

Cytogenetic characteristics of a murine in vitro model for the human anaplastic large cell lymphoma (ALCL)

C. Rudolph^a C. Bittner^b A.C. Feller^b H. Merz^b B. Schlegelberger^a^aInstitute of Cell and Molecular Pathology, Hannover Medical School, Hannover;^bDepartment of Pathology, Medical University of Luebeck, Luebeck (Germany)

Manuscript received 14 November 2005; accepted in revised form for publication by T. Liehr, 16 February 2006.

Abstract. Anaplastic large cell lymphoma (ALCL) is an entity of non-Hodgkin lymphomas (NHL) that often occurs in young children and adolescents. In the majority of cases, ALCL are of T-cell origin and contain the t(2;5)(p23;q35) leading to an NPM-ALK fusion or variant ALK translocations. In addition, there is an ALK-negative subtype of ALCL. The anaplastic lymphoid cell line TS1G6 established by interleukin (IL)-9 transfection of T-helper cells represents a murine model of this subtype. Here, we describe the cytogenetic features of this cell line using spectral karyotyping (SKY) and single-color fluorescence in situ hybridization (FISH). We show that TS1G6 cells exhibit a hypotetraploid karyotype

with complex structural alterations. Several unbalanced translocations involved the chromosomal region 14E5, and different translocation partners, i.e. X?A6, 3A3 and 8A1. FISH analysis using a BAC clone containing *c-myc* confirmed the presence of six copies, but also demonstrated that two loci were irregularly located, indicating that additional intrachromosomal rearrangements had occurred. Moreover, a duplication of the region XF2~3 was identified. Furthermore, six chromosomes 15 were found, representing a trisomy 15 in a tetraploid chromosome complement, indicating an altered gene dosage of the oncogene *c-myc* located in region 15D3.

Copyright © 2006 S. Karger AG, Basel

Anaplastic large cell lymphoma (ALCL) is an aggressive disease that represents about 5% of non-Hodgkin lymphomas (NHL) in adults and 15% of NHL in children. The most important immunohistochemical marker of ALCL is the expression of the CD30 antigen, a member of the nerve growth factor (NGF) gene family. Translocation t(2;5)(p23;q35) is a characteristic cytogenetic feature of ALCL and has been found in about 50% of the ALCL patients. This translocation results in the fusion of the nucleophosmin (*NPM1*) gene with the anaplastic lymphoma kinase (*ALK*) gene and the expression of the NPM-ALK fusion protein. Variant *ALK* translocations

involving *MSN* in Xq11, *TPM3* in 1q25, *AT1C* in 2q35, *TFG* in 3q21 and *CLTCL1* in 22q11 have also been described. Prognosis of the disease depends on the expression of ALK protein. Patients with ALK-negative ALCL show a considerably poorer prognosis compared to patients with ALK-positive ALCL, independent of the translocation partner.

Most ALCL arise from T-cells. In a few cases, no T-cell antigens or T-cell receptor gene rearrangements are detectable, suggesting an origin from precursor T- or B-cells. They are categorized as the 0-cell subgroup of ALCL. Rare ALK-positive cases have been found to be of B-cell origin (Gascoyne et al., 1999). In addition, some cases expressing the full-length ALK receptor have been described (Delsol et al., 1997). In the new WHO classification, these cases of B-cell origin are included in the category of diffuse large B-cell lymphoma (DLCL). There are only a few established in vivo and in vitro models for ALCL (Barbey et al., 1990; Drexler and Minowada, 1992; Drexler, 1993; Kinney et al., 1993; Pasqualucci et al., 1995; Dirks et al., 1996; Terenzi et al., 1996; Kuefer et al., 1997). However, so far no cytogenetic information is available.

This work was supported by grants from the European Union (Contract No. QL6 1-2000-00687) and the Deutsche Krebshilfe (Project No. 10-0992).

