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Review article

Transcription factors: Time to deliver

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ABSTRACT

Transcription factors (TFs) are at the center of the broad regulatory network orchestrating gene expression programs that elicit different biological responses. For a long time, TFs have been considered as potent drug targets due to their implications in the pathogenesis of a variety of diseases. At the same time, TFs, located at convergence points of cellular regulatory pathways, are powerful tools providing opportunities both for cell type change and for managing the state of cells. This task formulation requires the TF modulation problem to come to the fore. We review several ways to manage TF activity (small molecules, transfection, nanocarriers, protein-based approaches), analyzing their limitations and the possibilities to overcome them. Delivery of TFs could revolutionize the biomedical field. Whether this forecast comes true will depend on the ability to develop convenient technologies for targeted delivery of TFs.

1. Introduction

Since 1961, when Jacob and Monod in their seminal article [1] established principles of gene expression regulation and suggested the existence of endogenous factors that control gene expression, recent decades have witnessed an explosion of information related to different aspects of eukaryotic gene expression regulation, especially in the field of developmental biology and cancer. Gene expression control is fundamental to the vast majority of biological processes. Transcription factors are key regulatory molecules involved in this regulation, scanning the genome and modulating gene activity *via* binding specific elements [2,3]. TFs represent a group of proteins regulating gene expression by binding to regulatory DNA sequences in the cell genome and recruiting RNA polymerase and cofactors to target genes, resulting in transcription initiation. Being in physical contact with the DNA, TFs exert their functions in the cell nucleus and contain DNA-binding and transcription activation or repression domains. It is widely considered that deregulation of transcription factors is a driver of numerous diseases [4–6] and a hard nut to crack. A third of human developmental disorders are due to mutated TF [7]. Vaquerizas et al. identified at least 164 TFs responsible for 277 monogenic diseases [8]. Some disease-associated TFs are expressed in tissue-specific manner [9], necessitating efforts to alter their dysregulated activity in specific target cells.

Now one can observe a paradigm shift in understanding TFs not only as drug targets but as pharmaceuticals themselves [10–13], especially in the field of regenerative medicine. In the past 10 years, the regenerative medicine area has shown enormous progress in cellular reprogramming and transdifferentiation [14–16]. A key aspect of these advances is revealing the role of specific TFs that manipulate cell fate. However, for each defined TF to effectively reach the nuclei of target cells, an efficient and safe delivery method is needed. Recent studies on delivery of TFs for cellular reprogramming have been covered in several reviews [11,13,17,18]. In this review, we focus on delivery of TFs in the context of targeted regulation of intracellular processes. We pay attention to a small set of TFs, now generally accepted as master-regulators of different gene expression programs, which are vantage points for TF-based therapeutic intervention, still lacking due to the delivery issues. We underline several master-regulator TFs in the areas of reprogramming/transdiffentiation, stimulus-responsible TFs, tumor suppressor TFs. The criterium to combine these different TFs in one set is their instructive character, resulting in ability to influence expression of hundreds of responsive genes. This feature of key TFs make their delivery in target cells an appealing therapeutic strategy for different clinical indications and provides a new conceptual look at TFs not only as drug targets but as pharmaceuticals themselves. We briefly discuss the therapeutic impact of TF-based transcriptional regulation, concentrating on recombinant TF delivery technologies, their limitations, ways of overcoming them, and possible consequences of the technologies used.

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2. Clinical field for delivery of TFs

Cells respond to various signals through manipulation of complex interconnected regulatory networks [3] that operate the gene expression pattern. Numerous signaling pathways converge on TFs, orchestrating gene expression through binding to specific sites within the genome and modulating activity of specific genes. Some TFs are referred as master-regulators of specific gene expression programs such as embryogenesis [19], oxidative stress response [20,21] or even cell fate [22,23]. Different diseases including cancer [24], cardiovascular disease [25], neurological disorders [26,27], autoimmunity and inflammation [28], diabetes [29], infertility [30], and obesity [31] can be caused by dysfunction of TFs. Being at convergence hubs of multiple cellular signaling pathways, TFs are often dysregulated in cancer cells and are considered as attractive but "undruggable" targets for cancer therapy [32] because of major hurdles in specifically altering activity of TFs. The "undruggability" of TFs is based on several aspects [33,34]: a) for TF targeting, the drug should be delivered into the nucleus; b) most TFs (except nuclear receptors such as androgens, retinoic acid, glucocorticoid receptors, *etc.*, which have endogenous small molecule ligands [35]) lack a deep hydrophobic pocket for drug binding. Excepting for nutlin [36,37] developed for interrupting p53:MDM2 interaction, targeting TFs with small molecules remains an elusive task despite their enormous potential as therapeutic targets.

