Research Article
Volume 5:1, 2021

Journal of Genetics and Genomes

ISSN: 2684-4567 Open Access

Molecular Cytogenomic Characterization of Two Murine Liver Cancer Cell Lines: MH-22A and Hepa 1-6

Shaymaa Azawi[1], Katja Piaszinski[1], Milash Balachandran[1,2], Thomas Liehr[1*] and Martina Rincic[3]

[1]*Institute of Human Genetics, Jena University Hospital, Jena, Germany*

[2]*Department of Genetic Engineering, SRM Institute of Science and Technology, Kattankulathur, Chennai, India*

[3]*Croatian Institute for Brain Research, School of Medicine University of Zagreb, Salata, Zagreb, Croatia*

Abstract

Background: Murine liver tumor cell lines MH-22A and Hepa 1-6 are used regularly in studies working on better treatment options. Even though both cell lines have been established 40 to 50 year ago, the literature lacks details on their cytogenetic and cytogenomic constitution. Here the corresponding gap in the literature is closed, as both cell lines have been characterized in detail for their chromosome numbers and content.

Methods: Here we performed the first molecular cytogenetic characterization for the two murine liver tumor cell lines MH-22A and Hepa 1-6 using fluorescence in situ hybridization and molecular karyotyping.

Results: Both cell lines are (near-)triploid and show numerous chromosome rearrangements leading to expressed copy number alterations. The by molecular cytogenetics and molecular karyotyping obtained data of the cell lines MH-22A and Hepa 1-6 was in silico (= genome browser based-) translated to the human genome. This previously successfully applied approach enabled the characterization of MH-22A as a model for hepatoblastoma or early hepatocellular carcinoma and Hepa 1-6 for (advanced) hepatocellular carcinoma.

Conclusions: The cell lines MH-22A and Hepa 1-6 can now be applied in research in a justified way.

Keywords: Molecular cytogenetics • Murine HCC cell lines • Cholangiocarcinoma • Mutagenes

Abbreviations: Array Comparative Genomic Hybridization (ACGH); Fluorescence in Situ Hybridization (FISH); Hepatocellular Carcinoma (HCC); Hepatoblastoma (HB); Murine Multicolor Banding (MCB); Multicolor-FISH (mFISH)

Introduction

Cancer disorders are a major burden for human society; among them liver cancer is fifth most leading cause of death worldwide [1,2]. Hepatoma or hepatocellular carcinoma (HCC) is with ~80% the most common malignant primary liver tumor in adults [2-4], while hepatoblastoma (HB) can be found primarily in children with an incidence of 4.3% per year [5]. Further subgroups of adult liver cancer are fibrolamellar HCC [6], cholangiocarcinoma [7], angiosarcoma [8] and secondary liver cancer, i.e. liver metastases from other primary tumors. Chronic viral infection, overweight and alcohol consumption are the main risk factors for HCC; besides aflatoxin exposure, iron overload, smoking and oral contraceptives are thought to play a causative role in HCC initiation [4]. Differences in hormone-levels and life style may also play a role, as liver cancer is two-to four-fold more frequent in adult men than in adult women [9,10]. For HB hepatitis infection, maternal estrogen exposure, maternal alcohol consumption, maternal smoking, or maternal direct or indirect nitrosamines intake have been identified as risk factors, and outcome is in general much better than in HCC. In HB 10-year overall survival rate of >80% were already achieve before the year 1990, based on liver resection and transplantation and/or chemotherapy [11]. In contrast, treatment strategies for HCC are still limited and successful treatment means increasing overall survival of patients for few months only; accordingly, early HCC diagnosis has still the best influence on prognoses. In HCC surgical resection and liver transplantation are the only effective treatment options for patients with early stage HCC [12]. Besides, chemo and radiotherapy for the majority of patients with advanced HCC go together with severe side effects. Therefore, there is a need for more effective medication with less adverse effects for patients with liver cancer, especially HCC [2,13,14]. Also more research is necessary, as genetic heterogeneity in liver cancer is observed, also hampering individualized treatment [3,12,15].

