Mitotic Stability of Small Supernumerary Marker Chromosomes: A Study Based on 93 Immortalized Cell Lines

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Key Words

Cell bank · Cell lines · Epstein-Barr virus immortalization · Marker chromosome shape · Puberty · Small supernumerary marker chromosome

Abstract

Small supernumerary marker chromosomes (sSMC) are known for being present in mosaic form as 47,+mar/46 in >50% of the cases with this kind of extra chromosomes. However, no detailed studies have been done for the mitot-

ic stability of sSMC so far, mainly due to the lack of a corresponding in vitro model system. Recently, we established an sSMC-cell bank (Else Kröner-Fresenius-sSMC-cellbank) with >150 cell lines. Therefore, 93 selected sSMC cases were studied here for the presence of the corresponding marker chromosomes before and after Epstein-Barr virus-induced immortalization. The obtained results showed that dicentric inverted duplicated-shaped sSMC are by far more stable in vitro than monocentric centric minute- or ring-shaped sSMC. Simultaneously, a review of the literature revealed that a comparable shape-dependent mitotic stability can be found

in vivo in sSMC carriers. Additionally, a possible impact of the age of the sSMC carrier on mitotic stability was found: sSMC cell lines established from patients between 10–20 years of age were predominantly mitotically unstable. The latter finding was independent of the sSMC shape. The present study shows that in vitro models can lead to new and exciting insights into the biology of this genetically and clinically heterogeneous patient group.

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Small supernumerary marker chromosomes (sSMC) are an interesting finding in the human population. About 2/3 of the estimated ~3 million sSMC carriers in a human population of ~7 billion are clinically normal. Thus, these sSMC may be considered as a type of chromosomal heteromorphism in our species. Such view is supported by the similarities observed in sSMC and B chromosomes [Liehr, 2011, 2014]. However, in the remaining 1 million sSMC carriers, the extra chromosome is responsible for clinical problems. Thus, sSMC also have a clinical impact in genetic diagnostics.

sSMC may be present as inverted duplicated (inv dup or i)-, ring (r)-, or centric minute (min)-shaped derivatives. While min and r are usually monocentric, inv dup sSMC derivatives tend to be dicentric. It is known that (a) an euchromatic sSMC derived from chromosome 15 may lead to the inv dup(15) syndrome, (b) an i(12p) is associated with Pallister-Killian syndrome, (c) a der(22)t(11;22) is causative for Emanuel syndrome, (d) an i(18p) is responsible for the corresponding i(18p) syndrome, and (e) an inv dup(22)(q11.2) leads to cat-eye syndrome. During the last decade, progress has been made concerning genotype-phenotype correlations of sSMC that do not belong to any of the aforementioned syndromes. Furthermore, the pericentric regions' sizes, which are most likely dosage-insensitive, are currently subject to research studies. The impact of sSMC size, uniparental disomy, and mosaicism on phenotypical and/or clinical outcome should be considered when analyzing each successive study [Liehr, 2011, 2014].

Even though sSMC are an interesting example of numerical and at the same time structural aberrations of the human basic karyotype, in vitro models are scarce [Voet et al., 2001; Brecevic et al., 2006]. About 7 years ago, we started to establish a collection of immortalized sSMC cell lines [Tönnies et al., 2007] which is now denominated as 'Else Kröner-Fresenius-sSMC-cellbank' due to project funding by this foundation (http://www.fish.uniklinikum-jena.de/sSMC/sSMC+by+chromosome/EKF_cellbank.html). It comprises, at present, a collection of im-

mortalized cell lines derived from >150 sSMC carriers, partly also including their parents.

In this study, 93 selected cell lines from the Else Kröner-Fresenius-sSMC-cellbank were analyzed for mitotic stability of the corresponding individual sSMC.