Request reprints from Prof. Brigitte Schlegelberger
Institute of Cell and Molecular Pathology, Hannover Medical School
Carl-Neuberg-Strasse 1, 30625 Hannover (Germany)
telephone: +49 511-532 4522; fax: +49 511-532 4521
e-mail: Schlegelberger.Brigitte@mh-hannover.de

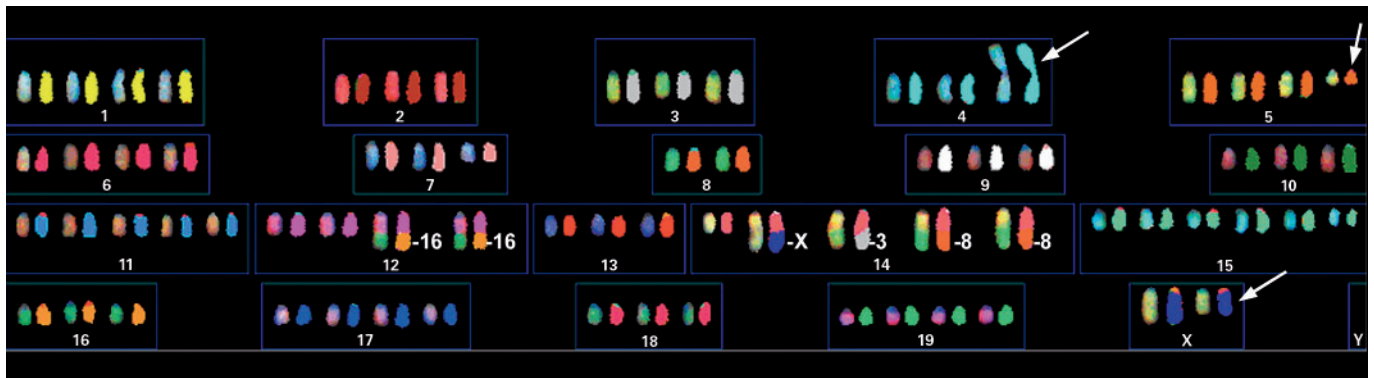


Fig. 1. SKY analysis of the cell line TS1G6 revealed a hypertriploid chromosome complement with structural chromosomal alterations. Unbalanced translocations involved chromosomes X, 3, 8, and 14. The breakpoint within chromosome 14 was consistently in the region 14E5. Furthermore, an isodicentric chromosome 4, deletions of chromosomes 5 and X (arrows), and two dicentric chromosomes involving chromosomes 12 and 16 were detectable. Deletion of one chromosome 7 was only present in this metaphase as non-clonal change.

The TS1G6 cell line has been generated by interleukin (IL)-9 transfection of a T-helper cell clone (TS1) and shows tumorigenicity when injected into C57Bl/6 mice (Uyttenhove et al., 1991). TS1G6 cells are positive for CD3, CD4, CD25, CD30, and CD71, thus resembling the typical immunophenotype of ALCL. They do not express ALK, and thus represent an ALK-negative subtype of the human ALCL (Bittner et al., 2000). Here, we aimed to characterize the murine anaplastic lymphoid cell line TS1G6 using spectral karyotyping (SKY) and single-color fluorescence in situ hybridization (FISH). Cytogenetic analysis revealed a complex karyotype with clonal numerical as well as structural chromosome alterations. Several unbalanced translocations involving the chromosomal region 14E5 were found. Moreover, additional chromosomes 15, resembling a trisomy 15, were found indicating an altered gene dosage of the oncogene *c-myc* located in region 15D3.

Materials and methods

Cell culture and chromosome preparation

Cells were cultured in RPMI 1640 containing 10% heat-inactivated fetal calf serum, 2 mmol/L L-glutamine, 50 IU/ml penicillin and 50 μ g/ml streptomycin at 37°C and 5% CO₂. Metaphase chromosomes were prepared by incubation with colcemid at a concentration of 0.035 μ g/ml for 1 h following a standard protocol (Frank et al., 2004). Well spread metaphase chromosomes were obtained by dropping the cell suspension onto a glass slide in a climate chamber (Polymer, Kassel, Germany) at 22°C and 48% humidity.

