

# Five human genes encoding F-box proteins: chromosome mapping and analysis in human tumors

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**Abstract.** Members of the F-box protein (Fbp) family are characterized by an approximately 40 amino acid F-box motif. SCF complexes (formed by Skp1, cullin, and one of many Fbps) act as protein-ubiquitin ligases that control the G<sub>1</sub>/S transition of the eukaryotic cell cycle. The substrate specificity of SCF complexes is determined by the presence of different Fbp subunits that recruit specific substrates for ubiquitination. Unchecked degradation of cellular regulatory proteins has been observed in certain tumors and it is possible that deregulated ubiquitin ligases play a role in the altered degradation of cell cycle regulators. We have recently identified a family of human Fbps. As a first step aimed at determining if FBP genes could be involved in human neoplasia, we have mapped the chromo-

some positions of 5 FBP genes by fluorescence in situ hybridization (FISH) to 10q24 (BTRC alias  $\beta$ -TRCP/FBW1a), 9q34 (FBXW2 alias FBW2), 13q22 (FBXL3A alias FBL3a), 5p12 (FBXO4 alias FBX4) and 6q25  $\rightarrow$  q26 (FBXO5 alias FBX5). Since most of these are chromosomal loci frequently altered in tumors, we have screened 42 human tumor cell lines and 48 human tumor samples by Southern hybridization and FISH. While no gross alterations of the genes encoding  $\beta$ -Trcp/Fbw1a, Fbw2, Fbx4 and Fbx5 were found, heterozygous deletion of the FBXL3A gene was found in four of 13 small cell carcinoma cell lines. This is the first evaluation of genes encoding Fbps in human tumors.

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The F-box motif is a stretch of approximately 40 amino acids present in an expanding family of eukaryotic proteins (Bai et al., 1996). The name F-box derives from one of the proteins in which this domain was first identified and characterized, cyclin F (Bai et al., 1996). F-box containing proteins (Fbps) play an important role in ubiquitin-mediated protein

degradation. In fact, some Fbps, together with three other subunits form protein ubiquitin ligase complexes called SCFs (Skp1, cullin, one of many Fbps and the recently identified Roc1). Fbps are the substrate-targeting subunits of these ubiquitin ligase complexes (reviewed in Koepp et al., 1999). In addition to the F-box necessary to interact with Skp1, Fbp subunits contain other interaction domains (WD-40 domains or leucine-rich repeats [LRRs]) that appear to be involved in their interaction with substrates.

