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# Cytogenetic Diversity in Primary Human Tumors

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Cytogenetic patterns from primary short-term culture of breast cancer, renal carcinoma, and tumors of the central nervous system are presented to illustrate the range of karyotypic diversity of human solid tumors as well as their biologic differences in culture systems that support their growth. These studies have illustrated several major issues. 1) Results vary with the tissue of origin: primary cultures from breast are almost uniformly diploid, while renal tumors are near-diploid, mosaic, and show clonal aberrations; and CNS tumors are heterogeneous: some diploid, some near-diploid and some highly aneuploid. 2) Results after short-term culture are selective, representing subpopulations from the heterogeneous cells that are detected on direct analysis of fresh tumors by cytogenetics or flow cytometry (FCM). It is not yet clear whether prognosis depends on the dominant population of the primary tumor or alternatively should be influenced by detection of small aneuploid subpopulations. 3) Evidence from all three tumor types supports the interpretation that cytogenetically normal diploid cells constitute part of some tumor populations, and may be better adapted to routine growth in culture than aneuploid subpopulations from the same primary tumors. These cells may also compose a major portion of the viable population of tumors in vivo and, therefore, could represent a useful model for studies of tumorigenesis and therapeutic regimens.

**Key words: renal carcinomas, cytogenetic heterogeneity, flow cytometry, CNS tumors, breast tumors**

Human tumors display a wide range of karyotypic diversity. Many tumors are characterized by extensive numerical and structural chromosome aberrations. Some, however, show minimal deviation from the normal karyotype, and a few tumors appear to be largely represented by normal diploid cells. In the leukemias and lymphomas, chromosome aberrations are relatively uniform throughout a tumor cell population. In contrast, many human solid tumors display great intratumoral cytogenetic heterogeneity. Moreover, tumors of the same morphologic subtype may display a wide range of karyotypic deviations.

Recent studies in the leukemias and lymphomas have demonstrated relatively uniform and probably causal relationships between certain chromosome rearrange-

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ments and specific tumors. One of the best examples of such a relationship is the 8; 14 translocation (or t(2:8) or 8;22) found in cells of Hodgkin's lymphoma. At the molecular level these translocations result in transposition of the myc oncogene into a region adjacent to a functioning immunoglobulin locus. In lymphoid cell lineages, the functional result of the transposition is oncogene activation, which presumably is responsible for acquisition of tumor-relevant properties. These and other similar relationships between chromosome rearrangements and specific tumors are discussed by Dr. Carlo Croce [1]. Their demonstration is facilitated when tumors have a near-diploid chromosome constitution.

Similarly, the diploid and near-diploid populations of cells from primary solid human tumors could represent a desirable model for the study of early events in tumorigenesis and for rational approaches to therapy. The cellular models used for experimental approaches to tumor therapy often utilize well-established tumor cell cultures. Such cultures, frequently derived from advanced stages of tumor in vivo, often undergo further evolution in culture [2]. Thus, the cultured cells may differ greatly from the original primary tumor against which therapy should be targeted.

We will present examples from primary human tumors which illustrate different spectra of cytogenetic patterns in short-term culture, intrinsic biologic differences in their ability to adapt to growth in culture, and differing responses to selective culture systems. Some selective factors are probably responsible for the reduced karyotypic diversity seen in short-term tumor cultures in comparison to that found by direct cytogenetic analysis of the fresh tissues. These differences are amply demonstrated in studies of breast cancer [3–5]. Several important sources of variability for the DNA content and karyotypic patterns of human tumors are shown in Figure 1. The source of tumor reflects the state of tumor progression to some extent. Metastases, whether solid, in marrow, or from effusion fluids, represent more advanced stages of tumor development than the primary lesion. Needle aspirates, in contrast to the solid primary tumor mass, are likely to include cells with less adhesive and presumably less differentiated characteristics. For example, in an epithelial tumor, intercellular bridges, a sign of differentiation, are likely to result in adherent cell masses. From any of the sources in Figure 1, the fresh tumor is likely to consist of a mixed population of cells of differing viability, and of both tumor and non-tumor origin. Both the transport medium and the first processing of the tumor by disaggregation methods may select differentially between cells of greater and lesser viability. If the cells are not analyzed

