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*This paper is dedicated to the memory of Professor Bronisław Filipowicz*
***Review***

# Transcription factors as targets of anticancer drugs*°

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**Several general and gene- and cell-selective transcription factors are required for specific transcription to occur. Many of them exert their functions through specific contacts either in the promoter region or at distant sequences regulating the initiation. These contacts may be altered by anticancer drugs which form non-covalent complexes with DNA. Covalent modifications of DNA by alkylating agents may prevent transcription factors from recognizing their specific sequences or may constitute multiple „unnatural" binding sites in DNA which attract the factors thus decreasing their availability in the cell. The anticancer drug-transcription factor interplay which is based on specific interactions with DNA may contribute to pharmacological properties of the former and provide a basis for the search for new drugs.**

RNA synthesis in eukaryotic cells is a very complex process. Several general and specific transcription factors and other regulatory proteins are required for specific transcription to occur. Many of them exert their functions through specific contacts either in the promoter region or at distant sequences enhancing or attenuating the initiation of transcription [1, 2].

The availability of transcription factors, purified or obtained by means of recombinant DNA technology, as well as the identification of DNA sequences which are recognized by these proteins, have greatly advanced studies

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**Abbreviations:** see Table 1 and 2.

of this aspect of regulation of gene expression. A method of choice to study the interaction of transcription factors with their binding sites is the electrophoretic mobility shift assay [3–29]. DNA–protein complexes migrate as discrete bands separated from free DNA. Several experiments have been recently undertaken to elucidate how specific contacts between transcription factors and their target sequences in DNA are affected by anticancer drugs and related compounds. This paper outlines this expanding field of research.

DNA-binding compounds are able to affect properties of nucleic acids through physicochemical interactions or covalent bond formation. Two types of non-covalent complexes may be distinguished if these interactions are defined in terms of steric relations between DNA and the drug: intercalation and external binding [30, 31]. Intercalation of the flat chromophore between the adjacent base pairs is often assisted by interactions of side substituents, usually in the minor groove [30–32]. These substituents often provide a basis for sequence specificity. The best known example is actinomycin D, which shows a nearly absolute specificity for d(GC) base pairs [30]. Some intercalative drugs (e.g. nogalamycin) have two side substituents interacting in both grooves [32]. There are bisintercalating drugs such as echinomycin, which bear two chromophores connected by a rigid linker of sufficient dimensions and suitable conformation to accomodate two DNA base pairs between the intercalating rings [30, 31].

A second class of ligand exists which do not intercalate, but instead form strong complexes with DNA by binding in the minor groove. Pyrrole antibiotics (e.g. netropsin and distamycin A) and 4′,6-diamidino-2-phenylindole (DAPI) represent this class [30, 31].

Anticancer drugs which form covalent bonds with DNA belong to chemically distinct classes from the simple inorganic drug cisplatin to a series of bisalkylating drugs developed from mustard gas [30, 31, 33]. A common feature of this group of compounds is that their molecules bind to nucleic acids and other macromolecules and form either one covalent bond (monoadduct) or two covalent bonds (diadduct). In the latter case they may span two sites in the same strand of DNA or in the complementary strands producing intrastrand or interstrand cross-links, respectively. Although monoadducts may represent a large fraction of the bound drug, this type of binding is of less therapeutic significance as it exhibits lower cytotoxic potential.

Drugs which have been assayed for their propensity to interact with transcription factors–DNA system are listed in Table 1 and their specificity of binding to DNA is outlined ([30–33] and references specified in Table 1). Transcription factors and the corresponding target sequences which were used as fragments of double-stranded oligo- or polynucleotides in experiments with different drugs are presented in Table 2. The comparison of drugs preference or specificity (Table 1) and primary structures of the binding sites of different transcription factors leads to a general conclusion that in most cases the effect of any drug may be predicted on the basis of specificity of its binding to DNA while steric properties of drug–DNA complexes play a minor role. Hence interactions of TATA binding protein (TBP), a component of TFIID [1], with its target sequences are abolished by non-covalently binding drugs distamycin A, netropsin, microgonotropens, DAPI and Hoechst 33258 [3, 18], which exhibit A·T specificity, and adduct-forming tallimustine, a drug combining A·T preference with bisalkylating propensity [4]. Sp1–DNA interaction is competitively inhibited by several drugs exhibiting d(G·C) preference: the intercalating ligands actinomycin D [14], adriamycin and mitoxantrone [15], and the non-intercalating ligand chromomycin [9, 12]. On the other hand, the interaction of Sp1 with DNA is not affected by tirolone, an intercalator exhibiting A·T preference [15], nor by ethidium bromide, proflavine and 9-aminoacridine carboxamide derivatives intercalating with low base