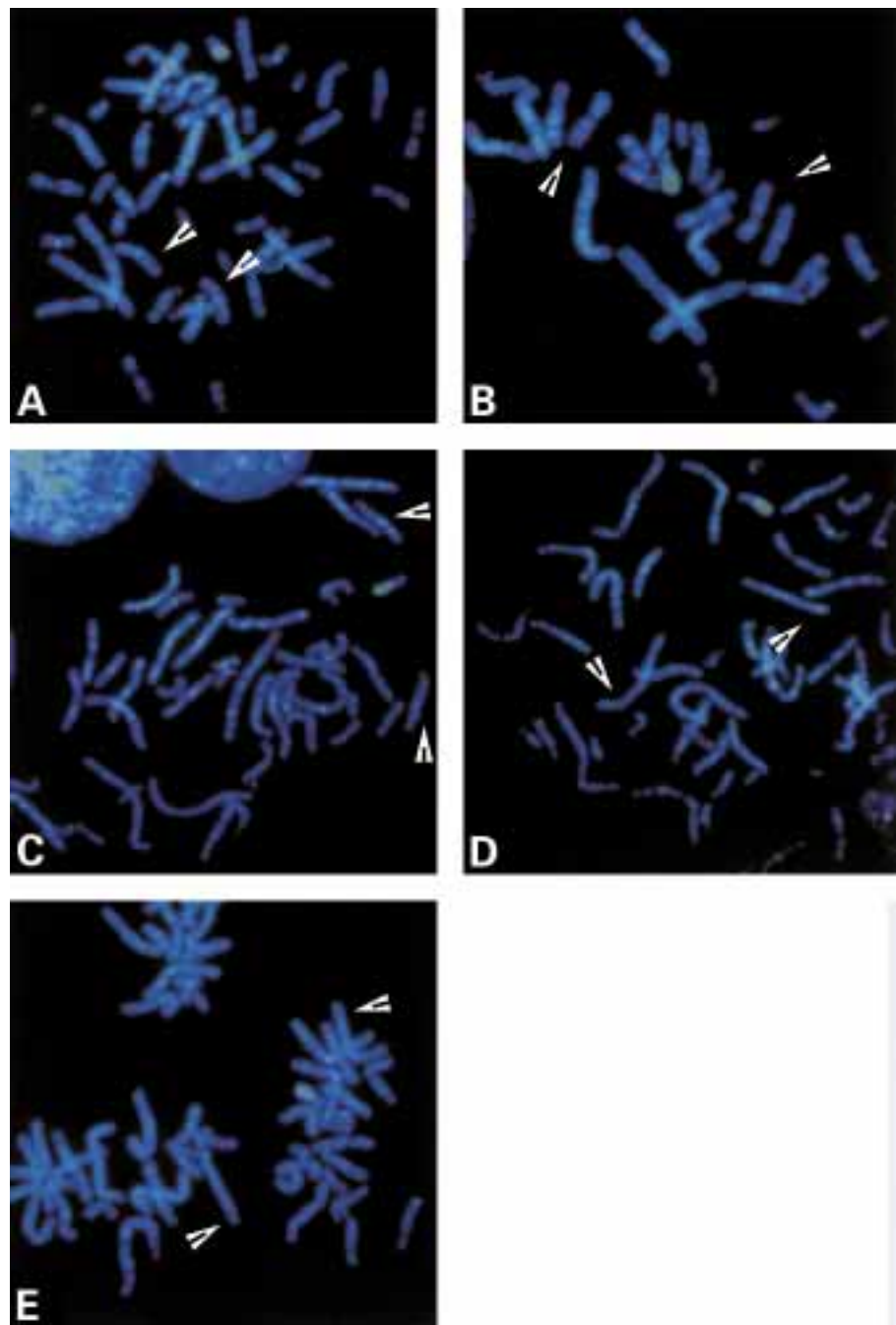
Until recently, only four human Fbps were known:  $\beta$ -Trcp,  $\beta$ -Trcp2, Skp2 and cyclin F. Using Skp1 as bait in a two-hybrid screen, we have recently identified seven human Fbps (Cenciarelli et al., 1999). Additionally, we have searched various databases with the amino acid sequences corresponding to the conserved residues of the F-box motif. We have so far identified a family of a total of 26 human Fbps, 25 of which are novel (Cenciarelli et al., 1999). Based on the presence of different pro-

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**Fig. 1.** FISH localization of FBP genes. Purified phage DNA containing a genomic probe was labeled with digoxigenin dUTP and detected with Cy3-conjugated antibodies. The signals corresponding to the locus of the genomic probe (red) are seen against the DAPI stained normal human chromosomes (blue-white) from actinomycin D-treated cells. **(A)** Localization of BTRC to 10q24. **(B)** Localization of FBXW2 to 9q34 **(C)** Localization of FBXL3A to 13q22. **(D)** Localization of FBXO4 to 5p12. **(E)** Localization of FBXO5 to 6q25 → q26. Arrows point to FBP-specific FISH signals.

tein motifs, we have agreed with other groups on a systematic nomenclature for vertebrate Fbps and named the Fbps that contain WD-40 domains Fbws, those containing LRRs Fbls, and the remaining ones Fbxs (Cenciarelli et al., 1999; Regan Reimann et al., 1999; Winston et al., 1999a). In accordance with the Guidelines for Human Gene Nomenclature, the genes encoding Fbws, Fbls and Fbxs have been named FBXLs, FBXWs and FBXOs, respectively (<http://www.gene.ucl.ac.uk/nomenclature/FBX.shtml>). We have further characterized representative members of these three classes of Fbps ( $\beta$ -Trep/Fbw1a, Fbw2, Fbl3a, Fbx4, Fbx5, and Fbx7) and for all of them

we have demonstrated the *in vivo* formation of novel active SCF ubiquitin ligase complexes which potentially target different substrates for ubiquitin-mediated degradation (Cenciarelli et al., 1999).