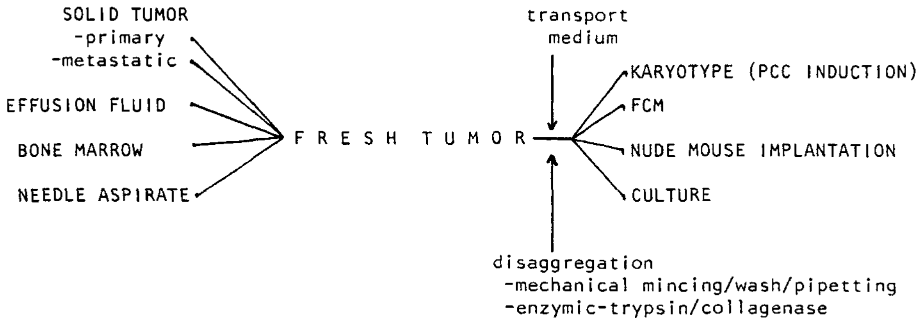


Fig. 1. Sources of fresh tumor are shown at left and processing techniques on the right. Premature chromosome condensation (PCC) is a method that permits visualization of chromosomes from interphase cells [18].

directly, either for karyotype or for DNA content by flow cytometry, then further selection among existing subpopulations will occur unobserved, as the tumor is processed either for tissue culture or for nude mouse implantation prior to study of DNA or karyotype. (Another modification which can be introduced is the induction through premature chromosome condensation of karyotypes from cells that were not dividing spontaneously within the tumor).

Culture of the tumor cell population prior to karyotypic study introduces a large number of new variables, many of which are intentionally selective or preferential for tumor cells. Table I lists a number of procedures that are commonly used either to deter the growth of normal cells or to enhance the concentration of tumor cells prior to culture. Table II presents modifications of the physical aspects of substrate, medium, or environment—all of which are potentially selective for specific subsets within a tumor cell population.

## BREAST CANCER

With these considerations as background, we will present results from primary cultures of three types of human solid tumors. The first set of data are derived from

**TABLE I. Pretreatment of Cells Prior to Culture**

| |
|---|
| Purposes |
| To deter fibroblast, monocyte or other normal cell contamination |
| To enhance concentration of tumor cells |
| Methods |
| Differential adhesion or response to trypsin |
| Passage in soft agar |
| Isolation by cloning (rings or wells) |
| Disaggregation; selection for single cells vs clumps |
| Ficoll or Percoll gradients |
| Elimination of special populations |
| Phagocytes by Fe uptake and magnets |
| Reaction with antibody-coated surfaces |

**TABLE II. Selection of Cell Type by Culture Conditions**

| |
|---|
| Monolayer vs suspension growth |
| Feeder layer |
| Cell source |
| Method of inactivation |
| Substrate modification |
| Laminin |
| Fibronectin |
| Collagen |
| Polylysine |
| Extracellular matrix |
| 3-D growth |
| Agar or collagen gel |
| Medium and environment |
| % oxygen |
| % serum |
| Conditioning of media by cultured cells |
| Supplements; growth factors, hormones, etc. |

breast cancers. Remarkably little cytogenetic data have been obtained from direct studies of human tumors or from cell cultures [6]. A recent review revealed fewer than 20 cases analyzed directly by banded methods and not all could be completely analyzed. Direct analyses of advanced breast cancer, mainly in the form of effusion fluids, also are few in number. Cell lines, almost all derived from effusion fluids, have shown cytogenetic instability and heterogeneity with unpredictable emergence of new clonal karyotypes. Lines that are widely used as models for therapeutic strategies, such as MCF-7, show great variability and marked karyotypic differences between cells grown in vivo and in vitro [2]. Until recently human breast tissue and tumors have been extraordinarily difficult to propagate in culture. Even now the growth of normal or tumor-derived breast epithelium is dependent on supplementation of culture media by hormones, growth factors, and other poorly defined additives, such as conditioned media from other cell culture sources. The cells we studied were grown by Dr. Helene Smith and co-workers [7,8] from fresh solid tumor after prolonged enzymic disaggregation, followed by removal of the single cell suspension and culture of the adherent cell masses termed "organoids." The cell masses attach and begin to divide in a medium supplemented with hormones, insulin, cholera toxin, and growth factors derived from existing cell cultures. Even under these conditions they are capable of very limited growth in culture. The cells are epithelial in morphology, form secretory domes, show junctional complexes, and have mammary milk-fat globule antigens. Chromosome analyses of 15 tumor-derived primary cultures in either first or second passage has yielded predominantly diploid cells [5]. Chromosomal banding at levels of 550 or higher resolution has not revealed structural aberrations. No clonal aberrations have been detected in the primary cultures although occasional nonclonal structural or numerical aberrations have been found. Morphologic, antigenic and functional markers indicative of breast cell origin are cited above but indicators of malignant characteristics are less decisive. Of the latter, the most convincing is the demonstration of invasion of amniotic membrane in vitro, a property not shared by breast cells derived from non-malignant sources [9]. Other evidence suggesting that the diploid cells represent subpopulations of tumor in approximately two-thirds of primary breast cancers has been reviewed [10].

