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First Cytogenomic Characterization of the Murine Testicular Tumor Cell Line I-10

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Abstract

After being established in 1967, the commercially available murine Leydig cell tumor line I-10 has been used in almost 50 published studies. I-10 has not been characterized, either at the chromosomal/ cytogenetic level or the genetic level, similar to many other murine tumor cell lines. In this study, we performed molecular karyotyping and multicolor banding-based molecular cytogenetics. A slightly hyperdiploid karyotype with 43 chromosomes was described. The main aberrations comprised several unbalanced translocations and three unusual rearrangements (two dicentric derivatives and one neocentric derivative). Nine regions showed copy number gains, and only five small chromosomal parts showed loss of copy numbers. A standardized translation of these imbalances in the human genome was performed, which showed a 63% overlap of the detected imbalances with testicular germ cell tumors, a 53% concordance with human spermatocytic seminomas and non-seminomas, and only a 36% overlap (approx.) of large copy number gains and losses were similar to the corresponding human Leydig cell tumors. However, no Y-chromosome was detected in this

male-derived cell line. Overall, the I-10 cell line was found to be a testicular germ cell tumor model and cannot be treated as a model that is specific to human Leydig cell tumors. At best, it might be suited as a model for an early onset of Leydig cell tumors.

Keywords
Murine cell line; testicular tumor; Leydig cell tumor (LCT); dicentric chromosome; neocentric chromosome; Y-chromosome loss.

1. Introduction

Leydig cell tumors (LCTs) in males are a kind of testicular mass that might lead to hormonal changes in up to a quarter of the cases [1, 2]. Although LCTs are rare, they are still the most common non-germ cell testicular tumors that cause adult feminization and premature childhood masculinization [1, 2]; they might cause gynecomastia or infertility. All childhood LCTs and ~90% of adult cases are benign. However, 10% of adult LCTs become malignant and often metastasize before diagnosis. Orchidectomy is the primary treatment option, but in most cases, hormonal changes remain unaffected. As identifying a malignant subset of LCTs is difficult at the early stages, cellular models are necessary for conducting more directed fundamental research to identify genetic markers and facilitate the development of treatment options [1, 2].

Interestingly, there has been only one murine LCT model available since 1967, which is the lutropin receptor-deficient cell line I-10 [3]. It was established from a testicular interstitial tumor, i.e., from the LCT-transplant H10119, which was introduced into a monolayer culture and injected into a BALB/cJ mouse. Using the single-cell plating approach, the I-10 cell line was established from the third culture passage [3]. Although available for around 55 years [4], I-10 has been used in only 45 studies. This might be because I-10 has not been characterized genetically, either at the chromosome level or at the DNA level.

To provide information regarding the genetic characteristics of I-10, we performed the first comprehensive cytogenomic characterization of cell line I-10 by performing murine multicolor banding (mcb), molecular karyotyping, and in silico translation of the obtained results into the human genome (as previously described [5]). We found that I-10 has only ~36% similarity with the imbalance patterns of LCTs but has 68% concordance with the imbalance patterns of testicular germ cell tumors.

2. Materials and Methods

2.1 Cell Line Work up and Molecular (Cyto) Genetics

After purchasing and directly cultivating the adherent murine I-10 cells following the manufacturer's instructions (American Type Culture Collection, ATCCR CCL83™; Wesel Germany), the cells were prepared in parallel cytogenetically to obtain the chromosomes and genetically to extract the whole genomic DNA [5]. The cell line was not used in any other studies by the authors of this study, and ATCC confirmed the identity of the cell line.