Material and Methods

sSMC cell lines were selected from the Else Kröner-Fresenius-sSMC-cellbank. Cell lines were established as previously reported using Epstein-Barr virus (EBV) induced immortalization of B-cells [Tönnies et al., 2007]. The cell lines are described on <http://ssmc-tl.com/ekf-cellbank.html>, and useful details for this paper are shown in table 1. Data for sSMC mosaicism (table 1) were obtained from previous standard cytogenetic studies of their T-lymphocytes or amniocytes when a prenatal diagnosis was carried out. For each cell line standard GTG-banding was performed, and 15–50 metaphases were evaluated. The origin and the size of the sSMC (table 1) have already been determined with primary material; for the applied FISH-approaches see Liehr [2014].

For this study, 2-color fluorescence in situ hybridization on standard cytogenetic preparations of the 93 sSMC-cell lines listed in table 1 was done. Commercially available centromeric probes for chromosomes 2, 3, 6, 7, 8, 9, 12, 13/21, 14/22, 15, 17, 18, and Y (Kreatech, Amsterdam, The Netherlands) or homemade whole chromosome painting probes for chromosomes 1, 5, 15, and/or 19 were applied. The probes were chosen according to the chromosomal origin of the sSMC; e.g. whole chromosome painting probe 19 was used since there is no unique centromeric sequence available for chromosome 19. Only one of the aforementioned probes was used in each sSMC case together with a control probe; a centromeric probe for chromosome 17. A total of 30–100 metaphase plates were studied per case. The obtained results, i.e. percentage of sSMC present in the cell lines, were compared to the mosaic rate observed in the original sample (blood or amnion) of the same patients. If >27% of the cells carried an sSMC after the immortalization, this was considered as a gain (class G); if <27% of the cells had a comparative increase or decrease of sSMC, this was rated as staying equal (class E); if a decrease of >27–80% was observed, it was rated as a moderate loss (class L); and if the loss was between 81–100% of the original cells present with sSMC after the immortalization, it was considered as a severe loss (class SL). These classifications were done based on the experimental data obtained in this study in order to facilitate analysis of the data.

Results

All 93 studied sSMC cases are summarized in table 1 including relevant clinical parameters and the observed percentage of sSMC mosaicism in T-cells or amniocytes before immortalization and in B-cells after immortalization. Of the 93 studied sSMC cases, 9 cell lines contained r-shaped, 32 min-shaped, and 52 inv dup-shaped sSMC.

