Chromosome Research **12**: 299–307, 2004.
© 2004 *Kluwer Academic Publishers. Printed in the Netherlands*

Karyotype stability of the DT40 chicken B cell line: Macrochromosome variation and cytogenetic mosaicism

Hong Chang & Mary E. Delany*
Department of Animal Science, 2131D Meyer Hall, One Shields Avenue, University of California, Davis, CA 95616, USA; Tel: (+1)530-754-9343; Fax: (+1)530-752-0175; E-mail: medelany@ucdavis.edu
**Correspondence*

Received 19 December 2003. Received in revised form and accepted for publication by Herbert Macgregor 23 January 2004

Key words: aneuploidy, chicken, chromosomes, cytogenetics, DT40, karyotype, mosaicism

Abstract

The DT40 transformed chicken B-cell line is an important and widely used vertebrate cell line. Knowledge of the 'wild-type' DT40 karyotype is a significant consideration for most research applications and for extrapolation of results to normal cells. Here we present data indicating that (1) modal karyotype differences exist between DT40 cultures from different sources, (2) DT40 cultures are cytogenetically mosaic, and (3) the mosaic features can change over time with media conditions influencing the degree and type of non-modal karyotypes that emerge. In addition to the previously reported trisomy for chromosome 2, a monosomy for chromosome 4 and a variant 4p– are described. The macrochromosomal variation and mosaic nature of the DT40 system should alert researchers using this important cell line to consider whether such variation will impact experimental results and interpretation. The results are discussed in regard to current knowledge of cytogenetic anomalies associated with chicken cancers.

Introduction

The DT40 cell line is a transformed chicken B-cell line. It is one of the most valuable vertebrate cell lines available for research and is used widely (Winding & Berchtold 2001). The original bursal lymphoma developed in a Hyline SC female chicken following infection with avian leucosis virus (ALV). The DT40 line was established following two *in vivo* transfers of tumor cell suspensions through young syngeneic recipients (Baba & Humphries 1984, Baba *et al.* 1985). Characteristics of DT40 include cell surface IgM, proviral sequence insertion upstream of *c-myc*, increased expression of *c-myc*, and continuous capacity for Ig light chain gene conversion (Baba *et al.* 1985, Buerstedde *et al.* 1990). An important feature of the DT40 cell line is its high frequency of homologous recombination providing great utility and opportunities for gene-targeting/gene-function experiments (Buerstedde & Takeda 1991). Studies in DT40 were pivotal to the elaboration of the gene conversion mechanism of immunoglobulin diversification (Kim *et al.* 1990) and vertebrate DNA repair mechanisms (Morrison & Takeda 2000), and have contributed to understanding chromatin organization (Nakayama & Takami 2001), centromeric protein function (Fukagawa & Brown 1997, Mackay *et al.* 1998, Fukagawa *et al.* 2001a, Fukagawa *et al.* 2001b, Nishihashi *et al.* 2002), and artificial chromosome biology (Dieken *et al.* 1996, Mills *et al.* 1999).

It is well known that chromosome aberrations are characteristic of many human cancers (Sanberg

1982, Roulston & Le Beau 1997, Thompson 1997) and that additional karyotype changes are common during *in vitro* culture of transformed cell lines; thus, experiments utilizing such cells are undertaken with that proviso. However, because the chicken chromosome complement contains numerous microchromosomes, accurate karyotyping is not easily accomplished. Thus, there is limited cytogenetic information on the nature and evolution of karyotype changes associated with chicken oncogenesis *in vivo* or transformed cells *in vitro*. The majority of the research in this area was conducted decades ago and prior to the advent of molecular cytogenetics; most of the studies focused predominately on Marek's disease (MD) tumors and MD-tumor derived transformed cell lines (Pontén 1963, Owen *et al.* 1966, Bloom 1970, Akiyama & Kato 1974, Yoon *et al.* 1976, Takagi *et al.* 1977, Moore *et al.* 1993, Moore *et al.* 1994).

In preparation for a DT40 knock-out experiment, we sought to confirm the report by Sonoda *et al.* (1998) that DT40 cells have a stable karyotype of $2n = 80$ (ZW) including trisomies for Gga-2 and an unidentified microchromosome. Here we present an analysis of the five largest pairs of the macrochromosome complement, Gga-1-4 and Gga-Z, of the DT40 cell line and provide evidence for cytogenetic variation between stocks from different sources. In addition, we found the DT40 culture systems to be cytogenetically mosaic wherein the extent or degree of mosaicism varied with culture media condition as well as over time.

