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Tumor progression and metastasis

Jun Yokota

Biology Division, National Cancer Center Research Institute, 1-1 Tsukiji 5-chome, Chuo-ku, Tokyo 104-0045, Japan

Email: jyokota@gan2.ncc.go.jp

It is now widely accepted that cancer is attributed to the accumulation of genetic alterations in cells. Thus, to understand the molecular mechanisms of cancer metastasis, it is indispensable to identify the genes whose alterations accumulate during cancer progression as well as the genes whose expression is responsible for the acquisition of metastatic potential in cancer cells. Molecular analyses of cancer cells in various stages of progression have revealed that alterations in tumor suppressor genes and oncogenes accumulate during tumor progression and correlate with the clinical aggressiveness of cancer. Comparative analyses of gene expression profiles between metastatic and non-metastatic cells have revealed that various genes are differentially expressed in association with the metastatic potential of cancer cells. A number of genes have been also identified as having functions in inducing or suppressing metastasis in experimental models. However, the association between causative genetic alterations and resulting phenotypic alterations with respect to the metastatic potential of cancer cells is not fully understood. Therefore, elucidation of genotype–phenotype correlation will be required to further understand a complex process of metastasis. Here, I review the progress on molecular studies of tumor progression and metastasis of the past 20 years and discuss the future direction in this field of science.

Introduction

Stepwise progression of human cancer has been clinically well recognized. Several types of pre-malignant lesions, such as dysplasia and hyperplasia, can be detected in diverse organs prior to the appearance of fully malignant invasive tumors. The pre-malignant lesions are caused either by genetic alterations which induce monoclonal expansion of the cells, or by environmental factors, such as viral infection, which induce polyclonal expansion of the cells. Subsequently, accumulation of genetic alterations occur in one (or a few) of the pre-malignant cells, and the cells convert into malignant ones of clonal origin and produce a primary tumor. However, at the early stage of primary tumor expansion, the cells are not invasive and metastatic. Then, new clones with invasiveness and metastatic ability appear as a result of further accumulation of genetic alterations in the cells. Thus, fully malignant cells are invasive and metastatic; however, only a restricted fraction of the cells in a primary tumor are considered to be highly metastatic. Namely, cells in a primary tumor are phenotypically and biologically heterogeneous, and such a heterogeneity is caused by the difference in the genes altered in each cancer cell. Therefore, highly metastatic cells often acquire alterations in more genes than non-metastatic cells, and various genes are differentially expressed between metastatic and non-metastatic cells. Such cells selectively produce a metastatic tumor in a distant organ; thus, cells in the metastatic tumor are considered to carry all the genetic alterations necessary to maintain malignant phenotypes of cancer cells, including invasiveness and metastatic ability (Figure 1).

Two different molecular approaches have been taken to identify such genes. One is the identification of genes whose alterations accumulate during cancer progression. The other is the identification of genes whose expression is responsible for the acquisition of metastatic potential in cancer cells. During the two decades of molecular biological studies on human cancer, a number of genes have been identified as being genetically or epigenetically altered, specifically in far-advanced and/or highly metastatic cancer cells. On the bases of those pieces of information, the process of invasion and metastasis has been understood in association with genetic and/or epigenetic alterations of defined genes in cancer cells.

In this review, I will summarize the major developments for the identification of genes involved in tumor progression and metastasis in the past 20 years, and summarize the genes whose alterations occur specifically in advanced cancer cells and the genes whose expression is responsible for the acquisition of metastatic activity in cancer cells.

Presence of multiple genetic alterations in human cancer cells

Since cancer is attributed to genetic alterations accumulated in the cells, it is indispensable to identify genes whose alterations accumulate during tumor progression to understand the molecular mechanisms of metastasis. Over the past 2 decades, a number of genes that are genetically altered in human cancer cells have been identified. Identification of several oncogenes in the early 1980s has opened the way to search for genetic alterations in human cancer cells and, up to now, nearly 100 oncogenes have been identified (1,2). Isolation of tumor suppressor genes in the late 1980s and 1990s has further accelerated the studies on genetic alterations in human cancer cells and provided us with a large amount of valuable information on understanding the underlying genetic alterations in human cancer cells.

In particular, in the early period of studies on tumor suppressor genes, restriction fragment length polymorphism (RFLP) analysis (3) was used extensively, and it has been shown by the RFLP studies that loss of heterozygosity (LOH) occurs frequently at multiple chromosomal loci in a variety of human cancers (4,5). Since LOH was considered as being a hallmark of gene inactivation in cancer cells, it was hypothesized that a number of tumor suppressor genes are inactivated by genetic alterations in human cancer cells (6). It was in

Abbreviations: HNPCC, hereditary non-polyposis colorectal cancer; LOH, loss of heterozygosity; MMP, microsatellite mutator phenotype; RFLP, restriction fragment length polymorphism; SCLC, small-cell lung carcinoma.

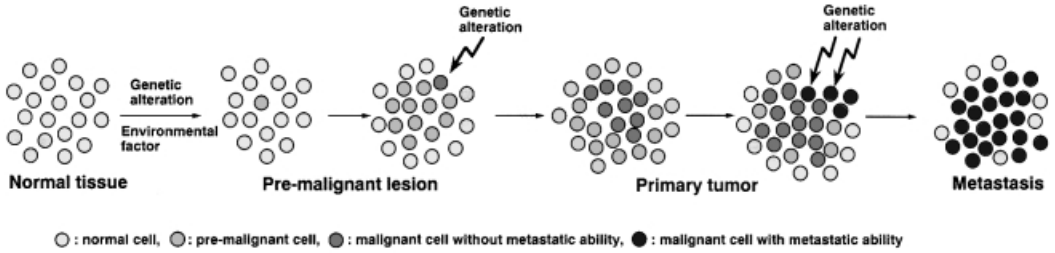

Fig. 1. Stepwise malignant progression of human cancer in association with accumulation of genetic alterations in cells.

