Eur J Cancer Clin Oncol, Vol. 22, No. 3, pp. 345–348, 1986.
Printed in Great Britain.

0277-5379/86$3.00+0.00
Pergamon Press Ltd.

Molecular Biology of Metastases: A Review of Recent Approaches

LANCE A. LIOTTA
Laboratory of Pathology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20205, *U.S.A.*

METASTASES are the major cause of treatment failure for cancer patients. There is a great need to develop new clinical methods to (a) predict the aggressiveness of a patient's individual tumor and (b) localize and destroy established metastases growing in internal body sites. Therefore, a major challenge to cancer scientists is to identify biochemical factors (specific gene products) which are augmented in metastatic tumor cells and are functionally associated with their malignant propensity. Measurement of the level of such factors may provide a predictive index of the aggressiveness of the patient's tumor. Antibodies to the same metastatic factors could be applied to radioscintography to localize clinically silent metastases. Finally, pharmacologic agents which bind to and/or block the metastatic factors may be ultimately useful in the therapy of metastases.

A metastasis is the end result of a complex series of steps through which tumor cells from the primary mass invade host tissue, enter the circulation, evade host defenses, arrest in the target organ, and invade and grow out in that organ to initiate a metastatic colony. It is apparent that this complex process must involve multiple gene products. Metastatic propensity is assumed to be distinctly separate from tumorigenicity alone. Defined 'oncogenes' have been identified which can induce the tumorigenic state. However, the role of oncogenes in the metastatic process has been unclear. A number of laboratories are now attempting to identify 'metastases genes' which elicit or augment the metastatic phenotype. The following major approaches are being used to identify metastases genes:

A. *Traditional molecular biology*
Cloning of genes which code for proteins known to be involved in at least one step in the metastatic process [1–5].

B. *Somatic cell hybridization*
Identification of augmented or suppressed gene products in hybrid tumor cells formed from normal cells × metastatic tumor cells or metastatic × nonmetastatic tumor cells [6, 7].

C. *DNA transfection*
Transfection of high molecular weight DNA fragments or isolated genes (including known oncogenes) into normal or nonmetastatic tumor cells in order to induce the metastatic phenotype [8–11].

D. *Differential cDNA screening*
Selection of specific genes which are differentially expresses in metastatic versus nonmetastatic tumor cells [12, 13].

Examples of recent progress using some of the above approaches will now be briefly reviewed.

A variety of biochemical factors have been associated with defined phases of invasion or metastases. Cell surface receptors for collagen, glycoproteins such as laminin, or proteoglycans are all postulated to facilitate tumor cell attachment, which is thought to be the first step of invasion [1]. Attachment may trigger the release of degradative enzymes which facilitate the penetration of the tumor cells through tissue barriers. Important enzymes include collagenases, serine proteases, and cathepsins. Once the tumor cell has entered the target tissue, specific growth factors may be required for colony formation. Throughout all steps of metastases, evasion of host immune defenses may involve a variety of tumor gene products.

The laminin receptor is a 67,000 KDa cell surface protein isolated in our laboratory and thought to play a role in tumor cell attachment to basement membranes [2–4]. Monoclonal antibodies to the human breast cancer laminin receptor have been used to isolate a *c*DNA clone for the receptor (Sobel *et al.*, submitted). The monoclonal antibody (LRmAb) was used to screen a lambda gtll expression vector *c*DNA library. When the

lambda phage infects bacteria it forms plaques. Since the inserted *c*DNA expressed the protein of its sequence, the *c*DNA encoding a specific protein inserted in the phage can then be identified using antibodies to that protein to screen the plaques. 1.6 Million plaques were screened and six plaques were selected which exhibited specific immunoreactivity with LRmAb. The *c*DNA clones were used to prepare a nick translated probe for the laminin receptor. The probe was hybridized to RNA from human tumor cells previously studied for their ability to specifically bind labeled laminin. The probe was specific for a single message of the size sufficient to code for the laminin receptor protein. Furthermore, the level of message correlated with the laminin binding activity of each tumor cell type.

Another example of a cloned gene for a protein involved in metastases is the gene for plasminogen activator (PA) [5]. This protease is thought to augment tumor invasion through the generation of plasmin which can degrade extracellular matrix proteins and activate other latent proteases. Analysis of the product of the urokinase PA gene has revealed that the enzyme is secreted as an inactive single-chain pro-urokinase zymogen. It is activated by proteolysis which removes lys residue to generate a two chain active molecule. The catalytic activity is located on the amino terminal portion (residues 1–135) of the chain. The regulation of PA function in tissues may be linked to the activation of the pro-urokinase, and this has a number of implications for tumor invasion.

