UNIVERSITI PUTRA MALAYSIA

EFFECTS OF BORTEZOMIB ON HIF-1 AND HIF-2 TRANSCRIPTIONAL ACTIVITIES

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MASTER OF SCIENCE
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By

NORAINI BINTI ABD AZIZ

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Master of Science

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

EFFECTS OF BORTEZOMIB ON HIF-1 AND HIF-2 TRANSCRIPTIONAL ACTIVITIES

By

NORAINI BINTI ABD AZIZ

November 2013

Chairman : Norazizah Shafee, PhD

Faculty : Biotechnology and Biomolecular Sciences

Bortezomib is the first proteasomal inhibitor (PI) to be used therapeutically in humans for treating relapse cases of multiple myeloma and mantle cell lymphoma. A proposed mechanism is that it prevents proteasomal degradation of pro-apoptotic proteins, leading to enhance apoptosis. Although the alpha subunit of hypoxia inducible factor 1 (HIF-1α) is not degraded, the heterodimeric HIF-1 fails to transactivate target genes. HIF-1 and HIF-2 are related hypoxia-inducible transcription factors that are important for survival of hypoxic tumor cells. Most reports have focused on the effects of bortezomib on HIF-1 but not HIF-2 transcriptional activities. In the present study, the effect of bortezomib on

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HIF-2 activity in cells with different levels of expression of the HIF-1α and HIF-2α subunits, was investigated. Results showed that bortezomib treatment suppressed the transcription and expression of *CA9*, a HIF-1-specific target gene, but had minimal effects on *EPO* and *GLUT-1*, which are the target genes of both HIF-1 and HIF-2. A similar dichotomy of responses was also seen with exogenously-introduced hypoxia response elements of *CA9* and *EPO*. These data led to a conclusion that bortezomib attenuates the transcriptional activity of only HIF-1 but not HIF-2. This novel finding on the lack of inhibitory effect of bortezomib on HIF-2 transcriptional activity will be important in the improvement of design and treatment modalities to enhance the efficacy of this and other proteasomal inhibitor drugs.

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Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

KESAN BORTEZOMIB TERHADAP AKTIVITI TRANSKRIPSI HIF-1 DAN HIF-2

Oleh

NORAINI BINTI ABD AZIZ

November 2013

Pengerusi : Norazizah Shafee, PhD

Faculty : Bioteknologi dan Sains Biomolekul

Bortezomib adalah perencat protesom yang pertama digunakan secara terapeutik pada manusia bagi merawat kes-kes berulang seperti mieloma berbilang dan limfoma sel mantel. Satu mekanisma yang dicadangkan adalah bortezomib berupaya menghalang degradasi protesom protein-protein pro-apoptosis yang menyebabkan peningkatan apoptosis. Walaupun subunit faktor induksi hipoksia 1α (HIF-1α) tidak didegradasi, heterodimer HIF-1 gagal untuk mengaktifkan gen sasaran. HIF-1 and HIF-2 adalah faktor induksi transkripsi hipoksia yang penting untuk kelangsungan hidup sel-sel tumor hipoksia. Banyak laporan terdahulu memberi tumpuan kepada kesan bortezomib terhadap aktiviti transkripsi HIF-1 sahaja, tetapi tidak HIF-2. Dalam kajian ini, kesan

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bortezomib terhadap aktiviti HIF-2 di dalam sel-sel yang mempunyai tahap ekspresi HIF-1 dan HIF-2 yang berbeza telah dikaji. Hasil yang diperoleh dalam kajian ini menunjukkan bahawa rawatan menggunakan bortezomib dapat merencatkan transkripsi dan ekspresi *CA9*, iaitu gen sasaran khusus bagi HIF-1. Walaubagaimanapun, ia mempunyai kesan minimum terhadap *EPO* dan *GLUT-1*. Tindak balas yang sama juga telah dilihat dalam unsur respon hipoksia yang dibawa secara eksogen bagi *CA9* dan *EPO*. Data-data ini membawa kepada kesimpulan bahawa bortezomib hanya merencatkan aktiviti transkripsi HIF-1, tetapi tidak HIF-2. Pengetahuan mengenai kekurangan kesan bortezomib terhadap aktiviti transkripsi HIF-2 boleh menjadi penyumbang ke arah strategi untuk peningkatan keberkesanan reka bentuk serta kaedah rawatan menggunakan dadah ini atau perencat protesom yang lain.

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I certify that a Thesis Examination Committee has met on 7 November 2013 to conduct the final examination of Noraini binti Abd Aziz on her thesis entitled "Effects of Bortezomib on HIF-1 and HIF-2 Transcriptional Activities" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently submitted for any other degree at Universiti Putra Malaysia or at any other institutions.

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