Delivery of therapeutic drugs in the form of a Trojan horse

When our immune system is infiltrated by pathogens and other disease-causing particles, the key response is usually inflammation and cell death. Cell death is also essential for fundamental physiological processes such as (embryonal) development and tissue homeostasis. In order to ensure the proper functioning of all organs in our body, millions of our approximately 1014 body cells are removed (and replaced) daily by apoptosis, a programmed form of evolutionarily conserved, non-immunogenic cell death that is tightly controlled at the genetic level. To avoid a deleterious immune response, the resulting dead cells are eliminated by phagocytes. For a long time, scientists believed that cell death was caused either via apoptosis or was considered to be unregulated (accidentally) and non-programmed. All forms of unregulated cell death were merged under the term “necrosis”, which is a process that finally results in the rupture of the plasma membrane and the uncontrolled release of intracellular contents. Therefore, this type of cell death (via necrosis) is profoundly immunogenic and triggers inflammatory responses in neighboring cells too. Recent research demonstrates that beside apoptosis, there exist other multiple forms of cell death that are also genetically programmed (summarized in Galluzzi et al., Semin Cell Dev Biol. 2014). Over the last decade, we have discovered and characterized diverse pathways of regulated necrosis that contribute in a combined fashion to a plethora of human diseases and organ failure, including sepsis, myocardial infarction and solid organ transplantation failure. Therefore, there is a significant amount of interest in the development of therapeutics to target these different pathways for clinical purposes, which will revolutionize clinical practice in the near future.

One of the main reasons that irreversible damage occurs is the long interval between a patient’s admission at the hospital and the successful application of effective therapy. Protein-based therapeutics may be a promising solution. This method involves the administration of directly useable bioactive proteins that can correct defects in proteins involved in a multiplicity of different genetic-metabolic disorders and diseases. Due to its unrivaled efficacy, protein-based therapeutics has the potential to become the medicine of the future. Unfortunately, since bioactive proteins are in the form of macromolecules, their uptake is selectively restricted by the hydrophobic phospholipid bilayer of cellular membranes.

There may be a solution to the impenetrability issue of bioactive proteins, which can be derived from the human immunodeficiency virus (HIV). This virus spreads across cells via a very small domain called TAT, which translocates into neighboring cells to modify gene transcription and spread the disease. In simple terms, it is the TAT domain that facilitates viral entry into the cell, and it is therefore designated as a cell penetrating peptide (CPP). The CPP domain is therefore like a spearhead that can potentially be used to transfer various types of molecules into cells. In other words, TAT can serve as a Trojan horse that can be used to deliver per se impervious large molecules (drugs) into (injured) cells. In the context of protein-based therapeutics, this large molecule would be a bioactive protein that would be able to prevent regulated cell death. This technology requires the synthesis of a fusion protein by linking the TAT transduction domain to a molecule of interest using a bacterial expression system and purification of this fusion protein. In

principle, this purified fusion protein can be directly injected into patients. So far, TAT fusion proteins and peptides have been used to treat mouse models of cancer, inflammation and other diseases. The success of this promising technology is illustrated impressively in Figure 1. In a clinically relevant model of fulminant liver failure (the degree of damage is indicated by the discoloration in the liver in the middle), we were able to prevent cellular apoptosis by administration of a TAT-crmA fusion protein. CrmA is the counterpart to caspase-8, a molecule that is activated during this cell death process, which is always fatal if left untreated.

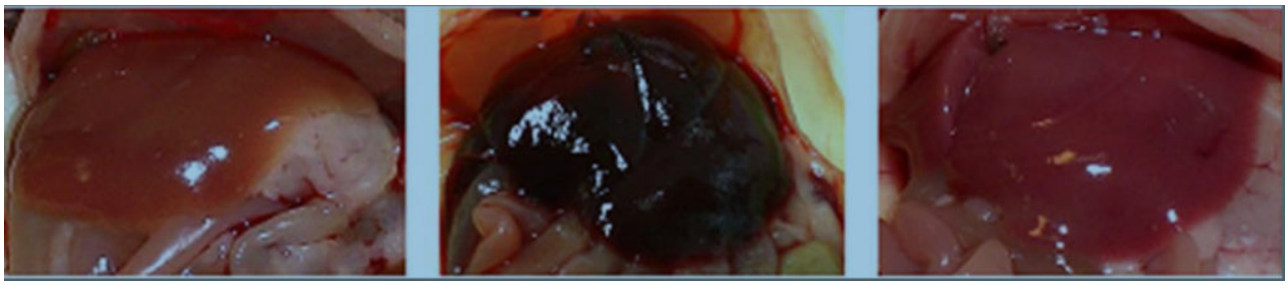

Fig. 1. The induction of apoptosis (middle) in the course of fulminant liver failure is prevented by concomitant treatment which a TAT-crmA fusion protein (right). A healthy control is represented on the left side (from Krautwald et al., J Biol Chem. 2010).

In the undermentioned article, we have delineated the current technology for CPP transfer and summarized studies on the most commonly used protein transduction domains and their potential as therapeutic agents for the treatment of cellular damage and the prevention of regulated cell death. Clinically feasible interference to attenuate organ injury may only be possible in situations in which reperfusion damage or the mode of regulated cell death can be anticipated. Therefore, cardiac surgery and particularly solid organ transplantation are promising therapeutic applications of this novel technology.

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Publication

Inhibition of regulated cell death by cell-penetrating peptides.
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