457e Design of Effective Cancer Treatment Strategies Using Systemically Delivered Sirna: Insights from in Vivo Studies and a Mathematical Model of RNA Interference

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RNA interference (RNAi) refers to the ability of double-stranded RNA molecules to cause sequence-specific degradation of complementary mRNA sequences. The effector molecules of RNAi are 21 to 23-nucleotide small interfering RNAs (siRNAs) that are anticipated to serve as novel therapeutics for the knockdown of pathogenic gene products associated with diseases including cancer, viral infections, and autoimmune disorders. However, systemic administration of siRNA molecules presents a significant challenge that has slowed the realization of RNAi-based therapies. Recently, we have demonstrated the successful use of transferrin (Tf)-targeted, cyclodextrin-based polycations (CDP) for systemic delivery of siRNA in a murine model of Ewing's sarcoma. Twice-weekly administration of siRNA at a dose of 2.5 mg/kg inhibited the growth of metastases after low-pressure tail-vein injection of 5x10^6 TC71 Ewing's sarcoma cells in NOD/scid mice. This raises the question of what the minimum dose and dose frequency would be to achieve this tumor growth inhibition. Given the relatively recent discovery of RNAi in mammalian cells, many details of its mechanism are still being elucidated and the design of treatment regimens to achieve gene knockdown depends heavily on trial and error. With the high cost of siRNA molecules and the relatively large quantities needed for in vivo application, experimental exploration of the parameter space for optimizing RNAi-based cancer treatment is prohibitive. Design of a mathematical model that could explore the impact of parameters such as siRNA dose, siRNA dose frequency, tumor (cell) doubling time, and delivery method (e.g., naked siRNA, formulated with vector, chemically modified) would save time and resources while accelerating the development of life-saving cancer therapies. Therefore, we designed a kinetic model that incorporates the key steps involved in RNAi-based therapy from the delivery of siRNA to the cells to cleavage of the target mRNA molecules. We show how mathematical modeling can be combined with in vitro and in vivo experimental data to rationally develop RNAi-based treatment regimens for cancer therapy. Cell lines engineered to constitutively express luciferase provide convenient model systems to study siRNA-mediated gene silencing in vitro and in vivo. We use siRNA against the luciferase gene and monitor the degree of gene silencing by bioluminescent imaging with the Xenogen IVIS imaging system. These cell lines can be used for in vitro studies or injected into mice to form subcutaneous or metastatic tumors. In addition to the murine model of Ewing's sarcoma in NOD/scid mice using luciferase-expressing TC71 cells, we have developed a syngeneic model of neuroblastoma using luciferase-expressing Neuro2A cells in A/J mice. Transgenic C57BL/6 mice with hepatocyte-specific luciferase expression provide a third experimental system. Systemic delivery of siRNA against luciferase in these mice will be used to investigate the effects of dose, dose frequency, and delivery method on the luciferase knockdown in the tumors (NOD/scid, A/J) or liver (C57BL/6 transgenic). Preliminary in vitro and in vivo experiments looking at the effect of cell doubling time illustrate our use of both experimental data and mathematical modeling to better understand siRNA-mediated gene silencing and to rationally design effective treatments. Using cell lines with different doubling times, we observed that gene silencing was shorter in more rapidly growing cells and the mathematical model's predictions closely matched the experimental data. This effect is also observed in vivo. While siRNA-mediated luciferase knockdown lasts only a few days in rapidly growing tumors, we observe gene silencing for over two weeks after delivery of siRNA to hepatocytes. Calculations with the mathematical model demonstrate that this discrepancy in the duration of gene silencing can be explained by the differences in cell doubling time between the tumors (~2-3 days) and the hepatocytes (~1 year). These results indicate that cell doubling time, or tumor growth rate, is a critical parameter to consider when designing a treatment schedule since rapidly growing cells will require more frequent dosing to maintain silencing of a target gene. Future experiments will look at the effect of other experimentally controllable parameters such as siRNA dose, dose frequency, and delivery method. This systematic study of siRNA-mediated gene silencing in vitro and in vivo will provide a foundation for designing therapeutic treatment regimens. Just as importantly,

it will allow us to validate the mathematical model by demonstrating that the model can accurately predict the observed trends. This will enable us to put the mathematical model to practical use as a tool for the design and modification of siRNA treatments. Because effective treatment of diseases, including some cancers, may require that a pathogenic gene product only be reduced below a threshold value to have the desired effect such as apoptosis of the cancer cells, the mathematical model can be used to guide modification of RNAi-based treatments to achieve a desired reduction in the target gene and its protein product. With the burgeoning interest in nucleic acid-based therapeutics such as siRNA, mathematical models that can aid in the design of effective treatment strategies will augment the understanding of this new class of therapeutics and expedite the development of clinically relevant therapeutics for disease treatment and management.