Abstract

The next generation of cancer therapeutics will specifically target processes responsible for the growth and survival of cancer cells. Among the most promising of these molecularly targeted therapeutics are small interfering RNAs (siRNAs). These siRNAs serve as the effectors of RNA interference, a naturally occurring and highly specific mechanism for regulating gene expression through sequence-specific degradation of messenger RNA. While these siRNAs have shown potential in vitro and in preclinical animal models, safe and effective systemic delivery remains one of the greatest challenges hindering their clinical application. This thesis describes an engineering approach to address the challenge of systemic delivery of siRNAs for cancer therapy.

Analysis of the kinetics of siRNA-mediated gene silencing reveals that gene inhibition by unmodified siRNAs can last for one week in rapidly dividing cells and up to one month in cells with minimal division. Additionally, chemical modifications to enhance siRNA nuclease stability do not prolong intracellular siRNA activity. These data, when used in combination with results from a mathematical model of siRNA function, demonstrate that dilution from cell division, and not intracellular nuclease stability, is the dominant factor governing the duration of gene inhibition by siRNAs.

Cyclodextrin-containing polycations (CDP) can self-assemble with siRNAs to form nanoparticles with desirable properties for systemic application. Characterization of these nanoparticles demonstrates that they can contain several thousand siRNAs, protect the siRNA payload from nuclease degradation, and be modified with transferrin targeting ligands that show multivalent binding to cell surface receptors.

Multimodality in vivo imaging with positron emission tomography (PET) and bioluminescent imaging (BLI) is used to monitor the biodistribution and function of the siRNA nanoparticles after intravenous administration in live mice. Attachment of targeting ligands to the surface of the nanoparticles enhances gene inhibition within the tumor, although the biodistribution and tumor localization are not dependent on the amount of targeting ligand. The targeting ligand likely serves to augment nanoparticle uptake by the tumor cells. When the siRNA nanoparticles are used to deliver therapeutic siRNAs to achieve tumor growth inhibition in disseminated and subcutaneous murine cancer models, schedule-dependent anti-tumor effects are observed.