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Specific Chromosome Aberrations in Senescent Fibroblast Cell Lines Derived from Human Embryos

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INTRODUCTION

Human fibroblasts have a limited lifespan in culture. After an initial period of growth during which a confluent sheet is formed, cells pass into a rapid cell growth phase followed by senescence [1]. Because of the lifespan variations observed in vitro, these cells may provide a model for studying aging at the cellular level [2, 3]. In cell lines established from adult skin biopsies, chromosomally abnormal cells are frequently seen at low passage [4, 5]; this is not true, however, in human embryo skin fibroblasts [5]. Human embryo lung fibroblasts remain essentially diploid with a small number of chromosome aberrations seen during rapid cell growth [1]; they are, however, characterized by a high level of chromosome aberrations, polyploidy and aneuploidy, during senescence [6, 7].

Senescence may be due to the accumulation of mutations or chromosome abnormalities [2], or a more general deterioration in cell functions involving essential enzymes. Transcriptional errors could result in defective proteins causing further mutation that leads to senescence [8]. Abnormal proteins have been found in senescent cells [9–11], although their significance is unclear [12]. Thompson and Holliday [7] concluded that the chromosome aberrations in senescent cells are probably a consequence of defective proteins and are not a major cause of death.

In the course of monitoring culture conditions, a high level of chromosome abnormalities was observed in senescent WI-38 human embryo fibroblasts. This report describes the specificity of these aberrations and those in senescent MRC-5 embryo fibroblasts.

MATERIALS AND METHODS

WI-38 human embryo lung fibroblasts were obtained from Flow Laboratories (Scotland) at passage 20; MRC-5 human embryo fibroblasts were kindly provided by Dr. R. Holliday at passage 43. The culture and cytogenetic procedures followed are as described by Harnden [13] using Ham's F10 medium with 10% fetal calf serum, 10% tryptose phosphate broth, penicillin (50 international units/ml) and streptomycin (50 international units/ml). A 1:2 split ratio was used throughout. Cells were judged totally senescent when they could not generate sufficient numbers to produce viable subcultures. Final chromosome preparations were made following trypsinization and reseeding without

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splitting. Aberrations were scored according to the method of Buckton and Pike [14]; banding was carried out using quinacrine mustard [15]. Cells were tested for mycoplasma using fluorescence microscopy [16] at passage levels equal to or above those at which chromosome preparations were made and found negative.

RESULTS

Table 1 summarizes the cytogenetic data for the senescent phase of cells. With WI-38 cells, a transition from an essentially normal diploid cell line to hypodiploidy was observed. At passage 54, 10 cells contained 45 chromosomes without any structural abnormality, all of which were lacking a G group chromosome. Banding revealed that the absent G was consistently chromosome 22. The cell line then became more aneuploid with chromosome modal numbers 44 (range 43–46, 38% of cells) and 86 (range 56–90, 62% of cells). At this stage many dicentrics were observed, although the number of other chromosome aberrations (fragments, rings, gaps, and breaks) remained relatively low. Figure 1 shows 50 dicentrics from senescent WI-38 cells. Existing recommended nomenclature [17] can only be used to describe translocations in which breakage and subsequent rejoining has occurred. Of the 100 chromosomes involved, rearrangement occurred in telomeric regions in at least 87. Chromosome 7 was more frequently involved than any other chromosome with both ends involved in joining. The short arms of chromosomes 16 and 12 and both arms of chromosome 19 were also preferentially involved (table 2). WI-38 cells could not be cultured beyond passage 55.

With senescent MRC-5 cells, a transition to hypodiploidy was not observed. However, an increase in the chromosome number, giving an essentially tetraploid line with an increased frequency of dicentrics, was seen. Figure 2 shows 50 dicentrics from these cells. Telomeric regions were again preferentially involved in at least 93 of the 100 chromosomes involved. Although the long arms of chromosome 7 and the short arms of chromosome 12 were again frequently implicated, the most common joining involved the long arms of chromosome 13. The long arms of chromosome 5 and short arms of chromosomes 17 and 22 were also selectively involved, while the involvement of the short arms of chromosomes 7, 16, and 19 seen in the WI-38 cell line was absent. MRC-5 cells could not be cultured beyond passage 56.

