FUNCTIONAL PROTEOMICS APPROACHES FOR HIGH-THROUGHPUT DETERMINATION OF CKIT AND SMALL MOLECULES

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Human KIT is a proto-oncogene that encodes for a trans-membrane receptor (cKIT) with intrinsic tyrosine-kinase (TK) activity with functions as the receptor for Stem Cell Factor (SCF). Expression of cKIT protein has been reported in a wide variety of cells, including mast cells, hematopoietic progenitors, germ cells or melanocytes. Alteration on Kit expression/activity are associated with several hematopoietic disorders, gastrointestinal stromal tumours (GIST), piebaldims, among other diseases. Several cKIT point mutations has been described in clinical samples (>150 patients), many of these clinical relevant proteins are associated with constitutional Kit phosphorylation and downstream activation, independent of the interaction with SCF. The recent advances in the field of molecular-targeted therapy with cell based assays allow us to select drugs on the basis of specific molecular abnormalities. Nevertheless, at present this studies are costly, have long response times and, in many diseases are difficult to since there´s a limited availability of samples. Here, we propose a high-throughput method for screening different small molecules that it will be used in Kit-related disorders, whether it´s mutated or not. We have design and prepared Nucleic Acids Programmable Array (NAPPA), which the content is wild-type cKit and all the clinical relevant point mutations of cKit described in a cohort of 150 patients. Using this kinase NAPPA array, we showed that the kinases on the array display auto-phosphorylation activity and interaction with SCF as reported *in vivo*. In addition, interactions of small molecules, such as ADP or staurosporine or imatinib, have been tested. The high-throughput interaction studies show different selectivity and efficiency among different point mutated cKit kinases; for example D816V mutant is not interacting with small molecules, suggesting non-inhibition capacity.

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