Investigations of Pyrrole-Imidazole Polyamide Effects on DNA Replication

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for Cesar.

Acknowledgments

The program that defines the parameters of our physical nature, our biology, is acquired from reproduction; our humanity, however, is born from the information we extract from our world. Based on the information we are given from the experience of countless lives before us and from sensory observations, and what we choose to believe, we learn how best to operate in our world. This information propagates and evolves through our interactions, both direct and indirect, with others. Thus, in expressing our unique humanity we are all models, whether we intend to be or not. Given the critical role of information in shaping our humanity, it is apparent that teaching and mentorship, which provide curated information, are extremely powerful means for shaping our world. Some individuals teach purposefully, and amongst those who have this intention some are better than others.

That some individuals are better teachers than others is not an indictment of any person. It is tantamount to a tautology. I make this point to express my incredulousness at my good fortune of having so many excellent mentors show up throughout my life at critical times. Though their individual styles were each unique and varied significantly, most of my mentors have been encouraging. They challenged and allowed me to continue asking for bigger mountains to climb, despite my penchant for biting off more than I could chew. It seems to me that in almost every case I can recall that my mentor chose to help me specifically, or at least more so than other pupils. Perhaps this interpretation is simply of a reflection of their skill as teachers, making each student feel special, or my vanity. In any case, they did more than just offer guidance. They pinned their hopes and

expectations on me. I could feel this. I could sense their recognition of an opportunity in me, though I often had no idea what the opportunity was. I think that they believed at some level that training me would allow them to shape the world in ways they could not with their own hands. The interesting thing, though, is that it was not the guidance or training that usually impacted me the most, but the belief in myself born from their faith in me. This, too, is the power of teaching.

Not all of my mentors have resonated with me so strongly, though. Some mentors created such a strong dissonance with my own values that I found it impossible to ignore. I am grateful to them as well—just as grateful as I am to the mentors whom I understood immediately. Both positive and negative experiences contribute to self-discovery and development of one's value system. Therefore, the mentors whom I could not understand or agree with still played significant roles in my journey to become a person that I could respect. It is also true that some of the mentors whose teachings felt so alien to me I eventually learned to understand long after I had left their tutelage. These experiences have often stayed with me the most.

If I have had any success up to this point in my life, it is because of my mentors and the people I choose to keep close to my heart. My humanity is a reflection of their humanity, and theirs of mine.

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Abstract

Pyrrole–Imidazole polyamides are programmable, cell-permeable small molecules that bind in the minor groove of double-stranded DNA sequence-specifically. Polyamide binding has been shown to alter the local helical structure of DNA, disrupt protein-DNA interactions, and modulate endogenous gene expression. Py–Im polyamides targeted to the androgen receptor-DNA interface have been observed to decrease expression of androgen-regulated genes, upregulate p53, and induce apoptosis in a hormone-sensitive prostate cancer cell line. Here we report that androgen response element (ARE)-targeted polyamides induced DNA replication stress in a hormone-insensitive prostate cancer cell line. The ATR checkpoint kinase was activated in response to this stress, causing phosphorylation of MCM2, and FANCD2 was monoubiquitinated. Surprisingly, little single-stranded DNA was exhibited, and the ATR targets RPA2 and Chk1 were not phosphorylated. We conclude that polyamide induces relatively low level replication stress, and suggest inhibition of the replicative helicase as a putative mechanism based on *in vitro* assays. We also demonstrate polyamide-induced inhibition of DNA replication in cell free extracts from *X. laevis* oocytes. In this system, inhibition of chromatin decondensation is observed, preventing DNA replication initiation. Finally, we show that Py-Im polyamides targeted to the ARE and ETS binding sequence downregulate AR- and ERG-driven signaling in a prostate cancer cell line harboring the TMPRSS2-ERG fusion. In a mouse xenograft model, ARE-targeted polyamide treatment reduced growth of the tumor.

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