Role of oncogenes in metastases

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Introduction
The mechanisms responsible for the induction of metastatic behavior in tumor cells have yet to be fully elucidated. A number of intriguing hypotheses have been put forward, but have not been confirmed. All depend on the observation that the ability to metastasize is not the same in all cells within a tumor, but varies markedly between cells within a tumor leading to the placement of metastasis within the general phenomenon of tumor heterogeneity (1,2). Some of these models assume that genetic alterations in the cell lead to metastasis, while others are based upon non-heritable fluctuations. Weiss (3) has long espoused models in which cells subject to as yet undefined environmental influences can transiently acquire the ability to metastasize. The most prominent model for tumor progression based on genetic events is that of Nowell (4) who had proposed that tumorigenic cells have an increased frequency of mutation and that these mutations are required to induce metastasis. While on first appearance, these models may seem to be contradictory, in fact both mechanisms might operate. For example, the genetic change could lead to the ability to respond to an external factor which then induces metastasis. Since only a small fraction of cells injected into a mouse or tested in an in vitro assay eventually metastasize, such a synthesis of the two models is appealing. Until recently, it has not been possible to systematically induce metastatic behavior in cells. However, as will be detailed below, the ras oncogene can serve such a function. While it is consistent with the genetic hypothesis that the introduction of an activated oncogene could lead to metastasis, this finding does not resolve the issue as to the extent of importance of environmental factors and in no way proves that genetic alterations lead to metastasis in actual human tumors.

Here we will summarize the evidence indicating that the ras oncogenes can induce metastatic potential and suggest how this information may further the study of tumor progression. The ras oncogene was originally identified as a gene capable of inducing transformation and tumorigenicity (5). It has also been shown by many workers, in experiments to be reviewed below, to induce the metastatic phenotype. Many workers have repeatedly documented the independence of metastatic potential from tumorigenicity (6). Thus, the ability of ras oncogenes to induce metastasis was unexpected. Nonetheless, the experimental evidence now clearly indicates a wide range of situations in which transfection by the rasH oncogene leads to metastasis as well as tumorigenicity.

This work is of particular importance for the study of metastasis because using the rasH oncogene it is possible to reliably and reproducibly introduce the metastatic phenotype into previously non-metastatic cells. Cells which have been rendered metastatic in this way should now provide a fertile medium for both biochemical and genetic analysis.

The first clue that rasH might influence metastasis was that 50% of the tumors induced by the Harvey sarcoma virus appeared metastatic in immunocompetent rats. After inoculation with the virus, highly invasive tumors arose, some of which seemed to have metastasized to distant sites (7). However, in these experiments, we cannot eliminate the possibility that virus rescue led to tumors at distant sites. Thus, more recent work has employed transfer of cloned DNA sequences to eliminate this possibility.

In vivo assays for metastasis
One of the most difficult aspects in attempting to study metastasis is the identification of suitable assays to determine if a cell line is metastatic. First, because one often wishes to test cells or tumors in non-syngeneic animals, immunological barriers may arise. In most of the experiments discussed below, nude mice are used to reduce such barriers. Furthermore, these mice must be young, <8 weeks old, because NK cell activity rises dramatically after that age (8,9).

Several different strategies are used for the injections. In the spontaneous metastasis assay, cells are injected s.c., a tumor forms, and later the animals are autopsied and evaluated for metastasis. In a variation on this assay, some workers will excise the tumor after it has been allowed to form to increase the time after which the animal can be evaluated. If the tumor is allowed to remain, it will usually kill the animal within months, or even sooner. Thus, after excision one can wait longer in order to evaluate for metastasis (see ref.4).

An alternate assay termed the experimental metastasis assay is also often employed. In this method, cells are injected i.v. The resultant lung nodules indicate the extent of metastatic potential. This assay may not appear to have much in common with the actual mechanism of metastasis, but it is apparent that metastasis involves the exodus of cells from the circulation into distant sites, and the formation of secondary tumors at those sites. The experimental assay duplicates those later steps. In fact, the results from this assay often, but not always, correlate with results in the spontaneous assay (10,11). The advantage of the experimental assay is that, unlike the spontaneous assay, it gives highly reproducible numbers for quantitative comparisons [for a review of assays for metastasis, see Nicolson and Poste (6)]. These two assays have been used in the majority of the studies evaluating the ability of rasH to influence metastasis.