Fluorescence in situ hybridization (FISH) was performed as described in previous studies. For multicolor-FISH (mFISH), whole chromosome paints ("SkyPaintTM DNA Kit M-10 for Mouse Chromosomes", Applied Spectral Imaging, Edingen-Neckarhausen, Germany) were used, while for FISH-banding, murine chromosome-specific multicolor banding (mcb) probe mixes [5] were used. At least 30 metaphases were analyzed for each set of probes (Zeiss Axioplan microscopy) equipped with the ISIS software (MetaSystems, Altlussheim, Germany). Also, 50 metaphases were analyzed for the presence of X and Y-chromosomes using the X and Y-specific BAC probes RP23–29K3 in XA2/RP23–71G11 in A6/RP23–257N12 in XC1~2 (all labeled in Spectrum-Orange) and RP24–95K23 in YA2/RP24–14O08 in YD/RP24–209O20 in YC2 (all labeled in Spectrum-Green). Chromosome microarray studies (CMA) were performed using SurePrint G3 Mouse CGH Microarray, 4 × 180K (Agilent Technologies, Waldbronn, Germany) [5].

Imbalances and breakpoints of I-10, based on the mcb and CMA data, were aligned to the human homologous regions using Ensembl and the UCSC Genome Browser, as previously described [5]. The data were compared to genetic changes known from human LCTs [6, 7], seminomas, non-seminomas, and testicular germ cell tumors [8-12].

2.2 Ethics Statement

According to the ethics committee (medical faculty) and the Animal Experimentation Commission of the Friedrich Schiller University, there are no ethical agreements necessary for studies involving murine tumor cell lines, such as I-10.

3. Results

This cell line showed a slightly hyperdiploid karyotype with many single-cell aberrations, as gains and losses of (derivative) chromosomes. The karyotype is shown below and in Figure 1. 43,der(X)t(X;16)(XA1→XE1::XE3→Xqter::16B4),-Y,idic(1)(A1),der(1)t(1;16)(C5;B4),t(3;9)(H4;F2),+del(4)(D2),der(5)t(5;17)(G2;C),+del(6)(B2),+del(6)(B2),del(7)(A3),+dic(7;17)(A;A),del(9)(A5B),+dic(10;13)(10qter->10C1::10A3->10A1::13A1->13qter),neo(12)(C1->qter),+der(12)t(12;15)(12pter->C1::15B->15D3::15E3->15qter),der(13)t(13;14)(C1;A3),-14,del(18)(A1B1).

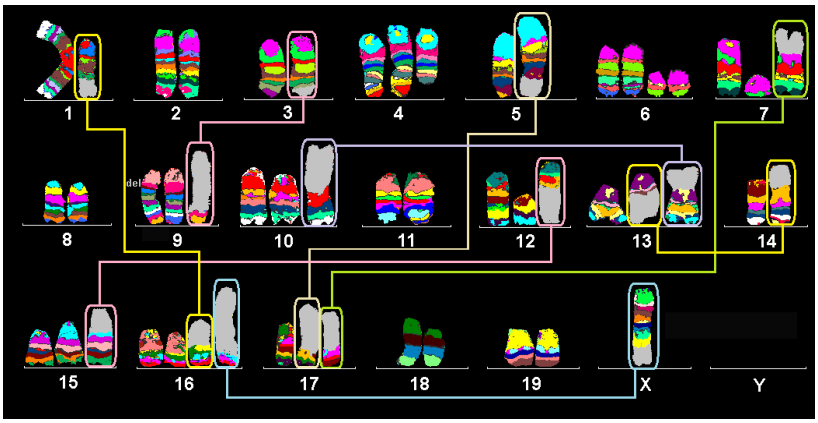

Figure 1 Pseudo-color banding depiction of 20 chromosome-specific murine multicolor banding experiments performed on the I-10 cell line. The del(9)(A5B) is highlighted by

the letters 'del' in gray. The derivative chromosomes, consisting of different chromosomes, are highlighted by frames and displayed twice.

Aberration-induced chromosomal imbalances were relatively rare (13) relative to 16 derivative chromosomes. The main aberrations were unbalanced translocations; one such aberration led to the formation of unbalanced translocations of chromosomes 12 and 15 and a neocentric derivative of chromosome 12. Also, a more simple dicentric as dic(7;17)(A;A), and a more complex dicentric as dic(10;13)(10qter->10C1::10A3->10A1::13A1->13qter) were found to be stable aberrations in this cell line.