In the work presented herein, fluorescence in situ hybridization (FISH) was used to map the chromosome location of the human FBP genes and consequently determine if these positions coincided with loci known to be altered in tumors or in inherited disease. A  $\lambda$  FIX II human placenta genomic library was screened by high-stringency screening using cDNA probes for the five FBP genes (BTRC and FBXL3A probes were two

*Hind*III restriction fragments (nucleotides 1–571 and 1–450, respectively), FBXW2, FBXO4, and FBXO5 probes were their respective full-length cDNAs). Phage clones were confirmed by high-stringency Southern blot hybridization and partial sequence analysis. At least two genomic clones for each FBP gene were isolated, labeled and used for FISH as described (Demetrick, 1994). BTRC was mapped and localized to 10q24 (Fig. 1A), FBXW2 to 9q34 (Fig. 1B), FBXL3A to 13q22 (Fig. 1C), FBXO4 to 5p12 (Fig. 1D) and FBXO5 to 6q25→q26 (Fig. 1E). While our work was underway, the localization of BTRC was reported by others (Fujiwara et al., 1999; Winston et al., 1999b).

FBP genes (particularly, BTRC, FBXL3A, and FBXO5) are located in chromosome loci frequently altered in tumors. In particular, loss of 10q24 (where BTRC is located) has been demonstrated in approximately 10% of human prostate tumors (Lundgren, 1991) and small cell lung carcinomas (SCLC) (Kim et al., 1998; Yokota et al., 1989), suggesting the presence of a tumor suppressor gene at this location. Although rarely, the 9q34 region (where FBXW2 is located) has been shown to be a site of loss of heterozygosity (LOH) in human ovarian and bladder cancers. LOH is also observed in the region between 13q12 and 13q22 (where FBXL3A is located) in approximately 75% of human SCLC (Yokota et al., 1989). Finally, 6q25→q26 (where FBXO5 is located) has been shown to be a site of loss of heterozygosity in human ovarian, breast and gastric cancers, hepatocarcinomas, Burkitt's lymphomas, and parathyroid adenomas. More references and details on these chromosome loci can be found in the Online Mendelian Inheritance in Man database (<http://www3.ncbi.nlm.nih.gov/omim/>).

We evaluated the presence of deletions and rearrangements of these five FBP genes in 42 different human tumor cell lines (representative of eight tumor cell line types) using Southern blot hybridization (Table 1). Comparing the patterns of bands and the signal intensities in tumors versus human diploid fibroblasts, no deletions, amplifications or translocations of FBP genes were identified. Interestingly, we found an under-represented FBXL3A signal relative to other FBP signals in four small cell carcinoma (SCC) cell lines (three in lung and one in prostate), suggesting the absence of one allele. The heterozygous deletion in these four SCC cell lines was then confirmed by FISH. In all four cases the remaining allele was wild type as determined by sequencing amplified cDNAs derived from these four cell lines.

Using FISH, we also analyzed BTRC in 22 samples from human prostate carcinomas that often show loss of 10q24, and in eight samples from breast carcinomas (Table 2). FBXW2, FBXL3A, and FBXO4 were analyzed in eight breast carcinomas and FBXO5 in ten ovary cancers since these tumors often show LOH at 6q25→q26. No deletions or amplifications of these FBP genes were identified by FISH in human tumor samples, confirming that gross genomic alterations in these genes are either absent or very rare in human cancers.

We and others have shown that human FbPs play a role in the ubiquitination of G<sub>1</sub> regulatory proteins as their homologs do in yeast (Carrano et al., 1999; Sutterlüty et al., 1999; Tsvetkov et al., 1999). One way for a tumor cell to obtain a growth advantage is to enhance proteolysis of cell cycle inhibitors and

**Table 1.** Analysis of FBP genes in human cancer cell lines<sup>a</sup>

Cell lines	Source <sup>b</sup>	BTRC, FBXL2 FBXO4, FBXO5 <sup>c</sup>	FBXL3A <sup>d</sup>
Prostate cancer:			
PC3	A	naf	naf
DU 145	A	naf	naf
LNCaP.FGC	A	naf	naf
TSUPr-1	B	naf	naf
Small cell prostate carcinoma:			
ICHIKAWA	C	naf	HD
Small cell lung carcinoma (SCLC):			
DMS114	A	naf	HD
DMS273	A	naf	naf
SHP77	A	naf	naf
NCI-H69	A	naf	HD
NCI-H82	A	naf	naf
NCI-H125	A	naf	naf
NCI-H157	A	naf	naf
NCI-H209	A	naf	HD
NCI-H526	A	naf	naf
NCI-H592	A	naf	naf
NCI-H841	A	naf	naf
NCI-H1092	A	naf	naf
Lung carcinoma (NSCLC):			
A549	A	naf	naf
NCI-H322	A	naf	naf
Breast cancer:			
MCF7	A	naf	naf
SK-BR-3	A	naf	naf
SK-BR-7	A	naf	naf
MDA-MB-231	A	naf	naf
MDA-MB-453	A	naf	naf
MDA-MB-468	A	naf	naf
MDA-MB-435S	A	naf	naf
T-47D	A	naf	naf
Acute T cell leukemia:			
Molt-4	A	naf	naf
Jurkat	A	naf	naf
C91	D	naf	naf
MT-2	D	naf	naf
T cell lymphoma:			
KARPAS 299	E	naf	naf
HUT 78	E	naf	naf
Burkitt lymphoma:			
Ramos	A	naf	naf
JD 38	E	naf	naf
KK125	E	naf	naf
Namalwa	A	naf	naf
AS282A	F	naf	naf
CW678	F	naf	naf
ST486	A	naf	naf
Pollik	F	naf	naf
BL113	F	naf	naf

