Biochimica et Biophysica Acta 1855 (2015) 183–192

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbacan

Review

Transcription factors and chromatin proteins as therapeutic targets in cancer

Simon J. Johnston, Jason S. Carroll *

Cancer Research UK Cambridge Institute, University of Cambridge, Robinson Way, Cambridge CB2 0RE, UK

ARTICLE INFO

Article history:
Received 9 January 2015
Received in revised form 9 February 2015
Accepted 9 February 2015
Available online 23 February 2015

Keywords:
Transcription factors
Cancer
Drugs
Small molecules
siRNA
Nuclear receptors

ABSTRACT

Targeting the factors that regulate gene transcription is a compelling strategy in cancer therapeutics. Traditionally, these have been considered intractable targets, but recent work has revealed novel strategies for the regulation of transcription factor activity in cancer. This review will highlight some of the emerging concepts and provide examples where agents that target transcription factors are being exploited clinically for cancer therapies.

Contents

1. Introduction

Targeting the factors that regulate gene transcription for cancer treatment, is an exciting but challenging concept. Cells respond to various stimuli *via* modulation of specific gene expression programmes,

* Corresponding author.
E-mail address: jason.carroll@cruk.cam.ac.uk (J.S. Carroll).

http://dx.doi.org/10.1016/j.bbcan.2015.02.002
0304-419X/

which culminates in the production of effector proteins and transcripts that elicit the biological response. Multiple upstream cellular pathways converge on transcription factors and alter their functional state, directly influencing the target genes regulated by these factors. Activated transcription factors function in the context of multi-protein complexes with cofactors, and bind directly to specific sites on accessible DNA to regulate gene expression *via* recruitment of the RNA polymerase II machinery [1].

In cancer, this process can be dysregulated at multiple levels, including alterations in upstream signals, or at the level of the transcription factor itself, or both — a common feature observed in acquired resistance secondary to treatment. In all cases, transcription of critical target genes can be considered a common endpoint in oncogenic cell signalling pathways. Thus transcriptional machinery can be viewed as "hubs" that influence the development of many of the hallmarks of cancer progression [2], and the factors that regulate transcription represent attractive therapeutic targets that could circumvent many of the issues of cross-talk, redundancy and resistance that are inherent in targeting upstream cell signalling enzymes.

Despite the motivation to directly modulate transcription factors in cancer therapeutics, they have proved to be challenging targets and are commonly termed 'undruggable' [3]. However, some of the most commonly prescribed and utilized treatments for both cancer and non-cancer related conditions work by directly targeting a specific class of transcription factors called nuclear receptors. For example, glucocorticoid receptor ligands (*e.g.* synthetic glucocorticoids such as hydrocortisone, dexamethasone, prednisolone and methylprednisolone) are widely utilized for their anti-inflammatory and immunosuppressive effect in conditions such as asthma, arthritis and auto-immune disorders — as well as for their pro-apoptotic effect in haematological malignancies such as multiple myeloma [4,5]. It is possible that nuclear receptors constitute an unusual class of transcription factors with substantially greater druggability; however, in recent years, an increased understanding of transcriptional regulation, coupled with technological advances, has made the direct or indirect targeting of other transcription factors realistic options.

Before a transcription factor can become a realistic and *bona fide* drug target, it is important that we understand and define the underlying biological properties of that protein, in order to identify the right family member to target, the correct clinical context when this factor is essential for cancer progression and any potential feedback mechanisms that might influence the efficacy of a targeted agent. Furthermore, because many transcription factors are expressed in a range of normal tissues where they play important physiological roles, it is important to anticipate and study potential unwanted effects of targeting oncogenic transcription programmes. Due to advances in 'omic' technologies, we have a greater understanding of transcription factor binding sites, the chromatin states that can influence binding dynamics, the associated proteins that contribute to transcription factor function and the key downstream target genes. This information has revealed the key factors regulating transcriptional activity that likely function as putative drug targets (summarized in Fig. 1), whilst also identifying less tractable options.

To target these factors, approaches include the use of small molecules, peptides and g-quadruplex regulatory mechanisms, all of which have resulted in the identification of compounds that are currently being assessed in clinical trials. Furthermore, RNA regulation has been explored as a way of targeting transcription factor function, by short-circuiting the key downstream targets of driving transcription factors.

This review aims to highlight specific examples of these evolving concepts that are being utilized to treat cancer by targeting its driving transcription factors (Table 1). In addition, we will detail some of the emerging concepts and technical advances that may reveal new opportunities for drugging these important regulatory factors in cancer.

2. Targeting transcription factors

2.1. Direct targeting of transcription factors

2.1.1. Nuclear receptors

Direct therapeutic modulators of nuclear receptor function work by physical interaction with the transcription factor (the nuclear receptor) itself, resulting in conformational changes that influence the interactions with co-activators or co-repressors [7]. The presence of a ligand binding domain pocket in nuclear receptors creates the opportunity for eliciting distinct conformation changes and therefore different gene expression programmes by using different ligands. As an example, the oestrogen receptor (ER), which is the defining and driving transcription factor in 75% of breast cancers [8] can be blocked by different classes of antagonists that have distinct mechanisms and different clinical efficacy [9,10].

Tamoxifen, an endocrine therapy introduced into the clinic in the 1980s to treat ER positive breast cancer, represents one of the most successful targeted cancer therapies to date, having a significant impact on survival rates in breast cancer patients [11]. Fulvestrant (ICI 182780) is a newer agent for the direct inhibition of ER protein levels, which can be an effective secondary transcription factor modulator when tumours have acquired resistance to tamoxifen [12]. Similarly a number of therapies exist for inhibition of the androgen receptor (AR), which functions as a driving transcription factor in prostate cancer. Direct AR antagonists include established agents such as bicalutamide and more recently, enzalutamide [13,14], both of which have had significant impacts on patient survival. Enzalutamide binds directly to the AR with greater affinity than bicalutamide, and targets AR-mediated gene expression by inhibiting its nuclear translocation and its ability to recruit cofactors and bind to DNA [15]. Drugs targeting ER and AR, the key transcription factors in breast and prostate cancers, respectively, have become the mainstays of treatment for these hormone dependent cancers.

Targeting nuclear receptors in hormone dependent cancers has had a substantial impact on patient survival rates. One reason why nuclear receptors are likely to be more amenable to drug targeting than most other transcription factors is the existence of a ligand regulated binding domain. Such a common druggable pocket that exists within an entire family of transcription factors is either lacking or uncharacterized in many other types of transcription factors. As such, it is not particularly surprising that there has been a notable absence of therapeutic compounds targeting other classes of transcription factors in the clinic. However, alternative strategies have been developed to target non-nuclear receptor transcription factors, both directly and indirectly. These are outlined below.

2.1.2. Non-nuclear receptor transcription factors

Beyond the nuclear receptor, there are relatively few examples of the direct targeting of other transcription factor proteins using small molecules, and efforts have focused on indirect targeting of transcription factor function *via* interacting molecules [7].

However, a precedent for the inhibition of non-nuclear receptor transcription factors by direct interaction was recently established in breast cancer cells using a natural product, thiostrepton [16]. This naturally-occurring antibiotic was demonstrated to bind directly and specifically to the forkhead box protein FoxM1 – an oncogenic forkhead box transcription factor upregulated in a wide range of cancers [17] – resulting in inhibition of genomic FoxM1–DNA interactions.

This proof of concept study may reinvigorate the effort to target transcription factors by direct interaction. Meanwhile, there has been considerable progress in targeting transcription factors *via* protein and DNA interactions, which is summarized below.

