Zeitschrift: Bulletin der Schweizerischen Akademie der Medizinischen Wissenschaften = Bulletin de l'Académie suisse des sciences médicales = Bollettino dell' Accademia svizzera delle scienze mediche

Herausgeber: Schweizerische Akademie der Medizinischen Wissenschaften

Band: 34 (1978)

Artikel: Genetic analysis of cell malignancy : evidence from somatic cell genetics

Autor: Klinger, Harold P.

DOI: https://doi.org/10.5169/seals-308167

Bull. Schweiz. Akad. Med. Wiss. 34, 377-388 (1978)

Department of Genetics, Albert Einstein College of Medicine Bronx, N.Y.

GENETIC ANALYSIS OF CELL MALIGNANCY - EVIDENCE FROM SOMATIC CELL GENETICS*

HAROLD P. KLINGER

Summary

When normal nontumorigenic cells are fused with tumorigenic cells some of the resulting hybrids are nontumorigenic in respect to their ability to grow in immune-deficient nude mice. Comparison of the chromosome content of nontumorigenic with tumorigenic hybrids, as well as with the cells of tumors which develop from the latter, reveals that in normal human x tumorigenic Chinese hamster hybrid crosses, two specific human chromosomes of the nontumorigenic parental line are very likely responsible for the suppressive effect. In some other hybrid crosses these and additional human chromosomes also seem to cause suppression. These findings suggest that a tumorigenic cell has lost at least two and possibly more gene functions which determine normal growth responses. The chromosomes from a normal cell can apparently correct these defects, although it is not yet clear if this is true genetic complementation or due to introduction of other genes which control cell growth or a cell's response to environmental growth regulatory stimuli. These findings led support to the view that genetic alterations are important in the process of malignant transformation and allow the development of a working hypothesis for the possible mechanisms involved. Some findings of other workers in this area also suggest that malignant cells may be producing some cell membrane proteins which are different from those of nontumorigenic cells. If this turns out to be true then it may ultimately be possible to develop immunotherapeutic procedures, i.e., to produce tumor cell specific antisera.

* Supported by NIH grant Nos. CA-16720, GM-19100 and GM 07001.

Zusammenfassung

Wenn normale gutartige mit malignen Zellen zur Fusion gebracht werden, so besitzt ein Teil der daraus resultierenden Hybridzellen nicht die Fähigkeit, in der immundefekten "nude mouse" zu Tumoren auswachsen zu können. Der systematische Vergleich der Chromosomensätze von solchen Hybridzellen mit und ohne diese Fähigkeit zum Tumorwachstum sowie auch von Zellen aus bereits entstandenen Tumoren ergab, dass zwei spezifische menschliche Chromosomen für den die Malignität unterdrückenden Effekt verantwortlich zu sein scheinen. Diese Aussage bezieht sich vorerst auf Hybridzellen, die aus der Fusion von normalen menschlichen und tumorerzeugenden Zellen des chinesischen Hamsters resultieren. In weiteren solchen Hybridzellen scheinen die gleichen sowie zusätzliche andere menschliche Chromosomen an der Unterdrückung des Tumorwachstums beteiligt zu sein. Diese Beobachtungen deuten darauf hin, dass entartete Zellen mindestens zwei, wenn nicht mehrere Genfunktionen verloren haben, welche das normale Zellwachstum kontrollieren. Chromosomen von normalen Zellen können diesen Verlust korrigieren, wobei allerdings noch nicht geklärt ist, ob es sich dabei um eine echte genetische Komplementation oder aber um die Einführung von anderen Genen handelt, welche ebenfalls direkt das Zellwachstum bestimmen oder aber für den Kontakt der Zelle mit exogenen, das Wachstum regulierenden Faktoren mitverantwortlich sind. Diese Beobachtungen stützen die Annahme, dass den genetischen Veränderungen eine zentrale Bedeutung beim Prozess der malignen Transformation zukommt und ermöglichen die Entwicklung von neuen Arbeitshypothesen über die bei der Transformation involvierten Mechanismen. Beobachtungen anderer Forscher deuten darauf hin, dass die malignen Zellen gewisse Zellmembranproteine produzieren, welche sich von solchen, die von nicht tumorigenen Zellen produziert werden, unterscheiden. Wenn sich diese Beobachtung bestätigen lässt, so besteht die Möglichkeit, die Krebskrankheit mit immunotherapeutischen Methoden, z.B. mit tumorzellspezifischen Antiseren anzugehen.

