Biochemical Pharmacology 107 (2016) 1–13

Contents lists available at ScienceDirect

Biochemical Pharmacology

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

Research update

Targeting transcription factors by small compounds—Current strategies and future implications

Judith Hagenbuchner[b], Michael J. Ausserlechner[a,*]

[a] Department of Pediatrics I, Medical University Innsbruck, Innrain 66, A-6020 Innsbruck, Austria
[b] Department of Pediatrics II, Medical University Innsbruck, Innrain 66, A-6020 Innsbruck, Austria

ARTICLE INFO

Article history:
Received 18 September 2015
Accepted 8 December 2015
Available online 11 December 2015

Keywords:
Drug targeting
Cancer therapy
BET proteins
p53
HIF1
MYC
FOXM1

ABSTRACT

Transcription factors are central regulators of gene expression and critically steer development, differentiation and death. Except for ligand-activated nuclear receptors, direct modulation of transcription factor function by small molecules is still widely regarded as "impossible".

This "un-druggability" of non-ligand transcription factors is due to the fact that the interacting surface between transcription factor and DNA is huge and subject to significant changes during DNA-binding. Besides some "success studies" with compounds that directly interfere with DNA binding, drug targeting approaches mostly address protein–protein interfaces with essential co-factors, transcription factor dimerization partners, chaperone proteins or proteins that regulate subcellular shuttling. An alternative strategy represent DNA-intercalating, alkylating or DNA-groove-binding compounds that either block transcription factor-binding or change the 3D-conformation of the consensus DNA-strand. Recently, much interest has been focused on chromatin reader proteins that steer the recruitment and activity of transcription factors to a gene transcription start site. Several small compounds demonstrate that these epigenetic reader proteins are exciting new drug targets for inhibiting lineage-specific transcription in cancer therapy.

In this research update we will discuss recent advances in targeting transcription factors with small compounds, the challenges that are related to the complex function and regulation of these proteins and also the possible future directions and applications of transcription factor drug targeting.

1. Introduction

For a long time the classical targets for improving cancer treatment have been receptors, kinases or other proteins involved in signal transduction, whereas transcription factors have long been considered as un-druggable targets. However, transcription factors are the central regulators of gene transcription and a large number of diseases, such as neurodegenerative disorders, diabetes and also cancer are associated with the deregulation of transcriptional networks. In fact it has been estimated that transcription factors account for 20% of oncogenes in cancer [1]. The understanding of these complex networks and pharmacological strategies to modulate the activity of distinct transcription factors will therefore be essential for the development of novel therapeutic approaches.

Most current strategies to modulate gene expression during *e.g.*, cancer treatment indirectly affect transcription factors activity, since the inhibition of upstream kinases by specific small molecules results in modulation of multiple downstream pathways and therefore usually does not affect one single transcription factor. To further improve specific therapeutic intervention, minimize side effects and develop a "patient-specific therapy" the interest in directly targeting transcription factors has

Abbreviations: bHLH, basic helix-loop-helix; BET, bromodomain and extra-terminal; DBD, DNA-binding domain; DNMT, DNA-methyltransferases; HSP, heat shock proteins; HAT, histone acetyltransferase; HIF1, hypoxia-inducible factor-1; HDAC, histone-deacetylase; KLF10, Kruppel-like factor 10; Mycro, *Myc* activity-reducing organic; PAS, Per-ARNT-Sim domain; PK083, PhiKan083; P-TEFb, positive transcription elongation factor b; RORγt, retinoic acid receptor-related orphan receptor gamma t; Pol II, RNA polymerase II; STAT3, signal transducers and activators of transcription 3; Th17, T-helper 17; TF, transcription factor.

* Corresponding author. Fax: +43 512 504 24680.
E-mail address: michael.j.ausserlechner@i-med.ac.at (M.J. Ausserlechner).

http://dx.doi.org/10.1016/j.bcp.2015.12.006
0006-2952/

increased, since effective manipulation of these regulators may allow a "transcriptome-specific" therapy.

A proof for the relevance of directly modulating transcription factors in various therapies, most prominent cancer therapy, has been provided since many years/decades by targeting nuclear receptors that contain a clearly defined ligand-binding pocket and are activated by natural ligands, such as retinoic acid-, glucocorticoid-, estrogen-, or androgen-receptors. Activation or inhibition of these transcription factors is a central aspect of many standard cancer therapies as it leads to tumor cell type specific cell death and growth inhibition and the primary response to these therapeutics is of significant prognostic value. In fact *e.g.*, the use of glucocorticoids in childhood leukemia represented a real therapeutic success story since its introduction 50 years ago. The use of these hormones/synthetic ligands is nowadays indispensable in cancer therapy as exemplified by the pronounced effects of glucocorticoids in leukemia, anti-estrogens in breast cancer or anti-androgens in prostate cancer therapy. However, almost all other transcription factors that lack pockets for activating ligands were ignored for drug discovery strategies and considered as "un-druggable"—a frequent comment from reviewers when grant proposals on this topic were rejected. One reason for neglecting these essential central regulators of life and death lies in the lack of a typical small binding pocket and the fact that usually the only clearly defined ligand is the cognate DNA-consensus sequence. In contrast to a steroid- or ATP-binding pocket this "ligand" requires a huge protein surface as interaction site on the transcription factor that is difficult to target with small compounds. Fortunately, our understanding of the transcription factor nucleosome complex has led to several "success stories" which have changed prevalent attitudes about targeting non-ligand transcription factors in drug discovery.

