Functional Characterization of “Two Hybrid Associated Protein 1 with RanBPM” (Twa1), a New Nuclear Protein Related to Cancer

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Overview. Twa1 is a recently discovered protein of unknown function. There are, however, several reasons to believe that it is involved in the cell cycle, and, therefore, it may play an essential role in cancerous cells. First, it was discovered through a two hybrid-associated protein screen with RanBPM, a protein involved in several cellular processes including mitosis and DNA replication. Second, Twa1 contains the LisH motif, known to be involved in microtubule dynamics and chromosome segregation. Third, Twa1 presents interactions with RNPS1 (RNA-binding protein with serine-rich domain 1), an RNA binding protein important in the translation process. Here, we propose a functional characterization of Twa1 through the study of its three-dimensional structure and its protein-protein interactions. This characterization is essential to validate Twa1’s potential as an anti-cancer target and enable the structure-based design of new anti-cancer drugs. Preliminary results. During the purification process, Twa1 showed a propensity to form unstable aggregates. Denaturation tests by circular dichroism together with size exclusion chromatography revealed a condition in which the protein remains as a stable dimer. However, Twa1 did not form crystals in any of the conditions tested. In response, we are trying two alternative approaches: (i) Increasing the protein’s stability; and (ii) using truncated forms of Twa1, which can be easier to crystallize or to study using nuclear magnetic resonance. In parallel, we are investigating the interaction between RNPS1 and Twa1, and its role in the cell cycle and cancer development.

Word Keys: Twa1, cancer, crystallography.

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The role of Twa1, however, is not well understood, but it is believed that it is involved in the cell cycle for several reasons. First, because these functions overlap with those suggested for the RanGTPase cycle. Second, because (RNA-binding protein with serine-rich domain 1)

For these reasons, we hypothesize that Twa1 can have an essential role in cancer cells and it can therefore be a potential anti-cancer target.

The final goal of this work open the possibility of based on the 3D structure of

Twa1 was purified by a sequence of chromatography columns: immobilized Ni-affinity, ion exchange, hydrophobic interaction and size exclusion finally. The protein is prone to form, and it has been started the production of truncated forms of Twa1, which could be more prone to crystallize, or to be studied by nuclear magnetic resonance. Moreover studies are being carried out to confirm, as well as how this interaction can result in the development of cancer.