Results in NIH3T3 cells
Thorgeirsson et al. (12) tested the metastatic potential of NIH3T3 cells which had been transformed via transfection with genomic DNA from a patient with acute myeloblastic leukemia. As judged by the experimental metastasis assay in athymic nude mice, those cells which were transformed with exogenous rasH sequences possessed metastatic potential. This was in contrast to spontaneous transformants which were negative in the assay. Rejection due

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to differing NK susceptibility was not the explanation for these results as both NIH3T3 cells, spontaneous transformants and the cells transformed by ras<sup>H</sup> were equally subject to NK killing in vitro and in fact there was substantial killing directed against each of these lines.

The possibility that additional genomic DNA was responsible for the induction of the metastatic phenotype was not eliminated by this work but many reports have now been published demonstrating that cloned, defined ras<sup>H</sup> oncogenes when transferred into NIH3T3 cells result in metastatic potential when the cells are tested in nude mice.

Muschel <i>et al.</i> (13) isolated multiple independent clones of NIH3T3 cells transformed with the ras<sup>H</sup> oncogene from the T24 bladder carcinoma cell line and tested for metastasis in the experimental metastasis assay in athymic nude mice. All of these clones were metastatic using the experimental metastasis assay. In addition, clones of NIH3T3 cells transformed by the viral ras<sup>II</sup> oncogene were also metastatic in the assay. These results were in concordance with those of Egan <i>et al.</i> (14) who demonstrated that the extent of metastasis, i.e. the number of lung colonies, was proportional to the levels of P21 in any given transformant. This group also found that the transformed NIH3T3 cells gave rise to metastasis in the spontaneous metastasis assay.

It was possible that the ability to induce metastatic behavior in NIH3T3 cells was merely a property of transformation; however, NIH 3T3 cells transformed by other oncogenes do not necessarily become metastatic. In Table I, we show representative data from our experiments indicating the extent of metastasis in the experimental metastasis assay by NIH3T3 cells transformed by various oncogenes. Those transformed by ras<sup>H</sup>, either the viral or the T24 oncogene, give rise to lung colonies. In contrast, NIH3T3 cells transformed by c-mos or v-sarc were highly tumorigenic, with rapidly growing tumors, but were much less metastatic. These results may appear to be in conflict with those of Egan <i>et al.</i> (14) who have suggested that all oncogenes which act as tyrosine kinases are capable of inducing metastasis in NIH3T3 cells, because after introduction of v-mos, v-sarc, v-raf and others the cells induced lung colonies. In these experiments, 3 x 10<sup>5</sup> cells were injected/mouse while in the experiments described above, 5 x 10<sup>4</sup> were used. It should be noted that these quantities of NIH3T3 cells rendered tumorigenic with myc or P53 did not form lung colonies at that dosage. Thus, there appears to be a quantitative difference with ras which is apparently the most effective.

Because the activated ras<sup>H</sup> oncogene could induce metastatic behavior in NIH3T3 cells, it became of interest to test the effect of elevated levels of the proto-oncogene. The ras<sup>H</sup> oncogene is distinguished from its normal counterpart by a point mutation (4). In the T24 bladder carcinoma, the mutation is at the position coding for the 12th amino acid, as it is in the viral oncogene. However, mutation at position 61 as well as other points will achieve the same effect. Although transformation of the proto-oncogene, i.e. the normal gene, does not result in transformation, Chang <i>et al.</i> (15) has shown that the proto-oncogene when joined to the long terminal repeat of the Moloney leukemia virus, a sequence including a powerful promoter and transcriptional enhancer, could now transform NIH3T3 cells although with a lower efficiency than the oncogene. The transformants were also tumorigenic. Muschel <i>et al.</i> (13) then tested cells transformed using a similar construction and, as expected, these transformants contained greatly elevated levels of normal P21. When tested for tumorigenicity, they formed tumors which grew at the same rate as NIH3T3 cells transformed by the ras<sup>H</sup> oncogene, but in the experimental metastasis assay, they were essentially negative (see Table I). In similar experiments, Bradley <i>et al.</i> (16) found that NIH3T3 cells transformed by the ras<sup>H</sup> oncogene were metastatic both in the experimental assay and in the spontaneous metastasis assay. In apparent contradiction to the results of

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**Table I. Metastatic potential of transformed NIH3T3 cells**

