Structural Insights on the oncoprotein Cripto CFC domain and its Alk4 receptor

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Motivation

Cripto is a membrane protein indicated as an important target for therapeutic intervention in the treatment of several widespread cancers, including breast, colon and lung carcinomas. The protein displays all the features of an oncogene being able to support survival, transformation, migration and proliferation in a large variety of cell lines. It is also highly over-expressed in many tumors, while it is poorly detectable in normal tissues. Cripto is the founding member of the extra-cellular EGF-CFC growth factors, which are composed of two adjacent cysteine-rich domains: the EGF-like and the CFC. It has been shown that each single domain of Cripto (EGF and CFC) can bind different protein partners. Cripto performs an obligatory role as co-receptor for the growth factor Nodal. Indeed, binding of Nodal to the activin receptor complex (Alk4/ActRIIB) only occurs in the presence of Cripto. In this process Cripto binds Nodal through the EGF domain and binds Alk4 through the CFC domain. Although both domains are involved in Cripto tumorigenic activity, the CFC domain plays a primary role. Through this domain Cripto interferes with the onco-suppressive activity of activins, either by blocking the receptor Alk4 or by binding soluble activin.

Methods

To investigate the interaction of Cripto CFC domain with the Alk4 receptor we have: 1) solved the 3D structure of the Cripto CFC domain by NMR analysis performed in water at different pH values, and 2) modelled by homology the extra-cellular domain (ECD) of Alk4 receptor, on the basis of the human receptor Alk3 crystal structure (sequence identity 26%) .

Results

Our NMR studies have led to the first experimental data on the Cripto CFC domain 3D structure. CFC molecular model shows a fold made of three antiparallel strands linked by disulfide bridges and connected through short loops. All the determined structures appear generally more structured at physiological pH values than under acidic conditions. Human Alk3 receptor was used as template for building a comparative model for the Alk4 receptor extracellular domain. Extracellular domains of human Alk3 and Alk4 match the Pfam family of activin receptors. These are hydrophilic cysteine-rich ligand-binding domains characterized by the consensus cysteine box: CCX{4-5}CN. The numerous conserved cysteine residues involved in disulfide bridges represented secure landmarks for obtaining an accurate sequence alignment between target and template and, consequently, a reliable Alk4 homology model. The structural insights we achieved on Cripto CFC and Alk4 extracellular domains constitute the basis for the study of their interaction, which is key to the Cripto tumorigenic activity. This study is now in progress.

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