Introduction

A long-standing debate has revolved around the question of whether the primary alteration which occurs when a normal cell becomes malignant is a genetic or nongenetic one. Of those who favor the genetic hypotheses some propose that cell malignancy behaves like a mendelian dominant characteristic whereas others believe that it behaves like a recessive trait. It will be the main objective of this review to show that although both these views may be correct to some degree they are probably oversimplistic. Because of the broad scope of this problem the discussion will be limited to information which has been obtained in the

last few years, and primarily to results from studies in somatic cell genetics. More general reviews can be found elsewhere (GERMAN, 1974; MULVIHILL, et al., 1977).

Experimental Approaches

The question of whether the malignant cell phenotype behaves like a dominant or recessive trait was approached by EPHRUSSI and collaborators who fused malignant with nonmalignant cells and found that all of these hybrids produced tumors when introduced into animals (EPHRUSSI, 1972). They thus concluded that malignancy behaved like a dominant trait because they presumed that the chromosomes of the nonmalignant parental cell were retained in the hybrid and yet failed to affect the tumorigenicity of the malignant partner. However, since they did not identify all the chromosomes of the hybrids before injection, nor of the resulting tumor cells, their interpretation was probably incorrect in the light of more recent findings. HARRIS and collaborators in fact found that hybrids between nontumorigenic and tumorigenic mouse cells which retain many chromosomes of the nontumorigenic partner were suppressed in their tumorigenic potential whereas those which lost chromosomes either prior to injection into animals, or while in the animal, did develop into tumors. Consequently, they concluded that the chromosomes of a diploid cell carry genes which suppress tumorigenicity and these behave in a dominant manner, implying that the genetic change in malignant cells is of a recessive nature (WIENER, et al., 1971; HARRIS, 1971; WIENER, et al., 1974; JONASSON, et al., 1977).

STANBRIDGE (1976) extended this work to intraspecific human cell hybrids and showed that tumorigenic cells derived from an established HeLa cell line were completely suppressed in their tumorigenic potential when fused with nontumorigenic diploid human cells. These intraspecific hybrids retained most, but generally not all of the chromosomes of both parental lines. Fusions between two different tumorigenic human cell lines did not result in suppressed hybrids. Independently, we obtained very similar results with an almost identical hybrid system (KLINGER, et al., 1978). Since none of Stanbridge's or our own original diploid human x heteroploid tumorigenic human cell hybrids were able to give rise to tumors it was not clear if the chromosomes of the diploid human parent are in fact responsible for the suppression or whether the loss of chromosomes or other information from the tumorigenic parent is occurring in the process of hybridization and this is why tumorigenicity disappears. Consequently, we forced elimination of chromosomes from the hybrids by chemical back selection and other procedures and found that some of these were now tumorigenic and the tumorigenic cells had in fact lost a sizeable number of chromosomes, many of them from

the nontumorigenic parental line (KLINGER and EUN, in press). This clearly demonstrates that the potential for tumorigenicity was maintained by the cells and that chromosomes of the diploid parent are very likely responsible for the suppression. If this is true then we have to assume that only certain chromosomes of the diploid carry suppressive information since not all were absent in those hybrids which could grow as tumors and not all were present in all the suppressed hybrids. This is difficult to demonstrate clearly in these intraspecific human hybrids because the parental origin of only some of the chromosomes can be determined on the basis of chromosome morphology or biochemical markers. Consequently we turned to systems of diploid human x tumorigenic heteroploid rodent (mouse and Chinese hamster) cell hybrids where the parenfal chromosomes can clearly be identified both on the basis of distinct chromosome banding patterns and biochemical chromosome markers. These studies are not yet complete but several points are clear (KLINGER, et al., 1978; KLINGER and EUN, in press):

