Chapter 2: High yield bacterial expression, purification and characterisation of full-length, bioactive human tousled-like kinase 1B involved in cancer

2.1 Abstract

Human Tousled-like kinases (TLKs) are highly conserved serine/threonine protein kinases responsible for cell proliferation, DNA repair, and genome surveillance. Their possible involvement in cancer via efficient DNA repair mechanisms have made them clinically relevant molecular targets for anticancer therapy. Innovative approaches in chemical biology have played a key role in validating the importance of kinases as molecular targets. However, the detailed understanding of the protein structure and the mechanisms of protein-drug interaction through biochemical and biophysical techniques demands a method for the production of an active protein of exceptional stability and purity on a large scale. In this chapter, we report the design of a bacterial expression system to express and purify biologically active, wild-type Human Tousled-like Kinase 1B (hTLK1B) by co-expression with the protein phosphatase from bacteriophage λ. We have obtained remarkably high amounts of the soluble and homogeneously dephosphorylated form of biologically active hTLK1B with our unique, custom-built vector design strategy. The recombinant hTLK1B can be used for the biochemical assays and structural studies and may further facilitate the development of new TLK inhibitors for anti-cancer therapy using a structure-based drug design approach.

Chapter 3: Evaluating the therapeutic viability of bacterially expressed human TLK1B-kinase domain for cancer drug design

3.1 Abstract

Protein kinases act as crucial players in the fundamental functions of the cell. Frequent alterations in the expression patterns of protein kinases often implicate human cancer initiation and progression. Treatment with small-molecule inhibitors targeting serine/threonine kinase signalling pathways to inhibit malignant transformation has shown significant promise in clinical cancer therapy. Hence, the new kinase drug development programmes have extended their search to identify a wide range of novel kinase targets within the family. Human Tousled-like kinases (TLKs) are an evolutionarily conserved family of serine/threonine kinases involved in DNA replication, transcription, DNA damage and repair, and chromosomal stability. The direct association of TLKs to cancer; amplification of both *TLK1/1B* and *TLK2* have made them viable molecular targets for anticancer therapy. Several reports demonstrate numerous functions of TLKs in development and disease via different interacting partners. However, a detailed understanding of its substrates and regulation has yet remained elusive. In this chapter, through preliminary biophysical and biochemical characterisation, we investigate and determine the usability of recombinant Human Tousled-like Kinase 1B_Kinase Domain (hTLK1B_KD) purified from *Escherichia coli* for structural, functional and first-stage *in-vitro* drug candidate screening studies. By illustrating hTLK1B_KD as an example, our attempts to generate a stable, homogenously dephosphorylated and catalytically active hTLK1B_KD in high yields utilising a bacteriophage λ protein phosphatase (LPP) coexpression system represents a fundamental step towards the structure-based design of TLK-specific inhibitors.

Chapter 4: Biological evaluation of new phenothiazine inhibitor-J54 with potent anti-TLK1B activity for prostate cancer therapy

4.1 Abstract

Specific phenothiazines (PTHs) were previously identified in a library screen as the inhibitors of TLK1B. Through the *in-vitro* kinase assays and molecular docking studies, we now report on the synthesis and biological evaluation of a new phenothiazine scaffold-J54, with a potent TLK1B inhibitory activity for the treatment of prostate cancer (PCa) still responsive to the androgen-deprivation therapy (ADT). Most PCa deaths result from the progressive failure in the standard of care, ADT, leading to the metastatic castration-resistant PCa (mCRPC). Treatments that can suppress the conversion to mCRPC have the best potential to be rapidly implemented into the clinics. ADT results in the increased expression of TLK1B, an essential kinase upstream of NEK1 and ATR, thereby, mediating the DNA-damage response (DDR) that typically results in a temporary cell-cycle arrest of the androgen-responsive PCa cells. *Singh et al.* (2019) recently established the existence of an ADT>TLK1B>NEK1>ATR>Chk1, DDR checkpoint pathway, while its abrogation leads to apoptosis. In this chapter, we studied J54 as a new and potent inhibitor of this axis, and as the mediator of apoptosis *in-vitro* and in the LNCaP xenografts, which has potential for clinical investigation as a PCa treatment in combination with ADT. J54 appears to have low affinity for the dopamine receptor (DR2) in the modelling studies and has weak detrimental behavioural effects in the mice and *C. elegans* models.