1986 that the first tumor suppressor gene, *RB*, was isolated from the human genome by molecular cloning (7), and more than 20 tumor suppressor genes have been identified to date (8,9). Molecular analyses of those genes in cancer cells have confirmed that multiple tumor suppressor genes are indeed inactivated in a single cancer cell by two mutational events (1,10,11). Furthermore, alterations in several oncogenes have been also detected in a subset of cancer cells. For instance, alterations in the *APC*, *K-ras* and *p53* genes are common in colorectal cancer (12), while those in the *p53*, *RB*/*p16*, *c-myc* and *K-ras* genes are common in lung cancer (11,13). In the studies on colorectal cancer, accumulation of LOH on chromosomes 5q, 17p and 18q was documented in 1988 (14), and the *APC* and *p53* genes on 5q and 17p, respectively, were identified later as being inactivated in cancer cells by two mutational events, including LOH, as proposed by Knudson in 1971 (15). In our studies on small-cell lung cancer, the frequent occurrence of LOH on chromosomes 3p, 13q and 17p was first shown by RFLP analysis in 1987 (16). Subsequently, inactivation of the *RB* gene on 13q and the *p53* gene on 17p was revealed by mutational analyses of those genes.

Accumulation of genetic alterations during tumor progression

The presence of multiple genetic alterations in cancer cells strongly indicated that those alterations accumulate in the cells in a stepwise manner during tumor progression. To prove this assumption, comparative analyses of genetic alterations between early and late stage tumors have been extensively performed in various types of human cancers. The number of genetic alterations in late stage tumors were usually more than those in early stage tumors in various types of cancers. The frequencies of alterations in a set of the genes analyzed were higher in late stage tumors than in early stage tumors, while those in another set of genes were high in both early and late stage tumors. Based on the results of those studies, genetic models for tumor progression have been constructed in various types of human cancers in association with accumulation of genetic alterations in cancer cells. Such a genetic model was first demonstrated on colorectal carcinoma, since tumors in various stages of progression, from small adenomas to large metastatic carcinomas, can be relatively easily obtained for analysis (12,14,17). Subsequently, similar genetic models have been constructed for various types of human cancers. Since stepwise accumulations of genetic alterations during progression have been observed in several tumor types, in particular in tumors of epithelial origin, the concept of multistage carcinogenesis is now widely accepted as being a consequence of multiple genetic alterations accumulated in cancer cells.

Then what are the genes responsible for the acquisition of metastatic potential in cancer cells? Based on the concept of multistage carcinogenesis, the metastatic activity of cancer cells should be defined by the genes whose alterations accumulate predominantly in late stage cancer cells. However, up to now, genes critical for the acquisition of metastatic potential in cancer cells have not yet been identified, even in colorectal carcinoma (12).

Genetic alterations predominantly detected in metastases

Several other types of comparative studies have been conducted to identify critical genes involved in metastasis. One of the most convincing approaches is a comparative analysis of genetic alterations between primary tumors and metastases. However, only a limited amount of information is available, probably because of difficulties in collecting a sufficient number of metastatic tumors for analysis. We and others previously performed comparative LOH studies of distant metastases and primary tumors of colorectal carcinoma, lung carcinoma and prostate cancer (18–24). The results clearly indicate that the number of chromosomes with LOH in metastases is significantly higher than that in primary tumors, and support the concept of multistage carcinogenesis in association with accumulation of genetic alterations during tumor progression. In addition, several chromosomal loci showed LOH more frequently in metastases, suggesting that some tumor suppressor genes are involved in acquisition of metastatic potential in cancer cells. In particular, frequent LOH on chromosome 14q has been consistently observed in metastatic and advanced colorectal carcinomas among the studies of three different groups (18,19,23,25), suggesting that chromosome 14q harbors a metastasis suppressor gene for colorectal carcinoma. Chromosomes 18q and 22q are also common targets of frequent LOH in both colorectal and lung cancers of aggressive phenotypes (21,22,26–28). However, target genes on these chromosome arms have not yet been identified.

Prognostic significance of oncogene and tumor suppressor gene alterations

In the field of clinical oncology, it is very important to identify molecular markers for the evaluation of prognosis in cancer patients. Thus, association between oncogene alterations in cancer cells and prognosis of patients has been extensively investigated in various types of human cancers. For instance, amplification of the *N-myc* oncogene is now a valuable prognostic marker for patients with neuroblastoma (29,30), and amplification/overexpression of the *c-erbB-2* oncogene is also a marker for the aggressiveness of ovarian and breast cancers (31,32). In general, oncogene amplification occurs late in tumor progression and correlates well with clinical aggressiveness of tumors (33,34). Point mutations of the *ras*

oncogenes, in particular of the *K-ras* gene, occur in a variety of human cancers, such as pancreatic cancer, colorectal cancer, lung adenocarcinoma and thyroid carcinoma. The prognostic significance of *ras* mutation has been documented in lung adenocarcinoma (35).

