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ORIGINAL ARTICLE

FIRST CYTOGENOMIC CHARACTERIZATION OF MURINE TESTIS TUMOR CELL LINE MLTC-1

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Abstract: The cell line MLTC-1 was established in 1982 as a transplantable Leydig cell tumor from a C57BL/6 mouse. The cell line has already been applied in >100 studies: still, the only information about its genetic content is given in the first description: MLTC-1 initially had a polyploid karyotype. Here, a molecular karyotyping and multicolor banding-based molecular cytogenetic study was done to provide the first chromosomal/ (molecular) cytogenetic characterization of MLTC-1. A hexaploid karyotype with 72 to 105 chromosomes was hereby characterized. Besides polyploidy, unbalanced two- and three-way translocations, dicentrics and one neocentric derivative were identified. Also, no Y-chromosome could be detected in this clearly male derived cell line. Overall, a completely unbalanced genome is present in MLTC-1 with ~20 regions being subject to copy number losses or gains. After translating these imbalances into the human genome in a standardized way, a 40% match of imbalances with human Leydig cell tumors was evident; however, about the same rate of concordance was detectable for human spermatocytic seminomas and non-seminomas as well as testicular germ cell tumor. Accordingly, MLTC-1 is better suited as an advanced testicular germ cell tumor model in general, rather than specifically for human Leydig cell tumors.

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INTRODUCTION

Among the rare group of male testicular tumors, Leydig cell tumors (LCTs) are the most common non-germ cell derived cancers. They may be first recognized as an abnormal testicular mass, and often induce hormonal changes leading to premature masculinization, adult feminization, gynecomastia or infertility. All childhood and a majority of adult LCTs are benign and non-invasive. Nevertheless, ~10% of adult LCTs can become aggressive, invasive and metastasizing. In these cases, orchidectomy is indicated; still, hormonal changes remain unchanged in most patients. As it would be desirable to pinpoint the malignant LCTs at early stages, more basic research is necessary. Besides animal models, (murine) tumor cell lines are ideally suited for such studies. [1, 2]

The murine tumor cell line MLTC-1 has been available since 1982 and was derived from an Leydig cell tumor M548OP transplantable in C57BL/6 mice.[3] According to Pubmed, MLTC-1 has been used in >100 studies, so far. Surprisingly, concerning the genetic constitution of MLTC-1, all that is known to date is that it had a polyploidy karyotype of 91 to 99 chromosomes, which was already established at its first description in 1982.[3]

Here the first comprehensive cytogenomic characterization of MLTC-1 cell line by murine multicolor banding (mcb), molecular karyotyping and in silico translation of obtained results into the human genome (as previously described[4] revealed that it has an ~40% similarity to human LCTs, testicular germ cell tumor as well as spermatocytic seminomas and non-seminomas.

MATERIAL AND METHODS

Adherent murine MLTC-1 cell line was processed according to the provider's instructions (American Type Culture Collection, ATCCR CRL-2065™; Wesel Germany), cells were at the same time worked up cytogenetically to get chromosomes, and molecular genetically to extract whole genomic DNA.[4] The cell line was only used for the here reported study, and ATCC guaranteed cell line identity.

As previously described, fluorescence in situ hybridization (FISH) was done: for multicolor-FISH (mFISH), whole chromosome paints ("SkyPaintTM DNA Kit M-10 for Mouse Chromosomes", Applied Spectral Imaging, Edingen-Neckarhausen, Germany) were used, and for FISH-banding, murine chromosome-specific multicolor banding (mcb) probe mixes[4] were used. At least 30 metaphases were analyzed for each probe set (Zeiss Axioplan, Jena, Germany) microscopy, equipped with ISIS software (MetaSystems, Altlussheim, Germany). In addition, 50 metaphases were analyzed for X and Y-chromosome presence using X and Y-specific BAC probes RP23-29K3 in XA2 / RP23-71G11 in A6 / RP23-257N12 in XC1~2 (all labelled in Spectrum-Orange) and RP24-95K23 in YA2 / RP24-14O08 in YD/ RP24-209O20 in YC2 (all labelled in Spectrum-Green). Chromosome microarray studies (CMA) were performed by "SurePrint G3 Mouse CGH Microarray, 4×180K" (Agilent Technologies, Waldbronn, Germany); as in previous studies, imbalances smaller than 3.5 Mb were not included, as they could not be aligned with FISH-data.[4]

Imbalances and breakpoints of MLTC-1 according to mcb and CMA data were aligned to human homologous regions using Ensembl and the UCSC Genome Browser, as previously done [4]. The obtained data was compared to genetic changes known from human LCTs[5, 6], testicular germ cell tumors[7] and seminomas.[8-11]

