The Gardner syndrome: increased tetraploidy in cultured skin fibroblast*

B. SHANNON DANES

Department of Medicine, Cornell University Medical College, New York, New York 10021, USA.

Summary. Tetraploidy was increased in skin fibroblast cultures grown in the laboratory at the same time under the same conditions and derived from 2 probands with the Gardner syndrome and 9 affected members of one family as compared to that occurring in cultures from 5 relatives by marriage and 10 normals. Tetraploidy was present at the first subculture (2 weeks after the initial biopsy was cultured), and for each line studied the percentage of dividing cells showing tetraploidy remained constant.

The relation of the observed tetraploidy to the increased risk of such patients to develop abnormal growths and cancer has not been established. The increased tetraploidy should be of value in identifying the presence of the gene for the Gardner syndrome in high risk families.

Determination of the inheritance of an autosomal dominant disorder may not be possible until clinical signs appear. For both clinical management and genetic counselling in families whose members are at risk for such autosomal dominant disorders, it would be beneficial to be able to detect the presence of such genes before their clinical expression.

The Gardner syndrome is such an autosomal dominant disorder characterized by four abnormal growths (multiple intestinal polyposis, osteomas, fibromas and sebaceous cysts) (Gardner, 1972; Pierce, 1972).

Cell culture offers an opportunity to study the isolated somatic cell to determine if in culture a cell carrying the Gardner gene will express an abnormality which reliably and reproducibly reflects the presence of this gene. If such a cell marker for the Gardner syndrome is expressed in the cultured cell, then cell culture studies on families at risk would be possible for identification of family members who inherited the Gardner gene.

Subjects and methods

Two probands with the Gardner syndrome and the family of one of these probands (No. 2) with 9 other members with the Gardner syndrome and 2 unaffected individuals in 3 generations have been studied (Table I). The diagnosis of the Gardner syndrome was made in each individual on the basis of the presence of skin growths by physical examination, bone lesions by x-rays, and colonic lesions found on colonoscopy followed by microscopic examination of colonic biopsies (Gardner, 1972; Pierce, 1972).

Split thickness biopsies were taken without anaesthesia from the extensor surface of the upper arm from the 2 probands, 11 members of the family, and 5 members by marriage as well as 10 normal, unrelated individuals age matched to the family members. The proband of the No. 2 family was biopsied three times in a 6-month period. Each biopsy was cut into 2 parts so that duplicate cultures were set up and grown in 2 different CO₂ incubators.

The establishment of the cell lines from skin biopsies by standard culture methods (Danes and Bearn, 1969) required only 2 weeks, so that the cells studied had been grown in culture for only 2½ weeks before the present studies. Fibroblast lines were established and maintained in Falcon plastic petri dishes in Eagle's minimum essential medium with 20% human AB serum and 5% beef embryo extract ultrafiltrate (Chu and Giles, 1959) in an atmosphere of 5% CO₂ in air. The pH of the medium was kept between 7.0 and 7.4 during the culture

Received 11 February 1975.

* This research was made possible through a grant from the National Large Bowel Cancer Project of the National Cancer Institute Division of Cancer Research (CA 15973) and supported in part by the Danes Medical Research Fund, Cornell University Medical College and the Zemmurray Foundation.

TABLE I
CLINICAL PHENOTYPES OF 2 INDIVIDUALS AND 10 AFFECTED MEMBERS OF 1 FAMILY
WITH THE GARDNER SYNDROME

Families Studied		Age at Biopsy (yr)	Clinical Evidence of the Gardner syndrome*					% of Dividing Cells Showing Tetraploidy
Number	Generation, No.		Intestinal Polyposis	Osteomas	Fibromas	Sebaceous Cysts	Other Clinical Data	
1	Proband	32	X	X			Died age 32, adenocarcinoma of colon	35
2	I.2						Died age 61; cancer of colon and stomach	
	I.6					X	Cysts in breasts; alive age 86	
	II.1	45	X				Deceased age 32; heart attack	18
	II.3						Melanoma on foot, colectomy	
	III.2 (proband)	38	X			X		11, 16, 18
	III.3	36	X			X		20
	IV.2	23	X			X		13
	IV.3	21	X					20
	IV.4	19	X (early)	X		X		22
	IV.5	17	X (?)			X		31
	IV.7	11	X (early)			X		17
	IV.8	12	X (early)				Supernumerary teeth	15
	IV.9	10	X (?)			X		14

* X = present.

period. All cultures studied had been grown in the same incubators at the same time using the same batch of medium in order to reduce environmental variables.

