P863 GENERATION OF A FIRST-IN-CLASS INHIBITOR FOR THE MASTER ONCOREGULATOR HNRNP K IN MULTIPLE MYELOMA

Topic: 13. Myeloma and other monoclonal gammopathies - Biology & Translational Research

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Background: Haematological malignancies constitute a plethora of different neoplasms including leukaemia, lymphomas and myeloma. Despite all of them having robust first-line treatments and a myriad of therapeutic alternatives, some remain incurable due to the resistance and refractoriness of tumour cells. The heterogeneous nuclear ribonucleoprotein K, hnRNP K, is a master oncoregulator that binds C-rich tracks of nucleic acids and is implicated in multiple biological functions such as splicing, polyadenylation and translation. Indeed, hnRNP K contributes to treatment resistance and poor outcomes in haematological malignancies. Compared to other hnRNPs, hnRNP K has the unique structural characteristic of containing three K-homology (KH) domains that allows it to bind both ssDNA and RNA sequences, as well as a K-interactive (KI) region that binds to multiple critical proteins such as Src, Fyn or Lyn amongst others. Interestingly, hnRNP K regulates the p53/p21 and c-Myc pathways, and our group has characterised hnRNP K as a driver of leukaemia so far, thus proving its role in haematological neoplasms.

Aims: We aim to develop a first-in-class inhibitor for hnRNP K and test its efficacy in circumventing resistance of haematological malignancies.

Methods: We have produced full-length (FL) hnRNP K protein in-house and verified that it was properly folded and could bind RUNX1 RNA and mRNA sequence-based ssDNA, using Tycho and fluorescence anisotropy technologies. We then carried out a high-throughput screen of over 7,000 small molecules (both FDA and non-FDA approved) using AlphaLISA technology and validated both chemically and biologically the inhibition of hnRNP K with those identified compounds. Lastly, we have performed phenotypical analysis of the effects of the hnRNP K inhibitors in Multiple Myeloma cell line AMO1 and L363 overexpressing hnRNP K through CRISPR synergistic activation mediator (SAM).

Results: We have successfully produced and purified the FL hnRNP K protein, with stable inflexion temperatures (Ti) as measured by Tycho. Furthermore, we corroborated that it maintained its ssDNA and RNA binding abilities through fluorescence anisotropy. Lastly, we set up the AlphaLISA system to screen a library of over 7,000 small molecules and identified circa 10 interesting hits, from which we validated the 5 best based on their inhibition capacity and specificity of hnRNP K. We verified the chemical properties of these small molecules. Lastly, we confirmed that they had a biological effect of decreasing the levels of hnRNP K downstream pathways as well as inducing changes in viability dose-response curves, using Multiple Myeloma cells (AMO1 and L363) genetically modified with CRISPR/SAM technology to overexpress hnRNP K. Likewise, we investigated the absence of such biological effect in hnRNP K-Knock Out (KO) AMO1 and L363 cells, obtained by CRISPR-Cas9.

Image:
Summary/Conclusion: We have identified a first-in-class hnRNP K inhibitor that specifically binds and blocks hnRNP K in modified Multiple Myeloma cell lines. This constitutes a novel and promising therapeutic approach that could help overcome the current drug resistance in haematological neoplasms currently found in the clinic.

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