**Table 1. DNA-binding anticancer drugs – mode of interaction and base and/or sequence specificity**

| Compound/structure | Abbreviation | Base/sequence specificity | Remarks | References |
|---|---|---|---|---|
| I. Non-covalently binding drugs | | | | |
| **Intercalating agents** | | | | |
| Mepacrine/quinacrine | Q | G·C (±) | | [30] |
| Amsacrine | mAMSA | A·T (±) | | [3] |
| Tilorone | TIL | A·T (±) | | [15] |
| Ethidium bromide | EB | G·C (±) | | [3] |
| Actinomycin D | ACT | G·C (++) | | [32] |
| Anthracycline antibiotics: | | | | |
| Adriamycin (doxorubicin) | ADR | G· C (±) | Fe(III)[1] | [15] |
| Nogalamycin | NOG | G·C (+), GCA | | [15, 32] |
| Other ligands: | | | | |
| Echinomycin | ECH | G·C (++) | bisintercalator | [3] |
| Hedamycin | HED | G·C (±) | | [15] |
| Mitoxantrone | MTX | G·C (+) | | [15] |
| **Non-intercalating, minor groove binding agents** | | | | |
| Pyrrole antibiotics and derivatives: | | | | |
| Distamycin A | DST | A·T (++) | | [15, 30, 31] |
| Netropsin | NT | A·T (++) | | [15, 30, 31] |
| Microgonotropens[2] | MGTs | A·T (++)/G·C (+) | minor & major | [19] |
| Other ligands: | | | | |
| Hoechst 33258 | | A·T (++) | | [3, 30] |
| 4′,6-Diamidine-2-phenylindole | DAPI | A·T (++) | | [3, 30] |
| Chromomycin $A_3$ | CHR | G·C (+) | Mg(II)[3] | [3, 30] |
| Mithramycin | MTR | G·C (+) | Mg(II)[3] | [30] |
| II Covalently binding drugs | | | | |
| Monofunctionally binding drugs (monoadduct forming) | | | | |
| Benzo(α)pyrene | BaP | G (++) | carcinogen | [10, 11, 30] |
| Pluramycin | PLR | G (++) | minor groove | [5] |
| CC-1065 | C1065 | A (++) | minor groove | [3] |
| 2-Chloroethyl ethyl sulphide | CEES | G>A | monofunctional binding; analogue of HD | [21] |
| Bifunctionally binding drugs: | | | | |
| Sulphur mustard | HD | G>A, GGG | | [21] |
| Nitrogen mustard | $HN_2$ | G>A, GGG | | [7, 30, 31] |
| Quinacrine mustard | QM | G>A, GGG | | [7] |
| Tallimustine mustard (FCE 24517) | TLM | A | minor groove | [7] |
| Melphalan | MFL | GGG | | [7, 31] |
| Cisplatin | cisPt | GG | | [17, 30, 31] |

[1]In some studies adriamycin covalently bound to DNA in the presence of dithiothreitol and Fe (III) ions was assayed [20]. [2]A series of related drugs with distamycin A moiety and polyamine tail, which interacts in the major groove and exhibits preference for G·C [19]. [3]Binding depends on Mg(II) [30]. Specificity of drugs is designated as weak (±); moderate (+) and strong (++).

pair specificity or moderate affinity to G·C sequences (M. Czyż, unpublished observation), and non-intercalating antibiotics distamycin A [9, 13, 15] and netropsin [14].