Chromosomes with closely associated telomeres were often observed. These were not scored as dicentrics unless continuous material could be seen between each chromosome. Telomeric union between sister chromatids did not arise. Union between single chromatids of different chromosomes were thought to occur only at a low frequency, although these could not be unambiguously distinguished from chromatids lying close to each other or from dicentrics with chromatid gaps. Although homologous chromosomes were frequently involved in dicentric formation, these appeared to be largely confined to cells where polyploidization had taken place. No diploid or hypodiploid cells were seen in which both homologues were bound together. In addition, there may have been an increased frequency of associations between telomeres and centromeres.

TABLE 1

SUMMARY OF CYTOGENETIC DATA ON TWO LINES OF HUMAN FIBROBLASTS AT PASSAGE LEVELS CORRESPONDING TO RAPID CELL GROWTH AND SENESCENCE

LINE AND PASSAGE	TOTAL CELLS EXAMINED	MITOTIC INDEX* (%)	CHROMOSOMES/CELL						NO. CELLS WITH FRAGMENTS	NO. CELLS WITH GAPS AND BREAKS	NO. CELLS WITH DICENTRICS	TOTAL DICENTRICS	FREQUENCY OF DICENTRICS†	GROWTH PHASE
			<44	44	45	46	47–50	>55						
WI-38:														
41	50	1.05	0	0	3	43	1	3	0	9	0	0	0	Rapid cell growth
52	50	1.65	2	3	12	27	0	6	2	6	8	8	3.1	Early senescence
54	50	0.95	7	9	14	8	0	12	4	4	20	26	9.3	Mid senescence
55	50	0.75	4	7	6	2	0	31	3	6	36	82	24.1	Late senescence
MRC-5:														
48	50	1.50	2	3	4	35	2	4	0	2	0	0	0	Rapid cell growth
51	50	1.55	1	5	9	27	0	8	0	5	7	8	3.1	Early senescence
54	50	0.60	1	0	1	2	0	46	2	7	19	21	5.0	Late senescence

* 2,000 cells scored at each passage.

† Total number of dicentrics/total number of chromosomes $\times 10^8$.