Table 1. Overview of the 93 cell lines studied

Case ID	Karyotype of sSMC	Size, Mbp	Gender	Age, years	Clinically affected	T-lymphocytes mosaic, %	B-lymphocytes mosaic, %	Class
EKF-#1-p11.1/1-r 05PO749	r(1)(:p21.1→q31.1::)	82	M	0	+	8 (amnion)	6	E
EKF-#1-p11.1/1-m 12L0084	min(1)(:p11.1→q12:)	~7	F	37	–	60	59	E
EKF-#2-p11.1/1-m 12L0052	min(2)(:p11.1→q11.2:)	8	F	38	–	40	2	SL
EKF-#2-p11.1/2-m 12L0107	min(2)(:p11.1→q11.2:)	~8	F	3	–	15 (amnion)	20	E
EKF-#2-p11.2/1-m 2010B113	min(2)(:p11.2→q11.1:)	~13	M	18	–	100	2	SL
EKF-#2-p11.2/2-m/r 06PO793	min(2)(:p11.2→q11.1:)[15]/ min(2)(:p11.2→q11.1::q11.1→ p11.2:)[4]/r(2)(:p11.2→ q11.1:)[4]/r(2)(:p11.2→ q11.1::p11.2→q11.1:)[11]	10	M	32	–	53	12	L
EKF-#3-p12.1/1-m/r 07PO736	r(3)(:p12.1→q11.1:)[14]/ min(3)(:p12.1→q11.1:)[5]/ min(3)(:p12.1→q11.1::q11.1→ p12.1:)[2]	~9	F	29	–	17	39	G
EKF-#5-p13.2/1-m 12L0054	min(5)(:p13.2→q11.1:)	~13	M	1	+	80	1	SL
EKF-#5-p12/1-m 12L0077	min(5)(:p12→q11.1:)	~10	F	43	–	100	54	L
EKF-#6-p11.1/2-i 11L013	min(6;6)(:p11→q11: :p11→q11:)	~10	M	35	–	100	1 or 11 (interphase)	SL
EKF-#7-p12/1-m 12L0211	min(7)(:p12→q11.1:) UPD(7)mat	12	M	4	+	8	0	SL
EKF-#7-p11.2/1-m 12L0080	min(7)(:p11.2→q11.1:) UPD(7)mat	19	F	15	+	100	53	L
EKF-#7-p11.2/1-m/r 05PO529	min(7)(:p11.2→q11.21:) [5]/r(7)(:p11.2→q11.21:)[2]/ r(7;7)(:p11.2→q11.21::p11.2→ q11.21:)[1]	8	F	13	+	81	25	L
EKF-#7-p11.1/1-r 12L0102	r(7)(:p11.1→q11.21:)[15]/ r(7;7)(:p11.1→q11.21: :p11.1→q11.21:)[4]/r(7;7;7;7) (:p11.1→q11.21::p11.1→ q11.21::p11.1→ q11.21::p11.1→q11.21:)[1]	18	F	11	+	65	42	L
EKF-#7-p11.1/1-m 06PO708	min(7)(:p11.1→q11.1:)	~6	F	5	+	50	16	L
EKF-#8-p11.21~11.22/1-r 2008B113	r(8)(:p11.21~11.22→q11.1:)	7	M	15	+	100	79	L
EKF-#8-p11/1-m 12L0230	min(8)(:p11→q11:)	~6	F	12	–	94 (amnion)	14	SL

Table 1 (continued)

Case ID	Karyotype of sSMC	Size, Mbp	Gender	Age, years	Clinically affected	T-lymphocytes mosaic, %	B-lymphocytes mosaic, %	Class
EKF-#8-p10/1-r 12L0256	r(8)(::p10→q23.3::)	~72	M	13	+	100	16	SL
EKF-#9-p12/3-m/r 12L0225	r(9)(::p12→q12::) min(9)(:p12→q12:)	~20	M	4	–	50 (amnion)	50	E
EKF-#9-p12/1-r 12L0117	r(9)(::p12→q12::)	~15	M	59	–	22	5	L
EKF-#9-p11.1/1-m 12L0174	min(9)(:p11.1→q12:)	~15	F	15	–	20	2	SL
EKF-#9-p10/1-m 12L0196	min(9)(:p10→q13:)	~20	F	40	–	95	0	SL
EKF-#12-p11.1/1-m 12L0200	min(12)(:p11.1→q12:)	~8	M	25	+	66	28	L
EKF-#12-p11.22/1-m 12L0161	min(12)(:p11.22→q11:)	~8	F	38	–	83	54	L
EKF-#13/21-q11/1-i 2010B211	inv dup(13or21)(q10)pat	~36	M	1	+(addition- al +21; Down syndrome)	100	100	E
EKF-#13/21-q11/1-i-father 2010B212	inv dup(13or21)(q10)	~36	M	adult	–	100	100	E
EKF-#13/21-q11/2-i 12L0113	inv dup(13or21)(q10)pat	~36	F	1	–	100 (amnion)	74	E
EKF-#13/21-q11/2-i-father 12L0114	inv dup(13or21)(q10)	~36	M	adult	–	100	84	E
EKF-#13/21-q11/4-i 12L0162	inv dup(13or21)(q10)mat	~36	F	3	–	100 (amnion)	87	E
EKF-#13/21-q11/3-i 12L0126	inv dup(13)(q11) or inv dup(21)(q11.1)mat	~36	F	1	–	6 (amnion) maternal contamination	0	SL
EKF-#13/21-q11/5-i 12L0170	inv dup(13or21)(q10)mat	~36	F	13	–	100	78	E
EKF-#14-q11/1-m 12L0235	min(14)(pter→q11.1:) UPD(14)mat	~19	M	34	+	87	51	L
EKF-#14-q11/1-i 12L0147	inv dup(14)(q11)	~38	F	37	–	50	35	L
EKF-#14-q11.1/1-i 06P0430	inv dup(14)(q11.1)	~38	F	29	–	100	55	L
EKF-#14-q11.1/3-i 11L004	inv dup(14)(q11.1)	~38	M	31	–	100	88	E
EKF-#14-q11.1/4-i 12L0086	inv dup(14)(q11.1)	~38	F	37	–	23	24	E

