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Drugging the Undruggable: Transcription Therapy for Cancer

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Abstract

Transcriptional regulation is often the convergence point of oncogenic signaling. It is not surprising, therefore, that aberrant gene expression is a hallmark of cancer. Transformed cells often develop a dependency on such a reprogramming highlighting the therapeutic potential of rectifying cancer-associated transcriptional abnormalities in malignant cells. Although transcription is traditionally considered as undruggable, agents have been developed that target various levels of transcriptional regulation including DNA binding by transcription factors, protein-protein interactions, and epigenetic alterations. Some of these agents have been approved for clinical use or entered clinical trials. While artificial transcription factors have been developed that can theoretically modulate expression of any given gene, the emergence of reliable reporter assays greatly facilitate the search for transcription-targeted agents. This review provides a comprehensive overview of these developments, and discusses various strategies applicable for developing transcription-targeted therapeutic agents.

Keywords

Cancer; targeted therapy; transcription; transcriptional regulation; transcription therapy; drug development

1. Introduction

As a fundamental cellular event converting the genetic information into messenger RNA for subsequent protein translation, transcription is tightly regulated by a plethora of proteins (*e.g.*, transcription factors, transcription co-regulators) that interact with *cis*-acting elements primarily residing in the 5' promoter region. Since fine control of transcription is essential for cellular homeostasis, it is not surprising that dysregulation of the transcriptional machinery is often fundamental to the pathophysiology of human disease. Indeed, the transformed phenotype is characterized by wide-spread aberrations in gene expression mainly caused by abnormal regulation of transcription. Whereas some oncogenic proteins (*e.g.*, Ras) activate pathways leading to altered transcription, others (*e.g.*, Myc) are themselves transcription factors that directly control the expression of genes essential for proliferation, survival, and/or metastasis. Conversely, a number of tumor suppressors (*e.g.*, p53) repress tumor growth by reactivating genes frequently lost in human cancers. Since cancer cells often develop a dependency on the specific changes in gene expression caused

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by oncogenic stimulations [1], it is generally believed that the development of therapeutic agents capable of correcting these abnormalities is a promising strategy for effective cure of this deadly disease.

Transcription therapy is an emerging strategy that intends to rectify aberrant gene expression in cancer cells through direct intervention in the transcription process. Although this term was coined only a decade ago [2], it is estimated that at least 10% of FDA-approved anti-cancer drugs that were not initially developed to target transcription actually regulate this process in one way or another [3]. Compared to molecules involved in cell signaling (*e.g.*, protein kinases), however, transcription is often considered “undruggable” due to the perception that (a) transcription is a nuclear event not readily accessed by therapeutic agents, and (b) many essential components involving transcription lack enzymatic activity suitable for chemical interventions. In recent years, however, great progress has been made in efforts to develop transcription-targeted therapeutic agents. Some of these agents are approved for clinical practice, or have entered clinical trials for cancer treatments. This review provides a comprehensive overview of these developments, and discusses various strategies applicable for developing transcription-targeted agents.

2. Transcriptional regulation and targeting strategies

Transcription is a complex process involving multiple proteins and their interactions with DNA. This process is catalyzed by RNA polymerase II assembled on core promoter regions with the assistance of gene-specific transcription factors, and regulated at three distinct levels [4](Fig 1A). First level control involves binding of transcription factors to defined promoter/enhancer regions with subsequent recruitment of the basal transcriptional machinery. Transcription factor activity is often altered in human cancers due to mutations, abnormal signaling, and/or posttranslational modifications and, thus, is the most frequent target for therapeutic interventions [5]. Whereas therapeutic agents may compete with transcription factors for DNA binding, they may also mimic *cis*-regulatory elements functioning as protein “traps” (Fig 1B). The second level of regulation is achieved through protein-protein interactions occurring between transcription factors and their regulatory proteins (often referred to as transcriptional co-regulators), or between different transcription factors. Transcriptional co-regulators do not necessarily contain DNA-binding domains, but can be recruited to promoters by interacting with DNA-bound transcription factors. These co-regulators often harbor enzymatic activities required for posttranslational modifications (*e.g.*, acetylation, methylation, and ubiquitination) of transcription factors, or co-exist with those modifying enzymes, thereby altering stability and activities of their interacting partners. One well-characterized transcriptional co-regulator is the histone acetyltransferase p300, which acetylates a number of transcription factors (*e.g.*, p53) promoting their activities [6]. MDM2, on the other hand, is recruited to p53-target promoters and directly represses p53 transcription activity, while also serving as the major E3 ubiquitin ligase for p53 facilitating p53 ubiquitination and subsequent proteosomal degradation [7]. MAML1 represents another class of transcriptional co-activators, which form a ternary complex with the transcription factor CSL and the intracellular domain of NOTCH1. Formation of this ternary complex allows for binding of CSL to DNA, and is required for activating NOTCH signaling [8]. Therapeutic agents can be developed to disrupt protein-protein interactions leading to altered gene expression (Fig 1C). This strategy mainly targets interactions between transcription factors and their co-regulators, but can also interfere with protein dimerization (Fig 1C). The latter is often required for DNA binding of many oncogenic transcription factors (*e.g.*, Myc) [9]. The third level of regulation is related to the epigenetic landscape including promoter methylation and posttranslational modifications to core histones. Whereas methylation of *cis*-regulatory elements can restrict the access of gene-specific promoters to transcription factors, various histone modifications (*e.g.*, acetylation)