1. Hybrids can be obtained in all these crosses which are nontumorigenic or suppressed. (The term "tumorigenic", when referring to our own work, will be used only to designate the ability of cells to grow as tumors in immunedeficient nude mice.)
2. Suppressed hybrids in general contain more human chromosomes than those which are not, but there are many exceptions, some of which are very informative, as will be discussed below.
3. No human chromosome in single copy appears to be able to affect the tumorigenicity of the rodent lines. This is also true for 240 of the 276 possible combinations of different chromosomes taken two at a time. However, in the suppressed hybrids certain combinations of human chromosomes appear which are rarely found in tumorigenic hybrids and these combinations are never found in the cells of tumors resulting from those rare hybrids which contain them. Although the body of data is not yet extensive enough to allow statistically significant correlations to be obtained for all the possible interactions of two or more chromosomes, some very conspicuous trends have emerged. Human chromosome Nos. 9 and 11 seem to impart suppression in human x hamster crosses, and in addition the No. 8, in combination with one or both of the former, acts as a suppressor in human x mouse crosses. Also, in the human x hamster crosses Nos. 13 and 17 in combinations with 9 and 11, and each other, can serve as alternate suppressor combinations (KLINGER, et al., 1978, and in preparation).
4. Human x Chinese hamster hybrids almost always retain the human No. 6 (KLINGER and EUN, 1978). Those which do not retain a suppressor chromosome combination but retain

the No. 6 are more tumorigenic than the parental hamster line in that they require fewer cells and less time to produce tumors. This also appears to be true in human x mouse (RAG line) hybrids but is less pronounced in the human x mouse A9 cell line crosses, possibly because the latter is already highly tumorigenic (FREEDMAN and SHIN, 1974).

5. Examination of the nontumorigenic and tumorigenic hybrids, as well as of resulting tumor cells for phenotypes which have been implicated as being specifically associated with tumorigenicity reveal that none correlate completely with the ability of the cells to form tumors and in fact seem to segregate independently from the tumorigenic phenotype and each other. These phenotypes are: (1) decreased contact inhibition (density-independent growth); (2) anchorage independence (ability to grow in semi-solid methylcellulose supplemented medium); (3) decreased serum requirement (ability to grow in medium with low serum concentration); and (4) ability to produce fibronectin which has also been misnamed large external transformation sensitive (LETS) protein.

Two of these phenotypes do correlate completely with tumorigenicity but in one direction only. All tumorigenic cells have decreased contact inhibition and are anchorage independent but some cells which are nontumorigenic also exhibit these phenotypes. STRAUSS, et al. (1976) and STANBRIDGE and WILKINSON (1978) also found independent segregation of what were formerly believed to be tumorigenicity related phenotypes from the tumorigenic one in cell hybrids.

Conclusions and Synthesis

The combined results of the somatic cell studies indicate that chromosomes of a nontumorigenic cell can impart to a tumorigenic one information which suppresses the tumorigenicity of the latter. Although there has been some evidence for a possible contribution of nonchromosomal components to this effect (JONASSON and HARRIS, 1977) there has also been evidence against cytoplasmic factors being involved (HOWELL and SAGER, 1978). Our observation of strong associations between specific human chromosome combinations and suppression do not formally exclude nonchromosomal factors. However, if nonchromosomal factors are involved they would have to consistently cosegregate with the implicated chromosomes. This seems very improbable.