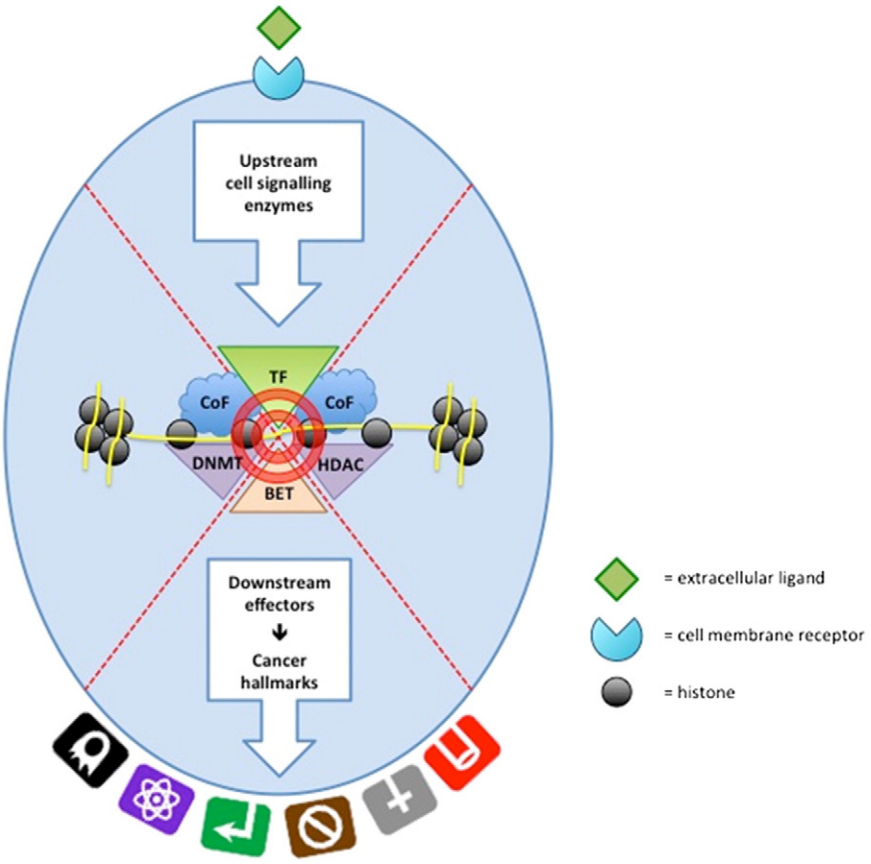

Fig. 1. Targeting transcription factors and chromatin proteins in cancer. TF = transcription factor; CoF = cofactor; HDAC = histone deacetylase; BET = bromodomain and extra-terminal; DNMT = DNA methyltransferase. Cancer hallmarks [6] that would be impacted by TF targeting include (from left to right): activating invasion and metastasis; deregulating cellular energetics; sustaining proliferative signalling; evading growth suppressors; resisting cell death; inducing angiogenesis.

2.2. Indirect targeting of transcription factors

2.2.1. Via inhibition of protein interactions

2.2.1.1. Small molecules. Targeting protein interactions has historically proven elusive because proteins generally offer relatively large and flat interacting surfaces that are not readily perturbed by small molecule drugs [18]. However, a precedent for targeting transcription factors via protein–protein interactions was established a decade ago with the development of a small molecule inhibitor, Nutlin, which perturbs the interaction between the p53 tumour suppressor (a transcription factor) and its negative regulator MDM2 [19,20].

Table 1
Examples of therapeutic agents designed to target a transcription factor pathway.

Mechanism		Class	Example	Target	Tumour	Development
Targeting transcription factors						
Direct	Nuclear receptor	NR modulator	Tamoxifen	ER	Breast	Approved (1977)
	Non-nuclear receptor	Small molecule	Thiostrepton	FoxM1	Breast	*In vitro*
Indirect	Protein interaction	Small molecule	RG7112	p53–MDM2	Liposarcoma; acute leukaemia	Phase I
		Stapled peptide	ALRN-6924	P53–MDM2–MDMX	Solid tumours (wild-type p53)	Phase I/II
		Peptidomimetic	ABT-199	BCL-2	Chronic lymphocytic leukaemia	Phase III
	DNA–protein interaction	Decoy oligonucleotide	STAT3 decoy	STAT3	Head and neck squamous cell carcinoma	Phase 0
		G-quadruplex stabilizer	TMPyP4	cMyc	Various	*In vitro*
	RNA degradation	Small interfering RNA	ALN-VSP02	*VEGF & KSP*[a]	Solid tumours with liver metastasis	Phase I
		Antisense oligonucleotide	AZD9150	STAT3	Lymphoma; various solid tumours	Phase I
	Direct enzymatic regulator	Kinase inhibitor	Ruxolitinib	STAT proteins	Myoproliferative neoplasms	Approved (2011)
Targeting chromatin regulator proteins						
DNA modification		DNA methyltransferase (DNMT)	5-azacytidine	DNMT1/DNMT3a/DNMT3b	Myelodysplastic syndromes	Approved (2004)
Histone modification		Histone deacetylase (HDAC) inhibitor	Vorinostat	HDAC	Cutaneous T-cell lymphoma	Approved (2006)
		Histone methyltransferase (HMT) inhibitor	EPZ-5676	DOT1L	MLL-fusion leukaemia	Phase I
		BET protein inhibitors	I-BET151	BRD3, BRD4, SEC	MLL-fusion leukaemia	*In vivo*

[a] Non-transcription factor targets.

Aberrations in p53 signalling are almost universal in human cancer [21]. Half of these aberrations are due to p53 mutation, whilst the other half are attributed to inactivation of wild type p53 by negative regulators such as MDM2 and MDMX — offering the potential to reactivate p53 to treat a range of cancers. Nutlin binds selectively to the MDM2 binding pocket of p53, inhibiting p53–MDM2 interaction and MDM2-mediated ubiquitination of p53, hence prolonging the half-life of activated p53 [19]. Nutlin demonstrated antitumour effects in multiple cancer cell line models and in mouse xenograft models of prostate cancer and osteosarcoma [19,22]. The Nutlin derivative compound RG7112 is undergoing clinical trial in liposarcoma [23] and leukaemia [24]. Phase 1 clinical trial of patients with acute leukaemia indicated single agent clinical efficacy at the minimum tolerated dose, with a remarkable 16% (5 out of 31) complete response rate in those with heavily pre-treated acute myeloid leukaemia who completed cycle 1 dosing (31 out of 43) [24]. These data confirm that protein–protein interactions between transcription factors and regulatory proteins can be blocked successfully with chemical inhibitors [25]. However, issues relating to the tolerability and toxicity of p53 reactivating agents such as RG7112 are not insignificant. For example, during the RG7112 clinical trial in liposarcoma, all patients experienced at least one adverse event, most commonly nausea and vomiting, and there were 12 serious adverse events in 8 patients, including neutropenia (6 patients) and thrombocytopenia (3 patients) [23].

In the last few years, several classes of potent, selective, and efficacious small molecule MDM2 inhibitors have been designed and developed, and several such compounds are being evaluated in clinical trials as new anticancer drugs [21,26,27]. Additionally, small-molecule MDMX inhibitors have been reported, with the goal of targeting other members of the p53 complex, for example in models of leukaemia and breast cancer [28,29].

Reactivation of mutated p53 is another therapeutic option that has been explored using a variety of approaches including small molecules [21]. Such compounds act to rescue wild-type p53 function by stabilizing specific tumour-associated p53 mutants in their active conformation [30,31]. Several p53 reactivating drugs, *e.g.* PRIMA-1, CP31398 and PhiKan083, have demonstrated efficacy in pre-clinical models [32]. Recently, a PRIMA-1 analogue (APR-246), entered phase Ib/II clinical trial for the treatment of recurrent high grade serous ovarian cancer in combination with standard platinum-based cytotoxic therapy.

2.2.1.2. Stapled peptides. An alternative approach for blocking protein–protein interactions is via peptide inhibitors. Early attempts at using peptides revealed problems with peptide stability, which was partially bypassed by the development and utilization of modified peptides with improved stability. Specifically, the introduction of a hydrocarbon backbone (staple) to short peptides maintains them in their active alpha-helical state. This approach has been shown to improve the pharmacologic performance of peptides, increase target affinity, proteolytic resistance and serum half-life whilst conferring higher levels of cell penetration through endocytic vesicle trafficking [33].

Such an approach has been explored *in vivo* to target BCL-2 family proteins (critical apoptosis regulators) using a stabilized alpha-helix of the essential BID death domain, BH3, with evidence of growth inhibition in a leukaemia xenograft model [34]. Subsequently, *in vivo* evidence of successful targeting of specific transcription factor protein interactions has been demonstrated from xenograft models of leukaemia (inhibition of NOTCH transcriptional complex formation), and choriocarcinoma (via p53-inhibitory protein interactions) [35,36].

Whilst technical reproducibility issues exist for stapled peptide approaches and their potential use in patients [37,38], there are peptide inhibitors of transcription factors that are moving into the early stages of clinical trial development. For example, the compound ALRN-7041 which stabilizes wild-type p53 by binding and inhibiting both MDM2 and MDMX, is currently being explored in pre-clinical models of osteosarcoma and breast cancer that overexpress MDM2 and MDMX [36,39]. More recently, a dual inhibitor of MDM2/MDMX called ALRN-6924 has moved into phase I clinical trial for patients with advanced solid tumours expressing wild-type p53.

2.2.1.3. Peptidomimetics. Other peptide approaches showing promise include the use of peptidomimetics: small organic molecules that target specific protein–protein interactions by arranging essential functional groups (pharmacophores) into three-dimensional conformations that are complimentary to the binding pocket in the protein [40]. In theory, peptidomimetics combine the advantages of both peptides (high efficacy and target selectivity) and small organic molecules (cell permeability, stability from protease-mediated proteolytic degradation, oral activity and bioavailability) [41]. However, in practice, issues of *in vivo* metabolic susceptibility and cellular permeability have hindered clinical translation [42], and resolution of these issues is essential for utilization of these approaches.