dictate whether the chromatin environment is permissive for transcription factor binding to *cis*-regulatory elements. Conversely, transcription factors can not access *cis*-regulatory elements if the DNA is condensed in closed chromatin marked by modified histones such as lysine 9-methylated histone H3 [10]. The fact that epigenetic alterations are often observed in human cancers [11] indicates that therapeutic targeting epigenetic modifications may alter gene expression leading to changes in cancer cell behavior (Fig 1D).

Therapeutic agents targeting these different levels of transcriptional regulation have been developed (Table 1) and will be reviewed in detail. Nuclear receptors (*e.g.*, retinoic acid receptors, RARs; androgen receptor, AR; estrogen receptor, ER) belong to a large family of transcription factors regulated by ligand binding [12] and play well-established roles in regulating development and progression of cancer, particularly leukemia and hormone-related cancers (*e.g.*, prostate and breast cancer). Agents binding to these receptors (*e.g.*, RAR agonists retinoids) or interfering with their binding to natural ligands (*e.g.*, the AR antagonist Bicalutamide, or the ER antagonist Tamoxifen) alter expression of target genes of these nuclear receptors. The development and application of these agents in cancer treatment were extensively reviewed [13–15], and will not be further discussed. Because therapeutic interventions of epigenetic mechanisms likely render global alterations in gene expression, it is arguable that molecules targeting the first level, *i.e.*, the binding of transcription factors to gene-specific promoters, would be more specific in modulating expression of a specific gene and, thus, likely to have fewer off-target effects [4]. These targeting strategies often require pre-characterization of the mechanism(s) responsible for aberrant expression of cancer-related genes. Emerging strategies such as the design of artificial transcription factors that bind specific DNA sequences can be developed without knowledge of specific proteins responsible for expression of the genes of interest. Moreover, unbiased high-throughput drug screens based on reporter assays are also applicable to identify transcription-targeted agents for further drug development.

3. Targeting binding of transcription factors to gene-specific promoters

3.1. DNA-binding small molecules

Binding of transcription factors to specific DNA sequences is a crucial step towards recruiting accessory proteins and the supporting machinery for transcriptional activation or repression. Many anti-cancer agents commonly used in clinical practice (*e.g.*, cisplatin) bind DNA and, therefore, regulate gene expression by interfering with protein-DNA interactions although DNA recognition is often not sequence-specific. Mithramycin and anthracyclines (*e.g.*, daunorubicin) preferably bind G/C-rich DNA sequences recognized by Sp1 [16] and repress Sp1 transcriptional activity, thereby inhibiting expression of genes essential for cancer cell growth or survival (*e.g.*, VEGF, TGFBR2, cyclin D1, Bcl-2) [17]. Indeed, mithramycin induces differentiation of leukemic cells by repressing expression of the oncogene *c-myc*, which has two Sp1 binding regions in its promoter [18]. Mithramycin also competes with Sp1 for binding to the core promoter of the dihydrofolate reductase (DHFR) gene, thereby inhibiting DHFR expression which is often at a high level in methotrexate-resistant cancer cells [19]. WP631 is a bisanthracycline (Fig 2) composed of two daunorubicin molecules linked through a *p*-xylenyl linker. This small molecule similarly down-regulates expression of the multidrug resistance protein (MRP-1) by inhibiting Sp1-activated transcription [20,21] and has potential in the treatment of cancer resistant to common therapeutic drugs. WP631 also binds the G/C-rich –148/–124 region of the urokinase receptor (uPAR) promoter, decreases uPAR expression, and subsequently inhibits migration of colon cancer cells [22]. Another antibiotic with the ability to intervene in Sp1-DNA binding is hedamycin, which inhibits survivin expression by binding a 21-bp G/C-rich site (–115/–195) in the survivin promoter [23]. Survivin is a multi-function protein commonly expressed in transformed cells [24]. The survivin promoter contains at least 7

