

The long and winding road of Kit juxtamembrane domains

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» **Background.** Mutations in the Kit receptor tyrosine kinase [RTK], which result in ligand-independent activation of the kinase, are associated with cancers such as gastrointestinal stromal tumors [GISTs] and mastocytosis. Kit mutations in GISTs most frequently occur in the noncatalytic Kit juxtamembrane [JXM] region, suggesting that this domain is crucial in regulation of kinase activity. Moreover, genetic and crystallographic studies have implicated the cytosolic JXM region of the Kit RTK as an autoinhibitory regulatory domain. In this study we propose a computational rationale for the role of wild-type and clinically relevant mutant Kit JXMs in controlling receptor autophosphorylation and its response to imatinib.

Materials and methods. We have used advanced molecular simulation techniques, based on the so-called self-guided molecular dynamics [SGMD] and molecular mechanics/Poisson-Boltzmann free energy calculations [MM/PBSA], to investigate the behavior of isolated wild-type and mutant Kit fragments formed by the JXM residues that fold into a beta-hairpin in the native protein structure.

Results. The beta-hairpin folding of the Kit wild-type and several mutant JXM domains was directly simulated in explicit water

at native folding conditions in three 300-ns SGMD simulations. Through structural and energetic analysis of the folding events, we answered some basic questions about the folding of these domains in water. The wild-type sequence folded into a series of beta-hairpin structures in our simulations, the major cluster of which agrees well with the X-ray experimental observation. On the contrary, altered structures were obtained, as function of the different type of mutation considered [i.e., missense and deletions]. Different intrapeptide interactions drive the JXM to misfolded conformations, and the solvation/entropic effects, which resist folding, are also shown to prevent the mutant sequences peptide from folding into wild-type like structures. These structures then act differently in keeping the Kit in its autoinhibited conformation. Finally, simulations of the entire protein with wild-type and mutant JXMs allowed to calculate the free energy of binding (and hence the IC50 value) of these RTK and Imatinib.

Conclusions. Our simulations contributed for the first time to highlight the possible effects exerted by the presence of Kit JXM mutations on the active/inactive structure of Kit and on its affinity towards Imatinib.

Novel class of akt/PI3k inhibitors based on glucidic scaffold

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» Phosphatidylinositol 3-kinase [PI3K] and Akt regulate many important cellular processes, controlling the balance of survival and apoptosis of mammalian cell. Akt is the major mediator of survival signals that protect cells from apoptosis, after activation in response to various stimuli, in a PI3K dependent manner [1]. Recent studies showed the importance of Akt-PI3K pathway in many human diseases. In addition, Akt is a critical player in the development, growth and therapeutic resistance of cancer, thus emerging as therapeutic target for cancer treatment [2].

Although much of the drug-development efforts have been focused on ATP-binding-site inhibitors, development of small molecule analogues of natural phosphoinositides is now considered as potential alternative for pathway interruption and therapeutic applications.

In this context, new glucose-based inositol analogues have been synthesised as potential inhibitors of the Akt-PI3K pathway. Carbohydrates are the most abundant chiral molecules available naturally. This characteristic, the low cost and easy with which they can be obtained in a pure state make these as prime candidates for the synthesis of chiral scaffolds with pharmaceutical and medical applications [3]. The structure of phosphatidylinositol-3-phosphate produced by PI3K, and natural substrate of Akt, can be easily reconducted to suitably modified D-glucose, where specific

functional groups have been introduced, taking into account the considerations already reported on structure/activity relationships of previously synthesised inhibitors [4].

The inhibitory activity of the synthesised compounds has been tested in inhibition assays on the well characterized murine dendritic cell line, D1. In order to rationalise the biological activity showed by the analogues synthesised, preliminary docking simulation studies on Akt were performed with the AutoDock 3.0 software package.

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