The effects of the histone deacetylase inhibitor, LBH589, on breast cancer in bone and on physiological bone remodelling

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Declaration

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Awards and publications arising from candidature

Awards

2011 **South Australian Young Woman of the Year Award Recipient**
South Australian Reunion Group of the Women of the Year Association

2010 **Prime Minister's Australia Asia Endeavour Award Recipient**
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University of Adelaide, Australia

Published abstracts from presentations to professional societies

M. Lee, G. Yue, E. Ko, V. Ponomarev, A. Evdokiou, P.C. Leung and C. Lau. *In vivo* investigation on the value of Chinese herbs in the management of bone metastasis. 2010 International Conference on Integrative Medicine Against Cancer cum The 6th Pong Ding Yuen International Symposium on Traditional Chinese Medicine, Nov. 26-28 2010, Hong Kong, SAR, China.

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Abbreviations

µ-CT	micro-computed tomography
ALP	alkaline phosphatase
AR	androgen receptor
BFR	bone formation rate
BLI	bioluminescence
BV	bone volume
CTCL	cutaneous T-cell lymphoma
Dkk	dickkopf
FAT	factor acetyltransferases
GCT	giant cell tumour
HAT	histone acetyltransferases
HDAC	histone deacetylase
HDI	histone deacetylase inhibitor
MAR	mineral apposition rate
MM	multiple myeloma
MS	mineralising surface
NHB	normal human bone
OCN	osteocalcin
OPG	osteoprotegerin
PBMC	peripheral blood mononuclear cells
RANKL	receptor activator of nuclear factor-κB ligand
SEM	scanning electron microscope
SRE	skeletal related event
Tb.Th	trabecular thickness
Tb.N	trabecular number
Tb.Sp	trabecular separation
TRAP	tartrate-resistant alkaline phosphatase

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Abstract

Histone Deacetylase Inhibitors (HDIs) are emerging as an exciting new class of potential anticancer agents for the treatment of solid and haematological malignancies. Despite the infancy of the field, there is now an impressive body of data describing the ability of these molecules to modulate a wide variety of cellular functions, including cell differentiation, cell cycle progression, apoptosis, cytoskeletal modifications, and angiogenesis. Over the past few years, results obtained from clinical trials and pre-clinical animal experiments demonstrate the ability of HDIs to selectively kill cancer cells with limited or no toxicity to normal tissues and organs. Amongst the HDIs now in clinical trials, SAHA (Vorinostat) is the first of its class to get approval from the U.S.A. Food and Drug Administration (FDA) for the treatment of cutaneous manifestations in patients with cutaneous T-cell lymphoma (CTCL). Although a number of early-phase clinical trials using different HDIs have demonstrated promising antitumour responses for a variety of cancer types, the effect of HDIs on skeletal malignancies, or the consequence of HDI treatment on the bone microenvironment, and in the context of osteoclast and osteoblast function, has not been reported. The studies undertaken in this thesis aimed to:

1). Investigate the anticancer efficacy of the HDI LBH589 in animal models of primary breast cancer, and on bone destruction caused by breast cancer growth in bone.

2). Investigate the effects by which LBH589 regulates normal bone metabolism in the context of osteoclast and osteoblast function both *in vitro* and *in vivo*.

In vitro, LBH589 treatment resulted in a dose and time-dependent increase in apoptosis in a panel of well established breast cancer cell lines. This was associated with the processing and activation of caspases-8, and -3, concomitant with the

activation of the Bcl_2 family protein, Bid and cleavage of the apoptosis target protein, PARP. LBH589 treatment leads to a marked increase in acetylated histone-H3 and induction of the p21 protein.

The highly aggressive MDA-MB231-TXSA human breast cancer cell line was used to evaluate the antitumour activity of LBH589 in murine models of breast cancer development and progression at both the orthotopic site and in bone. MDA-MB231-TXSA cells form aggressive, rapidly growing tumours when injected into the orthotopic site of the mammary fat pad of nude mice, and stimulate the formation of osteolytic lesions when injected into the tibial marrow cavity of nude mice. MDA-MB231-TXSA breast cancer cells were tagged with a triple reporter gene construct, which allows real-time monitoring of tumour growth in live animals. Tumour progression with and without LBH589 treatment was monitored in live animals, and in real-time using bioluminescence imaging (BLI). The development of breast cancer-induced osteolysis was measured using high resolution μ-CT and histology.

In vivo, LBH589 had no effect on tumour growth in the mammary fat pad or in bone, as demonstrated by BLI and histology. However, high resolution μ-CT analyses of the tibiae demonstrated significant protection from breast cancer-induced osteolysis with LBH589 treatment, associated with a marked reduction in the number of $TRAP^+$ osteoclasts lining the trabecular bone surface. Furthermore, the bone volume of the contralateral, non-tumour bearing tibiae was significantly increased with LBH589 treatment compared to the vehicle-treated group of animals. This effect was also seen in the tibiae of mice bearing mammary tumours, suggesting potential anabolic actions of LBH589.

The effect of LBH589 on osteoclast differentiation and bone resorption was then evaluated using three independent *in vitro* models of osteoclastogenesis. When human peripheral blood mononuclear cells and the RAW264.7 murine monocytic cells were cultured with the receptor activator of nuclear factor kappa B-ligand (RANKL), both formation of $TRAP^+$, multinucleated cells and bone resorption were increased compared with control cells that were cultured in the absence of RANKL. When added in combination with RANKL, LBH589 dose-dependently inhibited RANKL-induced osteoclastic differentiation and bone resorption. Similarly, bone resorption by mature osteoclasts that were isolated from human Giant Cell Tumours of bone was also inhibited by LBH589 treatment. The effect of LBH589 was selective for osteoclast differentiation and bone resorption since cell viability was not affected. The effect of LBH589 on osteoblast function was also investigated by using mineralised bone forming primary human osteoblast cultures. When osteoblasts harvested from normal human bone donors were cultured under osteogenic conditions, LBH589 significantly enhanced the ability for these cells to produce mineral. The enhanced mineralisation was associated with significant increases in Runx2, OPG and RANKL mRNA expression. The effects of LBH589 treatment on normal bone metabolism were further investigated in animals with an intact immune system and in the absence of tumours. In these experiments, the rate of new bone formation and bone remodelling *in vivo* was investigated using the fluorescent calcium-binding dye, calcein. Longitudinal live μ-CT analysis of the tibiae in these animals showed an increase in bone volume with LBH589 treatment. The increase in bone volume was attributed to an inhibition of the number of $TRAP^+$ osteoclasts lining the trabecular bone surface, and also, to an increase in the activity of osteoblastic cells. The observed increase in bone formation rate (BFR) was

attributable to an enhanced mineral apposition rate (MAR), suggesting that the effect of LBH589 was most likely appositional, with little evidence for new bone formation.

The data presented in this thesis demonstrate a lack of anticancer efficacy of LBH589 against breast cancer growth in the mammary tissue and in bone. However, the ability of LBH589 to modulate bone metabolism by regulating osteoclast and osteoblast function indicate new and previously unrecognised osteotropic properties of LBH589. Taken together, these results suggest that LBH589 can inhibit the activities of osteoclasts, whilst promoting osteoblast activity, with limited toxic side effects, therefore potentially providing a new avenue for the treatment of skeletal conditions in which bone loss is significant.

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