Establishing of Peptide-Kinase-Inhibitors via Phage Display

Dörthe Wirtz - (PhD-Thesis)

Abstract

Both, the insulin-like growth factor receptor (IGF-1R) and the insulin receptor (IR) are members of the transmembrane receptor kinases, which belong to the family of growth factor receptors. IGF-1R is responsible for development, normal growth, and differentiation of cells, but also for suppression of apoptosis. The ability of the receptor to simultaneously initiate proliferation and anti-apoptotic signals indicates, that IGF-1R might might be an interesting target for drug development. Generally, common kinase inhibitors are small molecule inhibitors which show competitive inhibition with respect to ATP. As the ATP site is highly conserved across kinases those inhibitors might lead to undesirable side effects. Especially in the case of IGF-1R a selective inhibition is essential as a simultaneous inhibition of the closely related IR might lead to severe consequences e.g. the formation of a diabetic phenotype. To avoid those effects, the aim of this PhD was to develop a specific peptide inhibitor for the IGF-1R kinase. According to the common opinion, dimerisation is necessary for the activation of a receptor kinase. Therefore, my approach was based on preventing dimerisation by competition with sequence-specific peptides. This concept was to be realized for two regions of the IGF-1R and IR: One target was the juxtamembrane region which is supposed to have an auto regulatory function as well as a role in the dimerisation process of the kinase. In addition, IR and IGF-1R display significant sequential differences in this region. As other target the αD-Helix/*hinge region* which is crucial for the transition of the inactive to the active conformation was chosen. To find a specific peptide ligand for both targets, a library of peptides with randomized amino acid sequences was screened by *phage display.*

To generate the peptide sequence a technique was developed to express small peptides in high scales and purify them by affinity chromatography.

Validation of the sequences derived from the *phage display* against the juxtamembrane region led to a peptide sequence which is able to inhibit the IGF-1R kinase in a µM range:

•Substrate- and autophosphorylation of the monomeric IGF-1-R kinase is significantly inhibited by the peptide.

•The inhibytory effect of the sequence is highly specific for the IGF-1 kinase. Even the closely related IR kinase is not affected by the peptide.

•Inhibition follows a non competitive mechanism with respect to ATP.

•The activity of the dimeric kinase is not impaired. The peptide binds selective to the inactive kinase and impairs dimerisation.

•Additionally, the inhibitory peptide degenerates the interaction of the phosphotyrosin binding

domain (PTB) of insulin receptor substrate-1 (IRS-1) with the kinase. Hence the peptide acts as a inhibitor of signal transduction. This inhibition is independent of the kinase conformation.

Phage display against a peptide composed of the αD-helix/hinge region of IGF-1R provided a peptide sequence which inhibitory effect was significantly stronger compared to the effect of the peptide against the juxtamembrane region:

•This peptide inhibits specific the inactive monomeric kinase of IGF-1R while the activity of the dimeric kinase is not impaired.

•Binding of the inhibitory peptide to the hinge region leads to a fixation of the kinase in an autoinhibited conformation.

•The inhibitory effect is specific for members of the insulin receptor family. However binding of the peptide to the IGF-1R kinase is much more stronger as the binding to the kinase of the IR.

•Inhibition follows a non competitive mechanism with respect to ATP.

Thus two peptide inhibitors could be generated, which bind to different regulatory regions of the unactivated kinase structure and inhibit the activation process. Additionally one peptide inhibits the signaltransduction independently of the kinase structure.