Interaction of murine cathepsin B and DARPin and its prospects

P-02.3-06

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Cysteine cathepsins are lysosomal proteases that influence many cellular processes. They are activated by other active cathepsins in the lysosomal acidic milieu by cleavage of the propeptide. They are essential for homeostasis in healthy tissue and are involved in processes such as bone remodeling, antigen processing and presentation, hormone processing, and overall protein turnover. Their aberrant expression and activity play major roles in many pathologies including in the progression, invasion, and metastasis of solid tumors. Among them, cathepsin B (CtsB) has been the most studied in the context of cancer. During cancer progression, it can be found at the surface of and in membrane invaginations of tumor cells where it was linked with extracellular matrix degradation and metastasis spread. Designed ankyrin repeat proteins (DARPins) are genetically engineered antibody mimetic proteins based on natural ankyrin proteins. DARPins can be used as diagnostic or therapeutic agents but also as agents for co-crystallization due to their high selectivity and affinity for the selected target. We designed a new inhibitory DARPin (4m3) that shows a high affinity for murine, but not human, cathepsin B. Currently, the structure of murine cathepsin B remains unresolved due to lack of success in crystallization efforts but DARPin 4m3 could be used for chaperone-assisted crystallization since its binding is expected to sufficiently stabilize murine CtsB. Due to similar biochemical and physiological properties between human and murine CtsB it is sensible to resolve the structure of murine variant which could help in structure-based drug design. Additionally, the resolved structure could help understand the potential difference between binding of antibodies and related proteins to murine and human CtsB, which could be helpful in the design of human CtsB-targeting molecules.