putative Sp1 binding sites [25] suggesting that this gene is a valid target for many G/C-binding small molecules. Tetrameprocol (Tetra-O-methyl-nordihydroguaiaretic acid, also referred to M4N or EM-142, Fig 2) is a derivative of a plant lignan that binds G/C-rich sequence and inhibits survivin expression resulting in apoptosis of transformed cells without apparent toxicity [26]. This small molecule is currently in clinical trials for treatments of refractory solid tumors, lymphoma and leukemia [27]. In spite of these successes, small-molecule DNA intercalators are unlikely to efficiently inhibit binding of a single transcription factor because of their limited sizes which render them incapable of binding to DNA spanning more than a few base pairs [28].

3.2. Polyamides

Polyamides containing N-methylpyrrole (Py) and N-methylimidazole (Im) are designed to bind minor grooves of gene-specific double-strand DNA based on a set of pairing rules (*i.e.*, Py/Py binds A·T or T·A while Im/Py and Py/Im recognizes GC and CG, respectively)[29]. In principle, any DNA sequence can be recognized by a polyamide. These pyrrole-imidazole polyamides bind target DNA sequences with nanomolar to subnanomolar affinities and, therefore, are useful in competing with transcription factors for DNA binding while subsequently inhibiting gene expression. One recent example is a cell-permeable polyamide designed to preferentially bind B sites (5'-WGGWW-3', or 5'-GGGWW-3', where W = A or T) recognized by the transcription factor NF B, an important regulator of immune responses and cell survival [30]. This polyamide (Fig 2) blocks NF B binding to the IL6 and IL8 promoters and inhibits TNF- α -induced expression of IL6, IL8 as well as other NF κ B target genes [30]. A similar polyamide was designed to specifically recognize the androgen response element (5'-WGWWCW-3') and inhibits expression of androgen-responsive genes essential for sustaining prostate cancer growth and progression [31]. In addition, polyamides that bind *cis*-regulatory elements for HIF-1, AP-1, glucocorticoid receptor, and NF-Y have been reported [32–35].

3.3. Transcription factor decoys

Double-strand oligodeoxynucleotides (ODNs) or hairpin single-strand ODNs that mimic binding sites for transcription factors appear to be effective decoys. These ODNs often contain consensus recognition sequences for targeted transcription factors, or contain modified sequences that allow for stronger protein-DNA interactions [36]. Transfected decoys bind corresponding transcription factors and prevent their interaction with target promoters, thereby regulating gene expression. Indeed, decoys targeting transcription factors including Sp1, AP-1, STAT3, and Ets-1 inhibit expression of cancer associated genes (e.g., VEGF, uPAR, Bcl-XL) and attenuate growth and metastasis of various cancer cells [37–40]. Interestingly, in spite of striking similarity in the consensus binding sequences between STAT1 and STAT3, a decoy was designed to specifically bind STAT3 but not STAT1 [41], demonstrating the feasibility of designing decoys capable of discriminating closely-related transcription factors. However, like other oligonucleotide-based agents, delivery of transcription factor decoys to target cells and *in vivo* stability are two major challenges for clinical applications.

4. Targeting protein-protein interactions involved in transcriptional regulation

Whereas transcription factors often bind DNA as dimers, they also interact with transcriptional co-regulators to tightly control gene expression. Disrupting dimerization and/or complex formation, therefore, is another effective strategy to manipulate transcription. However, targeting protein-protein interactions is challenging as protein-protein interactions often involve expansive protein surfaces and lack well-defined hydrophobic binding pockets

[42]. In spite of these difficulties, peptide mimetics or small molecules that bind transcription factors or their co-regulators have been developed as potential therapeutic agents.