For chromosome preparations, the cells were trypsinized into suspension and approximately 10^4 cells were suspended into 5 ml medium, inoculated into small Falcon petri dishes and incubated in an atmosphere of 5% CO_2 in air. Forty-eight hours later when a burst of mitoses was observed, chromosome preparations were made and stained in either aceto-orcein or Giemsa (Sumner, Evans, and Buckland, 1971).

Chromosome preparations were examined on duplicate cell lines established at the first and second subcultures by trypsinization from all individuals studied. To evaluate the influence of time in culture on mitotic

activity, cell lines derived from the 2 probands and 4 normals at the 6th, 10th, 14th, and 18th subcultures by trypsinization were studied.

Two slides of each chromosome preparation were made on 2 different cultures at each subculture examined. Mitoses on the entire slide were examined as it was found that bias could be introduced if only part of the slide was studied. Only if more than 50 mitoses were counted per slide was the preparation considered to reflect the mitotic activity of that cell line.

The number of chromosomes in each dividing cell was determined. Tetraploidy was recorded (Table II) as the number (expressed as a percentage) of dividing cells with more than 46 chromosomes per cell in the total number of dividing cells examined per culture.

TABLE II
PERCENTAGE OF DIVIDING CELLS SHOWING TETRAPLOIDY IN SKIN FIBROBLAST CULTURES DURING 4 CULTURE MONTHS FROM 4 NORMALS AND 2 PROBANDS WITH GARDNER SYNDROME

Subjects		Percentage of Dividing Cells Showing Tetraploidy									
		Subculture Number*									
		1		6		10		14		18	
	Duplicate Cultures	1†	2	1	2	1	2	1	2	1	2
Normals	1	0	0	0	0	0	0	2	2	6	5
	2	2	1	1	1	3	2	5	5	4	3
	3	1	2	3	3	2	1	4	3	4	4
	4	0	0	0	0	1	1	1	1	3	2
Gardner's syndrome											
	Proband of family										
	1	—	—	35	35	31	30	31	29	29	29
	2	11	12	18	17	18	16	18	20	16	16

* Subcultured by trypsinization once a week.

† Numbers refer to replicate culture dishes.

Results

At the first subculture skin fibroblast cultures established from the 10 normal, unrelated subjects and the 5 members by marriage of family No. 2 showed 0 to 4% of the cells in mitosis having more than 46 chromosomes. The number of chromosomes per cell was tetraploid (92) or hypotetraploid. Dividing cells with more than 92 chromosomes were not observed. Endoreduplication was observed once in 2000 divisions counted in cultures from normal subjects studied. No other aberration in mitosis was observed. The incidence of tetraploidy in each normal cell line remained relatively constant through 18 subcultures (Table II).

As compared with normal cultures, the cultures derived from the 2 probands showed increased tetraploidy at the first subculture. The number of chromosomes per cell in such cells was tetraploid (92) or hypotetraploid. Endoreduplication was seen in all lines from these 2 probands. No other abnormality in mitotic activity was noted. The percentage of tetraploidy was constant for each line and showed little variation through 18 subcultures (Table II). The lines established from the 3 biopsies taken from proband No. 2 in a 6-month period showed 11, 16, and 18% of dividing cells being tetraploid.

In the family studied (Fig.) the cultures derived from the 10 clinically affected patients had 11 to 31% of the dividing cells showing tetraploidy. There appeared to be no correlation between the age of the affected subject, the clinical expression of the Gardner syndrome, and the degree of tetraploidy in the cultures (Tables I and II).

FIG. Pedigree of family 2, with 10 members with the Gardner syndrome. Number in each symbol is the percentage of dividing cells showing tetraploidy. Arrow identifies proband.

The cultures derived from the family member (IV.6—Fig) without clinical evidence of the Gardner syndrome studied had 1% tetraploidy. The occurrence of tetraploidy (0–2%) in the cultures from the 5 family members by marriage was similar to that observed in normal cultures.