In this context in vitro and/or animal models for HCC but also for HB are applied [1], e.g. to gain new insights concerning development, metastasis, and treatment approaches like application of in immune checkpoint inhibitors and adaptive cell therapies [16,17]. Especially suited are murine tumor cell lines, like MH-22A or Hepa 1-6, which have the capacity allow new insight about regulation of tumor related genes and pathways in HB and HCC [2,17]. Even though the cell lines MH-22A and Hepa 1-6 were established decades ago, information about their cytogenetics/cytogenomics are scarce. Cell line MH-22A was established at the Institute of Cytology, Russian Academy of Sciences, St. Petersburg, Russia, from solid form of murine HCC 22A being induced by 3-methylcholantrene in C3HA mice [18,19]. Hepa 1-6 originates from cell line Hepa 1, which was taken into culture in 1980 from the BW7756 tumor, which arose spontaneously in the C57L/J mouse strain. Hepa 1 cells were then treated with mutagenes and thus, subclones were derived; chromosome numbers in Hepa 1 cells were around 67 including 2-4 biarmed chromosomes [20,21], Hepa 1-6 cells were never studied (cyto)genetically before.

Here the two murine liver cancer cell lines MH-22A and Hepa 1-6 were studied for the first time molecular cytogenomically. This data was in silico (= genome browser based-) translated to the human genome. This approach has been used successfully before [22-27], and characterized here MH-22A as a model for HB or early HCC and Hepa 1-6 as a model for (advanced) HCC.

Material and Methods

Murine HCC cell lines

***Address for Correspondence:** Thomas Liehr, Institute of Human Genetics, Jena University Hospital, Jena, Germany, Tel: ++49-3641-9396850,*

Fax: ++49-3641-9396852; E-mail: Thomas.Liehr@med.uni-jena.de

***Received date:** 03 December, 2020; **Accepted date:** 17 December, **Published date:** 24 December, 2020*

The murine cell line MH-22A (Passage +4) was purchased from European Collection of Authenticated Cell Cultures (Salisbury, UK – order# ECACC 96121721) and Hepa 1-6 (Passage 22) was obtained from Cell lines Service GmbH (Eppelheim, Germany – order# CLS 400474). Cells grow adherently in RPMI-1640 medium with 2 mM L-glutamine, 10 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), and 10% fetal-calf serum in the presence of antibiotics. For this study, the cells were divided into two portions. Portion 1 was worked up cytogenetically, and cells from portion 2 were used to extract whole-genomic DNA by Blood and Cell Culture DNA Midi Kit (Qiagen, Düsseldorf, Germany) as previously described [24].

According to written statements from the ethical committee (medical faculty) and the Animal Experimentation Commission of the Friedrich Schiller University, there are no ethical statements necessary for cases involving the use of murine tumor-cell lines, like MH-22A and Hepa 1-6.

Molecular cytogenetics

Fluorescence in situ hybridization (FISH) was performed as previously described [24]. "SkyPaintTM DNA Kit M-10 for Mouse Chromosomes" (Applied Spectral Imaging, Edingen-Neckarhausen, Germany) was applied for multicolor-FISH (mFISH) with whole chromosome paints, and murine chromosome-specific multicolor banding (mcb) probe mixes for FISH-banding. At least 30 metaphases were acquired and analyzed for each probe set on a Zeiss Axioplan microscope, equipped with ISIS software (MetaSystems, Altlussheim, Germany). Array-based comparative genomic hybridization (aCGH) was completed according to standard procedures with "SurePrint G3 Mouse CGH Microarray, 4 x 180 K" (Agilent Technologies, Santa Clara, CA, USA).

Data analysis

The regions of imbalances and breakpoints in MH-22A and Hepa 1-6 were characterized after analyses of aCGH and mcb data, and aligned with their human homologous regions using Ensembl Genome Browser, as previously described [24].

The data we obtained compared with the most known gene alteration for human HCC according to the literature [25-29].

Results

Molecular cytogenetics

MH-22A: In MH-22A metaphases were hypotriploid and had the following composite karyotype:

56~57<3n>,XX,-X or Y,der(2)(A1→B::B→A3::C3→H1::H1→H4),der(5)(A1→G3::G3→F::F1→12qter),+3,dic(5)(A1→C1::C1→A1:CEN:A1→B1~3:),der(5)t(5;12)(F1;F1),+dic(5;15)(12F2→12F1::5F→5G3::CEN::CEN::15A1→15qter),+dic(6;17)(A1;A1),der(12)(A1→B1::A1.2→B1:),del(13)(D1D2.3),der(14)(4A1→14D1::14C1→14D1::7D3→7E3::7?E3~F1::?7E1::14C2→14C3::18B3→18E4),-14,-16,der(16)(A1→C3::B1→C4),-18

A representative cytogenomic view on the MH-22A karyotype is shown in Figure 1.

