Biological Activity of a Py-Im Polyamide Androgen Receptor Antagonist

Thesis by

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To Valerie.

For all those late nights and long weekends.

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Abstract.

Py-Im polyamides are cell-permeable, programmable, sequence-specific, DNA minor groove-binding small molecules. When designed to bind a DNA sequence that matches the consensus DNA-binding sequence of a transcription factor, they can be used to block the binding of that transcription factor to its response element *in vitro* and in cell culture. We have used this approach to inhibit the genotropic activity of the endogenous transcription factors HIF1α, glucocorticoid receptor (GR), and androgen receptor (AR). In this work, we report the completion of a library of hairpin Py-Im polyamides targeted to all possible 5'-WGNNNW-3' (W = A or T) sequences. These compounds bind their target DNA sequences with high affinity. One compound from this set targets the sequence 5'-WGWWCW-3', which matches the DNA binding consensus sequence of GR and AR and has been shown to inhibit the gene regulatory activity of these proteins in cell culture. Herein, we show that a cyclic derivative of this compound maintains its activity against AR-driven gene expression in hormone-sensitive LNCaP prostate cancer cells. As androgen receptor signaling is crucial to prostate cancer growth and metastasis even in its recurrent form, we next examine the activity of the AR/GR antagonist in a tissue culture model of castration-resistant prostate cancer. In this model, the polyamide retains its activity against AR-driven mRNA expression, but it fails to inhibit the binding of AR to its response element. The polyamide-mediated repression is also accompanied by significant cell stress and cytotoxicity, which are explored in the final two chapters of this thesis. The former investigates a role for polyamides as inhibitors of DNA Topoisomerase II. Despite *in vitro* evidence indicating polyamides prevent Topoisomerase II binding, no evidence for this is found in cell culture. The final chapter reveals that polyamide-mediated cytotoxicity is likely due to inhibition of DNA synthesis. This occurs at concentrations similar to those used for transcription factor inhibition, suggesting that S-phase disturbance accompanies efforts to regulate gene expression with polyamides.

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