PROTEOSTASIS/DRUG DISCOVERY

A stress-free stress response

A new molecule that specifically activates a key protein homeostasis pathway has been identified. The ability to initiate the IRE1–XBP1s branch of the unfolded protein response opens up new avenues for basic research and treatment of disease.

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Perturbations of protein homeostasis can lead to the accumulation of unfolded proteins in the endoplasmic reticulum (ER), which is detrimental to cellular function. Defense against this form of stress is achieved by the unfolded protein response (UPR), a transcription–translation program consisting of three branches initiated by the proteins IRE1, PERK, and ATF6. The most evolutionarily conserved of these branches is mediated by the unfolded protein sensor IRE1, which can splice the mRNA of XBP1 to produce the active transcription factor XBP1s. The UPR is involved in many physiological processes, such as development and immunity, and has also been implicated in a diverse range of diseases, including neurodegenerative disorders, diabetes, and cancer.

Although the UPR is a topic of intense research, it has been difficult to separate the effects of the individual branches. The most commonly used pharmacological tools (tunicamycin and thapsigargin) activate all three branches in a non-specific manner by disturbing protein processing in the ER. Recently, a specific activator of the ATF6 branch has proven useful in dissecting the molecular outputs of this pathway and in alleviating pathology in some disease models[1,2]. However, thus far there has not been a pharmacological means to stimulate the IRE1–XBP1s branch with great specificity; existing activators that target the IRE1 kinase domain frequently cause off-pathway effects through binding to other kinases or promote cellular toxicity[3,4]. In addition, numerous groups have studied the effects of IRE1–XBP1s activation by overexpressing these proteins and inducing permanent transcriptional activation. However, a permanent genetic activation of a normally transient program is artificial and can cause cell death; it is also not easily achieved in a therapeutic setting. Even inducible genetic systems in which doxycycline administration can transiently activate XBP1s expression are limited by the need to deliver the exogenous gene. Now, Grandjean et al.[5] have discovered a highly specific IRE1 activator, by means of a high-throughput screen, followed by systematic transcriptional profiling that shows specific induction of XBP1s gene targets.

To find this elusive molecule, Grandjean et al.[5] performed a high-throughput compound screen in cells expressing an XBP1s splicing reporter, removing compounds that were toxic or only weakly active or that upregulated known targets of the heat shock response or other UPR branches (Fig. 1). The compounds selected spanned different structural classes, and those similar to known compounds that bind kinase active sites were removed, improving the chances of finding drugs that work by a different mechanism. The effects of the final three compounds were then characterized through RNA sequencing (RNA-seq) to better determine the specificity for XBP1s compared to that of ATF6- or PERK-dependent targets. Interestingly, the compounds that met these criteria only activated the XBP1s-dependent transcriptional outputs of the IRE1 branch and did not promote other functions of IRE1 activation, including c-Jun N-terminal kinase (JNK) activation and mRNA degradation

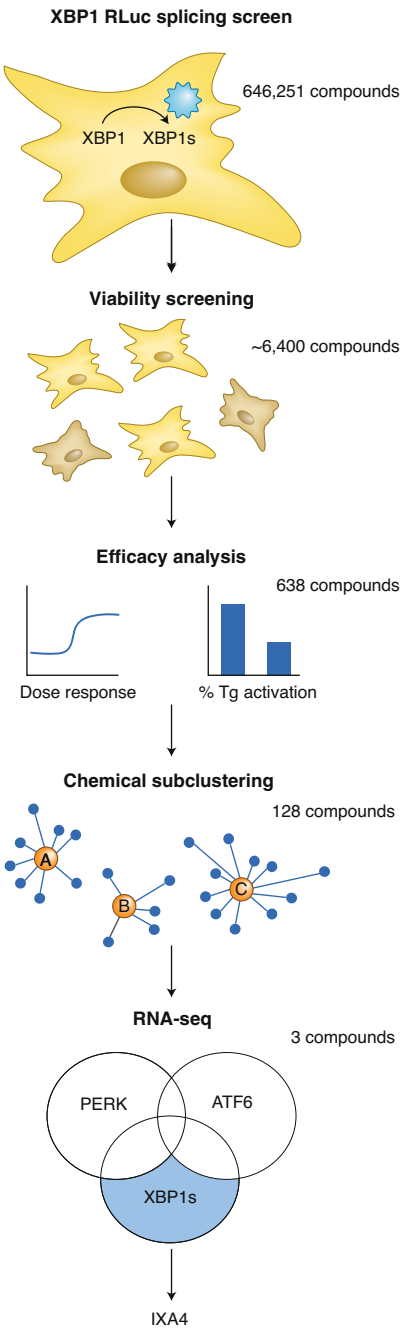

Fig. 1 | Screening pipeline for specific activators of the IRE1–XBP1s pathway. A luciferase-based XBP1 splicing reporter (RLuc) was used to screen the Scripps Drug Discovery Library. Compounds known to activate the heat shock response (HSR) and promiscuous compounds were removed. The remaining compounds were confirmed in triplicate and tested for toxicity. Known activators of ATF6 were eliminated, and surviving compounds were tested for efficacy (Tg, thapsigargin). This was followed by chemical subclustering. Compounds likely to activate the IRE1 kinase domain were removed, and the remaining compounds were tested for activation of the XBP1s target *DNAJB9* and the ATF6–PERK targets *BiP* and *CHOP*. The top three hits from this pipeline were then used for RNA-seq to characterize their selectivity in greater detail using gene sets that represent activation of each branch of the UPR.

through regulated IRE1-dependent decay (RIDD). The most selective compound, IXA4, was then used for phenotypic studies in mammalian cells, in which treatment reduced the levels of human mutant amyloid precursor protein (APP) and secreted amyloid-β. IXA4 treatment also alleviated mitochondrial dysfunction induced by APP. These experiments demonstrate the potential utility of this molecule for dissecting the role of the IRE1–XBP1 UPR branch in disease and for exploring future therapeutic avenues for diseases involving APP and amyloid-β.

The new compound will also be valuable for interrogating the function of this UPR branch in basic physiology and for evaluating its role and its potential as a therapeutic target in different disease models. The IRE1–XBP1 branch of the UPR is involved in development, immune responses, and some metabolic processes, so the ability to dissect the effects of this pathway independently of protein stress will be especially useful in these contexts. An additional piece of information that will be needed is how quickly XBP1s levels return to baseline following washout of IXA4. Ideally, the effect will be transient and reversible, but this has not yet been determined. Another key question remains regarding the mechanism of action of IXA4. Unlike most other IRE1-modulating drugs, this compound does not bind to the kinase active site, and canonical autophosphorylation of IRE1 is required for its splicing activity to be triggered. Further structural studies will likely be required to shed light on this issue.

Finally, elevating levels of XBP1s genetically has resulted in positive effects in neurodegenerative disease models and has also been shown to improve cognitive function and longevity[6–8]. It would therefore be exciting if IXA4 itself proves to be active and non-toxic in vivo, a possibility which must be high on the agenda for those pursuing research into this interesting and useful new pharmacological tool. ❐

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Competing interests

The authors declare no competing interests.