Commentary

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Are human cancers ever diploid—or often trisomic? Conflicting evidence from direct preparations and cultures

N.B. Atkin and M.C. Baker

Department of Cancer Research, Mount Vernon Hospital, Northwood, Middlesex (UK)

Is aneuploidy a *sine qua non* for human cancer? This question, which has long been debated, assumes a greater importance now that genomic changes in cancer cells have become a major focus of attention. However, problems of methodology have so far made it impossible to give a decisive answer. Ideally, the chromosomes of cancer cells that were in mitosis in vivo should be studied. However, dividing cells are often scanty in solid tumors, and, moreover, it is difficult to obtain good chromosome preparations from those that are present. Recourse has, therefore, often been made to culture methods to increase the number of analyzable metaphases.

We wish to point out some discrepancies in the results of chromosome analyses using direct preparations, as compared with culture techniques. We are concerned here only with malignant solid tumors, i.e., those that show evidence of ability to invade surrounding tissues and/or metastasize; it is not our purpose to consider the point in the development of tumors when clonal chromosome anomalies first occur, although there is, of course, abundant evidence that this takes place often before the stage of malignancy is reached. Neither are we concerned with leukemias, which, although once considered to be quite frequently diploid, have, with improvements in technique (Yunis, 1984; Testa et al., 1985), increasingly been shown to be aneuploid. Nor, finally, are we concerned with animal tumors, for which there have been several reports of malignant tumors with apparently diploid karyotypes (reviewed by Oshimura and Barrett, 1985).

Direct preparations of solid malignant tumors. Clonal chromosome abnormalities, usually with several numerical and/or structural changes, are the rule. Although a small number of diploid metaphases, presumed to be normal reactive cells and usually distinguishable by their good chromosome morphology, are commonly present, we are unaware of any studies using chromosome-banding techniques in which diploid metaphases are in the majority or otherwise strongly suspected of being tumor cells. From the several hundred tumors, including our own unpublished cases, that have now been studied, we can postulate that diploid human cancers, if they exist, must constitute significantly less than 1% of all cancers (with the proviso, of course, that relatively little is known about the chromosomes of the technically less favorable tumor types, such as skin carcinomas, which might, therefore, have a higher incidence of diploidy).

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Request reprints from Dr. N.B. Atkin, Department of Cancer Research, Mount Vernon Hospital, Northwood, Middlesex HA6 2RN (UK).

Chromosome studies on cells in culture. In striking contrast to the consistent findings of clonal chromosome abnormalities in direct preparations of malignant tumors, there have been a number of studies in which culturing of tumor cells for a week or longer resulted exclusively or predominantly in the growth of diploid cells, suggesting that the tumors themselves were diploid. Thus, the vast majority of cells in cultures of 15 primary breast carcinomas studied by Wolman et al. (1985) were diploid. The metaphases were from first- (after approximately 1 wk in culture) or second-passage cultures; similar diploid cultures of breast carcinomas were found to "retain their capacity for invasiveness" in an in vitro assay (Smith et al., 1985), suggesting that the cells were indeed malignant. Diploid metaphases also predominated in a more recent study on 40 breast carcinomas (Zhang et al., 1989). (However, about 75% of breast carcinomas are associated with desmoplasia, defined as excessive connective tissue stroma [Meissner and Warren, 1971], and it would not be surprising, perhaps, if the connective tissue elements in these tumors showed preferential growth in culture.) Gibas et al. (1985), discussing their own finding and that of another group (Jellinghaus et al., 1976) of only normal karyotypes in cultures of prostatic tumors, suggested, among possible interpretations, that the dividing cells were nonmalignant or that some primary prostatic tumors had not undergone any gross chromosomal change. (In contrast, in five consecutive prostatic tumors, including four carcinomas and one sarcoma, we were able to demonstrate clonal aneuploidy [Atkin and Baker, 1985].) Bullerdiek et al. (1985) compared direct preparations with cell cultures (mostly of 1–3 wk duration) from 29 carcinomas; although nearly all the cultures were diploid, all the direct preparations were aneuploid.

In studies of benign salivary gland tumors, Mark et al. (1988) found that the percentage of tumors yielding diploid cell lines varied with the culture technique (these authors favored the view, however, that the diploid cells were neoplastic).

Recent findings (Oshimura et al., 1989) on tumors induced after transfection of normal diploid hamster embryo cells with v-Ha-*ras* and v-*myc* oncogenes are relevant to the question of the identity of diploid metaphases in tumor cell cultures. Direct studies of the tumors induced in *nude* mice revealed only aneuploidy, but, when cultured, the tumors showed a highly variable proportion (2–94%) of diploid metaphases at the first passage. That the latter were normal cells was suggested by the morphology of the colonies in direct chromosome preparations made in situ.

