Optimization of siRNAs for the selective targetting of point mutations

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Background: Small interfering RNAs (siRNAs) direct the RNA-induced silencing complex (RISC), containing Argonaute2 (Ago2), to cleave complementary mRNA targets. Although recent structural and kinetic studies have clarified the molecular steps involved in target recognition and cleavage, a quantitative framework linking sequence features to cleavage efficiency remains lacking. This work presents a mechanistic mathematical model describing Ago2-mediated mRNA cleavage and its dependence on siRNA sequence. Method: A reaction mechanism was developed based on the sequential formation of enzyme–substrate complexes, including initial target binding, activation through central base pairing, irreversible mRNA cleavage, and product release. Steady-state kinetic equations were derived for conditions of substrate excess, enzyme excess, and the general case, yielding Michaelis–Mentenand Morrison-like rate equations. Model parameters were related to thermodynamic quantities governing target binding (Kb) and catalytic activation (Ka). Cleavage rate constants (kc) were predicted using a sequence-based linear regression model trained on experimentally measured cleavage rates with leave-one-out cross-validation (LOOCV). Additional models incorporated the effects of mismatches and RNA duplex thermodynamics on catalytic activation. Results: The proposed model reproduces the experimentally observed Michaelis–Menten behaviour under substrate excess and firstorder kinetics under enzyme excess. The sequence-based prediction of the cleavage rate constant achieved a correlation coefficient of 0.906 between predicted and experimental ln(kc) values in LOOCV. The framework also captures the influence of sequence composition, mismatch position, and thermodynamic stability on target cleavage, allowing quantitative prediction of mRNA silencing efficiency under physiological conditions. Conclusion: This mechanistic model integrates structural, thermodynamic, and kinetic aspects of Ago2-mediated RNA interference into a unified quantitative framework. By linking siRNA sequence features to catalytic efficiency, it provides a predictive tool for estimating mRNA cleavage rates and silencing efficacy, with potential applications in the rational design and optimization of therapeutic siRNAs.