<table>
<thead>
<tr>
<th>Transforming gene</th>
<th>Clone</th>
<th>No. of mice injected s.c./no. with tumors</th>
<th>Days to reach 2 cm diameter</th>
<th>No. of mice injected i.v./no. with metastases</th>
<th>Average no./mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>v-ras&lt;sup&gt;H&lt;/sup&gt;</td>
<td>A</td>
<td>7/7</td>
<td>6-13</td>
<td>5/5</td>
<td>97 + 12</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>3/3</td>
<td></td>
<td>73 + 9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>4/4</td>
<td></td>
<td>77 + 9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>11/11</td>
<td></td>
<td>70 + 9</td>
<td></td>
</tr>
<tr>
<td>c-ras oncogene</td>
<td>A</td>
<td>7/7</td>
<td>13-17</td>
<td>20/20</td>
<td>11 + 8</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>5/5</td>
<td></td>
<td>28 + 6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>5/4</td>
<td></td>
<td>4 + 3</td>
<td></td>
</tr>
<tr>
<td>c-ras&lt;sup&gt;II&lt;/sup&gt; proto-oncogene</td>
<td>A</td>
<td>7/7</td>
<td>12-19</td>
<td>16/1</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>10/1</td>
<td></td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>c-mos oncogene</td>
<td>A</td>
<td>7/7</td>
<td>15-19</td>
<td>8/0</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>8/0</td>
<td></td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>8/0</td>
<td></td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>src oncogene</td>
<td>A</td>
<td>7/7</td>
<td>14-20</td>
<td>8/1</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>8/1</td>
<td></td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>8/0</td>
<td></td>
<td>0</td>
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</tr>
</tbody>
</table>

The data for the v-ras<sup>H</sup>, c-ras<sup>II</sup> oncogene and proto-oncogene are taken from Muschel <i>et al.</i> (13). The NIH3T3 cells were transfected with plasmids containing the oncogenes. No carrier DNA was used. Transformants were isolated with cloning cylinders, grown up, their status was confirmed with Southern blotting to assess incorporation of the appropriate DNA and immunoprecipitation of ras P21 to verify gene expression. The metastasis assays were done in BALB/c nude mice females 4-6 weeks of age. For tumorigenicity, 5 x 10<sup>5</sup> cells were injected s.c. For the experimental assay, cells were trypsinized, allowed to incubate for 2 h in medium and single cell suspensions of 5 x 10<sup>4</sup> cells were injected into the tail veins. After 4 weeks the animals were autopsied. To test the c-mos oncogene, we used a plasmid containing the upstream deleted human c-mos clone which was described by Wood <i>et al.</i> (44). For src, a construction of the avian src oncogene linked to a Moloney leukemia virus LTR as described by Anderson and Scolnick (45) was used.
Muschel et al. (13), Bradley et al. (16) found that NIH3T3 cells transformed with elevated levels of the proto-oncogene were metastatic. A.H. Greenberg (personal communication) probably has provided the resolution of these reports in that moderate levels of P21 lead to transformation but not metastasis while higher levels yield metastatic cells. Muschel et al. were probably using cells with lower levels than those used by Bradley et al.

The experiments of Egan et al. (14) further confirm the effect of ras \(^{H}\) on metastasis by showing that the extent of metastatic potential is proportional to the level of ras \(^{H}\) in both spontaneous and experimental metastasis assays. In a first set of experiments, metastatic potential was found to correlate with the level of ras \(^{H}\) expression in NIH3T3 cells transformed with the ras \(^{H}\) from the bladder carcinoma. Furthermore, those rare metastatic variants isolated from the cells with barely detectable ras \(^{H}\) were found to have high levels of ras \(^{H}\) expression. At the DNA level, these cells were found to have undergone rearrangement or amplification of the introduced ras \(^{H}\) oncogene. Thus, the level of ras expression seemed to correlate directly with metastatic potential. Furthermore, the threshold level of ras \(^{H}\) required for tumorigenicity seems to be less than that for metastasis.

In a second set of experiments, they introduced a ras \(^{H}\) gene linked to a steroid-responsive promoter from the mouse mammary tumor virus. The number of metastases increased >2-fold after pretreatment with dexamethasone, further confirming the dose dependence of metastasis upon oncogene expression.