In contrast, the prognostic significance of tumor suppressor gene inactivation is still unclear or controversial in several types of cancers, although that of LOH on several chromosomes has been documented in a variety of cancers. This could be partly due to the technical difficulties in detecting various types of tumor suppressor gene alterations, including point mutations, intragenic insertions/deletions and homozygous whole-gene deletions, by a simple method. Methylational inactivation should be also considered as a mechanism of tumor suppressor gene inactivation, as in the case of the *p16* gene (36). Alternatively, it is also possible that most tumor suppressor genes are involved in the genesis rather than the progression of cancer, since those are also genes responsible for hereditary tumors (8,9). We should also consider that the biological significance of tumor suppressor gene alterations is different among types of mutations and among types of cancers. In particular, the *p53* gene has multiple functions and several mutant forms should still keep some normal functions, so it is likely that the phenotypes of cancer cells are different if the types of mutations are different. Therefore, it is now very important to elucidate more critically the association of cancer phenotypes with cancer genotypes for the analysis of tumor suppressor genes.

Tumor type specificity of genetic alterations

Molecular genetic analyses of various types of human cancers have also revealed that there are two groups of genes involved in human carcinogenesis. One is a group of genes which are genetically altered commonly in diverse tumor types, and the other is a group of genes which are altered specifically in particular tumor types (1,8). Examples of the former group are *p53*, *RB* and *p16*, and those of the latter are *VHL*, *RET* and various genes translocated in leukemia cells. Mutations of the *p53* gene have been found most widely in a variety of tumors (37,38), whereas *RB* and *p16* inactivation is somewhat limited to a more restricted group of tumors. Interestingly, the *RB* and *p16* genes are preferentially inactivated in small-cell lung carcinoma (SCLC) and non-SCLC, respectively, although both genes are involved in the same signaling pathway for the regulation of the G_1/S transition of the cell cycle (39,40). Mutations of the *VHL* and *RET* genes have been detected exclusively in renal cell cancer and thyroid cancer, respectively. What is the implication of tumor type specificity for genetic alterations? It implies that a set of responsible genes for carcinogenesis is different among cells of different histological types. Some genes are commonly involved in the malignant transformation of diverse cell types, whereas some other genes are involved in that of particular cell types.

If a set of genes altered in cancer cells is different among tumors of different histological types, it is highly possible that critical genetic alterations for the acquisition of metastatic potential are also different among cancers of different origin. At present, we do not know whether genes responsible for metastatic potential are common in all different histological types of tumors or unique and peculiar to particular tumor types. However, LOH studies on various types of advanced and metastatic cancers have indicated that the profiles of allelotypes are different among tumors of different origin, supporting the idea that genes for metastasis are also different among tumors of different origin. However, it is still unknown which genes are responsible for the acquisition of metastatic potential in any types of cancers. Thus, it is now very important to identify genes which are affected by LOH specifically in metastatic tumors.

Another important finding coming from molecular analyses of human cancers is the discovery of multiple pathways for carcinogenesis in tumors of the same histological type. In colorectal cancer, the microsatellite mutator phenotype (MMP) was discovered by the application of the arbitrarily primed PCR DNA fingerprinting method to the analysis of somatic genetic alterations in tumors (41,42). This phenotype is now known to be a consequence of somatic or germ-line inactivation in DNA mismatch repair genes (12,43). Hereditary non-polyposis colorectal cancer (HNPCC) is caused by hereditary mutations of a mismatch repair gene (12). Tumors with MMP exhibit a low frequency of mutations in the *K-ras*, *APC* and *p53* genes, but a high frequency of mutations in the genes with simple repeated sequences, such as the *TGF*-β receptor and *BAX* genes (44,45). These results indicate the existence of at least two distinct pathways for colorectal carcinogenesis. These two pathways should also exist in other types of HNPCC-associated cancers, such as cancer of the endometrium, although the prevalence of endometrial cancer with MMP would not be so high as that of colorectal cancer with MMP.

In cancers not associated with HNPCC, it is still unclear whether there are multiple pathways for carcinogenesis. If there are, the phenotype (biological behavior) of cancer cells should be different even among tumors derived from the same origin of precursor cells, because of the difference in a set of genes altered in cancer cells. Frequencies of *p53* mutations vary considerably among tumors of different histological types (46,47) and, in tumors without *p53* mutations, alterations in genes upstream or downstream of the *p53* gene cannot be always detected. Thus, it is possible that multiple pathways for carcinogenesis exist in those tumors. Accordingly, it has been suggested that there are several different sets of genes involved in metastasis even in the same histological types of cancers.

Tumor cell heterogeneity with respect to metastatic potential of cancer cells

Another approach in defining a set of genes involved in metastasis is a comparative study of cancer cells with metastatic potential and of those without metastatic potential. It was nearly 30 years ago that Fidler first demonstrated the heterogeneity of mouse melanoma cells with respect to metastatic potential (48). In other words, he successively selected sub-lines with different metastatic potential from a mouse melanoma cell line. His study clearly indicated that a primary tumor often contains sub-populations of metastatic and non-metastatic cancer cells. Since then, several similar animal models have been developed, and the concept of tumor cell heterogeneity with respect to the metastatic potential has now been well established and widely accepted (49). This concept has made the studies on metastasis genes more difficult, because highly metastatic cells are present as a small (or large) population in a primary tumor, based on this concept. If so, we have to find some specific marker to select the cells with high metastatic potential in the primary tumor. Such a marker should be a

specific genetic alteration, but one which is accumulated in cancer cells during tumor progression. For this reason, several transfection studies of DNA from highly metastatic human tumors have been performed (50,51); however, to date, no genes inducing or suppressing metastasis have been identified by this method.

