**Institutionen för Onkologi-Patologi, Karolinska Institutet, Stockholm, Sverige**

# F-box proteins as regulators of oncogenic pathways by ubiquitylation

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# ABSTRACT

F-box proteins are the substrate-recognition components of the SCF E3 ubiquitin ligases that catalyze the ubiquitylation of many key cell cycle regulators. Functional studies indicate that the ubiquitin-proteasome system participates in the control of nearly all cellular processes through the timely degradation of short-lived regulatory proteins. Accordingly, altered protein degradation due to defective E3 ligases has been shown to underlie many human diseases, such as cancer. The studies in this thesis have focused on the functional characterization of two F-box proteins, FBXW7/hCDC4 and FBXO28, in ubiquitylation and degradation of the cell cycle regulatory proteins, cyclin E and Myc, and their potential deregulation in cancer.

The tumor suppressor protein FBXW7/hCDC4 has been linked to human tumorigenesis through the targeted degradation of several important oncoproteins, including cyclin E. The ubiquitin-dependent turnover of cyclin E1 is regulated by phosphorylation and isomerization of cyclin E1, and executed by the concerted actions of the FBXW7/hCDC4-α and -γ isoforms. Our results demonstrate that this two-isoform dependence is not employed in conditions where cyclin E1 levels are elevated. Under these circumstances, cyclin E1 can be ubiquitylated by FBXW7/hCDC4-α alone, perhaps through an alternative pathway that does not require isomerization. In the second study, we report that cyclin E2 is targeted for ubiquitin-dependent proteolysis by $SCF^{FBXW7/hCDC4}$. Interestingly, we found that cyclin E1 enhances the ubiquitin-dependent proteolysis of cyclin E2, suggesting a mechanism by which cyclin E1 regulates the abundance of cyclin E2, allowing it to possibly perform non-redundant functions in cell cycle control.

In the last two studies we characterized the novel F-box protein, FBXO28, initially identified in an RNAi screen for F-box genes that regulate cell proliferation. We show that $SCF^{FBXO28}$ targets Myc for ubiquitylation, without altering Myc protein turnover. Instead, FBXO28 was found to be an important regulator of Myc-driven transcription through the ubiquitin-dependent recruitment of a transcriptional cofactor to Myc target gene promoters. In addition, we found that FBXO28 is a nuclear substrate for cyclin-CDK phosphorylation and that phosphorylation of FBXO28 is significantly associated with poor prognosis in patients with primary breast cancer. FBXO28 may thus constitute an important player in cell proliferation and Myc pathways during tumorigenesis.

In summary, in this thesis we present different mechanisms by which SCF-mediated ubiquitylation can regulate proliferation, thus linking ubiquitin-mediated processes to proliferative pathways often altered in human cancer.


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