The results of CMA for I-10 are summarized in Figure 2A. The imbalances detected in murine cell line I-10 (Figure 2A) were translated into the human genome (Figure 2B and Table 1); this enabled us to compare I-10 imbalances to such in human LCTs; we identified a ~36% overlap of the detected alterations (Table 2). Additionally, 63% and 53% concordance of imbalances were detected with testicular germ cell tumors and human spermatocytic seminomas and non-seminomas (Table 2). No Y-chromosome was detected in 50 correspondingly analyzed metaphases using X and Y-specific BAC probes. However, one (derivative) X-chromosome was detectable in all cells (results not shown).

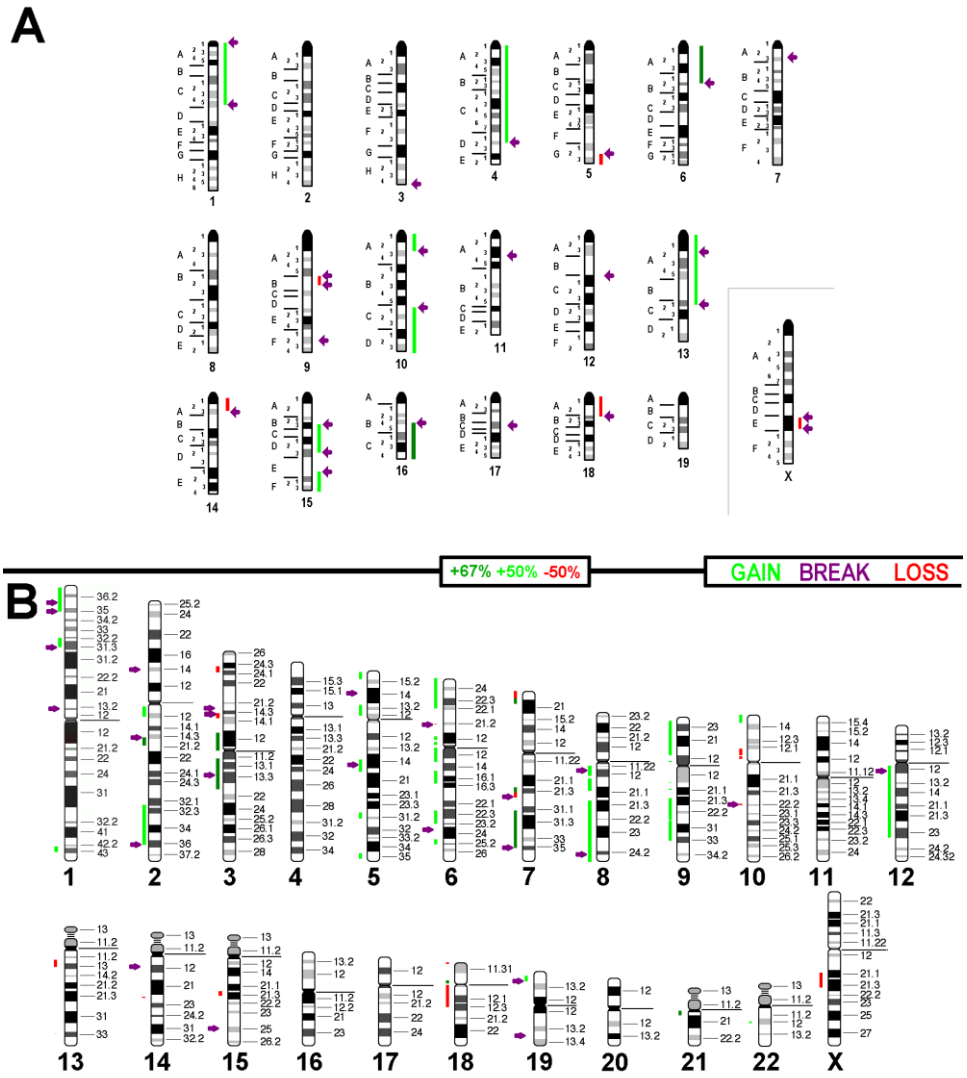

Figure 2 The results of the chromosome microarray (CMA) of the I-10 cell line are depicted based on a diploid basic karyotype. Gains are shown as green bars, losses are shown in red, and breaks are shown with arrows. (A) Imbalances observed in the cell line were depicted along a murine chromosome set. (B) The results were translated and projected along a human chromosome set.

