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Overexpression, purification, and characterization of the Hsp70.14 protein towards the discovery and development of new anti-cancer compounds

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Cancer remains one of the leading causes of death, with over 2 million cases worldwide. New therapeutic approaches are therefore under constant development, aimed at eliminating various pathways/mechanisms utilized by cancerous cells. Previous studies have investigated the molecular interaction between the Heat shock protein 70.14 (Hsp70.14) and the RING finger domain of Retinoblastoma binding protein 6 (RBBP6) to determine how this interaction contributes to the progression of cancer. Disruption of this interaction through the discovery and development of protein-protein interaction (PPI) modulators serves as one of the potential therapeutic approaches that can be used to reduce the development of cancer. Hence this present study aimed at the recombinant expression, purification, and characterization of the Hsp70.14 protein, one of the interacting partners of RBBP6. Hsp70.14 was expressed in competent Top10 E. coli cells, purified using affinity chromatography, and thereafter characterized using FTIR and Raman spectrometry. Additionally, in silico methods were used to computationally characterize and predict the structure of the protein. The results show that the protein predominantly contains hydrophilic residues, and its structure is made up of two alpha helices and three anti-parallel beta strands, which was successfully validated using a Ramachandran plot and a Qmean swiss model. FTIR results revealed a high number of carbonyl and hydroxyl groups present in the protein whilst Raman data displayed symmetric C-C stretching and CH2 twisting vibrations in the fingerprint region of the protein respectively. These characterizations provided the basis for the structural determination of the protein and the subsequent identification of the residues important in the interaction with this RING domain partner for the discovery and design of new anti-cancer biopharmaceuticals.

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