms play in *FBXW7* inactivation in human tumors. In many primary tumors and derived cell lines, *FBXW7* mutations frequently occur without a concomitant loss or

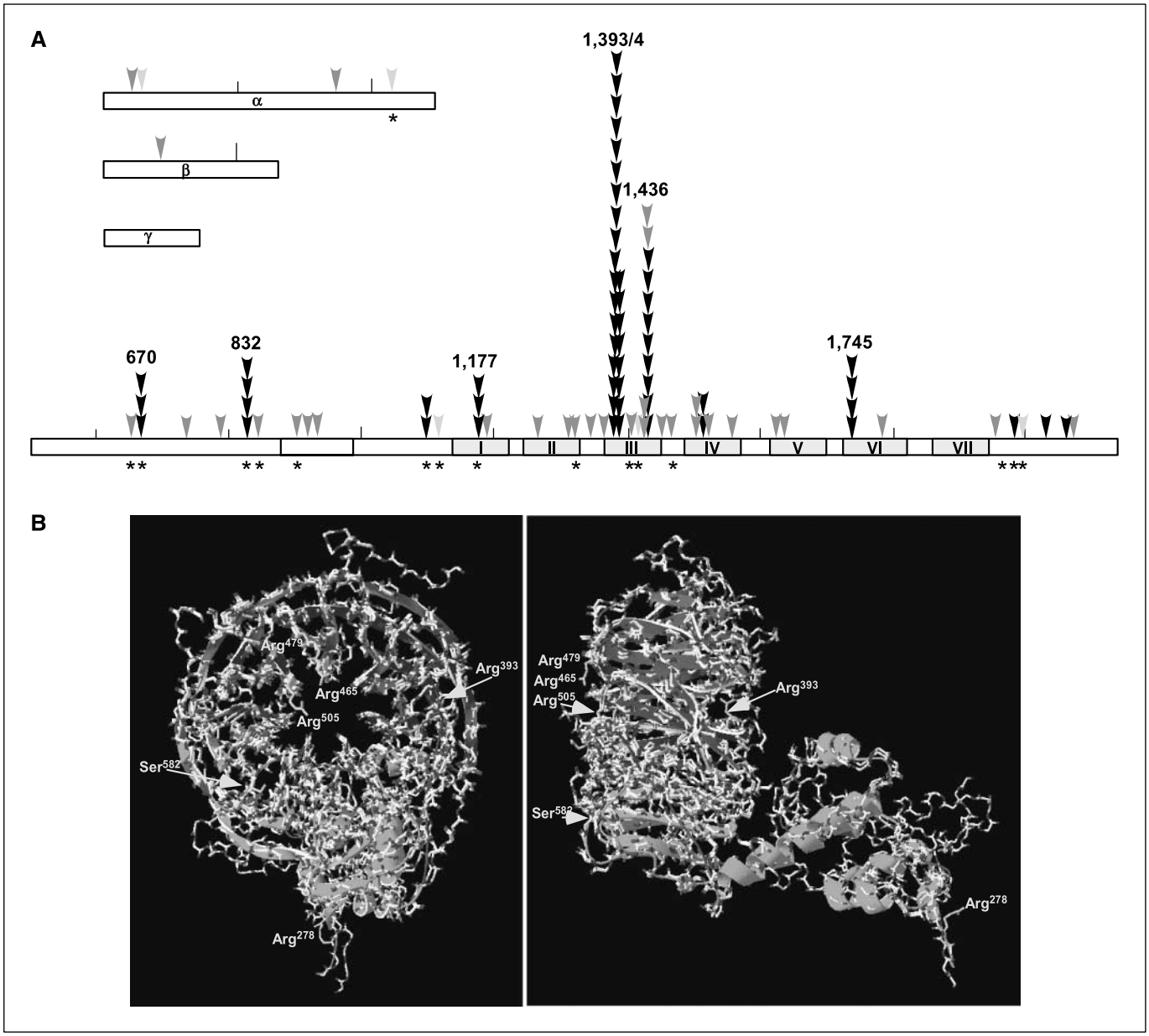

Figure 1. *FBXW7* mutations in primary human tumors. *A,* spectra of *FBXW7* mutations. Three 5′-exons (α, β, and γ) encoding the Fbxw7 isoforms are alternatively spliced to 10 common exons that encode the F-box domain (*hatched box*) and WD40 repeats (*boxes I–VII*). Each triangle represents an *FBXW7* mutation found in a primary human tumor. Mutation type is indicated by color (*dark gray,* point mutations; *light gray,* deletions/insertions; *black,* C→T or G→A point mutations at CG dinucleotides). Mutational hotspots are observed at nucleotides 670, 832, 1,177, 1,393, 1,394, 1,436, and 1,745. *, chain terminating mutations (nonsense). *B,* molecular structure of Fbxw7 diagramming mutational hotspots in human tumors. The seven WD40 repeats of Fbxw7 form a β-propeller structure that is involved in substrate recognition. Amino acids that correspond to mutational hotspots are highlighted in yellow. Arg^{465}, Arg^{479}, Arg^{505}, and Ser^{582} are localized to the surface or lumen of the β-propeller structure; Arg^{393} is located on the rear-face of the β-propeller; and Arg^{278} is located proximal to the F-box domain.