<sup>a</sup> For each cell line, 10–15 µg of genomic DNA was digested with the restriction enzymes indicated below, electrophoresed through a 0.8% agarose gel, denatured, neutralized and transferred overnight to nylon filters (Hybond-N, Amersham-Pharmacia). cDNA probes were labeled with α-[<sup>32</sup>P]dCTP by a random printing method. Hybridization was performed with the Quickhyb kit (Stratagene) according to the manufacturer's instructions. BTRC was analyzed by Southern blot using *Xba*I and *Bam*HI; FBXW2, FBXL3A, FBXO4 and FBXO5 were analyzed using *Xba*I and *Hind*III.

<sup>b</sup> A, ATCC, American Type Culture Collection; B, A. Senderowicz' lab; C, H. Okada's lab; D, W. Parks' lab; E, G. Inghirami's lab; F, R. Della Favera's lab.

<sup>c</sup> naf, no abnormalities found; nd, not determined.

<sup>d</sup> HD, heterozygous deletions. Heterozygous deletions in FBXL3A were confirmed by FISH.

**Table 2.** FISH analysis of FBS genes in primary human cancers<sup>a</sup>

Human tumors	Source <sup>b</sup>	BTRC	FBXW2 FBXL3A FBXO4	FBXO5
Prostate cancer 22 tumors	G	naf	nd	nd
Breast cancer 8 tumors	H	naf	nd	nd
8 tumors	G	nd	naf	nd
Ovarian cancer 10 tumors	H	nd	nd	naf

<sup>a</sup> naf, no abnormalities found; nd, not determined,  
<sup>b</sup> G, M. Loda's lab; H, D. Demetrick's lab.

block degradation of cell cycle activators. Altered disruption of cellular regulatory proteins may in fact result in deregulated proliferation typical of cancer cells (reviewed in Ciechanover, 1998). For example, abnormal ubiquitin-mediated degradation of the p53 tumor suppressor (reviewed in Prives, 1998), the putative oncogene  $\beta$ -catenin (reviewed in Peifer, 1997) and the Cki p27 (reviewed in Ciechanover, 1998) has been correlated with tumorigenesis, supporting the hypothesis that some genes encoding ubiquitinating enzymes may be mutated in tumors. In agreement with this hypothesis is the fact that the p53 specific ubiquitin ligase, Mdm2, is the product of an oncogene often found overexpressed in p53-wild type tumors. We have mapped the chromosome positions of five recently identified human FBP genes and found that BTRC, FBXL3A, and FBXO5 reside in regions of chromosome loss in human neoplasia.

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This, together with the possible role of Fbps in cell proliferation, is consistent with FBP genes being targets of genetic alteration in malignancy. However, in this first evaluation of five FBP genes in human tumors, we have not found evidence for any gross alterations. The only exception is the heterozygous deletion of the FBXL3A gene (with the remaining allele wild type) found in approximately 30% of the neuroendocrine-derived small cell carcinoma cell lines tested (three lung SCC and one prostate SCC).

There are a number of possible explanations for the lack of gross genomic alterations in the FBP genes in human tumors: (i) these genes are not mutated because their function is pleiotropic (e.g., as for BTRC) and therefore vital for the cell; (ii) FBP genes may be targets of micro-deletions or point mutations that are not detectable by our analysis; (iii) FBP expression could be modified in tumors, rather than by gene mutation, at a transcriptional, post-transcriptional or post-translational level; (iv) Genes encoding upstream Fbp regulators or downstream Fbp targets, rather than FBP genes themselves, may be altered in human cancer; (v) Disruption of Fbp functions does not have any effect on tumorigenesis. Our study was not designed to discern among these possibilities and future detailed analyses will be necessary to determine a possible role of Fbp genes in human malignancies.

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