4.1. Peptide mimetics and stapling peptides

As a major mediator of oncogenic signaling, phosphorylated STAT3 binds target promoters (e.g., the anti-apoptotic Bcl-2 family members) upon homodimerization through interactions between the phosphotyrosine and Src-homology 2 (SH2) domain [43]. A SH2-binding phosphopeptide PY*LKTK and its tripeptide derivatives (P/A)Y*L (where Y* represents phosphotyrosine) decrease the DNA-binding activity of STAT3 by inhibiting STAT3 dimerization [44]. A derived peptidomimetic compound S3I-M2001 containing only one residual amide bond (Fig 2) inhibits STAT3-mediated transcription and tumor growth [45]. A different strategy was applied to develop molecules inhibiting the NOTCH transcription factor complex. This ternary transcription factor complex is comprised of the intracellular domain of NOTCH1, the DNA-bound transcription factor CSL, and a member of the co-activator MAML family (e.g., NALM1). Based on a dominant-negative fragment of MAML1 (residues 13–74) that antagonizes the NOTCH signaling, a hydrocarbon-stapled, cell permeable peptide SAHM1 spanning residues 21 to 36 was designed, and specifically inhibits NOTCH1 target gene expression resulting in global suppression of NOTCH1 signaling [8]. Hydrocarbon Stapling restores α -helical structure of small peptides and can greatly improve binding affinity, proteolytic resistance and serum half-life while allowing cell penetration through endocytic vesicle trafficking [46]. This is the first example that stapled peptides can target seemingly intractable interactions between transcription factors and their co-regulators. A stapled peptide (SAH-p53-8) that stabilizes the p53 α -helical region required for MDMX binding was recently developed. This peptide preferably binds the p53 repressor MDMX as compared to MDM2, and subsequently activates p53 target gene expression resulting in inhibition of tumor growth in mice with no signs of toxicity [47].

4.2. Small molecules targeting protein-protein interactions

Small molecules that dissociate transcription factors from their co-regulators have also been developed. The oncogenic transcription factor c-Myc impacts gene expression by binding to target promoters upon dimerization with its unique partner Max. Through screening chemical libraries using *in vitro* protein-protein binding assays, several small molecules, including a peptidomimetic compound (IIA6B17) (Fig 2), that disrupts Myc-Max dimer formation [48,49] were identified. Similarly, a small molecule KG-501 (2-naphthol-AS-E-phosphate) (Fig 2) that interferes with the interaction between the transcription factor CREB and its coactivator p300 and several natural compounds that can dissociate the Tcf- β -catenin complex were also identified through library screening [50]. These small molecules inhibit expression of corresponding target genes as anticipated [50]. The effective concentrations of these small molecules, however, are rather high ($> 10\mu\text{M}$), consistent with the concept that protein-protein interactions are relatively refractory to chemical interventions. Nevertheless, more than 20 structurally-diverse small molecules that disrupt the interaction between p53 and its major repressor MDM2 at low micromolar levels or even nanomolar levels have been developed [51]. Most of these compounds bind the p53-binding cleft of MDM2. Nutlin-3a, for instance, is an imidazoline derivative (Fig 2) that displaces p53 from MDM2 *in vitro* with nanomolar potency ($\text{IC}_{50} = 90\text{ nM}$). This compound activates p53-dependent gene expression resulting in cell cycle arrest and tumor regression in a p53-dependent manner [52]. However, Nutlin-3a does not induce apoptosis in most cancer cell lines [53] and fails to activate p53 in MDMX-overexpressing cancer cells [54,55], limiting its therapeutic application. The spiro-oxindole MI-219 was later developed through a structure-based approach, and is currently the most potent inhibitor of the MDM2-p53 interaction

with a K_i of 5 nM [56]. This orally-active small molecule is 10,000-fold selective for MDM2 over MDMX, and activates p53 and induces apoptosis in tumor xenografts [56]. SJ-172550 (Fig 2), a small molecule which preferably disrupts the p53-MDMX interaction, was recently identified through screening ~300,000 compounds [57]. This MDMX inhibitor activates p53 and induces p53-dependent gene expression in MDMX-overexpressing cancer cells.

5. Epigenetic Interventions in Transcription

Changes in DNA methylation levels and/or histone modification states often result in overexpression of oncogenes or repression of tumor suppressor genes. It is not surprising, therefore, that these epigenetic alterations are frequent in human cancers [58,59]. Indeed, decreased expression of tumor suppressor genes (*e.g.*, Rb, VHL, p16, APC) is often caused by promoter hypermethylation catalyzed by DNA methyltransferases (DNMTs) [60]. Removal of acetyl groups from core histones associated with tumor suppressor genes (*e.g.*, p21) [61] by histone deacetylases (HDACs), or addition of methyl groups to histone lysine residues by histone methyltransferases (HMTs), can also convert chromatin to a condensed state that restricts access of transcriptional factors to DNA, thereby, inhibiting expression of tumor suppressor genes. Inhibitors of DNMTs, HDACs or HMTs capable of reversing cancer-associated epigenetic changes, therefore, are widely sought as effective anti-cancer agents [10,62].