Differential gene expression in association with metastatic potential of cancer cells

However, isolation of high- and low-metastatic subclones from a primary tumor has made it possible to elucidate the properties unique to high-metastatic cells. High- and low-metastatic cells are different from each other in various aspects, and those differences have been extensively investigated at the molecular levels during the last 2 decades. The cDNA differential or subtractive hybridization method was often used in the 1980s and the mRNA differential display method (52) is now more commonly used. The results indicated that various genes are differentially expressed between metastatic and non-metastatic cells. Among the genes differentially expressed between metastatic and non-metastatic cells, several genes have the effect of inducing or suppressing metastasis. For instance, the *nm23* and *Elm1* genes have been shown to suppress the metastatic activity of cancer cells by forced expression in cancer cells, while the *p9Ka/mta1* gene has a function of inducing metastasis (53–56). Several other genes, such as *KAI1*, *KiSS-1* and *Tiam-1*, which were isolated by using other molecular methods, have also been shown to have functions in suppressing or inducing metastasis (57–59). Thus, those genes have been considered to have critical functions in regulating metastatic activity in human cancer cells. However, no genetic alterations of those genes have been found in human cancer cells, and expression profiles of those genes in human cancer cells do not always correlate with the metastatic potential of the cells. Therefore, it is still unclear whether the expression profiles of those genes are clinically valuable for the prediction of metastatic potential in cancer patients.

Association of genetic alterations with metastatic phenotype of cancer cells

To understand how cancer cells acquire metastatic potentials, it is necessary to clarify the causative genetic alterations for metastatic transformation of normal cells and resulting epigenetic alterations unique to cancer cells with metastatic ability (60,61). We now know that a number of oncogenes and tumor suppressor genes are genetically altered in cancer cells and that those alterations accumulate during tumor progression. Thus, it goes without saying that alterations of those genes are causative events for multistage carcinogenesis, although we still do not know which genes are responsible for the acquisition of invasiveness and metastatic potential in cancer cells (Figure 2). In the meantime, the number of candidate genes whose expression could possibly change the metastatic potential of human cancer cells has been increasing. Thus, by analyzing genotype–phenotype correlation with respect to metastatic potential, it should be possible to identify genetic alterations responsible for metastasis.

Suitable assay systems are required for the functional analysis of gene products with respect to metastasis. NIH 3T3 cells have often been used as recipients of transfection with various oncogenes. Interestingly, the metastatic phenotype was accomplished by transfection of mutated *ras* oncogenes into

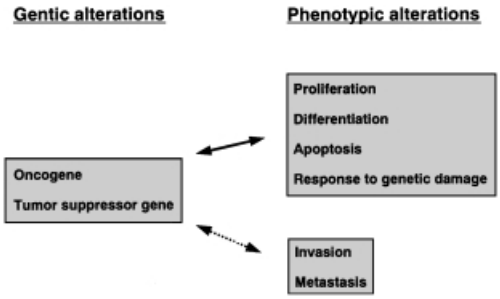

Fig. 2. Crossroads between genetic and phenotypic alterations in human cancer. Oncogenes and tumor suppressor genes are known to have functions to regulate proliferation, differentiation, apoptosis and responses to genetic damages; however, their involvement in invasion and metastasis remains unclear.

NIH 3T3 cells (60). However, clinically, *ras* mutation occurs in the early stage of progression in several types of cancers, in particular in colorectal carcinoma. Correlation of *ras* mutation with prognosis has been documented only in lung adenocarcinoma as mentioned before, and such a correlation is not clear in other types of cancers. Thus, it is still questionable whether mutated *ras* genes have a function of enhancing the metastatic potential of cancer cells. Several problems of this assay system have previously been documented by Nicolson (60). Kerbel *et al.* (61) have also pointed out the importance of assay systems which evaluate the biological function of oncogenes, and recommended the use of reconstituted organ culture systems rather than the use of tissue culture systems.

Tumor suppressor genes constitute key points in many complex cellular pathways that regulate proliferation, differentiation, apoptosis and response to genetic damage (8,9). To date, there has been no evidence that any of the known tumor suppressor genes are involved specifically in the stage of invasion and metastasis (Figure 2). However, some tumor suppressor genes may have a function partly in the stage of tumor progression. For instance, loss of E-cadherin function in tumors results in the rapid progression of relatively benign adenomas to invasive, metastatic carcinomas. Germline mutation of the E-cadherin gene predisposes to diffuse, poorly differentiated gastric cancer, and its downregulation in sporadic tumors is associated with poor clinical prognosis (62). Recent studies have indicated that angiogenesis may be regulated, in part, by *p53* tumor suppressor gene function (63–65). As mentioned before, there are several specific chromosomal regions which are preferentially deleted in advanced and metastatic cancers. Thus, it is highly possible that there are several unknown tumor suppressor genes involved specifically in tumor progression and metastasis.