Table 1 The detailed results of murine CMA and translation to the human genome.

region	gain	homolog region in human	
		cytoband	position (GRCh37/hg19)
1pter-1C5	+1	8q11.21-q12.1	8:50767106–56535248
		8q13.1-q21.11	8:67336477–76107163
		6p12.3-p12.2	6:49796129–52568703
		6q11-q13	6:61967179–73920868
		6p12.1-p11.2	6:56223874–58686221
		2q14.3-q21.1	2:128848553–131914911
		2q11.2-q12.2	2:97151065–106819719
		13q33.1	13:103237605–103533914
		2q32.1-q32.2	2:189007277–190504466
		2q32.2-q32.3	2:190506076–196592789
		2q32.3-q36.3	2:196592790–227130883
4pter-4D2	+1	8q12.1-q12.3	8:56650304–62695565
		8q21.3-q22.1	8:87057363–97246782
		8q12.3	8:63094926–64018516
		6q14.3-q16.2	6:87793887–100245013
		9p21.2-p13.1	9:27325073–38472099
		9q22.33-q33.2	9:100037894–123488942
		9q21.31-q21.32	9:82993521–85697078
		9q21.32	9:85856924–86154717
		9p24.1-p21.2	9:6847129–27220407
		1p32.1-p31.3	1:59120351–67562260
		1p36.33-p35.3	1:868618–29650818
6pter-6B2	+2	7q21.2-q21.3	7:92745197–97502117
		7p22.1-p21.3	7:7132996–12536829
		7q31.1-q35	7:112138919–144893609
10pter-10A3	+1	6q25.1-q25.2	6:150394771–154997844
		6q22.31-q23.2	6:123289910–133789230
		19p13.3	19:2030908–4172050
10C1–10qter	+1	12q23.3	12:104359309–108176937
		22q12.3	22:32783299–33472414
		12q13.2-q23.3	12:55351591–104351507
		10p15.3-p15.1	10:138698–5865622
		1q42.3-q43	1:235330060–240084659
13pter-13C1	+1	7p14.2-p13	7:36524506–43605930
		6p22.3-p22.1	6:20065223–28502803
		6p25.3-p23	6:181261–15099150
		6p23-p22.3	6:15104709–20060798

		9q22.1-q22.32	9:91031851–97067712
		5q35.2-q35.3	5:173750964–177039611
		5q31.1-q31.2	5:134073478–137090938
		9q21.32-q21.33	9:86231955–90340399
		9q22.32-q22.33	9:97320957–99417669
		9p13.1	9:38810965–40707569
		9q12-q13	9:65585614–65901647
		9p11.2	9:43623473–43941731
		8q22.1	8:97247028–97373828
		5p15.33-p15.31	5:191425–7935441
		5q14.3	5:84566270–96144383
15B-15D3	+1	5p13.3-p12	5:30309359–42888975
		8q22.1-q24.3	8:97446632–146158346
15E3–15qter	+1	12q12-q13.2	12:41591549–55049456
		3q12.3-q21.2	3:101882967–125343459
		3p12.3-p11.1	3:75865702–90309600
16B4–16qter	+2	21q11.2-q22.3	21:15515528–43438088
		21q11.2	21:14535253–14714360
		18p11.21	18:15016525–15155234
		2q21.1	2:132604281–132757591
region	**loss**	**homolog region in human**	
		cytoband	position (GRCh37/hg19)
		7q22.1	7:100092419–102191754
5G2–5qter	-1 [50%]	7p22.3-p22.1	7:169204–6771649
		7q21.3-q22.1	7:97598308–99229367
		13q12.13-q13.2	13:26784894–34260463
9A5–9B	-1	15q21.2-q21.3	15:52189618–55943472
		3p14.3-p14.1	3:57993765–64009700
		3p24.3-p24.1	3:23146386–27721393
14pter-14A3	-1	14q22.1	14:52272055–52598781
		6p21.2	6:39069766–39266486
		10q22.1-q22.2	10:74870164–75850522
		10p11.21	10:35284099–35521818
		10p12.1-p11.22	10:28950711–32678701
18pter-18B1	-1	10p12.1	10:27747786–28722506
		10p11.21	10:35676708–37094546
		18p11.32	18:112543–599224
		18q11.1-q12.3	18:18528605–41073893
		Xq21.1–21.32	X:80524112–93428087
XE1-E3	-1	Yp11.31–11.2	Y:2925306–6001979
		Xq21.32-q21.33	X:93448016–94928263
Ypter-qter	-1	Yq11.21	Y:14832491–14945874
region	**breakpoint**	**homologue region in human**	
		cytoband	potential tumor associated genes