Materials and methods

Culture sources and conditions

The DT40 cell line was obtained from two sources: the American Type Culture Collection (ATCC, CRL-2111, lot no. 1151670), designated here as ATCC-DT40, and from J. M. Buerstedde (Heinrich-Pette-Institute, Hamburg, Germany), designated JMB-DT40. The ATCC-DT40 accession was deposited by the originator of the line E.H. Humphries, in 1994. Since deposition, the line was occasionally cultured for quality control purposes but not for any great length of time, usually a month or less (personal communication, ATCC technical support staff). The ATCC DT40 cells utilized for this study were from a vial frozen in 1999. The ATCC-DT40 and JMB-DT40 cells were grown separately, using two types of media, designated I and II, in the same incubator at 40°C and 5% CO_2. Cells were passaged every two to three days. Media I consisted of 500 ml of DMEM (high glucose/with L-glutamine, BioWhittaker: 12-604F) supplemented with 50 ml fetal bovine serum (GIBCO: 16000-044), 10 ml chicken serum (GIBCO: 16110-082), 10 ml penicillin/streptomycin (5000 U penicillin, 5000 μg streptomycin/ml, BioWhittaker: 17-603E), and 5 mg 2-mercaptoethanol (Fisher: O3446I-100). Media II consisted of RPMI-1640 (with L-glutamine, BioWhittaker: 12-702F) supplemented as described above and including 5 ml L-glutamine (200 mmol/L, GIBCO: 25030-149). The formulations of DMEM and RPMI differ in pH, osmolality, inorganic salts, amino acids, vitamins, and other components, e.g., glucose, pyruvate.

Chromosome harvest and karyotype analysis

Three chromosome harvests were performed on day 8, 31 and 57 following culture initiation. Prior to this study, JMB-DT40 cells were cultured for 171 days in the lab using media II and then aliquots were frozen in liquid nitrogen. The two culture systems used for this study were initiated from a single frozen vial and day one was counted from that initiation. Colcemid (0.075 μg/ml media, GIBCO: 15210-040) was added to the cell cultures for one hour. Standard hypotonic treatment of 0.56% KCl for 30 min was followed by several fixations using 3:1 methanol: glacial acetic acid. Slide preparations were stained by 6% Gurr's R66 Giemsa (BDH Chemicals Ltd, Poole, England: 35086). Metaphases were viewed with an Olympus BX60 microscope and images were captured using Simple PCI software (Compix, Inc). Metaphase cells were analyzed for the presence of the five largest pairs of macrochromosomes, Gga-1-4,-Z. As a technical control, an analysis was also conducted on a primary culture of chicken embryo fibroblasts (CEF2 at passage 3, Swanberg and Delany 2003).

Results

Chromosomal harvests were performed on DT40 cells from two sources cultured in two types of media, at three different time points. Modal and second-most-frequent macrochromosomal complements for Gga-1-4 and Gga-Z are presented in Table 1, along with the karyotype results for a non-transformed primary CEF culture.

The ATCC-DT40 cells grew well in media I from the initiation of the culture. Cells from the first two harvests exhibited the same modal macrochromosomal complements of 2 Gga-1, 3 Gga-2 (trisomy), 2 Gga-3, 1 Gga-4 (monosomy) and 1 Gga-Z (hemizygous) (see Table 1 and Figure 1a). A high frequency of the modal karyotype cells at harvest 1 (8d) and 2 (31d), 60.9% and 72.7%, respectively, exhibited a variant Gga-4 possessing a smaller-than-normal p arm (4p–) (see arrowhead in Figure 1a). The second-most-frequent karyotype at harvest 1 was similar to the modal karyotype except for a disomic condition for Gga-2. From harvest 2, two second-most-frequent karyotypes emerged, one as indicated above and the other trisomic for Gga-2 and disomic for Gga-4 (see Table 1). By the third harvest (57d), the ATCC-DT40 cell population cultured in media I was almost entirely mosaic for the macrochromosome complement. No more than three cells shared the same karyotype. The cells exhibited highly variable aneuploid conditions, e.g., combinations of polysomy, monosomy and nullosomy. Forty-five percent of the cells exhibited a combined total of six to eight copies of Gga-1 and Gga-2 (see Figure 1c–d).