At present no single mouse or human chromosome alone appears to carry information adequate for suppression, at least not in those systems which have been tested to date. (See also AVILES, et al., 1977). That at least two, and in some crosses possibly more chromosomes are

required, suggests a multigenic effect. This would be compatible with the suggestion which has been made that a cell must suffer at least two genetic defects to achieve the tumorigenic state (KNUDSON, et al., 1973). However, our observation that some of the same human chromosomes act as suppressors of established cell lines of completely different origin makes it seem unlikely that suppression is due to genetic complementation of defective genes in the classical sense since this would require the unlikely assumption that all the cells of these different strains have achieved the tumorigenic state because of mutations of similar (homologous) genes. As KLEIN (1976) has suggested, it seems more likely that the suppressive effect is due to the introduction via specific chromosomes of elements that have become nonfunctional in the tumorigenic cell. These elements, which may be structural or regulatory genes, or both, would provide a cell with normal responsiveness to its environment. When the segregation of chromosomes in hybrids occurs spontaneously, or is fostered by selection procedures, then the tumorigenic phenotype reappears, clearly demonstrating that the cell's tumorigenic potential has not been lost permanently and again providing support for the view that chromosomal genes regulate the suppressive phenomenon. Thus the mutations which cause the change to the tumorigenic state are possibly recessive but they could also be deletions or regulatory alterations and the added information in the hybrids is not acting like a dominant in the classic sense. It is for this reason that it would probably be better to discard the concept of dominant versus recessive gene action in tumorigenic cells until the precise mechanisms are understood.

As noted, we have some evidence which suggests that some human chromosomes may have an effect opposite to those which are suppressors. These may carry information for what have been called household cell functions and may thus enhance a cell's ability to grow. However our evidence for such "tumorigenicity promoter" chromosomes, or genes, is as yet less rigorous than that for suppressors.

From the foregoing considerations and the knowledge gained from other related studies one can construct a genetic hypothesis of malignancy. This hypothesis assumes that a cell suffers a series of consecutive gene mutations either spontaneously, or induced by environmental agents such as chemicals, radiation, viruses, etc. When mutations of genes which regulate cell growth take place then the cell gains a selective growth advantage but it is not yet a tumorigenic cell. Loss of normal contact inhibition or anchorage dependence might be examples of loss of such growth regulatory functions. But it is clear that such changes alone are not enough to make a cell tumorigenic since there are examples of such cells which will not form tumors (FREEDMAN and SHIN, 1977; KLINGER, et al., 1978; STANBRIDGE and

WILKINSON, 1978). Ability to grow at low serum concentrations is another phenotype often associated with tumorigenicity, as are a host of other phenotypes, yet none of these are expressed by all tumorigenic cells. This indicates that although such phenotypes may initially impart to a cell a selective growth advantage, they are clearly not the factors which are responsible for the ability of a cell to grow into a tumor.

There is another class of mutations whereby the cell loses genetic information for a function essential for the differentiation of the organisms as a whole but not for the cell. Here again the loss of such differentiated function might give the cell a selective advantage but in itself not make it tumorigenic. Fibronectin production may be an example here. A cell which loses the ability to produce fibronectin can perhaps better utilize its metabolic machinery for producing other proteins which are more important for its own growth. This may explain why many tumorigenic cells are nonproducers. Clearly, however, the loss of this phenotype is not essential for tumorigenicity since as we have shown, some tumor cells derived from either established cell lines, or hybrids, are very good fibronectin producers (KLINGER and EUN, in press).

Thus a cell which has suffered one or more mutations may gain a selective advantage and proliferate faster than its fellows but it may not yet be able to produce an invasive tumor. However, the selective growth advantage, by increasing the size of the mutant cell population raises the probability that a cell of this clone will accummulate another critical mutation which would result in the loss of yet another phenotype directly or indirectly restrictive to growth. The affected cell or cells of this subclone may now be able to proliferate more effectively, i.e., invasively (not necessarily faster, since some tumors in fact grow quite slowly) possibly because it no longer responds to growth regulatory systems normally present in all organisms.

