Chapter 1

Introduction: DNA as a therapeutic target in cancer

1.1 Background and significance.

The human genome consists of approximately 20,000 protein coding genes and many more genes that encode non-coding RNA with crucial cellular functions.(1) The regulatory networks that govern gene expression are immensely complex and work cooperatively to control cellular function and cellular response to environmental stimuli. It is due to this intricate regulation of gene expression that cells of the same genetic material can differentiate into various phenotypes in the human body to perform specialized tasks.

As a result of numerous DNA dependent processes, corruption to the DNA code can result in aberrant cellular behavior.(2) Thus, essential DNA dependent processes such as transcription and replication participate in DNA damage repair to ensure genomic stability.(3, 4) (Fig. 1.1) Transcription coupled nucleotide excision repair (TC-NER) is a mechanism that relies on elongating RNA polymerase II (RNAP2) to identify lesions or blockages in the DNA. Once the RNAP2 holoenzyme encounters a blockage on the transcribed DNA strand it recruits the proteins CSA, CSB, XAB2, and HMGN1 to repair the DNA lesion. If the DNA damage cannot be repaired, persistent blockage to RNAP2 elongation will trigger p53 dependent and independent apoptosis.(3, 5-8) Similarly, DNA lesions are recognized by replicating DNA polymerase in the S phase.(4) If the lesion cannot be repaired, persistent block to replication will also trigger cell death. While most instances of DNA damage are efficiently repaired, some escape as mutations and are retained in the genetic code. Over time these mutations accumulate and cause altered patterns of gene expression, which ultimately lead to genetic diseases like cancer.

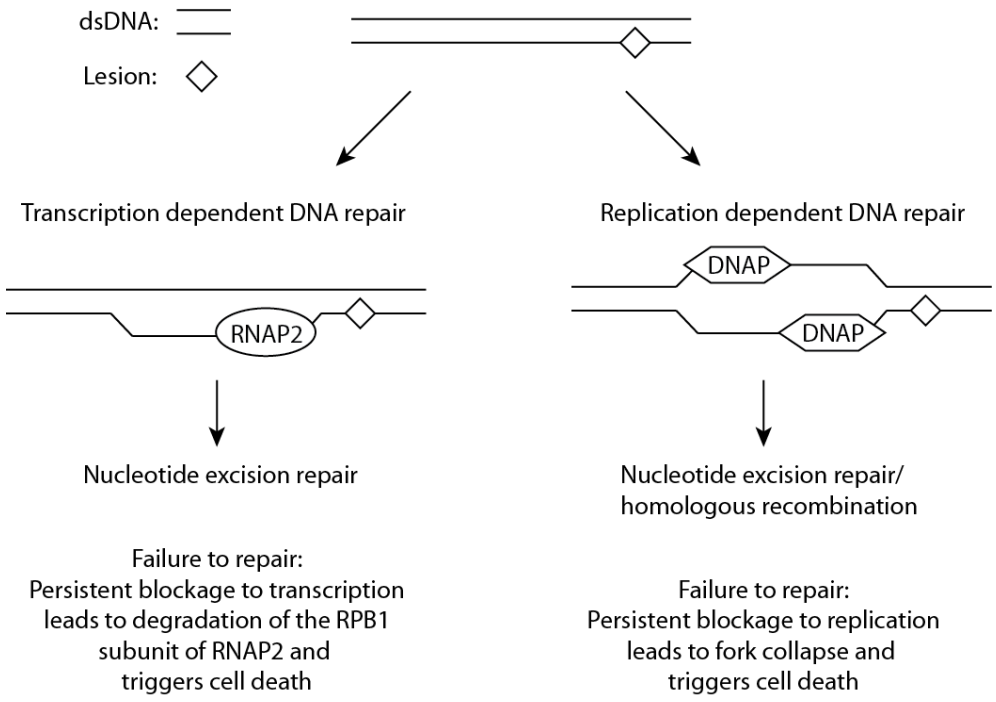

Figure 1.1. Transcription and replication dependent mechanisms of DNA repair.