5.1. DNMT inhibitors

Azacytidine (5-azacytidine or Vidaza) and 2'-deoxy-5-azacytidine (Decitabine or Dacogen) are two cytidine analogs that potently inhibit DNMTs by trapping the enzymes for proteosomal degradation [58]. Treatment of cancer cells with these compounds results in DNA hypomethylation and subsequent re-expression of tumor suppressor genes, thereby attenuating tumor growth. Vidaza and Decitabine are approved for the treatment of high-risk myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) [60]. However, these DNMT inhibitors are unstable due to *in vivo* deamination by cytidine deaminase. SGI-110 (S100) (Fig 2), a recently-developed dinucleotide and Decitabine analog resistant to metabolic inactivation, induces both expression of the tumor suppressor p16 and tumor growth inhibition [63], and is currently in clinical trials for treatment of MDS and AML patients [60]. Another major limitation of cytidine analogs for clinical application is their toxicity at high concentrations due to their incorporation into DNA. Non-incorporating DNMT inhibitors such as the quinoline derivative SGI-1027 and the antibiotic nanaomycin A [64,65] may overcome this limitation and offer a larger therapeutic window [60]. The latter two small molecules decrease cellular cytosine methylation and up-regulate the tumor suppressor genes p16, MLH1, TIMP3, and RASSF1A in various cancer cells [64,65].

5.2. HDAC and HMT inhibitors

The catalytic activity of a HDAC is largely dependent on Zn^{2+} , a relatively easy target for small molecules [10]. More than 20 structurally-distinct small molecules with common metal-binding moieties to chelate Zn^{2+} and inhibit HDAC activity have been developed; many are in clinical trials for various hematological and solid tumors [62]. These include pan-HDAC inhibitors such as suberoylanilide hydroxamic acid (SAHA, Vorinostat, or Zolinza) (Fig 2) and Panobinostat (LBH589), as well as isoform-specific inhibitors such as Valproic acid (VPA or Depakote) (inhibiting classes I and IIa HDAC) and Romidepsin (Istodax) (targeting HDAC1 and HDAC2) [62]. SAHA and Romidepsin are approved for treating cutaneous T cell lymphoma. HDAC inhibitors up-regulate p21 expression, leading to cell cycle arrest [61], or increase expression of death receptors and their ligands (*e.g.*, DR5, TRAIL, FAS and FasL) [66] or other pro-apoptotic genes Bim, Bax and Bak [67],

thereby, inducing cancer cell apoptosis. HDAC inhibitors also stimulate mitotic or autophagic cell death or inhibit angiogenesis; these effects are more likely achieved through modulating functions of non-histone HDAC substrates [68]. Chaetocin, a fungal metabolite, is the first compound to inhibit the HMT SUV39H1 and decrease histone H3 lysine 9 (K9) methylation [69] while inducing p21 expression leading to cell cycle arrest [70] and activating expression of the tumor suppressors p15INK4B, E-cadherin, as well as proapoptotic genes (*e.g.*, Fas, FasL, DR4) [71,72]. Other small molecules that inhibit HMT G9a (*e.g.*, BIX-01294 and its analog UNC0638) or Dot1 (*e.g.*, EPZ004777) (Fig 2) also regulate transcription [73,74]. Due to crosstalk between DNA methylation and histone modifications, co-treatments of cancer cells with both DNMT and HDAC inhibitors synergistically reactivates expression of tumor suppressor genes (*e.g.*, MLH1, TIMP3, and p16) [75], suggesting potential clinical benefits of combination regimes.