The inhibition of transcription factors–DNA binding may depend on sequence specificity of the drug. This seems to be the case for different effects of actinomycin D on Sp1 and NFκB (Table 2). A G-rich sequence occurs in the binding site of the latter [7, 14], and its binding to DNA is prevented by bisalkylating agents reacting with purines, particularly with guanines: nitrogen and quinacrine mustards or cisplatin [7]. In contrast to Sp1 the binding of NFκB to its recognition site is not affected by actinomycin D [14]. A plausible

**Table 2. Transcription factors – recognized sequences and the effect of DNA binding drugs**

| Transcription factors | | | Effect of DNA binding drugs[1] |
|---|---|---|---|
| Abbreviation | Full name | Recognized sequence | |
| TBP (TFIID) | TATA binding protein; a component of TFIID [1, 5, 6] | TATAAAAA [3, 4] | DST (↓) [3,4]; NT (↓) [3]; DAPI (↓) [3]; CHR (↓) [3]; Hoechst 33258 (↓) [3]; HED (↓) [3]; NOG (↓) [3]; CC-1065 (↓) [3]; mAMSA (0) [3]; ADR (↓) [3]; TLM (↓) [4]; PLR(↑) [5]; cisPt(↑) [6]; MGT[2] (↑) [18]; |
| Sp1 | split protein [10, 38] | GGGAGG [13] GGGCGGG [7, 11, 12] GATCGAGGGCGTGGC [15] | MFL (0) [7]; CC-1065 (↓) [8]; CHR (↓) [9,12]; ACT (↓) [14]; TIL (0) [15]; ADR (↓) [15]; MTX (↓) [15]; DST (0) [9, 13, 15]; NT (0) [14]; BaP (↑) [10], (↓) [11]; |
| NFκB | nuclear factor κB [2] | GGGACTTT [7] | Q (0) [7]; $HN_2$ (↓) [7]; QM (↓) [7]; cisPt (↓) [7]; MFL (↓) [7]; ACT (0) [14]; |
| E2F1 and E2F4 | positive regulators of genes involved in DNA synthesis [16, 19] | TTTCGCGCCAAA [15] TTTCGCGCC [18] | TIL (↓) [15]; ADR (↓) [15]; NOG (↓) [15]; MTX (↓) [15]; DST (↓) [15,19]; NT (↓) [15]; MGT[2] (↓) [18, 19]; HED (↓) [15]; BaP (↑) [16]; |
| Oct 1 N-oct-3,5 | octamer binding protein, POU family [20] | ATTTGCAT [7] ATGCAAAT [20] | Q (↓) [7]; DST (↓) [7]; QM (↓) [7]; TLM (↓) [7]; ADR+Fe(III)[3] (↓) [20]; ADR (0↓) [20]; |
| AP2 | activating protein 2 [2] | GCCCGCGGC [21] | HD (↓) [21]; CEES (↓) [21]; |
| SRF | serum response factor [7] | CCAAATAAGG [13] | DST (↓) [13]; |
| Myo D | muscle specific transcription factor [13] | CAACTG [13] | DST (0) [13]; |
| MEF 2 | myocyte specific enhancer binding factor [13] | CTATATTTAT [13] | DST (↓) [13] |
| EGR 1 | early growth response protein [3] | CGCCCCCGC [3] | mAMSA (0) [3]; ACT (0) [3]; ECH (↓) [3]; HED (↓) [3]; NOG (↓) [3]; EB (↓) [3]; CHR (↓) [3]; cisPt (↓) [3]; ADR (0) [3]; CC-1065 (0) [3]; DAPI (0) [3]; DST (0) [3]; NT (0) [3]; Hoechst 33258 (0) [3]; MGT (0 ↓) [18], (0) [19]; |
| NFE 1 = GATA1 | erythroid specific | GATAAG [7] | DST (↓) [7]; TLM (↓) [7]; |
| HMG 1, 2 | high mobility group [22, 27] | | cisPt (↑) [22, 28]; transplatin (0) [24]; |
| SRY | sex determining region Y protein [17, 22] | AACAAAG [17] | cisPt (↑) [17, 22]; |
| ARBP | attachment region binding protein [29]; | TGCAGGTGTCCTTAA [29] | Hoechst 33258 (↓) [29]; NT (↓) [29]; |

Binding of protein to DNA in the presence of drug: increased (↑), decreased (↓), no effect (0), slightly affected (0↓).
[1]See Table 1 for the abbreviations. [2]Dithiothreitol/Fe(III)-activated, covalently bound adriamycin to DNA [20].

explanation of the difference is that this site does not contain the GpC sequence which is considered to be a preferential binding site for this antibiotic [30]. The nucleotide sequence recognized by Sp1 does contain a GpC site [14], see also Table 2.

