Abstract

Palmitoylation is a common and important posttranslational modification. It is defined as the addition of palmitic acid to internal cysteines. Interestingly, in contrast to other lipid modifications, it is reversible. Given that palmitoylation can regulate protein-protein interactions, stabilize proteins or control their localization, control over the palmitoylation/depalmitoylation cycle provides indirect control over protein activity and function. While palmitoylation is catalyzed by the huge family of DHHC proteins, APT1 and APT2 (acyl-protein thioesterase 1 and 2) are the only enzymes known to be responsible for the process of depalmitoylation. Two death receptors, TRAIL-R1 and Fas, are reported to be palmitoylated. In addition, CLL (chronic lymphocytic leukemia) cells are known to be resistant to death receptor-mediated apoptosis. Therefore, it might be possible to regulate death receptor function by the regulation of death receptor depalmitoylation. One aim of this project was to elucidate the regulation of the palmitoylation/depalmitoylation cycle in CLL in more detail. In addition, the role of palmitoylation in death receptor signaling and its impact on apoptosis-resistant CLL cells should be investigated. It could be shown that the global palmitoylation cycle in CLL is significantly deregulated. There are indications that in particular depalmitoylation is increased in these cells. In line with this, APT1 and APT2 were found to be significantly overexpressed. Five miRNAs were identified as downregulated in CLL and as potential regulators of APT1 and APT2. And indeed, overexpression of four of those miRNAs (miR-138, miR-200a, miR-424 and miR-125a-5p) led to downregulation of both enzymes in malignant cells on protein level. Importantly, different interaction assays revealed that most probably both death receptors, Fas and TRAIL-R1, are directly depalmitoylated by APT1 and APT2. Additionally, it was shown that the mobility of Fas on the plasma membrane is palmitoylation-dependent. Increased palmitoylation induced death receptor aggregation. Therefore, mobility of Fas might be restricted by protein-protein interactions. On the other hand, enhanced palmitoylation of TRAIL-R1 increased the receptor's localization in DRMs, leading to more activated death receptor. Most remarkable, pharmacological inhibition of depalmitoylation sensitized resistant CLL cells, CLL-like Mec1 cells and HeLa cells to death receptor-mediated apoptosis. In addition, it was confirmed by siRNA and miRNA experiments that APT1 and APT2 are responsible for the sensitization of these cells. These results show that the interaction between thioesterases and death receptors is essential for proper apoptotic signaling. It reveals how important palmitoylation is for the induction of apoptosis by death receptors and offers exciting new insights into the pathogenesis of CLL.