1.2 DNA as a target for cancer therapy.

As the underlying source of cancer, some of the oldest and most effective anticancer agents are targeted to the DNA. Historically, the development of DNA targeted chemotherapeutics began as circumstantial observation to the side effects of chemical warfare during World War II.(9) Physicians examining sailors exposed to mustard gas, after a shipment of M47A1 mustard gas bombs leaked from the damaged *SS John Harvey,* noticed signs of lymphoid and myeloid suppression.(10) It was reasoned that the high proliferation rate of bone marrow cells made them susceptible to the alkylating effects of mustard gas, thus cancers with similarly high proliferations rates, such as leukemias and lymphomas, may also be targeted by such agents.(11) In a clinical study by Goodman et al. in 1946, it was found that treatment with nitrogen mustards indeed caused remission in patients with lymphoma.(12)

Intercalation Binding Compound	Selectivity	Action	Clinical use
Daunomycin	5'-WCG	inhibition of topoisomerase II	acute myeloid leukemia
Doxorubicin	5'-WCG	inhibition of topoisomerase II	breast cancer, stomach cancer, cervical cancer, non-Hodgkin's lymphoma, adult acute leukemia, endometrial cancer, rhabdomyosarcoma
Idarubicin	5'-WCG	inhibition of topoisomerase II	acute myeloid leukemia
Epirubicin	5'-WCG	inhibition of topoisomerase II	breast cancer
Actinomycin D	5'-PyGCPu	inhibition of topoisomerase II	Ewing's sarcoma, Wilms' tumors, soft-tissue sarcomas
Topotecan	ND	inhibition of topoisomerase I	ovarian, lung, cervical cancer
Irinotecan	ND	inhibition of topoisomerase I	ovarian, lung, cervical cancer
Etoposide	ND	inhibition of topoisomerase II	testicular cancer, small-cell lung cancer
Teniposide	ND	inhibition of topoisomerase II	acute lymphocytic leukemia
Mitoxantrone	5'-PuPy	inhibition of topoisomerase II	leukemias, breast cancer, ovarian cancer, prostate cancer
Minor Groove Binding			
Bleomycin	5'-GC, 5'-GT	ds DNA cleavage	testicular cancer, non-Hodgkin's lymphoma
Mitomycin C	5'-CG	alkylation/crosslinking	stomach, gi, anal, bladder, breast, cervical, colorectal, head and neck, and non-small cell lung cancer
Mithramycin	GC-rich	inhibits RNA synthesis	testicular cancer
Other			
Cisplatin	ND	crosslinking	testicular, ovarian, head and heck cancers
Temozolomide	ND	alkylation	astrocytoma and melanoma
Decitabine	ND	DNMT inhibitor	acute myeloid leukemia, medulloblastoma
5-fluoruracil	ND	thmyidylate synthase inhibitor	colon, recturm, head and neck cancers
Cytarabine	ND	inhibits DNA and RNA synthesis	acute myeloid leukemia

Table 1.1. Selection of FDA approved compounds that affect DNA dependent processes.[13, 14]

The utility of alkylating agents for the treatment of lymphoma opened the way for the development of new DNA targeted agents with novel mechanisms.(13, 14) (Table 1.1) Many of these drugs form covalent interstrand crosslinks, stabilize protein-DNA complexes of topoisomerases I and II, or inhibit DNA and RNA synthesis.(4, 13, 14) These modifications to the DNA introduce blockages to many DNA dependent processes including transcription and replication, which in turn triggers apoptosis in diseased cells.(4, 14-17) However, because transcription and replication are common to cancerous

and normal cells alike, systemic treatment with DNA targeted therapeutics can be very toxic to the patient as well.

