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Institutionen för Cell- och Molekylärbiologi

Characterization of F-box proteins and their target substrates in cancer

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ABSTRACT

Protein degradation, by means of ubiquitylation tagging for subsequent degradation by the ubiquitin-proteasome system (UPS), has opened up a newfound way of protein degradation some three to four decades back. Termed the 'kiss of death', this field of study has since sparked off the quest for substrates for the main enzymes executing ubiquitylation, the E3 ligases. Ubiquitylation of proteins have been implicated in a wide variety of biological processes, many of which whose dysregulation lead to tumorigenesis. One major subgroup, the SCF-type of E3 ligases, utilizes a variable component, an F-box protein, for substrate recognition. However, with more than 70 F-box proteins in our genome, most of them poorly characterized, it remains a challenge to unravel the biological significance of each of these proteins. In this thesis, we seek to expand the understanding of two of such SCF-type E3 ligases, namely, Fbw7 and FBXO28 and their substrates in processes such as cyclin E regulation by Fbw7, MYC-mediated transcription and tumorigenesis by FBXO28 and cell motility with the focus on ßPIX as a substrate of FBXO28.

Previous work has demonstrated that the SCF(Fbw7/Cdc4) complex is responsible for the ubiquitin-dependent degradation of cyclin E1. In the first study (Paper I), we show that a cooperation between Fbw7α and Fbw7γ is required for driving ubiquitylation and degradation of cyclin E1 in the nucleolus. Specifically, we show that Fbw7α acts as a cofactor for Pin1 and aids in isomerization of the cyclin E1 phosphodegron and subsequent translocation and targeting of cyclin E1 for degradation in the nucleolus by Fbw7γ.

In the two other studies, we investigate the function of FBXO28. In **Paper II**, we identify a previously uncharacterized cell cycle-regulated F-box protein, FBXO28, and explore its role in cancer. We show that the CDK1/2 phosphorylated FBXO28 protein assembles a SCF^{FBXO28} ubiquitin ligase that targets MYC for non-proteolytic ubiquitylation and demonstrate that this is important for MYC-driven transcriptional activity. Furthermore, expression of a non-functional FBXO28 mutant or silencing FBXO28 leads to impairment in MYC-driven transcriptional activity, transformation and tumorgenesis. Lastly, we show that high FBXO28 expression and phosphorylation are indicators for poor prognosis in breast cancer. In **Paper III** we find that FBXO28 is able to interact with a group of proteins, the PAK1-ßPIX-GIT1 complex, that are key players in cell migration. FBXO28 is found to localize to the cell-matrix complex upon treatment with EGF and ubiquitylates ßPIX in a non-proteolytic but phosphorylation-dependent manner. Additionally, we show that FBXO28 positively regulates the formation of PAK1-ßPIX-GIT1 complexes, and a depletion of FBXO28 leads to an impairment in cell migration and invasion of metastatic cancer cells. Furthermore, we demonstrate a poor prognosis for breast cancer patients with membranous staining of FBXO28.

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