Spectral karyotyping (SKY)

For SKY analysis, chromosomes were denatured at 74°C in 70% formamide, 2 \times SSC for 1.5 min. The SKY probe mixture for mouse chromosomes (Applied Spectral Imaging, Ltd., Migdal HaEmek, Israel) was denatured at 80°C for 7 min and preannealed at 37°C for 1 h. The hybridization took place for 72 h in a humidified chamber. Signal detection was carried out according to the manufacturer's instructions (Applied Spectral Imaging, Ltd., Migdal HaEmek, Israel). For signal acquisition and analysis of chromosomes, the SpectraCube™ system combined with an epifluorescence microscope, CCD camera and SKY-View™ software was used. In total, 20 metaphases were analyzed.

Single-color fluorescence in situ hybridization (FISH)

FISH analysis was performed on metaphase chromosomes using a SpectrumGreen-labeled probe generated from BAC clone RP23-382J5, hybridizing to region F2~3 within the murine X chromosome. The *c-myc* oncogene was analyzed using a probe generated from BAC clone RP24-488H15.

For chromosome and probe denaturation, slides were heated for 10 min on an 80°C heating plate and hybridized overnight at 37°C. After washing with 0.4 \times SSC for 2 min at 72°C, chromosomes were counterstained with 4,6-diamidino-2-phenylindole (DAPI) and mounted with Vectashield (Vector Laboratories, Inc., Burlingame, CA, USA). Signals were acquired using an epifluorescence microscope equipped with CCD camera and FISHView software (Applied Spectral Imaging, Ltd., Migdal HaEmek, Israel).

Results

Chromosomal alterations detected by SKY

SKY analysis of the cell line TS1G6 showed numerical as well as structural chromosomal alterations. The hypotetraploid chromosome complement with chromosome numbers of 71 chromosomes indicated that mainly four copies of each chromosome (4n) were present, except for chromosome 15, which was present in six copies, and chromosome 11, present in five copies. This represents a trisomy 15 in a diploid chromosome complement. Moreover, loss of chromosomes X, 2, 3, 7, 8, 9, 10, 13, 16 and 18 were detected as clonal changes. Clonal structural alterations included unbalanced translocations, dicentric as well as isodicentric chromosomes and deletions. A representative karyotype is shown in Fig. 1.

Chromosome 14 was involved in four different translocations. Translocation partners were chromosomes X, 3, and 8 with breakpoints in X?A6, 3A3, and 8A1. The breakpoints of chromosome 14 consistently involved band 14E5. Furthermore, we found two copies of a dicentric chromosome Dic(12F;16cen), an isodicentric chromosome 4 and a deleted chromosome 5.

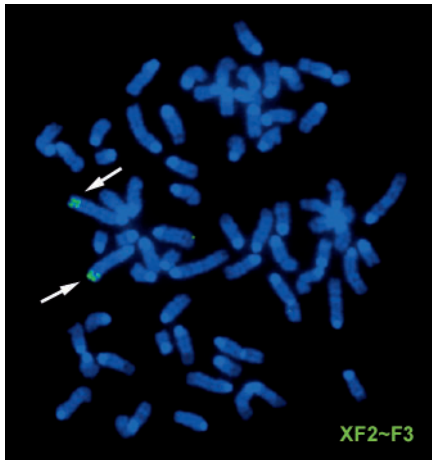


Fig. 2. FISH analysis using a probe specific for chromosomal region XF2~F3 showed double signals for the regular chromosome X and the Der(14)T(X?A6;14E5), indicating a duplication of at least region XF2~F3.