Upon initiation of the ATCC-DT40 cells in media II, the cells grew poorly and experienced a long recovery phase. At the first harvest, the slow cell growth resulted in low cell recovery with no mitotic cells. Although the cells continued to grow slowly and exhibited unusual growth features, such as numerous cellular extensions and an oval rather than round cell shape, the second chromosome harvest was successful. Forty-three percent of the cells showed the modal karyotype of 2 Gga-1, 3 Gga-2 (trisomy), 2 Gga-3, 1 Gga-4 (monosomy) and 1 Gga-Z, and among this population, 47.1% of the modal-karyotype cells exhibited the variant Gga-4p–. The second-most-frequent karyotype from harvest 2 differed from the modal by exhibiting a monosomy for Gga-1. By the third harvest, the cells were growing very well in media II; the same modal macrochromosomal complement was evident as in harvest 2 and among this population, 94.4% of the cells exhibited Gga-4p–. The second-most-frequent karyotype identified at harvest 3, differed from the modal by exhibiting a disomic condition for Gga-4 (see Table 1).

The JMB-DT40 cells grew well in both media I and in media II. The modal macrochromosomal complement of 2 Gga-1, 3 Gga-2 (trisomy), 2 Gga-3, 2 Gga-4 and 1 Gga-Z was observed from all three harvests for both culture media systems (Figure 1b). The proportion of JMB-DT40 cells exhibiting the modal karyotype was generally higher in the media II cultures than the media I cultures, at the same harvest. For example, 66.7% of the cells cultured in media II exhibited the modal karyotype whereas 51.5% of the cells in media I exhibited the modal karyotype, at harvest 1. In media I, the second-most-frequent karyotype varied from the modal by exhibiting a monosomy for Gga-4 (harvest 1 and 2) or a monosomy for Gga-3 (harvest 3). In media II, the second-most-frequent karyotype varied from the modal by exhibiting a monosomy for Gga-3 or a monosomy for Gga-1 (harvest 1), a monosomy for Gga-4 (harvest 2), and disomy for Gga-2 (harvest 3) (see Table 1). The 4p– variant was not observed in the JMB DT40 cultures.

Eighty-two percent of the CEF2 cells exhibited the normal chicken macrochromosomal diploid karyotype with one or two copies of Gga-Z which was expected since the cell culture was created by pooling cells from both female and male embryos. Of the cells exhibiting non-modal karyotypes, none shared the same karyotype. The non-modal karyotypes contained single monosomies for different macrochromosomes suggesting non-specific chromosome loss during the chromosome harvest/slide making procedures. One tetraploid cell was observed.

Qualitative observations on the cellular morphology of the cultures indicated that the ATCC-DT40 cells grew in clusters and clumps with larger clusters in media I as compared to media II (Figure 2a–b). In contrast, the JMB-DT40 cells typically grew as long chains in media I or predominantly as single-cells in media II. The JMB-DT40 cells grown in media I exhibited a generally larger cell size than JMB-DT40 cells grown in media II (Figure 2c–d).

Table 1. Macrochromosomal variation found in DT40 cells from different sources, cultured in different media over time.[a]

Culture	Harvest	Modal karyotype 1	2	3	4	Z	% cells (#)	Second-most-frequent karyotype 1	2	3	4	Z	% cells (#)	Total # cells	Days in culture
ATCC-DT40	1	2	3	2	1[b]	1	51.1 (23)	2	2	2	1	1	15.6 (7)	45	8
(media I)	2	2	3	2	1[c]	1	62.9 (22)	2	2	2	1	1	8.6 (3)	35	31
								2	3	2	2	1	8.6 (3)		
	3	– Mosaic karyotype[d] –												33	57
ATCC-DT40	1	– ND[e] –												ND	8
(media II)	2	2	3	2	1[f]	1	43.6 (17)	1	3	2	1	1	12.8 (5)	39	31
	3	2	3	2	1[g]	1	48.6 (18)	2	3	2	2	1	13.5 (5)	36	57
JMB-DT40	1	2	3	2	2	1	51.5 (17)	2	3	2	1	1	12.1 (4)	33	8
(media I)	2	2	3	2	2	1	35.1 (13)	2	3	2	1	1	18.9 (7)	37	31
	3	2	3	2	2	1	40.4 (19)	2	3	1	2	1	10.6 (5)	47	57
JMB-DT40	1	2	3	2	2	1	66.7 (26)	2	3	1	2	1	5.1 (2)	39	8
(media II)								1	3	2	2	1	5.1 (2)		
	2	2	3	2	2	1	57.6 (19)	2	3	2	1	1	9.1 (3)	33	31
	3	2	3	2	2	1	56.4 (22)	2	2	2	2	1	10.3 (4)	39	57
CEF2	1	2	2	2	2	1 or 2	82.4 (28)	– None –						34	14

[a]Both the modal and the second-most-frequent macrochromosomal complement (Gga-1-4 and Gga-Z) are indicated.
[b]Among the 23 cells showing the modal karyotype, 60.9% of the cells showed Gga-4p–.
[c]Among the 22 cells showing the modal karyotype, 72.7% of the cells showed Gga-4p–.
[d]No more than 3 cells exhibited the same macrochromosomal complement; 45.5% of the cells polysomic for both Gga-1 and Gga-2 (three to five copies, each).
[e]ND: not determined (see Results for detail).
[f]Among the 17 cells showing the modal karyotype, 47.1% of the cells showed Gga-4p–.
[g]Among the 18 cells showing the modal karyotype, 94.4% of the cells showed Gga-4p-4p–.