## RENAL CARCINOMA

In contrast to the modifications necessary to culture breast cancer, some human tumors grow well in simple culture systems. A high proportion of renal cell carcinomas grow well on standard plastic surfaces with unmodified serum-supplemented media, after either mincing or collagenase dissociation of the fresh tumor material. The cultures have yielded chromosome harvests from over 2/3 of the samples received. Most of those which we were unable to culture were either extremely limited in sample volume or had reduced viability because of tumor infarction.

Within the first 2–3 weeks the cultured cells show a highly characteristic, relatively uniform morphology. They form a monolayer which is typically epithelial and pavement-like. Individual cells are commonly triangular in two-dimensional appearance, and have characteristic heavy granulation in the immediate perinuclear region. Electron microscopy of the fresh surgical specimen is similar to that of the cultured cells after 2–5 weeks. Cells from both sources share characteristics of extensive cytoplasmic vacuolization, circumferential papillation of the cytoplasmic

border, and dense reticulated chromatin patterns in the one or two nucleoli of each nucleus. Thus, there is morphologic evidence that the cultured cells are tumor-derived. The cytogenetic analyses of these cultures, presented in Table III and IV, are divided into cultures from which harvests were obtained in 2 weeks or less, and those with prolonged culture prior to chromosome analysis. The results do not differ qualitatively, but the frequency of clonally aberrant chromosome populations was higher when harvests were obtained relatively early. Three patterns are evident. Two cultures showed exclusively aberrant cytogenetic patterns, while a few cultures contained exclusively normal diploid cells. Among those that were diploid, several cases may be inappropriate for inclusion in the study; for example, cases 4959 and 4749 were reclassified as an adenoma and as an oncocytoma, respectively. The remaining cases included both a normal diploid population and a clonally aberrant population. Cases with aberrant populations were generally marked by chromosome losses and gains. Of these, seven contained clones missing the Y chromosome and seven, some overlapping, showed trisomy for chromosome 7. In cases 5151 and 5512, the clonal aberrations were structural. In summary , 10 of the 19 cultures showed clonal chromosome aberrations. These cultures, therefore, showed not only a high

**TABLE III. Cytogenetics of Cultured Renal Tumors With 1st Harvest $\leq$14 Days**

| Case no. | Harvest time (days) | No. metaphase cells analyzed | Karyotype |
|---|---|---|---|
| 4632 | 9 | 29 | 47,X,−Y,+7,+10/50,X,<br>−Y,+7,+10,+12,+16,+17 |
| 4667 | 8 | 6 | 46,XY |
| 4674 | 12 | 18 | 46,XY/46,X,−Y,+7/<br>43,XY,+7,+ others |
| 4823 | 12 | 30 | 46,XY/45,X,−Y/<br>46,X,−Y,+7/<br>47,X,−Y,+7,+12 |
| 4942 | 13 | 14 | 46,XX/47,XX,+7 |
| 4959 (adenoma) | 10 | 13 | 46,XX |
| 5151 | 10 | 13 | 46,XY/46,XY,16q− |
| 5188 | 10 | 25 | 46,XY/45,X,−Y |
| 5300 | 14 | 28 | 46,XY/45,X,−Y/<br>46,X,−Y,+7 |

