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

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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 unrelated 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.

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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 (NPM) 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, ATIC 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 B-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.
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 μg/ml streptomycin at 37°C and 5% CO₂. Metaphase chromosomes were prepared by incubation with colcemid at a concentration of 0.035 μg/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× 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 SKYView™ software was used. In total, 20 metaphases were analyzed.

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, 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 karyotype of the cell line is described as:
71<4n>X,–X,–X,Del(XD),–2,–3,–iDic(4cen),Del(5D),–7,–8,–9,–10,+11,Del(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–F3, 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–F3 (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

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.
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.

Trisomy 15 represents a characteristic chromosomal alteration in murine T-cell leukemia (Wiener et al., 1978; Spiria 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 cyogenetically 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.

References


