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STRUCTURAL STUDIES OF HUMAN ACIDIC FIBROBLAST GROWTH FACTOR MUTANTS TO BE USED IN ANTICANCER THERAPY

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Lectins are carbohydrate-binding proteins present ubiquitously in nature. They play a role in biological recognition phenomena involving cells and proteins. The interaction lectin-carbohydrate is highly specific, and can be exploited for the development of nanoparticles containing on their surface specific lectins that are directed to carbohydrate residues present only on malignant cells and absent on healthy ones [1].
Lectins have been found to possess several anticancer properties and they are proposed as therapeutic agents, binding to cancer cell membrane receptors, causing cytotoxicity, apoptosis and inhibition of tumor growth. Some lectins are able to prevent the proliferation of malignant tumor cells because they recognize the T-antigen (Gal β 1–3GalNAc) found specifically on the surface of tumor cells [2]. The main problem is that their use as a detection agent for the T-antigen in clinical studies is not possible because the immune system can recognize them as foreign molecules and develop an immune response.
Previous studies in our laboratory have characterized a lectin found in *Boletus edulis* mushrooms called BEL β-trefoil which has antiproliferative activity on tumor cell lines, because it contains three binding sites for the T-antigen. Unlike other lectins with this property, BEL β-trefoil shows structural homology with a human protein, acidic Fibroblast Growth Factor (FGF1) [3]. Superposition of the two structures suggests that the human protein could be mutated to contain at least one of the binding sites for the T-antigen. Such mutations should create in FGF1 the potential capacity of recognizing tumor cells with less immunogenicity than the fungal protein.
FGF1 is a mitogenic and chemotactic protein that mediates cellular functions by binding to transmembrane receptors, which are activated by ligand-induced dimerization requiring heparin as co-receptor.
To reach our purpose FGF1 cDNA was cloned into a bacterial plasmid and then mutated in two positions to prevent its binding to the natural receptor, thus suppressing its physiological activity. Loss of function was tested in fibroblast growth tests and then site-directed mutagenesis was performed in three specific positions to produce an FGF1 capable to bind T-antigen. Ligand-protein binding affinity was measured using fluorimetric and isothermal titration calorimetric techniques. Attempts to crystalize the mutants of FGF1 were made using the hanging drop technique with the final aim to carry out their structural characterization by X-ray diffraction analysis of the crystals.

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