B-D-1

An *in vitro* Study Examining the Effect of Sub-Lethal QS 755nm Lasers on the Expression of p16INK4a on Melanoma Cell Lines

Henry HL Chan,[#] Leihong Xiang,[#] Joseph CK Leung,[*] Kenneth WT Tsang,[@] Kar-neng Lai[*]
[#]Division of Dermatology; [*]Division of Nephrology; [@]Division of Respiratory Medicine; Department of Medicine, University of Hong Kong

Introduction: Q-switched lasers had been used in the treatment of lentigo maligna but their role remains controversial. While previous studies have addressed the change in adhesion molecule expression after sub-lethal laser damage, no study has addressed the impact of sub-lethal laser damage at a molecular level. The p16 gene has been proposed as the candidate gene for melanoma. Our objective is to examine the effect of sub-lethal laser damage on p16 expression in melanoma cell lines.

Methods: Three human melanoma cell lines – HTB 66, Sk-mel-24 (HTB 71), and G361 – were irradiated by a Q-switched 755nm Alexandrite laser at fluences that ranged from 0.85 to 2.0 J/cm^2. HTB 66 was the only cell line with significant expression of p16INK4a while the other 2 cells lines were p16INK4a negative and served as negative control. Protein and mRNA expression for p16 were assessed by flow cytometry and RT-PCR respectively.

Results: The level of p16INK4a protein in cell line HTB 66 increased significantly after laser irradiation as compared with non-irradiated cells. The level of p16INK4a protein did not change in p16INK4a-negative cell lines (Sk-mel-24 and G361). However, there was only a slight increase in the percentage of G0/G1 phase cells.

Conclusion: We concluded that sub-lethal laser damage could increase DNA damage leading to an increase in p16 expression, and such effect would be particularly undesirable for patients with p16 mutation. Further studies are warranted to examine the role of sub-lethal laser damage in inducing p16 mutation.

B-E-1

Establishment of a Mouse Model of Autoimmune Thyroiditis and Identification of Thyroiditogenic Epitpoe on Mouse Thyroid Peroxidase (MTPO)

HP Ng, AWC Kung
Department of Medicine, University of Hong Kong, Queen Mary Hospital, Hong Kong

Autoimmune Thyroid Disease (AITD) is a common condition affecting mostly female subjects. Thyroid peroxidase (TPO) is a well-characterized autoantigen in AITD. Autoantibodies and autoreactive T lymphocytes to TPO are believed to play a major role in the pathogenesis of Hashimoto's Thyroiditis (HT). To understand the mechanism of AITD and the role of TPO, we attempted to establish a mouse model of HT by immunizing C57Bl/6(H-2^b) x CBA (H-2^k) F1 mice with recombinant mTPO (rmTPO). rmTPO ectodomain 1-837 was produced from E. coli using pGEX-4T-1 expression vector. To establish the mouse model of HT, mice were immunized with 10μg rmTPO in Complete Freund Adjuvant. Antibodies against mTPO were detected in all mice. At day 50, thyroiditis with infiltration of mononuclear cells and destruction of thyroid follicles were seen in 3 out of 5 mice. Moreover, 4 mice showed decreased total T4 level relative to 88 ± 14 pmol/l (mean ± SD) of the control animals. To identify the thyroiditogenic epitope, animals were immunized with a synthetic mTPO peptide 587-606. This peptide sequence corresponded to human TPO 599-617, which is a critical part of the conformational epitope within the human TPO molecule. Antibodies were detected in all animals immunized with the mTPO peptide, however the titer was much weaker when compared to that of mouse immunized with rmTPO ectodomain. Thyroiditis with infiltration was seen in 4 out of 6 animals, but there was no follicular destruction. Immunotyping of the cellular infiltrate showed predominantly CD4+ T cells and B220+ B cells. None of the control mice immunized with adjuvant alone developed antibodies and thyroiditis. In conclusion, a model of HT induced with mTPO was established and peptide 587- 606 was identified to be one of the thyroiditogenic epitopes on mTPO.