Very recently, a cancer model in which dominantly acting oncoproteins are somatically regulated *in vivo* has been developed, and it was shown using such a model that melanoma genesis and maintenance are strictly dependent on expression of an activated *H-ras* gene in a doxycycline-inducible mutated *H-ras* mouse melanoma model null for the *p16* gene (66). This kind of mouse model system could be suitable for the functional analysis of various metastasis-related genes *in vivo*. On the other hand, different susceptibility to malignant transformation by oncogenes has been shown between rodent cells and human cells. According to the paper by Hahn *et al.* (67), one important difference between rodent and human cells comes from their telomere biology. Murine somatic cells express telomerase activity and have much longer telomeres

than their normal human counterparts, which lack telomerase activity. It was shown that the ectopic expression of the telomerase catalytic subunit (*hTERT*) gene in combination with two oncogenes resulted in direct tumorigenic conversion of normal human epithelial and fibroblast cells. This method can be also applied for biological analysis of metastasis-associated human genes. However, genetic activation of the *hTERT* gene has not been identified in human cancer to date; thus, elucidation of the mechanism for *hTERT* activation is necessary to understand the process of multistage carcinogenesis in human cells.

Future directions

The most devastating aspect of cancer is the emergence of metastases in organs distant from the primary tumor, since most deaths from cancer are due to metastases. Thus, to understand the molecular mechanisms of metastasis is one of the most important issues in cancer research. Recent advances in molecular and cellular biology have been breathtaking and have made it possible to identify genetic and epigenetic determinants for tumor progression and metastasis. In fact, various genes which are involved in these processes have been identified in the last 2 decades. Here, I summarized our current understanding in this field of science, and also summarized the genes whose alterations accumulate during human tumor progression and the genes whose expression could control the metastatic potential of cancer cells. Lastly, I will discuss future directions of molecular studies on tumor progression and metastasis and the possible ways of applying this knowledge in cancer clinics.

According to the report by Duggan *et al*. (68), more than 1.1 million expressed sequence tagged sites (ESTs), corresponding to 52 907 unique human genes have been catalogued; however, the function, expression and regulation of $>80\%$ of them has yet to be determined. Deletion mapping studies have defined more than 30 regions dispersed on 21 different chromosome arms as candidate tumor suppressor loci for lung cancer; however, only a few genes have been identified as the targets of chromosomal deletions (11). Thus, we can speculate that we now have only 10–20% of the information about genetic and epigenetic determinants for metastasis in human cancer. A complete human genome sequence will be available in a few years, and known genes and EST markers are being mapped systematically in the human genome (69,70). The information of the whole human genome sequence will facilitate the studies on genetic alterations in human cancer and make it possible to grasp all the genetic alterations accumulated in cancer cells. The whole set of genes whose expression is altered specifically in metastatic cells will also be identified more easily when all the genes in the human genome are cloned and mapped. DNA and cDNA arrays will be promising ways to survey structural alterations and differential expression of a total of 100 000 genes systematically (71). By applying those technologies for detection of genetic and epigenetic determinants for tumor progression and metastasis, it will be possible to diagnose the clinical aggressiveness of cancer at bedside in future.

According to the concept of tumor cell heterogeneity, highly metastatic cells are present as a sub-population in a primary tumor. At present, it is impossible to divide the cells in a primary tumor into metastatic and non-metastatic cells, since we still do not know how to divide the cells with respect to metastatic potentials. However, once we obtain molecular markers for prediction of metastatic potential of cancer cells, several methods can be applied in clinics (72). One of the most valuable and attractive technologies applicable in clinics will be the establishment of a method to comprehensively analyze the whole genome in a single cell (73). Then, in the near future, it will be possible to identify all types of genetic alterations and to see the expression profile of the whole 100 000 genes in each single cell in a tumor. The information obtained by those analyses will definitely contribute to the creation of new principles for prevention, diagnosis and therapy of metastasis.