Covalent modifications of DNA, either monoalkylation or bisalkylation with several drugs result in a loss of factors affinity to the modified sites (Table 2). Even O6 methylation of guanines in DNA, causing a mutagenic lesion, prevents Sp1, NFκB, EGR1 and SRF from binding [7]. Methylation of cytosine at C5 which is a naturally occuring modification may also decrease Sp1 binding [34].

There are data indicating that sequence specificity is not the only feature determining the effect. Nogalamycin and hedamycin, which are non-specific intercalators, and chromomycin $A_3$, which exhibits G·C specificity, effectively inhibit TBP binding to DNA [3]. The latter observation may be explained by distortion of the recognition site by the drug bound at the adjacent sequences. This is a case of CC-1065 which alkylates N3 of adenine and inhibits Sp1 binding to the neighbouring sequences [3].

However, the most interesting observations are these which are not consistent with the inhibition mode of drug–transcription factors interactions. This is the case of pluramycin, an anthraquinone derived antibiotic which forms covalent adducts with N7 of guanine [5]. TBP interacting with DNA facilitates intercalation of the drug downstream of the TATA box and adduct formation. The adduct in turn stabilizes the TBP–DNA complex [5]. Cisplatin which forms intrastrand cross-links at neighbouring purines in GpG or GpA sequences as prevailing lesions [33] abolishes NFκB [7] and EGR1 [3] binding to DNA. It was found, however, that platinated DNA strongly binds TBP in the TFIID complex [6], SRY protein [17] and hUBF (human upstream binding factor) [35]. TBP binding occurs at modified DNA sequences obviously unrelated to the consensus sequence of TBP. The affinity of TBP to cisplatin adducts according to Vichi *et al.* [6] is due to a striking similarity between a distorted DNA structure at the cisplatin diadduct and the three dimensional DNA structure of TATA box induced by TBP binding. Similarly, two structural chromatin proteins, high mobility group (HMG1 and 2) [22, 24] and histone H1 [36] exhibit increased affinity to DNA modified with cisplatin. The shielding of cisplatin adducts by HMG protein and histone H1 or by transcription factors protects the lesion from repair [6]. Alternatively, HMG1 protein bound to the adduct site blocks replication [27]. Most interestingly, adducts of transplatin, the biologically inactive isomer of cisplatin, do not increase the affinity of HMG1 protein [24] and exhibit lower affinity to histone H1 [36]. These differences between the two isomers together with the observations of different effects on transcription and replication (reviewed in [31] and [33]) may contribute to the explanation of the high biological activity of cisplatin *versus* its *trans* isomer. Parenthetically worth to be mentioned that the carcinogen benzo(*a*)pyrene–DNA adducts increase the affinity of Sp1 for DNA sequences which are not its specific binding sites [10].

The studies reviewed here indicate that apart from topoisomerases/DNA system [37], DNA and RNA polymerases [33] the anticancer drugs may alter regulation of gene expression by interfering with multiple transcription factors and consequently preventing binding of regulatory proteins. Alternatively, covalent modifications of DNA may increase the affinity of a transcription factor to its sequence thus slowing dissociation of the protein–DNA complex or create new unnatural binding sites. The latter mechanism which seems to be common for some anticancer drugs and carcinogens [5, 6, 11, 12, 16, 17] decreases the effective concentration of transcription factors. Covalently modified DNA just hijacks transcription factors [38].

The observations that anticancer drugs modulate transcription factors–DNA interac-

tions open new perspectives in the search for drugs targeted to specific sequences recognized by transcription factors which are overrepresented in the cancer cell [2]. As angiogenesis is a critical process involved in solid tumor growth [39], any drug which may specifically prevent hypoxia inducible factor 1 (HIF-1) from binding to its target sequences would be promising. It may be speculated that the drug should be site-specific and preferably undergo reductive activation in hypoxic cells [40]. A predictable manner of competition between a regulatory protein and a low-molecular mass ligand for critical DNA sequences may contribute to future progress.

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