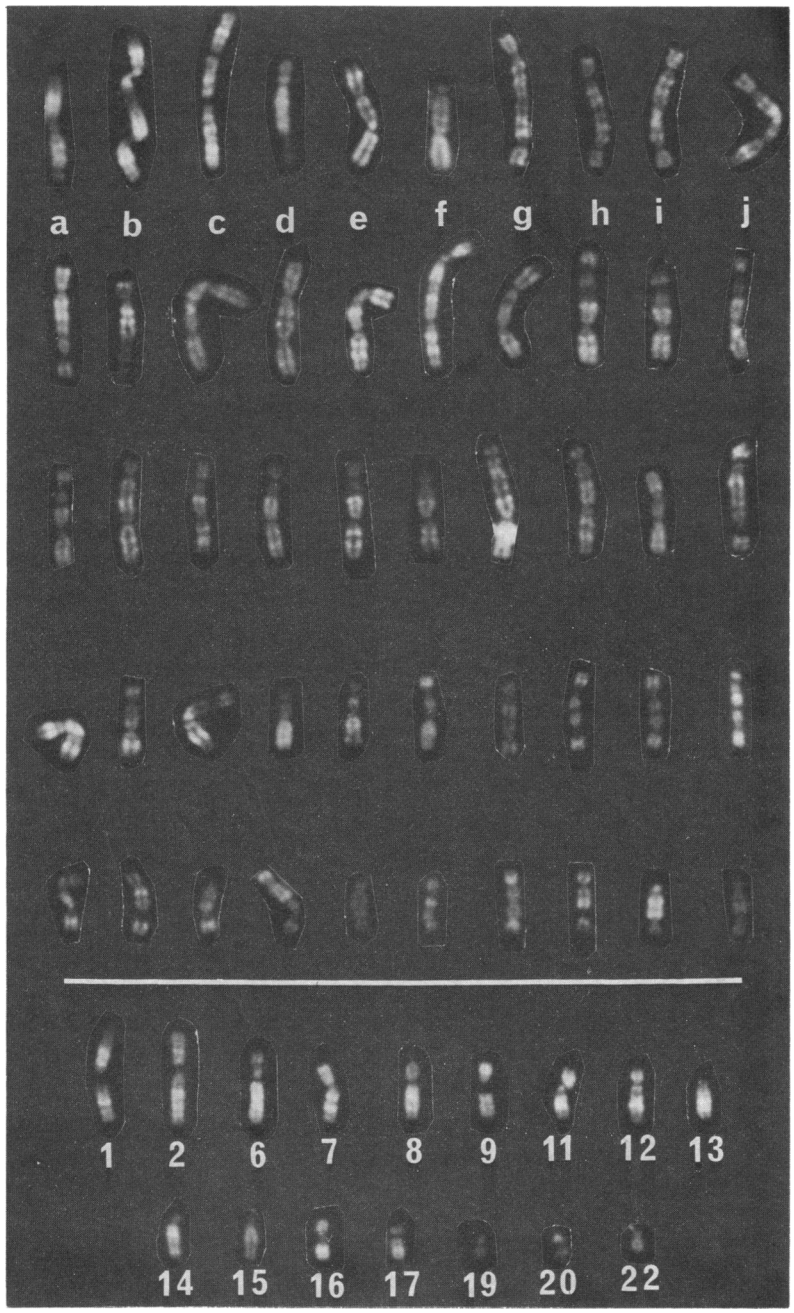

FIG. 1.—50 dicentrics from 36 senescent WI-38 cells with the normal chromosomes thought to be involved as whole chromosomes presented below. Duplicated dicentrics in polyploid cells are included once only. Dicentrics are described using a minor modification of the nomenclature of Hsu et al. [18] (i.e., p = short arm; q = long arm; k = centromere; T = telomere;

TABLE 2

DISTRIBUTION OF TELOMERIC FUSIONS FOR EACH CHROMOSOME ARM IN THE TWO LINES OF SENESCENT HUMAN FIBROBLASTS (50 DICENTRICS FOR EACH LINE)

	WI-38	MRC-5		WI-38	MRC-5
	p,q	p,q		p,q	p,q
1.	1,1	1,2	14.	1,—	—,1
2.	1,—	—,1	15.	1,—	—,1
3.	—,—	—,1	16.	19,2	2,—
4.	—,—	—,—	17.	—,2	6,—
5.	—,—	—,5	18.	—,—	—,1
6.	2,1	—,—	19.	5,5	1,2
7.	18,9	—,11	20.	3,2	3,—
8.	1,—	—,—	21.	—,—	1,—
9.	1,—	3,2	22.	—,1	5,3
10.	—,—	3,—	x	—,—	—,—
11.	—,1	—,—	y	—,—	—,—
12.	8,1	11,3	Other identifiable	1	1
13.	1,—	—,24	Unknown	12	6

NOTE.—p = short arm involvement; q = long arm. Sites other than telomeres are included in "other identifiable" and "unknown."

DISCUSSION

These results demonstrate cellular senescence in vitro characterized by highly specific chromosome aberrations, in particular dicentric formation. These observations are consistent with those reported by Saksela and Moorhead [6] and Thompson and Holliday [7] who found polyploidy, aneuploidy, and an increased frequency of dicentrics in WI-38 and MRC-5 cells. The greater number of abnormalities may reflect a later stage in senescence.

