THE HEMOPEXIN DOMAIN AS A NEW TARGET FOR DRUG DISCOVERY

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Matrix metalloproteinases (MMPs) are zinc-dependent enzymes which constitute a family (23 in human) within the metzincin clan of metalloendopeptidases.[1] They break through the underlying basement membrane in order to intravasate into surrounding blood or lymphatic vessels promoting tumor angiogenesis, and facilitating cancer cell migration and metastasis.

The human membrane type-I matrix metalloproteinase (MT1-MMP) can degrade a number of ECM macromolecules such as collagens, gelatin, laminins, fibronectin, vitronectin, aggrecan, fibrin and lumican; MT1-MMP also activates pro-MMP2 and pro-MMP13, and is implicated in protein-protein interactions (e.g., CD44).[2] Thus MT1-MMP is one of the factors that influence the cellular microenvironment and thereby affect cell-signaling pathways and eventually alters cellular behavior.

Cancers with metastatic spread are frequently resistant to conventional chemotherapeutic approaches, underlining the urgent need for novel treatment in these diseases. As it is notable that the hinge region is critical for MT1-MMP-mediated pro-MMP2 activation, and MT1-MMP mutants without the hemopexin domain (HPX) completely lost their ability to promote cell invasion into type-I collagen matrix; we have chosen a construct based on the hinge and the HPX to evaluate the protein druggability.

The aim of this project is to solve the 3D structure in solution of a protein construct containing the linker region and the HPX domain of MT1-MMP (MMP14). To this end, we have designed the protocol for expressing and purifying this construct and we have been able to obtain the soluble and folded protein, both in LB and minimal media. We have acquired a set of NMR triple resonance experiments for sequence specific assignment, using both single (^{15}N) and double labelled samples (^{13}C, ^{15}N), and for structure determination. Currently, we are working on the assignment of the protein resonances and performing ^{15}N relaxation studies. The final goal would be to exploit this information to identify inhibitors of protein-protein interactions involving the HPX domain.

REFERENCES

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