BIOLOGICAL ACTIVITY OF RHODIUM METALLOINSERTORS AND THE DESIGN OF BIFUNCTIONAL CONJUGATES

Thesis by

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Abstract

The Barton laboratory has established that octahedral rhodium complexes bearing the sterically expansive 5,6-chrysene diimine ligand can target thermodynamically destabilized sites, such as base pair mismatches, in DNA with high affinity and selectivity. These complexes approach DNA from the minor groove, ejecting the mismatched base pairs from the duplex in a binding mode termed metalloinsertion. In recent years, we have shown that these metalloinsertor complexes also exhibit cytotoxicity preferentially in cancer cells that are deficient in the mismatch repair (MMR) machinery.

Here, we present evidence to support the notion that mismatches in genomic DNA are the primary biological target of rhodium metalloinsertors and the source of their cell-selectivity. A structure-activity study on a family of ten metalloinsertor complexes revealed a highly sensitive relationship between the lipophilicity of the non-inserting ancillary ligands and the biological activity of the complex. Complexes with hydrophilic ligands were found to be highly cell selective, exhibiting preferential cytotoxicity in MMR-deficient cells at low concentrations and short incubation periods, whereas complexes with lipophilic ligands displayed poor cell-selectivity. ICP-MS studies were carried out to determine the cellular uptake and localization patterns of the ten compounds. The lipophilic complexes displayed enhanced cellular uptake compared to the more polar compounds, and their uptake patterns were indicative of a passive diffusion mechanism. Curiously, there was no correlation between cellular uptake of rhodium and selectivity for MMR-deficient cells. In fact, the complexes with the most selective activity exhibited low cellular accumulation overall. It was also discovered that

all of the complexes localized to the nucleus in concentrations sufficient for mismatch binding; however, highly lipophilic complexes also exhibited high mitochondrial uptake, consistent with the previous study. This relationship between subcellular localization and cell-selective biological activity confirms that mitochondrial DNA is not the desired target of metalloinsertor complexes; rather, these complexes recognize mismatches in genomic DNA.

We have also explored the potential for metalloinsertors to be developed into more complex structures with multiple functionalities that could either enhance their overall potency or impart mismatch selectivity onto other therapeutic cargo. We have constructed a family of bifunctional metalloinsertor conjugates incorporating *cis*-platinum, each unique in its chemical structure, DNA binding interactions, and biological activity. Attachment of a potent oxaliplatin derivative to a metalloinsertor through the leaving group ligand afforded an intrinsically metastable complex with high cytotoxicity in MMR-deficient cancer cells as well as enhanced cellular uptake properties. Additionally, we developed a bimetallic complex derived from a new family of potent and selective metalloinsertors containing an unusual Rh—O axial coordination. This complex also incorporates a platinum center containing only one labile site for coordination of DNA, rather than two, which leads to nonclassical platinum adduct formation selectively at mismatched DNA. Finally, we synthesized a mixed metal dinuclear Rh(III)/Pt(II) complex, wherein both the rhodium and platinum centers are coordinated to a bridging aromatic ligand capable of interaction with the DNA base stack through either intercalation or insertion. These complexes bind DNA mismatches from

the minor groove through metalloinsertion, situating the reactive platinum metal center directly at the mismatched site.

In the development of metalloinsertor-*cis*-platinum conjugates, we have acquired a diverse repertoire of bifunctional complexes with mismatch recognition capability as well as the ability to form covalent adducts. Although we have yet to achieve cell-selective toxicity in MMR-deficient cells, we almost universally observe potency surpassing that of the FDA-approved chemotherapeutic cisplatin in a variety of human cancer cell lines. Moreover, a significant finding in our study of these conjugates has been the discovery that these complexes induce apoptotic cell death, rather than the necrotic pathway typically triggered by rhodium metalloinsertors. It appears that rerouting to the apoptotic pathway is incongruous with the uniquely selective biological activity observed for metalloinsertors. This result suggests that there is a critical response to mismatch recognition in a cellular environment that leads to cell-selective activity.

We further explored the underlying mechanisms surrounding the biological response to mismatch recognition by metalloinsertors in the genome. Immunofluorescence assays of MMR-deficient and MMR-proficient cells revealed that a critical biomarker for DNA damage, phosphorylation of histone H2AX (γH2AX) rapidly accumulates in response to metalloinsertor treatment, signifying the induction of double strand breaks in the genome. Significantly, we have discovered that our metalloinsertor complexes selectively inhibit transcription in MMR-deficient cells, which may be a crucial checkpoint in the eventual breakdown of the cell via necrosis. Additionally, preliminary *in vivo* studies have revealed the capability of these compounds to traverse the complex environments of multicellular organisms and accumulate in MMR-deficient

tumors. Our ever-increasing understanding of metalloinsertors, as well as the development of new generations of complexes both monofunctional and bifunctional, enables their continued progress into the clinic as promising new chemotherapeutic agents.

Table of Contents

List of Figures, Tables, and Schemes