DESIGN AND TESTING OF RIBOZYMES FOR CANCER GENE THERAPY

James S. Norris, Brian Hoel, Dale Voeks, Friderikj Maggouta, Michael Dahm\textsuperscript{1}, Weihua Pan\textsuperscript{2}, and Gary Clawson\textsuperscript{2}

Department of Microbiology and Immunology
Medical University of South Carolina
171 Ashley Avenue
Charleston, SC 29425
\textsuperscript{1}HEXAL Gentech Forschungs GmbH
Industriestr, 25
D-83607 Holzkirchen
Germany
\textsuperscript{2}Department of Pathology C7768
Penn State University
500 University Drive
Hershey, Pennsylvania 17033

INTRODUCTION

The following chapter will cover the subject of the development of a series of ribozymes that target the AC40 subunit of RNA polymerase I (RNA pol I), an essential cellular gene that, when inactivated, leads to cell death. The ribozymes are designed to be delivered in a virus or a liposome as a therapy for prostate cancer. In the course of development of these ribozymes it became clear that target selection in any given mRNA was subject to rules that were not easily defined. Therefore, the following chapter will describe the efforts of this laboratory to develop ribozymes against one of the essential subunits of RNA pol I first using an \textit{in vitro} target selection system coupled with computer modeling followed by functional analyses of release and target cleavage activities.

RNA POLYMERASE I

Yeast and higher eukaryotes contain three nuclear RNA polymerases. RNA pol I is used to transcribe rDNA genes, while the other two are involved in transcription of...
messenger RNA, 5S, tRNAs, U6 and 7SK RNAs and certain viral RNAs. RNA pol I consists of at least 14 subunits of which ten appear to be essential for the function of the enzyme. Two subunits AC40 and AC19 are thought to be essential and likely play a role in subunit assembly. The AC40 subunit is also shared by RNA pol III (Lanzendorfer et al., 1997). The mouse and human homologue of AC40 are cloned (Song et al., 1994; unpublished).

Where studied, ribosomal RNA genes have been found to be tandemly arrayed with 100–1,000 copies spanning several million base pairs (Wallace and Birnstiel, 1966). RNA pol I transcribes these genes and the primary transcripts are processed to form 18S, 5.8S and 28S ribosomal RNA which form the core of the ribozyme. Ribosomal genes account for 40% of the total cellular transcription and 80% of the RNA content of the cell while constituting only 1% of the genome (Moss and Stefanovsky, 1995). Mature ribosomal RNA and ribosomal proteins form the ribosome complex. We hypothesized that any disruptions of ribosome complex formation would likely result in dysregulation of translation and/or death of the cell. The retinoblastoma gene product has been shown to disrupt RNA pol I mediated transcription by sequestering upstream binding factor UBF, thus down-regulating cellular proliferation via disruption of ribosome assembly (Cavanaugh et al., 1995; Voit, Schafer, and Grummit, 1997).

**PROSTATE CANCER**

Prostate cancer is now the most frequently diagnosed cancer in US males and the second leading cause of death (Proctor et al., 1997). Already a major health problem, increases in incidence and mortality are predicted in the next 15 years as the population ages. The molecular mechanisms behind disease initiation and progression still remain largely a mystery. The overall value of screening and treatment also remains controversial. A sensitive marker for screening and monitoring prostate cancer is prostate-specific antigen (PSA). Concerns have been raised about the diagnostic accuracy of this molecule and its predictive ability although newer methodology measuring bound and free PSA have been developed and may be more predictive.

Therapies for prostate cancer are largely defined by stage of disease and include radical prostatectomy, radiation therapy, and cryotherapy. If the disease is not detected until it is more advanced, distant metastases have usually occurred and watchful waiting, radiation therapy, hormonal ablation therapy and limited chemotherapy have been employed, none with any great success. The occurrence rates and mortality of non-localized disease is very high (Cersosimo and Carr, 1996). Further, about one-third of patients at diagnosis are in an advanced stage of disease, thus, additional therapeutic options are clearly required before successful treatment of prostate cancer can be achieved.

There are a number of gene therapeutic approaches being tested for prostate cancer, including trials that involve stimulation of the immune system and trials where delivery of herpes simplex virus thymidine kinase gene is used to cause a bystander effect following application of gancyclovir. Other clinical trials are in progress. Presently the most serious deficiency in the application of gene therapy is the lack of delivery vehicles. This continues in 1998 and until such delivery systems have been developed, successful treatment of prostate cancer will be problematic, unless immunotherapy or successful bystander therapy are developed.