

Structural studies on the urokinase plasminogen activator receptor (uPAR), a key mediator of cancer invasiveness. L. Iuzzolino^a, A. Andolfo^b, N. Sidenius^b, F. Blasi^c & M. Degano^a. ^a*Biocrystallography Unit and* ^c*Molecular Genetics Unit, DIBIT San Raffaele Scientific Institute, Milan, Italy, and* ^b*IFOM FIRCC Institute of Molecular Oncology, Milan, Italy.*

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Plasminogen activators, their inhibitors, and their cell surface receptors play a key role in many physiological and pathological processes like cell migration, tissue remodelling, cancer invasion and metastasis [1]. The urokinase Plasminogen activator (uPA) can bind to and cleave the specific high-affinity receptor uPAR. uPAR is composed of three homologous domains (D1, D2 and D3) and is linked to the cell surface through a glycosyl phosphatidylinositol (GPI) anchor. After binding to uPA and release of the aminoterminal fragment D1, uPAR is converted to its active form, the D2D3 fragment, endowed with high chemotactic activity [2]. Using model systems, many laboratories have demonstrated that the inhibition of the uPA activity or the block of the uPA/uPAR interaction, lead to a decrease of the invasive properties of tumors [3, 4]. The determination of the X-ray structure of the full receptor and of its activated form (D2D3) is the key-point to understand the urokinase mediated activation and the structural basis of the functional diversity between both forms. Moreover knowledge of the three-dimensional architecture of uPAR, together with the known structure of uPA [5, 6] and the complete structural characterization of uPA-uPAR interaction is expected to aid understanding the basis of tumor invasiveness. The structures will represent a framework for the rational design of receptor antagonists that may lead to potential drug candidates. We successfully expressed in High Five cells recombinant baculovirus for the soluble variant of uPAR and D2D3, and for the aminoterminal fragment of uPA, which is the binding domain to uPAR. We obtained milligrams of soluble, highly purified proteins combining affinity and gel filtration chromatography. Using ELISA test and gel filtration chromatography we verified that the receptor expressed in insect cells is functional with respect to its ability to interact with uPA. After mixing uPAR and ATF in molar ratio 1:1 the complex formation is quantitative. We monitored the absence of aggregated protein using gel filtration and dynamic light scattering (DLS) at the working concentrations. The good DLS profile was predictive of high probability for the protein to crystallize but the quality of the crystals is still not satisfying. A possible hindrance for crystallization could be heterogeneous glycosylation. Enzymatic deglycosylation with PNGase F and inhibition of glycosylation with tunicamycin did not yield protein suitable for crystallization. Therefore we introduced in uPAR selective mutation of asparagine residues carrying N-linked glycosylation to glutamine to completely remove glycosylation. After characterization of the quality and activity of the recombinant protein we will proceed for binding studies and further crystallization trials.

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