NEW CONCEPTS IN CANCER

Metastasis, Oncogenes and Growth Factors

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NEW CONCEPTS IN CANCER

Metastasis, Oncogenes and Growth Factors

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Preface

Metastasis of tumor cells remains a major clinical problem and an obstacle to curative therapy. In recent years, investigations have been carried out to delineate factors associated with the metastatic process, in the hope of delineating important determinants or sites for therapeutic interference. Other approaches have focused on the identification of qualitative and quantitative differences that may exist between the primary and metastatic tumor tissues. Although differences in the biochemical and molecular properties of some metastatic cells have been reported, the clinical relevance of these findings remains relatively unproven.

The aims of the symposium were threefold: (1) to define and review the clinical status of metastatic disease and the present status of response to radiation and chemotherapy; (2) to identify factors associated with and/or responsible for tumor metastasis; (3) to evaluate the role of various growth factors in metastasis and therapy.

The overall plan of the symposium was to discuss the principles and functions associated with tumor cell metastasis in model systems and to illustrate their relevance to the clinical situation. In recent years, considerable data have been accumulated on clinical materials concerning, for example, the association of certain oncogenes with specific tumor types. Early results suggest a possible relationship between the over-expression of oncogenes and clinical resistance to chemotherapy. Once these observations are confirmed, the task in the future will be to design target-site-specific drugs and to evaluate their therapeutic potential. These and other therapeutic strategies were discussed during the two-day symposium.

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New Therapeutic Approaches in Recurrent Metastatic Disease

M. Boiron

1.1 Introduction

One of the major obstacles limiting successful cancer therapy is the ability of malignant cells to overcome the immune defense system and form resistant foci of metastatic tumor cells in different locations. Until very recently the appearance of metastases meant a high probability of death. Although there is no single clinically useful agent that could be defined as a specific antimetastatic drug, advances have been made in the treatment of some metastatic cancers during the last decade.

The focus of the first day of this symposium is to define the progress that has been made experimentally and clinically to date and to propose new strategies for developing new drugs or therapeutic approaches for the treatment of metastasis.

I will begin this ambitious programme by summarizing the main advances that have been made, separating them into four different approaches, as follows:

(a) better definition and precise quantification of metastatic foci;
(b) better knowledge of the fundamental mechanisms leading to metastatic cells;
(c) progress in cytoreductional chemotherapy;
(d) above all, evidence that some biological response modifiers (BRMs) wield remarkable power in the control of metastatic states.

1.2 Advances in Diagnosis

Imaging techniques such as high-resolution tomodensitometry, nuclear magnetic resonance, external and transluminal echography, body scans and monoclonal antibody labelling allow a better definition and more precise quantification of the modalities involved in the spreading of certain cancers such as lymphomas, breast cancer and small-cell lung cancer. They therefore contribute to a better understanding of the therapeutic activity.

In the same way, the characterization of the heterogeneity of metastases helps to set up therapeutic strategies.

1.3 Advances in Research

During the past few years, impressive progress has been made in our understanding of the basic mechanisms involved in metastatic states. Whatever their scope (oncogenes, antioncogenes, proteases, lipoxygenases) they will certainly contribute to new therapeutic strategies. One of the most important observations to emerge is that tumor cells seem to be heterogeneous not only in their metastatic ability to spread but also in their homing capacity. Not only is the spontaneous generation of phenotypic heterogeneity an inherent feature of tumor cells, but a specific cell phenotype seems to be an important determinant of the probability that a cell will form a metastasis.

Gene amplification is associated with drug resistance as evidenced by DHFR, TS and MDR-1 genes and it could also be involved in tumor progression and metastatic spread. Although the mechanisms underlying such genetic amplification are as yet poorly understood, they already emerge as a possible target for new therapeutic approaches.

1.4 Advances in Chemotherapy

Although chemotherapy has led to a significant increase in the life span of patients suffering from metastatic cancers—for example breast cancer—on the whole, attempts to control such cancers by chemotherapy have been disappointing. The new fact in the field is that a sophisticated strategy of chemotherapy intensification associated with bone marrow autograft allows a significant increase in long-term disease-free survival for some types of cancers: testicular cancers, ovarian cancers, neuroblastomas and breast cancer. In our experience, this increase can be 20 to 30% higher than the level observed with conventional chemotherapy.

Another recent advance deals with the development of new molecules with potential anti-cancer activity such as ellipticins, fotemustine and vinorelbine. This last molecule is a new synthetic derivative of vinca alkaloids that displays strong activity against metastatic breast cancer, epidermoid cancer of the lung and Hodgkin's disease. In addition, new families with original mechanisms of action have been developed: taxanes are strong antagonists of tubulin polymerization that have demonstrated activity in advanced ovarian and small-cell lung cancer. Cyclophosphazenes may well prove to be interesting drugs and specific inhibitors of topoisomerases open a new field to drug therapy.

1.5 Advances in BRM

A fundamental advance in the control of metastatic states is linked to the use of some biological response modifiers such as interferons and interleukin-2.

