Abstract

Numerous promising drug candidates exhibit, owing to their size and hydrophilic nature, a poor bioavailability and consequently, their clinical application is limited. An attractive way to increase the effectiveness of such drug molecules is to deliver them specifically to their site of action in order to prevent unwanted side effects. Over the years, many strategies evolved, including the use of so-called cell-penetrating peptides (CPPs) as a tool to overcome the obstacle of poor cell-permeability of many biologically active molecules. CPPs have the capability to translocate across cellular membranes without destroying their integrity and to carry a broad variety of cargoes, which can either be attached electrostatically or covalently.

The first part of this thesis deals with the optimization of CPPs concerning their uptake efficiencies. In particular, an alanine-scan of the CPP sC18 was performed to study the structure-function relationship of the peptide yielding two peptides which were further characterized. In the first case, an increase in net charge and an improved amphipathic structure resulted in significantly higher internalization rates compared to the native peptide. The other peptide sequence demonstrated decreased uptake efficiencies and was used as a control. These two novel peptides and sC18 were decorated to the surface of silica nanoparticles of different sizes (50-300 nm) *via* electrostatic interactions. Cellular uptake studies revealed an internalization, which was mainly dependent on the size of the particle and the used CPP. The obtained data highlight that the key driving force for these differences was the net charge of the peptide and generally allowed deeper insights to achieve the greatest possible uptake efficiencies of such CPP-NP conjugates.

Nowadays, since it is known that many diseases are caused by dysfunctions at the subcellular level, the delivery of therapeutic molecules to a defined intracellular destination is of increasing interest. Therefore, the second part of this work focuses on the combination of CPPs with specific sorting signals to achieve sub-cellular targeting. First, different mitochondrial-targeting sequences (MTS) were fused to sC18 to overcome cellular membranes and to reach the mitochondrial organelle. The biological characterization of the novel peptide hybrids demonstrated that not every chosen MTS was suitable for the targeted delivery into mitochondria and that also the CPP moiety plays a crucial role for the uptake into the matrix of this organelle. The cytostatic agent chlorambucil was conjugated to the most promising chimera and anti-proliferative assays revealed a more pronounced cell growth arrest, pointing out the great potential of combining CPPs with further peptide moieties. Therefore, the last part of this thesis, is dedicated to the incorporation of another targeting sequence, in particular the CaaX-box relevant for protein prenylation. The latter is a post-translational modification mediated by prenyltransferases, which is used by eukaryotic cells to attach isoprenoid lipids to proteins and enables the association to membranes. Herein, a shortened version of sC18

was elongated with the *C*-terminal region of different Ras-proteins to verify if the prenylation machinery can be used to target specific membrane compartments. It turned out that the cellular uptake of these novel CaaX-peptides is dependent on the intracellular recognition of the CaaX-motif and that they have the ability to directly interact with prenyltransferases. Moreover, one of the CaaX-peptides affected downstream signaling of Ras-proteins in pancreatic cancer cells, which highlights the ability to potentially act as mimetics for such proteins by competing for prenylation.