Discussion

A mutation can be detected only if an abnormality identifies its presence. It has been demonstrated (Mellman, 1973) that human genetic mutations not detectable *in vivo* can often be reliably identified when the somatic cell with the mutation is grown in culture. Skin fibroblasts which appear normal *in vivo* have been shown to reveal the presence of a mutated gene in culture. An autosomal recessive or X-linked recessive disorder becomes dominant in culture with the cell marker in the heterozygous cell, with 1 mutated gene being indistinguishable from that in the homozygous cell with 2 such genes.

As 3 cell systems (bone, gastrointestinal tract, and skin) show abnormal growths in the patient with the Gardner syndrome (Gardner, 1972; Pierce, 1972), it appeared that any somatic cell with the Gardner gene might manifest a cell marker which would identify the presence of this gene.

Colonic polyps without evidence of neoplastic changes from patients with the Gardner syndrome have been reported (Mark *et al*, 1973) to show chromosome abnormalities, including tetraploidy, similar to that reported in this paper for the cultured skin fibroblast with the Gardner genotype (Table II and Fig.). In the family studied in detail (Fig.) IV.5 had no skin lesions and the presence of colonic polyps was questionable. The cultures from this individual showed 31% tetraploidy, whereas those from his sister who had both skin lesions and colonic polyps at 11 years of age showed only 17% tetraploidy. Therefore, the degree of clinical expression of the Gardner gene did not influence the presence of, or the degree of, tetraploidy observed in the cultured fibroblasts.

Since the biopsies were taken from areas of the skin which did not have sebaceous cysts or fibromas, the cells represented somatic cells before any abnormal growth activity. As tetraploidy was present at the first subculture and the percentage of dividing cells showing tetraploidy did not increase in 4 culture months (Table II), it did not seem likely that the tetraploidy was secondary to ageing in culture or to viral infection during this culture period. However, if an undetected viral contamination existed in the laboratory, both cells with and

without the Gardner gene were exposed with only the Gardner cells showing increased tetraploidy.

In 1914 Boveri postulated that chromosomal aberrations were important in the aetiology of malignancies. Since that time chromosomal abnormalities have been described in a variety of tumours including colonic tumours. It is not known if chromosomal abnormalities including tetraploidy are important in the pathogenesis or if they result secondarily from the malignant change. Knudson, Strong, and Anderson (1973) proposed that every human cancer occurs in a genetic and a nongenetic form. In the genetic form, the mutation predisposes the cell to a subsequent change which results in alterations in cellular functions resulting in malignant growth. The observations presented above suggested that the cell with the Gardner gene is predisposed to tetraploidy when grown in culture.

Chromosomal instability has been observed in some rare autosomal recessive disorders associated with increased risk of cancer. Chromosome instability was reflected in the cultured cells in the Bloom syndrome by a high frequency of chromatid interchanges and rearrangements (German, 1969), in Fanconi's anaemia by chromatid gaps and breaks (Schroeder and Kurth, 1971), and in the Louis-Bar syndrome by chromosome breakage (Hecht *et al.*, 1966). The cultured skin fibroblasts from the Gardner syndrome showed no increased tendency for chromosome breakage or rearrangements similar to that seen in these 3 disorders. In xeroderma pigmentosum, the cultured skin fibroblasts showed pseudodiploidy with reciprocal translocations (German *et al.*, 1970; German, 1972), whereas in the Gardner syndrome the cultured cells showed tetraploidy or hypotetraploidy.

In endoreduplication, chromosome replication occurs without an intervening mitosis resulting in a tetraploid nucleus. Endoreduplication occurs in cultured cells from normal individuals but at a very low incidence (Obe, 1965; Turner and Wald, 1965). The percentage of tetraploid cells has been reported to be below 6% for fibroblast cultures derived from normal individuals (Todaro and Martin, 1967). In the 10 lines studied from normal individuals in the present study, 1 cell with endoreduplication was observed in 2000 divisions examined and tetraploidy was present (0 to 4%) (Table II).

In cultures derived from patients with such chromosomal abnormalities as trisomy 21-22, the percentage of cells showing heteroploidy has been reported to be increased (Ferrier and Ferrier, 1966; Todaro and Martin, 1967). The cell with the Gardner genotype showed increased tetraploidy,

with endoreduplication being observed more frequently than in cultures from normals. Such abnormal divisions would lead to alterations in chromosomal content per cell and probably chromosomal aberrations.