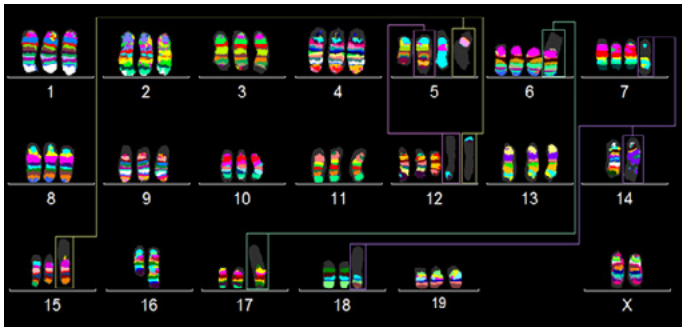

Figure 1. Murine multicolor banding (mcb) as applied on cell line MH-22A. The typical pseudocolor banding for all 20 different murine chromosomes is shown. This figure depicts the summary of 20 chromosome-specific fluorescence in situ hybridization (FISH)-experiments. Four translocations consisting of two different chromosomes, each, are highlighted by frames in this summarizing karyogram.

Hepa 1-6: Hepa 1-6 cell line was mitotically more unstable than MH-22A and had a hypertriploid karyotype comprising 68-71 chromosomes per metaphase; composite karyotype (representative cytogenomic result see Figure 2) reads as follows:

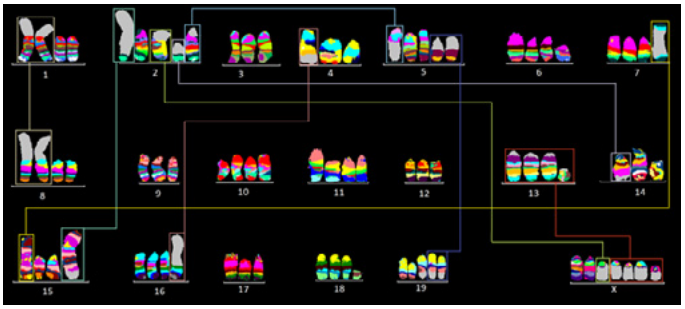

Figure 2. Murine mcb as applied to the murine Hepa 1-6 cell line.

68~71<3n>,X,del(X)(A3A7),+der(X)t(X;2)(A3;A3),+der(X)t(X;13)(A3;A1),+der(X)t(X;13)(A3;A1),+der(X)t(X;13)(A3;A1),+der(X)t(X;13)(A3;C1),dic(1;8)(A1;A1),+dic(1;8)(A1;A1),-2,-2,der(4)t(4;16)(4A1→4C3::4C7→4E2::16B5→16Cqter),del(4)(D2E2)x1~2,der(5)t(2;5)(A2;G2),+del(6)(A2F1),+6,-8,+10,+11,-13,-13,-13,der(14)t(2;14)(H2;D2),del(14)(A3E4)x1~2,dic(15)(15qter→15F1:cen:15A1→15F3::15F3→15A1::2G1→2qter),+idic(15)(7qter→7F2::15F1→15A1::15A1→15A1::7F2→7qter),+18,+del(18)(D1),der(19)t(5;19;5)(F;D2),+der(19)t(5;19;5)(F;D2),+der(19)t(5;19)(F;D2). aCGH

After comparison with aCGH data which was in concordance with FISH results, the final karyotype with the gains, loss and breakpoints for MH-22A and Hepa 1-6 cell lines could be summarized in Figure 3. These results were translated to the corresponding homologous regions in the human genome as depicted in Figure 4. All included imbalances in the evaluation were larger than 3.5 mega base pairs.

