46,XY/45,X mosaicism in an amniotic fluid cell culture: suppression of abnormal cell line after subcultivation

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SUMMARY An abnormal cell population, 45,X, appeared in 3 of 4 cell lines established from an amniotic fluid specimen obtained from a normal mid-trimester pregnancy. Two of the cell lines were subjected to repeated chromosome analyses until VII passage. The abnormal cells were suppressed after repeated trypsinisations; simultaneously, fibroblast-like cells outgrew the cultures, which were previously predominated by epithelial-like cells. Polyploidy was found in 0 to 12% of the cells, the highest level existing in the early passages. The question of whether chromosomally abnormal cells present in primary cultures and the early subcultures reflect the karyotype of the fetus is discussed.

Polyploidy is regularly found among cultured amniotic fluid cells. More recently, chromosomal mosaicisms have been reported in a number of cases without confirmation of the abnormal chromosome constitution in cells from the fetus or from the live born child. A few cases of true mosaicism have also been published. A survey of the mosaic findings has been published by Hsu *et al.* (1976). Later publications confirm that pseudomosaicism is occasionally found (Aula *et al.*, 1978, Rodriguez *et al.*, 1978). The diagnostic dilemma is obvious. Firstly, is the mosaic constitution of the amniotic fluid cells representative of the karyotype of the fetus, or is it an anomaly that arises *in vivo* or *in vitro* without any consequences for the child? Secondly, if the mosaicism found reflects the condition of the fetus, how would it influence the phenotype of the child? This paper, in which a case of 46,XY/45,X mosaicism is reported, deals with the first problem.

In order to obtain a rapid diagnosis, primary cultures or second to third passage cultures are generally used for chromosome analysis. In the present amniotic fluid specimen, repeated chromosome analyses were performed, and the karyotype of the cells was followed until the seventh passage. Usually the primary cultures have a predominance of epithelial-like cells which have a short life span and disappear after a few trypsinisations; subsequently other cell types predominate in the cultures. If the aberrant cells of a mosaic amniotic fluid cell culture

are also lost after a few passages, it might be possible to relate the chromosome abnormality to cell type. The consequences with respect to the cytogenetic diagnosis are discussed.

Materials and methods

The amniotic fluid specimen was obtained by transvaginal amniocentesis from a second trimester pregnancy before induced abortion. The fluid was part of normal material analysed with respect to the effect of culture conditions on the behaviour of amniotic fluid cell cultures; therefore, a chromosome analysis was not made on the fetus.

The following day, the amniotic fluid was centrifuged for 10 min at 260 g, and the cells were resuspended in 6 ml medium composed of Eagle's MEM with Hank's salt solution supplemented with 20% fetal calf serum and glutamine. Penicillin and streptomycin were added as antibiotic agents. Four cell lines were started by division of the cell suspension into four Leighton tubes with cover slips. After the first trypsinisation, the cells were transferred to 30 ml plastic flasks (Nunc, Denmark) with a growth surface area of 25 cm². The living cells were examined with an inverted phase contrast microscope (Leitz-Diavert). Cultures were harvested for chromosome analysis after trypsinisation. The slides were stained with the conventional Giemsa technique and with the quinacrine fluorescence technique (Q-banding).

Results

Two cell populations, 46,XY and 45,X, were found in cultures from the amniotic fluid. Most of the cells had a normal karyotype but the 45,X chromosome constitution was found in cells from three of the four cell lines. The Y chromosome, when present, had a normal bright fluorescence. The cytogenetic findings are summarised in the Table. All the cells analysed from line 3, III passage had a Y chromosome. Thirty mitoses from cell line 1 were analysed in V passage; only one of these was 45,X. Cell line 4 was karyotyped in II, V, VI, and VII passage. The abnormal 45,X cells amounted to 8 of 70 cells analysed from passage II. During the following passages, the aberrant cell population decreased. One of 19 cells from V passage was lacking the Y chromosome. Sixty metaphases were analysed from passage VI and 95 metaphases from passage VII, but no cells with the aberration were found among them. The 45,X cells were most frequently found among cells from cell line 2, passage III, but here, too, the abnormal cell population was reduced after subcultivations, as only 2 of 69 cells in passage VII had this karyotype.

Chromosomal aberrations of different types appeared sporadically in all the lines. Thus, a new clone with the chromosome constitution 46,XY, -D, +G emerged in cell line 2 from the III to VI passage.

The proportion of polyploid cells in each passage is stated for about 200 cells when possible. Generally 0 to 3% of the cells showed polyploidy, but in two cultures the frequency was as high as 12%.

The morphology of the cells, epithelial-like or fibroblast-like, was scored before harvest of the cells. The term fibroblast-like includes cells with a real fibroblast-shape as well as AF-cells, as they have been defined by Hoehn *et al.* (1974). The epithelial-like cells predominated only in the very early stages of growth; after III passage the fibroblast-like cells overgrew the cultures.

