Ubiquitination in apoptosis signaling
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Citation for published version (APA):

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SUMMARY

Background and aims

Apoptosis signaling is tightly regulated. Most pro-apoptotic proteins are normally inactive, and require transcriptional upregulation, posttranslational modification or translocation to become active. In addition, they can be held in check by anti-apoptotic proteins. Therefore, the balance between pro-apoptotic protein activity and anti-apoptotic protein activity determines whether a cell goes into apoptosis after exposure to stress stimuli. Only when the stress is severe enough, the threshold is overcome and the cell dies by apoptosis.

Cancer cells encounter many stress stimuli during their transformation, rapid growth and metastasis. Such stimuli would induce apoptosis in normal cells, but a hallmark of cancer cells is a deregulated apoptosis signaling pathway as a result of genetic lesions they acquired during transformation. This resets the apoptotic threshold such that cancer cells can just survive the stress they normally encounter, but paradoxically also makes cancer cells very sensitive to any additional apoptosis-inducing stimuli. From a molecular point of view, it means that the reservoir of anti-apoptotic proteins is completely saturated by active pro-apoptotic proteins. This cancer cell state is known as ‘primed for death’. Initiating apoptosis in tumor cells is therefore a promising anti-cancer strategy that has reached the clinic, as exemplified by the BH3 mimetic ABT-263 and the death ligand TRAIL. To understand how the apoptotic threshold is reset in tumor cells, and to design therapies to overcome this threshold specifically in tumor cells, it is essential to understand the regulation of apoptosis protein activity in molecular detail.

A posttranslational modification that can strongly affect protein activity is ubiquitination, which can regulate protein level, protein location, and protein interaction. Ubiquitination is not only important in apoptosis signaling, but regulates most signaling pathways in the cell. Canonically, ubiquitin was described to be conjugated to the ε–amino-group of lysine residues, or in rare cases to the α–amino-group of the N-terminal residue. However, our lab and others have demonstrated that cysteine, serine and threonine residues can be targeted for an unconventional type of ubiquitination. The list of substrates modified by unconventional ubiquitination is steadily increasing, but what mammalian ubiquitination enzymes can mediate this ubiquitin conjugation is unknown.

Ubiquitination is one of the factors regulating protein stability, but on its own does not explain the extreme variation in protein turnover time that can vary from minutes to days. Regulation of protein degradation is essential, because together with protein synthesis rate, it will determine the protein levels in the cell. It has been suggested that N-terminal acetylation affects protein degradation rate, but it is disputed whether it stabilizes a given protein or rather targets it for degradation.

The work described in this thesis focuses on ubiquitination and protein degradation, with an emphasis on how these processes regulate apoptosis signaling. More specifically, our aims were:
1. To increase the understanding of ubiquitin-mediated regulation of apoptosis signaling
2. To identify the E3 ubiquitin ligase targeting the apoptosis protein tBidN for unconventional ubiquitination
3. To delineate the role of N-terminal acetylation in protein degradation

Main findings and implications
We have identified two novel ubiquitination events that regulate apoptosis signaling. In chapter 2, we demonstrate that the anti-apoptotic protein Bcl-B is modified by K48-linked ubiquitination on a lysine residue in a loop that is unique within the group of anti-apoptotic Bcl-2 family members. This targets Bcl-B for proteasomal degradation, and therefore regulates its protein level at steady state. Ubiquitination thereby regulates the capacity of Bcl-B to protect against cell death as induced by a variety of anti-cancer therapeutics. Clinically, cancer cells can depend on the high expression of Bcl-B, and its expression level can be used as biomarker to predict tumor aggressiveness. Our data indicate that Bcl-B expression should be assessed on the protein rather than the mRNA level. In addition, modulating the activity of the enzymes regulating Bcl-B ubiquitination, for example with small molecules, might be a feasible anti-cancer therapy.

In chapter 3, we show that ubiquitination regulates cell surface expression, and therefore pro-apoptotic activity, of the death receptor TRAIL-R1. We identify the transmembrane ligase MARCH-8 as the enzyme targeting TRAIL-R1 for ubiquitination and subsequent degradation in lysosomes. TRAIL-R1 cell surface expression was increased by knockdown of MARCH-8, suggesting that inhibition of MARCH-8 function might be an efficient strategy to sensitize tumor cells to TRAIL therapy. From a more fundamental perspective, TRAIL-R1 is the first identified substrate of endogenous MARCH-8, whose molecular characteristics and physiological functions are largely unknown.

We previously identified the N-terminal fragment of caspase-8 cleaved Bid (tBidN) as a substrate for unconventional ubiquitination. This ubiquitination is required to target tBidN for proteasomal degradation, and therefore relieves the inhibition that tBidN imposes on tBidC-mediated apoptosis. We aimed to identify the (de-)ubiquitination enzymes modulating tBidN ubiquitination status and therefore stability (chapter 4). To this end, we developed an RNAi-based screening approach that allows to read-out protein stability by flow cytometry, of three different proteins at once. In addition, we analyzed the tBidN-interactome by mass spectrometry. The N-end rule ligase UBR2 was found among the interactors, and the interaction was confirmed by co-immunoprecipitation studies. The interaction involved the hydrophobic type II binding site in UBR2, and most likely involved an internal region in tBidN. UBR2 targeted tBidN for ubiquitination, possibly including unconventional ubiquitination by esterification. These data suggest that UBR2 is a novel regulator of apoptosis signaling, and could be one of the first identified mammalian E3 ligase that can mediate unconventional ubiquitin conjugation.

In chapter 5, we study the role of N-acetylation in protein degradation. We found that N-acetylation inhibits protein degradation by two different mechanisms. First, it blocks N-terminal ubiquitination of protein substrates and thereby reduces their ubiquitin-mediated targeting to the proteasome. Second, it could block proteasomal degradation directly, independent of its effect on N-terminal ubiquitination. Hence, N-acetylation is an important determinant of protein stability.
REFERENCES