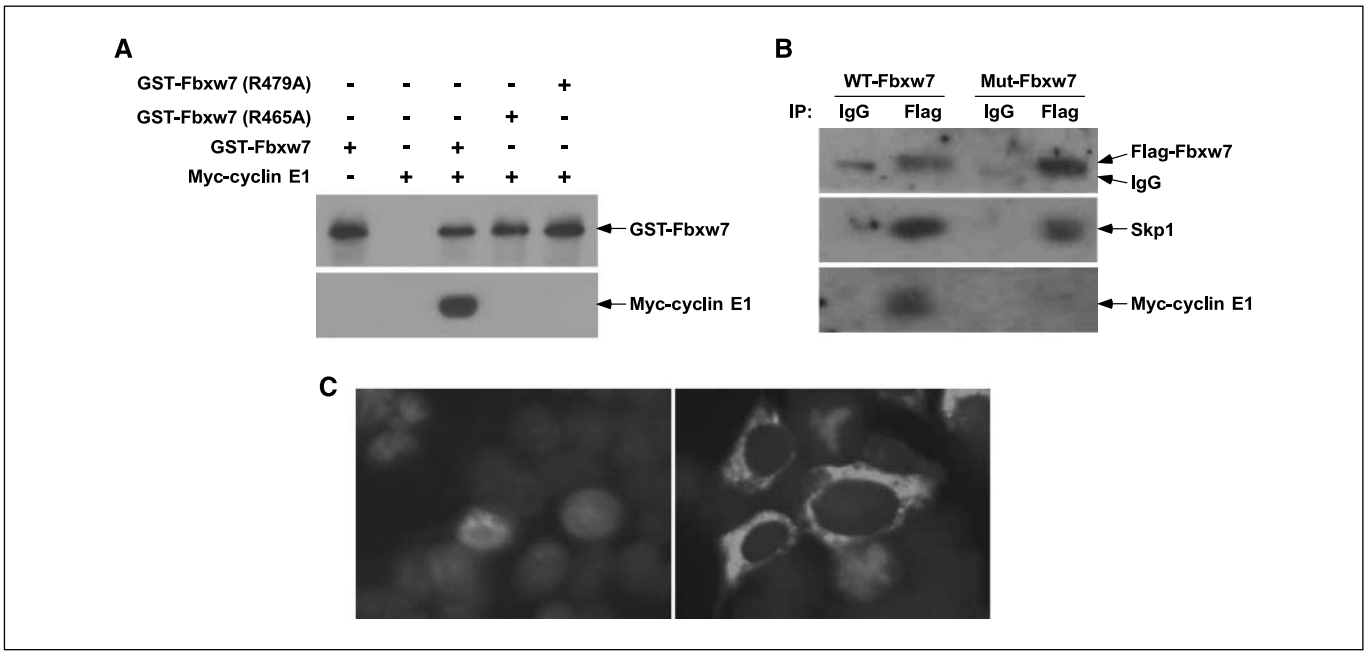

Figure 2. Functional analysis of Fbxw7 mutants. *A,* effect of hotspot mutants Arg^{465} and Arg^{479} on binding to cyclin E1 substrate. HEK 293T cells were cotransfected with plasmids that express Myc-cyclin E1 and GST-α-Fbxw7 or mutants GST-α-$Fbxw7^{R465C}$ or GST-α-$Fbxw7^{R479Q}$, and complexes precipitated and analyzed by Western blot analysis. *B,* functional analysis of α-isoform–specific mutant α-$Fbxw7^{+p16}$ isolated from a prostate tumor specimen. HEK 293T cells were cotransfected with Myc-cyclin E1 and Flag-α-Fbxw7 or Flag-α-$Fbxw7^{+p16}$ and extracts immunoprecipitated with anti-Flag antibodies. *C,* α-$Fbxw7^{+p16}$ mutant mislocalizes to the cytoplasm. HEK 293T cells were transfected with plasmids that express Flag-α-Fbxw7 (*left*) or Flag-α-$Fbxw7^{+p16}$ (*right*) and protein location was assessed by immunofluorescence with anti-Flag-FITC antibodies.

