

Molecular cytogenetic characterization of the urethane-induced murine lung cell line LA-4 as a model for human squamous cell lung cancer

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Abstract. The murine tumor cell line LA-4 (also known as LA4 or LA 4) has been extensively used in ~70 studies from 1975 to present. However, the genetic characteristics have not been comprehensively delineated, apart from a single solid-stain cytogenetic study, which reported an average of 116 chromosomes per cell. LA-4 was created via urethane induction in an A/He mouse and demonstrated characteristic features of lung adenoma cells. In the present study, multicolor banding-based molecular cytogenetics was combined with molecular karyotyping to characterize ploidy, copy number alterations and chromosomal breakpoints of LA-4. A hyper-tetraploid karyotype with 85-93 chromosomes per cell, three distinct (pseudo-) dicentric derivatives, two neocentrics, four unbalanced translocations, two chromosomes with terminal deletions and one chromosome with a balanced inversion were detected. The results were translated into the human genome and a comparison with the literature revealed that LA-4 is well-suited as a murine model for human squamous cell lung cancer.

Introduction

Human lung malignancies are one of the leading causes of cancer-associated death and account for ~17% of annual new tumor diagnoses worldwide. Lung cancers are categorized into the following subtypes: Lung squamous cell lung carcinoma (L-SCC; accounting for ~30%) and non-SCCs, which include e.g., lung adenocarcinomas (L-AC; ~40%) and large cell carcinomas (~10%) (1,2). Approximately 90% of L-SCCs

are induced by mutagens, particularly toxins inhaled during smoking (1,3).

Accordingly, clinicians and oncologists are confronted with high numbers of lung cancer patients seeking treatment and cure. There are numerous therapeutic options available, including cytotoxic chemotherapy with carboplatin or cisplatin, or erlotinib or cetuximab to target epidermal growth factor, ipilimumab or nivolumab (inhibitors of the immune system) and ramucirumab (antiangiogenetic mechanism of action) (1). Ongoing research aims to target and interfere with additional metabolic pathways in cancer cells to increase sensitivity and response to therapies in lung cancer patients; numerous studies were performed using murine lung cancer cell lines (4-6).

The majority of commonly used murine cancer cell lines were established decades ago. More uniform in shape than human chromosomes, murine chromosomes are difficult to characterize by banding cytogenetics, which resulted in a lack of detailed genetic characterization despite the widespread use of these cell lines (7,8). The urethane-induced murine lung cancer cell line LA-4 (also known as LA4 or LA 4), utilized in ~70 studies in the literature, exemplifies the cytogenomic under-characterization of murine tumor cell lines. LA-4 was established in 1975 (9) from a lung adenoma induced in a (most likely female) A/He mouse. Cells from a primary tumor were cultured and 50 cells were cytogenetically analyzed; on average, 114 (range, 54-133) chromosomes per metaphase were counted. Stoner *et al* (9) reported that the subclones LA-1 to LA-6 were established from the primary tumor; however, further details were only reported for LA-4 and at present, the other 5 cell lines are no longer available. Reports of LA-4 documented epithelial morphology with slower growth compared to the primary tumor. In addition, LA-4 cells did not have any tumorigenic potential in nude mice. Solid-stain cytogenetics revealed 116 (range, 87-132; 80% of cells within the range of 106-122) chromosomes (9). According to the American Type Culture Collection (ATCC) webpage (accessed from Germany, but cell stocks are the same worldwide if purchased from ATCC, https://www.lgcstandards-atcc.org/Products/All/CCL-196.aspx?geo_country=de#characteristics), LA-4 cells have 38-256 chromosomes per cell, with rearranged chromosomes in ~12% of the metaphases.

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As comprehensive cytogenomic characterization of murine tumor cell lines may be performed using murine multicolor banding (mcb) combined with molecular karyotyping (8), the present study provided the first karyotype (including ploidy level) for the LA-4 cell line, with an overview of chromosomal imbalances and *in silico* translation to regions of homology within the human genome; thus, it was possible to determine for which human tumor type this cell line may be used as model.

