Redox Responsive Polymeric Nanocapsules for Protein Delivery

Muxun Zhao, Yi Tang
Department of Chemical and Biomolecular Engineering, University of California, Los Angeles

Statement of Purpose: Virtually all human cancer cells have elaborate anti-apoptotic strategies to overcome apoptosis, which is a vital anti-neoplastic mechanism to obstruct tumor progression. Therefore, the ability to rapidly resurrect the apoptosis circuitry in tumor cells can be a highly effective option for cancer treatment. Although different strategies have been developed to counter the individual inactivation mechanisms, a more potent chemotherapy option is to directly arm the cancer cells with executioner proteins or apoptosis-inducing proteins that are not targeted by these aforementioned anti-apoptotic maneuvers. From a therapeutic perspective, protein-based approaches are safer than gene therapy because no random or permanent genetic changes are involved, and only transient actions of proteins are needed for the desired results. However, compared to small molecule therapeutics, proteins suffer from serum instability and are inefficient or unable to penetrate the cell membrane. As a result, protein-based antitumor options still remain elusive, despite its numerous therapeutic targets and vast potential.

Apoptin is a small protein (121 amino acids) isolated from chicken anemia virus (CAV) that induces p53-independent apoptosis in a tumor-specific way. In a variety of tumor cell lines, apoptin becomes phosphorylated, enters the nucleus, and induces apoptosis. In sharp contrast, apoptin is unphosphorylated in normal cells and stays in the cytoplasm. An important feature of apoptin is that it can recognize early stages of oncogenesis and it can induce apoptosis. Due to its high selectivity and potency, apoptin has become an attractive antitumor target for gene therapy approaches. We have developed a simple and effective method of delivering proteins intracellularly. Using this method, apoptin has been delivered to cancer cells and in xenograft mouse tumors with its function and selectivity preserved (*1*).

Methods: To deliver apoptin protein directly, we first encapsulated recombinant maltose-binding-protein (MBP) apoptin fusion protein in a degradable polymeric nanocapsule (*2*). The nanocapsule is formed by first electrostatically depositing acrylamide-based monomers on to the surface of the MBP-apoptin complex (MBP-apoptin forms a 30-40 mer multimeric complex with an estimated size of 35 nm). Followed by addition of a redox-sensitive crosslinker (*3*), *in situ* polymerization is initiated with the free radical initiators and proceeded for one hour to form a thin, positively charged polymer layer that encapsulates the MBP-apoptin. Particles formed are characterized by DLS, followed by *in vitro* cellular uptake and cytotoxicity assays, as well as *in vivo* MCF-7 xenograft studies.

Results: The MBP-apoptin particles are found to be uniform in size and charge. When added to cancer cell lines, the nanocapsule readily penetrated the cell membranes, disintegrated to release the MBP-apoptin and induced rapid apoptosis of the cells. In contrast, the nanocapsule had no apoptotic effect on noncancerous cell lines such as HFF, highlighting the differential targeting on tumor cells only. Confocal microscopy of rhodamine labeled MBP-apoptin showed that the protein entered the nucleus of cancer cells only, while remained in cytosol of HFF. When injected intratumorally into mice carrying xenografted MCF-7 tumor, the nanocapsules significantly inhibited the progression of tumor growth in comparison to the controls, demonstrating the potential utility of this polymer-protein complex as a cancer therapeutic.

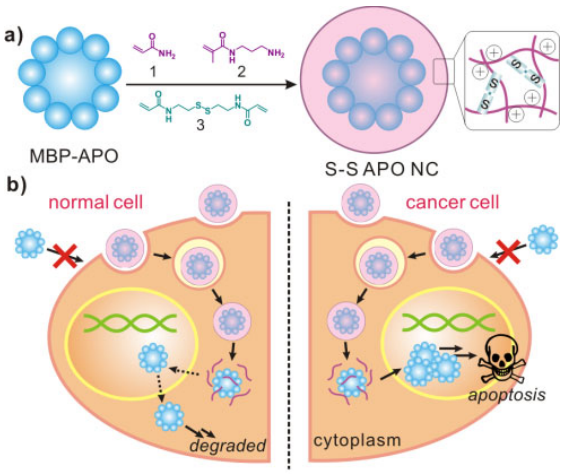

Figure 1. Degradable nanocapsules for apoptin delivery. a-b) Schematic diagram of synthesis of degradable apoptin nanocapsules (S-S APO NC) and delivery into tumor cells to induce apoptosis.

Conclusions: We were able to deliver the high molecular weight complex of the tumor-selective MBP-APO using a redox-responsive polymeric nanocapsule *in vitro* and *in vivo*. The choice and design of the sub-100 nm nanocapsule is well-suited for diverse protein targets because of its mild preparation conditions, completely reversible encapsulation and efficient cell membrane penetration/release of the protein cargo in the cytoplasm. Our application here further illustrates how intracellular protein delivery using nanoscale system can provide new possibilities for achieving selective cancer therapy.

References:

(*1*) Zhao M. *Nano Today* **2012**; in revision.
(*2*) Gu Z. *Nano Lett.* **2009**; 9:4533-4538.
(*3*) Zhao M. *Biomaterials* **2011**; 32:5223-5230.