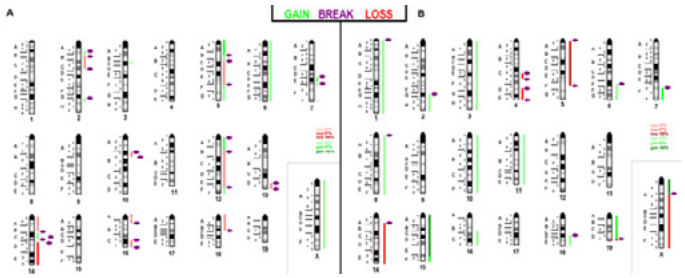

Figure 3. aCGH results for MH-22A and Hepa 1-6. Copy number variations detected in MH-22A (A) and Hepa 1-6 (B) are summarized here with respect to a triploid-basic karyotype. Gains are depicted as green bars (one more copy=light green; two more copies=dark green), loss of one copy is depicted as a red bar and loss of two copies is depicted as a dark-red bar. Breaks are registered here as arrows.

Data-analyses

The common aberrations and cytogenetic changes that frequently occur in HB (Table 1), HCC (Table 2) [28,29], cholangiocarcinoma (Table 3) [7], and fibrolamellar HCC (Table 4) [6] were compared to the copy number alterations detected in Hepa 1-6 and MH-22A (Figure 4; Suppl. Table). No corresponding data was available for angiosarcoma, and liver metastases originating from other primary cancers were not taken into account here.

