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Phenotypic Heterogeneity within Clones of Fetal Human Cells

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SUMMARY

The heterogeneity of cell morphology characteristics of some colonies of human fetal kidney and amniotic fluid cells has been analyzed by biochemical and cell-cloning techniques. All the presumed subclones derived from dimorphic colonies were initially epithelioid, but some cells became fibroblastlike as the cell density increased. To determine if the observed heterogeneity occurred within clonal populations of cells, we determined the isozyme phenotype of dimers from renal cells heterozygous for glucose-6-phosphate dehydrogenase (G6PD). Colonies showing mixed cellular morphology expressed only a single G6PD isozyme, thus revealing their single-cell origin. Our results indicate that cell morphology is influenced by the cellular density within the clone, and that a single human renal cell in vitro can yield progeny of two morphological types.

INTRODUCTION

Transformation from one cellular phenotype to another has been observed for diploid cells in vitro [1–3], and such changes have been correlated with the appearance of novel cell properties [4, 5] and new differentiated functions [6, 7]. However, the evidence that such a morphological change regularly occurs within *clones* of cells is weak. With the exception of myoblast fusion studies [8], the single-cell origin of colonies containing two distinguishable cell types has not been established. The single-cell origin of such pleiomorphic colonies has not been established biochemically in any case.

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The cellular mosaicism in heterozygotes carrying G6PD variants provides the means to determine if populations of cells have a single-cell origin. Analyses of tumors from heterozygous females reveal that some are derived from single cells while others have a multicellular origin [9, 10].

We used the cellular mosaicism at the G6PD locus to analyze colonies of human fetal kidney cells that exhibit morphological heterogeneity (table 1, fig. 1*A* and *B*). Our studies indicate that these colonies of mixed morphology *are* clones and not mixed colonies, and that the heterogeneity is a consequence of increasing intraclonal cell density.

MATERIALS AND METHODS

Tissue from seven dead fetuses of 10–20-weeks gestation were obtained according to protocol approved by the Committees for Clinical Investigation and Fetal Research of The Johns Hopkins Medical Institutions. Clones were established either directly from dissociated cells or from cell cultures established from explants. Cells prepared directly from kidney by treatment with collagenase (0.2%; Sigma Type II, Sigma, St. Louis, Mo.) and trypsin (0.25%; Gibco, Grand Island, N.Y.) were plated at densities of $0.1—5 \times 10^3$ into 60-mm plastic petri dishes containing Eagle's MEM (Gibco) supplemented with nonessential amino acids and 20% fetal calf serum. Alternatively, cells emanating from 3–8-day outgrowths of explants derived from fetal kidney, lung, heart, liver, and skin were replated at low densities (10–100 cells per 60-mm dish). Colonies of amniotic fluid cells were established by distributing the cellular pellet from 10-ml fluid obtained by midtrimester amniocentesis into two 60-mm petri dishes.

At 10-days incubation, clones in petri dishes were fixed and stained with toluidine blue or Giemsa to determine cellular morphology. In some cases, individual colonies were isolated within a cloning cylinder [11] and transferred to individual petri dishes. The resulting clones were analyzed for G6PD phenotype on cellulose acetate gels [12] or were used as a source of subclones.

RESULTS

All the cells of colonies derived from human fetal skin, lung, heart, and testes were entirely fibroblastic (i.e., spindle-shaped cells with bipolar processes, in parallel array). No heterogeneity was observed (table 1). In contrast, the colonies obtained from human fetal kidney and amniotic fluid cells were of several varieties (table 1). Some consisted entirely of fibroblasts, and others were composed entirely of epithelioid cells (randomly oriented round cells without processes). In addition, there were heterogeneous colonies that seemed to contain fibroblasts as well as epithelioid cells. Serial examination of these dimorphic colonies revealed that they were initially homogeneous, consisting purely of epithelioid cells, but that fibroblastlike cells appeared in the center of the colony as the colony increased in size. These spindle-shaped cells streamed over the epithelioid cells, subdividing the presumed clone into compartments (fig. 1).