Despite this, there are a number of peptidomimetic compounds in pre-clinical and clinical development that target transcription factors. For example, STAT3 (Signal Transducer and Activator of Transcription 3), which is an oncogenic transcription factor commonly found constitutively activated in breast cancer cell lines and tumour specimens [43–46], has been targeted for the treatment of breast cancer using peptidomimetics [47]. A similar approach has been used to target nuclear receptor transcription factor function via co-regulator interactions. As an example, interaction of ER with steroid receptor co-activators (SRC) can be selectively inhibited with potential application in breast cancer [48]. In prostate cancer, there is pre-clinical evidence that targeting the interaction between AR and the co-regulator PELP1 using peptidomimetics may be a viable treatment option for patients with advanced disease [41].

In BCL-2 dependent haematological cancers, the BH3-mimetic ABT-199 has reached phase III clinical trial for the treatment of relapsed/refractory chronic lymphocytic leukaemia (CLL) in combination with existing therapy (rituximab). In contrast to previous small molecule approaches that inhibit both BCL-2 and BCL-2-like 1 (BCL-XL) [49], the peptidomimetic ABT-199 is selective for BCL-2. Since BCL-XL suppression causes dose-limiting thrombocytopenia in patients and is not required for therapeutic effect, ABT-199 offers greater potential for clinical utility in the treatment of CLL and other haematological malignancies (*e.g.* diffuse large B-cell lymphoma), as well as in solid tumours such as lung cancer [50,51].

2.2.2. Via DNA–protein interactions

2.2.2.1. Decoy oligonucleotides. Decoy oligonucleotides are short, double-stranded DNA molecules bearing the consensus binding motif of a specific target transcription factor. Binding of a transcription factor to its decoy oligonucleotide has the potential to inhibit transcriptional activation by competitive inhibition of binding to gene promoter or enhancer elements, resulting in the removal of the bound *trans*-factor from the endogenous *cis*-element [52–54]. A major limitation of the decoy approach has been the rapid degradation of phosphodiester oligonucleotides by serum and intracellular nucleases. To address this issue, nuclease-resistant oligonucleotides have been developed; specifically, replacement of a non-bridging oxygen in phosphate linkages with sulphur has resulted in greater stability of DNA oligonucleotide decoys [54].

Recently, a “first-in-human” phase 0 trial of a phosphorothioate-modified duplex oligonucleotide targeting STAT3 in head and neck squamous cell cancer (HNSCC) was reported [55]. Intratumoural injection of a STAT3 decoy DNA target resulted in decreased STAT3 target gene expression, as assessed by immunohistochemical staining of tissue microarray, suggesting that addition of STAT3 binding DNA decoys can effectively downregulate the transcriptional activity of STAT3 on endogenous target genes. Further work using a cyclic STAT3 decoy – designed to avoid 3′ end nuclease degradation by circularizing the duplex

oligonucleotide using a hexa-ethyleneglycol linkage – demonstrated improved stability, evidence of STAT3-specific targeting and growth inhibition in HNSCC xenograft models [55]. These studies may prove a significant step forward in the systemic administration of oligonucleotide therapy to target the function of transcription factors.

2.2.2.2. G-quadruplex stabilizers. G-quadruplexes are secondary DNA structures formed by nucleic acid sequences containing several short runs of guanine nucleotides. Guanine-rich regions with the potential to form G quadruplexes are over-represented in telomeres and transcriptional start sites [56–58] — especially within genes involved in transcription and DNA replication, such as oncogenes [59]. There is emerging evidence that G-quadruplex motifs and structure formation modulate transcription factor binding. Enrichment in G-quadruplexes has been found in computationally predicted transcription factor binding sites [60,61]. Small molecules that stabilize G-quadruplexes in promoter regions of transcription factors have been explored [59], with the goal of destabilizing or perturbing transcription factor function by indirectly affecting the structure and consequent accessibility of DNA to regulatory proteins.

One of the most well studied transcription factors in cancer is the oncogene c-Myc, which is deregulated in many haematological and solid cancer types [62–64]. The transcriptional expression of c-Myc can be repressed by stabilization of a G-quadruplex in the *MYC* promoter region using a small molecule cationic porphyrin TMPyP4 [65]. There is evidence that TMPyP4 acts via the c-Myc transcriptional activation protein, NM23H2, preventing it from binding to and unwinding the G-quadruplex [66], thereby modulating the expression of c-Myc mRNA. This would imply that perturbation of specific G-quadruplexes function to influence a transcription factor that is critical for tumour progression via modification of a functionally linked regulatory transcription factor. Other transcription factors demonstrating potential to be targeted via G-quadruplexes include HIF1α in renal cancer [67] and Bcl-2 in lymphoma and colorectal cancer [68].

2.2.3. Via RNA degradation

2.2.3.1. Small interfering RNA. Another possible way to specifically block a driving transcription factor of interest is via destabilization of the mRNA of that factor. RNA expression of specific genes can be downregulated by the delivery of small interfering RNA (siRNA) molecules into cells. These short (usually 21 base pair) double-stranded RNAs directly incorporate into the RNA-induced silencing complex (RISC) [69], where the strands are separated by the catalytic protein, argonaute [70]. One strand then guides RISC to the complementary mRNA strands of the target gene, suppressing its expression and translation into the protein product [71].

Although siRNA is a standard laboratory based tool for mRNA regulation, applying this novel approach to targeting transcription factors and other proto-oncogenes has been limited by issues related to the short *in vivo* half life of siRNA, problems associated with delivery to the organs of interest, and uptake into the cytoplasm of target cells. Novel strategies to overcome these issues include the use of liquid nanoparticle delivery systems and the combination of two complementary gene targets. For example, to inhibit angiogenesis in solid tumours, co-targeting of both vascular endothelial growth factor (VGEF) and kinesin spindle protein (KSP) is thought to have greater efficacy. Phase 1 trial data for a joint VEGF and KSP siRNA therapy (ALN-VSP02) recently reported anti-VEGF activity in advanced solid tumours with liver involvement, and is the first study to report a therapeutic anti-tumour response following systemic administration of siRNA therapy [72].

Disease in the liver is a prime target of the siRNA approach because larger molecules (up to 200 nm in diameter) including drug delivery nanocarriers are able to pass readily into the liver from the vasculature [73]. By contrast, in many other tissues, molecules greater than 5 nm diameter do not readily cross the vascular endothelial barrier, implying that liver-related diseases are likely to be a major therapeutic focal area of this type of technology.

An alternative approach, with the potential to improve on the half-life of siRNA, is the use of short hairpin (sh) RNA. This utilizes a double stranded RNA that is expressed in the target cell following insertion of a DNA construct encoding the shRNA of interest, leading to more durable gene silencing [74]. The shRNA transcript is processed by the enzyme Dicer in the cytoplasm, and incorporated into RISC — whereupon the process continues in the same way as for siRNA [71]. Future pre-clinical and clinical trials will attest to the effectiveness of using shRNA-based strategies. Meanwhile, technological advances such as the advent of CRISPR-associated RNA-guided endonuclease Cas9 technology, may make it possible to achieve stable gene silencing or accurate gene editing for cancer therapeutic applications [75].

2.2.3.2. Antisense oligonucleotides. Another approach for targeting transcription factors via gene expression is the use of antisense oligonucleotides. These have two alternative mechanisms of action: oligonucleotide-assisted RNase H-dependent reduction of targeted RNA expression; and the steric-blocker oligonucleotides, which physically prevent or inhibit the progression of splicing or the translational machinery [76].

As an example, AZD9150 (ISIS-STAT3Rx or ISIS 481464), a synthetic antisense oligonucleotide against STAT3, has undergone phase 1 clinical trial in patients with advanced lymphoma and solid tumours [77]. Preliminary findings from fifteen heavily pre-treated patients, composed of six advanced lymphoma cases (three diffuse large B cell lymphoma (DLBCL), two Hodgkin's lymphoma, one mantle cell lymphoma) and nine cases with various solid tumours were recently published. Partial response was observed in two out of three DLBCL patients, both resulting in a greater than 50% reduction in tumour size. No responses were observed in any of the patients with solid tumours. A phase 2 dose-expansion study is currently underway in patients with DLBCL and other advanced lymphomas [78].

2.2.4. Via direct enzymatic regulators

Deregulated cell signalling enzymes that function upstream of transcription factors can lead, ultimately, to many of the classic cancer hallmarks via alteration of transcription factor function and gene expression patterns. Multiple enzyme cascades converge on transcription factors and catalyze the addition and removal of specific post-translational modifications (PTMs). Alteration of PTMs can affect a range of transcription factor activity: for example subcellular localization, protein–protein interactions, and sequence-specific DNA binding [79].