6. Artificial Transcription Factors for Gene-Specific Transcriptional Regulation

Because a single transcription factor may recognize promoters for many genes and epigenetic factors often induce global changes in gene expression, targeting one gene without altering expression of collateral genes remains challenge. Artificial transcription factors that theoretically can target any given specific DNA sequence have emerged in recent years. The prototypes of these designed gene-specific DNA-binding proteins is a group of natural transcription factors containing multiple, tandem Cys2-His2 zinc fingers, each of which contains approximate 30 amino acids but uses only a few key residues to bind 3 or 4 successive bases in the major groove of DNA [76]. Altering residues on the recognition site of a zinc finger transforms the DNA-binding motif to allow recognition of a distinct sequence. Since zinc finger motifs recognizing various 3-bp DNA were identified using phage display or other screening methods, it is possible to stitch several zinc finger domains in tandem to generate an artificial transcription factor that can bind any pre-determined DNA sequence [77]. Fusing transcriptional activation domains (*e.g.*, VP16 domain from herpes simplex virus, or p65 transactivation domain) to these designed zinc-finger proteins (ZFP) allows for activation of gene-specific expression, whereas adding transcriptional repression domains (*e.g.*, KRAB domain, Suv39H1 protein methyltransferase, or DNA methyltransferases M.SssI or DNMT3a) can repress expression of genes bound by the engineered proteins [78,79]. A ZFP generated by joining 6 zinc fingers recognizes a 18 bp site, which theoretically should occur only once in the human genome [76], can provide virtually single gene specificity [80].

ZFP-based strategies were successfully applied to target cancer-associated genes. An early example is a ZFP containing 5 zinc fingers and designed to bind a region (−69 to −41) harboring the EGR1, Sp1, and WT1 recognition sites in the MDR1 promoter [81]. This ZFP was fused to two copies of the KRAB repressor domain decreasing MDR1 expression and sensitizing drug-resistant NCI/ADR-RES breast cancer cells to doxorubicin [81,82]. Similarly, a ZFP fused to the VP16 activation domain that binds to a region overlapping the p53 recognition site in the Bax promoter induces Bax expression resulting in apoptosis of Saos2 human sarcoma cells [83]. Maspin is a prognostic marker for various cancers (*e.g.*, breast cancer) and represses tumor growth and metastasis [84]. A designed 6-finger ZFP targeting a region (−126 to −108) of the maspin promoter and fused to a strong transcription activator VP64 increases maspin expression, while exerting multiple anti-cancer effects including decreased invasiveness and growth of xenografted breast cancer cells [85]. VEGFA is another cancer-related gene successfully targeted by designed artificial transcription factors. ZFPs capable of binding the VEGFA promoter and fused to the vErbA repression domain down-regulate VEGFA expression in glioblastoma cells [86]. Delivery of

a VEGFA-targeted ZFP through adenoviral vectors represses tumor growth and increases animal survival in a human glioblastoma xenograft model [87].

In spite of these successes, application of ZFP-based agents for cancer treatment faces significant challenges including efficient delivery to specific cells or tissues. While viral-based approaches remain mainstream, the efficacy of fusing a short cell-penetrating peptide to ZFPs [88] remains to be tested. Application of ZFPs in targeted cancer therapy is also been hindered by the resource intensive and empirical nature of design of zinc-finger proteins recognizing gene-specific DNA sequences [89]. Recent emergence of transcription activator-like (TAL) effectors of plant pathogenic bacteria containing a modular DNA binding domain may provide an alternative strategy for developing artificial transcription factors targeting gene-specific DNA sequences [89]. These TAL effectors contain tandem, polymorphic amino acid repeats which can specifically recognize single, contiguous nucleotides in DNA. Engineering a protein containing varying combinations of amino acid repeats would allow recognition of a gene-specific DNA sequence [89].

7. Unbiased high-throughput drug screening

Recent availability of chemical libraries containing hundreds of thousands of structurally-diverse compounds facilitates drug discovery in a high-throughput manner [90]. However, success in high-throughput screening (HTS) for small-molecule transcriptional regulators is rather limited due to the lack of assays that can be used for rapid, accurate and cost-efficient measurements of changes in gene expression. Although mRNA and their encoded proteins can be accurately measured by Northern/Western blotting, quantitative RT-PCR, or ELISA, these approaches are neither convenient nor cost-efficient. Bioluminescent reporter technologies provide a convenient and cost-efficient means to monitor alternations in gene expression and are useful in large-scale searches for small-molecule transcriptional regulators [91–93]. Typically, a bioluminescent reporter (*e.g.*, firefly luciferase) fused to a cloned or synthetic promoter harboring essential *cis*-regulatory elements is integrated into cultured cells (Fig 3A), providing a platform for high-throughput screening amenable to robotic manipulations [91]. This approach is superior in that (a) luciferase activity can be easily measured with current instrumentation, (b) the assay is sensitive and has a broad linear range (7–8 logs), and (c) the luciferase protein has a short half-life (<2h) thus accurately reflecting the activity of an upstream promoter [94,95]. Earlier applications of this strategy led to identification of small molecules that regulate activities of transcription factors such as CEPB α , p53, HIF-1, STAT3 using reporters controlled by tandem repeats of *cis*-regulatory elements recognized by these transcription factors [91–93,96]. Recently, reporters driven by promoters cloned from individual genes were developed for high-throughput screening (HTS). YM-155 (Fig 2), for instance, was identified employing cells stably expressing a firefly luciferase gene under the control of a 2.7 kb survivin promoter [97]. YM-155 was validated to down-regulate expression of survivin but not other apoptosis suppressors (*e.g.*, c-IAP2, XIAP, Bcl-2, and Bad), inducing apoptosis and exerting anti-cancer effects in animal models [97]. This small molecule is currently in phase II clinical trials for refractory diffuse large B-cell lymphoma and advanced non-small lung carcinoma.