Table 1 (continued)

Case ID	Karyotype of sSMC	Size, Mbp	Gender	Age, years	Clinically affected	T-lymphocytes mosaic, %	B-lymphocytes mosaic, %	Class
EKF-#14-q11.1/5-i 12L0134	inv dup(14)(q11.1)	~38	M	51	–	100	100	E
EKF-#14-q11.1/6-i 12L0222	inv dup(14)(q11.1)	~38	F	29	–	100	100	E
EKF-#15-q10/1-i 12L0090	inv dup(15)(q10)	~36	M	6	–	100 (amnion)	90	E
EKF-#15-q11/1-m 12L0087	min(15)(:p11→q11:)	~8	F	50	–	100	58	L
EKF-#15-q11/1-i 12L0105	inv dup(15)(q11)	~36	M	12	–	20 (amnion)	9	L
EKF-#15-q11.1/1-r 12L0094	r(15)(::p11.1→q11.1::)	~8	F	17	+	54	26	L
EKF-#15-q11.1/1-m 12L0123	min(15)(:p11.1→q11.1:)	~8	F	8	+	35 (amnion)	0	SL
EKF-#15-q11.1/1-i 07PO019	inv dup(15)(q11.1)	~36	M	34	–	100	19	SL
EKF-#15-q11.1/2-i 12L0091	inv dup(15)(q11.1)	~36	F	9	–	30 (amnion)	28	E
EKF-#15-q11.1/3-i 12L0112	inv dup(15)(q11.1)	~36	F	7	–	100 (amnion)	99	E
EKF-#15-q11.1/4-i 12L0183	inv dup(15)(q11.1)	~36	M	39	–	100	100	E
EKF-#15-q11.1/5-i 12L0169	inv dup(15)(q11.1)	~36	M	42	–	64	87	G
EKF-#15-q11.1/7-i 12L0133	inv dup(15)(q11.1)	~36	M	13	+	100	57	L
EKF-#15-q11.1/8-i 12L0212	inv dup(15)(q11.1)	~36	F	45	–	100	100	E
EKF-#15-q11.1/8-i-son 12L0213	inv dup(15)(q11.1)mat	~36	M	n.a.	–	100	100	E
EKF-#15-q11.1/8-i-daughter 12L0214	inv dup(15)(q11.1)	~36	F	n.a.	–	100	100	E
EKF-#15-q11.1/9-i 12L0142	inv dup(15)(q11.1)	~36	F	adult	–	100	100	E
EKF-#15-q11.1/10-i 12L0143	inv dup(15)(q11.1)	~36	F	adult	–	100	100	E
EKF-#15-q11.1/11-i 12L0221	inv dup(15)(q11.1)	~36	F	0	–	100 (amnion)	98	E
EKF-#15-q11.1/12-i 12L0305	inv dup(15)(q11.1)	~36	F	0	–	100 (amnion)	100	E
EKF-#15-q11.2/2-i 12L0101	inv dup(15)(q11.2)	~40	M	45	–	100	97	E

Table 1 (continued)