1.3 Limitations of DNA targeted therapy.

Most DNA targeted therapeutics preferentially affect cancerous cells due to their high proliferation rate and genomic instability, but benign cells can also be affected. Normal cells can tolerate basal levels of DNA damage generated by exogenous chemicals and by by-products of cellular metabolism. However, the endogenous DNA repair mechanisms are often overwhelmed by DNA targeted therapeutics.(18) Studies of patients treated daunomycin and cytarabine shortly after their introduction in the 1960s documented the presence chromosomal abnormalities associated with DNA fragmentation in normal cells.(19, 20) The extensive DNA damage caused by chemotherapeutic treatment has been linked to the acquisition of resistance towards chemotherapy and the development of secondary cancers.(21-23)

A recent study on the effects of chemotherapy in the tumor microenvironment indicates genotoxic stress can cause normal cells to promote tumor survival, which further complicates the long term utility of DNA targeted drugs. In the study by Sun et al. treatment of prostate fibroblasts with DNA damaging agents such as bleomycin, mitoxantrone, and ionizing radiation was found to activate WNT16B expression in a NF-κB dependent manner.(24) Interestingly, the expression of WNT16B was not significantly increased when prostate cancer cells were treated with the same genotoxic agents. As a secreted signaling protein, WNT16B activates the Wnt expression program in tumor cells, which in turn promotes survival and metastasis.

As a consequence of the numerous side effects of DNA targeted therapeutics, research in the field has waned in favor of therapeutic agents with more specific molecular targets and less systemic toxicity.(14) However, despite their limitations DNA targeted therapies remain a staple in most treatment regimens. Thus, development of a new class of DNA targeted molecules, without genotoxic side effects, could circumvent the problems associated with current therapies.

1.4 Noncovalent minor groove binders as anticancer agents.

DNA minor groove binders consist of molecules that permanently modify DNA in a covalent manner and those that interact with DNA noncovalently. The latter group of molecules interferes with DNA dependent process in a reversible manner. This group of molecules includes DAPI, pentamidine, berenil, Hoechst, distamycin A, netropsin, and their synthetic derivatives.(25)

Clinically, diarylamidines, consisting of DAPI, pentamidine, and berenil, have been used for the treatment of several protozoa related diseases.(26) (Fig. 1.2) The minor groove binder DAPI inhibits DNA and RNA polymerases by binding to A/T rich tracts of DNA.(27-30) While DAPI is active against *Trypanosome Congolese,* undesirable side effects have limited its clinical use. Pentamidine is clinically used to treat infections of *Trypanosoma brucei gambiense*, *Leismania donovani*, and *Pneumocystis carinii*. Berenil is used to treat trypanosomiasis in veterinary medicine.(25)

Bisbenzimidazoles are Hoechst-like compounds that bind to A/T rich DNA sequences.(31, 32) (Fig. 1.2) They have been shown to interfere with DNA dependent process in cell culture without causing DNA damage.(33) Furthermore, a symmetric

bisbenzimidazole has demonstrated antitumor activity against CH1 human ovarian carcinoma xenografts *in vivo*.(34)

Distamycin A and netropsin are tripyrrole and dipyrrole oligomers, respectively, and bind to A/T tracts. Both compounds bind to the minor groove in a 1:1 fashion.(35, 36) (Fig. 1.2) Distamycin has been shown to also bind in a 2:1 manner.(37) Similar to other noncovalent minor groove binders, these compounds inhibit DNA and RNA polymerases.(6, 28, 38)