Unlike transcription factors, traditionally, deregulated enzymes have been viewed as eminently druggable. However, targeting the enzymes upstream of transcription factors allows for greater opportunity for cross-talk between enzymatic cascades, and greater risk of acquired drug resistance via escape pathways, *e.g.* as an example of signalling pathways that can lead to drug resistance, upregulation or alterations in the PI3K/AKT/mTOR signalling pathway can contribute to ligand independent ER positive breast cancer [80]. One proven way to promote effectiveness is to specifically target deregulated enzymes that directly alter specific transcription factor PTMs.

Ruxolitinib is a tyrosine kinase inhibitor used in the treatment of patients with myeloproliferative neoplasms (MPNs). It was developed following the discovery of a high frequency of activating Janus kinase-2 (*JAK2*V617F) mutations in cases of MPN that do not harbour a BCR-ABL1 translocation. JAK signalling activates the STAT family of proteins, which includes a number of latent transcription factors that, when phosphorylated on tyrosine residues by the JAKs, drive the expression of genes involved in proliferation, apoptosis, migration and differentiation, as well as the production of angiogenic and inflammatory proteins [81,82].

If the goal is to target a transcription factor in cancer, via modulation of a key upstream regulatory enzyme, it is important to identify the functionally relevant PTMs on the target transcription factor or transcription

factor associated co-factor in order to specifically modulate these via their regulatory enzymes. As an example, FoxA2 is negatively regulated by insulin via Akt-mediated phosphorylation at T156, and FoxA2 can be rendered constitutively active by inducing a genetic mutation at this locus (T156A) [83]. By linking PTMs with transcription factor function, the possibility exists to target a DNA regulatory protein of interest via selective modulation of specific upstream regulatory pathways.

In ER + breast cancer and in prostate cancer, it is well established that DNA interactions and transcriptional potential of the driving transcription factors (ER and AR) are influenced by the pioneer factor FoxA1, which thereby plays an essential role in determining tumour growth and progression [84–87]. The identification of PTMs on FoxA1 that are essential for its function would potentially create an opportunity for indirect inhibition of ER or AR function in breast and prostate cancer, respectively, via modulation of the important associated transcription factor. However, in order to anticipate potential side effects that result from FoxA1 inhibition, it would be necessary to explore the physiological functional redundancy mechanisms that exist between FoxA1 and FoxA2 and the role of FoxA1 in non-target organs such as the liver: notably glucose homeostasis and fat metabolism. Furthermore, the specificity of this approach is limited by how many proteins the putative upstream enzymatic target can regulate, beyond the transcription factor in question.

3. Targeting chromatin regulator proteins

A key issue related to transcription factor activity is the accessibility of chromatin for transcriptional activation or inhibition. Transcription factors tend to bind to DNA at regulatory elements that are open and accessible, implying that the factors that modulate chromatin accessibility may be important factors that assist or inhibit transcription factor activity and may in fact be *bona fide* drug targets themselves.

Substantial work has revealed the importance of chromatin regulatory proteins that have the capacity for modifying histone proteins (around which DNA is wrapped) including enzymatic "writers" and "erasers" of epigenetic marks [88] (see Fig. 2). Other chromatin proteins such as the bromodomain and extra-terminal domain (BET) family are involved in the recognition of histone marks (termed the "readers", Fig. 2), and provide a platform for transcriptional cofactors [89]. In recent years, several inhibitors that target these chromatin regulatory proteins have reached the clinic for the treatment of various haematological malignancies and it is likely that these examples represent a new direction for oncology treatment.

This section summarizes novel concepts in targeting chromatin proteins and outlines the progress being made in translating these into effective cancer treatments.

3.1. Targeting DNA methyltransferases

Cancer cells are characterized by global DNA hypomethylation leading to genomic instability, alongside hypermethylation of a subset of gene promoters contained within CpG dinucleotide islands, which leads to stable transcriptional silencing of tumour suppressors [90].

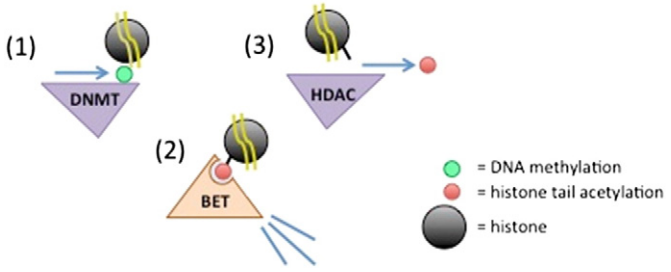

Fig. 2. Chromatin regulator proteins. (1) Writers, (2) readers and (3) erasers of epigenetic marks. *e.g.* DNMT = DNA methyltransferase; BET = bromodomain and extra-terminal; HDAC = histone deacetylase.

DNA methyltransferases DNMT1, DNMT3a and DNMT3b coordinately regulate the methylation state of DNA [91] and therefore represent attractive targets for cancer therapy. Azanucleoside compounds such as 5-azacytidine and decytabine are cytidine analogues that incorporate into DNA and form covalent complexes with DNA methyltransferases, resulting in the depletion of all three active DNMTs [92]. This leads to the reversal of aberrant DNA methylation and the transcriptional reactivation of many genes, including tumour suppressor genes [92–94].

Originally tested as an anti-cancer compound in the 1970s, 5-azacytidine had encouraging clinical efficacy, but was limited by excess gastrointestinal toxicity [95]. In recent years, the utility of 5-azacytidine as an anticancer agent has been re-explored at lower doses [96] — leading to its approval for use in patients with myelodysplastic syndromes (MDS) [97]. Low dose 5-azacytidine has been demonstrated to increase the time to conversion of MDS to acute myeloid leukaemia (AML), improve quality of life in MDS patients and has become the first therapy to improve survival of patients with MDS compared to conventional therapy such as cytarabine [98,99].

3.2. Targeting histone modifications

Enzymes that regulate transcription factor function by writing or erasing epigenetic marks on chromatin proteins include histone acetylases and deacetylases (HATs and HDACs), and histone methyltransferases (HMTs) and demethylases (*e.g.* lysine demethylase, KDM).

HDACs "erase" lysine acetylation on histone tails, repressing transcriptional activation by promoting a shift towards a more condensed chromatin state [100,101]. Therapeutic targeting of HDAC enzymes can reverse the transcriptional repression of genes that promote apoptosis and cell differentiation, while inhibiting cell cycle progression and therefore cell division [102 (Fig. 3). Two HDAC inhibitors have demonstrated clinical utility in treating cancer, namely romidepsin and vorinostat, both of which have been approved for the treatment of cutaneous T-cell lymphoma [103,104].

The mechanism of action of HDAC inhibitors is the subject of much debate. The overall anti-tumour activity of HDAC inhibitors may be due to modulation of a range of molecular processes beyond transcription, via regulation of acetylation status
of both histone and non-histone proteins — leading to a diverse effect on cellular physiology [100]. Proposed mechanisms of HDAC inhibitor activity include induction of apoptosis via altered transcription of proteins involved in the intrinsic and extrinsic pathways and induction of proteosomal degradation [105]. With improving knowledge of the pleiotropic anticancer effects of HDAC inhibitors, rational combination therapies with, for example, proteasome inhibitors [106] are being explored in order to expand their role to treat a broader range of haematological and solid malignancies.

Chromatin proteins that "read" epigenetic modifications on histones include the BET (Bromodomain and Extra-Terminal) family. These recognize polyacetylated lysine residues of histone tails, providing a framework for transcriptional effector complexes, and promoting transcriptional elongation. Translocation of the BET protein BRD4 has been shown to be a driver event in an incurable subtype of paediatric squamous cell carcinoma [107,108], whilst in mixed-lineage leukaemia (MLL)-fusion leukaemia, translocation partners including BRD4 are often members of transcriptional elongation complexes [109]. Compounds that modify the function of BRD4 as a critical transcriptional regulator have therefore been sought. The experimental compound JQ1 has been established as a potent and selective inhibitor of the bromodomain protein BRD4, with its antitumour effect in these settings linked to reduction in c-Myc overactivity [110]. The bromodomain family inhibitor compound I-BET151 has demonstrated *in vivo* preclinical efficacy in murine models of MLL-fusion leukaemia, with parallel