Case ID	Karyotype of sSMC	Size, Mbp	Gender	Age, years	Clinically affected	T-lymphocytes mosaic, %	B-lymphocytes mosaic, %	Class
EKF-#15-q11.2~12/1-i 06P0226	inv dup(15)(q11.2~q12)	~42	F	adult	–	100	96	E
EKF-#15-q12/1-i 12L0141	inv dup(15)(q12)	~48	M	24	+	100	100	E
EKF-#15-q12~13/1-i 12L0258	inv dup(15)(q12~13)	~50	M	10	+	100	98	E
EKF-#15-q13/1-i 12L0265	inv dup(15)(q13)	~54	F	9	+	25	30	E
EKF-#15-q13.1/1-r 12L0269	r(15)(:p11.2→q13.1::)	~17	F	5	–	79 (amnion)	61	E
EKF-#15-q14q13.2q13.3/1-i 11L369	inv dup(15)(pter→q13.1~13.2: :q13.3→q14::q14→q13.3: :q13.1~13.2→pter)x2	~65	M	18	+	100	100	E
EKF-#15-q14/1-i 12L0131	inv dup(15)(q14)	~70	F	13	+	100	98	E
EKF-#18-pter/1-i 12L0053	inv dup(18)(q11.1)	~35	F	5	+	100	99	E
EKF-#18compl#8/1-m 06PO222	der(18)t(8;18) (p23.2~23.1;q11.1)	23	M	1	+	100	100	E
EKF-#19-p13.1/1-m 12L0085	min(19)(:p13.12~13.11→q12:)	~21	M	7	+	63	16	L
EKF-#19-p13.11/1-m 05P0067	min(19)(:p13.11→q12:)	~20	F	0	?	25 (amnion- mosaic?)	0	SL
EKF-#19-p13.11/2-m 12L0140	min(19)(:p13.11→q11~12:)	~18	F	7	–	73 (amnion)	38	L
EKF-#19-p13.11/2-r 2008B073	min(19)(:p13.11→q11:)[3]/ r(19)(:p13.11→q11:)[9]/ r(19)(:p13.11→q11: :p13.11→q11:)[2]	~18	F	28	–	60	0	SL
EKF-#19-p12/1-m 12L0089	min(19)(:p12→q12:)	~9	M	5	+	75	18	L
EKF-#19-p12/2-m 12L0127	min(19)(:p12→q13.11~13.12:)	~12	M	17	+	77	8	SL
EKF-#19-p12/3-m 12L0253	min(19)(:p12→q13.1?1:)	~10	M	6	+	50	15	L
EKF-#19-p11.1/1-m 05P0277	min(19)(:p11.1→q13.1:)	~7	M	31	–	25	0	SL
EKF-#21-q21.1/1-i 12L0092	inv dup(21)(q11.2)	~28	M	6	–	100 (amnion)	100	E
EKF-#21-q11.2~21.1/1-m 06P0503 and 06P0503-A	min(21)(pter→q11.2~21.1:)	~30	M	1	+	33	0	SL
EKF-#22-q11.1/1-m 12L0093	min(22)(:p11.1→q11:) maternal UPD 22	~7	F	10	–	22	15	L

Table 1 (continued)

