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Author(s)	Lau, TYH; Liao, X; Shum, DKY; Cheung, A; Tipoe, GL
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NUCLEAR AND CELL MEMBRANE SURFACE AREA ALTERATION IN HEXAMETHYLENE BISACETAMIDE (HMBA) INDUCED HUMAN COLONIC CARCINOMA CELL LINE (LOVO)

T.Y.H. Lau, X. Liao*, D.K.Y. Shum*, A.L.M. Cheung, G.L. Tipoe
Department of Anatomy and Department of Biochemistry*, The University of Hong Kong, Hong Kong.

The aim of the present study was to determine the effect of HMBA on the morphology of the nuclear and cell membrane of LOVO (CCL-229) using electron microscopical and stereological techniques. 2mM and 4mM of HMBA were included in the culture medium for a period of 7 days and then for a further 3 days HMBA-containing medium was omitted. Control flasks were never exposed to HMBA. Cell numbers were counted on days 0,1,3,5,7 and 10 using a haemocytometer and cells were processed for electron microscopy. Inhibition of the growth of CCL-229 cells was produced by 2mM and 4mM HMBA treatment during the first 7 days of culture but growth resumed after HMBA was withdrawn on day 7 and there was an increase in cell number until day 10. The nuclear to cytoplasmic ratio (N/C) and surface-to-volume ratio (S/V) of the nuclear and plasma membranes were estimated using simple point-counting techniques; the nuclear volume (V_N) was estimated from point-sampled intercepts. From these data cytoplasmic volume (V_{CYT}), cell volume (V_{CELL}), surface area of plasma membrane ($S_{PM,CELL}$) and nuclear membrane ($S_{NM,N}$) were calculated. Statistical analyses revealed significant increases in V_{CELL} and V_{CYT} with a decrease in N/C in HMBA-treated group on day 7 when compared with control, but no significant alterations in V_N. The values of V_{CYT} and N/C were maintained up to day 10 despite the absence of HMBA. The $S/V_{NM,N}$ significantly decreased in 4mM HMBA-treated cells on day 1 and with 2mM HMBA-treated group on day 7 when compared with the control. The $S/V_{PM,CELL}$ also decreased in both HMBA-treated group on day 1 and in the 2mM HMBA-treated group on day 3 when compared with the control. Generally, $S_{PM,CELL}$ progressively decreased in the control group from day 1 to day 10 and had lower values when compared with both HMBA-treated groups. The $S_{PM,CELL}$ significantly increased in cells treated with 2mM and 4mM HMBA on day 7 and persisted till day 10 when compared with the control group. The $S_{NM,N}$ increased in the 4mM HMBA-treated group on day 7 when compared with the controls. We conclude that HMBA suppresses cell proliferation and induces an increase in cell volume and surface area of plasma membrane in the later stages of the culture period.

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Effect of material deprivation on Epstein-Barr virus infection in Hodgkin's disease: preliminary analysis of a West Midlands population

Karen Flavell[$], Christothea Constandinou[$], Derek Lowe[$], Kenneth Scott[~], Stuart Haldene[~], Claire Newey[@], David Evans[@], Ralph Smith[#], John Crocker[^], Lawrence S Young *, Paul Murray*[$].

[$]School of Health Sciences, University of Wolverhampton, Wolverhampton, WV1 1DJ, [~]Department of Histopathology, New Cross Hospital, Wolverhampton, WV10 0QP, [@]Department of Histopathology, Russells Hall Hospital, Dudley, West Midlands, DY1 2HQ, [#]West Midlands Cancer Intelligence Unit, The Public Health Building, The University of Birmingham, Birmingham, B15 2TT, [^]Department of Histopathology, Birmingham Heartland's Hospital, Bordesley Green East, Birmingham, B9 5SS, and *Institute for Cancer Studies, University of Birmingham, Edgbaston, Birmingham, B15 2TT.

The Epstein-Barr virus (EBV) is detectable in Hodgkin/Reed-Sternberg (HRS) cells in a percentage of Hodgkin's disease (HD) tumours. However, the level of EBV-positivity in HD is variable and depends on several factors including age, sex and country of residence. In particular, EBV positive HD tumours appear to be less common in developed populations, with percentages of between 40-50% for North American and European cases, 57% for HD in China, but much higher rates in underdeveloped countries such as Peru and Kenya. Although we are able to speculate that in the UK, the level of EBV infection in Hodgkin's tumours is likely to be higher in underprivileged populations, data supporting this assumption is lacking.