Somatic cell hybridization is another major approach to study genes involved in metastases. The results from this type of approach must be analyzed carefully since hybrid cells may be unstable and the exact karyotypic features of each hybrid clone may be different. However, it is possible to reliably interpret the data derived from an individual hybridization system in regard to the correlation of a specific gene product with a biologic phenotype. Specifically, the metastatic propensity of individual hybrid cell lines has been compared with their secretion of proteases. In one such study, fusion of tumor cell lines of high and low metastatic propensity resulted in maintenance or enhancement of the high metastatic phenotype [6]. When the metastatic cells were fused with normal cells, the metastatic phenotype was suppressed. The secretion of basement membrane degrading type IV collagenase in these hybrids correlated well with their metastatic behavior. Thus for this system, type IV collagenase may be genetically linked with the expression of the metastatic phenotype. Another proteolytic enzyme, PA, has also been studied in tumor cell hybrids. Ramshaw *et al.* [7] demonstrated that non-metastatic hybrids expressed low levels of PA. However, as the hybrids reverted to the matastatic phenotype during culture *in vitro*, the PA level was still found to be low. Thus in the latter system, high levels of PA were apparently not required for metastases.

A series of recent reports have demonstrated that transfection of tumor DNA into suitable nontumorigenic and nonmetastatic recipient cells can induce those cells to become fully metastatic. Studies from our laboratory [8] as well as those of Bernstein and Weinberg [9] and Koestler *et al.* [10] have shown that NIH-3T3 cells transfected with human tumor DNA containing the ras^H oncogene are metastatic when injected intravenously or subcutaneously into nude mice. The ras^H oncogene, by itself, transfected into NIH-3T3 cells can induce these cells to be fully metastatic in nude mice. Further studies by Muschel *et al.* [11] have shown that the activated ras^H but not the proto-oncogene (normal cellular counterpart of the ras oncogene) can induce NIH-3T3 cells to be fully metastatic. A plasmid construct of the proto-oncogene which induces large amounts of the normal p21 protein will induce NIH-3T3 cells to be fully tumorigenic but not metastatic in nude mice. Consequently, tumour growth potential alone was not sufficient to induce the metastatic behavior in nude mice. Diploid fibroblasts transfected with the T24 ras^H also produced extensive spontaneous metastases. Thus, induction of the complete metastatic phenotype by ras^H does not require the use of aneuploid or otherwise 'unusual' recipient cells such as NIH-3T3 cells.

More than one hypothesis can be set forth to explain how a single transforming gene can induce the complex metastatic process in the appropriate cell type. The oncogene, in an additive fashion, could confer growth potential on cells which are already expressing all the necessary gene products for metastases. A second explanation is that the integrated oncogene induces a genetic instability. This instability then results in the production of metastatic variants which are selected *in vivo*. A third possibility is that transformation by the ras oncogene induces a cascade of cellular gene products normally expressed by migrating cells during embryogenesis or tissue remodeling. Evaluation of these alternative hypotheses will be the subject of future research.

We can conclude that ras^H oncogene transformation may provide a useful model system to identify metastatic genes. Through transfection or *c*DNA library screening, genes can now be identified which are altered in their expression in the metastatic ras^H transformants compared to the benign counterparts. It remains unknown as to whether the ras oncogene plays a necessary role in human tumors. To date, the data is conflicting. Some investigators have found increased express-

ion of ras in invasive metastatic tumors compared to benign counterparts [14]. Other investigators have found no difference in ras content in metastases compared to the primary tumor [15, 16]. Unfortunately, most of these studies could not distinguish the activated ras from the proto-oncogene form.

The molecular genetics approach may be valuable to study the tumor cell host interactions. Bernstein and Weinberg [9] have found that ras^H transformed 3T3 cells which are fully metastatic in nude mice are only tumorigenic in an immunocompetent strain of mice. This is not unexpected since host immune factors are well known to modulate the metastatic process. This group went further to transfect a human tumor DNA segment which could restore the metastatic capacity in this alternate strain of mice. This gene may therefore play an important role, not in the intrinsic aggressiveness of tumor cells, but in their interaction with the host. Genes which regulate major histocompatibility antigens on the tumor cell surface have previously been shown to affect the metastatic process [17]. Whether the Bernstein and Weinberg gene is related to MHC antigen expression, remains to be determined.