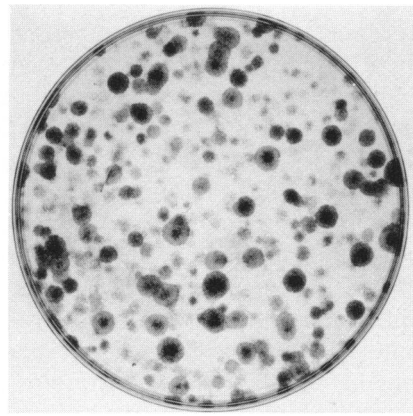
To determine if the fibroblastlike cells in the dimorphic colonies were in fact progeny of epithelioid cells and not merely fibroblast contaminants, we isolated the dense center of one of these renal cell colonies with a cloning cylinder. The resultant cell suspension, enriched for spindle-shaped cells, was diluted with nutrient medium and plated sparsely (20–300 cells per 100-mm dish) into a series of

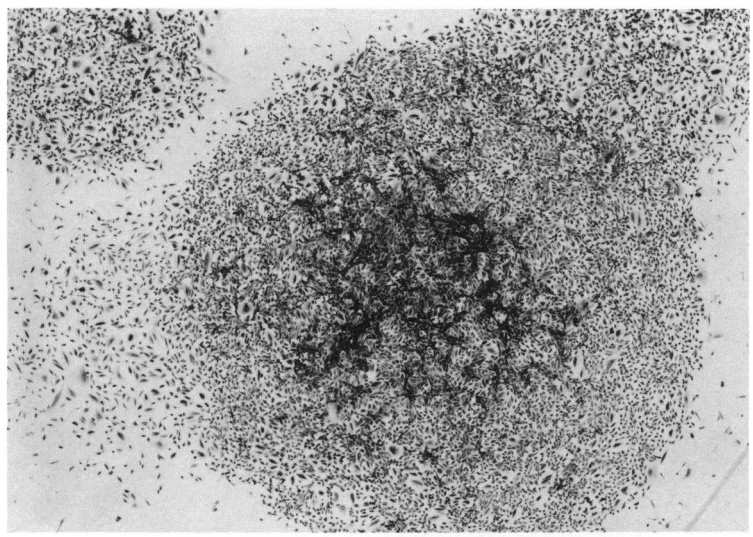
TABLE 1

MORPHOLOGY OF COLONIES DERIVED FROM FETAL TISSUES

Tissue	Subject	Age*	Source of colonies	Cloning efficiency	No. colonies	MORPHOLOGY† (% TOTAL)		
						Fibroblast	Epithelial	Mixed‡
Kidney	Fe 13	12 wks	3-day outgrowth from explant		15	13	67	20
	E 14	12½ wks	Direct plating from tissue§	.01	30	16	60	24
	Fe 15	13 wks	Direct plating from tissue	.02	78	5	83	12
	Fe 19a	14 wks	Direct plating from tissue	.01	15	6	27	67
	Fe 19b	...	8-day outgrowth from explant	.05	22	39	55	6
	Fe 19c	...	Clone of mixed morphology	.06	140	0	29	71
	Fe 20	18 wks	5-day outgrowth from explant	.16	22	5	23	72
	Fe 25	16 wks	7-day outgrowth from explant	.15	51	4	22	74
Lung	Fe 15	13 wks	3-day outgrowth from explant	.72	201	100	...	...
	Fe 19	14 wks	First subculture from explant	.33	83	100	...	...
Heart	Fe 15	13 wks	3-day outgrowth from explant	.26	191	100	...	...
Liver	Fe 21	10 wks	6-day outgrowth from explant	.06	30	100	...	...
Skin	K 12	14 wks	3-day outgrowth from explant	.35	92	100	...	...
Amniotic fluid		18 wks	Direct plating from pellet	...	29	7	86	7

* From conception.
† Analyzed at 10–12 days after plating.
‡ Dense-centered colonies of dimorphic morphology.
§ Dissociated cells.





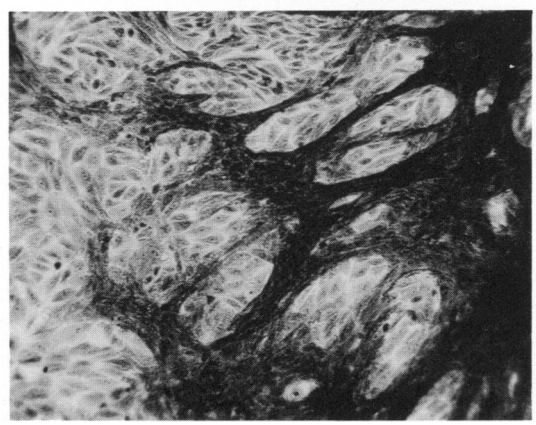


FIG. 1.—(*A, top*), Photomicrographs of subclones derived from a clone of fetal kidney origin (Fe 19c) showing prominence of colonies with dense centers. Close-up of dimorphic clones (*B, center*) 25 ×, and (*C, bottom*) 100 × (close-up of center).

