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'Small Molecule Screening Towards New Therapeutics' Review Series

A new era for small molecule screening: from new targets, such as JAK2 V617F, to complex cellular screens

Stefan N. Constantinescu*

Guest Editor

Abstract

Traditionally reserved to research and development in pharmaceutical companies, screening of small molecule libraries is rapidly becoming an approach undertaken by academic laboratories. Novel cellular assays, sensitive systems to probe function, emerging new molecular targets are just some of the reasons explaining this shift. Targets of small molecules identified in cellular screens begin to be amenable to identification by elegant genetic approaches, such as probing toxicity of candidate small molecules on libraries of genetically modified yeast strains. Several new targets, such as JAK2 V617F, an activated JAK2 (Janus Kinase 2) mutant genetically associated with the majority of human myeloproliferative neoplasms, are being actively pursued. In this Review Series, we will learn how libraries of small molecules are harnessed to identify novel molecules, that alone or in combination, have the ability to alter cell fate, cell signalling, gene expression or response to extracellular cues.

Kinase inhibitors

Perhaps the most attractive targets for small library screening are kinases, as the ATP pocket usually bound by such small molecules is well understood at the structural level. Imatinib, an inhibitor of tyrosine kinases Abl, KIT and platelet-derived growth factor (PDGF) receptor, has changed the landscape of therapeutic intervention in several diseases, most notably chronic myeloid leukaemia (CML), where the constitutively active breakpoint cluster region-Abelson (BCR-ABL) tyrosine kinase exerts a major pathogenical role [1], but also in other diseases where KIT is constitutively active, such as gastrointestinal stromal tumours [2]. Small molecules able to modulate KIT activity at different levels, *i.e.* dimerization *versus* kinase activation [3], might also complement the use of heterozygous $KIT^{+/-}$ cells for the investigation of the mechanistic role of KIT in the function of several cell types, such as the interstitial cells of Cajal [4].

JAK2 V617F

The majority of human BCR-ABL negative myeloproliferative neoplasms (MPNs), polycythemia vera, essential thrombocythemia (ET) and primary myelofibrosis (PMF) have recently been linked to the presence of an activated mutant of JAK2, where a point mutation in the pseudokinase domain (V617F) leads to dysregulated kinase activity (for review see [5]). Inhibitors of JAK2, such

*Correspondence to: Stefan N. CONSTANTINESCU,
Ludwig Institute for Cancer Research & de Duve Institute,
Université catholique de Louvain, Avenue Hippocrate 74,
UCL 75-4, Brussels B1200, Belgium.
Tel.: 322-764-7540; Fax: 322-764-6566
E-mail: stefan.constantinescu@bru.licr.org

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as the tyrphostin AG490 [6], have been used to identify JAKs as responsible for constitutive signalling in primary cells from MPN patients [7]. But these inhibitors may target several other tyrosine kinases [8].

Several inhibitors of JAK2 are now being tested in clinical trials. These molecules exhibit a certain degree of specificity for JAK2 *versus* the other three other JAKs (JAK1, Tyk2, JAK3), but do not discriminate between wild-type and JAK2 V617F [9–11]. Potential unwanted effects would be myelosuppression, given the key role of JAK2 in blood formation. An inhibitor specific for JAK2 V617F, would spare normal haematopoiesis, but would be much more difficult to obtain. Unexpectedly, a histone deacetylase inhibitor (ITF2357) was found to specifically target primary cells from PV and ET patients harbouring the JAK2 V617F mutation [12], suggesting that transformation by JAK2 V617F might require epigenetic events. In support of this possibility, $CD34^+$ cells from myelofibrosis patients are specifically inhibited by treatment with DNA methyltransferases and histone deacetylases [13].

MPL (TpoR) mutants

TpoR mutants where juxtamembrane W515 is replaced by leucine or lysine are present in 8% of ET and PMF patients, that are negative for JAK2 V617F mutation [14, 15]. W515 is part of a hydrophobic juxtamembrane cytosolic motif that is required to maintain un-liganded TpoR inactive [16]. Because JAK2 V617F signals from complexes with TpoR [5, 17] and because TpoR W515 mutants are linked to myelofibrosis induction, screening for small molecules targeting this receptor may lead to compounds that can be useful in treatment of MPNs.

JAK1 mutants

The homologous JAK2 V617F mutations in JAK1 (V658F) and Tyk2 (V678F) also lead to constitutive activation [18]. Recently, it was shown that $>$20% of human T acute lymphoblastoid leukaemia harbour activating mutations in JAK1 [19], including in the pseudokinase domain and the JAK1 V658F [20]. More such mutations are expected to be identified in cancer and other pathologies in the future, making members of the Janus kinase-signal transducer and activator of transcription (JAK-STAT) pathway attractive targets for inhibition by small molecules.

Designing inhibitors

In an ideal setting, computation should be able to help identify small molecules that bind to a particular segment of a protein. This holds the advantage of targeting with very high resolution a particular segment or module of a protein. In addition, such level of protein targeting is often necessary when a hit emerges from a random screen, and chemical groups must be added or substituted for lead optimization. A new docking software, EADock, is emerging as such a tool for protein targets that have been crystallized [21]. Validation was accomplished for the ability of EADock to predict binding modes, by the successful docking of the RGD cyclic pentapeptide on the $\alpha V\beta 3$ integrin [21].

Successful cellular screens and target identification

Screening for small molecules able to modify cell behaviour has been viewed with scepticism, because identification of the actual target of a hit is quite difficult. A recent study, however, proved that cellular screening can lead to isolation of small molecules that can rapidly be ascribed an intracellular target [22]. Small molecules (tenovins) that activate p53 were isolated from a cellular screen and found to act *via* inhibition of the protein de-acetylating activities of two members of the sirtuin family, SirT1 and SirT2 [22]. The small molecule candidates were then tested on a collection of diploid *Saccharomices cerevisiae* strains that each are heterozygous for a specific gene deletion. Toxicity of one particular compound is higher on a strain that is heterozygous for the gene that codes for the protein that is targeted by the tested compound [23, 24]. Thus, combining cellular screens with compound-induced haploinsufficiency can be highly successful. Interestingly, SirT1 exerts different functions in different cell types, and was reported to regulate skin aging and skin response to ultraviolet (UV) [25].

Perspectives

Small molecule screening will likely target not only signalling pathways or enzymes, but also newer players, such as microRNAs, proteins involved in epigenetic regulation or those involved in instating stemness. Coupled to ongoing advances in computation, docking, proteomics and systems biology, small molecules are likely to occupy centre stage in future biomedical research.

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