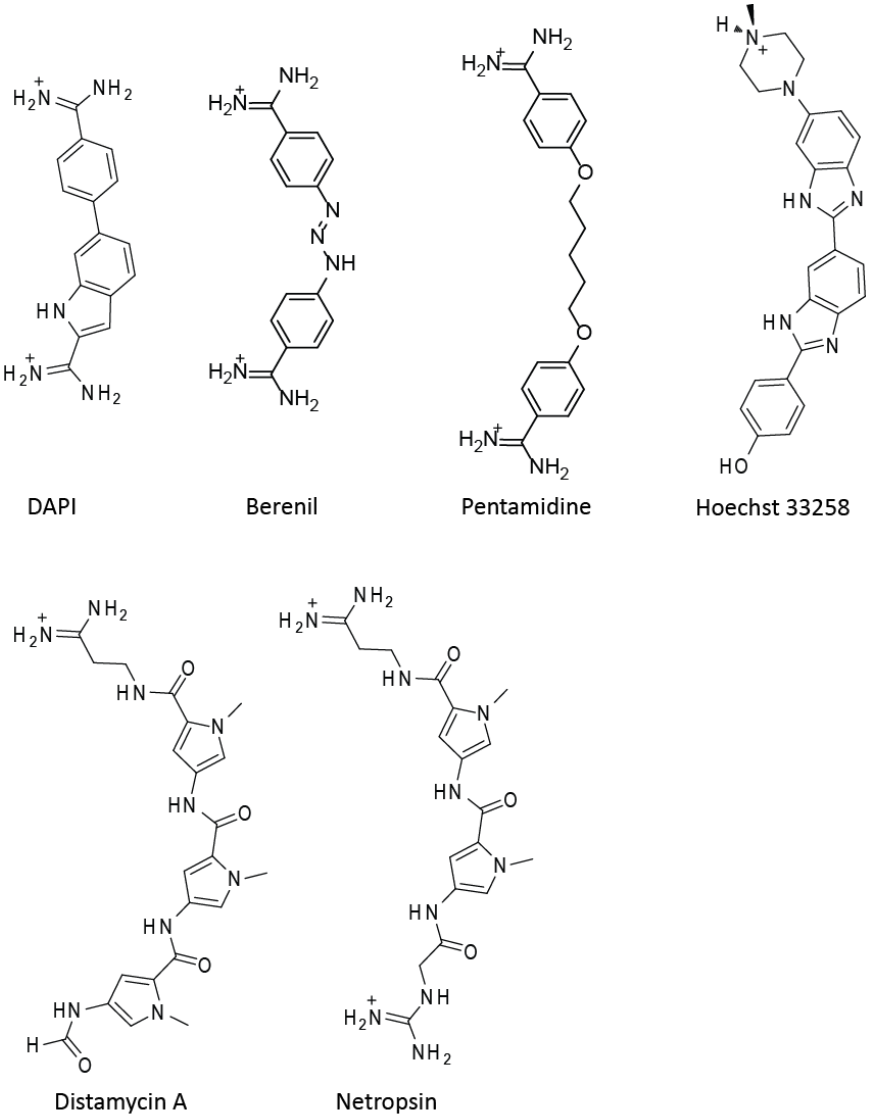

Figure 1.2. Select noncovalent sequence specific minor groove binders.

Py-Im polyamides are synthetic oligomers based on the structures of distamycin A and netropsin. Research in the Dervan lab have improved the DNA binding affinity of polyamides by linking two oligomers with a turn unit and enforcing 2:1 binding as a hairpin.(39) Sequence recognition by polyamides has also been expanded by incorporation of new aromatic heterocycles that discriminate between A/T and G/C base pairs through the antiparallel pairing of these amino acids.(40, 41) (Fig. 1.3) Additionally, conjugation of fluorescein or isophthalic acid to the C-terminal tail of polyamides significantly improves their nuclear localization.(42, 43)