petri dishes. At 7 days after plating, the resulting colonies (obtained with an efficiency of 6%) were homogeneously epithelioid so that fibroblast contamination was not a likely explanation for the mixed morphology. On continued proliferation, spindle-shaped cells reappeared centrally, forming the same patterns noted in the original colonies (table 1—Fe 19c and fig. 1).

To demonstrate the single-cell origin of these dimorphic colonies, studies were undertaken of renal cells from a female fetus heterozygous for the common electrophoretic variant of the X-linked enzyme G6PD. Cells obtained from the outgrowth of a kidney explant were placed at low density (10–100 cells per 60-mm dish), and discrete colonies were obtained for kidney explants with an efficiency of 12%. Approximately one-half of these had a dimorphic morphology. Chromosome analyses of two of the mixed colonies revealed the expected 46XX karyotype. Ten colonies, including seven of mixed morphology, were isolated and analyzed for G6PD phenotype. The results of electrophoresis shown in table 2 and figure 2 revealed that each colony tested expressed either $G6PD_A$ or $G6PD_B$; none expressed both isozymes.

The presence of intraclonal heterogeneity, although dependent upon the density of cells within the clone, was independent of the number of clones in the petri dish; these patterns evolved even in dishes where colonies were sparse. However, clonal density alone did not induce the heterogeneous morphology as colonies of the same density, but entirely epithelioid, were also observed from the same specimen (table 1).

DISCUSSION

Clearly, the dimorphic colonies are in fact clones derived from single cells. Cells from the mixed colony give rise to colonies of entirely epithelial morphology that become heterogeneous as intraclonal density increases—indicating that the morphological phenotype of the parent was transmitted accurately to the progeny.

TABLE 2

CHARACTERISTICS OF CLONES DERIVED FROM CELLS OF RENAL ORIGIN, HETEROZYGOUS FOR $G6PD_A$ (Fe 20, CLONING EFFICIENCY = .17)

(Presumed) clone	Morphology	G6PD phenotype
3	Pure epithelial	A
5	Pure epithelial	B
6	Mixed	B
7	Mixed	B
10	Mixed	B
12	Mixed	B
13	Mixed	A
22	Pure epithelial	B
24	Mixed	A
25	Mixed	A

NOTE: Only a single isozyme is expressed in each clone. There is no correlation between G6PD phenotype and morphology.

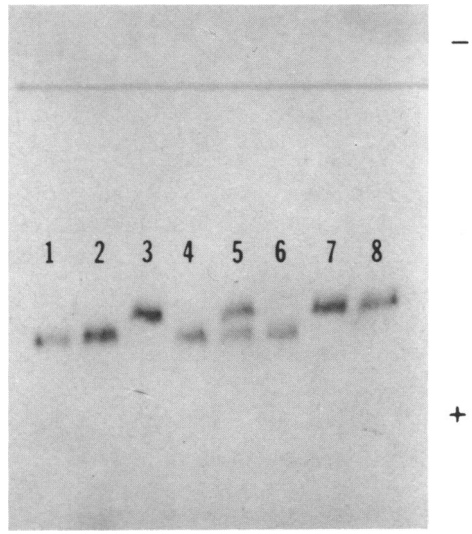


FIG. 2.—G6PD phenotypes of dimorphic clones. *Slot 5*: extracts of uncloned cells from heterozygous Fe 20 expressing both A and B isozymes. *Slots 1–4, 6–8*: extracts from mixed colonies expressing single isozyme, A or B.

Furthermore, the presence of a single isozyme of G6PD in each of the "mixed" clones tested is compelling evidence for the single-cell origin of the colonies. A cell heterozygous for G6PD expresses only a single form of the enzyme because it has only a single active X, and the isozyme expressed is stably transmitted to all progeny [13]. Therefore, if these colonies of mixed morphology originated from more than one cell, some should have expressed two forms of the enzyme. The probability that all seven of the mixed colonies have a single isozyme merely because the mixture originated from two cells that by chance have the same form of the enzyme is $(.5)^7$, or less than 1%.