**TABLE IV. Cytogenetics of Cultured Renal Tumors With Successful Harvest After 14 Days**

| Case no. | Harvest time | No. metaphase cells analyzed | Karyotype |
|---|---|---|---|
| 4635 | 2 months | 9 | 45,X,−Y |
| 4661 | 1 1/2 months | 8 | 46,XY |
| 4673 | 40 days | 10 | 46,XX |
| 4712 | 2 months | 10 | 46,XX |
| 4731 | 1 month + | 24 | 46,XY |
| 4749 | 33 and 38 days | 11 | 46,XX |
| 5111 | 21 days | 18 | 46,XX |
| 5199 | 19 days | 36 | 46,XY |
| 5310 | 20 days | 26 | 46,XY/45,X,−Y/?<br>45,X,−Y,+7,−20 |
| 5512 | 22 days | 18 | 46,XY/47,XY,+7/<br>48,XY,+7,+7 |

proportion of karyotypic abnormality but also considerable specificity for particular chromosomally aberrant clones. Recent work suggests that with modifications such as addition of growth factors, we can retrieve higher frequencies of chromosomally aberrant cells, particularly those with structural alterations.

What is not clear is whether the diploid cells are truly representative of tumor subsets or result from contamination of cultures by normal cell populations. This question is under investigation using cytochemical markers, but markers that clearly, reliably, and uniformly distinguish stromal or normal kidney from tumor-derived cells are not yet available to us. Recent results of direct analysis and short-term cultures from other laboratories have emphasized frequent rearrangement of chromosome 3 [11–13], an observation of great interest because of the family in which inheritance of a balanced translocation between chromosomes 3 and 8 was closely associated with the appearance of renal tumors [14]. Direct harvest also appears to yield a higher frequency of aberrant karyotypes [15].

We have attempted to pursue further the question of intratumoral heterogeneity in renal carcinoma by flow cytometry. The results, shown in Table V, appear to support the cytogenetic results based on cell culture. Most of the karyotypic abnormalities diagnosed would not be detectable by whole cell flow cytometry. The limits of resolution of the instrumentation used (Ortho Cytofluorograph 504 interfaced with a model 2150 computer) preclude detection of differences from the normal karyotype of less than 5 chromosomes (unpublished observations). In two instances, cases 4673 and 4674, flow cytometry of the fresh tumors showed both diploid and aneuploid peaks. The aneuploid cells in both cases were not recovered from culture. In case 4673, the fixed paraffin-embedded tissues were also analyzed by flow cytometry. The flow diagrams shown in Figure 2 are taken from two sections, one of which was almost exclusively tumor while the other was half tumor and half normal kidney. Despite the large differences in sampling, the flow diagrams did not differ substantially in the sizes of their diploid and aneuploid peaks. These results indicate the strong probability that a diploid subpopulation existed within the tumor.

To summarize the data obtained thus far, we have been able to culture cells from renal cell carcinomas with relative ease. We have defined clonal karyotypic abnormalities in more than 50% of the tumors analyzed, with apparent specificity for trisomy 7 and for loss of the Y chromosome. Although similarities of morphology

**TABLE V. Renal Tumor Study**

| Case no. | Karyotype | Flow cytometry |
|---|---|---|
| 4632 | 47,X,−Y,+7,+10/ 50,X,−Y,+7,+10, +12,+16,+17 | Diploid |
| 4635 | 45,X,−Y | Diploid |
| 4667 | 46,XY | Diploid |
| 4673 | 46,XY | Diploid, aneuploid |
| 4674 | 46,XY/46,X,−Y,+7 | Diploid, aneuploid |
| 4712 | 46,XX | Diploid |
| 4749 (oncocytoma) | 46,XX | Diploid |
| 4823 | 46,XY/45,X,−Y/47,X,−Y,+7,+12 | Diploid |
| 5512 | 46,XY/47,XY,+7/48,XY,+7,7 | Diploid |