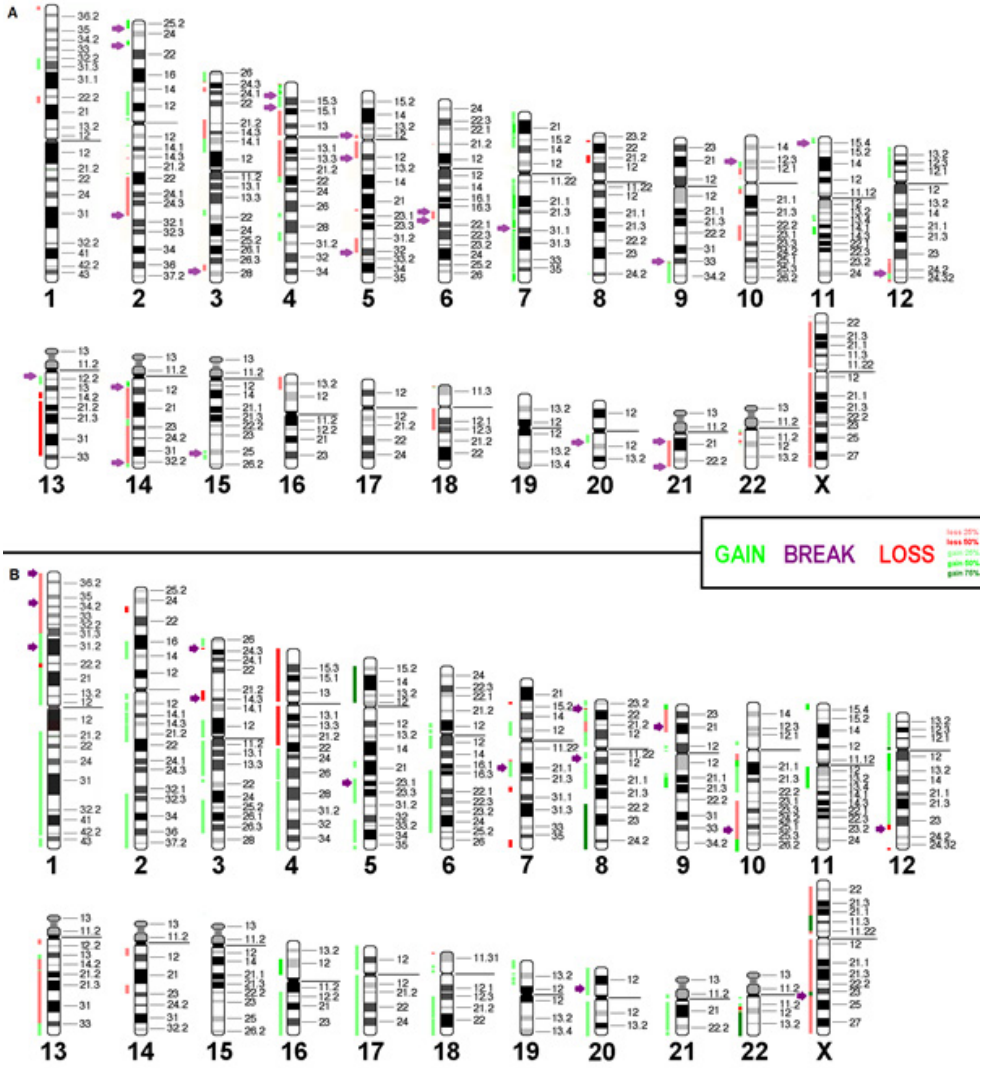

Figure 4. Results of in silico translation for MH-22A and Hepa 1-6 to human genome. Imbalances present in in MH-22A (A) and Hepa 1-6 (B) were 'translated' to the corresponding homologous regions in human. As before, gains are depicted as green bars (one more copy=light green; two more copies=dark green) loss are depicted as red bars (one more copy=light red bar; two more copies=dark-red). Breaks are registered here as arrows.

Accordingly, MH-22A has 5/9 CNVs (55%) being typical for HB and 6/12 being typical for HCC (50%), while Hepa 1-6 has 7/9 copy number alterations typical for HB (78%) and 8/12 for HCC (67%). Also MH-22A and Hepa 1-6 have 6/23 CNVs (26%) and 13/23 (56%) being typical for cholangiocarcinoma (Table 3), and 4/14 (27%) and 8/15 (53%) like in fibrolamellar HCC (Table 4).

Chromosome	Type of aberration	MH-22A	Hepa 1-6
1q	gain	-	+
2	gain	(+)	+
4q	loss	(+)	(+)
7q34	gain	+	-
8q	gain	-	+
14q11.2	gain	+	-
17q	gain	-	+
20q	gain	(+)	+
22	gain	-	+
Sum of concordance of CNVs for HB		5/9	7/9

Table 1. MH-22A and Hepa 1-6 compared to human hepatoblastoma. The copy number alterations in MH-22A and Hepa 1-6 are compared to those observed in HB (according to [28] and [29]). Abbreviations: += full concordance; (+)= partial concordance (still to be evaluated as concordance); -= no concordance.

Chromosome	Type of aberration	MH-22A	Hepa 1-6
1p	loss	-	+
1q	gain	-	+
4q	loss	(+)	(+)
6q	loss	(+)	(gain)

7q31	gain	+	-
8p	loss	(+)	(+)
8q	gain	-	+
11q13	gain	(+)	+
13q	loss	+	+
16q	loss	-	(gain)
17p	loss	-	(gain)
17q	gain	-	+
Sum of concordance of CNVs for HCC		6/12	8/12

Table 2. MH-22A and Hepa 1-6 compared to human hepatocellular carcinoma. The copy number alterations in MH-22A and Hepa 1-6 are compared to those observed in HCC (according to [28] and [29]). Abbreviations: + = full concordance; (+) = partial concordance (still to be evaluated as concordance); - = no concordance; (gain) = instead of loss here observed a gain.

Chromosome	Type of aberration	MH-22A	Hepa 1-6
1p13.3~21	gain	-	+
1q21	gain	-	+
1q31.1~32.1	gain	-	+
5q34~qter	gain	-	+
7p22	gain	+	-
7q11.2	gain	+	-
7q22.1	gain	+	-
9q33~qter	gain	+	-
11q12.2~13.4	gain	-	+
11q23.1~qter	gain	-	-
12p13	gain	+	+
12q13	gain	-	+
12q23qter	gain	(loss)	-
16	gain	-	+
17	gain	-	+
19p	gain	-	+
19q	gain	-	-
20q	gain	(+)	+
21q22.2~qter	gain	(loss)	+
22q	gain	-	+
1q25.3~35.2	loss	-	-
6	loss	-	-
17q21.3~22	loss	-	-
Sum of concordance of CNVs for cholangiocarcinoma		6/23	13/23

Table 3. MH-22A and Hepa 1-6 compared to human cholangiocarcinoma. The copy number alterations in MH-22A and Hepa 1-6 are compared to those observed in cholangiocarcinoma (according to [7]). Abbreviations: += full concordance; (+)= partial concordance (still to be evaluated as concordance); -= no concordance; (loss)= instead of gain here observed a loss.