Interferons

Recombinant α-interferon is active mainly in certain malignant hematological diseases such as hairy-cell leukemia (90% control, prolonged survival of patients) and chronic myelocytic leukemia (70% hematological remission, cytogenetic conversion, either complete or partial in 50% of cases, prolongation of the chronic phase of the disease). In certain solid tumors such as metastatic renal cancer or disseminated melanomas, interferon is active in a small but definite number of cases (around 15% of response with a few complete responses). When IFN is associated with chemotherapy (vinblastine for renal cancer, DTIC for melanomas), there is an additional action and the response percentage can reach 25%.

Interleukin-2

The most impressive results concern the evidence of antitumoral activity of an immunotherapy associating IL-2 and LAK in metastatic cancers. Indeed this discovery has been a remarkable example of tenacity. From 1981 to 1984 no response was noted in the first 66 patients treated either by cells alone or by IL-2 alone. It was only when high doses of IL-2, alone or associated with LAK, were employed that responses were observed in metastatic patients known to be refractory to conventional therapy (as shown by Table 1.1, derived from Rosenberg's data).

Table 1.1 Treatment of metastatic cancers by IL-2 or IL-2+LAKs*

	IL-2	IL-2+LAKs	Total
r	177	119	296
RR	20	43	63 (21%)
CR	4	14	18 (6%)

*Data from Rosenberg, assembled by Maraninchi (1988).

Table 1.2 Results of immunotherapy according to the type of cancer*

Type	IL-2	IL-2+LAKs	Total	%RR
	PR−CR/total	PR−CR/total	PR−CR/total	
Renal	11−4/55	25−8/72	36−12/127	28
Melanoma	9−0/37	10−4/49	19− 4/ 86	22
Colorectal	0−0/13	5−1/30	5− 1/ 43	12
Lymphomas	0−0/ 6	3−1/ 6	3− 1/ 12	25
Lung ADK	0−0/ 1	0−0/ 5	0− 0/ 6	—
Breast	0−0/ 2	0−0/ 2	0− 0/ 4	—
Sarcomas	0−0/ 1	0−0/ 6	0− 0/ 7	—

*Data from Rosenberg, assembled by Maraninchi (1988).

Actually, as shown in Table 1.2, also derived from Rosenberg's data, response is very variable according to the cancer type. One should note that minimal responses or responses not completely measurable for evaluation are not included in Rosenberg's statements. In other words, criteria for response are very stringent.

The mean duration of responses is 10 months; the mean duration of complete responses is higher than 17 months; responders have a better survival rate. Although the percentage of objective responses remained low, these provocative results have generated a number of questions and new trials.

Role of LAK cells

On the whole, it seems that the response rate and, above all, the complete response rate, are maximum when IL-2 is associated with LAK; LAK alone have no effect. But a randomized study was mandatory to assess this point correctly. This study has been performed by Rosenberg: it seems that the adjunction of LAK has no major impact on antitumoral activity of IL-2 provided that IL-2 is given at very high doses which, in passing, probably generate noticeable LAK activity *in vivo*. It remains, however, that there are more complete remissions with LAK+IL-2 and that the less immunogenic cancers, such as renal cancer, are better responders to LAK than the more immunogenic ones such as melanomas.

Role of non-LAK cells: TILs

TILs are a subpopulation of activated T-lymphocytes CD8+DR+, which are generated from tumor specimens, cultivated *in vitro* and reinjected to patients at a very high number ($>10^{11}$). TILs generation depends on a pretreatment by cyclophosphamide. In experimental models, TILs are 50 to 100 times more powerful than LAK for inducing an antitumor response. In man, the role of TILs is mainly important in melanomas.

Association of IL-2 with other BRM

The goal of these trials is to enhance the antitumoral activity of IL-2:

(a) by adjunction of another interleukin with tumoricidal activity, TNF, IFN-α or -γ;
(b) by potentialization of IL-2 activity, for example up-regulation of MHC on tumor cells (IFN-α or -γ);
(c) by suractivation of lymphoid populations (IL-2+IL-4);
(d) by stimulation of non-lymphoid cells playing a role in the antitumor response (IL-2+GM-CSF).

Preliminary results of these associations show that the adjunction of TNF to IL-2 does not significantly enhance the antitumor response of IL-2.

On the other hand, the combination of IL-2 and IFN-α looks promising, mainly in renal cancers.

Preliminary results indicate that metastatic breast cancer could be another candidate for IL-2+IFN-α. A great advantage of this strategy would be a substantial reduction in cost due to the fact that LAK would no longer be useful.

Association of IL-2 with chemotherapy

Despite immunosuppression induced by chemotherapy, it seems worthwhile to try to associate IL-2 and chemotherapy in metastatic cancers. Furthermore, cyclophosphamide (and total-body irradiation) potentializes more than 50 times the IL-2 action by blocking generation of suppressor cells against LAK or TILs. Several protocols are currently being carried out but it is too early to analyze the results.

IL-2 in adjuvant therapy

Instead of treating refractory and metastatic patients with a high burden of

tumor cells, it could be interesting to put IL-2 in adjuvant therapy after surgery of the tumor (melanoma, colorectal cancers). These trials are under study.