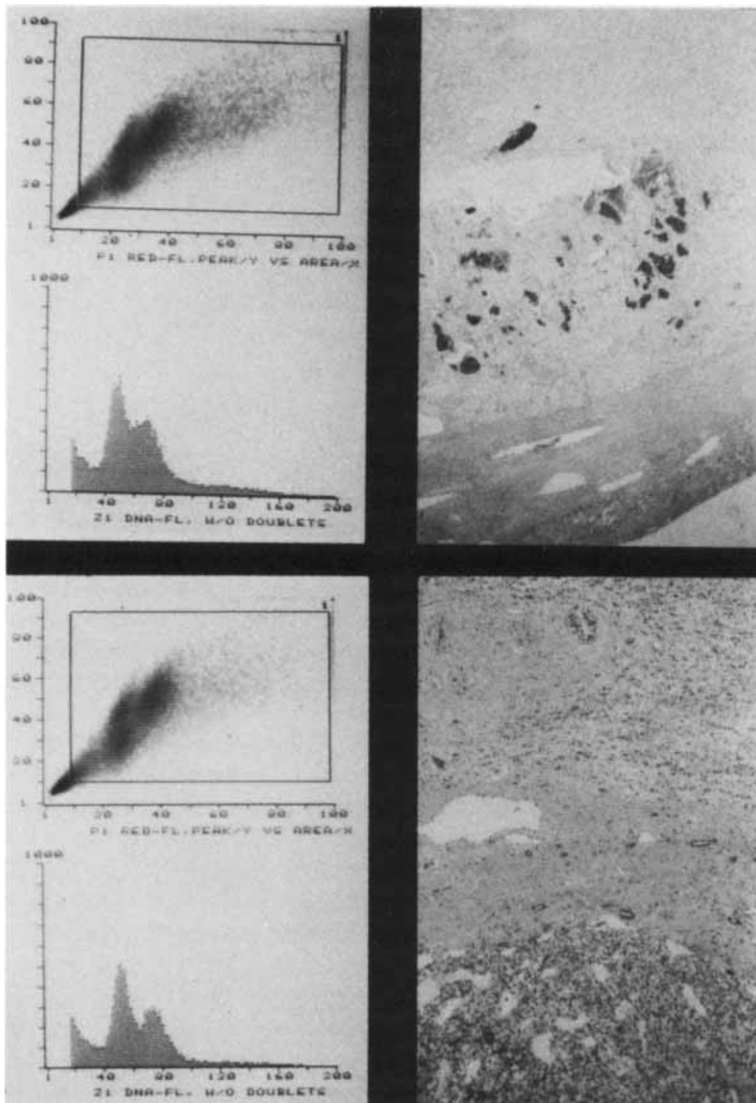

Fig. 2. On the left are the peak vs area scatter and flow histograms for two samples of the same renal carcinoma (case 4673). The paraffin blocks from which the flow samples were prepared are represented by the photomicrographs to the right of each. The histograms do not differ significantly, although the upper sample was more than 90% tumor, while the lower consisted of equal amounts of tumor and normal kidney.

suggest that the diploid cells in culture originate from tumor, we do not yet have clear proof that this is the case. In a few cases, it appears that aneuploid populations do not survive in this culture system.

## CENTRAL NERVOUS SYSTEM TUMORS

Tumors of the central nervous system, in contrast to the previous two tumor types, have been cultured with considerable frequency by other investigators [16,17].

The most consistent aberrations reported in glial tumors have been loss of a sex chromosome or trisomy 7, although many karyotypically normal cells were also found. Structural rearrangements and extensive chromosome aberrations were far more common in highly malignant tumors. Since normal brain tissue is nondividing and since the supporting stromal cells of the central nervous system can only be cultured with great difficulty, problems of potential contamination with normal cell populations are more remote. Cellular morphology in culture is also highly characteristic and aids in identification of the growing populations. In culture there is almost invariably a mixed population, including bipolar cells that are characteristic of glial origin admixed with stellate and pavement cells. We have successfully cultured 28 of 29 samples received. Of these, only 11 cytogenetic results are available at present. Table VI shows that some of these tumors have grown as pure diploid populations; others have shown loss of the second sex chromosome; some are a mixture of diploid and aneupolid cells. For example, case 5464 is aneuploid for a population that has lost the second sex chromosome while case 5674 is aneuploid for a population which is highly abnormal with multiple translocations. In case 5786 we have found only karyotypically abnormal cells in culture; one clone is pseudodiploid, while the other is markedly aneuploid. Thus, in this system where contamination by normal cells is unlikely, the results thus far, similar to those for renal carcinoma, include a number of cases that are diploid or show loss of a sex chromosome. The overall success rate of growth in culture is high in defined or modified culture media (additives such as zinc, etc).