Chromosome	Type of aberration	MH-22A	Hepa 1-6
1q	gain	-	+
6q27	gain	-	-
8q24	gain	-	+
17q25.3	gain	-	+
22q	gain	-	+
1p36.33	loss	+	+
4p	loss	(+)	+
4q	loss	(+)	(+)
8p21.3	loss	+	+
11p15.5	loss	-	-
17	loss	-	(gain)
19p	loss	-	(gain)
19q	loss	-	-
20	loss	-	(gain)
22q	loss	-	(gain)
Sum of concordance of CNVs for fibrolamellar HCC		4/15	8/15

Table 4. MH-22A and Hepa 1-6 compared to human fibrolamellar hepatocellular carcinoma. The copy number alterations in MH-22A and Hepa 1-6 are compared to those observed in fibrolamellar HCC (according to [6]).

Discussion

The study performed here in murine liver cancer cell lines was based on molecular cytogenetics and aCGH. Based on molecular cytogenetics the modal chromosome numbers of the cell lines could be determined as triploid. In aCGH it is technically impossible to determine the ploidy status without cytogenetic information and data-interpretation for aCGH can only be done correctly by combining results from the two approaches as done here and previously [22-27]. Accordingly, first lesion to learn from this study in connection with "Studies on the Pathogenesis of Chromosome Rearrangement" being the topic of this special issue is to remember, that no cytogenomic approach can stand alone. Each approach has its restrictions and can only be interpreted correctly in connection with others.

Liver cancer has several subtypes, specified as HCC, fibrolamellar HCC, cholangiocarcinoma, angiosarcoma, secondary liver cancer and HB [5-8,11,28,29]. As available animal models like the liver tumor cell lines MH-22A and Hepa 1-6 were never characterized in detail genetically before, and also they were established 40 to 50 years ago and karyotype evolution could have taken place, this study was undertaken. Its goal was to provide insights for which liver cancer subtype the 2 cell lines may be suited as models. The here the applied scheme was successfully applied already in several previous studies [22-27].

Clinically most important liver cancer are childhood HB and adult HCC. According to the data from Tables 1-4 one can carefully deduce that MH-

22A, being established from a tumor reported as HCC at least 50 years ago, shows more genetic similarities to HB than HCC. In detail, MH-22A has aberration in common to human HB to 55%, human HCC to 50%, human fibrolamellar HCC to 27% and human cholangiocarcinom to 26%. So the best match of MH-22A is to human HB, even though (early stage) HCC could also be considered.

For Hepa 1-6, an about 40 year old cell line, this comparison shows that it can be best considered to be a model for HCC. This is even as Hepa 1-6 has 7/9 copy number alterations typical for HB (= 78%) and only 7/12 (67%) in common for HCC. However, in Hepa 1-6, there are 8 versus 7 frequent chromosomal rearrangements in common for HCC and HB, respectively. Additionally, chromosomal aberrations are much less frequent in HB than in HCC; according to Buendia [28] and Luo [29] it would be very unusual for an HB to show the 8 in Table 1 mentioned aberrations simultaneously as present in Hepa 1-6, and besides also many other chromosomal rearrangements. The rates of common copy number variations of Hepa 1-6 with cholangiocarcinoma (56%) and fibrolamellar HCC (53%) are even smaller than for HCC (67%). Overall, Hepa 1-6 is interpreted to be most likely a model for advanced HCC.

Interestingly, the chromosome constitution of Hepa 1-6 seems not to differ significantly from that described for Hepa-1 by Darlington et al. in 1980 [21]: (i) here in this study 68 to 71 chromosomes were found, while in that research from 1980 [21] 67 chromosomes were described; (ii) in 1980 2-4 biarmed chromosomes were reported [21] – here again 4 such chromosomes could be found as two times dic(1;8)(A1;A1), dic(15)(15qter→15F1:cen :15A1→15F3::15F3→15A1::2G1→2qter), and idic(15)(7qter→ 7F2::15F1→15A1::15A1→15A1::7F2→7qter). Accordingly, it is surprising that murine tumor cell lines shown much correspondence to human tumors in terms of chromosomal imbalances, but the pathogenesis of chromosome rearrangements seems to be a fundamentally different one [30]. Especially the chromosomal stability of murine tumor cell lines is striking and has been shown repeatedly [22-27]. For human tumors and tumor cell lines tremendous chromosome instability with ongoing karyotype evolution is reported [31]. Similarly human tumor cell lines being established decades ago are never chromosomally stable over longer times [31-33]; this is even the case for human non-tumor cell lines [34-36].

