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Fax Communication

Non-tumorigenicity of Bloom's Syndrome Lymphoblastoid Cell Lines in Immunodeficient Mice

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INTRODUCTION

THOSE FACTORS that contribute to malignancy in mature B cells remain to be fully defined. However, over expression of the c-*myc* gene and infection by Epstein–Barr virus (EBV) appear to make a contribution and it has been suggested that these two factors alone are sufficient for the acquisition of tumorgenicity in immunodeficient mice [1]. However, in studies of B cells expressing a c-*myc* gene driven by an immunoglobulin enhancer, other oncogenes, such as N-*ras* and K-*ras*, c-*raf*-1, v-*abl* and *bcl*-2, have been shown to occasionally contribute to lymphomagenesis [2–4]. In addition, two reports indicate that overexpression of the c-*myc* gene together with immortalisation by EBV are not sufficient for full tumorigenicity in immunodeficient mice [5, 6]. Apparently benign over expression of the c-*myc* gene has been observed in EBV-immortalised lymphoblastoid cell lines derived from cancer-prone Bloom's syndrome (BS) patients [7]. Thus BS cell lines provide an ideal system in which to study lymphomagenesis, since they express high levels of the c-*myc* gene and are infected with EBV.

METHODS AND RESULTS

Initially we injected exponentially growing human lymphoblastoid cultures (LCLs) [7] derived from patients with inherited diseases, healthy individuals, patients with Burkitt's lymphomas (BL) and a patient with a colon adenocarcinoma (Colo 320) into immunodeficient mice (Table 1). During 6 months of daily monitoring, no tumours were observed in the first two groups. However, cell lines derived from the colon adenocarcinoma in which there is an amplification of the c-*myc* gene [8] caused the appearance of tumours at the injection site after only 2 weeks. Cell lines representative of BL occasionally gave small tumours in the lymph nodes that always regressed.

We then mutagenised two BS cell lines with methylnitrosourea (MNU). Previous experiments have indicated that mutagenesis with N-methyl-N′-nitrosoguanidine and 4-nitro-quinoline-N-oxide results in BS lymphocytes that are able to form tumours in immunodeficient mice [9]. We attempted to repeat these experiments by mutagenesis of the BS lymphocytes GM3403 and W-674. Exponentially growing cultures of each cell line were exposed to 0.4 μg/ml MNU, a dose sufficient to kill 70% of the culture. The cells were regrown for 2 weeks and the procedure repeated to select two cultures (GM3403/MNU and W-674/MNU), which had a doubling-time of 24 h. Exponentially growing BS lymphocytes normally double in cell number every 48 h [10]. Each culture was injected into immunodeficient mice. No tumours formed over a 6 month observation period (Table 1).

DISCUSSION

Thus in the BS system, over-production of the c-*myc* protein and immortalisation by EBV are insufficient to cause the formation of tumours in nude mice. Three explanations are possible: (1) EBV immortalisation plus c-*myc* expression are indeed sufficient for transformation yet a threshold level of c-*myc* protein is required for this oncogene to augment other cellular changes; this is considered unlikely since the c-*myc* protein levels

Table 1. Tumorigenicity of various LCLs in nude mice

Cell line	Line	No. tumours/ no. mice†
Bloom's syndrome (−/−)*	W-674	0/5
Bloom's syndrome (−/−)	1004	0/5
Bloom's syndrome (−/−)	GM3403	0/5
Bloom's syndrome (−/−)	D 8612	0/5
Bloom's syndrome (−/+)	AA874	0/5
Ataxia telangiectasia	GM1526	0/5
Fanconi's anaemia	GM4510A	0/5
Werner's syndrome	AG3829	0/5
Xeroderma pigmentosum (group C)	GM2249	0/5
Burkitt's lymphoma	Raji	0/5‡
Burkitt's lymphoma	Ramos	0/5‡
Colon adenocarcinoma	Colo320DM	5/5§
Colon adenocarcinoma	Colo320 HSR	5/5§
Mutagenised Bloom's syndrome	GM3403/MNU	0/5
	W-674/MNU	0/5

*−/− = homozygote, −/+ = heterozygote.
†5 Balb/c (nu/nu) mice were injected with either 2×10^7 cells at one site subcutaneously or 10^6 cells per site at four sites subcutaneously and one site intraperitoneally.
‡After 6 weeks small tumours appeared at lymph nodes; tumours subsequently regressed.
§Injection of Colo 320 (DM or HSR) resulted in transplantable tumours after 2 weeks.

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in BS are 3.5 times higher than those in LCLs derived from healthy individuals and similar to the levels observed in BL [7]; (2) activation of other oncogenes, such as N-*ras* and K-*ras*, c-*raf*-1, v-*abl* or *bcl*-1, is required; or (3) a deficiency in the activity of DNA ligase I in BS [11] suppresses the path to transformation in EBV/c-*myc* overexpressing cell lines (i.e. reversion of the DNA ligase I phenotype may allow expression of the transformed phenotype).

Whilst overexpression of the c-*myc* gene and immortalisation by EBV may play a predisposing role, it is likely that other cellular changes are necessary for LCLs derived from BS patients to progress towards malignancy.

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