A possible application that emerges from the transcription regulation conception *via* TF level modulation is delivery of TFs with tumor suppressor activity into cells with a corresponding inactivated gene [38]. The p53 protein, dysfunctional in over half of human cancers [39,40], is an obvious example of this approach. Nicknamed as a "guardian of the genome" [41,42], p53 is activated in response to diverse genotoxic stimuli and regulates cell cycle, directing cell toward apoptosis, senescence or cell-cycle arrest [22,43]. p53 modulates expression of more than three hundreds genes, acting mainly as transcriptional activator, although some genes are downregulated through indirect mechanisms [44]. Exerting anti-cancer activity, p53 is frequently mutated in many tumors and restoration of its impaired activity in cancer cells has been considered as valuable therapeutic strategy [45,46]. Besides p53 other TFs, such as early growth response-1 (EGR1) or interferon regulatory factor-1 (IRF-1) exert tumor suppressor activity in some types of cancer [47–49]. Indeed, this approach could circumvent numerous issues of upstream cell signaling pathway redundancy and cross-talk. Because signaling cascades always converge at TFs, direct delivery of TFs diminishes drug resistance acquisition risk (*via* alternative pathways) inherent in upstream signaling molecule inhibition.

Besides cancer, there is a growing list of disease-associated TFs [6,9,50]. Restoration of desirable TF activity may have great therapeutic potential in the treatment of disorders caused by lack of a specific TF activity. Among the practical consequences of the implementation of this approach is the ability to effectively influence pathogenesis of various diseases mediated through dysregulation in a particular cell type, for example, by means of delivery of a TF that activates cellular response to oxidative stress during inflammatory processes [51]. On the opposite side, the inhibition of undesirable TF activity, for instance promoting tumor maintenance and progression, could be done through delivery of a dominant negative TF [52], blocking the activity of an endogenous TF. The best characterized example is Omomyc, a bHLH-Zip domain of c-Myc with four amino acid substitutions, preventing Myc homodimerization and disrupting Myc/Max interactions [53]. Another possible approach to specifically repress defined transcription is through an artificial TF with a repression module [54]. For example, Bailus et al., developed a zinc finger-based artificial TF with a KRAB transcriptional repression domain and showed that the approach could silent unwanted expression *in vivo*[55]. Thus, the development of the tool for direct influence on cellular regulation through modulation of TFs is an important scientific problem of modern molecular biology with possible breathtaking biomedical applications.

There are several ways aimed at pharmacological manipulation of TFs: through small molecule interventions of TFs, and indirect (transfection-based) and direct (protein- and nanocarrier-based) approaches. The disadvantage of small molecule regulators [34,56–60] is the necessity for their distribution throughout the body and saturation of the organism to obtain sufficient concentrations in specific cells. The latter leads to high concentration of these regulators in undesirable cells and tissues and various side effects. In this case cellular and tissue specificity is typically low, leading to small molecule dilution throughout the organism and concentration decline in target cells below the level sufficient for influence on a specific cellular process [61,62]. Cell-type specific interaction utilizing signature surface receptors triggering intracellular cascades [63] is another pathway. Indirect and direct approaches more suitable for *in vivo* application are reviewed below.

The mechanism of action of some commonly used drugs involves transcription modulation [64,65]. Nonetheless, selectivity of action of small molecules leaves much to be desired. Macromolecular regulators such as TFs, developed over millions of years by nature to control a gene expression program, could be a much too sensitive and specific tool for transcription regulation. TFs being delivered into the nuclei of target cells are able to amplify their signal, prompting the cell machinery to express numerous mRNAs. Such signal amplification is almost impossible for small molecules, exerting theirs actions stoichiometrically: one molecule inhibits one target. This restriction could be circumvented using upstream elements of signal pathways, but this involves a risk of affecting other cellular processes.

The appropriateness of TFs for therapeutic modulation comes from the ability of a single TF to integrate a variety of signals and, for some cases, activate expression of hundreds of responsive genes (Fig. 1). For some cell types only a few TFs (from the total of 2000–3000 encoded in the human genome [34]) are instructive factors determining cell state and fate [66,67]. Identification of such key TFs could revolutionize our tools for cell regulation manipulation and create opportunities for development of new therapeutics. On the other hand, the precise delivery into cells of a given type could be even more important for TFs that control the expression of many genes, because these TFs can induce more side effects.

In addition to p53 exemplified above, we highlight other groups of key TFs, which delivery in the nuclei of target cells might create new opportunities for exogenous cell regulation for therapeutic purposes. TFs, directing cell reprogramming/transdifferentiation, are hot topic in regenerative medicine, which develop remedies, capable to replace cells and tissue, damaged due to ageing or disease, and normalize disturbed cognitive functions during neurodegenerative processes [68,69]. Induced pluripotent stem cells (iPS), products of cell reprogramming, are able to differentiate into different types and make unnecessary the use of human fertilized oocytes: source of human embryonic stem cells and ethical concerns in many countries [70,71]. iPS can be obtained with the use of a set of several TFs (see below, "TFs for pluripotent stem cells induction" section) from different sources such as person's skin and could generate more than 200 human cell types [69], creating the basis for personalized therapy with reduced risk of immune side effects. *In vivo* cell transdifferentiaton or direct conversion from one cell type to another represents another way for self-repair [72,73] and might be a non-invasive option for clinical indications with urgent need for specific cell type. The list of such diseases is rather long, starting from diabetes with dysfunction of pancreatic β-cells and ending with diseases of central nervous system with disability of neurons of different subtypes. First results in this field are very encouraging and show *in vivo* proof-of-principle for cell transdifferentiating with defined key TFs [73].

The evolution of our cells has resulted in development of defense systems, which main task is to protect the cell against variety of physiological and environmental insults and to prevent cellular damage, critical to survive. The examples of such insults are oxidative, toxic and hypoxic stresses. Interestingly, these stresses are hallmarks of serious