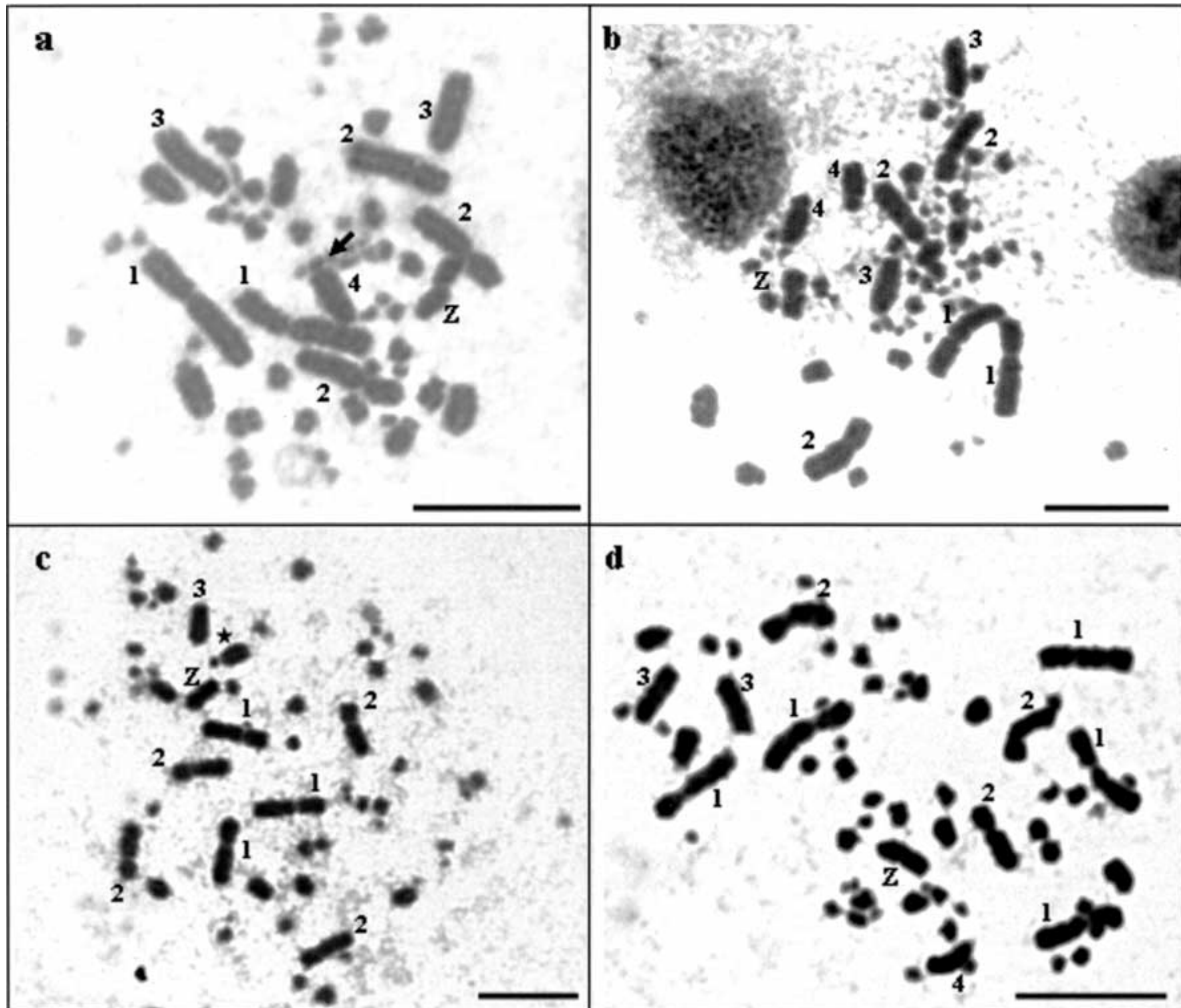

Figure 1. Metaphase cells illustrating the modal and highly aneuploid macrochromosomal complements of the various DT40 culture systems. (**a**) ATCC-DT40 (media I) cell illustrating the modal karyotype of 2 Gga-1, 3 Gga-2, 2 Gga-3, 1 Gga-4 and 1 Gga-Z. Arrowhead indicates Gga-4 with the variant 4p–. (**b**) JMB-DT40 (media II) cell with the modal karyotype of 2 Gga-1, 3 Gga-2, 2 Gga-3, 2 Gga-4 and 1 Gga-Z. (**c**) ATCC-DT40 (media I, harvest 3) cell with a karyotype of 3 Gga-1, 4 Gga-2, 1 Gga-3, 1 Gga-4 and 1 Gga-Z. The asterisk (*) marks the position of two closely located chromosomes, one small and one medium-sized microchromosome, that gives the mistaken appearance of a chromosome 4. (**d**) ATCC-DT40 (media I, harvest 3) cell with a karyotype of 5 Gga-1, 3 Gga-2, 2 Gga-3, 1 Gga-4 and 1 Gga-Z. Bars indicate 10 μm.

Discussion

Generous distribution from the originators and developers of the DT40 line along with subsequent sharing among many laboratories has led to essentially world-wide distribution of the DT40 cell line. The