The rapid rise in dicentric frequency seen in this study was unmatched by increased numbers of other chromosome aberrations (i.e., fragments, gaps, and breaks; table 1). Dicentrics often occurred in cells when there were no visible fragments present and appeared to involve joining at terminal bands. Identical

? = part of a chromosome of uncertain origin). Largest complete chromosome in each dicentric is described first. *First row:* (*a*) 1pk1qT–?, (*b*) 1qk1pT–T12pk12q, (*c*) 1qk–T2pk2q, (*d*) 6pk6qT–T19qk19p, (*e*) 6qk6pT–T12pk12q, (*f*) 6qk6pT–T17qk17p, (*g*) 7pk7qT–T7qk7p, (*h*) 7pk7qT–T7qk7p, (*i*) 7pk7qT–T7qk7p, (*j*) 7pk7qT–T12pk12q. *Second row:* (*a*) 7pk7qT–T16pk16q, (*b*) 7pk7qT–T19qk19p, (*c*) 7qk7pT–T7pk7q, (*d*) 7qk7pT–T7pk7q, (*e*) 7qk7pT–T9pk9q, (*f*) 7qk7pT–T11qk11p, (*g*) 7qk7pT–T12pk12q, (*h*) 7qk7pT–T16pk16q, (*i*) 7qk7pT–T16pk16q, (*j*) 7qk7pT–T16pk16q. *Third row:* (*a*) 7qk7pT–T16pk16q, (*b*) 7qk7pT–T16qk16p, (*c*) 7qk7pT–T19pk19q, (*d*) 7qk7pT–T19pk19q, (*e*) 7qk7pT–T19pk19q, (*f*) 7pk7qT–T19qk19p, (*g*) 7pk7qT–?, (*h*) 7pk7qT–?, (*i*) 8qk8pT–?, (*j*) 12pk12qT–T16pk16q. *Fourth row:* (*a*) 12qk12pT–T12pk12q, (*b*) 12qk12pT–T16pk16q, (*c*) 12qk12pT–T16pk16q, (*d*) 13qk13pT–?, (*e*) 14qk14pT–T20pk20q, (*f*) 15qk15pT–T16pk16q, (*g*) 16qk16pT–T16qk16p, (*h*) 16qk16pT–T16pk16q, (*i*) 16qk16pT–T16pk16q, (*j*) 16qk16pT–T17qk17p. *Fifth row:* (*a*) 16qk16pT–T19pk19q, (*b*) 16qk16pT–T20pk20q, (*c*) 16qk16pT–T22qk22p, (*d*) 16qk16pT–?, (*e*) 19pk19qT–T19qk19p, (*f*) 19qk19pT–T20pk20q, (*g*) 20pk20qT–T20qk20p, (*h*) ??, (*i*) ??, (*j*) ??.