Bondy et al. (18) have demonstrated the ability of ras \(^{H}\)-transformed cells to behave aggressively in another assay of metastasis, that using the chicken chorioallantoic membrane. In this assay, cells are injected into the veins of that membrane and metastasis is measured by looking for colonies within the liver of the chicken embryo. NIH3T3 cells transformed by ras \(^{H}\) are positive in this assay. Sarc-transformed cells are also positive in this study.

Greig et al. (19) also studied the effect of the ras \(^{H}\) oncogene upon metastatic potential. They obtained a clone of NIH3T3 cells which had been transfected with the ras \(^{H}\) oncogene, and found these cells to be metastatic in nude mice. Unlike other workers, they also found that native NIH3T3 cells were positive in metastasis assays, although in some cases only after 6 months. As they point out, and as was meticulously described by Todaro and Green when they described the development of this type of cell line, the method of passing these cells greatly influences the conversion of NIH3T3 cells to tumorigenic cells. The methods used in culturing these cells might account for the differences between the results of Greig et al. and those of other groups cited here (see also Van Roy et al. (20)). However, this work points out the difficulty of extrapolating from results in NIH3T3 cells to other cells. Because of their ready susceptibility to transformation, their aneuploidy and other unique features, results obtained in NIH3T3 cells may not be generalizable to other cell types. Thus, it was important to ascertain the effect of the ras \(^{H}\) oncogene on metastasis in other cell types.

Effect of ras \(^{H}\) on diploid cells

The ras oncogene also induces metastatic behavior in transformed primary fibroblasts. Muschel et al. (13) tested experimental and spontaneous metastasis by several lines of diploid rodent primary cells which had been transformed by Spandios and Wilkie (21) using the ras \(^{H}\) gene linked to an enhancer. All four lines tested were metastatic including transformed rat skin cells, rat muscle cells and Chinese hamster lung fibroblasts. Pozzatti et al. (22) extended this work by examining a series of rat embryo cells which had been transformed by either ras \(^{H}\) in co-transfection with a dominant selectable marker (pRSVneo) or by ras \(^{H}\) linked to an SV40 enhancer also in a co-transfection experiment. These clones were all highly metastatic in both spontaneous and experimental metastasis assays. In one case, the cells were tested as early as the third passage and were found to be metastatic even at that time. Thus, the ability of the ras \(^{H}\) oncogene to induce metastasis is not limited to NIH3T3 cells, but occurs even after transformation of primary, diploid fibroblasts.

Since the cells used initially were diploid, it was of interest to determine the nature of the karyotypic changes which might have developed during transformation. Surprisingly, Muschel et al. (23) found that some of these lines were apparently diploid after trypsin—Giemsa banding. Even those cell lines in which some chromosomal aberrations were found had near diploid karyotypes with a trisomy or a single translocation and duplication. The metastases which had arisen from the diploid cells were also diploid without any karyotypic abnormalities noted after banding.

These experiments confirmed the ability of the ras \(^{H}\) oncogene to induce metastasis, even after transformation of primary, diploid cells. Pozzatti et al. (22) described one exception. When ras \(^{H}\) was used in conjunction with the cooperating oncogene adenoviral gene E1A, the resultant cells were never metastatic in the spontaneous assay and only occasionally positive in the experimental assay. The explanation for this seemingly inhibitory effect of AdE1A on metastasis is not known at this time. It is possible that it may be an effect of a surface alteration in the cells leading to immune recognition, even in nude mice, but against this explanation is the observation that the intrinsic capacity of the cells to secrete type IV collagenase activity has been altered (23). Type IV collagenase is an enzyme which specifically degrades the basement membrane collagen, type IV collagen, and whose activity has been implicated in metastasis (24, 25). Metastatic cells often secrete high levels of this enzyme and inhibitors of proteases, which inhibit this enzyme’s activity, also eliminate the capacity of cells to invade into chicken chorioallantoic membranes. While rat embryo cells transformed with ras \(^{H}\) or ras \(^{H}\) plus v- myc secreted easily detectable amounts of this enzyme, the cells transformed with ras \(^{H}\) AdE1A secreted only baseline levels of activity, correlating with their low metastatic potential. This result implies that the intrinsic capacity of the cells for metastasis had been affected by the co-transfection with AdE1A.