Trisomic cell lines in cultures of tumors and normal tissues. Although it may be uncertain whether the cells that proliferate in a

Table I. Trisomies in cultures of tumor and normal tissue from cancer patients

Material	Duration of culture or number of subcultures	Type of growth	Chromosome findings	Reference
Six cystadenomas of ovary with evidence of early malignant change	7–12 days	Epithelial (?)	Trisomy 10 in 5/6	Knoerr-Gaertner et al. (1977)
Three cervical carcinomas	Six subcultures	Fibroblastic	Trisomy 7 in 75% of metaphases in one carcinoma (other two were diploid)	Lin et al. (1973)
Adenosquamous carcinoma of lung	10 days	?	Trisomy 12 in 5/25 metaphases	Liang et al. (1986)
Non-small cell lung cancers (10 patients) and normal lung samples (8/10 patients)	13 days	Apparently epithelial	Various trisomies, particularly trisomy 7, in tumor and normal samples from four patients and in tumor samples only from two patients	Lee et al. (1987)

given culture are normal or malignant, the finding of aneuploidy is commonly taken as grounds for assuming that the cells are neoplastic. However, even this might be called into question, unless the cells in culture can be compared with those in direct preparations of the tumor (a comparison that has infrequently been made). Certainly, such comparisons are desirable to eliminate the possibility that at least some of the changes have occurred in culture. We have noted several examples in the literature of cultures of malignant tumor tissue in which the only change was a single trisomy. Four are shown in Table I. Since a trisomy, as the only change, is rather unusual in malignancies, although trisomies are more common in premalignant lesions, it seems that these might indeed have arisen from preinvasive neoplastic cells still present in the tumor or that they might be examples of nonmalignant cells in which a chromosome change has occurred in vitro (or, speculatively, in vivo in the abnormal environment provided by the neoplastic cell population). In a study of lung cancers, Lee et al. (1987) suggested that trisomy 7 was an early change in lung cancer development, having found it in cultures derived from both tumor and normal lung tissue in four patients. This could also be true of trisomy 10 in the cultures of "borderline" ovarian carcinomas studied by Knoerr-Gaertner et al. (1977).

However, trisomic clones may also arise in cultures of cells from individuals without cancer. Nichols et al. (1987) found trisomy 11, as well as a deletion of 13q, in a culture of endothelial cells from the abdominal aorta. The latter observation clearly shows that caution should be exercised in the interpretation of chromosome changes in cultures derived from tumor tissue.

Discussion. Studies on uncultured tumor material support the view that human cancers are always aneuploid. The chromosome changes are frequently complex and, moreover, show considerable variability, even among histologically similar tumors from the same site. Part of this variability seems to stem from the finding that, while a particular chromosome may undergo nonrandom structural changes, the precise change (including the breakpoints in translocations and deletions) varies from tumor to tumor; moreover, in some tumors no change is seen (where the relevant genomic change has been brought about by an "invisible" event, such as a point mutation or somatic recombination?). Given that two or more genomic changes are essential for malignancy, could it happen that, in some tumors, all of these changes are brought about by "invisible" events, so that there is no change in the karyotype? The absence of diploid cancers would then suggest that aneuploidy per se has some particular significance for malignancy.

The question of whether cancers are ever diploid is thus far from academic. That aneuploidy plays an essential role was suggested some 15 years ago (Ohno, 1974) and reiterated more recently (Duesberg, 1987). The precise nature of this role must await further study at the molecular and chromosomal levels.

In regard to the latter, it clearly will be necessary to assemble comprehensive data on a wide range of tumors. This will necessitate considerable improvements in the techniques used in their study. Because of the increased numbers of morphologically superior metaphases available, culture technique will no doubt continue to be used (for a recent review of the technical aspects of tumor cell culture, see Teyssier, 1989). While, as already mentioned, there may be little doubt that where a particular chromosome anomaly is seen in a series of tumors, this anomaly was indeed present in vivo, there must always be the suspicion that this is not the whole picture—that, for instance, some of the other changes seen in the cultures have occurred in vitro. Obviously, care should be taken to minimize any technical deficiencies that might promote chromosomal changes, such as contamination by mycoplasma (Stanbridge et al., 1969; Romano et al., 1970), which is difficult both to detect and to eradicate (Hay et al., 1989). In addition, we suggest the following guidelines:

1. Both direct and culture techniques should be used wherever possible on the material. In many cases, the results of the direct procedure will provide assurance that cells with the same karyotypes are dividing in culture, and while, for instance, it may be concluded that the same rearranged chromosome is present in both direct and culture preparations, the latter may provide the better resolution and, therefore, enable more precise assignment of the breakpoints.

2. At least two cultures separated by a reasonable time interval should be studied. Such serial studies should indicate the degree of stability of the cell population and may show or suggest that some of the changes have occurred in vitro.

3. If the direct chromosome preparations are unsatisfactory, use should be made, whenever possible, of in situ hybridization probes (Devilee et al., 1988; Hopman et al., 1988) that could verify that the expected numbers of particular chromosomes (normal and/or derivative) are present in interphase tumor cells. Although only a few such probes are now available, the number should in-

crease in the near future. DNA measurements, although of limited use, may indicate whether there are wide discrepancies between the direct and culture material.

4. Particularly where they are diploid or near-diploid, the nature of the cells that are dividing in culture should be determined, e.g., by using monoclonal antibodies (Berger and Flandrin, 1984).

5. Culture techniques that allow verification that a particular clone, if represented on more than one coverslip preparation, was probably present in vivo (Jin et al., 1988) should be used wherever possible.

6. Authors should state whether other tumors from the same site or of similar histopathology were also investigated, and whether they yielded any results.

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