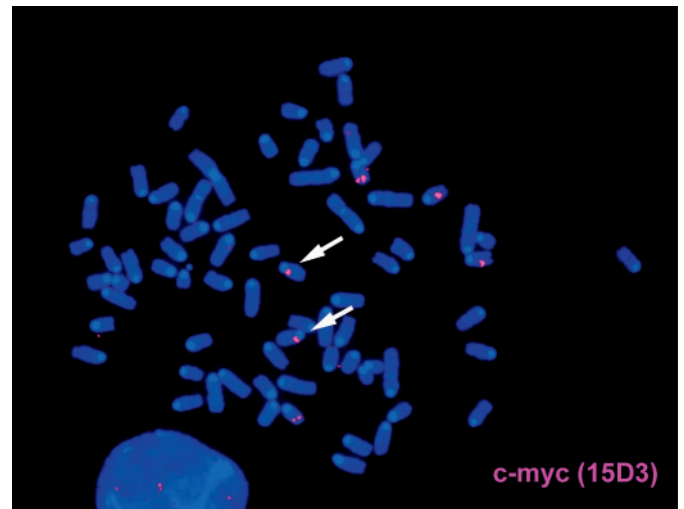


Fig. 3. FISH analysis of *c-myc* oncogene revealed six copies of the gene, thus resembling a trisomy 15. Four of these signals showed a normal localization whereas two signals (arrows) were irregularly localized, thus indicating an intrachromosomal rearrangement.

The karyotype of the cell line is described as:

71<4n>X,-X,-X,Del(XD),-2,-3,-iDic(4cen),Del(5D),-7,-8,-8,-9,-10,+11,Dic(12F;16cen) × 2,-13,Der(14)T(X?A6;14E5),Der(14)T(3A3;14E5),Der(14)T(8A1;14E5),+Der(14)T(8A1;14E5),+15,+15,-16,-16,-18[20]

Single-color fluorescence in situ hybridization

FISH analysis was performed to further characterize chromosomal alterations of chromosomes X and 15. Using a probe specific for the chromosomal region XF2~3, a duplication of this band was detectable in the normal chromosome X in SKY analysis. Furthermore, one of the derivative chromosomes 14, involved in an unbalanced translocation between chromosomes X and 14, also showed a duplication of band XF2~3 (Fig. 2).

FISH using a probe specific for the oncogene *c-myc* showed six single signals corresponding to six chromosomes 15 detected by SKY analysis. Four of these signals showed a regular localization whereas two irregularly localized signals indicated that additional chromosome rearrangements may have taken place (Fig. 3).

Discussion

There are only a few murine models of ALCL. In a paper by Kuefer et al. (1997), NPM-ALK expression by retrovirus-mediated gene transfer led to the development of a large-cell lymphoma of B-cell origin. In contrast, Chiarle et al. (2003) described the spontaneous formation of T-cell lymphomas and plasma cell tumors in an NPM-ALK transgenic mouse model. Up to now, there is no cytogenetic information available about these mouse models. Here, we describe the first cytogenetic investigation of a murine model of human ALK-

negative ALCL. The investigated cell line TS1G6 was established by (IL)-9 transfection and is tumorigenic after injection into immunocompetent mice. The cell line, morphologically and immunohistochemically characterized by Bittner et al. (2000), shows similarities to the human ALK-negative ALCL. SKY analysis showed a hypotetraploid chromosome complement with complex structural aberrations. Polyploidy and a high number of structural changes occur frequently in Hodgkin and Reed-Sternberg cells, but also in high-grade T-cell lymphomas or diffuse large B-cell lymphomas.

The *Alk* gene is localized on chromosome 17E2 in the mouse genome. No alteration of chromosome 17 was detectable by SKY, confirming the results of Bittner et al. (2000). However, dicentric as well as isodicentric chromosomes were detected. They may occur due to fusions of telomeric or near-telomeric regions of genetically unstable cells with critically short telomeres (Hande et al., 1999; Boukamp et al., 2005).

