

High Throughput Cellular Screen and Profiling Technologies and Their implication in Target and Drug Discovery

Markus Warmuth

Kinase Platform and Department of Lead Discovery, Genomics Institute of the Novartis Research Foundation (GNF), 10675 John Jay Hopkins Dr., San Diego, CA 92121, USA

Protein kinases and their role in cancer have been a main focus of research in both academia and pharmaceutical industry ever since the discovery of the first kinase oncogenes more than two decades ago. Tremendous progress has been made in targeting protein kinases with specific small molecule kinase inhibitors, several of which have either been approved for clinical application or are currently in clinical trials. Early reports on successful clinical trials with these drugs have spurred efforts in developing follow-up compounds as well as inhibitors of novel kinase targets. This will significantly increase the need for rapid and accurate cellular profiling tools for kinase drug discovery to aid all steps of drug development from identifying lead compounds for medicinal chemistry, to discovering unexpected cross-reactivities or to elucidating off-target effects of compounds currently under clinical evaluation.

This presentation will focus on the use of Ba/F3 cells, a murine hematopoietic cell line, as tool in automated high throughput profiling of kinase directed MedChem libraries. in kinase drug discovery. Ba/F3 is a murine, IL-3 dependent, hematopoietic cell line. Over the past years it has become a popular system for exploring both kinases and their small molecule kinase inhibitors. Ba/F3 cells were first characterized by Palacios and Steinmetz in an effort to isolate IL-3 dependent pro-B cells from bone marrow of Balb/c mice.

The utility of Ba/F3 in kinase research and drug discovery is primarily based on the following characteristics of these cells. (1) Ba/F3 cells are a fast growing suspension cell line that facilitates rapid experimental turn-around. (2) Ba/F3 cells are easy to be transfected by electroporation and are readily susceptible to infection by commonly used retro- and lentiviral expression systems. (3) Ba/F3 cells proliferate and survive in the absence of IL-3 when expressing a constitutively active tyrosine kinase or other oncogenes. The “addiction” of Ba/F3 cells to ectopically expressed kinase oncogenes has been demonstrated with multiple examples, including NPM-Alk, Flt3 mutants (both ITD and Y835D), Tel-PDGFR and Tel-Jak2. The possibility to elicit “oncogene addiction” in Ba/F3 cells has made them an ideal tool to test for the differential cytotoxicity of drug candidates or whole compound libraries. Once dependent on an oncogene such as a constitutively active tyrosine kinase, inhibition of such oncogene or kinase results in growth arrest and apoptosis that can be rescued by the introduction of IL-3 into the growth media. This assay principal is now widely used by many different investigators and has been of great value to not only test the potency of small molecule kinase inhibitors against wildtype kinases but also against clinically observed inhibitor resistant

mutants. In an effort to adapt the use of Ba/F3 differential cytotoxicity assays for high throughput screening and profiling of medicinal chemistry derived compounds, we have recently published an approach to generate Ba/F3 cells transformed by artificially activated tyrosine kinases. This approach was based on the well established fact that the fusion of tyrosine kinases to genes capable of inducing dimerization can lead to their constitutive activation and hence transformation of various cell types. Based on this finding we generated fusions of a total of 90 tyrosine kinases to parts of the TEL gene. TEL is a transcription factor that has been found as a natural fusion partner for several different kinases and for various transcription factors in both hematopoietic malignancies as well as soft tissue carcinomas. Transduction of this fusion kinase library into Ba/F3 cells gave rise to IL-3 independent Ba/F3 cell lines representing 33 individual kinases. Profiling of these cells against a collection of specific and non-specific reference compounds and comparisons with secondary assays on full length kinases revealed the value of this system. First, a close match was seen in IC_{50} values between artificial TEL/kinase fusions and naturally occurring kinase oncogenes such as Bcr-Abl, NPM-Alk and Flt3-ITD. Also, while the assay system seems to be advantageous over traditional biochemical profiling (see below), a highly significant match with various secondary assays on full length kinases was found. Finally, by culturing 33 representative cell lines on an automated tissue culture and profiling system (ACP) we could demonstrate the possibility to adopt Ba/F3 profiling to fully automated high throughput screening and profiling processes.

If used in high throughput format this newly generated Ba/F3 panel can turn into an important tool for guiding medicinal chemistry efforts in addition to being a tool for profiling preclinical drug candidates. Based on the rapid growth of Ba/F3 cells in suspension, it is an ideal line for growth in automated culture systems and a cellular potency and selectivity profile can be generated with a turnaround time of 1-2 weeks [45]. This data can provide chemists with a uniform and global view of the potency and selectivity of compounds and enables “kinase hopping” – the ability to use a given chemical scaffold for multiple different targets.

Even though the potency and selectivity of a given compound can be assessed by using traditional *in vitro* biochemical assays, the Ba/F3 differential cytotoxicity assay has several distinct advantages. First of all, Ba/F3 assays can accurately measure the activity of inhibitors that preferentially bind to the so-called inactive conformation of kinases. In contrast, traditional *in vitro* kinase assays dependent on the length of the recombinant protein, the activation of the kinase and the reaction conditions, may fail to accurately reflect the potency of such compounds. Indeed, while cellular assays are able to represent most relevant kinase conformations, biochemical assays often represent non-physiological conformations that result in strange structure activity relationships. Secondly, in contrast to the artificial, usually very small peptides used in *in vitro* assays, the kinase substrate(s) in a Ba/F3 assay is likely to be a biologically relevant substrate(s) required for cell proliferation and survival. Thirdly, a cellular assay system allows addressing issues such as membrane penetration and potency shifts due to plasma protein binding. Finally, the selectivity data generated from Ba/F3 differential cytotoxicity assays

is from the same cellular background and is generated at physiological cellular ATP concentration, which makes the data much more predictive of their in-vivo activity. In contrast, IC_{50} s generated in biochemical assays are at or around the K_m ATP for each kinase. Even though these IC_{50} s are a good indication of the affinities of the inhibitors to each kinase, the large variation of the K_m ATP among different kinases (can range from high nM to high uM) complicates the interpretation of the selectivity data generated using biochemical assays.