Early HTS assays utilize engineered cells carrying reporter genes at random genomic sites. However, randomly-integrated reporters are often epigenetically silenced by flanking condensed chromatin [98], leading to failures in the development of HTS assays for transcription-targeted agents. Since reporter genes residing in an open genomic site characterized by high levels of active histone modifications are actively expressed [98], a strategy to circumvent this limitation is to integrate reporter genes site-specifically into pre-characterized open genomic locations (Fig 3B) [99]. This strategy is dependent on homologous recombination mediated by recombinases such as the yeast-derived Flp

[100,101] and has been successfully applied to identify transcription inhibitors [99,102]. Indeed, a benzimidazole derivative MPBD (Fig 2) was identified to down-regulate expression of matrix metalloproteinase-9 (MMP-9) and inhibit MMP-9-dependent invasion of oral cancer cells [103]. Similarly, a high-throughput screen using a site-specifically integrated reporter led to identification of XI-006 (Fig 2), a benzofuroxan derivative, as an inhibitor of MDMX expression [102]. This compound activates expression of p53 target genes as anticipated, leading to breast cancer cell apoptosis [102].

Reporter-based HTS assays often generate a large number of false positive hits, making subsequent validation labor-intensive and challenging. A major underlying reason is related to the fact that reporter genes are controlled by cloned promoters lacking essential *cis*-regulatory elements (*e.g.*, enhancers) far-removed from transcription start sites, and are integrated into “foreign” genomic locations where the chromatin environment alters reporter expression in a manner atypical of the natural gene. Indeed, expression of a firefly luciferase gene directed by a cloned MMP-9 promoter and integrated in an open genomic location was shown to qualitatively, but not quantitatively, reproduce endogenous gene expression [100]. Theoretically, a strategy to circumvent these limitations is to integrate reporter genes into endogenous gene loci thereby allowing reporter gene expression under the control of endogenous gene promoters and native chromatin environments (Fig 3C). This can be achieved through a high-efficient gene targeting approach based on adenoassociated virus (AAV)-mediated homologous recombination (Fig 3C)[104]. Indeed, this method was used to insert a *Renilla* luciferase reporter at the immediate downstream of the transcription start site of the TNF- α gene [105]. However, it remains to be established whether such “*in situ*” reporters (1987) would more faithfully reflect endogenous gene expression and false rates of HTS assays based on these reporters would be significantly reduced.

8. Conclusion

Cancer-associated aberration in transcription is a promising drug target for anti-cancer therapeutics. Although transcription is traditionally considered undruggable, recent advances in the development of transcription-targeted agents demonstrate that rectifying abnormal gene expression is a feasible approach to cancer treatment. Importantly, with the continuous improvement of reporter-based assays, more reliable HTS assays will soon be available for rapid, large-scale identification of transcription-targeted molecules. Challenges remain with regard to development of therapeutic agents targeting transcription at the single gene level. Whereas ZFP-based agents have single-gene specificity [80], off-target effects of most of transcription-targeted agents have not been extensively explored. However, it is arguable whether there is a real need to develop single-gene targeted agents considering that the growth and survival of cancer cells are often driven by aberrant expression of a group of related genes that are often co-regulated by common transcription factors such as p53. Moreover, because gene expression levels are also determined by mRNA/protein stability that often differs between genes, it is possible to identify agents that can alter expression of targeted genes but exert little effects on other transcriptionally co-regulated genes. Nevertheless, the fact that several transcription-targeted agents have been approved or have entered clinical trials for cancer treatment support the increasingly-prevailing contention that transcription-targeted agents have potential in treating human cancers.