This study has analysed 123 cases of histologically confirmed Hodgkin's disease from the West Midlands, UK, for the presence of EBV in HRS cells using both *in situ* hybridisation to detect the EBERs and immunohistochemistry for the demonstration of LMP1. EBV status determined in this way for each patient was correlated with Townsend Score which was used as a measure of material deprivation. Townsend Scores, between -10 (less deprived) and 10 (most deprived), were provided by the West Midlands Cancer Intelligence Unit for individual patients based on data from the 1991 OPCS Census.

26/123 patients were identified as EBV-positive. The majority of EBV-positive patients (20/26) had Townsend scores above zero (indicating higher levels of material deprivation), whereas only 56/97 EBV-negative patients had scores above zero. Median Townsend scores for EBV-positive and EBV-negative groups were 2.3 and 0.5, respectively and the Mann-Whitney test gave a p value of 0.34. Similar findings were obtained when the lymphocyte predominant and/or the lymphocyte depletion subtype were excluded from the analysis. Although these results are not statistically significant they suggest that EBV-positive Hodgkin's disease in the West Midlands is more likely in patients from materially deprived areas. Further analysis of a larger series of patients is required to improve statistical power.

Is expression of p21WAF1/CIP1 related to EBV status in Hodgkin's disease?

Christothea Constandinou[$] Zdenek Kolar[&], Jan Martin[$], Kenneth Scott[~], Stuart Haldene[~], Borek Vojtesek**, John Crocker[^], Lawrence Young*, Paul Murray*[$].

[$]School of Health Sciences, University of Wolverhampton, Wolverhampton, WV1 1DJ, [&]Centre of Molecular Biology and Medicine, Palacky University, Olomouc, Czech Republic, **Masaryk Memorial Cancer Institute, Brno Czech Republic, [~]Department of Histopathology, New Cross Hospital, Wolverhampton, WV10 0QP, [@]Department of Histopathology, Russells Hall Hospital, Dudley, West Midlands, DY1 2HQ, [#] West Midlands Cancer Intelligence Unit, The Public Health Building, The University of Birmingham, Birmingham, B15 2TT, [^]Department of Histopathology, Birmingham Heartland's Hospital, Bordesley Green East, Birmingham, B9 5SS, and *Institute for Cancer Studies, University of Birmingham, Edgbaston, Birmingham, B15 2TT.

p21WAF1/CIP1 (p21) is a nuclear protein inhibitor of several cyclin-dependent kinases (cdks). It is a component of the quaternary complexes that control the cell cycle and includes cyclin D1, cdks, and the proliferating cell nuclear antigen (PCNA). This study has utilised immunohistochemistry to analyse the expression of p21 in Hodgkin/Reed-Sternberg (HRS) cells of 63 cases of histologically confirmed Hodgkin's disease in relation to Epstein-Barr virus (EBV) status. EBV status was determined by in situ hybridisation for the detection of the EBERs or by immunohistochemistry for LMP1. p21 nuclear expression was demonstrated in the majority of cases but intensity of expression was variable. High level expression of p21 (>50% HRS cells stained or >20% HRS cells strongly stained) was demonstrated in 8/18 EBV-positive cases but only in 7/45 EBV-negative tumours. All EBV positive cases showed some p21 staining in HRS cells. Four EBV-negative tumours did not express detectable levels of p21. An association between high level expression of p21 and decreased survival was found in EBV-negative patients. No such trend was noted for EBV-positive patients, which may reflect the relatively low numbers of patients in this group.

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Mapping t(2:5)(p23;q35) Nucleophosmin-Anaplastic Lymphoma Kinase Breakpoints in Anaplastic Large Cell Lymphoma Cell Lines.

JH Pringle, JA Shaw, L Potter, L Primrose, A Gillies, P Hickenbotham and I Lauder.
Department of Pathology, University of Leicester.

The t(2;5)(p23;q35) translocation fuses the nucleophosmin gene (NPM) on chromosome 5q35 to a neuronal protein kinase gene, anaplastic lymphoma kinase (ALK) on chromosome 2p23. The translocation is characteristic of a sub-group of CD30+ anaplastic large-cell non-Hodgkin's lymphomas (ALCL). Previous studies have used long range amplification of genomic DNA, isolated from fresh tissue biopsy material, to detect any translocations. We have cloned and sequenced the wild type NPM and ALK introns and the Karpass 299 translocation intron and have subsequently mapped the distinct intron-specific translocation breakpoints in three ALCL cell lines (SU-DHL-1, SUP-M2 and Karpass 299) by sequence comparison. This has enabled the design of NPM and ALK exon and intron-specific primer pairs to amplify across the translocation breakpoint in archival fixed paraffin-embedded material. The resulting amplified fragments are of a size suitable for direct sequencing.