Discussion

The presence of an aberrant cell clone in amniotic fluid cell cultures can be explained in several ways: (1) The anomaly arose *in vivo*, as a result of irregular mitoses in the fetus, in the amnion, or in the cells exfoliated to the amniotic fluid. (2) The anomaly arose *in vitro*, as a result of culture conditions. The latter hypothesis is excluded if the clone is observed in cultures from successive amniocenteses of the same pregnancy, or if the clone, as in the present case, is found in cultures from different cell lines, or in different primary cultures. With respect to the first hypothesis, the exfoliated cells do not seem to divide, as no mitoses were observed among uncultured cells prepared immediately after amniocentesis, and no cells were found to be labelled by autoradiographic examination after incubation for 4 h with ^3H thymidine (T. S. Clausen, 1978, unpublished data). Similarly, Hoehn *et al.* (1974) found no labelled cells among 2×10^{-3} cells examined after incubation for 24 h with ^3H thymidine and medium. So the cell division which gave rise to the abnormal clone would have taken place in the amnion or in the fetus itself, a fact which does not help us to make a correct chromosomal diagnosis of the child. In this connection, repeated amniocentesis is not expected to give any new information. This has been confirmed by the observations published so far, for example by Milunsky and Atkins (1976), who found only cells with a normal karyotype from the second amniocentesis, whereas a 46,XY/47,XXY constitution of cells from the first amniocentesis was also found in different tissues from the newborn infant. On the other hand, Sutherland *et al.* (1977) described a case of mosaicism in two successive amniocenteses, but fetal fibroblasts showed only normal karyotypes.

Cells belonging to a cell population with the abnormal karyotype 45,X were found in 3 of 4 cell lines from the present amniotic fluid specimen. As stated above, tissue from the fetus was not obtained,

Table Cytogenetic results from 4 cell lines originating from one amniotic fluid specimen

Cell line	Passage	Cell type*	46,XY	45,X	Other aberrations	Total	% polyploid	Total
1	V	FE	27	1	2	30	1	213
2	III	EF	126	51	0	177	2	200
	VI	F	6	0	1†	7	0	137
	VII	F	58	2	9†	69	1	168
3	III	EF	28	0	2	30	12	200
4	II	EF	59	8	3	70	12	100
	V	F	18	1	0	19	1	221
	VI	F	58	0	2	60	3	204
	VII	F	94	0	1	95	1	211

*E, epithelial-like; F, fibroblast-like. The predominating cell type is mentioned first.

†Cells with the karyotype 46,XY, -D, +G.

and the mosaic condition could not be verified. Mosaics with this karyotype have been described before in amniotic fluid cell cultures. Hsu *et al.* (1976) mentioned that 3 cases had been reported, including their own finding, where the anomaly was confirmed by culturing cells from the fetus. The mosaic conditions found at prenatal diagnosis generally include the same chromosome abnormalities seen frequently among liveborn infants, that is 45,X, monosomy G, and trisomy D and G. Therefore, discrimination between real mosaics and pseudomosaics is not possible from the type of aberration.

In the 2 cell lines where several passages were analysed, the 45,X cell population was considerably diminished after repeated subcultivations. As chromosome preparations used for prenatal diagnosis are made on primary cultures or after one subcultivation, little is known of the fate of the abnormal cells in the mosaic cases published so far. Sutherland *et al.* (1975) karyotyped 5 cases of mosaicism in both the primary culture and a subculture originating from it after trypsinisation. In 2 instances, the chromosome analysis revealed abnormal cells only in the primary cultures. In the present experiment, the disappearance of the cells with epithelial-like morphology paralleled the disappearance of the chromosome abnormality. The epithelial-like cells are supposed to originate from various parts of the fetus, for example, the skin, the respiratory tract, the urogenital tract, and the amnion. Cultures from the amnion are composed of epithelial-like cells with a short lifespan; cell lines from this tissue are difficult to establish (Boué *et al.*, 1975), and special culture conditions are required (Conover and Hirschhorn, 1971). The difficulty in establishing amnion cell cultures indicates that the origin of the fibroblast-like cells in amniotic fluid cell cultures is not the amnion, but the fetus itself, and consequently the chromosome constitution of these cells should be representative of the fetus.

A high incidence of polyploidy is frequently found in amniotic fluid cell cultures. The number of polyploid cells were recorded in all the cultures harvested for chromosome analysis; the results were ambiguous but the incidence tended towards a higher level in the first passages. A similar observation was made by Kohn and Robinson (1970), who found 13% polyploid metaphases after 20 days in culture, but no polyploid cells after 35 days. It is possible that this chromosome anomaly is also associated with the epithelial-like cells. Hahnemann (1974) found tetraploidy in up to 10% of cells from amnion cultures, but the frequency of polyploid cells in cultures from human fetal lung, skin, and kidney is at an equal

level, from 0.5 to 9% (Earley *et al.*, 1976). The origin of the polyploid cells in amniotic fluid cell cultures is thus unsolved.

If the epithelial-like cells show an unstable chromosome constitution, then it is inexpedient to prepare primary cultures for cytogenetic analysis, and the karyotyping would more usefully be carried out in a later passage on fibroblast-like cells. However, an extended culture time is unfortunate from a diagnostic point of view, and adds new problems to cytogenetic analysis, for instance, the selection of a clone or the emergence of new clones. Further investigations of the origin and the fate of the cell types found in the cultures will be necessary to clarify whether it is possible to discriminate between a real mosaic condition and pseudomosaicism.

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