RESULTS

According to molecular cytogenetics, MTLC-1 has a near pentaploid karyotype with many single cell aberrations; a basic karyotype with about 99 chromosomes per cell was identified; however, loss or gain of single chromosomes can be observed in almost each cell. The karyotype, as shown in Figure 1, can be written as: 72-105<6n>,XX,-Y,-Y,dic(1;1)(A1;A1), der(2)t(2;9)(E5;C),+del(2)(A2E5),+der(2)(pter->B::A1 ->B),der(3)(3pter-3A1::3F1->3G::14E2->14qter), der(3)(pter-A1::F1->G:),del(4)(A1A5),del(4)(A1A5), del(4)(A1A5),del(5)(E5),-5,-5,dic(6;6)(A1;A1),del(6) (D),-7,-7,-9,dic(11;12)(12qter->12E::12F2->12A1-> 12::11A1->11E2::17B-17qter),neo(11)(E1->qter),-11, -11,der(12)(pter->A3::B->qter),-12,-12,-13,-13, dic(15;15)(A1;A1),+15,+15,der(17)t(17;18)(B1;E1),- 18,-18,der(19)t(6;19)(A1;D1),-19,-19,-19.

Interestingly, a complete absence of the Y-chromosome could be confirmed in overall 50 correspondingly analyzed metaphases using X and Y-specific BAC probes, while two (derivative) X-chromosome were detectable in all cells (results not shown).

In Fig. 2A, the results of CMA for MTLC-1 are summarized. The FISH-results are in agreement with the aCGH data. Translation of detected imbalances in MTLC-1 (Figure 2A) into the human genome (Figure 2B, Supplement Table 1) enabled a comparison with imbalances present in human LCTs, spermatocytic seminomas and non-seminomas and testicular germ cell tumors, which identified a ~40%, ~40% and 36% overlap of detected alterations, respectively (Table 1).

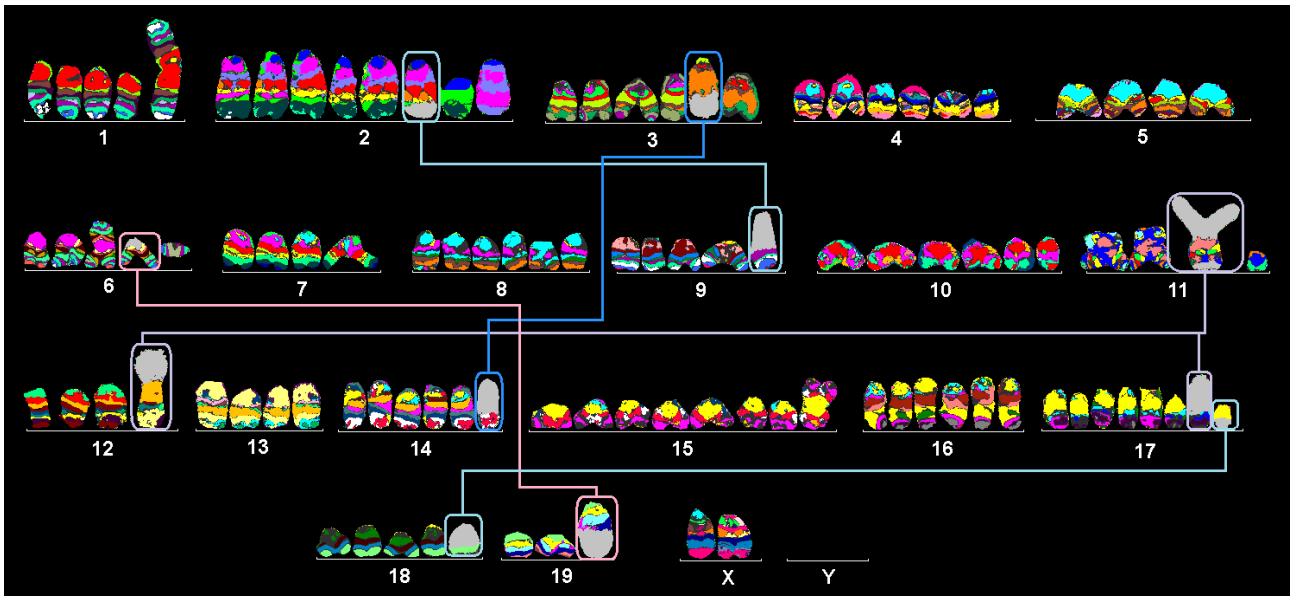

Figure 1. Pseudo-color banding depiction of 20 chromosome-specific murine multicolor banding experiments applied in cell line MLTC-1. Here, derivative chromosomes consisting of different chromosomes are also highlighted by frames and displayed two or three times.