Aside from the arguments just presented it seems very unlikely that one mutational event would be adequate in most cases to cause transformation because of a simple and plausible consideration. If one mutation were enough and we assume a modest gene mutation rate of 10^{-7} per cell generation, then hardly any of us would survive much beyond birth without developing a tumor. Proponents of the immune surveillance hypothesis will argue that potentially malignant cells may in fact be arising frequently but are eliminated. However, the observation that immune-deficient nude mice have no greater incidence of spontaneous tumors than other strains, and many other considerations, make it doubtful if immune defense is the primary protective system. It may simply be one of the ways the organism rids itself of some potentially malignant cells. (See KLEIN, 1975, 1976; KLEIN and KLEIN, 1977, for a

more extensive discussion of this area.) Even if this system is important it would not be incompatible with the proposed hypothesis since one of the mutational events a cell may have to undergo to be able to grow progressively is a loss of those antigenic properties which would allow it to escape the immune system.

Thus cellular transformation to the malignant state can be visualized as a series of two, and more likely many mutational events, most of which would result in the loss of expression of a series of phenotypes and this would give them a selective growth advantage. These need not be the same types of changes in cells of different tumors since one can easily envisage many different changes, like those discussed earlier, which could confer such an advantage. This possibility of different phenotypic changes leading stepwise to the malignant state would fit well with the observations and suggestion made by ROWLEY (1974), that each class of mutagenic agents produce a different pattern of chromosome abnormalities (and hence genetic changes) within cells of susceptible tissues leading to different tissue specific tumors.

Most mutations in a diploid cell of the type discussed earlier would be expected to be heterozygously recessive and therefore not expressed. OHNO (1974) has suggested plausible mechanisms whereby a cell by undergoing abnormal chromosome segregations can achieve hemizygosity and hence express recessive genes. In fact it is perhaps a single gene or an initial chromosome mutation which, by interfering with the process of chromosome segregation is one of the postulated mutational events required for malignant transformation. Since chromosome replication and segregation are complex processes requiring many different physiologic changes and the formation of complex cell structures, they must be under the influence of many genes, the mutation of any of which might well result in abnormalities of replication, somatic crossing-over or segregation. The increasing number of tumor type specific chromosome alterations which are being discovered in human neoplasias of the hematopoetic system (ROWLEY, 1975) and other human and animal cancers (MITTLEMAN and LEVAN, 1976; LEVAN, et al., 1977), some even specific to small chromosome segments (SUGIYAMA, et al., 1969; MITTELMAN, et al., 1972; ROWLEY, 1977), make it seem ever more likely that chromosome mutations are in fact important in the origin of at least some malignancies. In this respect it is interesting to note that human chromosome Nos. 8, 9, 11, 13 and 17 which our work implicates as carrying tumorigenicity suppressive information are frequently found to be altered numerically or structurally in human neoplasias. This correlation may be coincidental but this seems unlikely when it is considered that five different chromosomes are involved. It is also interesting that, as ROWLEY (1977) has pointed out, many of these chromosomes carry genes related to carbohydrate or nucleic acid metabolism.