1A1	idic	8q12.1	8:56014949–56454613 (*XKR4*)
1C5	del/t	2q36.3	2:228844666–229046361 (*SPHKAP*)
3H4	t	1p31.3	1:68564142–68698803 (*WLS*)
4D2	del	1p35.3	1:28525967–28559536 (*DNAJC8*)
5G2	del	7q22.1	7:102113565–102119354 (*POLR2J*)
6B2	t	7q35	7:145813453–148118090 (*CNTNAP2*)
7A2	dic/del	19q13.32	19:47421933–47508334 (*ARHGAP35*)
9F2	t/del	1p13.2	1:113245236–113254055 (*PPM1J*)
9A5	del	15q25.1	15:78632666–78640572 (CRABP1)
9B	del	1p36.11	1:24018269–24022915 (*RPL11*)
10A3	del	6q24.1	6:139224630–139309398 (*REPS1*)
10C1	del	19p13.3	19:3708107–3750811 (*TJP3*)
11A3	t [50%]	2p14	2:65215611–65250999 (*SLC1A4*)
12C1	t	14q12	14:31569318–31677010 (*HECTD1*)
13A2	idic	no homologues	no homologues
13C1	t	5q14.3	5:90664541–90679176 (*ARRDC3*)
14A3	del	10q22.2	10:75910960–76469061 (*ADK*)
15B	del	5p14.3	5:19473060–20575982 (*CDH18*)
15D3	del	8q24.23	8:139142266–139509065 (*FAM135B*)
15E3	del	12p12	12:41582250–41968392 (*PDZRN4*)
16B4	t/del	3q13.31	3:115521235–117716095 (*LSAMP*)
17C	t	6p21.2–21.2	6:40359325–40555204 (*LRFN2*)
18B1	del	2q14.3	2:128698791–128785694 (*SAP130*)
XE1	del	Xq21.2	X:85116185–85302566 (*CHM*)
XE3	del	Xp22.2	X:9935392–9936134 (*AC002365.1*)

Table 2 The copy number changes associated with molecular subtypes of human LCTs and seminomas, according to previous studies [6-12], compared to the copy number variants (CNVs) in cell line I-10. Concordances with human CNVs are highlighted in bold.

chromosomal region	I-10	Human LCTs [6, 7]	Seminoma/non-seminoma [8-11]	Testicular germ cell tumors [12]
1pter-p35	gain	**(gain)**	**(gain)**	**(gain)**
1p32.2-p31.3	gain	**(gain)**	**(gain)**	**gain**
2q11.2-q12	gain	loss	(loss)	(loss)
2q14.3-q21.1	gain	loss	no CNV	(loss)
2q32.2-q36	gain	loss	**gain**	**gain**
3p24.2–24.1	loss	loss	(gain)	gain
3p14.2-p14.2	loss	loss	(gain)	gain
3p12-q21	gain	loss	no CNV	**gain**
5pter-qter	gain	**(gain)**	**(gain)**	loss
6pter-qter	gain	no CNV	(loss)	**(gain)**
7pter-p22	loss	**(loss)**	(gain)	(gain)
7p22-p21	gain	**(loss)**	**(gain)**	**gain**
7q21.2-q21.3	gain	no CNV	**gain**	**gain**
7q21.3-q22	loss	no CNV	(gain)	gain
7q31.1–35	gain	no CNV	**(gain)**	**gain**
8q11.1-qter	gain	no CNV	**gain**	**gain**
9pter-q33	gain	**(gain)**	**(gain)**	**gain**
10pter-p14	gain	(loss)	**(gain)**	**(gain)**
10p11.2-p11.2	loss	**(loss)**	(gain)	**loss**
10q22.2-q22.2	loss	**(loss)**	no CNV	**loss**
12q11-q23	gain	**gain**	**gain**	**gain**
13q12-q13	loss	(gain)	**(loss)**	**loss**
15q21.2-q21.3	gain	**(gain)**	(loss)	(loss)
18pter-18p13.3	loss	no CNV	**(loss)**	**loss**
18p11.2-p11.1	gain	no CNV	loss	loss
18q11.1-q12.3	loss	(gain)	**loss**	**loss**
19p13.3-p13.3	gain	**gain**	**(gain)**	**(gain)**
21q11.1-q11.2	gain	no CNV	**(gain)**	**gain**
22q12-q12	gain	(loss)	(loss)	loss
Xq21.1-q21.3	loss	gain	gain	gain
OVERALL	**30**	**11/30**	**16/30**	**19/30**