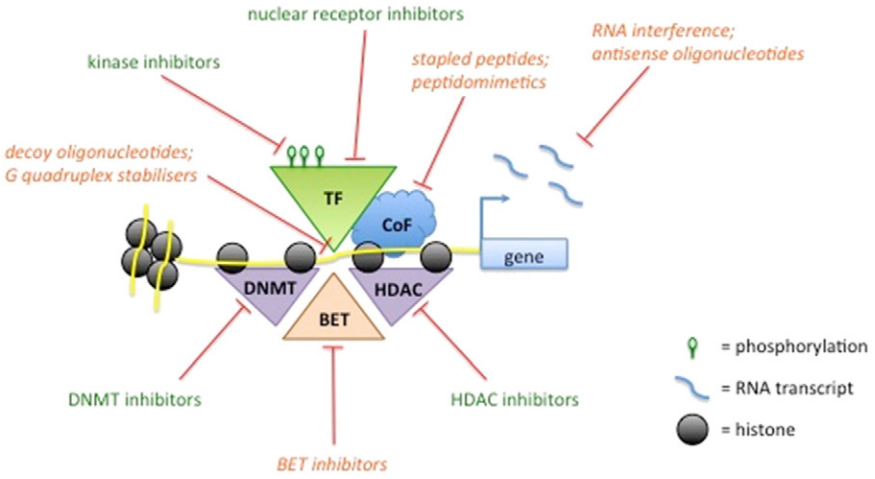

Fig. 3. Targeting transcription factor function: a mechanistic summary. TF = transcription factor; CoF = co-factor; HDAC = histone deacetylase; BET = bromodomain and extra-terminal; DNMT = DNA methyltransferase. Red T-bars indicate examples of inhibitory mechanisms, with compounds approved for clinical use/in clinical trial (highlighted in green/orange respectively).

experiments in cell lines demonstrating that I-BET151 causes displacement of bromodomain proteins BRD3, BRD4 and SEC from the chromatin, leading to transcriptional repression of key oncogenes such as BCL-2 and c-Myc [108].

Another therapeutic target in MLL-fusion leukaemia is the histone methyltransferase DOT1L, which is recruited by MLL-fusion proteins to drive the transcription of leukemogenic target genes via hypermethylation at H3K79. The small molecule DOT1L inhibitor, EPZ-5676, is currently in early clinical trial in this context [111].

Other chromatin protein targets in cancer include those that "write" epigenetic marks on histones such as the HMT, Enhancer of Zeste Homolog 2 (EZH2), which has an activating mutation in DLBCL [112] and is overexpressed in melanoma, breast and prostate cancer [113]. EZH2 has been targeted using small molecules and peptide approaches [114,115]. Histone demethylase targets include lysine-specific demethylase-1 (KDM1), which is overexpressed in neuroblastoma, colon, breast and prostate cancer [116] and has been targeted using small molecules and monoamine oxidase inhibitors [117,118].

Whilst there remain issues relating to the specificity of drugs targeting epigenetic modifiers of DNA and chromatin proteins, such compounds have gained a prominent role in the treatment of haematological cancers and their role is likely to expand to solid tumours, potentially in combination with the standard of care, or as second line treatment in the resistant context.

4. Future directions

One important variable that requires exploration is the importance of changes in protein sequence and function that occurs in cancer. It is becoming well known that some of the most commonly mutated genes in cancer are transcription factors and chromatin regulatory proteins [65,119–121]. This increasing body of mutational information has helped pin-point crucial transcription factors within a specific cancer of interest, but also reveals additional parameters that must be considered if attempting to target transcription factors. As an example, similar to what is observed for growth factors, mutant transcription factors may be inert to drugs that modulate activity of the wild type version, an important consideration when aiming to perturb a single driving transcription factor.

As previously mentioned, ER transcriptional activity can be effectively blocked with endocrine agents, but recent observations have shown that ER is commonly mutated in the metastatic context [122–124], with an enrichment for cells that have acquired mutations in the ligand binding domain, altering the efficacy of current treatments. Again, an understanding of these possible changes and events is of paramount importance for maximizing our ability to effectively inhibit cancer cell growth and tumour progression by targeting these specific types of proteins. Equally an understanding of these mutations may allow targeting of the mutant form of the protein only, thus improving the efficacy of the drug and restricting non-specific effects that result from inhibition of the wild type version of the protein.

However, whilst the traditional paradigm for a good therapeutic protein target is that it should be mutated or over-expressed in a cancer, recent examples highlight notable exceptions (*e.g.* BRD4 and HDACs), which are often discovered via unbiased screens of compound libraries. This highlights the variety of available mechanisms by which critical regulators of transcription factor and chromatin protein targets can be manipulated for therapeutic advantage, and the importance of ongoing study into the complex nature of transcriptional regulation.

5. Summary and conclusions

Transcription factors, their associated proteins and the variables that dictate chromatin accessibility have traditionally been thought of as intractable drug targets. However they constitute the downstream machinery that signalling pathways feed into, to ultimately drive the gene expression programmes that influence tumour formation, cancer progression and drug response. A step change is required to view the transcription factors as genuine therapeutic targets. By inhibiting the downstream transcription factors, redundancy mechanisms that exist within complex signalling pathways can be minimized, potentially improving the potency of targeted therapeutic approaches. Despite the fact that transcription factors are commonly considered undruggable, some of the most commonly utilized therapies (for both oncological diseases and other conditions) target a class of transcription factors called nuclear receptors. Nuclear receptors may be unusually amenable to therapeutic intervention because of the existence of a ligand binding pocket [125,126], but they epitomize the effectiveness and durability of successfully targeting transcription factors to improve disease outcome.

There have been numerous recent advances that involve targeting of transcription factors, either directly or indirectly (Fig. 3). Some of these have been summarized in this review and include direct inhibition with small molecules, modulation of the DNA targets, destabilization of the mRNA precursors of transcription factors and inhibition of upstream regulatory pathways that feed into the structure and function of transcription factors. Some of these have entered clinical trials, attesting to the potential impact of the diverse approaches explored. Numerous years ago, kinases were considered difficult targets [127,128] yet they

now constitute some of the most effective and malleable drug targets in cancer.

It is hoped that in years to come, momentum and confidence will be gained around the concept of targeting driving transcription factor pathways for cancer treatment. This will require better understanding of the key transcription factors in cancer, the post-translational modifications that influence the function of these transcription factors, the redundancy that exists between family members of specific transcription factors and the potential effects of modifying transcription factor function on their wider physiological roles. Through continued effort and scientific progress, the enormous untapped potential for improving the lives of cancer patients using treatments that target transcription factors and their associated factors and pathways will become reality.

Transparency document

The Transparency document associated with this article can be found, in the online version.

Acknowledgements

We thank Kelly Holmes, Aisling Redmond, Adam Nelson and Sarah Welsh for reading the manuscript. We would like to acknowledge the support of the University of Cambridge, Cancer Research UK and Hutchison Whampoa Limited.