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References

1. Bishop,J.M. (1991) Molecular themes in oncogenesis. *Cell*, **25**, 235–248.
2. Land,H., Parada,L.F. and Weinberg,R.A. (1983) Cellular oncogenes and multistep carcinogenesis. *Science*, **222**, 771–778.
3. Cavenee,W.K., Dryja,T.P., Phillips,R.A., Benedict,W.F., Godbout,R., Gallie,B.L., Murphree,A.L., Strong,L.C. and White,R.L. (1983) Expression of recessive alleles by chromosomal mechanisms in retinoblastoma. *Nature*, **305**, 779–784.
4. Ponder,B. (1988) Gene losses in human tumours. *Nature*, **335**, 400–402.
5. Rodriguez,E., Sreekantaiah,C. and Chaganti,R.S. (1994) Genetic changes in epithelial solid neoplasia. *Cancer Res.*, **54**, 3398–3406.
6. Knudson,A.G.Jr (1985) Hereditary cancer, oncogenes and antioncogenes. *Cancer Res.*, **45**, 1437–1443.
7. Friend,S.H., Bernard,R., Rogelj,S., Weinberg,R.A., Rapaport,J.M., Albert,D.M. and Dryja,T.P. (1986) A human DNA segment with properties of the gene that predisposes to retinoblastoma and osteosarcoma. *Nature*, **323**, 643–646.
8. Fearon,E.R. (1997) Human cancer syndromes: clues to the origin and nature of cancer. *Science*, **278**, 1043–1049.
9. Haber,D. and Harlow,E. (1997) Tumor suppressor genes: evolving definitions in the genomic age. *Nature Genet.*, **16**, 320–322.
10. Yokota,J. and Sugimura,T. (1993) Multiple steps in carcinogenesis involving alterations of multiple tumor suppressor genes. *FASEB J.*, **7**, 920–925.
11. Kohno,T. and Yokota,J. (1999) How many tumor suppressor genes are involved in human lung carcinogenesis? *Carcinogenesis*, **20**, 1403–1410.
12. Kinzler,K.W. and Vogelstein,B. (1996) Lessons from hereditary colorectal cancer. *Cell*, **87**, 159–170.
13. Sekido,Y., Fong,K.M. and Minna,J.D. (1998) Progress in understanding the molecular pathogenesis of human lung cancer. *Biochim. Biophys. Acta*, **1378**, 21–59.
14. Vogelstain,B., Fearon,E.R., Hamilton,S.R., Kern,S.E., Preisinger,A.C., Leppert,M., Nakamura,Y., White,R., Smith,A.M. and Bos,J.L. (1988) Genetic alterations during colorectal-tumor development. *N. Engl. J. Med.*, **319**, 525–532.
15. Knudson,A.G.Jr (1971) Mutation and cancer: statistical study of retinoblastoma. *Proc. Natl Acad. Sci. USA*, **68**, 820–823.
16. Yokota,J., Wada,M., Shimosato,Y., Terada,M. and Sugimura,T. (1987) Loss of heterozygosity on chromosomes 3, 13 and 17 in small-cell lung carcinoma and on chromosome 3 in adenocarcinoma of the lung. *Proc. Natl Acad. Sci. USA*, **84**, 9252–9256.
17. Fearon,E.R. and Vogelstain,B. (1990) A genetic model for colorectal tumorigenesis. *Cell*, **61**, 759–767.
18. Ookawa,K., Sakamoto,M., Hirohashi,S., Yoshida,Y., Sugimura,T., Terada,M. and Yokota,J. (1993) Concordant p53 and DCC alterations and allelic losses on chromosomes 13q and 14q associated with liver metastasis of colorectal carcinoma. *Int. J. Cancer*, **53**, 382–387.
19. Ishimaru,G., Ookawa,K., Yamaguchi,N., Sakamoto,M., Hirohashi,S., Muto,T. and Yokota,J. (1994) Allelic losses associated with the metastatic potential of colorectal carcinoma. *Int. J. Oncol.*, **5**, 267–273.
20. Malkosyan,S., Yasuda,J., Soto,J.L., Sekiya,T., Yokota,J. and Perucho,M. (1998) Molecular karyotype (amplotype) of metastatic colorectal cancer by unbiased arbitrarily primed PCR DNA fingerprinting. *Proc. Natl Acad. Sci. USA*, **95**, 10170–10175.

