

The structural nature of chromosomal instability in colon cancer cells¹

MARIA RIBAS,^{*,†} LAIA MASRAMON,^{*} GEMMA AIZA,^{*} GABRIEL CAPELLÀ,[‡] ROSA MIRÓ,[†] AND MIGUEL A. PEINADO^{*,2}

^{*}Institut de Recerca Oncològica, Hospital Duran i Reynals, L'Hospitalet, Barcelona, Spain;

[†]Departament de Biologia Cel·lular, Fisiologia i Immunologia, Institut de Biotecnologia i Biomedicina, Universitat Autònoma de Barcelona, Bellaterra, Barcelona, Spain; and [‡]Institut Català d'Oncologia, Hospital Duran i Reynals, L'Hospitalet, Barcelona, Spain

SPECIFIC AIMS

To characterize chromosomal instability in cancer cells, we analyzed genetic clonal divergence in three colon cancer cell lines (LoVo, HCT116, and SW480) regarded as archetypes in cancer research. A dynamic setting using comprehensive analyses at chromosomal level (G banding cytogenetics, comparative genomic hybridization, and centromeric fluorescence in situ hybridization, or FISH) was designed to allow the calculation of mutation rates.

PRINCIPAL FINDINGS

1 Cytogenetic characterization of cell lines

LoVo and HCT116 are diploid cells with a modal number of 49 and 46 chromosomes, respectively. SW480 are aneuploid cells showing a very complex karyotype. Its modal chromosome number was 57 with several chromosome markers in all metaphases. Due to the heterogeneity observed among metaphases, no major subpopulations were identified. All karyotypes agreed with previous reports and the ATCC catalog.

2 Rates of chromosomal instability in cell clones

The appraisal of chromosomal instability as a mutation rate is feasible only in dynamic settings. We have only considered the de novo mutations occurring in a defined interval of time (number of cell divisions here). Conservative (minimum rate, minr) and less strict (maximum rate, maxr) estimates of chromosomal instability were calculated. The maximum rate only takes into account nonclonal alterations and assumes they took place in the last generation (maxr=number of nonclonal alterations/number of cells). This assessment is an indicator of genetic instability irrespective of the viability of the cells harboring the novel genetic alterations that may or may not be transmitted to further generations. The minr assumes the accumulation of clonal and nonclonal alterations through gen-

erations with the same viability (minr=number of alterations/number of cells/number of generations).

Regarding numerical alterations, all three cell lines showed low rates (**Table 1**). On average, SW480 clones showed a ~fivefold increase in the minr (0.005 ± 0.001) compared with the HCT116 and LoVo clones (0.001 ± 0.002) (Mann-Whitney test, $U=25$, $P=0.036$). These differences were not observed in the maxr. At the structural level, cell lines displayed different rates of instability. LoVo clones showed a stable karyotype. In HCT116 cells, a certain degree of heterogeneity was observed in all clones, with 7–27% of the metaphases displaying novel chromosomal abnormalities, most of them unbalanced and nonclonal. The rates of chromosomal alterations in the clones of these two cell lines were of the same order for structural and numerical alterations (**Table 1**). All clones of SW480 showed novel structural rearrangements in all metaphases analyzed, resulting in rates ~100-fold higher than those found in LoVo and 10- to 50-fold more than HCT116 cells (**Table 1**). Differences in the minr and maxr of the three cell lines were statistically significant (Kruskal-Wallis nonparametric ANOVA test, minr KW=10.08 $P<0.0001$). Structural alterations largely surpassed numerical alterations in SW480 cells (~8-fold in parental cells and ~20- to 70-fold in their clones) (minr paired t test, $P=0.002$). Altogether, our observations suggest that the relative contribution of structural chromosomal instability to cell heterogeneity may be of special relevance in colorectal cancer cells.

3 Prevalence of structural over numerical instability

CGH analysis confirmed the karyotypes obtained by G banding in HCT116 and LoVo cells. SW480 cells displayed a very complex pattern that affected most chromosome profiles. In contrast to the striking heterogeneity observed in G banding analyses, CGH revealed a

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² Correspondence: Institut de Recerca Oncològica, Hospital Duran i Reynals, Granvia km 2,7, L'Hospitalet, 08907 Barcelona, Spain. E-mail: mpeinado@iro.es

TABLE 1. Chromosome alterations in clones of cell lines (G banding analysis)