Materials and methods

Cell line. The murine LA-4 cell line (no. CCL-196™; ATCC), were grown via adherent culture as per the supplier's protocol in Ham's F-12K medium, supplemented with fetal bovine serum and dimethyl sulfoxide (all from Thermo Fisher Scientific, Inc.). Subsequently, tandem cytogenetic analysis and whole genomic DNA extraction were performed (10) and analyses were performed as outlined below.

Molecular cytogenetics and karyotyping. Fluorescence *in situ* hybridization (FISH) was performed as previously described using whole-chromosome paints ('SkyPaint™ DNA kit M-10 for Mouse Chromosomes'; Applied Spectral Imaging) for multicolor-FISH and murine chromosome-specific mcb probe mixes for FISH-banding (10). At least 30 metaphases were analyzed for each probe set (Zeiss Axioplan microscopy; Zeiss AG), equipped with ISIS software v2.86 (MetaSystems). Array comparative genomic hybridization (aCGH) was performed via standard procedures using the 'SurePrint G3 Mouse CGH Microarray, 4x180K' (Agilent Technologies, Inc.) (10).

Data analysis. Imbalances and breakpoints of LA-4 were determined from the mcb and aCGH data and aligned to human homologous regions using Ensembl (<https://www.ensembl.org/info/website/tutorials/grch37.html>) and the University of California Santa Cruz (UCSC) Genome Browser (<http://genome-euro.ucsc.edu/cgi-bin/hgGateway?hgsid=95241316&clade=vertebrate&org=Human&db=hg18&redirect=manual&source=genome.ucsc.edu;GRCh37/hg19>), as previously described (10). The data were compared to genetic changes associated with human cancers (2,11,12).

Results

FISH and aCGH. The LA-4 cell line had a hyper-tetraploid karyotype with several numerical and structural aberrations. The following karyotype was observed in 88% of cells (Fig. 1): 85~93<4n>.X,dic(X;5)(Xqter>XA1::5G2->5qter),-X,-X,-4,-4,der(4)t(3;4)(G;E2),der(5)t(4;5)(A4;C3),del(5)(G2),inv(6)(A1C),+neo(7)(:F3->qter),+dic(7;11)(7A1->7F3::11A2->11qter),+8,+8,del(10)(B5),+idic(10)(A1;A1)x3,neo(11)(:B3->qter),-13,-14,+15,+der(15)t(13;15)(C2;D3),+17,+17,der(18)t(6;18)(B1;E3)x2,+19,+19,+19.

In 12% of LA-4 cells, identical structural aberrations, but random loss of single chromosomes was observed; chromosome numbers ranged from 67 to 72. Preferentially lost chromosomes were 1, 2, 6, 9, 15, 18 and 19.

Overall, the FISH results agreed with the aCGH-results, as summarized in Fig. 2A. *In silico* translation to the human

genome (only imbalances >3.5 mega base pairs were included) identified the corresponding homologous regions (Fig. 2B). Details of aCGH and translation of data are summarized in Table SI; the original data of the aCGH experiment are provided in Table SII.

Comparison with the literature. The corresponding translated homologous copy number variations (CNVs) for LA-4 (Fig. 2B) were compared with common imbalances in related human cancers (2,11,12) (Table I). In human L-AC, only 16/36 regions (44%) were affected by CNVs, similar to the cell line LA-4. Of note, 18/36 (50%) and 21/36 (58%) of CNVs were concordant with human L-SCCs and head and neck SCC (HNSCC).

Discussion

The LA-4 cell line is derived from a mutagen (urethane)-induced primary tumor. In 1975, LA-4 cells were demonstrated to have a karyotype with 87-132 chromosomes and 80% of the cells had chromosome numbers ranging from 106-122; no obvious structural aberrations were observed at that time (9). The only other cytogenetic analyses performed revealed structural aberrations in 12% of the cells with chromosome numbers ranging from 38 to 256 (https://www.lgcstandards-atcc.org/Products/All/CCL-196.aspx?geo_country=de#characteristics).