attributes of a high homologous recombination rate, ease of culture and abundant resources (see ⟨http://swallow.gsf.de/dt40.html⟩) contributes to the use of DT40 for numerous applications. Thus, in addition to recombination, Ig diversification, and gene function studies, uses of DT40 now include cytogenetic mapping, analysis of genome organization and chromosome-protein associations, as well as RNA and protein profiling, and analysis of signaling pathways (Konrad *et al.* 1999, Okamura *et al.* 2001, Wang & Leung 2002).

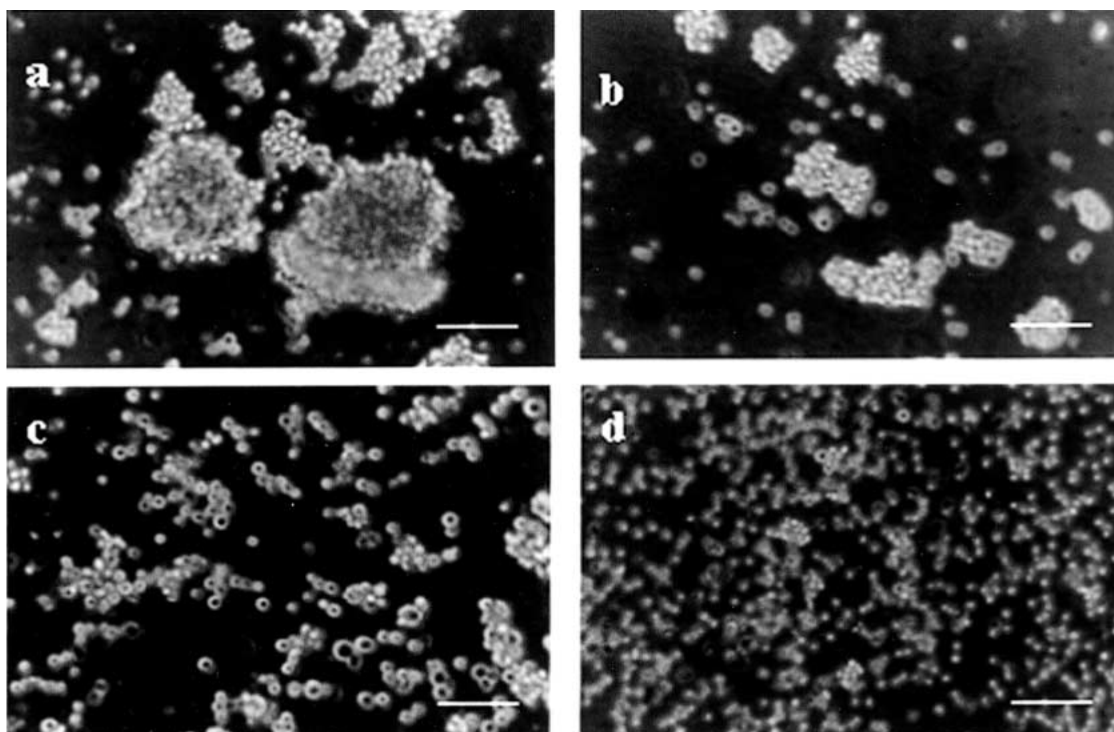

Figure 2. Morphological features of ATCC-DT40 and JMB-DT40 cell cultures. In general, ATCC-DT40 cells grew in clusters, whereas JMB-DT40 cells grew in chains or as single cells (see Results for detail). Cell culture photographs were obtained using a Nikon TMS Microscope (20x lens) outfitted with a Nikon F3 camera and using Ilford HP5 400 BW film. (**a**) ATCC-DT40 cells in media I. (**b**) ATCC-DT40 cells in media II. (**c**) JMB-DT40 cells in media I. (**d**) JMB-DT40 cells in media II. Bars indicate 100 μm.