4. Discussion

The first cytogenomic characterization of the tumor cell line I-10 revealed a slightly hyperdiploid karyotype of 43 chromosomes, with relatively few imbalances and breakpoints for a cell line that is in cell culture for around 55 years. Interestingly, I-10 is a tumor cell line with dicentric chromosomes, which are normally considered to be unstable [5, 13-25]. It is one of the few cell lines with a stable

neocentric chromosome [20, 21]. Murine multicolor banding (mcb), along with CMA, enabled a detailed characterization of numerical and structural changes in the I-10 cell line. This study highlighted the importance of understanding the ploidy grade, the individual chromosome numbers, and the rearrangements involved before using a cell line in further, more sophisticated experiments. Data on these parameters might allow well-informed studies to be performed. According to this study, I-10 is not a suitable model to be used for knockout studies of genes located in regions that are present in three copies in this cell line; this refers to murine chromosome 4A-D2, 6A-B2, 7A1-A3, 10A1-A3, and 10C1-qter.

This male cell line of testicular origin lost its Y-chromosome in all cells. The loss of Y-chromosome has been previously reported in other murine cell lines but never in a testicular-derived murine cell line. However, Y-chromosome instability has been found in rat and human testicular tumors [26-28], while the loss of the Y-chromosome was reported in the human testicular cell lines JKT-1 and TCam-2 [29, 30], as well. The reason for this tumor-associated sex-chromosome loss remains undetermined. Tumor-relevant genes might not be present on the Y-chromosome.

Here, a comparison of translated I-10-specific imbalances was performed, similar to that performed for other murine cell lines [5, 13-25]. Sequencing analyses might also be fruitful in the future, as subtype-specific differences in somatic mutations were found in testicular germ cell tumors [31, 32]. Clear differences in the mutation patterns between seminomas and non-seminomas were found [31, 32]. However, chromosomal imbalances in both subtypes were similar [8-11]. Thus, only considering copy number alterations summrized in this study (Table 2), seminomas and non-seminomas could be regarded as one group in common.

Regarding the gains and losses present in I-10, the highest concordance of 63% was for human testicular germ cell tumors, while the lowest concordance of ~36% was for human LCTs. No overrepresentation of sequences homologous to the short arm of chromosome 12 could be found in I-10, which might be related to the invasive growth of human testicular seminomas and non-seminomas [33]. This indicated that I-10 might be used as a model for early-onset LCT. However, it might also be used as a testicular germ cell tumor model.

5. Conclusions

The I-10 cell line is more suited as a model for human testicular germ cell tumors rather than for LCTs in particular. However, I-10 should not be used for studying metastasizing LCTs or testicular tumors. Moreover, studies on gene expression and approaches to introduce or remove gene copies need to consider the specific copy number alterations in this diploid tumor cell line.

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Author Contributions

TL conceived the study and obtained funding. SA, LMB and SK performed the FISH analysis. MR performed the CMA 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.

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Competing Interests

The authors have declared that no competing interests exist.

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