2. Current strategies for transcription factor targeting

Gene transcription results not from the activation of a single protein, but requires a complex system of protein–protein interaction and, in part, chromatin remodeling that finally leads to the assembly of a transcriptome complex. In principal, besides the inhibition of transcription factor expression there are four major strategies to modulate the activity of transcription factors with small compounds or peptide-mimetics:

- The first strategy focuses on inhibition of protein/protein interactions since many transcriptions factors act as homo- or heterodimers and depend on co-factors for appropriate function. The advantage lies in small, clearly defined protein surfaces that, if appropriate structural data is available, can be also targeted by structure base virtual screening approaches (Fig. 1A and B).
- The second one targets and manipulates the transcription factor DNA binding domain directly by peptidomimetics or small molecules and changes its conformation or prevents DNA binding. In this case usually high throughput screening approaches are applied that use the transcription factor DNA-binding domain (DBD) as bait for identifying DBD-interacting compounds (Fig. 1C).
- Most recent approaches target chromatin remodeling/epigenetic reader proteins, which are essential for DNA access of transcription factors. Blocking the function of these proteins that recognize specific acetylated lysine residues on histones surprisingly provides highly specific inhibition of otherwise un-druggable oncogenic transcription factors such as MYC (Fig. 2A).
- The fourth approach is based on blockage of protein/DNA-binding by compounds that compete with transcription factors for consensus sequence interaction or change the 3D conformation of target DNA sequences in a way that they cannot be recognized (Fig. 2B).

In the following we will briefly give some examples for successful small drugs, discuss recent advances and speculate about further improvements that can be expected in the future.

2.1. Modulation of transcription factor activity by targeting protein–protein interfaces to prevent homo- or heterodimerization

Many transcription factors either form complexes consisting of identical or different transcription factor proteins and only these homo- or heterodimers are capable for specific DNA recognition. Typical examples are the transcription factors p53 that associates in tetramers, MYC/MAX dimers, STAT3 and HIF1. In addition, sequence selectivity and affinity is further modulated by co-factors that steer DNA-binding. Importantly, transcription factors may modulate gene transcription either directly, by binding their cognate consensus sequences in the promoters of target genes, or indirectly by being recruited to promoter sites through interaction with other transcription factors. For example, FOXO transcription factors may cooperate with SMAD, p53 or MYC and in part modulate target gene promoters that lack typical FOXO sites. The same is also true vice versa. Due to their importance on transcription factor function these protein–protein interaction surfaces represent possible sites for drug-mediated inhibition.

P53 inhibits cell cycle progression and induces cell death upon DNA damage and was the first tumor suppressor protein to be identified. As it is mutated in the majority of advanced cancers (reviewed in Ref. [2]), significant effort was undertaken to identify drugs that re-activate the death-inducing/anti-proliferative transcriptional activity of this gate keeper protein. In healthy cells p53 is permanently ubiquitinated by the E3-ubiquitine ligase MDM2 and thereby marked for degradation. Therefore, one strategy is to increase cellular p53 levels by inhibiting the p53/MDM2 interaction. Several substances were identified that target the hydrophobic amino acids Phe19, Trp23 and Leu26 of p53 which are responsible for MDM2 binding. Among others compounds, RITA [3] or nutlin-3 [4] (Fig. 1A) have already been shown to stabilize p53 and induce p53-mediated cell death. Other strategies use d-peptide antagonists with increased binding to MDM2, thereby allowing the release of p53 [5], which in turn stabilizes and activates p53. Of note, such strategies to reactivate p53 are only relevant for cancer cells with wild-type p53. As outlined below, some hot-spot mutations not simply impair the transcriptional activity of p53 but confer a "gain of function mutation" and convert p53 into an oncogene as discussed in Section 2.3.

A similar strategy as for p53 stabilization can be applied also to indirectly inhibit MYC transcriptional activity by modulating the formation of MYC/MAX heterodimers. The substance NSC13728 (Table 1) stabilizes MAX homodimers leading to a decrease in MYC/MAX dimers [6]. Other compounds like the *Myc* activity-reducing organic substances Mycro1 and Mycro2 (Fig. 1B) specifically inhibit MYC/MAX dimerization with low activity toward other basic helix-loop-helix (bHLH) transcription factors [7]. Mycro1 and 2 were identified by screening of large chemical libraries, as MYC lacks of a clear secondary/tertiary structure [8], which excludes the use of structure-based *in silico* screening approaches.

Another oncogene that is frequently hyper-activated in cancer is the cytoplasmic transcription factor Signal transducers and activators of transcription 3 (STAT3). Its activation leads to increased expression of VEGF thereby facilitating angiogenesis, to induction of pro-survival proteins like the BCL2-family members BCL-XL and MCL1, as well as BIRC5/survivin making STAT3 to an attractive target for cancer therapy. A large number of inhibitors for STAT3 have been developed by various approaches (reviewed in Ref. [9]). Inhibition of STAT3 was achieved directly by blocking its SH2 domain to prevent protein–protein interaction with upstream regulators, its DNA-binding domain (see below) or its dimerization