In the present study, the majority of LA-4 cells had 85 to 93 chromosomes, i.e., a hyper-tetraploid karyotype. There were three distinct (pseudo-)dicentric derivatives, two neocentrics, four unbalanced translocations, two chromosomes with terminal deletions and one chromosome with a balanced inversion. Thus, it was not possible to confirm the original observation that LA-4 harbored no structural rearrangements (9). Also, in contrast to the information from ATCC, these derivative chromosomes were present in 100% and not limited to only 12% of the cells, a result confirmed by the aCGH results (https://www.lgcstandards-atcc.org/Products/All/CCL-196.aspx?geo_country=de#characteristics). However, as observed by Stoner *et al* (9), there was a small subpopulation with only 67-72 chromosomes per cell. The observed dicentric and neocentric chromosomes are unusual and only rarely reported as stable derivatives in tumor cell lines; of note, they have also been observed in human cancer cell lines (13). In previous studies of 23 murine tumor cell lines, these phenomena were only observed in the colorectal cancer cell line CMT-93 [for an overview see (14)].

These results indicate that LA-4 underwent a karyotype evolution during the past 46 years of cell culture. However, the LA-4 cells examined in the present study had an overall stable karyotype, with a tendency towards chromosomal loss in ~12% of cells. Of note, tetraploidization was an early event that was present in the original tumor (9). Previously documented cell line evolution, e.g., for HeLa (15), suggests that variant clones of LA-4 may be present in different laboratories. Thus, it is highly recommended that an aCGH or cytogenetic study of the locally available and used LA-4 cells is performed to compare their chromosomal/genetic constitution with that reported in the present study prior to further experimentation.



Figure 1. Summary of 19 chromosome-specific murine multicolor banding experiments applied in the LA-4 cell line depicted as pseudo-color banding. Derivative chromosomes consisting of different chromosomes are highlighted by frames and displayed twice in this summary karyogram.

