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Development and Application of in vitro Assays for the Identification and Characterization of Kinase Inhibitors

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Aberrant kinase activation has been linked to severe diseases like cancer, inflammatory and neurodegenerative disorders. Small molecule inhibitors can be used to block undesired kinase activity. Approximately twenty kinase inhibitors are marketed, mainly for oncological indications. However, the lack of selectivity of kinase inhibitors as well as the low predictability of recombinant kinase assays for cellular or *in vivo* activity of inhibitor molecules are key bottlenecks in kinase drug discovery, especially for chronic indications.
In this thesis, assays were developed to identify novel inhibitors against several kinases and to characterize their selectivity and their interaction with the expressed proteome in cell lysates and in intact cells. Moreover, a method was established to assess the cellular penetration of compounds in any cell type including primary cells, enabling a better correlation between an inhibitor´s affinity for kinases and its effect on cellular phenotypes.

A large part of this thesis was centered on TANK binding kinase 1 (TBK1), which is a key player in several inflammatory signalling pathways. The work included the adaptation and optimization of chemoproteomic assays to enable screening of compound libraries against TBK1 as well as detailed studies on compound-target interactions. First, novel TBK1 inhibitors were identified by screening a library of over 39,000 compounds against TBK1 in a chemoproteomic assay, utilizing cell lysates and a kinase-binding matrix. The advantage of this method over frequently used activity-based biochemical assays is that the affinity of compounds is evaluated for endogenous kinases under conditions that preserve post-translational modifications and protein complexes. Using the chemoproteomic assay, more than 200 novel TBK1 inhibitors of different chemotypes and potencies were identified.
Secondly, kinase-binding matrices were used to selectively capture TBK1 depending on its activation state. The activation state of a kinase is often determined by autophosphorylation or phosphorylation through another kinase. The resulting conformational changes may modify the binding affinity of small molecule kinase inhibitors for the kinase. The developed assay led to the identification of compounds that selectively bind TBK1 either from activated or from unstimulated cells.

Another innovation presented in this thesis was the development of a chemoproteomic assay to measure the cell permeability of kinase inhibitors. Discrepancies between biochemical and cell-based activities of compounds are often not well understood since the ability of a given compound to enter cells is rarely directly measured. The clinical kinase inhibitor NVP-BEZ235 has been reported to inhibit PI3Kγ and other lipid kinases. However, this small molecule was not efficacious when tested in PI3Kγ-dependent cell-based assays. Using the cell-permeability assay developed in this work, NVP-BEZ235 was indeed shown to be cell permeable but its binding affinity was stronger for other endogenous lipid kinases than for PI3Kγ. The same assay was also used to investigate two newly identified LRRK2 inhibitors, demonstrating that these inhibitors were cell-penetrant, albeit to a different degree.

Finally, a chemoproteomic assay was established using human cells isolated from small amounts of blood. Because the physiology of primary cells and the intrinsic individual variability of clinical samples are not reflected in tumor-derived cell lines, it is important to use human primary tissues for the discovery of novel drugs. The developed method enabled the measurement of binding affinities of compounds to PI3Kδ and PI3Kγ in blood cells isolated from distinct donors.

In summary, this thesis describes the development and application of *in vitro* assays to evaluate kinase inhibitors under close to physiological conditions. These assays range from high-throughput screening formats to the study of inhibitors in primary human cells, including detailed studies of structure-affinity-relationships of compounds for different activation states of a kinase. Altogether, these methods enable a better prediction of kinase inhibitor action from hit identification throughout chemical optimization to *in vivo* and clinical testing.