It is unlikely that one or two genes alone can be responsible for expression of the complete metastatic phenotype. Instead, it would seem more likely that a few key genetic changes (which may include activation of an oncogene) can induce a cascade of genetic expression for multiple gene products necessary for all the steps in metastases. This is supported by recent studies of differential messenger RNA levels in metastatic vs. nonmetastatic tumor cells derived from the same parent [12, 13]. The studies revealed some genes which were expressed in greater amounts in the metastatic compared to the nonmetastatic tumor cells and other genes preferentially expressed in the nonmetastatic tumor cells. It should be possible to clone these genes and study their products. The hope is that analysis of the products of such genes and their regulation may provide strategies for tumor diagnosis and metastases therapy.

REFERENCES

1. Liotta LA. Mechanisms of cancer invasion and metastases. In: Devita, Hellman, Rosenberg. eds. *Important Advances in Oncology* 1985. Philadelphia, JB Lippincott, 1985, 28–41.
2. Terranova V, Rao C, Kalebic T, Margulies I, Liotta LA. Laminin receptor on human breast carcinoma cells. *Proc Natl Acad Sci USA* 1983, **80**, 444–448.
3. Liotta LA, Hand P, Rao C, Bryant G, Barsky S, Schlom J. Monoclonal antibodies to the human laminin receptor recognize structurally distinct sites. *Exp Cell Res* 1985 **156**, 117–126.
4. Wewer U *et al.* Preliminary characterization of a laminin receptor *c*DNA clone. Abstract presented at The International Symposium on Biology and Chemistry of Basement Membranes. Mishima, Japan, June, 1985.
5. Blasi F. Plasminogen activator genes: Structure and regulation. Fogarty International Center Conference. NIH, March, 1985.
6. Turpeenniemi-Hujanen T, Thorgeirsson UP, Hart IR, Grant S, Liotta LA. Expression of collagenase IV in murine tumor cell hybrids which differ in metastatic potential. *J Natl Cancer Inst.* 1985, **75**, 99–103.
7. Ramshaw IA, Carlsen S, Wang HC, Badenoch-Jones P. The use of cell fusion to analyze factors involved in tumor cell metastases. *Int J Cancer* 1983, **32**, 471–478.
8. Thorgeirsson U, Hujanen T, Williams J, Westin E, Heilman C, Talmadge J, Liotta-LA. NIH-3T3 cells transfected with human tumor DNA containing activated ras oncogenes express the metastatic phenotype in nude mice. *Mol Cell Biol* 1985, **5**, 259–262.
9. Bernstein S, Weinberg R. Expression of the metastatic phenotype in cells transfected with human metastatic tumor DNA. *Proc Natl Acad Sci USA* 1985, **82**, 1726–1730.
10. Koestler TP, Sweet R, Yokoyama S, Corwin S, Greig R, Poste G. Metastatic properties of ras transfected NIH-3T3 cells. *Proc 75th Annual Meeting of the American Association for Cancer Research* 1984, Abstr. No. 234, **49**.
11. Muschel R, Williams J, Lowy D, Liotta LA. Harvey ras induction of metastatic potential depends upon oncogene activation and type of recipient cell. *Am J Pathol* 1985, **121**, 1–8.
12. Steeg P, Kalebic T, Claysmith A, Liotta L, Sobel M. Levels of specific mRNAs are altered in metastatic cells. *Federation of American Society for Experimental Biology, Proceedings*, Vol. 44, No. 5, Abstr. 5413.
13. Nicolson *et al.* Biochemistry and molecular biology RAW117 large cell lymphoma. In: Liotta LA, Lapis, Rabson eds. *Biochemistry and Molecular Genetics of Cancer Metastases.* The Hague, Martinus Nijhoff, 1985. (in press).
14. Vousden K, Marshall CJ. Three different activated ras genes in mouse tumors: evidence for oncogene activation during progression of a mouse lymphoma. *EMBO J.* 1984, **3**, 913–917.

15. Gallick G, Kurzrock R, Kloetzer W, Arlinghaus R, Gutterman J. Expression of p21 ras in fresh primary and metastatic human colorectal carcinomas. *Proc Natl Acad Sci USA* 1985, **82**, 1975–1799.
16. Kris RM, Avivi A, Bar-Eli M, Alon Y, Carmi P, Schlessinger J, Raz A. Expression of ki-ras oncogene in tumor cell variants exhibiting different metastatic capabilities. *Int J Cancer* 1985, **35**, 227–231.
17. Wallich R, Bulbuc N, Hammerling GJ, Katzav S, Segal S, Feldman M. Abrogation of metastatic properties of tumor cells by de novo expression of H-2K antigens following H-2 gene transfection. *Nature* 1985, **315**, 301–305.