Sachs and collaborators proposed that it is the balance between two types of chromosomes that determine the expression of malignancy or its suppression (YAMAMOTO, et al., 1973; AZUMI and SACHS, 1977). This would fit well with our own findings and the hypothesis proposed here. The chromosomes which they found had to be lost for tumorigenicity to appear could be those carrying suppressor information, and the ones they believe have to be retained, or in some cases duplicated, might be promoters analogous to the human No. 6 which appears to foster the tumorigenicity in our human x Chinese hamster hybrids. Here again we have an indication that the tumorigenic cell has undergone several genetic alterations and that it may be operating under a complex system of genetically determined cell regulatory mechanisms which cannot readily be fit into a simple dominant versus recessive concept. CROCE and KOPROWSKI (1974, 1975; KOPROWSKI and CROCE, 1977) are not in agreement with this view since they believe to have demonstrated positive (dominant) control of the transformed phenotype by showing that human chromosomes Nos. 7 or 17, of an SV40 transformed human cell, when transferred by cell fusion to a diploid mouse cell, causes the latter to transform and become tumorigenic. The SV40 genome integrates into chromosomes 7 and 17. Although their interpretation is a likely one their findings do not exclude mechanisms other than positive genetic control of malignancy because not enough is known about what happens when the viral genome integrates in a chromosome. Perhaps it inactivates growth regulatory genes of the host cell at the integration site (Nos. 7 and 17 are frequently abnormal in human neoplastic cells) or at other sites via its gene products. It is also possible that the viral genome is causing transformation of the host cell by mechanisms not yet understood. The aneuploid karyotypes KOPROWSKI and CROCE (1977) find in the transformed initially diploid mouse cell is compatible with the latter interpretation. It is also important to stress the fact that different mechanisms may well be operating in different types of transformed or malignant cells. Possibly the mechanism in viral transformation is in fact positive, i.e., like a dominant one, whereas in other cases, particularly where the genome of a virus is not involved, mechanisms like those outlined earlier are operating (KLEIN and KLEIN, 1977). The work of GATEFF (1978) further demonstrates the possible diversity of genetic mechanism responsible for neoplasia. She demonstrated that in Drosophila recessive lethal mutations of single genes regulating development can result in tissue-specific malignant transformation. Thus we have evidence which suggests that in some cases a single gene mutation may be enough to cause malignant transformation. However, this may only apply to insects and these specific cell types. Also if we want to be rigorous in our analysis of this date we must consider the possibility that the identified mutations are not the only ones which the cells

which form tumors have undergone. Possibly the initial mutation predisposes the cell to further ones or, more likely, is one of the crucial ones for expressing the malignant phenotype similar to the situation postulated by KNUDSON, et al., (1973) for familial retinoblastoma and other hereditary malignancies. Thus any cell suffering a subsequent mutation at another tumorigenicity related locus may give rise to a tumor along the lines postulated earlier. The chromosomal instability which GATEFF observed in the tumor cells hints at the possibility that these cells carry several mutations. Her finding that the tumorigenicity related genes are recessive mutations of developmentally important genes is in good agreement with our working hypothesis.

Future Prospects

Although we have a considerable body of circumstantial evidence to support a genetic hypothesis we are still unable to define the specific genetic changes nor the precise mechanisms which are responsible for the tumorigenic state. BRAMWELL and HARRIS (1978) have attempted to identify one such malignancy associated mechanism. By comparing the cell membrane proteins of tumor cells with those of nonmalignant variants selected from these cells by the use of a lectin, they believe to have identified a membrane glycoprotein which is present in larger quantities in several different types of tumor cells than in nontumorigenic cells. Also they have some evidence that it may be structurally abnormal in the tumorigenic cells. Additional evidence and independent confirmation of this finding is outstanding but it seems likely that similar examinations of such nearly identical tumorigenic cells and their nontumorigenic derivatives will allow identification of gene products intimately associated with the malignant cell. This should provide valuable clues as to the mechanisms operating in these cells, an understanding of which is almost certain to lead to successful therapeutic approaches. The identification of proteins quantitatively different, or better still, qualitatively specific to malignant cells might, as BRAMWELL and HARRIS (1978) indicate, allow development of immunotherapeutic procedures, i.e., the production of tumor cell specific antisera.

It is clear that somatic cell genetic and cytogenetic approaches to the problem of what has gone wrong with a malignant cell are providing much information. Some of this data is contradictory, difficult to fit into one unifying concept, and none of it has as yet allowed identification of specific mechanisms. Nonetheless, some parts of the puzzle seem to fit, as I hope to have shown, and it seems likely that the picture will become complete fairly soon.