21. Shiseki,M., Kohno,T., Nishikawa,R., Sameshima,Y., Mizoguchi,H. and Yokota,J. (1994) Frequent allelic losses on chromosomes 2q, 18q and 22q in advanced non-small cell lung carcinoma. *Cancer Res.*, **54**, 5643–5648.
22. Shiseki,M., Kohno,T., Adachi,J., Okazaki,T., Otsuka,T., Mizoguchi,H., Noguchi,M., Hirohashi,S. and Yokota,J. (1996) Comparative allelotype of early and advanced stage non-small cell lung carcinomas. *Genes Chromosom. Cancer*, **17**, 71–77.
23. Thorstensen,L., Qvist,H., Nesland,J.M., Giercksky,K.-E. and Lothe,R.A. (1996) Allelotype profiles of local recurrences and distant metastases from colorectal-cancer patients. *Int. J. Cancer*, **69**, 452–456.
24. Saric,T., Brkanac,Z., Troyer,D.A., Padalecki,S.S., Sarosdy,M., Williams,K. and Abadesco,L. (1999) Genetic pattern of prostate cancer progression. *Int. J. Cancer*, **81**, 219–224.
25. Young,J., Leggett,B., Ward,M., Thomas,L., Buttenshaw,R., Searle,J. and Chenevix-Trench,G. (1993) Frequent loss of heterozygosity on chromosome 14 occurs in advanced colorectal carcinomas. *Oncogene*, **8**, 671–675.
26. Takei,K., Kohno,T., Hamada,K., Takita,J., Noguchi,M., Matsuno,Y., Hirohashi,S., Uezato,H. and Yokota,J. (1998) A novel tumor suppressor locus on chromosome 18q involved in the development of human lung cancer. *Cancer Res.*, **58**, 3700–3705.
27. Jen,J., Kim,H., Piantadosi,S., Liu,Z.-F., Levitt,R.C., Sistonen,P., Kinzler,K.W., Vogelstein,B. and Hamilton,S.R. (1994) Allelic loss of chromosome 18q and progression in colorectal cancer. *N. Engl. J. Med.*, **331**, 213–221.
28. Muraoka,M., Konishi,M., Kikuchi-Yanoshita,R., Tanaka,K., Shitara,N., Chong,J.M., Iwama,T. and Miyaki,M. (1996) p300 gene alterations in colorectal and gastric carcinomas. *Oncogene*, **12**, 1565–1569.
29. Brodeur,G.M., Seeger,R.C., Schwab,M., Varmus,H.E. and Bishop,J.M. (1984) Amplification of N-*myc* in untreated human neuroblastomas correlates with advanced disease stage. *Science*, **224**, 1121–1124.
30. Seeger,R.C., Brodeur,G.M., Sather,H., Dalton,A., Siegel,S.E., Wong,K.Y. and Hammond,D. (1985) Association of multiple copies of the N-*myc* oncogene with rapid progression of neuroblastomas. *N. Engl. J. Med.*, **313**, 1111–1116.
31. Slamon,D.J., Clark,G.M., Wong,S.G., Levin,W.J., Ullrich,A. and McGuire,W.L. (1987) Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science*, **235**, 177–182.
32. Slamon,D.J., Godolphin,W., Jones,L.A. *et al.* (1989) Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. *Science*, **244**, 707–712.
33. Yokota,J., Tsunetsugu-Yokota,Y., Battifora,H., Le Fevre,C. and Cline,M.J. (1986) Alterations of *myc*, *myb* and *ras*^{Ha} proto-oncogenes in cancers are frequent and show clinical correlation. *Science*, **231**, 261–265.
34. Yokota,J., Wada,M., Yoshida,T., Noguchi,M., Terasaki,T., Shimosato,Y., Sugimura,T. and Terada,M. (1988) Heterogeneity of lung cancer cells with respect to the amplification and rearrangement of *myc* family oncogenes. *Oncogene*, **2**, 607–611.
35. Rodenhius,S. and Slebos,R.J. (1992) Clinical significance of *ras* oncogene activation in human lung cancer. *Cancer Res.*, **52**, 2665–2669.
36. Herman,J.G., Merlo,A., Mao,L., Lapidus,R.G., Issa,J.P., Davidson,N.E., Sidransky,D. and Baylin,S.B. (1995) Inactivation of the *CDKN2/p16/MTS1* gene is frequently associated with aberrant DNA methylation in all common human cancers. *Cancer Res.*, **55**, 4525–4530.
37. Nigro,J.M., Baker,S.J., Preisinger,A.C. *et al.* (1989) Mutations in the *p53* gene occur in diverse human tumour types. *Nature*, **342**, 705–708.
38. Harris,C.C. (1993) p53: at the crossroads of molecular carcinogenesis and risk assessment. *Science*, **262**, 1980–1981.
39. Otterson,G.A., Kratzke,R.A., Coxon,A., Kim,Y.W. and Key,F.J. (1994) Absence of p16^{INK4} protein is restricted to the subset of lung cancer lines that retains wild type RB. *Oncogene*, **9**, 3375–3378.
40. Kelley,M.J., Nakagawa,K., Steinberg,S.M., Mulshine,J.L., Kamb,A. and Johnson,B.E. (1995) Differential inactivation of CDKN2 and Rb protein in non-small-cell and small-cell lung cancer cell lines. *J. Natl Cancer Inst.*, **17**, 756–761.
41. Ionov,J., Peinado,M.A., Markhosyan,S., Shibata,D. and Perucho,M. (1993) Ubiquitous somatic mutations in simple repeated sequences reveal a new mechanism for colonic carcinogenesis. *Nature*, **363**, 558–561.
42. Perucho,M. (1996) Cancer of the microsatellite mutator phenotype. *Biol. Chem.*, **377**, 675–684.
43. Lengauer,C., Kinzler,K.W. and Vogelstein,B. (1998) Genetic instabilities in human cancers. *Nature*, **396**, 643–649.
44. Markowitz,S., Wang,J., Myeroff,L. *et al.* (1995) Inactivation of the type II TGF-β receptor in colon cancer cells with microsatellite instability. *Science*, **268**, 1336–1338.
45. Rampino,N., Yamamoto,H., Ionov,Y., Li,Y., Sawai,H., Reed,J.C. and Perucho,M. (1997) Somatic frameshift mutations in the *BAX* gene in colon cancers of the microsatellite mutator phenotype. *Science*, **275**, 967–969.
46. Hollstein,M., Sidransky,D., Vogelstein,B. and Harris,C.C. (1991) *p53* mutations in human cancer. *Science*, **253**, 49–53.
47. Greenblatt,M.S., Bennet,W.P., Hollstein,M. and Harris,C.C. (1994) Mutations in the *p53* tumor suppressor gene: clues to cancer etiology and molecular pathogenesis. *Cancer Res.*, **54**, 4855–4878.
