Probing the effects of Retinoblastoma Binding Protein 6 (RBBP6) knockdown on the sensitivity of cisplatin in cervical cancer cells

Harshini Mehta[1†], Melvin Ambele[2,3], **Pontsho Moela[1†*]**

[1]Division of Genetics, Department of Biochemistry, Genetics and Microbiology, Faculty of Natural and Agricultural Sciences, University of Pretoria, Hatfield, Pretoria, 0002, South Africa

[2]Institute for Cellular and Molecular Medicine, Department of Immunology and SAMRC Extramural Unit for Stem Cell Research and Therapy, Faculty of Health Sciences, University of Pretoria, Prinshof, Pretoria 0001, South Africa

[3]Department of Oral Pathology and Oral Biology, School of Dentistry, Faculty of Health Sciences, University of Pretoria, Prinshof, Pretoria 0001, South Africa

***Correspondence: Pontsho Moela pontsho.moela@up.ac.za**

†These authors contributed equally to this work and share first authorship

RBBP6 is considered a potential cancer biomarker due to its association with cell proliferationand the fact that it is overexpressed at cervical cancer sites where there is marked apoptosis and elevated p53. More information is emerging regarding the role of RBBP6 in cancer treatment, specifically its potential to sensitize cancer cells to radiation and certain chemotherapeutic agents via BCL-2 gene regulation. Cisplatin is an FDA-approved chemotherapeutic agent that still presents with acquired resistance in certain cervical cancer cases through p53 repression and BCL-2 upregulation, which is why there is ongoing researchin trying to understand the molecular mechanisms involved in the response of cervical cancer cells to this drug. The present study therefore aims to investigate the possible relationship between cisplatin and RBBP6 expression in cervical cancer cells. RBBP6 was silenced in Hela,CaSki and Vero cells using the RNAi technology, followed by measurement of wild-type p53and BCL-2 at mRNA level using qPCR. Cells co-treated with cisplatin and siRBBP6 were therefore analyzed for apoptosis induction and real time growth monitoring using flow cytometry and the xCELLigence system, respectively. Cancer cells in the co-treatment group showed a reduction in apoptosis compared to cisplatin-only group and real time growth monitoring revealed a reduced growth rate in RBBP6-knockdown cells treated with cisplatin. Although wild-type p53 remained unchanged in the co-treatment group of cancer cells, BCL- 2 was completely repressed, suggesting that RBBP6 is necessary for the responsiveness of cervical cancer cells to via BCL-2 downregulation. Findings from this study suggest that *RBBP6* expression promotes sensitivity of HeLa cells to cisplatin through BCL-2downregulation. Knockdown of *RBBP6* limits apoptosis induction and delays cell growth inhibition in response to cisplatin. The knowledge obtained here has the potential to help improve cisplatin efficacy through personalized administration based on the expression profileof RBBP6 among individual patients.

Keywords: RBBP6, p53, BCL-2, Cisplatin, HeLa, Vero, Apoptosis Abstract