CONCLUSION

Molecular cytogenetic studies to determine chromosome numbers and rearrangements, including genomic imbalances, are necessary and fundamental studies, without them more sophisticated studies dealing with tumor initiation, progression or even treatment cannot be carried out in a meaningful way.

Ethics Approval and Consent to Participate

Not applicable

Consent for publication

Not applicable

Competing interests

The authors declare no conflict of interest.

Funding

Supported by grant#2013.032.1 of the Wilhelm Sander-Stiftung.

Authors' contributions

TL developed the idea for the study and got funded for it; KP, MB and SA did the FISH-studies; MR performed aCGH studies and did pre-evaluation; KP, MB and SA performed the overall data interpretation; TL and SA did final paper drafting; all authors agreed on final draft.

Acknowledgements

The technical support of Nadezda Kosyakova (Jena, Germany) is kindly acknowledged.

Data and material availability

All data is included in the present paper and suppl. data.

References

1. ZJ, Brown, Heinrich B and Greten TF. “Mouse models of hepatocellular carcinoma: an overview and highlights for immunotherapy research." Nat Rev Gastroenterol Hepatol 15 (2018): 536-554.
2. HE, Zhang, Henderson JM and Gorrell MD. “Animal models for hepatocellular carcinoma.” Biochim Biophys Acta Mol Basis Dis 1865 (2019): 993-1002.
3. C, Ozen, Yildiz G and Dagcan AT. “Cevik D; Ors A; Keles U; Topel H; Ozturk M. Genetics and epigenetics of liver cancer.” N Biotechnol 30 (2013): 381-384.
4. J, Balogh, Victor D 3rd, Asham EH and Burroughs SG, et al. “Hepatocellular carcinoma: a review.” J Hepatocell Carcinoma 3 (2016): 41-53.
5. J, Feng, Polychronidis G, Heger U and Frongia G, et al. “Incidence trends and survival prediction of hepatoblastoma in children: A population-based study.” Cancer Commun (Lond) 39 (2019): 62.
6. H, Cornella, Alsinet C, Sayols S and Zhang Z, et al. “Unique genomic profile of fibrolamellar hepatocellular carcinoma.” Gastroenterology 148 (2015): 806-818.
7. SC, McKay, Unger K, Pericleous S and Stamp G, et al. “Array comparative genomic hybridization identifies novel potential therapeutic targets in cholangiocarcinoma.” HPB (Oxford) 13 (2011): 309-319.
8. MI, Khan, Medhat O and Bonnet G. “Angiosarcoma in father and son: A case report and literature review.” N Z Med J 117 (2004): U1103.
9. J, Guy and Peters MG. “Liver disease in women: the influence of gender on epidemiology. Natural history. and patient outcomes.” Gastroenterol Hepatol (NY) 9 (2013): 633-639.
10. EM, Wu, Wong LL, Hernandez BY and Ji JF, et al. Gender differences in hepatocellular cancer: disparities in nonalcoholic fatty liver disease/ steatohepatitis and liver transplantation. Hepatoma Res 4 (2018): 66.
11. JD, Buckley, Sather H, Ruccione K and Rogers PC et al. “A case-control study of risk factors for hepatoblastoma. A report from the Childrens Cancer Study Group.” Cancer 64 (1989): 1169-1176.
12. MF, Yuen, Cheng CC, Lauder IJ and Lam SK, et al. “Early detection of hepatocellular carcinoma increases the chance of treatment: Hong Kong experience.” Hepatology 31 (2000): 330-335.
13. L, He, Tian DA, Li PY and He XX. “Mouse models of liver cancer: Progress and recommendations.” Oncotarget 6 (2015): 23306-23322.
14. ZM, Jilkova, Kurma K and Decaens T. “Animal Models of Hepatocellular Carcinoma: The role of immune system and tumor microenvironment.” Cancers 11 (2019): 1487.

15. P, Mirabelli, Coppola L and Salvatore M. “Cancer cell lines are useful model systems for medical research.” Cancers 11 (2019): 1098.

16. E, Li, Lin L, Chen CW and Ou DL. “Mouse models for immunotherapy in hepatocellular carcinoma.” Cancers 11 (2019): 1800.