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Aberrations

AR	androgen receptor
DHFR	dihydrofolate reductase
DNMT	DNA methyltransferase
ER	estrogen receptor
HDAC	histone deacetylase
HMT	histone methyltransferase
HTS	high-throughput screening
MDR1	multidrug resistance protein 1
ODN	oligodeoxynucleotides
RAR	retinoic acid receptor
TF	transcription factor
ZFP	zinc finger protein

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Fig 1.

Transcriptional regulation and targeting strategies. (A) Transcription is regulated at distinct levels. (B) Small molecules or polyamides compete with transcription factors to bind *cis*-regulatory elements, whereas decoys bind transcription factors preventing them from binding to promoters. (C) Peptide mimetics or small molecules disrupt dimerization of transcription factors, or interactions between transcription factors and their co-regulators. (D) Inhibition of DNA or histone modifying enzymes changes the epigenetic landscape with subsequent effects on gene expression. (E) Artificial transcription factors fused with transcriptional activation or repression domains bind to promoters, modulating transcriptional outcomes. TF, transcription factor; GTF, general transcription factor; Pol II, RNA polymerase II; CoR, transcription co-regulator; DNMT, DNA methyltransferase; HDAC, histone deacetylase; HMT, histone methyltransferase; I, transcription-targeted agents; D, transcription factor decoy; Me, methyl group; Ac, acetyl group; ZFP, zinc-finger protein; A/R, transcriptional activation or repression domain.

Fig 2.
Chemical structures of transcription-targeted agents.

Fig 3.

Reporter assays for high-throughput drug screening. (A) A bioluminescence reporter (Luc) driven by a cloned or synthetic promoter is randomly integrated into the genome for drug screening. (B) The recombinase Flp mediates homologous recombination between FRT (F) sites allowing site-specific integration of a reporter gene driven by a clone promoter into a pre-characterized open genomic location. (C) AAV mediated homologous recombination results in integration of a reporter gene into the native gene locus, allowing the reporter gene expression under control of the endogenous promoter and native chromatin environment. The selection gene is removed by Cre-mediated excision.

Table 1

Agents targeting transcription at different levels

Agent	Target	Regulated genes	Clinical stage
TF-DNA binding			
Small molecule			
Mithramycin	G/C-rich DNA (Sp1)	c-Myc, DHFR	treatment of advanced testicular carcinoma
WP631	G/C-rich DNA (Sp1)	MRP-1, uPAR	
Hedamycin	G/C-rich DNA (Sp1)	survivin	Phase II (leukemia, solid tumors and lymphoma)
Tetrameprocol	G/C-rich DNA (Sp1)	survivin	
Polyamides	TF binding sites		
TF decoy	TF		
Protein-protein interaction			
Peptide mimetic			
S3I-M2001	STAT3 dimerization	Bcl-xL	
SAHM1	NOTCH1 complex	HES1, Myc, DTX1, HEY1, Nrarp	
SAH-p53–8	p53-MDMX interaction	MDM2, p21, MIC-1	
Small molecules			
IIA6B17	Myc-Max dimerization		
KG-501	CREB-p300 interaction	NR4A2, aCG, c-fos, RGS2	
Nutlin-3a	p53-MDM2 interaction	p21, MDM2	
MI-219	p53-MDM2 interaction	p21, MDM2, PUMA	Phase I
SJ-172550	p53-MDMX inteaction	p21	
Epigenetic modifications			
DNA methylation			
Azacytidine	DNMT		treatment of myelodysplastic syndromes
Decitabine	DNMT		treatment of myelodysplastic syndromes
SGI-110	DNMT	p16, MLH1, TIMP3, RASSF1A	Phase I/II (MDS and AML) *
Histone actylation			
SAHA	HDAC	p21, DR5	treatment of cutaneous T cell lymphoma
Romidepsin	HDAC	TRAIL, FAS, Bim, Bax, Bak	treatment of cutaneous T cell lymphoma
Histone methylation			
Chaetocin	SU (VAR)3–9	p21, E-cadeherin, Fas, DR4	
BIX-01294	G9a	mage-a2, Bmi1, Serac1	
EPZ004777	DOT1L	HOXA9, MEIS1	
ZFP	gene-specific sequence	MDR1, Bax, Maspin, VEGFA	
Agents identified by HTS			
YM-155		survivin	phase II (lymphoma and lung carcinoma)
MPBD		MMP-9	
XI-006		MDMX	

* MDS, myelodysplastic syndromes; AML, acute myeloid leukemia