Clone ID ^a	No. of generations	No. of metaphases	Altered metaphases	De novo structural alterations		De novo numerical alterations		Rate of structural alterations		Rate of numerical alterations	
				Clonal	Nonclonal	Clonal	Nonclonal	minr ^b	maxr ^c	minr ^b	maxr ^c
LoVo		20	3	0	0	0	3				0.150
L1	47	21	1	0	0	0	1	—	—	0.001	0.048
L3	33	17	1	0	0	0	1	—	—	0.001	0.059
L4	43	20	3	0	1	0	2	0.001	0.050	0.002	0.100
L5	29	16	0	0	0	0	0	—	—	—	—
L6	25	15	0	0	0	0	0	—	—	—	—
HCT116		15	1	0	1	0	1		0.067		0.067
H1	52	15	4	0	8	0	0	0.010	0.533	—	—
H3	52	17	2	0	3	0	0	0.003	0.176	—	—
H4	53	16	5	1	1	0	5	0.002	0.062	0.005	0.312
H5	63	15	3	0	2	0	1	0.002	0.133	0.001	0.066
SW480		18	16	2	16	1	2		0.889		0.111
S1	43	15	15	11	63	2	2	0.114	4.200	0.006	0.133
S2	42	15	15	9	72	1	2	0.128	4.800	0.004	0.133
S4	50	15	15	14	71	1	1	0.113	4.733	0.004	0.066

^a First letter of clone's name identifies cell line of origin: L: LoVo, H: HCT116, S: SW480. ^b Minimum rate (minr) indicate number of alterations (clonal and non clonal) per cell per generation. Dash in mutation rates indicates absence of alterations. ^c Maximum rate (maxr) indicate number of alterations (only non clonal) per cell assuming all of them took place in the last cell division. Dash in mutation rates indicates absence of alterations.

consistent profile for all clones. As a whole, these results imply that chromosomal reorganizations resulting in new chromosome markers in derivative clones do not involve substantial gains or losses of DNA.

The low prevalence of numerical alterations was confirmed by centromeric FISH of interphase cells using probes for chromosomes 3, 7, and 15. Numerical chromosome instability in this assay is revealed as heterogeneity with regard to the modal chromosome number of each clone (Table 2). Parental cells presented modal chromosome numbers in agreement with the G banding and CGH data. Since the cells were grown in chamber slides and processed in situ, it was possible to determine whether the changes observed in

chromosome number corresponded to independent mutational events or were clonal and propagated. In such a case, they were considered only once because they represented a single mutational event. All clones maintained the modal chromosome number of parental cells, except for chromosome 7 in one of the clones of SW480 cells (tetrasomy in parental cells and trisomy in the clone). In concordance, most of the remaining variations in chromosome number consisted of losses, with infrequent gains that affected principally chromosome 3 (Table 2). As a whole, the adjusted mutation rate was 0.002 ± 0.002 per chromosome and per generation in LoVo and HCT116 cells vs. 0.005 ± 0.003 in SW480 cells (Mann-Whitney test, $U=86.5$, two-tailed $P=0.027$).

TABLE 2. Chromosome number alterations analyzed by interphase centromeric FISH in colon cancer cell lines^a

Cell	Chromosome 3				Chromosome 7				Chromosome 15			
	-1	modal ^b	+1	rate ^c	-1	modal ^b	+1	rate ^c	-1	modal ^b	+1	rate ^c
LoVo		99.3 (2)	0.7	0.002		100.0 (3)		—	3.0	97.0 (2)		0.003
HCT-116		100.0 (2)		—		97.8 (2)	2.2	0.005	1.0	99.0 (2)		0.002
SW480	13.3	84.3 (3)	1.9	0.007	5.9	93.0 (4)		0.005	16.6	83.3 (5)		0.002
S-1	16.0	80.0 (3)	4	0.012	31.1	65.0 (4)		0.012	7.8	92.1 (5)		0.002
S-2	20.0	78.0 (3)	2	0.007	17.7	78.5 (4)		0.007	14.0	95.0 (5)		0.004
S-4	13.8	83.1 (3)	2.9	0.010	5.6	94.2 (4)		0.002	7.1	92.8 (5)		0.002
S-5	6.0	93.0 (3)	1	0.005	4.0	95.0 (4)		0.005	13.5	89.4 (5)		0.002
S-6	11.0	88.0 (3)	1	0.005	2.9	96.0 (3)	0.9	0.005	9.7	90.2 (5)		0.002

^a Cells were grown in chamber slides for 4–5 cell doublings and processed in situ. A minimum of 100 cells were analyzed per cell line and clone. ^b Numbers indicate percentage of cells displaying the modal chromosome number (in parentheses) for each clone. Percentages of cells lacking one copy or with an additional copy in regard to the respective modal number are shown in columns “-1” (chromosome loss) and “+1” (chromosome gain). ^c Rate shows number of alterations per chromosome per generation. For assessment of this rate, clonal alterations (deviations of the modal chromosome number shared by several cells within each colony) were counted only once. Dash indicates absence of alterations.

