Endogenous Gene Regulation by DNA Binding Polyamides

Thesis by

Nicholas George Nickols

In Partial Fulfillment of the Requirements
for the Degree of
Doctor of Philosophy

California Institute of Technology

Pasadena, California

2008

(Defended 5 June 2007)

For my family

Acknowledgements

I would like to express gratitude to my advisor, Professor Peter Dervan, for the privilege to work under his guidance in a research group that has been and continues to be filled with exceptionally intelligent and motivated individuals. His scientific vision and intellectual leadership are models for all of us who have studied with him. During my time in graduate school I have had the enviable opportunity to collaborate with a number of outstanding people. Tim Best, Ben Edelson, Bobby Arora, Bogdan Olenyuk, Jim Puckett, Claire Jacobs, Michelle Farkas, Ryan Stafford, Mike Brochu, Mazen Karaman, Joe Hacia, John Phillips, and Katy Muzikar: it has been my pleasure to work side by side with all of you. In addition to all of the people mentioned above, I would also like to thank Ray Doss, Michael Marques, Eric Fechter, Adam Kerstien, Justin Cohen, Jim Sanchez, Dave Chenoweth, Christian Dose, Sherry Tsai, Carey Hsu, Mareike Goeritz, Dan Harki, Julie Poposki, Shane Foister, Jason Belitsky, Victor Rucker, and Professor Michael Warring for valuable insight into science and for their friendship.

However, most of all I would like to extend huge love and appreciation for my family. Family is the most important thing in the world. That's it.

Abstract

Cells integrate stimuli through networks of proteins that affect programs of gene expression. The expression of genes is controlled by transcription activators and repressors that recruit or inhibit the recruitment of transcription complexes to the promoters of target genes. Molecules that can specifically modulate these protein-DNA interfaces could have significant applications in human medicine. Polyamides containing *N*-methylimidazole (Im) and *N*-methylpyrrole (Py) comprise a class of programmable DNA-binding ligands capable of binding to a broad repertoire of DNA sequences with affinities and specificities comparable to those of natural DNA-binding proteins. Numerous polyamide-dye conjugates localize in the nuclei of cultured cells. Multiple studies have shown that polyamides are capable of displacing DNA binding proteins from their target sites in a sequence specific manner. Early experiments in viral systems showed that polyamides can be active in cell culture. This thesis describes the use of polyamides to modulate the activity of two transcription activators: hypoxia inducible factor 1 (HIF-1) and androgen receptor (AR). Direct inhibition of the HIF-1-DNA and AR-DNA interfaces has relevance to human cancer. A polyamide that targets the hypoxia response element (HRE) binds its target site with high affinity and inhibits the expression of Vascular Endothelial Growth Factor (VEGF) and other HIF-1 induced genes in cultured cells. Similarly, a polyamide that targets the androgen response element binds its target site with high affinity and inhibits the expression of prostate specific antigen (PSA) and other androgen-induced genes in cultured cells. In both systems, the inhibition of target genes is consistent with a decrease in the occupancy of the transcription factor at relevant loci. The genome-wide effect of the HRE-targeted polyamide was compared to that of siRNA against HIF-1α. Remarkably, a comparable number of genes were affected by the HRE targeted polyamide as by the siRNA. The effect of the ARE targeted polyamide was compared to the synthetic antiandrogen bicalutamide. The polyamide and bicalutamide had a comparable effect on PSA expression, and genome-wide expression analysis reveals that both affected a similar number of transcripts. In addition, HIF-1α siRNA and bicalutamide affected nearly all genes induced by hypoxia or androgen, respectively, while the HRE- and ARE- targeted polyamides affected a subset of each pathway that could be consistent with the DNA binding preferences of the polyamides. The data suggest that polyamides can be used to modulate the activity of selected transcription factor pathways.

Table of Contents

List of Figures and Tables

Chapter 1 Page

Chapter 2

Chapter 3

Chapter 4

Chapter 5

Chapter 6

Chapter 7

Appendix A

Appendix B