References

[1] I. Carrera, J.E. Treisman, Message in a nucleus: signaling to the transcriptional machinery, Curr. Opin. Genet. Dev. 18 (5) (2008) 397–403.
[2] D. Hanahan, R.A. Weinberg, The hallmarks of cancer, Cell 100 (1) (2000) 57–70.
[3] J.E. Darnell Jr., Transcription factors as targets for cancer therapy, Nat. Rev. Cancer 2 (10) (2002) 740–749.
[4] S. Greenstein, K. Ghias, N.L. Krett, S.T. Rosen, Mechanisms of glucocorticoid-mediated apoptosis in hematological malignancies, Clin. Cancer Res. 8 (6) (2002) 1681–1694.
[5] S.V. Rajkumar, Treatment of multiple myeloma, Nat. Rev. Clin. Oncol. 8 (8) (2011) 479–491.
[6] D. Hanahan, R.A. Weinberg, Hallmarks of cancer: the next generation, Cell 144 (5) (2011) 646–674.
[7] A.N. Koehler, A complex task? Direct modulation of transcription factors with small molecules, Curr. Opin. Chem. Biol. 14 (3) (2010) 331–340.
[8] D.C. Allred, P. Brown, D. Medina, The origins of estrogen receptor alpha-positive and estrogen receptor alpha-negative human breast cancer, Breast Cancer Res. 6 (6) (2004) 240–245.
[9] B. Thurlimann, A. Keshaviah, A.S. Coates, et al., A comparison of letrozole and tamoxifen in postmenopausal women with early breast cancer, N. Engl. J. Med. 353 (26) (2005) 2747–2757.
[10] J. Cuzick, I. Sestak, M. Baum, et al., Effect of anastrozole and tamoxifen as adjuvant treatment for early-stage breast cancer: 10-year analysis of the ATAC trial, Lancet Oncol. 11 (12) (2010) 1135–1141.
[11] V.C. Jordan, Tamoxifen: a most unlikely pioneering medicine, Nat. Rev. Drug Discov. 2 (3) (2003) 205–213.
[12] S.J. Johnston, K.L. Cheung, Fulvestrant — a novel endocrine therapy for breast cancer, Curr. Med. Chem. 17 (10) (2010) 902–914.
[13] R.J. Santen, Clinical review 37: endocrine treatment of prostate cancer, J. Clin. Endocrinol. Metab. 75 (3) (1992) 685–689.
[14] G. Attard, J. Richards, J.S. De Bono, New strategies in metastatic prostate cancer: targeting the androgen receptor signaling pathway, Clin. Cancer Res. 17 (7) (2011) 1649–1657.
[15] H.I. Scher, K. Fizazi, F. Saad, et al., Increased survival with enzalutamide in prostate cancer after chemotherapy, N. Engl. J. Med. 367 (13) (2012) 1187–1197.
[16] N.S. Hegde, D.A. Sanders, R. Rodriguez, S. Balasubramanian, The transcription factor FOXM1 is a cellular target of the natural product thiostrepton, Nat. Chem. 3 (9) (2011) 725–731.
[17] S.S. Myatt, E.W. Lam, The emerging roles of forkhead box (Fox) proteins in cancer, Nat. Rev. Cancer 7 (11) (2007) 847–859.
[18] M.R. Arkin, J.A. Wells, Small-molecule inhibitors of protein–protein interactions: progressing towards the dream, Nat. Rev. Drug Discov. 3 (4) (2004) 301–317.
[19] L.T. Vassilev, B.T. Vu, B. Graves, et al., In vivo activation of the p53 pathway by small-molecule antagonists of MDM2, Science 303 (5659) (2004) 844–848.
[20] C. Klein, L.T. Vassilev, Targeting the p53–MDM2 interaction to treat cancer, Br. J. Cancer 91 (8) (2004) 1415–1419.
[21] C.J. Brown, S. Lain, C.S. Verma, A.R. Fersht, D.P. Lane, Awakening guardian angels: drugging the p53 pathway, Nat. Rev. Cancer 9 (12) (2009) 862–873.
[22] C. Tovar, B. Graves, K. Packman, et al., MDM2 small-molecule antagonist RG7112 activates p53 signaling and regresses human tumors in preclinical cancer models, Cancer Res. 73 (8) (2013) 2587–2597.
[23] I. Ray-Coquard, J.Y. Blay, A. Italiano, et al., Effect of the MDM2 antagonist RG7112 on the P53 pathway in patients with MDM2-amplified, well-differentiated or dedifferentiated liposarcoma: an exploratory proof-of-mechanism study, Lancet Oncol. 13 (11) (2012) 1133–1140.
[24] M. Andreeff, K.R. Kelly, K. Yee, et al., Results of the phase 1 trial of RG7112, a small-molecule MDM2 antagonist, in acute leukemia, 54th Annual Meeting of the American Society of Hematology. Georgia, Atlanta, 2012.
[25] A.M. Redmond, J.S. Carroll, Defining and targeting transcription factors in cancer, Genome Biol. 10 (7) (2009) 311.
[26] Y. Zhao, S. Yu, W. Sun, et al., A potent small-molecule inhibitor of the MDM2–p53 interaction (MI-888) achieved complete and durable tumor regression in mice, J. Med. Chem. 56 (13) (2013) 5553–5561.
[27] Y. Zhao, A. Aguilar, D. Bernard, S. Wang, Small-molecule inhibitors of the MDM2–p53 protein–protein interaction (MDM2 inhibitors) in clinical trials for cancer treatment, J. Med. Chem. 58 (3) (2015) 1038–1052.
[28] Y. Zhao, D. Bernard, S. Wang, Small molecule inhibitors of MDM2–p53 and MDMX–p53 interactions as new cancer therapeutics, Biodiscovery 8 (4) (2013) 1–15.
[29] M. Wade, Y.C. Li, G.M. Wahl, MDM2, MDMX and p53 in oncogenesis and cancer therapy, Nat. Rev. Cancer 13 (2) (2013) 83–96.
[30] A.C. Joerger, H.C. Ang, A.R. Fersht, Structural basis for understanding oncogenic p53 mutations and designing rescue drugs, Proc. Natl. Acad. Sci. U. S. A. 103 (41) (2006) 15056–15061.
[31] F.M. Boeckler, A.C. Joerger, G. Jaggi, T.J. Rutherford, D.B. Veprintsev, A.R. Fersht, Targeted rescue of a destabilized mutant of p53 by an in silico screened drug, Proc. Natl. Acad. Sci. U. S. A. 105 (30) (2008) 10360–10365.
[32] F. Essmann, K. Schulze-Osthoff, Translational approaches targeting the p53 pathway for anti-cancer therapy, Br. J. Pharmacol. 165 (2) (2012) 328–344.
[33] G.L. Verdine, G.J. Hilinski, Stapled peptides for intracellular drug targets, Methods Enzymol. 503 (2012) 3–33.
[34] L.D. Walensky, A.L. Kung, I. Escher, et al., Activation of apoptosis in vivo by a hydrocarbon-stapled BH3 helix, Science 305 (5689) (2004) 1466–1470.
[35] R.E. Moellering, M. Cornejo, T.N. Davis, et al., Direct inhibition of the NOTCH transcription factor complex, Nature 462 (7270) (2009) 182–188.
[36] F. Bernal, M. Wade, M. Godes, et al., A stapled p53 helix overcomes HDMX-mediated suppression of p53, Cancer Cell 18 (5) (2010) 411–422.
[37] T. Okamoto, K. Zobel, A. Fedorova, et al., Stabilizing the pro-apoptotic BimBH3 helix (BimSAHB) does not necessarily enhance affinity or biological activity, ACS Chem. Biol. 8 (2) (2013) 297–302.
[38] H.M. Lamb, J.M. Hardwick, Unlatched BAX pairs for death, Cell 152 (3) (2013) 383–384.
[39] Y.S. Chang, B. Graves, V. Guerlavais, et al., Stapled alpha-helical peptide drug development: a potent dual inhibitor of MDM2 and MDMX for p53-dependent cancer therapy, Proc. Natl. Acad. Sci. U. S. A. 110 (36) (2013) E3445–E3454.
[40] J.M. Ahn, N.A. Boyle, M.T. Macdonald, K.D. Janda, Peptidomimetics and peptide backbone modifications, Mini Rev. Med. Chem. 2 (5) (2002) 463–473.
[41] P. Ravindranathan, T.K. Lee, L. Yang, et al., Peptidomimetic targeting of critical androgen receptor–coregulator interactions in prostate cancer, Nat. Commun. 4 (2013) 1923.
[42] M. Furqan, A. Akinleye, N. Mukhi, V. Mittal, Y. Chen, D. Liu, STAT inhibitors for cancer therapy, J. Hematol. Oncol. 6 (2013) 90.
[43] R. Garcia, C.L. Yu, A. Hudnall, et al., Constitutive activation of Stat3 in fibroblasts transformed by diverse oncoproteins and in breast carcinoma cells, Cell Growth Differ. 8 (12) (1997) 1267–1276.
[44] R. Garcia, T.L. Bowman, G. Niu, et al., Constitutive activation of Stat3 by the Src and JAK tyrosine kinases participates in growth regulation of human breast carcinoma cells, Oncogene 20 (20) (2001) 2499–2513.
[45] T.N. Dechow, L. Pedranzini, A. Leitch, et al., Requirement of matrix metalloproteinase-9 for the transformation of human mammary epithelial cells by Stat3-C, Proc. Natl. Acad. Sci. U. S. A. 101 (29) (2004) 10602–10607.
[46] J.F. Bromberg, M.H. Wrzeszczynska, G. Devgan, et al., Stat3 as an oncogene, Cell 98 (3) (1999) 295–303.
[47] J. Chen, L. Bai, D. Bernard, et al., Structure-based design of conformationally constrained, cell-permeable STAT3 inhibitors, ACS Med. Chem. Lett. 1 (2) (2010) 85–89.
[48] T.R. Geistlinger, R.K. Guy, Novel selective inhibitors of the interaction of individual nuclear hormone receptors with a mutually shared steroid receptor coactivator 2, J. Am. Chem. Soc. 125 (23) (2003) 6852–6853.
[49] C. Tse, A.R. Shoemaker, J. Adickes, et al., ABT-263: a potent and orally bioavailable Bcl-2 family inhibitor, Cancer Res. 68 (9) (2008) 3421–3428.
[50] A.J. Souers, J.D. Leverson, E.R. Boghaert, et al., ABT-199, a potent and selective BCL-2 inhibitor, achieves antitumor activity while sparing platelets, Nat. Med. 19 (2) (2013) 202–208.
[51] L. Gandhi, D.R. Camidge, M. Ribeiro De Oliveira, et al., Phase I study of navitoclax (ABT-263), a novel Bcl-2 family inhibitor, in patients with small-cell lung cancer and other solid tumors, J. Clin. Oncol. 29 (7) (2011) 909–916.
[52] A. Bielinska, R.A. Shivdasani, L.Q. Zhang, G.J. Nabel, Regulation of gene expression with double-stranded phosphorothioate oligonucleotides, Science 250 (4983) (1990) 997–1000.
[53] R. Morishita, G.H. Gibbons, M. Horiuchi, et al., A gene therapy strategy using a transcription factor decoy of the E2F binding site inhibits smooth muscle proliferation in vivo, Proc. Natl. Acad. Sci. U. S. A. 92 (13) (1995) 5855–5859.
[54] R. Crinelli, M. Bianchi, L. Gentilini, M. Magnani, Design and characterization of decoy oligonucleotides containing locked nucleic acids, Nucleic Acids Res. 30 (11) (2002) 2435–2443.