Effect of ras \(^{H}\) on tumorigenic, non-metastatic cells lines

Further confirmation of the ability of the ras \(^{H}\) oncogene to induce metastatic behavior in non-metastatic cells has come from experiments in which ras \(^{H}\) has been introduced into transformed, tumorigenic but non-metastatic cells, and has converted these cells into metastasizing populations. Vosdien et al. (26) reported an experiment of nature in a lymphoma cell line of which one subclone was metastatic while the parent was not. The metastatic subclone turned out to carry an activated ras \(^{H}\) gene unlike the parent. Vosdien et al. (27) later amplified on this result by introducing ras \(^{H}\) into a highly tumorigenic cell line derived from a murine mammary carcinoma. While the parent cell had been weakly metastatic, the derivatives which had been transfected with ras were all highly metastatic. Most convincingly, they isolated cells from the lung nodules and again tested those isolated for metastatic ability. While most of the clones proved to be equally metastatic when tested a second time, one of the clones was not. Upon further analysis, this clone was found to have lost the introduced oncogene, thus further proving that the intro-
duction of this gene had been responsible for the induction of metastatic potential.

Collard *et al.* (28) had similar results when they introduced ras into T lymphoma cells. These cells acquired invasiveness and they metastasized in the experimental metastasis assay. The extent of metastasis was again proportional to the level of ras$^H$-specific mRNA.

Waghorne *et al.* (29) and Kerbel *et al.* (30) performed similar experiments using the SPI cell, a cell line isolated from a murine mammary carcinoma. They introduced the ras$^H$ oncogene and all clones which had incorporated ras$^H$ now were metastatic in the spontaneous metastasis assay. These cells were already positive in the i.v. assay. In addition, they found occasional metastatic clones arising in control transfections. For this cell line, exposure to Ca$^{2+}$ seems to result in occasional metastatic clones but most of the cells so exposed do not become metastatic. This ability of Ca$^{2+}$ to trigger metastatic behavior has not been generally observed. In contrast, every cell line which had ras$^H$ became metastatic. Using the proto-oncogene, they found that the normal gene was unable to duplicate the dramatic increase in metastatic capacity which followed introduction of the oncogene.

Introduction of ras$^H$ into many types of tumorigenic but non-metastatic cells results in metastatic cells. These results then raise questions about whether ras$^H$ is involved in the induction of metastasis in human tumors.

**Relevance to carcinogenesis**

At this time, it is not clear if ras$^H$ involvement is significant in the development of metastasis in human tumors. In general, the data is consistent with the position that ras may be important but other genes or gene functions must be involved in addition to ras. The results in neuroblastoma that clinical stage directly correlates with N-myc amplification is one such factor (31). The experimental evidence we will consider includes measurements of ras levels in actual tumors and the behavior of tumors which develop after treatment with carcinogens which activate ras oncogenes.

ras oncogenes have been sought in natural human tumors at the DNA, RNA and protein level. Using transfection of genomic DNA into NIH3T3 cells and assaying for transformation, various workers have estimated that between 10 and 30% of human tumors have activated oncogenes (32). Of these, the majority prove to be in the ras family, either Harvey, Kirsten or N. Other workers have screened human colon carcinomas and the precursor lesion, adenomatous polyps, techniques which can distinguish single base changes. These methods allow the detection of the point mutations which can activate ras genes. With these methods, it has been reported that up to 30% of colon carcinomas have activated ras (33,34). However, the activated gene is found in the non-invasive adenomas as well as the carcinomas. Furthermore, there was no correlation with metastasis and the presence of the gene. Of course, in an immunocompetent individual, additional changes may be required for a metastasis to escape immune surveillance.

Several groups have measured ras protein levels in colorectal tumors. They found no correlation of ras levels with biological behavior of the tumor. However, these workers and others did not distinguish the activated from the proto-oncogene. As was noted earlier, the proto-oncogene is only weakly active in inducing metastasis, so these results are difficult to interpret.

Although other workers have found 30% of colon carcinomas with mutated oncogenes (33,34), Gallick (35) did not detect any aberrantly moving P21. The point mutations which lead to activation of the oncogene also result in a mobility shift of the protein, with the mutated protein running slower than the proto-oncogene. Thus, using gel electrophoresis, it is possible in theory to determine if activated ras oncogenes are present. The discrepancy between this work and that at the DNA level remains to be explained.