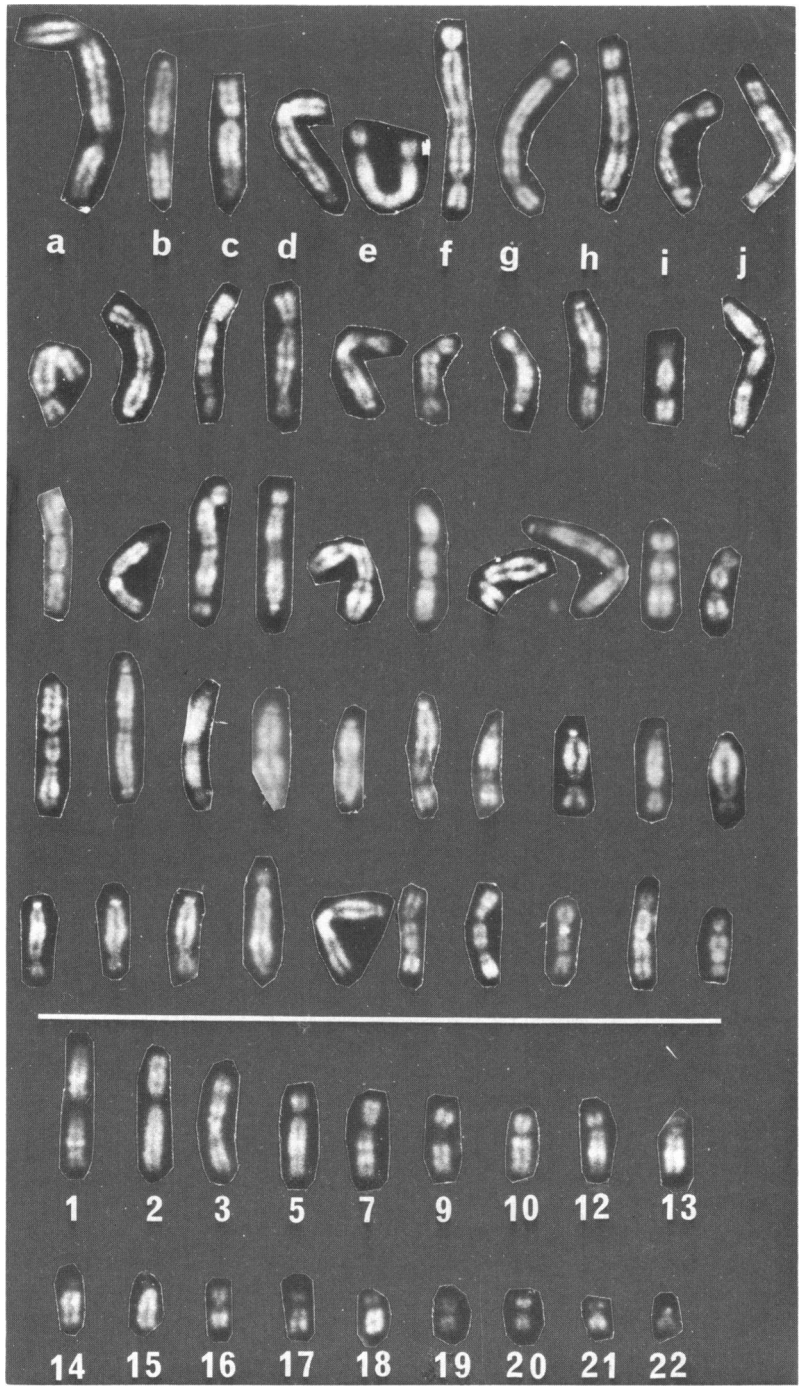

FIG. 2.—50 dicentrics from 48 senescent MRC-5 cells with the normal chromosomes thought

dicentrics could sometimes be seen in polyploid cells suggesting that they were true dicentrics rather than telomerically associated chromosomes. Although the same chromosomes were sometimes involved in different cells, the range of involvement and the frequent occurrence only in very senescent cells supports the idea that these are not stable dicentrics carried through successive cell generations [18]. These dicentrics, therefore, seem to occur spontaneously in senescent fibroblasts. A similar phenomenon has been described in SV-40 virus treated human fibroblasts [19, 20]. The term "telomeric binding" has been used to distinguish these from classical dicentric formation where dicentrics are usually accompanied by fragments in damaged cells [21].

The mechanism by which "telomeric binding" is produced is unknown. In this study culture conditions were in no way exceptional; both lines were free of mycoplasma, and the same batch of serum was used for cells in rapid growth and senescence. For each line, particular chromosomes seemed to be involved more often than others. This indicates that telomeric binding is a result of changes specific to those chromosomes rather than depletion of a factor essential for the cells. Classical studies on chromosome rearrangement suggest that telomeres are stable and breakage must occur for chromosome rearrangement [22]. The results presented here suggest that either this may not always be the case or that minute deletions do occur with terminal sequences showing increased instability in aging fibroblasts.