17. NP, Santos, Colaço AA and Oliveira PA. “Animal models as a tool in hepatocellular carcinoma research: A Review." Tumour Biol 39 (2017): 1010428317695923.

18. VI, Gelstein. “The growth of murine transplantable hepatomas.” Tsitologiya 23 (1971): 03-14.

19. YT, Alexanyan, Basmadjan ME, Movsesyan KS and Manuhyan LA. “A cell line obtained from transplantable murine hepatoma.” Byulleten Eksperimental’noi Biologii i Meditsiny 5 (1972): 94-95.

20. GJ, Darlington, “Liver cell lines.” Methods Enzymol 151 (1987): 19-38.

21. GJ, Darlington, Bernhard HP, Miller RA and Ruddle FH. “Expression of liver phenotypes in cultured mouse hepatoma cells.” J Natl Cancer Inst 64 (1980): 809-819.

22. C, Leibiger, Kosyakova N, Mkrtchyan H and Glei M, et al. “First molecular cytogenetic high resolution characterization of the NIH 3T3 cell line by murine multicolor banding”. J Histochem Cytochem 61 (2013): 306-312.

23. K, Guja, Liehr T, Rincic M and Kosyakova N, et al. “Molecular cytogenetic characterization identified the murine B-cell lymphoma cell line A-20 as a model for sporadic Burkitt’s lymphoma.” J Histochem Cytochem 65 (2017): 669-677.

24. E, Kubicova, Trifonov V, Borovecki F and Liehr T, et al. “First molecular cytogenetic characterization of murine malignant mesothelioma cell line AE17 and in silico translation to the human genome.” Curr Bioinform 12 (2017): 11-18.

25. H, Rhode, Liehr T, Kosyakova N and Rincic M, et al. “Molecular cytogenetic characterization of two murine colorectal cancer cell lines.” OBM Genetics 2 (2018): 037.

26. R, Steinacker, Liehr T, Kosyakova N and Rincic M, et al. “Molecular cytogenetic characterization of two murine cancer cell lines derived from salivary gland.” Biol Commun 63 (2018): 243-255.

27. S, Azawi, Liehr T, Rincic M and Manferrari M. “Molecular cytogenomic characterization of the murine breast cancer cell lines C-127I.; EMT6/P and TA3 Hauschka.” Int J Mol Sci 21 (2020): 4716.

28. MA, Buendia. “Genetic alterations in hepatoblastoma and hepatocellular carcinoma: common and distinctive aspects.” Med Pediatr Oncol 39 (2002): 530-535.

29. M, Luo, Conrad M and Lin HC. “Genetics of pediatric hepatoblastoma and hepatocellular carcinoma and their clinical application.” Am J Digest Dis 1 (2014): 97-111.

30. N, Kato and Kitamura T. “Cytogenetic abnormalities and gene mutations in myeloid leukemia.” Nihon Rinsho 67 (2009): 1875-1879.

31. A, McCormack, Fan JL, Duesberg M and Bloomfield M, et al. “Individual karyotypes at the origins of cervical carcinomas.” Mol Cytogenet 6 (2013): 44.

32. A, Kuechler, Weise A, Michel S and Schaeferhenrich A, et al. “Precise breakpoint characterization of the colon adenocarcinoma cell line HT-29 clone 19A by means of 24-color fluorescence in situ hybridization and multicolor banding.” Genes Chromosomes Cancer 36 (2003): 207-210.

33. J, Lemke, Claussen J, Michel S and Chudoba I, et al. “The DNA-based structure of human chromosome 5 in interphase.” Am J Hum Genet 71 (2002): 1051-1059.

34. M, Volleth, Zenker M, Joksic I and Liehr T. “Long-term culture of EBV-induced human lymphoblastoid cell lines reveals chromosomal instability.” J Histochem Cytochem 68 (2020): 239-251.

35. NL, Lopez Corrales, Mrasek K, Voigt M and Liehr T, et al. “Comprehensive characterization of genomic instability in pluripotent stem cells and their derived neuroprogenitor cell lines.” Appl Transl Genom 1 (2012): 21-24.

36. T, Liehr, Starke H, Heller A and Kosyakova N, et al. “Multicolor fluorescence in situ hybridization (FISH) applied to FISH-banding.” Cytogenet Genome Res 114 (2006): 240-244.