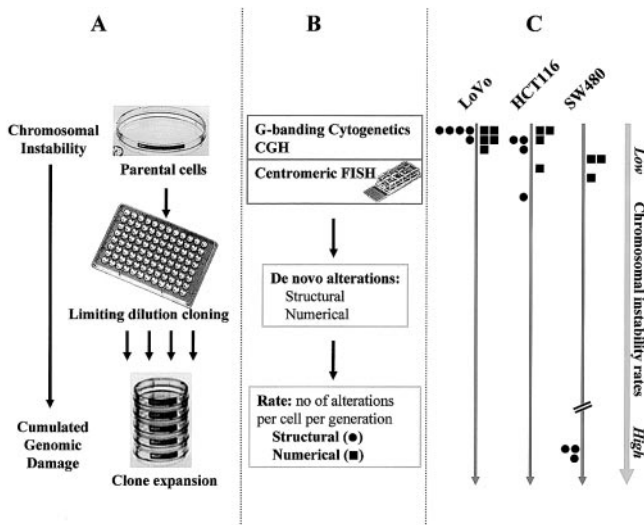


Figure 1. Schematic diagram. Chromosomal instability in cancer cells results in cumulated genomic damage and genetic divergence in clones derived from parental cells (A). A dynamic setting allowing calculation of mutation rates was applied to three colon cancer cell lines (LoVo, HCT116, SW480). Comprehensive analyses at chromosomal level (B) of parental cells and derived clones revealed distinctive patterns of genetic divergence (de novo alterations) in the three cell lines. The mutation rates for structural (circles) and numerical (squares) chromosomal alterations were calculated and are graphically depicted (C). SW480 cells displayed structural alterations as the main outcome of chromosomal instability.

CONCLUSIONS AND SIGNIFICANCE

Our results reveal the existence of a type of instability characterized by multiple translocation events with a low variability in chromosome number. Structural instability is extremely high in SW480 (average $\text{minr}=0.118$), low in HCT116 (average $\text{minr}=0.004$), and absent in LoVo cells (average $\text{minr} < 0.001$). We also show that aneuploid instability, understood as a high rate of gains and losses of whole chromosomes, occurs at low rate in the two near-diploid cell lines and is ~2.5- to 5-fold higher in the aneuploid cell line SW480.

To understand our estimates of chromosomal instability and how close they are to genuine rates, it is necessary to emphasize some considerations we made when analyzing the data. Actual numerical alterations appear in the form of aneuploidy or polyploidy. In assessing numerical changes (by G banding and FISH

analyses), we did not take into account polyploid endoreduplications because it is a well-characterized process driven by different mechanisms to aneuploidy. Clonal populations displayed a striking homogeneity in chromosome number, suggesting the clonal nature of most changes. Therefore, the heterogeneity in the chromosome number distribution is largely explained by sparse numerical mutations that are propagated by clonal expansion of stable cells.

Part of the confusion in the interpretation of chromosomal alterations arises from the biased reading of molecular data: aneuploidies and polyploidies are usually mixed and unbalanced rearrangements are frequently interpreted as numerical changes, precluding the identification of the underlying cause of the so-called chromosomal instability. The complex CGH profiles of most chromosomes in SW480 cells are suggestive of multiple rearrangements, and other studies have also noted the intricacy of the patterns of allelic loss and retention in different chromosomes in human colorectal cancer.

Chromosome instability can actually be rated only in dynamic settings such as the one described here. This type of assessment is precluded by undefined elapsed time and unknown generation number in *in vivo* studies of human cancer. Moreover, mutation rate is likely to be different according to the environmental conditions, which obviously are quite different in *in vivo* and *in vitro* settings. In consequence, our results can not be directly extrapolated to obtain estimates of chromosomal instability in primary tumors *in vivo*. Despite these limitations, our findings may help to interpret data generated in the genetic analysis of biopsies from cancer patients.

In summary, genetic instability is a landmark in colorectal cancer, but there is no straightforward approach to its analysis. The use of single methodologies and the entanglement in the interpretation of genetic alterations detected in molecular approaches have confounded the characterization of chromosomal instability. A realistic picture of the ongoing genetic processes taking place in tumor cell evolution can be obtained only in dynamic settings like the one reported here. Concomitant application of multiple and comprehensive methodologies is indispensable. We demonstrate that the nature of the chromosomal instability in the SW480 cell line is in essence structural. FJ