[55] M. Sen, S.M. Thomas, S. Kim, et al., First-in-human trial of a STAT3 decoy oligonucleotide in head and neck tumors: implications for cancer therapy, Cancer Discov. 2 (8) (2012) 694–705.
[56] M.L. Bochman, K. Paeschke, V.A. Zakian, DNA secondary structures: stability and function of G-quadruplex structures, Nat. Rev. Genet. 13 (11) (2012) 770–780.
[57] J. Eddy, N. Maizels, Gene function correlates with potential for G4 DNA formation in the human genome, Nucleic Acids Res. 34 (14) (2006) 3887–3896.
[58] A. Verma, K. Halder, R. Halder, et al., Genome-wide computational and expression analyses reveal G-quadruplex DNA motifs as conserved cis-regulatory elements in human and related species, J. Med. Chem. 51 (18) (2008) 5641–5649.
[59] S. Balasubramanian, L.H. Hurley, S. Neidle, Targeting G-quadruplexes in gene promoters: a novel anticancer strategy? Nat. Rev. Drug Discov. 10 (4) (2011) 261–275.
[60] J. Eddy, N. Maizels, Conserved elements with potential to form polymorphic G-quadruplex structures in the first intron of human genes, Nucleic Acids Res. 36 (4) (2008) 1321–1333.
[61] A.K. Todd, S. Neidle, The relationship of potential G-quadruplex sequences in cis-upstream regions of the human genome to SP1-binding elements, Nucleic Acids Res. 36 (8) (2008) 2700–2704.
[62] C.E. Nesbit, J.M. Tersak, E.V. Prochownik, MYC oncogenes and human neoplastic disease, Oncogene 18 (19) (1999) 3004–3016.
[63] N. Meyer, L.Z. Penn, Reflecting on 25 years with MYC, Nat. Rev. Cancer 8 (12) (2008) 976–990.
[64] C.V. Dang, MYC on the path to cancer, Cell 149 (1) (2012) 22–35.
[65] A. Siddiqui-Jain, C.L. Grand, D.J. Bearss, L.H. Hurley, Direct evidence for a G-quadruplex in a promoter region and its targeting with a small molecule to repress c-MYC transcription, Proc. Natl. Acad. Sci. U. S. A. 99 (18) (2002) 11593–11598.
[66] D. Sun, L.H. Hurley, The importance of negative superhelicity in inducing the formation of G-quadruplex and i-motif structures in the c-Myc promoter: implications for drug targeting and control of gene expression, J. Med. Chem. 52 (9) (2009) 2863–2874.
[67] S.J. Welsh, A.G. Dale, C.M. Lombardo, et al., Inhibition of the hypoxia-inducible factor pathway by a G-quadruplex binding small molecule, Sci. Rep. 3 (2013) 2799.
[68] H. Sun, J. Xiang, Y. Shi, et al., A newly identified G-quadruplex as a potential target regulating Bcl-2 expression, Biochim. Biophys. Acta 1840 (10) (2014) 3052–3057.
[69] T.A. Rand, K. Ginalski, N.V. Grishin, X. Wang, Biochemical identification of Argonaute 2 as the sole protein required for RNA-induced silencing complex activity, Proc. Natl. Acad. Sci. U. S. A. 101 (40) (2004) 14385–14389.
[70] C. Matranga, Y. Tomari, C. Shin, D.P. Bartel, P.D. Zamore, Passenger-strand cleavage facilitates assembly of siRNA into Ago2-containing RNAi enzyme complexes, Cell 123 (4) (2005) 607–620.
[71] Y. Deng, C.C. Wang, K.W. Choy, et al., Therapeutic potentials of gene silencing by RNA interference: principles, challenges, and new strategies, Gene 538 (2) (2014) 217–227.
[72] J. Tabernero, G.I. Shapiro, P.M. Lorusso, et al., First-in-humans trial of an RNA interference therapeutic targeting VEGF and KSP in cancer patients with liver involvement, Cancer Discov. 3 (4) (2013) 406–417.
[73] K.A. Whitehead, R. Langer, D.G. Anderson, Knocking down barriers: advances in siRNA delivery, Nat. Rev. Drug Discov. 8 (2) (2009) 129–138.
[74] D.D. Rao, J.S. Vorhies, N. Senzer, J. Nemunaitis, siRNA vs. shRNA: similarities and differences, Adv. Drug Deliv. Rev. 61 (9) (2009) 746–759.
[75] P.D. Hsu, E.S. Lander, F. Zhang, Development and applications of CRISPR-Cas9 for genome engineering, Cell 157 (6) (2014) 1262–1278.
[76] N. Dias, C.A. Stein, Antisense oligonucleotides: basic concepts and mechanisms, Mol. Cancer Ther. 1 (5) (2002) 347–355.
[77] D.S. Hong, A. Younes, L. Fayad, et al., A phase I study of ISIS 481464 (AZD9150), a first-in-human, first-in-class, antisense oligonucleotide inhibitor of STAT3, in patients with advanced cancers, J. Clin. Oncol. 31 (2013) (supplement, abstract 8523).
[78] NCT01563302, Phase 1/2, open-label, dose-escalation study of ISIS-STAT3Rx, administered to patients with advanced cancers, ClinicalTrials.gov2012.
[79] T.M. Filtz, W.K. Vogel, M. Leid, Regulation of transcription factor activity by interconnected post-translational modifications, Trends Pharmacol. Sci. 35 (2) (2014) 76–85.
[80] Gil E.M. Ciruelos, Targeting the PI3K/AKT/mTOR pathway in estrogen receptor-positive breast cancer, Cancer Treat. Rev. 40 (7) (2014) 862–871.
[81] K. Shuai, B. Liu, Regulation of JAK-STAT signalling in the immune system, Nat. Rev. Immunol. 3 (11) (2003) 900–911.
[82] J.S. Fridman, P.A. Scherle, R. Collins, et al., Preclinical evaluation of local JAK1 and JAK2 inhibition in cutaneous inflammation, J. Investig. Dermatol. 131 (9) (2011) 1838–1844.
[83] C. Wolfrum, D. Besser, E. Luca, M. Stoffel, Insulin regulates the activity of forkhead transcription factor Hnf-3beta/Foxa-2 by Akt-mediated phosphorylation and nuclear/cytosolic localization, Proc. Natl. Acad. Sci. U. S. A. 100 (20) (2003) 11624–11629.
[84] J.S. Carroll, X.S. Liu, A.S. Brodsky, et al., Chromosome-wide mapping of estrogen receptor binding reveals long-range regulation requiring the forkhead protein FoxA1, Cell 122 (1) (2005) 33–43.
[85] A. Hurtado, K.A. Holmes, C.S. Ross-Innes, D. Schmidt, J.S. Carroll, FOXA1 is a key determinant of estrogen receptor function and endocrine response, Nat. Genet. 43 (1) (2011) 27–33.
[86] Y. Imamura, S. Sakamoto, T. Endo, et al., FOXA1 promotes tumor progression in prostate cancer via the insulin-like growth factor binding protein 3 pathway, PLoS One 7 (8) (2012) e42456.
[87] C. Zhang, L. Wang, D. Wu, et al., Definition of a FoxA1 cistrome that is crucial for G1 to S-phase cell-cycle transit in castration-resistant prostate cancer, Cancer Res. 71 (21) (2011) 6738–6748.
[88] A. Tarakhovsky, Tools and landscapes of epigenetics, Nat. Immunol. 11 (7) (2010) 565–568.
[89] D. Gallenkamp, K.A. Gelato, B. Haendler, H. Weinmann, Bromodomains and their pharmacological inhibitors, ChemMedChem 9 (3) (2014) 438–464.
[90] S.B. Baylin, P.A. Jones, A decade of exploring the cancer epigenome — biological and translational implications, Nat. Rev. Cancer 11 (10) (2011) 726–734.
[91] P.A. Jones, G. Liang, Rethinking how DNA methylation patterns are maintained, Nat. Rev. Genet. 10 (11) (2009) 805–811.
[92] A. Quintas-Cardama, F.P. Santos, G. Garcia-Manero, Therapy with azanucleosides for myelodysplastic syndromes, Nat. Rev. Clin. Oncol. 7 (8) (2010) 433–444.
[93] V.L. Wilson, P.A. Jones, R.L. Momparler, Inhibition of DNA methylation in L1210 leukemic cells by 5-aza-2′-deoxycytidine as a possible mechanism of chemotherapeutic action, Cancer Res. 43 (8) (1983) 3493–3496.
[94] R.L. Momparler, J. Bouchard, N. Onetto, G.E. Rivard, 5-aza-2′-deoxycytidine therapy in patients with acute leukemia inhibits DNA methylation, Leuk. Res. 8 (2) (1984) 181–185.
[95] D.D. Von Hoff, M. Slavik, 5-azacytidine—a new anticancer drug with significant activity in acute myeloblastic leukemia, Adv. Pharmacol. Chemother. 14 (1977) 285–326.
[96] H.C. Tsai, H. Li, L. Van Neste, et al., Transient low doses of DNA-demethylating agents exert durable antitumor effects on hematological and epithelial tumor cells, Cancer Cell 21 (3) (2012) 430–446.
[97] L.R. Silverman, E.P. Demakos, B.L. Peterson, et al., Randomized controlled trial of azacitidine in patients with the myelodysplastic syndrome: a study of the cancer and leukemia group B, J. Clin. Oncol. 20 (10) (2002) 2429–2440.
[98] P. Fenaux, L. Ades, Review of azacitidine trials in Intermediate-2-and high-risk myelodysplastic syndromes, Leuk. Res. 33 (Suppl. 2) (2009) S7–S11.
[99] A.B. Kornblith, J.E. Herndon II, L.R. Silverman, et al., Impact of azacytidine on the quality of life of patients with myelodysplastic syndrome treated in a randomized phase III trial: a Cancer and Leukemia Group B study, J. Clin. Oncol. 20 (10) (2002) 2441–2452.
[100] J.E. Bolden, M.J. Peart, R.W. Johnstone, Anticancer activities of histone deacetylase inhibitors, Nat. Rev. Drug Discov. 5 (9) (2006) 769–784.
[101] O.A. O'connor, M.L. Heaney, L. Schwartz, et al., Clinical experience with intravenous and oral formulations of the novel histone deacetylase inhibitor suberoylanilide hydroxamic acid in patients with advanced hematologic malignancies, J. Clin. Oncol. 24 (1) (2006) 166–173.
[102] S. Minucci, P.G. Pelicci, Histone deacetylase inhibitors and the promise of epigenetic (and more) treatments for cancer, Nat. Rev. Cancer 6 (1) (2006) 38–51.
[103] S.J. Whittaker, M.F. Demierre, E.J. Kim, et al., Final results from a multicenter, international, pivotal study of romidepsin in refractory cutaneous T-cell lymphoma, J. Clin. Oncol. 28 (29) (2010) 4485–4491.
[104] E.A. Olsen, Y.H. Kim, T.M. Kuzel, et al., Phase IIb multicenter trial of vorinostat in patients with persistent, progressive, or treatment refractory cutaneous T-cell lymphoma, J. Clin. Oncol. 25 (21) (2007) 3109–3115.
[105] M. Dickinson, R.W. Johnstone, H.M. Prince, Histone deacetylase inhibitors: potential targets responsible for their anti-cancer effect, Investig. New Drugs 28 (Suppl. 1) (2010) S3–S20.
[106] M. Rahmani, E. Reese, Y. Dai, et al., Cotreatment with suberanoylanilide hydroxamic acid and 17-allylamino 17-demethoxygeldanamycin synergistically induces apoptosis in Bcr-Abl+ Cells sensitive and resistant to STI571 (imatinib mesylate) in association with down-regulation of Bcr-Abl, abrogation of signal transducer and activator of transcription 5 activity, and Bax conformational change, Mol. Pharmacol. 67 (4) (2005) 1166–1176.
[107] J. Zuber, J. Shi, E. Wang, et al., RNAi screen identifies Brd4 as a therapeutic target in acute myeloid leukaemia, Nature 478 (7370) (2011) 524–528.
[108] M.A. Dawson, R.K. Prinjha, A. Dittmann, et al., Inhibition of BET recruitment to chromatin as an effective treatment for MLL-fusion leukaemia, Nature 478 (7370) (2011) 529–533.
[109] C. Lin, E.R. Smith, H. Takahashi, et al., AFF4, a component of the ELL/P-TEFb elongation complex and a shared subunit of MLL chimeras, can link transcription elongation to leukemia, Mol. Cell 37 (3) (2010) 429–437.
[110] J.A. Mertz, A.R. Conery, B.M. Bryant, et al., Targeting MYC dependence in cancer by inhibiting BET bromodomains, Proc. Natl. Acad. Sci. U. S. A. 108 (40) (2011) 16669–16674.
[111] S.R. Daigle, E.J. Olhava, C.A. Therkelsen, et al., Potent inhibition of DOT1L as treatment of MLL-fusion leukemia, Blood 122 (6) (2013) 1017–1025.
[112] M.T. Mccabe, H.M. Ott, G. Ganji, et al., EZH2 inhibition as a therapeutic strategy for lymphoma with EZH2-activating mutations, Nature 492 (7427) (2012) 108–112.
[113] S. Varambally, Q. Cao, R.S. Mani, et al., Genomic loss of microRNA-101 leads to overexpression of histone methyltransferase EZH2 in cancer, Science 322 (5908) (2008) 1695–1699.
[114] S.K. Knutson, S. Kawano, Y. Minoshima, et al., Selective inhibition of EZH2 by EPZ-6438 leads to potent antitumor activity in EZH2-mutant non-Hodgkin lymphoma, Mol. Cancer Ther. 13 (4) (2014) 842–854.
[115] W. Kim, G.H. Bird, T. Neff, et al., Targeted disruption of the EZH2–EED complex inhibits EZH2-dependent cancer, Nat. Chem. Biol. 9 (10) (2013) 643–650.
[116] G.R. Sareddy, B.C. Nair, S.K. Krishnan, et al., KDM1 is a novel therapeutic target for the treatment of gliomas, Oncotarget 4 (1) (2013) 18–28.
[117] R. Ueda, T. Suzuki, K. Mino, et al., Identification of cell-active lysine specific demethylase 1-selective inhibitors, J. Am. Chem. Soc. 131 (48) (2009) 17536–17537.
[118] V. Cortez, M. Mann, S. Tekmal, et al., Targeting the PELP1–KDM1 axis as a potential therapeutic strategy for breast cancer, Breast Cancer Res. 14 (4) (2012) R108.
[119] A. Balakrishnan, F.E. Bleeker, S. Lamba, et al., Novel somatic and germline mutations in cancer candidate genes in glioblastoma, melanoma, and pancreatic carcinoma, Cancer Res. 67 (8) (2007) 3545–3550.