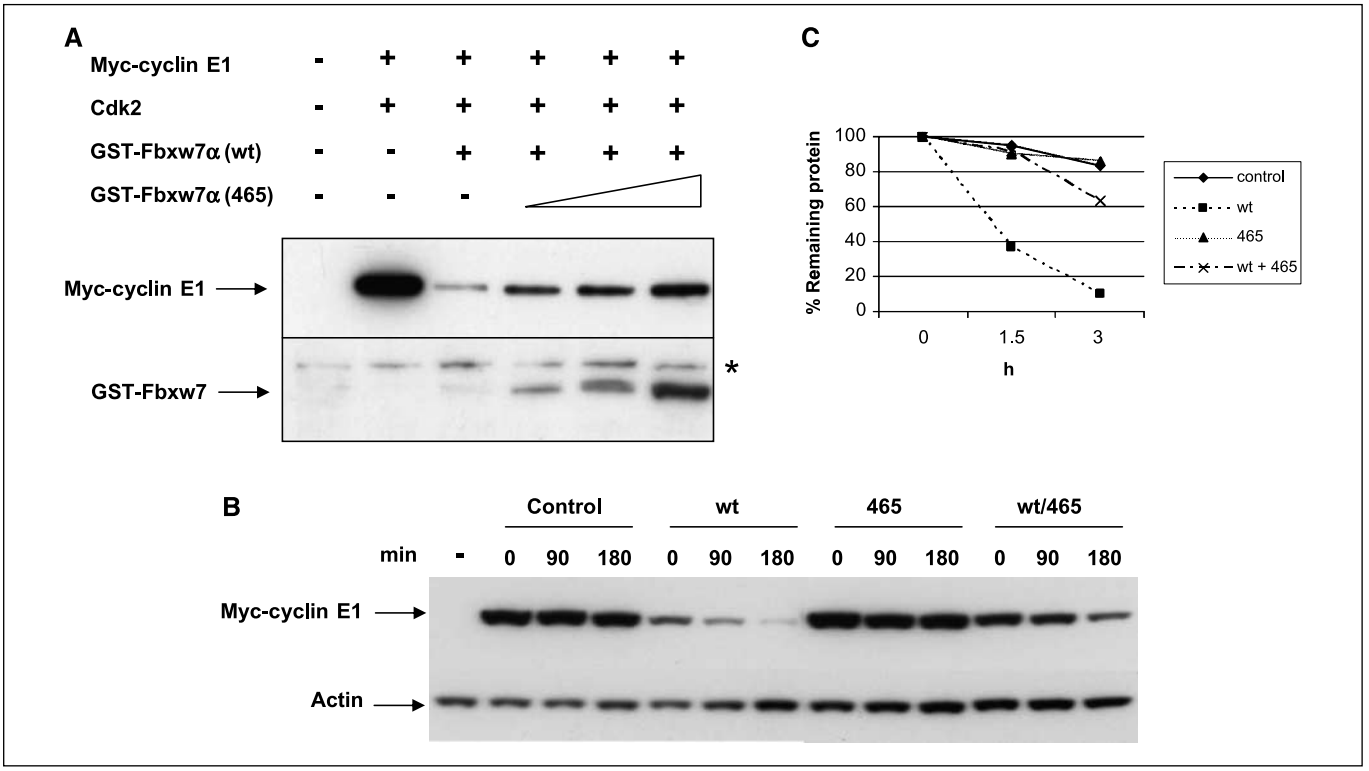

Figure 3. Hotspot mutant $Fbxw7^{R465C}$ acts dominant negatively to inactivate wild-type Fbxw7 function. *A,* expression of α-$Fbxw7^{R465C}$ interferes with degradation of cyclin E1 by wild-type α-Fbxw7. $HCT116^{Fbxw7-/-}$ cells were cotransfected with plasmids that express Myc-cyclin E1, Cdk2, wild-type GST-α-Fbxw7, and increasing amounts of mutant GST-α-$Fbxw7^{R465C}$. Lysates were subjected to Western blot analysis. Note the increase in cyclin E1 level in cells expressing wild-type α-Fbxw7 + α-$Fbxw7^{R465C}$ compared with wild-type α-Fbxw7 alone. An equal amount of total plasmid DNA was transfected in each lane. *, background band. *B,* coexpression of wild-type α-Fbxw7 + α-$Fbxw7^{R465C}$ leads to an increase in cyclin E1 half-life. $HCT116^{Fbxw7-/-}$ cells were cotransfected with plasmids that express wild-type α-Fbxw7 (*wt*), (*wt*), α-$Fbxw7^{R465C}$, or wild-type α-Fbxw7 + α-$Fbxw7^{R465C}$, and cyclin E1 half-life was determined by cycloheximide-chase experiments. Western blot for actin is shown as a loading control. *C,* graphical representation of results for cycloheximide-chase experiments.

mutation of the remaining allele, suggesting that *FBXW7* may not follow the classic "two-hit" model of tumor suppressor gene inactivation. It is possible that reduced expression of the wild-type allele might be sufficient to abrogate tumor suppressor activity or that *FBXW7* mutations could act dominant negatively. The former possibility is supported by a study that showed that loss of a single *FBXW7* allele can accelerate tumor development in $p53^{+/-}$ mice (4), suggesting *FBXW7* is a p53-dependent haploinsufficient tumor suppressor. However, we analyzed *FBXW7* and *p53* mutations in gastric cancers and failed to detect cooperation between these proteins (Supplementary Table S1). It remains to be determined whether *FBXW7* expression is reduced or silenced in tumors through epigenetic mechanisms such as promoter methylation.

