Elucidating the molecular action of synthetic heat shock protein 90 inhibitors in prostate cancer

by

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Abstract

One of the most common causes of cancer-related deaths for men in the developed world is prostate cancer (PCa), occurring in 1 in 5 men by the age of 85 years. Despite significant improvements in overall survival and quality of life for men suffering from PCa, approximately 10-20% of patients inevitably develop incurable castration resistant prostate cancer (CRPC). Although the androgen receptor (AR) remains a key player in disease progression, PCa is also known to depend on multiple other protein pathways. Therapies targeting the molecular chaperone Hsp90 offer advantages for the treatment of PCa as they are capable of targeting multiple protein pathways involved in PCa progression. Specifically, Hsp90 is responsible for the stabilisation, maturation and activation of over 300 client proteins, many of which are involved in PCa. First-in-class Hsp90 inhibitors, such as 17-allylamino-demethoxygeldanamycin (17-AAG), were ineffective in clinical trials however, new rationally-designed synthetic Hsp90 inhibitors, such as AUY922, have shown greater potency than 17-AAG in both cell lines and animal models. Our laboratory previously demonstrated only AUY922 and not 17AAG or DMSO is capable of reducing proliferation and inducing apoptosis in cell lines and tumour tissues cultured as explants.

The intention of this thesis was to elucidate differential molecular mechanisms of action of Hsp90 inhibitors to better understand the basis of their improved efficacy and assist in streamlining future development of inhibitor compounds for PCa. Proteomic analysis of human prostate tumour tissues cultured as explants revealed key proteins differentially regulated in response to AUY922 vs 17-AAG and DMSO and implicated cytoskeletal organization as a significant pathway involved in AUY922 efficacy. Specifically, AUY922 inhibits secretion of fibronectin due to degradation of microtubule networks which results in inhibition of secretory vesicle trafficking. This insight into the molecular mechanism of action underlying AUY922 anti-tumour activity in PCa provides a basis for further investigation into the role that AUY922 associated cytoskeletal organisation plays in inhibitor efficacy in PCa.

Investigation of mechanisms of Hsp90 inhibitor resistance focussed on examining the involvement of cytoprotective chaperone proteins Hsp27 and Hsp70, commonly upregulated in response to Hsp90 inhibitors. The techniques of co-immunoprecipitation and the Duolink *In Situ* system were optimised for use in evaluating the chaperone requirements of wild-type and variant forms of the AR, as AR variants do not contain an Hsp90 binding site and are therefore implicated in potential mechanisms of inhibitor resistance. Due to factors beyond control, only basic optimisation of these techniques was possible and further research will be required to test this hypothesis. Interestingly, use of a new series of Hsp90 inhibitors, SM253

and SM258, for the treatment of PCa cell lines and tumour explant tissues demonstrated promising pre-clinical efficacy without inducing expression of Hsp27 or Hsp70. Overall, the results provided in this thesis afford a better understanding of cellular response to specific inhibitors which will result in improved biomarkers and aid in improving our foresight into potential mechanisms of resistance which can speed the process of discovery of compounds that may be promising in the clinic.

Declaration

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