**TABLE VI. Cytogenetics of Cultured CNS Tumors**

| Case no. | Tumor diagnosis | Karyotype |
|---|---|---|
| 5464 | Medulloblastoma | 46,XY/45,X,−Y |
| 5521 | Glioblastoma Gr.IV | 46,XX |
| 5536 | Astrocytoma Gr. III | 46,XX |
| 5556 | Oligo-dendroglioma/ ependymoma | 46,XY |
| 5583 | Glioma, astrocytoma Gr. IV | 46,X,−X |
| 5674 | Glioblastoma, astrocytoma Gr. IV | 46,XY/abnormal clone with multiple aberrations |
| 5786 | Regrowth medulloblastoma (?) | 46,X,?Y,+multiple aberrations/55,X,?Y,+ multiple X aberrations |
| 5889 | Low-grade astrocytoma (?) | 46,XX |
| 5901 | Pineablastoma | 46,XX |
| 5965 | Hemangioblastoma | 46,XX |
| 6070 | Medulloblastoma | 46,XY |

## CONCLUSIONS

These studies of different primary human tumors illustrate several major problems. First, results appear to vary depending upon the tissue of origin. Simple unspecialized culture systems will not support the growth of cells from primary breast cancers or indeed cells from normal breasts, while they will support the growth of a large fraction of tumors of renal origin and almost 100% of tumors from the central nervous system. Moreover, primary cultures from breast are composed almost uni-

formly of normal diploid cells, while those of renal and CNS tumors include diploid and near-diploid cells. Both appear to contain mosaic populations and both show clonal aberrations in near-diploid cells. Unmodified and nonspecialized culture media do not appear to support highly aneuploid populations from renal tumors. Special media are necessary for the growth of cells from breast cancers and improve success in the culture of cells from CNS tumors. There is additional evidence that short-term culture conditions are highly selective in that the heterogeneous or multiclonal populations that historically have been detected using direct cytogenetic techniques have not been found in our cell cultures from breast and renal tumors.

Flow studies on the same tumors in a few cases suggest that subpopulations of renal tumors are not growing in culture. Evidence from all tumor types suggests that cytogenetically normal diploid cells constitute part of some tumor populations. The evidence is stronger for cultures derived from breast and central nervous system tumors, while further work is necessary to support that interpretation for the renal carcinoma cultures. It is clear that diploid and near-diploid cells from all three types of tumor appear better adapted to routine growth in culture than do aneuploid subpopulations from the same primary tumors.

In some respects we are very much like the blind men attempting to describe an elephant with our tools limited to investigation of one part or another of the entire beast. It is important to remember that for many or most primary solid tumors of man, the tumor has encompassed 7/8 of its entire life span prior to clinical detection. Considerable evolution and development of diversity can have occurred during this period and our studies of solid tumors in culture suggest that this is indeed the case. It is likely in some human tumors that at least one subset of the tumor cell population is karyotypically diploid. These cells, less altered than the remainder of the tumor cell population, are less vulnerable to therapeutic attack and, therefore, better fitted to carry on and survive as the tumor stem line. If some tumor populations are indeed cytogenetically normal, what significance can we attribute to the clonal karyotypic abnormalities that appear to be specific to solid tumor subtypes? We suggest that the primary and important events in tumor initiation and progression most often occur at the level of the gene rather than the chromosome, but that these consistent and apparently specific chromosome aberrations may serve as signposts for genes that are critically oncogenic for particular cell lineages or stages of differentation. Since the pathways for significant genetic alteration may depend on the unique functioning portions of specialized cell types, it is reasonable to expect that the clonal cytogenetic aberrations that characterize specific tumor types will differ. It is also reasonable to expect that evolution within a primary tumor or its metastases will be associated with new biological attributes which may in turn depend upon chromosomal rearrangement, loss, or reduplication. Thus it is reasonable to expect considerable cytogenetic diversity within individual tumors as well among different tumors. These differences must be explored and described for individual tumor types before they can be exploited for tumor therapeutic efforts.

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