Case ID	Karyotype of sSMC	Size, Mbp	Gender	Age, years	Clinically affected	T-lymphocytes mosaic, %	B-lymphocytes mosaic, %	Class
EKF-#22-q11.1/2-i 2008B140	inv dup(22)(q11.1)pat	~32	M	0	–	100 (amnion)	78	E
EKF-#22-q11.1/3-i 2008B136	inv dup(22)(q11.1)	~32	M	35	–	100	92	E
EKF-#22-q11.1/4-i 2008B246	inv dup(22)(q11.1)	~32	F	32	–	100	100	E
EKF-#22-q11.1/6-i 12L0104	inv dup(22)(q11.1)mat	~32	F	adult	–	100	100	E
EKF-#22-q11.1/6-i-mother 12L0103	inv dup(22)(q11.1)	~32	F	adult	–	100	100	E
EKF-#22-q11.1/8-i 12L0108	inv dup(22)(q11.1)mat	~32	M	4	–	100	100	E
EKF-#22-q11.1/8-i-mother 12L0109	inv dup(22)(q11.1)	~32	F	adult	–	100	96	E
EKF-#22-q11.1/9-i 12L0115	inv dup(22)(q11.1)mat	~32	M	10	–	100	51	L
EKF-#22-q11.1/9-i-mother 12L0116	inv dup(22)(q11.1)	~32	F	38	–	100	86	E
EKF-#22-q11.1/10-i 12L0118	inv dup(22)(q11.1)	~32	F	8	+	100	100	E
EKF-#22-q11.1/11-i 12L0121	inv dup(22)(q11.1)mat	~32	M	2	–	100 (amnion)	100	E
EKF-#22-q11.21/1-i 05PO209	inv dup(22)(q11.21)	~35	M	2	+	100	96	E
EKF-#22-q11.21/2-i 12L0088	inv dup(22)(q11.21)fam	~35	M	adult	–	100	48	L
EKF-#22-q12.1/1-m 06P0775	min(22)(pter→q12.1:)	~40	M	1	+	79	82	E
EKF-#22compl#11/1-m 12L0098	der(22)t(11;22) (q23;q11.2)	27	F	23	+	100	97	E
EKF-Y-q11.22/1-i 12L0110	inv dup(Y)(q11.22)	~50	M	20	+	86	70 (30%: 45,X)	E

G = Gain; L = loss; SL = severe loss of sSMC in the cell line; E = no major change in sSMC frequency.

Gain, loss, or the same frequency of sSMC were determined by comparing mosaic rates before and after EBV immortalization. According to the 4 classes introduced in the ‘Material and Methods’ section, 2 cases belonged to class G (gain), 50 to class E (equal), 24 to class L (moderate loss), and 17 to class SL (severe loss). The latter class included 8 cases with complete loss of the sSMC after immortalization. In figure 1, the 4 classes are aligned with the sSMC shape, showing that >80% of sSMC with an inv dup shape were mitotically stable, whereas only slightly

>20% of the min-shaped sSMC displayed this feature. Only 9 r-shaped sSMC cell lines were available here – they seem to be slightly less stable than min-shaped sSMC, but the overall behavior was similar.

In figure 2, the mitotic sSMC stability was aligned with the age of the sSMC carrier at the time the cell lines were established. Surprisingly, the sSMC were mitotically most unstable in B-cells within the age group of 10–20 years – i.e. during puberty.

Fig. 1. Mitotic stability of sSMC as determined in 93 cell lines including r-, min-, and inv dup-shaped sSMC. Schematic representations on how the 3 groups of sSMC look like are given in the legend. G = Gain; L = loss; SL = severe loss of sSMC in the cell line; E = no major change in sSMC frequency.

Other factors such as gender of the carrier, amount of euchromatin or heterochromatin on the sSMC, sSMC size, or co-occurrence of the sSMC with clinical symptoms (data not shown) showed no correlation with the mitotic stability of the studied sSMC.

Discussion

The mitotic stability of sSMC has not been studied systematically so far. This was mainly due to the lack of a corresponding in vitro system. Here, the mosaic state of sSMC present in 93 cell lines selected from the Else Kröner-Fresenius-sSMC-cellbank were assessed and compared to their original mosaic states after a short-term cell culture. Four different types of mitotic stability were observed: gain, loss, severe loss of sSMC, and no gross changes of sSMC percentages. These findings are surprising, taking into account that lymphoblastoid cell lines established by EBV transformation are considered to represent an identical chromosome constitution as present in the patient, also including additional chromosomes [Miyoshi et al., 1976; Abruzzo et al., 1986]. Deviations from original cytogenetic conditions were previously reported but were found exclusively in tumor-derived cell lines [Steel et al., 1971; Povey et al., 1980].