Telomeric binding may also occur in vivo. Dicentric formation by end-to-end chromosome fusion has recently been reported in a study on lymphocytes of patients with ataxia telangiectasia [23]; many of the translocations found in these cells appear to involve telomeric regions [24]. In chronic myeloid leukemia, the translocation resulting in the Philadelphia chromosome usually involves the terminal band of chromosome 9, although other sites have been described [25]. The rearrangements which are observed at low passage in fibroblasts from adults [5] also frequently appear to involve terminal regions (P. A. Benn, unpublished observations). Translocations in tumor cells [26, 27] and lymphocytes from healthy individuals [28] have also been reported in which telomeric regions appear to be involved. The apparently deleted chromosomes are stable at their broken ends,

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to be involved presented below. *First row:* (*a*) 1pk1qT–T1qk1p, (*b*) 1qk1pT–T22qk22p, (*c*) 2pk2qT–T22qk22p, (*d*) 3pk3qT–T15qk15p, (*e*) 5pk5qT–T5qk5p, (*f*) 5pk5qT–T7qk7p, (*g*) 5pk5qT–T7qk7p, (*h*) 5pk5qT–T13qk13p, (*i*) 7pk7qT–T7qk7p, (*j*) 7pk7qT–T7qk7p. *Second row:* (*a*) 7pk7qT–T7qk7p, (*b*) 7pk7qT–T13qk13p, (*c*) 7pk7qT–T19qk19p, (*d*) 7pk7qT–T22pk22q, (*e*) 9pk9qT–T13qk13p, (*f*) 9pk9qT–T20pk20q, (*g*) 9pk9qT–T22qk22p, (*h*) 9qk9pT–T13qk13p, (*i*) 9qk9pT–T20pk20q, (*j*) 10qk10pT–T12pk12q. *Third row:* (*a*) 10qk10pT–T12pk12q, (*b*) 10qk10pT–T18qk18p, (*c*) 12pk12qT–T12qk12p, (*d*) 12pk12qT–T13qk13p, (*e*) 12qk12pT–T12pk12q, (*f*) 12qk12pT–T12pk12q, (*g*) 12qk12pT–T13qk13p, (*h*) 12qk12pT–T14qk14p, (*i*) 12qk12pT–T21pk21q, (*j*) 12qk12pT–T22pk22q. *Fourth row:* (*a*) 12qk12pT–8p(band 21)k8q, (*b*) 13pk13qT–T13qk13p, (*c*) 13pk13qT–T13qk13p, (*d*) 13pk13qT–T13qk13p, (*e*) 13pk13qT–T13qk13p, (*f*) 13pk13qT–T16pk16q, (*g*) 13pk13qT–T17pk17q, (*h*) 13pk13qT–T17pk17q, (*i*) 13pk13qT–T17pk17q, (*j*) 13pk13qT–T19qk19p. *Fifth row:* (*a*) 13pk13qT–T20pk20q, (*b*) 13pk13qT–T22pk22q, (*c*) 13pk13qT–T22pk22q, (*d*) 13pk13qT–?, (*e*) 13pk13qT–?, (*f*) 16qk16pT–T17pk17q, (*g*) 17qk17pT–T17pk17q, (*h*) 19qk19pT–T22pk22q, (*i*) ??, (*j*) ??.

possibly as a result of a "healing" process [22]. In the case of ataxia telangiectasia, the disease has been associated with premature aging [29], and in the other examples cited, cellular aging may be an important factor.

Further observations of telomeric binding in experimental conditions may help clarify the nature of the telomere and the translocations which appear to involve terminal bands.

SUMMARY

In senescent fibroblast cell lines derived from human embryos, the number of chromosome aberrations were found to increase rapidly. In addition to an increase in aneuploidy and polyploidy, a high frequency of dicentrics occurred, but the number of other chromosome abnormalities remained approximately constant. Banding revealed that many of the dicentrics appeared to be end-to-end fusions of whole chromosomes. The involvement of chromosomes was nonrandom. This "telomeric binding" may reflect a progressive decrease in the stability of telomeric sequences or associated enzymes which may also occur in vivo.

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