Computational Evaluation of New Homologous Down Regulators of Translationally Controlled Tumor Protein (TCTP) Targeted for Tumor Reversion

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Abstract: The Translationally Controlled Tumor Protein (TCTP) has been investigated for tumor reversion and is a target of cancer therapy. Down regulators which suppress the expression of TCTP can trigger the process of tumor reversion leading to the transformation of tumor cells into revertant cells. The present investigation is a novel protein-protein docking approach to target TCTP by a set of proteins similar to the protein: sorting nexin 6 (SNX6) which is an established down regulator of TCTP. The established down regulator along with its set of most similar proteins were modeled using the PYTHON based software - MODELLER v9.9, followed by structure validation using the Procheck Package. Further TCTP was docked with its established and prospective down regulators using the flexible docking protocol suite HADDOCK. The results were evaluated and ranked according to the RMSD values of the complex and the HADDOCK score, which is a weighted sum of van der Waal's energy, electrostatic energy, restraints violation energy and desolvation energy. Results concluded the protein sorting nexin 6 of Mus musculus to be a better down regulator of TCTP, as compared to the suggested down regulator (Homo sapiens snx6).

Key words: Translationally Controlled Tumor Protein, TCTP, tumor reversion, down regulators, sorting nexins, homology modeling, protein-protein docking, MODELLER, HADDOCK.

1 Introduction

The “Translationally Controlled Tumor Protein (TCTP)” is expressed throughout the eukaryotic world in a wide range of organisms including both animals and plants. It is named as it was first described in mouse tumor cells as a growth related protein whose synthesis is regulated at the translational level. Initially this protein was considered to be only a growth regulator (Brioudes et al., 2010; Cao et al., 2010), but now diverse functions have been attributed to it such as calcium binding activity; microtubule binding and stabilization (Bommer and Thiele, 2004; Cao et al., 2010); regulation of apoptosis, in which it functions as an anti-apoptotic factor (Susini et al., 2008; Brioudes et al., 2010). It plays a very important role in the development of normal functioning of eukaryotes, and this is the reason behind the high degree of conservation of this protein (Chen et al., 2007). Researchers have proven that the over expression of TCTP leads to alterations in cell morphology and retards cell growth (Gachet et al., 1999). It is also involved in malignant transformation and is a target of cancer therapy (Yoon et al., 2007). TCTP also functions as an essential factor for cell proliferation (Chen et al., 2007; Brioudes et al., 2010). TCTP interacts with several proteins inside the human body, some notable examples of which are tubulin protein, mammalian polo like kinase (Barr et al., 2004) (plays a role in tumorigenesis), translation elongation factors, Na-K ATPase. The fact that TCTP interacts with these proteins shows that it plays an important role in cell cycle progression (Gachet et al., 1999) and protein synthesis. TCTP is present in the cytoplasm of a cell during interphase and remains localized with tubulin during mitosis. Earlier, the synthesis of this protein was thought to be controlled at the translational level only, but subsequent research has proven that TCTP is a protein which is regulated both at the transcriptional and post-transcriptional (translational) levels, by Ca2+ concentration (Xu et al., 1999).

2 TCTP: A therapeutic target

Tumor reversion is defined as the process by which
some tumor cells lose their malignancy (Tuynder et al., 2002). It may also be defined as a biological process in which a cancer or tumor cell is transformed into a revertant cell by the activation of some specific genes and deactivation of some other specific genes (Tuynder et al., 2002) (Fig. 1). Revealing studies from last decade have reported a total of 263 genes to be involved in tumor reversion, either by getting activated or inhibited during the process (Tuynder et al., 2002). Hence at the molecular level tumor reversion can be considered as a process which involves an entire cellular reprogramming of cancer cells. Tumor reversion events are extremely rare and involve the proteins SIAH1, presenilin, TSAP6 and TCTP (Telerman and Amson, 2009; Tuynder et al., 2004).

TCTP plays an important role in tumor reversion (Zhu et al., 2008; Telerman et al., 2010). It is a potential target of cancer therapy. By down regulating the activity of TCTP, tumor can be reverted and tumor cells can be converted to revertants. Revertant cells are such cells which have acquired the molecular know-how of the mechanisms to escape malignancy. Hence they can be used to study the molecular and genetic mechanisms by which a cancer/tumor cell can escape cancer (Tuynder et al., 2004).

As mentioned earlier TCTP is a target of tumor reversion (Tuynder et al., 2004), because the down regulation of TCTP through gene silencing, either by antisense cDNA technology or by siRNA interference technology results in suppression of the malignancy, which ultimately leads to reversion of tumor. In addition, gene silencing of TCTP also inhibits growth of cancer cells and induces apoptosis in them (Gnanasekar et al., 2009). In another independent research a protein sorting nexin 6 was identified as a potential negative regulator of TCTP (Yoon et al., 2006). Research has shown that the over expression of TCTP results in an alteration of microtubular cytoskeleton (Gachet et al., 1999).

3 Methodology and approach

Protein-protein interactions form the most important factor in the performance and regulation of many physiological processes and their accompanying biochemical reactions (Lee et al., 2008; Ritchie, 2008; Szilágyi et al., 2005). Some notable examples include signal transduction, intracellular trafficking, chemical coordination etc.

As mentioned earlier, the down regulation of TCTP can trigger the process of tumor reversion. Hence we searched all possible down regulators of TCTP. The search revealed that apart from a protein called “sorting nexin 6” (Yoon et al., 2006) of Homo sapiens there’s no other established down regulator of TCTP. Hence we decided to perform protein-protein docking. In rigid or bound docking the interacting proteins are treated as rigid bodies and thus they cannot undergo conformational changes as they interact with each other (Lee et al., 2008; Parks et al., 2001). In contrast, flexible docking algorithms treat the interacting proteins as flexible bodies thereby allowing them to undergo conformational changes either in the side chains or in the backbone or in both (Bonvin, 2004). Since proteins undergo changes in their conformation while forming a complex (Betts and Sternberg, 1999), as is explained by Koshland’s induce-fit hypothesis, flexible docking is closer to the in vivo environment and is thus considered to be a better computational analysis and representation of the interaction between two proteins. Also, it is very important to include flexibility in docking if the docked structures were homology modeled (Andrusier et al., 2008), as in our analysis. Thus we have performed flexible protein-protein docking to characterize the interaction between TCTP and its established and prospective down regulators. As an investigation, our work deals with protein-protein docking and the main goal of performing this procedure is to find which molecule of the sorting nexin family can best inhibit TCTP.

4 Structure of human TCTP

TCTP in humans is encoded by an 829 bp long gene located on chromosome No. 13 (13q14), called TPT1. As per the SCOP classification for TCTP, it belongs to the class of “All beta Proteins”, “Mss-4 like” superfamily and the “Translationally Controlled Tumor Protein-Histamine Releasing Factor” family. The 3D structure of human TCTP was taken from the Protein Data Bank (PDB ID 2HR9, 1A chain). The structure of the weak calcium binding site of TCTP as well as the entire structure of this protein has been determined using multidimensional NMR spectroscopy. The complete structure of human TCTP consists of a well-folded core and a very long flexible loop, connected by a short