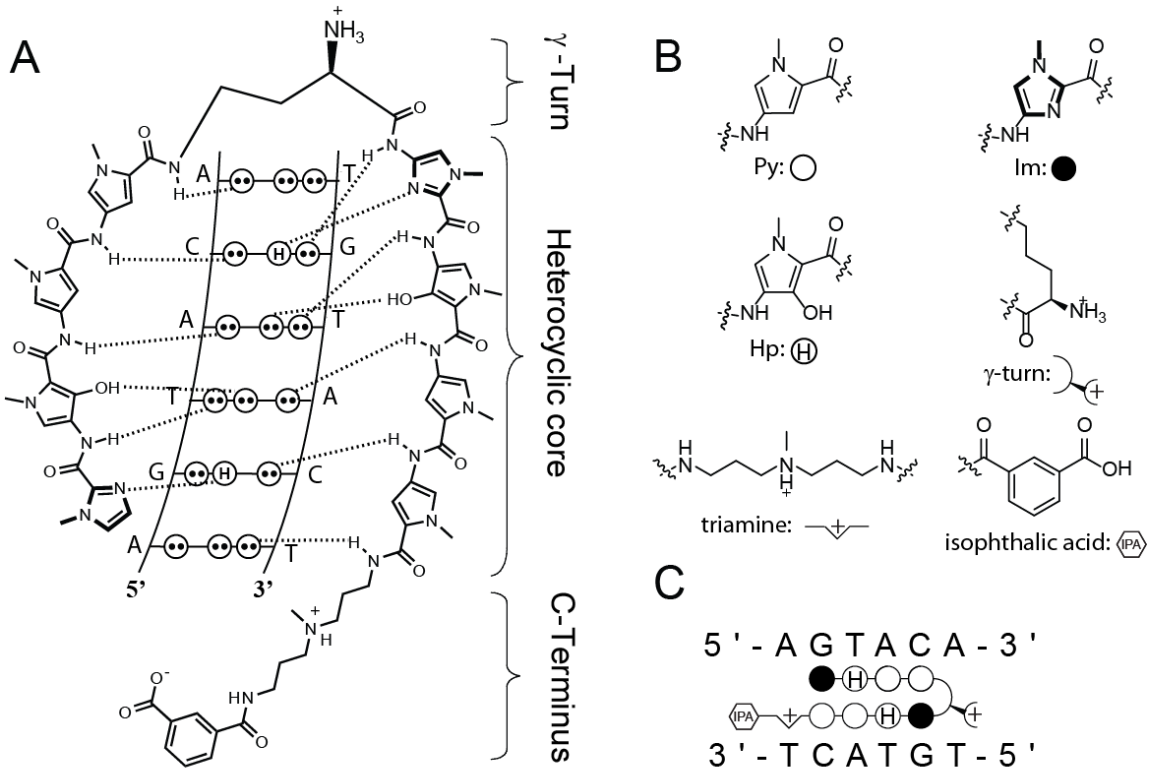

Figure 1.3. Sequence recognition by hairpin Py-Im polyamides. **(A)** DNA base pair discrimination on the minor groove floor is achieved by the antiparallel pairing of aromatic heterocycles. Py/Im pairing recognizes G/C base pairing, Hp/Py pairing recognizes T/A pairing, and Py/Py pairing is degenerate for T/A or A/T. The γ-turn and the c-terminus tail are degenerate for T/A or A/T. **(B)** Ball and stick representation of hairpin components. **(C)** A ball and stick representation of a polyamide bound to its target sequence.

Historically, these compounds were found to exhibit antifungal activity in yeast through a DNA dependent mechanism that did not cause genotoxicity.(44) In cell culture, Py-Im polyamides are able to regulate gene expression in inducible transcription systems(45-49), and are toxic to a variety of cancer cell lines.(50) Animal experiments have shown Py-Im polyamides are bioavailable through multiple forms of administration(51-55), and can affect gene expression in target tissues *in vivo*.(56, 57) These characteristics make Py-Im polyamides ideal candidates for development as novel DNA targeted therapeutics.

1.5 Scope of this work.

The work presented here focuses on the characterization of Py-Im polyamides as non-genotoxic antitumor agents that are active against prostate cancer xenografts. Chapter 2 details the pharmacokinetic and animal toxicity analysis of two hairpin polyamides targeted to the 5'-WGWWCW-3' sequence found in the androgen response element. In this study it was found that the polyamide with an α amino turn was much less toxic to animals than the compound with a β acetamide turn.(55) The less toxic polyamide is further characterized in chapter 3 as a non-genotoxic DNA binder that interferes with RNAP2 elongation, and causes cell death in human prostate cancer cells in cell culture and in xenografts.(58) Chapter 4 revisits the difference in rodent toxicity that stems from the γ-turn. By using 4 polyamides that vary at the turn (α amino, β amino, α acetamide, and β acetamide), we assessed differences in animal toxicity and determined the target organs of pathology. From this study we identified a structural analog to the parent compound that retains antitumor activity without causing animal toxicity.

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