[120] M. Muraoka, M. Konishi, R. Kikuchi-Yanoshita, et al., p300 gene alterations in colorectal and gastric carcinomas, Oncogene 12 (7) (1996) 1565–1569.
[121] G. Van Haaften, G.L. Dalgliesh, H. Davies, et al., Somatic mutations of the histone H3K27 demethylase gene UTX in human cancer, Nat. Genet. 41 (5) (2009) 521–523.
[122] W. Toy, Y. Shen, H. Won, et al., ESR1 ligand-binding domain mutations in hormone-resistant breast cancer, Nat. Genet. 45 (12) (2013) 1439–1445.
[123] D.R. Robinson, Y.M. Wu, P. Vats, et al., Activating ESR1 mutations in hormone-resistant metastatic breast cancer, Nat. Genet. 45 (12) (2013) 1446–1451.
[124] K. Merenbakh-Lamin, N. Ben-Baruch, A. Yeheskel, et al., D538G mutation in estrogen receptor-alpha: a novel mechanism for acquired endocrine resistance in breast cancer, Cancer Res. 73 (23) (2013) 6856–6864.
[125] D. Moras, H. Gronemeyer, The nuclear receptor ligand-binding domain: structure and function, Curr. Opin. Cell Biol. 10 (3) (1998) 384–391.
[126] P. Huang, V. Chandra, F. Rastinejad, Structural overview of the nuclear receptor superfamily: insights into physiology and therapeutics, Annu. Rev. Physiol. 72 (2010) 247–272.
[127] T.R. Tritton, J.A. Hickman, How to kill cancer cells: membranes and cell signaling as targets in cancer chemotherapy, Cancer Cells 2 (4) (1990) 95–105.
[128] G. Powis, Signalling targets for anticancer drug development, Trends Pharmacol. Sci. 12 (5) (1991) 188–194.