48. Fidler,I.J. (1973) Selection of successive tumor lines for metastasis. *Nature*, **242**, 148–149.
49. Fidler,I.J. (1990) Critical factors in the biology of human cancer metastasis: twenty-eighth G. H. A. Clowes memorial award lecture. *Cancer Res.*, **50**, 6130–6138.
50. Bernstein,S.C. and Weinberg,R.A. (1985) Expression of the metastatic phenotype in cells transfected with human metastatic tumor DNA. *Proc. Natl Acad. Sci. USA*, **82**, 1726–1730. [Published erratum appears in *Proc. Natl Acad. Sci. USA* (1988) **85**, 5581.]
51. Hayle,A.J., Darling,D.L., Taylor,A.R. and Tarin,D. (1993) Transfection of metastatic capability with total genomic DNA from human and mouse metastatic tumour cell lines. *Differentiation*, **54**, 177–189.
52. Liang,P. and Pardee,A.B. (1992) Differential display of eukaryotic messenger RNA by means of the polymerase chain reaction. *Science*, **257**, 967–971.
53. Leone,A., Flatow,U., King,C.R., Sandeen,M.A., Margulies,I.M.K., Liotta,L.A. and Steeg,P.S. (1991) Reduced tumor incidence, metastatic potential and cytokine responsiveness of nm23-transfected melanoma cells. *Cell*, **65**, 25–35.
54. Hashimoto,Y., Shindo-Okada,N., Tani,M., Nagamachi,Y., Takeuchi,K., Shiroishi,T., Toma,H. and Yokota,J. (1998) Expression of the *Elm1* gene, a novel gene of CCN family, suppresses *in vivo* tumor growth and metastasis of K-1735 murine melanoma cells. *J. Exp. Med.*, **187**, 289–296.
55. Davies,B.R., Davies,M.P.A., Gibbs,F.E.M., Barraclough,R. and Rudland,P.S. (1993) Induction of the metastatic phenotype by transfection of a benign rat mammary epithelial cell line with the gene for p9Ka, a rat calcium-binding protein, but not with the oncogene EJ-*ras*-1. *Oncogene*, **8**, 999–1008.
56. Toh,Y., Pencil,S.D. and Nocolson,G.L. (1994) A novel candidate metastasis-associated gene, *mta1*, differentially expressed in highly metastatic mammary adenocarcinoma cell lines. *J. Biol. Chem.*, **269**, 22958–22963.
57. Dong,J.T., Lamb,P.W., Rinker-Schaeffer,C.W., Vukanovic,J., Ichikawa,T., Issac,J.T. and Barret,J.C. (1995) *KAI1*, a metastasis suppressor gene for prostate cancer on human chromosome 11p11.2. *Science*, **268**, 884–886.
58. Lee,J.-H. and Welch,D.R. (1997) Suppression of metastasis in human breast carcinoma MDA-MB-435 cells after transfection with the metastasis suppressor gene, *KiSS-1*. *Cancer Res.*, **57**, 2384–2387.
59. Habets,G.G.M., Scholtes,E.H.M., Zuydgeest,D., van der Kamman,R.A., Stam,J.C., Berns,A. and Collard,J.G. (1994) Identification of an invasion-inducing gene, *Tiam-1*, that encodes a protein with homology to GDP–GTP exchangers for Rho-like proteins. *Cell*, **77**, 537–549.
60. Nicolson,G.L. (1987) Tumor cell instability, diversification and progression to the metastatic phenotype: From oncogene to oncofetal expression. *Cancer Res.*, **47**, 1473–1487.
61. Kerbel,R.S., Viloria-Petit,A., Okada,F. and Rak,J. (1998) Establishing a link between oncogenes and tumor angiogenesis. *Mol. Med.*, **5**, 286–295.
62. Guilford,P. (1999) E-cadherin downregulation in cancer: fuel on the fire? *Mol. Med. Today*, **5**, 172–177.
63. Van Meir,E.G., Polverini,P.J., Chazin,V.R., Su Huang,H.J., de Tribolet,N. and Cavenee,W.K. (1994) Release of an inhibitor of angiogenesis upon induction of wild type p53 expression in glioblastoma cells. *Nature Genet.*, **8**, 171–176.
64. Nishimori,H., Shiratsuchi,T., Urano,T., Kimura,Y., Kiyono,K., Tatsumi,K., Yoshida,S., Ono,M., Kuwano,M., Nakamura,Y. and Tokino,T. (1997) A novel brain-specific p53-target gene, *BAI1*, containing thrombospondin type 1 repeats inhibits experimental angiogenesis. *Oncogene*, **15**, 2145–2150.
65. Bouvet,M., Ellis,L.M., Nishizaki,M., Fujiwara,T., Liu,W., Bucana,C.D., Fang,B., Lee,J.J. and Roth,J.A. (1998) Adenovirus-mediated wild-type *p53* gene transfer down-regulates vascular endothelial growth factor expression and inhibits angiogenesis in human colon cancer. *Cancer Res.*, **58**, 2288–2292.
66. Chin,L., Tam,A., Pomerantz,J., Wong,M., Holash,J., Bardeesy,N., Shen,Q., O'Hagen,R., Pantginis,J., Zhou,H., Horner,J.W.II, Cordon-Cardo,C., Yancopoulos,G.D. and Depinho,R.A. (1999) Essential role for oncogenic *ras* in tumour maintenance. *Nature*, **400**, 468–472.
67. Hahn,W., Counter,C.M., Lundberg,A.S., Beijersbergen,R.L., Brooks,M.W. and Weinberg,R.A. (1999) Creation of human tumour cells with defined genetic elements. *Nature*, **400**, 464–468.

68. Duggan,D.J., Bittner,M., Chen,Y., Meltzer,P. and Trent,J.M. (1999) Expression profiling using cDNA microarrays. *Nature Genet.*, **21**, 10–14.
69. Marra,M.A., Hillier,L. and Waterston,R.H. (1998) Expressed sequence tags—ESTablishing bridges between genomes. *Trends Genet.*, **14**, 4–7.
70. The Sanger Centre and the Washington University Genome Sequence Center (1998) Towards a complete human genome sequence. *Genome Res.*, **8**, 1097–1108.
71. Lander,E.S. (1999) Array of hope. *Nature Genet.*, **21**, 3–4.
72. Sidransky,D. (1997) Nucleic-acid based methods for the detection of cancer. *Science*, **278**, 1054–1058.
73. Klein,C.A., Schmidt-Kittler,O., Schardt,J.A., Pantel,K., Speicher,M.R. and Riethmuller,G. (1999) Comparative genomic hybridization, loss of heterozygosity and DNA sequence analysis of single cells. *Proc. Natl Acad. Sci. USA*, **96**, 4494–4499.

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