DT40 karyotype features and stability are of significance for most research applications and data interpretation. No literature exists regarding the cytogenetic status of the cell line at inception or during its initial characterization and early usage. Within a study on chromosome-break accumulation in Rad51-deficient DT40 cells Sonoda and colleagues (1998) reported that the 'wild-type' DT40 exhibited a stable karyotype with $2n$ of 80 chromosomes (ZW), including trisomy for both Gga-2 and an unidentified microchromosome. Recently, Wang and Leung (2002) reported a DT40 ZZW sex chromosome variant.

Here we present data that indicate modal karyotype differences exist between DT40 cultures from different sources, that DT40 cultures are cytogenetically mosaic, and that the degree of cytogenetic mosaicism can change over time with media conditions having a significant impact on the degree of mosaicism and the type of non-modal karyotypes that emerge. Specifically, macrochromosome complement variation involved predominately Gga-2 as reported previously, as well as Gga-4, and to a lesser extent Gga-1 and Gga-3. Numerical or structural variation for the Z was not observed. Additionally, the cellular phenotype of the DT40 cultures suggested differential response of the cells to the growth conditions afforded by the media components. The results from the media systems used here are generally applicable to culture procedures used by many laboratories as DMEM (media I) and RPMI (media II) are commonly used reagents for DT40 cells.

The proportion of JMB-DT40 cells exhibiting the modal macrochromosomal karyotype (disomic for Gga-1, -3, -4, trisomic for Gga-2, hemizygous for Gga-Z) ranged from 35.1% to 66.7% for both media culture conditions. The proportion of cells with the modal karyotype declined over time indicative of an increasingly mosaic population. The proportion of ATCC-DT40 cells that exhibited the

modal karyotype (disomic for Gga-1, -3, trisomic for Gga-2, monosomic for Gga-4, hemizygous for Gga-Z) ranged from 43.6% to 62.9%. In media I the cells became highly mosaic over time with numerous copies of Gga-1 and -2 whereas in media II initial growth was slow and the latter two harvests were similar for modal-cell proportions. As a comparison, the proportion of CEF2 cells that exhibited the modal karyotype (disomic for Gga-1, -2, -3, -4 and one or two copies of the Z) was 82%. A key issue in cytogenetic studies lies in establishing how much of the observed variation is contributed by technical artifact. For example, chromosome loss during preparation can not be discounted as making some contribution to hypodiploid karyotypes. The values indicated above provide an index for the extent of cytogenetic mosaicism versus technical artifact. Using the non-transformed CEF2 results as a guide, an estimate or expectation for non-modal karyotypes, especially hypodiploid, due to technical artifact would be ~15% of the cells, as in this study the CEF2 analysis resulted in 82% modal diploid cells, 3% non-modal tetraploid (1 of 34 cells), and 15% (5 of 34 cells) non-modal, hypodiploid cells.

Chromosome studies were not conducted on the original tumor or the DT40 cell line during the early stages of its development, therefore, it is unknown if the Gga-2 trisomy reflects an original feature of the tumor or a derived feature that developed *in vitro*. The derivation of the monosomy for Gga-4 is also uncertain; it was a feature of the modal karyotype in the ATCC but not the JMB modal karyotype, although half of the JMB non-modal karyotypes exhibited a monosomy for Gga-4. Over culture time, an increasing majority of the ATCC Gga-4 chromosomes exhibited a smaller p arm in both media systems but this variant was not found in the JMB cells. One speculation is that Gga-4 is susceptible to numerical and structural alterations *in vitro*, perhaps favored by certain culture conditions.

The consistent numerical aberration of Gga-2 (trisomy) in DT40 allows for potential gene-dosage dysregulation of Gga-2 loci. Of particular interest, *c-myc* maps to Gga-2q (⟨http://www.poultry.mph.msu.edu/resources/Conmap/consensus⟩ Schmid *et al.* 2000). The *c-myc* proto-oncogene encodes a transcription factor known for its multi-faceted control of cell proliferation and differentiation, and involvement in both human and chicken cancers. Inappropriate over expression of *c-myc* usually induces p53 which leads to cell death; notably, however, p53 expression is absent in DT40 cells (Takao *et al.* 1999). The very initial analysis of DT40 showed the ALV proviral DNA sequence integrated 5′ to the *c-myc* locus and high levels of *c-myc* RNA expression (Baba *et al.* 1985). Recently, microarray studies indicated 18-fold higher *c-myc* expression in DT40 (as compared to normal bursal samples) correlating with the expression of hundreds of mRNAs (note: the microarray was developed from DT40 cDNAs) (Neiman *et al.* 2001). Such myc-induced alterations in gene expression due to viral integration and coupled with a trisomic condition could impact a number of pathways involving control of genome stability (and thus chromosome constitution) in the DT40 cells.