The loss of 5E4-5 due to Del(5D) relates to a loss of the syntenic region 1p22 in two human ALK-negative ALCL due to i(1)(q10). The net loss of XA1-?A6 and XD-Ter due to Del(X)(D), Der(14)T(X?A6;14E5) and the loss of two chromosomes X in the tetraploid clone is comparable to monosomy X as reported in one human ALK-negative ALCL. The gain of 14A1-E5 due to the supernumerary Der(14)T(8A1;14E5) translates into gain of chromosome regions 3p21→p14 and 3p24, which is comparable to trisomy 3 found in two human ALCL, gain of chromosome region 10q22, which is comparable to trisomy 10 found in one human ALCL, and gain of chromosome regions 8p21 and 8p23, which is comparable to trisomy 8 found in three human ALCL (<http://cgap.nci.nih.gov/Chromosomes/Mitelman> and <http://www.ensembl.org>).

Interestingly, translocations mainly involved chromosome 14. Although three different translocation partners underwent a rearrangement with this chromosome, the breakpoint on

chromosome 14 was always the same, 14E5. The murine chromosomal band 14E5 is homologous to regions 13q32→q33 in the human genome. No alterations of this region or other regions homologous to breakpoints in the cell line TS1G6 have been described in ALK-negative ALCL patients.

In the TS1G6 cell line, two dicentric chromosomes Dic(12F;16cen) were detected. The oncogene *tcl1* is located in the region 12F1. Virgilio et al. (1998) reported the development of T-cell leukemias in a *tcl1* transgenic mouse model. Overexpression of *tcl1* resulting from chromosomal aberrations can be found in T- and B-cell neoplasms in humans, but have not been described in ALCL. It cannot be ruled out that the *tcl1* gene is activated due to the Dic(12F;16cen) in the TS1G6 cell line.

TS1G6 contains six copies of chromosome 15 corresponding to a trisomy 15 in a diploid chromosome complement.