Aviles D., Jami J., Rousset J-P. and Ritz E.: Tumor x host cell hybrids in the mouse: Chromosomes from the normal cell parent maintained in malignant hybrid tumors. J.natn.Cancer Inst. 58: 1391-1399 (1977).

Azumi J-I. and Sachs L.: Chromosome mapping of the genes that control differentiation and malignancy in myeloid leukemic cells. Proc.natn.Acad.Sci., USA 74: 253-257 (1977).

Bramwell M.D. and Harris H.: An abnormal membrane glycoprotein associated with malignancy in a wide range of different tumours. Proc.R.Soc.Long.B. 201: 87-106 (1978).

Croce C.M. and Koprowski H.: Somatic cell hybrids between mouse peritoneal macrophages and SV40-transformed human cells. J.Expl.Med. 140: 1221-1229 (1974).

Croce C.M. and Koprowski H.: Assignment of gene(s) for cell transformation to human chromosome 7 carrying Simian virus 40 genome. Proc.natn.Acad.Sci., USA 72: 1658-1660 (1975).

Ephrussi B.: Hybridization of Somatic Cells. (Princeton University Press, Princeton, N.J., 1972).

Freedman V.H. and Shin S.I.: Cellular tumorigenicity in nude mice: correlation with cell growth in semi-solid medium. Cell 3: 355-359 (1974).

Freedman V.H. and Shin S.I.: Isolation of human diploid cell variants with enhanced colony-forming efficiency in semisolid medium after a single step chemical mutagenesis. J.natn.Cancer Inst. 58: 1873-1875 (1977).

Gateff E.: Malignant neoplasms of genetic origin in Drosophila melanogaster. Science 200: 1448-1459 (1978).

German J. (ed.): Chromosomes and cancer. (John Wiley and Sons, N.Y., 1974.)

Harris H.: Cell fusion and the analysis of malignancy. The Croonian Lecture. Proc.R.Soc.B 179: 1-20 (1971).

Howell A.N. and Sager R.: Tumorigenicity and its suppression in hybrids of mouse and Chinese hamster cell lines. Proc.natn.Acad.Sci., USA 75: 2353-2362 (1978).

Jonasson J., Povey S. and Harris H.: The analysis of malignancy by cell fusion. VII. Cytogenetic analysis of hybrids between malignant and diploid cells and of tumors derived from them. J.Cell Sci. 24: 217-254 (1977).

Jonasson J. and Harris H.: The analysis of malignancy by cell fusion. VIII. Evidence for the intervention of an extra-chromosomal element. J.Cell Sci. 24: 255-263 (1977).

Klein G.: Mechanisms of carcinogenesis. Internat. Congress of Radiation Research, Vth Seattle, 1974 pp. 869-878 (Academic Press, N.Y., 1975).

Klein G.: Analysis of malignancy and antigen expression by cell fusion. Fed.Proc. 35: 2202-2204 (1976).

Klein G. and Klein E.: Immune surveillance against virus-induced tumors and nonrejectability of spontaneous tumors: Contrasting consequences of host versus tumor evolution. Proc.natn.Acad.Sci., USA 74: 2121-2125 (1977).

Klinger H.P., Baim A.S., Eun C.K., Shows T.B. and Ruddle F.H.: Human chromosomes which affect tumorigenicity in diploid human x heteroploid human and rodent cell hybrids. IN: Human Gene Mapping 4: Fourth International Workshop on Human Gene Mapping. Birth Defects: Original Article Series (1978, in press) The National Foundation, New York. Also in Cytogenet. Cell Genet. Vol. 22 (1978, in press).

Klinger H.P. and Eun C.K.: Mass production of human x rodent cell hybrids in the nude mouse - extent of chromosome and gene marker stability. Cytogenet.Cell Genet. 20: 373-385 (1978).