Observations compatible with two morphologic types within a single clone have been reported for embryonic chick cartilage [14], human fetal muscle [8], and amniotic fluid cells [15]; however, the single-cell origin of these putative clones has not been established by biochemical criteria. The single G6PD isozyme observed in these dimorphic colonies not only reveals their clonal nature but indicates that at least some cells of renal origin give rise to daughter cells of two phenotypes in response to increasing intraclonal cell density. The intraclonal heterogeneity is evidence that this adaptive behavior is heritable. Furthermore, our observations lend support to the evidence [16–19] that cell density is important for the expression or maintenance of new cell phenotypes during development.

REFERENCES

1. LEWIS WH: Mesenchyme and mesothelium. *J Exp Med* 38:257–262, 1923
2. KAIGHN ME, PRINCE AM: Production of albumin and other serum proteins by clonal cultures of normal human liver. *Proc Natl Acad Sci USA* 68:2396–2400, 1971

3. SLAVINSKY EA, AUERSBERG N: Propagation in vitro of functional rate adrenal cortical cells: modification of the differentiated state by culture conditions. *In Vitro* 9:260–269, 1974
4. WILLINGHAM MC, YAMADA KM, YAMADA SS, POUYSSEGUR J, PASTAN I: Microfilament bundles and cell share are related to adhesiveness to substratum and are dissociable from growth control in cultured fibroblasts. *Cell* 10:375–380, 1977
5. ALI IU, MAUTNER V, LANZA R, HYNES RO: Restoration of normal morphology, adhesion, and cytoskeleton in transformed cells by addition of a transformation-sensitive surface protein. *Cell* 11:115–126, 1975
6. EGUCHI G, OKADA TS: Differentiation of lens tissue from the progeny of chick retinal pigment cell cultures in vitro: a demonstration of a switch of cell types in clonal cell culture. *Proc Natl Acad Sci USA* 70:1495–1499, 1973.
7. VON DER MARK K, GAUSS V, VON DER MARK H, MULLER P: Relationship between cell shape and type of collagen synthesized as chondrocytes lose their cartilage phenotype in culture. *Nature* 267:531–533, 1977
8. HAUSCHKA SD: Clonal analysis of vertebrate myogenesis III: Developmental changes in muscle-colony-forming cells of human fetal limb. *Dev Biol* 37:345–368, 1974
9. LINDLER D, GARTLER SM: Glucose-6-phosphate dehydrogenase mosaicism: utilization as a cell marker in the study of leiomyomas. *Science* 150:67–69, 1965
10. FRIEDMAN JM, FIALKOW PJ: Viral "tumorigenesis" in man: cell markers in condylomate acuminata. *Cancer* 17:57–61, 1976
11. HAM RG, PUCK TT: in *Methods in Enzymology*, edited by COLOWICK SP, KAPLAN NP, New York, Academic Press, 1962, p 90
12. MIGEON BR, KENNEDY JF: Evidence for the inactivation of an X chromosome early in the development of the human female. *Am J Hum Genet* 27:233–239, 1975
13. DAVIDSON RG, NITOWSKY HM, CHILDS B: Demonstration of two populations of cells in the human female heterozygous for glucose-6-phosphate dehydrogenase variants. *Proc Natl Acad Sci USA* 50:481–485, 1963
14. LAVIETES BB: Cellular interactions and chondrogenesis in vitro. *Dev Biol* 21:584–610, 1970
15. HOEHN H, BRYANT EM, KARP LE, MARTIN GE: Cultivated cells from diagnostic amniocentesis in second trimester pregnancies I. Clonal morphology and growth potential. *Pediatr Res* 8:746–754, 1974
16. MORRIS JE, MOSCONA AA: Induction of glutamine synthetase in embryonic retina: its dependence on cell interactions. *Science* 167:1736–1738, 1970
17. DESCHATRETTE J: Differentiated variants of a rat hepatoma: partial reversion induced by cell aggregation. *Cell* 22:501–511, 1980
18. MARTIN GR, EVANS MJ: Differentiation of clonal lines of teratoma cells: formation of embryoid bodies *in vitro*. *Proc Natl Acad Sci USA* 72:1441–1445, 1975
19. MORRIS JE, MOSCONA AA: The induction of glutamine synthetase in cell aggregates of embryonic neural retina: correlations with differentiation and multicellular organization. *Dev Biol* 25:420–444, 1971