Targeting YB-1 controls drug response via distinct mechanisms in malignant pleural mesothelioma

Thomas Johnson1,2,3, Karin Schelch1,2, Kadir Sarun1, Yuen Y Cheng1,2, Annette Lasham4, Nico van Zandwijk2, Glen Reid1,2
1The Asbestos Diseases Research Institute, Sydney; 2The University of Sydney, 3Sydney Catalyst, 4University of Auckland

Introduction
Malignant pleural mesothelioma (MPM) is an aggressive malignancy and current therapy is essentially limited to palliative care. Y-box binding protein-1 (YB-1) is a multifunctional oncoprotein widely accepted to play a role in the growth of many tumours. High expression of YB-1 is associated with poor patient outcome in tumours including NSCLC and is related to increased chemoresistance. YB-1 has been implicated in suppressing apoptotic pathways in breast cancer and disrupting the cell-cycle via transcriptionally regulating cyclins A, B1 and D1 in multiple other malignancies. Here we investigate the mechanisms behind the role of YB-1 in MPM cell growth and subsequent effects on drug resistance.

Results
(I) YB-1 is frequently overexpressed in MPM cell lines
YB-1 protein was over-expressed in most MPM cell lines compared to the immortalised mesothelial cell line MeT-5A. Heightened YB-1 expression was also seen in tumour tissue from patients suffering MPM compared to nearby healthy tissue (B).

(II) YB-1 knockdown inhibits growth in MPM cell lines, but not always through apoptosis
siRNA-mediated YB-1 knockdown significantly inhibited the growth of 2 MPM cell lines over 120 hours. An increase in early and late apoptotic MSTO cells was observed in response to YB-1 knockdown 72 hours after transfection, but no change was seen in VMC23 cells, using TALI apoptosis assays.

(III) YB-1 knockdown causes G0-G1 cell cycle arrest in MPM cells that do not undergo apoptosis
VMC23 cells displayed an increase in cells in G0-G1 after YB-1 knockdown compared to controls, while MSTD cells showed an increase in subG1 population, consistent with the data in Fig. II.

(IV) YB-1 knockdown either decreases or increases innate MPM drug resistance, depending on the mechanism of YB-1 growth inhibition
MSTD cells (which undergo apoptosis in response to YB-1 knockdown) were sensitised to cisplatin and vinorelbine over 96 hours after transfection with YB-1 siRNA, whereas VMC23 exhibits an increase in resistance to these chemotherapy drugs, likely due to YBX1-siRNA induced G0-G1 cell cycle arrest.

(V) YB-1 is overexpressed in MSTO MPM cells with acquired chemoresistance
YB-1 protein was expressed at higher levels in MSTO cells with acquired resistance to cisplatin, gemcitabine and vinorelbine compared to parental MSTO cells.

Conclusions and future directions
Here we show that downregulating the Y-box binding protein-1 inhibits MPM cell growth via the induction of either apoptosis or G0-G1 cell cycle arrest. Cells undergoing YB-1 knockdown induced apoptosis are sensitised to cisplatin and vinorelbine treatment, while those arresting at G0-G1 show increased resistance. We also found that YB-1 is overexpressed in cell lines with acquired drug resistant, suggesting it plays an important role in both the innate and acquired chemoresistance of MPM cells. In the future, The mechanisms behind the anti-apoptotic and cell-cycle driving activity of YB-1 will be investigated. Additionally, ethics approval is in place to conduct YB-1 knockdown experiments alone or in combination with chemotherapy in an in vivo intraperitoneal mouse model using MPM cells.

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Contact details
E: tjoh9110@uni.sydney.edu.au
T: +61430843711