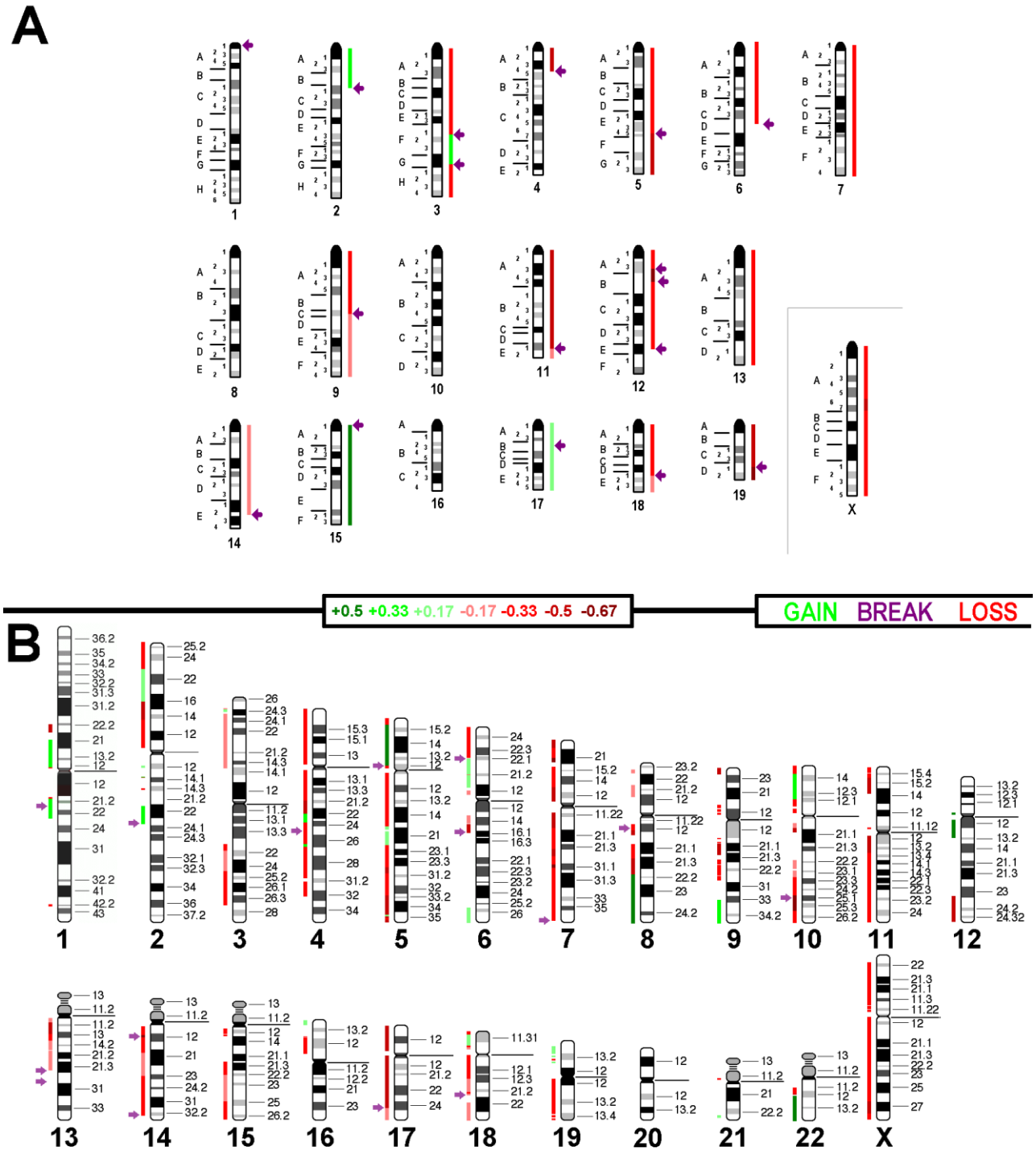

Figure 2. Chromosome microarray (CMA) results of MLTC-1 cell line are depicted with respect to a diploid-basic karyotype.
Gains are indicated as green bars, losses in red and breaks with arrows. (A) Imbalances observed in the cell line depicted along a murine chromosome set. (B) Results translated and projected along the human chromosome set.

DISCUSSION

The first cytogenomic characterization of the tumor cell line MTLC-1 reported here revealed a hexaploid karyotype of 99 chromosomes with many imbalances and breakpoints. As a numerically variant karyotype with ~95 chromosomes was already reported in 1982[3], it can be concluded that the MTLC-1 cell line remained relatively stable during the last 40 years, as also reported for other murine cell lines before.[4, 12-24] Like most of

Table 1. Copy number changes associated with molecular subtypes of human LCTs and seminomas according to[5-11], compared with the copy number variants (CNVs) in cell line MLTC-1.