Klinger H.P. and Eun C.K.: Suppression of tumorigenicity in somatic cell hybrids. I. Suppression and reexpression of tumorigenicity in diploid human x D98AH2 hybrids demonstrating separation of the tumorigenic from other associated phenotypes. Cell (in press).

Klinger H.P., Eun C.K. and Shows T.B.: Suppression of tumorigenicity in somatic cell hybrids. II. Identification of human chromosomes which carry suppressive information in diploid human x heteroploid tumorigenic Chinese hamster cell hybrids. (in preparation.)

Knudson A.G., Strong L.C., Anderson D.E.: Heredity and cancer in man. In: Progress in Medical Genetics (Steinberg AG, Bearn AG, eds.) vol. 9 (Grune and Stratton, New York, 1973).

Koprowski H. and Croce C.M.: Tumorigenicity of simian virus 40-transformed human cells and mouse - human hybrids in nude mice. Proc.natn.Acad.Sci., USA 74: 1142-1146 (1977).

Levan A., Levan G. and Mittelman F.: Chromosomes and cancer. Hereditas 86: 15-30 (1977).

Mittleman F. and Levan G.: Clustering of aberrations to specific chromosomes in human neoplasms. II. A survey of 287 neoplasms. Hereditas 82: 167-174 (1976).

Mittleman F., Mark J., Levan G. and Levan A.: Tumor etiology and chromosome pattern. Science 176: 1340-1341 (1972).

Mulvihill J.J., Miller R.W. and Fraumeni J.F. Jr. (eds.): Genetics of human cancer (Raven Press, N.Y., 1977).

Ohno S.: Aneuploidy as a possible means employed by malignant cells to express recessive phenotypes. In: Chromosomes and cancer, J. German, ed. (John Wiley and Sons, N.Y. 1974).

Rowley J.D.: Do human tumors show a chromosome pattern specific for each etiologic agent? J.natn.Cancer Inst. 52: 315-320 (1974).

Rowley J.D.: Nonrandom chromosomal abnormalities in hematologic disorders of man. Proc. natn.Acad.Sci., USA 72: 152-156 (1975).

Rowley J.D.: Mapping of human chromosomal regions related to neoplasia: Evidence from chromosomes 1 and 17. Proc.natn.Acad.Sci., USA 74: 5729-5933 (1977).

Stanbridge E.J.: Suppression of malignancy in human cells. Nature 260: 17-20 (1976).

Stanbridge E.J. and Wilkinson J.: Analysis of malignancy in human cells: Malignant and transformed phenotypes are under separate genetic control. Proc.natn.Acad.Sci., USA 75: 1466-1469 (1978).

Straus D.S., Jonasson J. and Harris H.: Growth in vitro of tumor cell x fibroblast hybrids in which malignancy is suppressed. J.Cell Sci. 25: 73-86 (1976).

Sugiyama T., Kurita Y., Nishizuka T.: Biological studies on 7,12-di-methylbenz(c) anthracene-induced rat leukemia with special reference to the specific chromosomal abnormalities. Cancer Res. 29: 1117-1124 (1969).

Wiener F., Klein G. and Harris H.: The analysis of malignancy by cell fusion. III. Hybrids between diploid fibroblasts and other tumor cells. J.Cell Sci. 8: 681-692 (1971).

Wiener F., Klein G. and Harris H.: The analysis of malignancy by cell fusion. V. Further evidence of the ability of normal diploid cells to suppress malignancy. J.Cell Sci. 15: 177-183 (1974).

Yamamoto T., Rabinowitz A. and Sachs L.: Identification of the chromosomes that control malignancy. Nature New Biol. 243: 247-250 (1973).

Address of author: H.P. Klinger, M.D., Ph.D., Department of Genetics, Albert Einstein College of Medicine, Eastchester Road and Morris Park Avenue, Bronx, N.Y. 10461.