HEPARANASE AS AN ATTRACTIVE TARGET FOR ANTICANCER THERAPEUTICS AND NOVEL DRUG DESIGN

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Cancer cells require the ability to degrade the extracellular matrix (ECM) in order to turn into invasive and metastatic cancer cells. Heparanase, is an endo-b-D-glucosidase capable of specifically degrading one of the ECM components, heparan sulfate. It has been found that heparanase expression is increased in numerous cancer processes (1, 2) and there is a direct correlation between the production of heparanase and the invasiveness of tumor cells. Furthermore, it has been reported that in serum from patients and animals carrying metastatic tumors the level of the enzyme is very high (2, 3). The implication of heparanase in cancer progression makes it a very attractive target in antitumor treatments, emphasized by the fact that it appears to be a unique enzyme, in contrast to the multiple proteases implicated in the same phenomena.

The practical utility of heparanase, in relation to the design of inhibitors for tumor treatment, has been limited by the absence of a 3D structure of the enzyme. In this sense, the possibility of identifying independent domains of smaller size would allow the structural study of each of them by Nuclear Magnetic Resonance. Thereby, one of the aims of the present work is the determination of the three-dimensional structure of the heparanase enzyme in solution through NMR techniques. The delineation of the structure will provide insights about the mechanisms of binding to the substrate, about the catalytic activity of heparanase and will facilitate the design of inhibitors of the interaction between heparanase and HS.

To undertake this task, we have worked intensively in the identification of a heparanase construct suitable for NMR studies. The behaviour of heparanase in solution is not ideal for NMR studies due to its low solubility. The use of detergent during the lysis and purification process has been necessary to avoid the precipitation of the protein. Additionally, we have assayed an extensive range of conditions (pH, buffers, temperature) in order to achieve optimal solubility, concentration and stability of the sample. The presence of detergent does not preclude the interaction between heparanase and known inhibitors of the enzyme such as suramin or heparin. This interaction is specific and selective, validating this heparanase construct as target for the search of new inhibitors.

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