

# Fbw7 Eludes the Two-Hit Hypothesis

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Ever since Alfred G. Knudson Jr. published his seminal statistical analysis of retinoblastoma, scientists have embraced the two-hit hypothesis, which suggested that both copies of tumor suppressor genes have to be mutated in order to drive cancer initiation. However, in some cases, tumor suppressors do not follow the two-hit rule. A recent Fred Hutch review article, co-authored by graduate student Ryan Davis, Staff scientist Dr. Markus Welcker and Principal Investigator Dr. Bruce Clurman (Human Biology Division), uses the ubiquitin ligase component and tumor suppressor Fbw7 as a model to illustrate this point. This review concisely presents the most current research on Fbw7 and entices us with potential therapeutic opportunities provided by cancers in which Fbw7 is mutated.

Ubiquitin-mediated proteolysis has been implicated in a wide array of biological processes and components of the ubiquitin-proteasome system are mutated in many cancers. Large-scale sequencing of cancer genomes and mouse modeling experiments have shown that the F-box protein Fbw7 is a potent tumor suppressor. "The Fbw7 tumor suppressor is part of a ubiquitin ligase that degrades key oncoproteins, including c-Myc, c-Jun, Notch, and cyclin E. Fbw7 binds to substrates after they become phosphorylated, which results in their ubiquitylation and subsequent destruction by the proteasome", explained Dr. Clurman. Fbw7 is a part of the SCF<sup>Fbw7</sup> ubiquitin ligase (see figure). Within SCF<sup>Fbw7</sup>, Fbw7 functions as an adaptor, providing substrate specificity to the SCF<sup>Fbw7</sup> complex. Substrates that are phosphorylated on a motif known as the Cdc4 phosphodegron (CPD) bind Fbw7, and three key arginine residues (R465, 479 and R505; hereafter Fbw7<sup>ARG</sup>) on Fbw7 that bind CPD are mutational hotspots in cancer. "The inactivating Fbw7 mutations commonly found in cancers allow oncogenic substrates to accumulate and drives carcinogenesis," added Dr. Clurman.

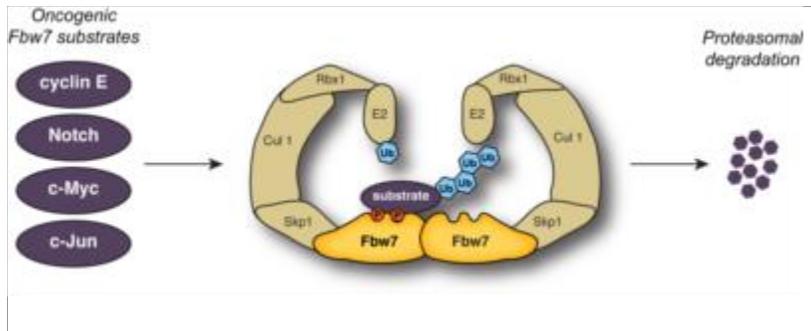
Many of the well-characterized Fbw7 oncoprotein substrates are transcription factors with established or emerging roles in development and tumorigenesis, which highlights Fbw7's broad reach. For instance, c-Myc proteins are known to have profound effects on cancer at least in part through its transcriptional control of proliferation, cell growth, metabolism and protein synthesis. Importantly, the CPD on c-Myc is mutated in some human Burkitt's lymphomas, highlighting a potential pathological consequence of impaired c-Myc degradation. The authors also point out that

mutations in the Notch CPD are mutually exclusive with Fbw7 mutations that occur in T-cell acute lymphoblastic leukemia (T-ALL), which emphasizes the impact of Fbw7-dependent Notch degradation in T-ALL pathogenesis.

Because the prognostic value of Fbw7 mutations had remained unclear despite extensive studies, the authors performed a meta-analysis (a computational study of previous research) of The Cancer Genome Atlas (TCGA), a widely used public database of cancer biology. This analysis confirmed a high prevalence of Fbw7<sup>ARG</sup> mutations, particularly in cancer types where Fbw7 mutations are found in more than 10% of cases, such as T-ALL. Notably, the second Fbw7 allele often remained intact, contrasting with the classical two-hit scenario. The phenotypes of Fbw7<sup>ARG</sup> mice are consistent with Fbw7<sup>ARG</sup> acting in a dominant-negative fashion, but why there has been a strong biological selection for Fbw7<sup>ARG</sup> mutations, compared to nonsense or null mutations, is still unclear. Because Fbw7 functions as a dimer *in vivo*, the authors propose that Fbw7<sup>ARG</sup> dominantly inhibits the protein encoded by the wild-type allele (Fbw7<sup>WT</sup>) and that Fbw7<sup>ARG</sup>-Fbw7<sup>WT</sup> dimers may have substrate-specific effects.

The investigators conclude the review by proposing therapeutic strategies to target the Fbw7 pathway in cancer. The first approach would be to develop small molecule agonists to increase substrate affinity towards Fbw7<sup>ARG</sup>. Second, the oncoprotein substrates themselves could be targeted, and a previous study showed that pharmacologic inhibition of c-Myc in T-ALL to be a potentially viable strategy. Third, a synthetic lethal approach could be employed to identify pathways that are essential for the survival of Fbw7-defective, but not normal cells. Finally, a more general strategy would be to reduce, but not eliminate Fbw7 function, in such a way to eradicate cancer-initiating cells of the hematopoietic system, while preserving Fbw7's tumor-suppressive function in solid tissues. Overall, this review showed that our understanding of Fbw7's tumor suppressive function has progressed tremendously over the past 5 years, through identification of substrates, mouse modeling, and studying the unique biological and oncogenic properties of Fbw7<sup>ARG</sup> mutations. The network of oncogenic pathways regulated by Fbw7 offers a glimpse into how to target this pathway in the clinic.

[Davis RJ, Welcker M, Clurman BE](#). 2014. Tumor Suppression by the Fbw7 Ubiquitin Ligase: Mechanisms and Opportunities. *Cancer Cell*, 26(4), 455-64.



*Image provided by Mr. Ryan Davis.*

The Fbw7 protein binds both to the remainder of the SCF complex (Skp-1, Cullin-1, Rbx1 and an E2 enzyme) and its oncogenic substrates (purple ovals). This binding leads to substrate ubiquitylation (light blue hexagons) and degradation by the proteasome (purple hexagons). This degradation is impaired in many Fbw7-mutated cancers.