In poultry, there are a number of virally-induced oncogenic diseases which are significant for their impact on the poultry industry; further, these conditions provide valuable experimental models for vertebrate oncogenesis. Unfortunately there is limited information on their cytogenetic attributes because although studies of chromosome constitution in virally-induced chicken neoplasias date back to the late 1950s (Rubin & Temin 1958) relatively few cytogenetic studies of chicken tumor cells and lines have been conducted. The majority of the cancer-related cytogenetic literature comes from Marek's disease tumors and derived cell lines with limited information on karyotypes from chemically or other virally-induced tumors and transformed cell lines (Pontén 1963, Owen *et al.* 1966, Bloom 1970, Yoon *et al.* 1976, Takagi *et al.* 1977). Recent studies consistently implicate aberrations involving Gga-1 in MD cell lines although the aberration may occur late in oncogenesis or perhaps *in vitro* (Takagi *et al.* 1977, Moore *et al.* 1993, 1994). It would be interesting to know if, as identified in the ALV-derived DT40 cell line, trisomy for Gga-2 and monosomy for Gga-4 are characteristic of ALV-induced tumors or derived cell lines *in vitro*.

In summary, the results of this study indicate levels of macrochromosomal variation and mosaicism suggestive of instability of the DT40 karyotype, at least for the accessions studied here; such cytogenetic instability has the potential to affect research results, interpretation and

extrapolation and should be given significant consideration when utilizing the DT40 cell line.

Acknowledgements

We are grateful to Dr. J.-M. Buerstedde (Heinrich-Pette-Institute, Hamburg, Germany) for providing the JMB-DT40 cells.

References

Akiyama Y, Kato S (1974) Two cell lines from lymphomas of Marek's disease. *Biken J* **17**: 105–116.

Baba TW, Humphries EH (1984) Differential response to avian leukosis virus infection exhibited by two chicken lines. *Virology* **135**: 181–188.

Baba TW, Giroir BP, Humphries EH (1985) Cell lines derived from avian lymphomas exhibit two distinct phenotypes. *Virology* **144**: 139–151.

Bloom SE (1970) Marek's disease: chromosome studies of resistant and susceptible strains. *Avian Dis* **14**: 478–490.

Buerstedde JM, Reynaud CA, Humphries EH, Olson W, Ewert DL, Weill JC (1990) Light chain gene conversion continues at high rate in an ALV-induced cell line. *EMBO J* **9**: 921–927.

Buerstedde JM, Takeda S (1991) Increased ratio of targeted to random integration after transfection of chicken B cell lines. *Cell* **67**: 179–188.

Dieken ES, Epner EM, Fiering S, Fournier RE, Groudine M (1996) Efficient modification of human chromosomal alleles using recombination-proficient chicken/human microcell hybrids. *Nat Genet* **12**: 174–182.

Fukagawa T, Brown WR (1997) Efficient conditional mutation of the vertebrate CENP-C gene. *Hum Mol Genet* **6**: 2301–2308.

Fukagawa T, Mikami Y, Nishihashi A *et al.* (2001a) CENP-H, a constitutive centromere component, is required for centromere targeting of CENP-C in vertebrate cells. *EMBO J* **20**: 4603–4617.

Fukagawa T, Regnier V, Ikemura T (2001b) Creation and characterization of temperature-sensitive CENP-C mutants in vertebrate cells. *Nucleic Acids Res* **29**: 3796–3803.

Kim S, Humphries EH, Tjoelker L, Carlson L, Thompson CB (1990) Ongoing diversification of the rearranged immunoglobulin light-chain gene in a bursal lymphoma cell line. *Mol Cell Biol* **10**: 3224–3231.

Konrad JP, Mills W, Easty DJ, Farr CJ (1999) Cloning and characterization of the chicken gene encoding the telomeric protein TRF2. *Gene* **239**: 81–90.

Mackay AM, Ainsztein AM, Eckley DM, Earnshaw WC (1998) A dominant mutant of inner centromere protein (INCENP), a chromosomal protein, disrupts prometaphase congression and cytokinesis. *J Cell Biol* **140**: 991–1002.