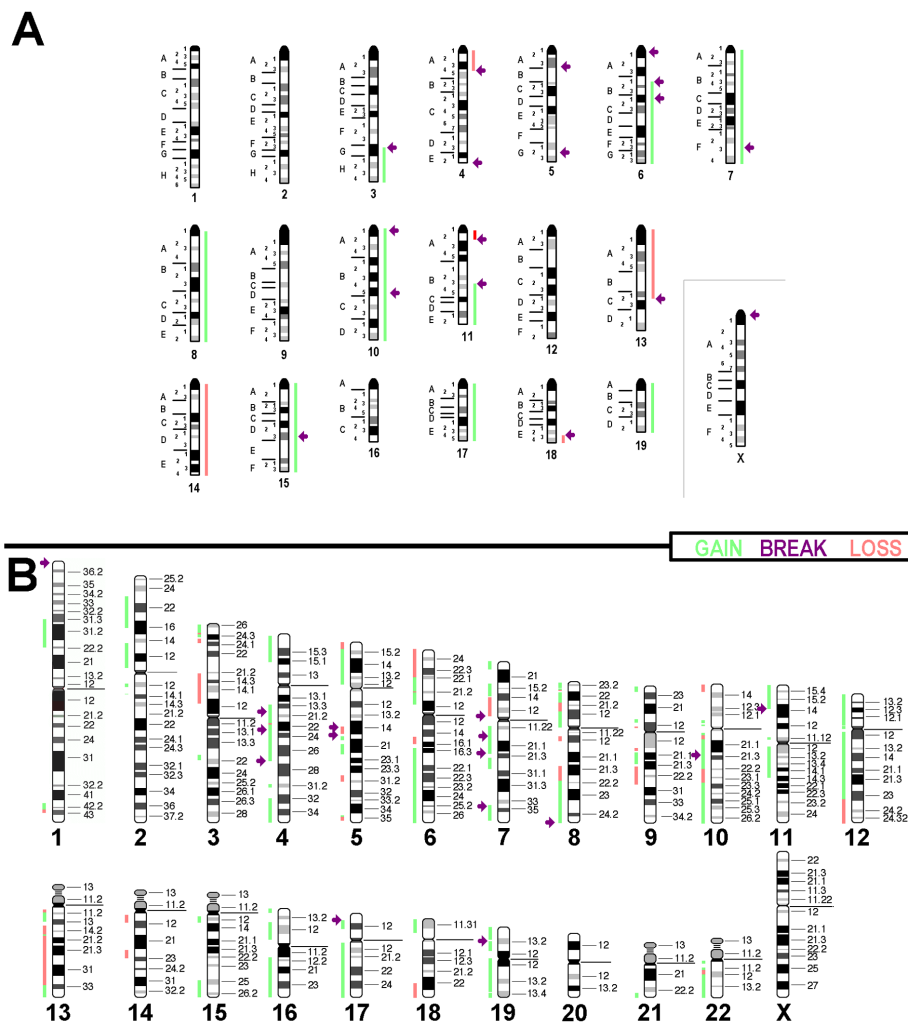


Figure 2. Summary of array comparative genomic hybridization results and copy number variations detected in the cell line LA-4, depicted with respect to a diploid-basic karyotype. Gains are indicated as green bars, losses in red and breaks with arrows. (A) Imbalances observed in the cell line depicted along a murine chromosome set. (B) Results translated and projected along the human chromosome set.

LA-4 was established from a benign lung cancer, an adenoma. As reported in the present study, these genetic data establish that LA-4 cells have evolved *in vitro*, through the acquisition of compounding genetic changes, which were not initially present in 1975 (9). However, the comparison in Table I, which aligned LA-4 with specific human lung tumor

types, was rather inconclusive at first observation. Aberrations typical for human L-ACs were ~44% concordant and human L-SCCs were ~50% concordant. Of note, LA-4 cells exhibited ~58% concordance with imbalances observed in human HNSCCs. Thus, it may be cautiously proposed that LA-4 cells demonstrate characteristic (genetic) features most consistent

Table I. Copy number changes associated with molecular subtypes of human L-AC, L-SCC and HNSCC (2,11,12) compared to those in LA-4 (translated to human).

Chromosomal region	LA-4	Human L-AC	Human L-SCC	Human HNSCC
1p31.3-p22.3	Gain	Gain	Loss	Loss
2p23~2q12	Gain	(Gain)	Gain	Gain
3pter~p24.3	Gain	-	Loss	Loss
3p21.2-3p12	Loss	-	Loss	Loss
~4	Gain	Gain	Loss	-
~5p	Gain	Gain	Gain	Gain
5q14q14	Loss	Gain	Loss	Loss
5q15~q22	Gain	Gain	Loss	Loss
6pter-p22.1	Loss	Gain	Gain	Gain
6p22.1-6q12	Gain	Gain	Gain	Gain
6q15-q15	Loss	Loss	Gain	Loss
6q16.3-qter	Gain	(Loss)	Gain	Gain
~7pter-p14	Gain	Gain	Gain	Gain
7p14-p10	Loss	Gain	Gain	Gain
~7q	Gain	Gain	Gain	Gain
~8p	Gain	-	Loss	-
~8q12-q22.1	Loss	Gain	Gain	Gain
8q22.1-qter	Gain	Gain	Gain	Gain
9q21.2-q22.3	Loss	-	Gain	Gain
~10q	Gain	-	Loss	Gain
~11pter-q14.3	Gain	Gain	-	Gain
12pter-q23	Gain	Gain	Gain	Gain
12q23-qter	Loss	Gain	-	Gain
~13	Loss	Gain	Loss	Gain
14q10-q12	Loss	Gain	Gain	Gain
14q22-q23	Loss	Gain	-	Gain
15q25-qter	Gain	-	Gain	Gain
~16	Gain	Gain	Gain	Gain
~17p	Gain	Loss	Loss	Gain
~17q	Gain	Gain	Gain	Gain
18pter-p11.2	Gain	-	Gain	Gain
18q22-qter	Loss	Loss	Loss	Gain
~19	Gain	-	Gain	Gain
21q22.3-qter	Gain	-	-	Gain
~22	Gain	-	Gain	Gain
Overall		16/36	18/36	21/36

L-AC, lung adenocarcinomas; LSCC, lung squamous cell carcinoma; HNSCC, squamous cell carcinoma of head and neck.

with human SCC cells. As their lung origin is indubitable and they are mutagen-induced, similar to the majority of human L-SCCs, LA-4 may be considered a well-suited model for non-metastatic human L-SCC.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article and in Tables SI and SII.

Authors' contributions

TL conceived the study and obtained funding. SA, MB, FK and SK performed the FISH analysis. MR performed the aCGH study and pre-evaluation. SA performed the overall data interpretation. TL and SA checked and approved the authenticity of the raw data and drafted and edited the manuscript. All authors read and agreed to the final draft of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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