Chromosomal region	MLTC-1	LCTs[5-6]	Testicular germ cell tumor[7]	Seminoma/ non-seminoma[8-10]
1p22.2-p22.1	loss	**loss**	gain	gain and loss
1p21.2-p12	gain	loss	**gain**	gain and loss
1q21.1-q23	gain	loss	**gain**	**gain**
1q42-1q43	loss	**loss**	gain	gain
2pter-p23	loss	**(loss)**	gain	(gain)
2p23-p16	gain	(loss)	**gain**	**(gain)**
2p16-p11.2	loss	**(loss)**	gain	(gain)
3p24.2-p14.2	loss	**loss**	gain	(gain)
3q21-q27	loss	**loss**	gain	(gain)
4pter-q32	loss	no CNV	**loss**	**loss**
5p15.3-p13.1	gain	**(gain)**	loss	no CNV
5p12-q15	loss	(gain)	**loss**	no CNV
5q15-q23.1	gain	**(gain)**	loss	no CNV
5q23.1-qter	loss	(gain)	**loss**	no CNV
6pter-p22.1	loss	no CNV	gain and loss	no CNV
6p22.1-p12	gain	no CNV	gain and loss	no CNV
6p12-q15	loss	no CNV	gain and loss	no CNV
6q25.3-qter	gain	no CNV	gain and loss	loss
7pter-qter	loss	**(loss)**	gain	**loss**
8p23.1-q22.1	loss	**loss**	gain	gain
8q22.1-qter	gain	no CNV	**gain**	**gain**
9pter-q22.3	loss	(gain)	gain	gain
9q33-qter	gain	(loss)	**gain**	**gain**
10p14-p12.2	gain	no CNV	loss	**(gain)**
10p12.2-qter	loss	no CNV	**loss**	(gain)
11pter-qter	loss	**(loss)**	**loss**	**(loss)**
12p11.2-q13.2	gain	**(gain)**	**gain**	**(gain)**
12q23-qter	loss	(gain)	gain	(gain)
13q11.2-q21.3	loss	(gain)	**loss**	**loss**
14q11.2-qter	loss	no CNV	gain	gain
15q21.3-qter	loss	(gain)	gain and loss	**loss**
16p13.1-p11.2	loss	**(loss)**	gain and loss	**loss**
17pter-qter	loss	**(loss)**	gain	(gain)
18p11.31-p11.2	gain	no CNV	loss	**gain**
18p11.2-qter	loss	**(loss)**	**loss**	**loss**
19p13.3-p12	gain	**gain**	gain and loss	(loss)
19q12-qter	loss	gain	**loss**	**(loss)**
22q12-qter	gain	no CNV	loss	loss
OVERALL	**38**	**15/38**	**14/38**	**15/38**

Legend: Concordances with human CNVs are highlighted in bold

these cell lines, MTLC-1 also showed several dicentric chromosomes normally considered to be instable; also, MTLC-1 is one of the few cell lines with a stable neocentric chromosome.[19, 22] By the molecular cytogenetic characterization scheme applied here, a detailed characterization of numerical and structural changes of MTLC-1 was performed, which enabled the determination of ploidy-grade, individual chromosome numbers and involved rearrangements. Only by combining the FISH method (which detects balanced and unbalanced rearrangements) and the CMA approach (which detects only unbalanced rearrangements), it was possible to characterize the genome of the cell line in a comprehensive way. Based on that data further studies with this cell line can now be done in a justified way. It is clear that gene-knock-in and -knock-out studies make no sense in this cell line, as for most genomic regions, 6 chromosome copies are present.

Interestingly, the fact that MTLC-1, as a male cell line of testicular origin, lost its Y-chromosome completely is not that surprising. Considering the literature, human Y-chromosome instability has been reported before in testicular tumors[25], and Y-chromosome loss in human testicular cell lines.[26, 27] Possibly, this tumor associated sex-chromosome loss may be due to the fact, that no tumor-relevant genes are present on the Y-chromosome. A comparison of translated gains and losses with human LCTs, spermatocytic seminomas and non-seminomas as

well as human testicular germ cell tumors revealed that MTLC-1 showed a 36 to 40% agreement with all of them. Still, no overrepresentation of homologous regions human 12p could be found in MTLC-1. The latter gain of copy numbers is typically observed in invasively growing human testicular seminomas and nonseminomas[28]; the absence of gain of regions homologous to human 12p could support the idea that MTLC-1is a model of advanced LCT. However, it could also be argued that MTLC-1 is suited as a model for advanced testicular tumors in general.

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