Abstract

Maintaining the integrity of the plasma membrane is essential for cells in order to survive. With the increased motility, membrane dynamics and a need to navigate through dense extracellular matrix for tissue invasion, membrane stress poses big challenges for invasive cancer cells and thus they depend on an efficient plasma membrane repair (PMR) machinery to cope with this challenge. The underlying molecular mechanisms used to repair membrane lesions in eukaryotic cells are not well characterized. Furthermore, the potential role of PMR in cancer metastasis and disease progression are not yet investigated. Metastasis is responsible for about 90% of cancer deaths, thus novel and innovative strategies for its inhibition are needed.

In this thesis the role of PMR in invasive breast cancer cells versus non-invasive breast cancer cells was investigated using a human metastatic breast cancer cell model overexpressing epidermal growth factor receptor-2 (ErbB2/HER2). Using this cell model system, we found that the cell membranes of invasive breast cancer cells are injured more frequently than those of non-metastatic cells. These cells compensate efficiently by up-regulating their PMR machinery, implicating annexin- and S100 proteins, which are often found up-regulated in metastatic cancers. We show that the Ca^{2+}-binding protein S100A11 in a complex with the Ca^{2+}- and phospholipid- binding protein annexin A2 (ANXA2) are important for efficient PMR in invasive breast cancer cells. We also provide the first evidence for the existence of this complex *in vivo*. RNAi-mediated depletion of S100A11 compromises membrane wound healing after laser and glass beads induced injury, and restricts the invasiveness of ErbB2 positive breast cancer cells. Following injury to the cell membrane and Ca^{2+}-flux into the cytoplasm, S100A11 and ANXA2 are recruited and co-accumulate at the site of injury within 15-45 seconds. This co-accumulation was mutually dependent. Our results suggest that S100A11 in a complex with ANXA2 is involved in PMR, in part by facilitating polymerization of cortical filamentous actin (F-actin) and excision of the damaged part of the membrane.

Prompted by these findings, we investigated potential proteins involved in the PMR system in ErbB2 positive breast cancer cells by quantitative proteomics analysis. Here, several annexin family members were recruited to the plasma membrane upon injury, including annexin A1-7, A9, and A11. We sought to characterize the potential role and function of the Ca^{2+} - and phospholipid-binding protein annexin A7 (ANXA7) in PMR. Upon laser induced membrane injury, we found that ANXA7 translocates to the site of repair within 15-30 seconds and stays until the plasma membrane is resealed. RNAi-mediated depletion of ANXA7 compromises membrane wound healing after laser and detergent induced injury and restricts the invasiveness of ErbB2 positive breast cancer cells. Furthermore, we found that upon local membrane disruption, ANXA7 binds Apoptosis Linked Gene-2 (ALG-2) in a Ca^{2+}-dependent manner. Depletion of ANXA7 by siRNAs or knockout by CRISPR/cas9 delayed translocation of ALG-2 to the site of injury and delayed assembly of the Endosomal Sorting Complex Required for Transport III (ESCRT III). This effect inhibited the shedding activity around the wounded membrane. Our results suggest that ANXA7 positions and anchors ALG-2 to the injured membrane to initiate assembly of the ESCRT III complex at the site of repair to excise damaged membrane, which is required for wound healing in invasive breast cancer cells.

Our findings show that S100A11, ANXA2 and ANXA7 are involved in PMR and identify them and PMR in general as potential targets for cancer therapy of metastatic cancers.