The relation, if any, between the increased incidence of tetraploidy observed in skin fibroblast cultures from patients with the Gardner syndrome and their increased risk to abnormal growths and cancer in all 3 tissues (bone, skin, and gastrointestinal tract) is, as yet, unknown. The increased incidence of tetraploidy in the cultured skin fibroblast should be of value as a cell marker for identification of the Gardner gene in families at risk.

The author is indebted to Drs Victor A. McKusick and Rulon W. Rawson for interesting her in this group of genetic disorders and to Ann J. Krush for making the members of family 2 available for this study. The proband of family 1 was a patient of Drs Paul Sherlock and Martin Lipkin of the Sloane-Kettering Memorial Center. The assistance of Beth K. Rottell is gratefully acknowledged.

REFERENCES

- Boveri, T. (1914). *Zur Frage der Entstehung maligner Tumoren*. G. Fisher, Jena.
- Chu, E. H. Y. and Giles, N. H. (1959). Human chromosome complements in normal somatic cells in culture. *American Journal of Human Genetics*, **11**, 63-79.
- Danes, B. S. and Bearn, A. G. (1969). Cystic fibrosis of the pancreas. A study in cell culture. *Journal of Experimental Medicine*, **129**, 775-793.
- Ferrier, P. E. and Ferrier, S. A. (1966). Chromosome abnormalities in hematologic disorders. *New England Journal of Medicine*, **274**, 914.
- Gardner, E. J. (1972). Discovery of the Gardner syndrome. *Birth Defects: Original Article Series*, **13**, 48-51.
- German, J. (1969). Bloom's syndrome I. Genetical and clinical observations in the first twenty-seven patients. *American Journal of Human Genetics*, **21**, 196-227.
- German, J. (1972). Genes which increase chromosomal instability in somatic cells and predispose to cancer. In *Progress in Medical Genetics*, vol. 8, pp. 61-101. Ed. by A. G. Steinberg and A. G. Bearn. Grune and Stratton, London and New York.
- German, J., Gilleran, T., LaRock, J., and Regan, J. D. (1970). Mutant clones amid normal cells in cultures of xeroderma pigmentosum cells. *American Journal of Human Genetics*, **22**, 10a.
- Hecht, F., Koler, R. D., Rigas, D. A., Dahnke, G. S., Case, M. P., Tisdale, V., and Miller, R. W. (1966). Leukemia and lymphocytes in ataxia-telangiectasia. *Lancet*, **2**, 1193.
- Knudson, A. G., Jr., Strong, L. C., and Anderson, D. E. (1973). Heredity and cancer in man. In *Progress in Medical Genetics*, vol. 9, pp. 113-158. Ed. by A. G. Steinberg and A. G. Bearn. Grune and Stratton, London and New York.
- Mark, J., Mitelman, F., Dencker, H., Norrby, C., and Tranberg, K. G. (1973). The specificity of the chromosomal abnormalities in human colonic polyps. *Acta Pathologica et Microbiologica Scandinavica*, **81**, 85-90.
- Mellman, W. J. (1973). Human cell culture techniques in genetic studies. In *Medical Genetics*, pp. 157-165. Ed. by V. A. McKusick and R. Claiborne. HP Publishing Co., New York.
- Obe, G. (1965). Chromosome endoreduplication in normal controls. *Lancet*, **1**, 59.
- Pierce, E. R. (1972). Pleiotropism and heterogeneity in hereditary intestinal polyposis. *Birth Defects: Original Article Series*, **13**, 52-62.

- Schroeder, T. M. and Kurth, R. (1971). Spontaneous chromosomal breakage and high incidence of leukemia in inherited disease. *Blood*, **37**, 96–112.
- Sumner, A. T., Evans, H. J., and Buckland, R. A. (1971). New technique for distinguishing between human chromosomes. *Nature New Biology*, **232**, 31–32.
- Todaro, G. J. and Martin, G. M. (1967). Increased susceptibility of Down's syndrome fibroblasts to transformation by SV₄₀. *Proceedings of the Society for Experimental Biology and Medicine*, **124**, 1232–1236.
- Turner, J. H. and Wald, N. (1965). Endoreduplication and disease. *Lancet*, **1**, 915.