Our data show that Fbxw7 mutants corresponding to the major mutational hotspots in primary tumors (Arg^{465} and Arg^{479}) can act dominant negatively to abrogate wild-type Fbxw7 function. Coexpression of wild-type α-Fbxw7 and α-$Fbxw7^{R465C}$ was found to increase the steady-state level of cyclin E1 as well as its half-life, compared with expression of wild-type α-Fbxw7 alone. The different isoforms of Fbxw7 interact *in vivo* through association of their D-box motifs located in the NH_2-terminal region of the protein, and this interaction enhances SCF^{Fbxw7}-associated ubiquitylation activity (19). If Fbxw7 predominantly functions as a homodimer or heterodimer *in vivo*, then expression of a single mutant allele may be sufficient to functionally inactivate its tumor suppressor function. This could explain why most primary tumors contain only a single mutant *FBXW7* and retain a wild-type allele. Further study is needed to determine whether other Fbxw7 mutations also act dominant negatively and whether expression at physiologic levels is sufficient to exert the dominant negative effects on wild-type Fbxw7.

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Correction: The *FBXW7* Gene Is Mutated in a Variety of Human Tumors

In the article on how the *FBXW7* gene is mutated in a variety of human tumors in the October 1, 2007 issue of *Cancer Research* (1), the correct name of the ninth author is Dimitra Dafou.

1. Akhoondi S, Sun D, von der Lehr N, Apostolidou S, Klotz K, Maljukova A, Capeda D, Fiegl H, Dafou D, Marth C, Mueller-Holzner E, Corcoran M, Dagnell M, Nejad SZ, Nayer BN, Zali MR, Hansson J, Egyhazi S, Petersson F, Sangfelt P, Nordgren H, Grander D, Reed SI, Widschwendter M, Sangfelt O, Spruck C. *FBXW7/hCDC4* is a general tumor suppressor in human cancer. Cancer Res 2007;67:9006–12.

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FBXW7/hCDC4 Is a General Tumor Suppressor in Human Cancer

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