Thor *et al.* (37) examined ras levels in colorectal tumors by making sections and then staining with immunoperoxidase techniques. They found an increased level of ras at the invasive fronts of tumors. However, this work has not been verified by others. Ohuchi *et al.* (38) found no difference in staining in differing portions of gastric tumors. Ohuchi *et al.* (38) also found no difference in mammary cancers. The antibodies used in both these studies do not distinguish between activated and normal genes. Other investigators have found a correlation between ras expression and breast cancer stage (40-43). Hand *et al.* (42) examined ras levels in tumors by making sections and then staining with immunoperoxidase techniques. They found an increased level of ras at the invasive fronts of tumors.

Thus, activated ras and elevated P21 is clearly found in a significant number of tumors. Whether this finding is important in terms of metastasis is uncertain at this time. It may depend on tumor histologic type.

**Activation of ras$^H$ with carcinogens**

Dimethylbenz[a]anthracene (DMBA) has long been established as a tumor initiator in the development of skin papillomas in appropriate strains of mice. When the application of DMBA to the skin is followed by treatment with a tumor promoter such as croton oil, the mice develop papillomas. Only rarely, ~1% of the time, do they develop carcinomas. Balmain *et al.* have demonstrated that the vast majority of the papillomas have an activated ras$^H$ gene (44,45). Thus, the papillomas, which are benign, non-invasive, non-metastatic tumors, have an activated ras$^H$ oncogene. Clearly in this case, in an animal host the acquisition of the activated ras$^H$ oncogene is not sufficient to induce metastasis. Although the results from the studies of colon carcinoma are less decisive, they lead to the same conclusion, that the presence of an activated ras$^H$ gene is not sufficient to induce metastasis. In contrast, the work of Harper *et al.* (46) led to the opposite conclusion. They took cell lines which formed benign papillomas upon introduction onto the skin of mice and introduced the ras oncogene by transfection with calcium phosphate DNA precipitates into these cells. They were now malignant in behavior (46).

The work of Muschel *et al.* (13) does describe one experimental situation in which an analogous result occurred. When the activated ras$^H$ oncogene was introduced into C127 cells, these cells were highly tumorigenic but were not metastatic. Even when ras$^H$ expression was boosted to very high levels, these cells still remained non-metastatic. Apparently in many natural tumors, the presence of the oncogene ras$^H$ is not sufficient to induce metastasis as it seems to be in many tissue culture systems.

**Is it possible to reconcile the ability of ras$^H$ to induce metastasis with the concept of heterogeneity of tumors?**

Over the last 20 years, it has repeatedly been found that upon subcloning, the cells from a tumor will often prove to be different even though that tumor was presumably of a clonal origin. Thus, when cells from a metastatic tumor are subcloned, some of the clones are indeed metastatic but some will be non-metastatic.
(1,10). This result has been interpreted to suggest that tumors first arise without metastatic potential but later acquire the ability to metastasize. This acquisition of new characteristics during the growth of a tumor occurs with respect to many characteristics, including growth rate, drug resistance and karyotype. In the case of markers which confer a selective advantage, the emergence of cells with these characteristics is perhaps not surprising. The genetic mechanisms for the development of some of these features is known. Drug resistance for both methotrexate resistance and multiple drug resistance has been attributed to amplification of genes which code for proteins which can inactivate the effect of the drug.

With regard to characteristics such as metastasis for which a selective advantage is not apparent, the mechanisms which may be responsible for the selection of such clones are not known. Cillo et al. (47) have demonstrated that the highly metastatic B16 melanoma cell when exposed to methotrexate develops resistance at a 1000-fold greater rate than the weakly metastatic cell. The development of resistance in this system is due to amplification of the dihydrofolate reductase gene. These workers suggest that the development of metastasis in one cell line might be due to the increased ability to amplify DNA. The argument against this interpretation is that one would expect that all cells which had amplified the appropriate, and of course unidentified, DNA should be substantially more metastatic than those cells which had not yet amplified that DNA.

Since many of these tumors are highly aneuploid, the continual non-disjunction which must be occurring may also play an important role in the emergence of variant clones. Thus, within many tumorigenic populations, clones which have newly acquired the ability to metastasize will spontaneously occur. The mechanism through which these clones arise is unknown. The ras oncogene is able to induce metastasis uniformly in such cells as well as in non-transformed cells. Nonetheless, it is apparent that activation of the ras oncogene does not initiate metastatic behavior in many spontaneous tumors, although there may be some in which this is an important factor. Studies of the mechanism through which the ras oncogene can induce metastasis will perhaps lead to information which will help explain the induction of metastasis in spontaneous tumors.

References


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