References

- Barbey S, Gogusev J, Mouly H, Le Pelletier O, Smith W, Richard S, Soulie J, Nezelof C: DEL cell line: a 'malignant histiocytosis' CD30+ T(5;6)(q35;p21) cell line. *Int J Cancer* 45:546–553 (1990).
- Bittner C, Feller AC, Renaud JC, Lange K, Pietrzik R, Jenetzky C, Briese J, Gaiser T, et al: An animal model for anaplastic large cell lymphoma in the immunocompetent syngeneic C57BL/6 mouse. *Lab Invest* 80:1523–1531 (2000).
- Boukamp P, Popp S, Krunic D: Telomere-dependent chromosomal instability. *J Invest Dermatol Symp Proc* 10:89–94 (2005).
- Chiarle R, Gong JZ, Guasparri I, Pesci A, Cai J, Liu J, Simmons WJ, Dhall G, Howes J, Piva R, Inghirami G: NPM-ALK transgenic mice spontaneously develop T-cell lymphomas and plasma cell tumors. *Blood* 101:1919–1927 (2003).
- Delsol G, Lamant L, Mariame B, Pulford K, Dastugue N, Brousset P, Rigal-Huguet F, al Saati T, et al: A new subtype of large B-cell lymphoma expressing the ALK kinase and lacking the 2;5 translocation. *Blood* 89:1483–1490 (1997).
- Dirks WG, Zaborski M, Jager K, Challier C, Shiota M, Quentmeier H, Drexler HG: The (2;5)(p23;q35) translocation in cell lines derived from malignant lymphomas: absence of t(2;5) in Hodgkin-analogous cell lines. *Leukemia* 10:142–149 (1996).
- Drexler HG: Recent results on the biology of Hodgkin and Reed-Sternberg cells. II. Continuous cell lines. *Leuk Lymphoma* 9:1–25 (1993).
- Drexler HG, Minowada J: Hodgkin's disease derived cell lines: a review. *Hum Cell* 5:42–53 (1992).
- Frank O, Rudolph C, Heberlein C, von Neuhoff N, Schrock E, Schambach A, Schlegelberger B, Fehse B, et al: Tumor cells escape suicide gene therapy by genetic and epigenetic instability. *Blood* 104:3543–3549 (2004).
- Gascoyne RD, Aoun P, Wu D, Chhanabhai M, Skinner BF, Greiner TC, Morris SW, Connors JM, et al: Prognostic significance of anaplastic lymphoma kinase (ALK) protein expression in adults with anaplastic large cell lymphoma. *Blood* 93:3913–3921 (1999).
- Gaudet F, Hodgson JG, Eden A, Jackson-Grusby L, Dausman J, Gray JW, Leonhardt H, Jaenisch R: Induction of tumors in mice by genomic hypomethylation. *Science* 300:489–492 (2003).
- Hande MP, Samper E, Lansdorp P, Blasco MA: Telomere length dynamics and chromosomal instability in cells derived from telomerase null mice. *J Cell Biol* 144:589–601 (1999).
- Inghirami G, Macri L, Cesarman E, Chadburn A, Zhong J, Knowles DM: Molecular characterization of CD30+ anaplastic large-cell lymphoma: high frequency of *c-myc* proto-oncogene activation. *Blood* 83:3581–3590 (1994).
- Kinney MC, Collins RD, Greer JP, Whitlock JA, Sioutos N, Kadin ME: A small-cell-predominant variant of primary Ki-1 (CD30)+ T-cell lymphoma. *Am J Surg Pathol* 17:859–868 (1993).
- Kuefer MU, Look AT, Pulford K, Behm FG, Patten-gale PK, Mason DY, Morris SW: Retrovirus-mediated gene transfer of *NPM-ALK* causes lymphoid malignancy in mice. *Blood* 90:2901–2910 (1997).
- Pasqualucci L, Wasik M, Teicher BA, Flenghi L, Bolognesi A, Stirpe F, Polito L, Falini B, Kadin ME: Antitumor activity of anti-CD30 immunotoxin (Ber-H2/saporin) in vitro and in severe combined immunodeficiency disease mice xenografted with human CD30+ anaplastic large-cell lymphoma. *Blood* 85:2139–2146 (1995).
- Spira J, Wiener F, Klein G: Robertsonian translocation studies on the significance of trisomy 15 in murine T-cell leukemia. *Cancer Genet Cytogenet* 9:45–49 (1983).
- Terenzi A, Bolognesi A, Pasqualucci L, Flenghi L, Pileri S, Stein H, Kadin M, Bigerna B, et al: Anti-CD30 (BER = H2) immunotoxins containing the type-1 ribosome-inactivating proteins momordin and PAP-S (pokeweed antiviral protein from seeds) display powerful antitumor activity against CD30+ tumour cells in vitro and in SCID mice. *Br J Haematol* 92:872–879 (1996).
- Uyttenhove C, Druetz C, Renaud JC, Herin M, Noel H, Van Snick J: Autonomous growth and tumorigenicity induced by P40/interleukin 9 cDNA transfection of a mouse P40-dependent T cell line. *J Exp Med* 173:519–522 (1991).
- Virgilio L, Lazzeri C, Bichi R, Nibu K, Narducci MG, Russo G, Rothstein JL, Croce CM: Deregulated expression of *TCL1* causes T cell leukemia in mice. *Proc Natl Acad Sci USA* 95:3885–3889 (1998).
- Wiener F, Ohno S, Spira J, Haran-Ghera N, Klein G: Cytogenetic mapping of the trisomic segment of chromosome 15 in murine T-cell leukaemia. *Nature* 275:658–660 (1978).

Trisomy 15 represents a characteristic chromosomal alteration in murine T-cell leukemia (Wiener et al., 1978; Spira et al., 1983). Gaudet et al. (2003) showed that genome-wide hypomethylation may induce murine T-cell lymphomas that consistently had a trisomy 15. Interestingly, the proto-oncogene *c-myc* is located in region 15D3 of the murine genome. Trisomy 15 leads to an altered gene dosage of *c-myc*. This may be pathogenetically important, since Inghirami et al. (1994) reported on the high frequency of *c-myc* oncogene activation in human CD30+ ALCL.

In conclusion, using SKY and FISH we were able to cytogenetically characterize the murine anaplastic lymphoid cell line TS1G6. Our results may be the basis to further dissect the molecular changes responsible for the development of ALK-negative ALCL.