Mills W, Critcher R, Lee C, Farr CJ (1999) Generation of an approximately 2.4 Mb human X centromere-based minichromosome by targeted telomere-associated chromosome fragmentation in DT40. *Hum Mol Genet* **8**: 751–761.

Moore FR, Schat KA, Hutchison N, Leciel C, Bloom SE (1993) Consistent chromosomal aberration in cell lines transformed with Marek's disease herpesvirus: evidence of genomic DNA amplification. *Int J Cancer* **54**: 685–692.

Moore FR, Calnek BW, Bloom SE (1994) Cytogenetic studies of cell lines derived from Marek's disease virus-induced local lesions. *Avian Dis* **38**: 797–799.

Morrison C, Takeda S (2000) Genetic analysis of homologous DNA recombination in vertebrate somatic cells. *Int J Biochem Cell Biol* **32**: 817–831.

Nakayama T, Takami Y (2001) Participation of histones and histone-modifying enzymes in cell functions through alterations in chromatin structure. *J Biochem (Tokyo)* **129**: 491–499.

Neiman PE, Ruddell A, Jasoni C *et al.* (2001) Analysis of gene expression during *myc* oncogene-induced lymphomagenesis in the bursa of Fabricius. *Proc Natl Acad Sci USA* **98**: 6378–6383.

Nishihashi A, Haraguchi T, Hiraoka Y *et al.* (2002) CENP-I is essential for centromere function in vertebrate cells. *Dev Cell* **2**: 463–476.

Okamura A, Pendon C, Valdivia MM, Ikemura T, Fukagawa T (2001) Gene structure, chromosomal localization and immunolocalization of chicken centromere proteins CENP-C and ZW10. *Gene* **262**: 283–290.

Owen JJ, Moore MA, Biggs PM (1966) Chromosome studies in Marek's disease. *J Natl Cancer Inst* **37**: 199–209.

Pontén J (1963) Chromosome analysis of three virus-associated chicken tumors: Rous sarcoma, erythroleukemia, and RPL12 lymphoid tumor. *J Natl Cancer Inst* **30**: 897–921.

Roulston D, Le Beau MM (1997) Cytogentic analysis of hematologic malignant diseases. In: Barch MJ, Knutsen T, Spurbeck JL, eds. *The AGT Cytogenetics Laboratory Manual, Third Edition*. Philadelphia: Lippincott-Raven Publishers, pp 325–374.

Rubin H, Temin HM (1958) Infection with the Rous sarcoma virus *in vitro*. *Fed Proc* **17**: 994–1003.

Sanberg AA (1982) Chromosomal changes in human cancers: specificity and heterogeneity. In: A.H. Owens, D.S. Coffey, S.B. Baylin, eds. *Tumor Cell Heterogeneity: Origins and Implications*. New York: Academic Press, pp 367–397.

Schmid M, Nanda I, Guttenbach M *et al.* (2000) First report on chicken genes and chromosomes 2000. *Cytogenet Cell Genet* **90**: 169–218.

Sonoda E, Sasaki MS, Buerstedde JM *et al.* (1998) Rad51-deficient vertebrate cells accumulate chromosomal breaks prior to cell death. *EMBO J* **17**: 598–608.

Swanberg SE, Delany ME (2003) Dynamics of telomere erosion in transformed and non-transformed avian cells *in vitro*. *Cytogenet Genome Res* **102**: 318–325.

Takagi N, Sasaki M, Ikuta K, Kato S (1977) Chromosomal characteristics of six cultured lymphoblastoid cell lines originating from Marek's disease lymphomas. *Biken J* **20**: 21–28.

Takao N, Kato H, Mori R *et al.* (1999) Disruption of ATM in p53-null cells causes multiple functional abnormalities

in cellular response to ionizing radiation. *Oncogene* **18**: 7002–7009.

Thompson FH (1997) Cytogenetic methods and findings in human solid tumors. In: M.J. Barch, T. Knutsen, J.L. Spurbeck, eds. *The AGT Cytogenetics Laboratory Manual, Third Edition*. Philadelphia: Lippincott-Raven Publishers, pp 375–430.

Yoon JW, Kim SN, Hahn EC, Kenyon AJ (1976) Lymphoproliferative diseases of fowl: chromosome breaks caused in lymphocytes by JM-V herpesvirus. *J Natl Cancer Inst* **56**: 757–762.

Winding P, Berchtold MW (2001) The chicken B cell line DT40: a novel tool for gene disruption experiments. *J Immunol Methods* **249**: 1–16.

Wang X, Li J, Leung FC (2002) Partially inverted tandem repeat isolated from pericentric region of chicken chromosome 8. *Chromosome Res* **10**: 73–82.