One problem of the present study is that the percentage of cells with sSMC before EBV transformation could only be established from a T-cell blood fraction or from amniocytes of sSMC carriers; these data were then com-

Fig. 2. Frequency of groups G (gain) and E (no change) compared to frequency of groups L (loss) and SL (severe loss) from figure 1 plotted against the age of the sSMC carriers at the time of providing a blood sample for EBV transformation. min- and r-shaped sSMC (a) are compared to inv dup-shaped ones (b). In c all 3 sSMC shapes are summarized.

pared to data obtained from the B-cell fraction of the identical patient. Overall, it is a general assumption that the cells with sSMC determined in a tissue are somehow representative for the cells with sSMC in all other tissues of the patient studied [Papoulidis et al., 2012]. However,

Fig. 3. Mosaicism as reported for all sSMC-cases reported in the literature [Liehr, 2014] is shown for min- and r-shaped sSMC on the left and for inv dup-shaped sSMC on the right.

this conjecture could only be more checked in detail in exceptional cases. There are studies showing complete absence of an sSMC in some tissues [Papoulidis et al., 2012] or a high variance of the presence of sSMC in different tissues [Fickelscher et al., 2007]. Still, in the majority of cases in which more than one tissue was studied, the presence of sSMC could be shown in a comparable amount of them [Liehr, 2014].

The most interesting finding of this present study was that a correlation between sSMC shape and stability was uncovered. As depicted in figure 1, sSMC with inv dup shape are by far more mitotically stable than r- or min-shaped ones. In order to check if these results were not just an artifact of the cell culture (establishment), a summary of mosaicism present in sSMC with inv dup shape in comparison with r or min shape was performed according to Liehr [2014] (fig. 3). min- and r-shaped sSMC are found in mosaic form in 70% of the cases, while only ~22% of inv dup sSMC are. In other words, these findings reflect a similar mitotic stability in vivo as is found here in this in vitro study.

As discussed earlier [Guilherme et al., 2012], it is suggested that inv dup-shaped sSMC should be considered mitotically unstable as they are the most frequent dicentric derivatives. At the same time, min- and especially r-shaped sSMC, which are monocentric, should be more stable than inv dup-shaped ones. However, it was shown here that it is the other way round. Taking into account that (a) inv dup-shaped sSMC are always stabilized by the presence of 2 intact telomeric regions, (b) min- and r-shaped sSMC might lack at least one telomere, and (c) min-shaped sSMC might even have no telomeres at all

[Guilherme et al., 2012], the possibility of an increased influence on the mitotic stability based on telomere presence or absence rather than multi-centromeres is highly probable. However, the influence of the centromere on the mitotic stability of sSMC is far from being understood. As demonstrated recently, dicentric sSMC show different patterns of centromeric activity, rather implicating enhanced chromosome instability due to dicentricity than enhanced stability [Ewers et al., 2010].

Data summarized in figure 2 can be cautiously interpreted as a hint to a possible influence of puberty on sSMC stability. It could be further speculated that pubertal hormone activation may interfere with mitotic stability of sSMC; however, some in vitro tests would be necessary to prove the validity of this idea. On the other hand, no correlation of the latter could be found with the gender of the carrier, amount of euchromatin or heterochromatin on the sSMC, sSMC size, or co-occurrence of the sSMC with clinical symptoms (data not shown). However, previous studies revealed that during meiosis there is selection against sSMC in males but not in females [Dalpra et al., 2005; Liehr, 2006]. Thus, changes of mitotic stability of sSMC in connection with different hormonal statuses may not be as farfetched as one would initially think, especially as an influence of estrogen on kinetochore assembly has already been demonstrated [Kabil et al., 2008]. Still, all the aforementioned speculations are based on the present results of the in vitro study which showed that sSMC are mitotically unstable if B-cells of juvenile donors are EBV transformed.

Overall, we present the first in vitro study based on the Else Kröner-Fresenius-sSMC-cellbank. Mitotic stability of sSMC has been shown to be dependent on sSMC shape, but this assumption needs to be considered in more detail in future reports, especially as it is often not reported by array-comparative genomic hybridization based results.

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