The design of novel RNAi vectors to study the effect of silencing genes in the ceramide biosynthesis pathway and apoptosis.

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The study of ceramide induced apoptosis has revealed a surprising array of candidate targets and a range of drugs or candidate drugs, but it is difficult to determine which of these are attractive as candidates for the development of drugs to inhibit ceramide signalling.

Our aim is to develop siRNA based technology to determine for which of the candidate genes is ablation of expression compatible with viability. We are adopting a strategy of generating two or more novel siRNA constructs for each target, to test these for cell viability and then for the effect on ceramide sensitivity.

To date, we have generated approximately 50 vector constructs containing targets to several genes thought to be involved in apoptosis, in particular in ceramide induced apoptosis. We have also designed siRNA targets against additional mutants from our CTL resistant library. These include siRNAs directed against the genes for endozepine, GEMIN4, LIAS and cyclophilin A. Initial studies performed with a set of constructs, showed no effects of toxicity in the 3T3 cell line tested. APOPercentage assays performed on these selected constructs showed that no apoptosis was triggered upon the silencing of these genes. The efficiency of gene silencing using this system is currently being assayed using quantitative PCR. Further studies are underway to test all the constructs and assay the effect of silencing, the selected genes, on the apoptosis induced by ceramide.