Toxicity

Finally, I would like to mention some problems related to toxicity of IL-2. At the massive doses used to obtain antitumor response, IL-2 is formidably toxic. However, in a suitable environment these treatments are certainly feasible at a high scale. If the toxicity is decreased by association of IL-2 with cells stimulated *ex vivo* or by continuous perfusions of IL-2 then the therapeutic efficacy is partially lost. Actually, it is necessary to always work with the maximal tolerated dose, which can vary from patient to patient.

In brief, intensive stimulation of the human immune system by supra-physiological doses of IL-2 allows one to obtain tumoral regression superior or equal to the best cytotoxic chemotherapy (Table 1.3).

Table 1.3 Antitumoral response after IL-2 (High doses) in 377 patients*

Treatment	Renal cancer	Melanoma	Colorectal cancer		
	RR−CR/total	RR−CR/total	RR−CR/total	RR/total	%
IL-2	11−4/55	9−0/37	0−0/13	20/105	19
IL-2−LAK	25−8/72	10−4/49	5−1/30	40/151	26
TNF+IL-2	2−0/ 7	1−1/14	0−0/ 2	3/ 23	13
Cy+IL-2	—	2−0/13	—	2/ 13	15
Cy+IL-2 +TILS	0−0/ 8	11−1/20	—	11/ 28	39
α-IFN+IL-2	7−4/15	3−0/14	1−0/ 2	11/ 31	35
Fu-FoL+IL-2	—	—	6−0/21	6/ 21	29
RR/total	45/157	36/147	12/68	93/372	
(%)	(29)	(24)	(18)	(25)	

*Data from Rosenberg, assembled by Maraninchi (1988).

Provided one has the support of an intensive-care unit it is reasonable to give IL-2 at maximum tolerable doses. IL-2 is definitely active in patients without a prestimulation of target cells *ex vivo*. The activity score can be enhanced by association of IL-2 with:

other modulators of the biological response;
antitumor chemotherapy.

At Saint-Louis Hospital we are trying phase I of IL-2 associated with intensive radiochemotherapy and bone marrow autografting in early or late phases of Hodgkin and non-Hodgkin lymphomas.

1.6 Conclusions

The main point as regards the curability of metastatic/diffuse cancers seems to be related to the degree of genetic heterogeneity of the tumor.

If this degree is low then results obtained by conventional chemotherapy are rather good: testicular cancers, 85% cured; lymphomas, 60% cured; embryonnal sarcomas, more than 60% cured.

If this degree is high then results remain poor: metastatic breast cancer, 10% cured; metastatic colony cancer, practically no cure; ovarian cancer, 25 to 30% cured in diffuse forms; no cure in truly metastatic forms.

It is clear that the understanding of the heterogeneity of cancer stem cells, and of their spreading ability, and the comprehension of the role of cytotoxic lymphoid and non-lymphoid cells allow for adequate design of therapy with biological response modifiers and cytotoxic drugs. It is also clear that substantial progress should derive from this strategy. In this way, some of the most sensitive metastatic cancers (malignant melanoma, brain metastases, renal cell carcinoma) have already been shown to respond to such approaches.

Finally, even if some obvious progress has already been made a lot of questions remain. It will require more dialogue between clinicians and experimental scientists to develop effective antimetastatic therapies. This is what we shall try to do throughout this symposium.

Reference

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2

Features of Tumor Progression in H-*ras* transformed Rat Embryo Cells

Ruth-J. Muschel, Martha Sack, Marisa C. Weiss, Vincent J. Bakanauskas and W. Gillies McKenna

2.1 Introduction

The initial description of oncogenes was based on the ability of modified cellular genes to induce transformation in tissue culture or tumorigenicity when cells expressing those genes were introduced into animals. Since the ability of a tumor to metastasize is an additional property of a tumor cell, distinct from the formation of a tumor (Fidler and Hart, 1982; Kripke *et al.*, 1978), one might expect that oncogenes would play no role in metastasis or in tumor progression in general. On the other hand, activated *ras* oncogenes are found frequently in some types of human carcinoma such as colon (Vogelstein and Gillespie, 1979) and pancreatic carcinoma (Almoguera *et al.*, 1988) and in the case of colorectal carcinoma the activation of *ras* may be a late event in the development of the tumor, suggesting that the actual effect of *ras* may be to influence tumor progression rather than the initiation event. Thus, studies of the effect of oncogenes upon metastasis and other parameters of tumor progression as studied in the laboratory might help to evaluate the potential influence these genes may have in tumor progression *in vivo*.

Our approach was to develop *in-vitro* transformation systems using various cloned oncogenes in order to test the effects of these genes upon aspects of tumor progression, such as metastasis or resistance to radiation. In an early set of experiments, we transformed NIH 3T3 cells with the *ras* oncogene and demonstrated that these cells acquired the capacity to metastasize while cells transformed in other ways did not (Muschel *et al.*,

1985). Since NIH 3T3 cells are highly aneuploid (Todaro and Green, 1963), heterogeneous in regard to transformation (Katz, 1986) and readily transform spontaneously, the possibility remained that the induction of metastatic potential in these cells might reflect some of their intrinsic cellular characteristics rather than being a direct effect of the transfected oncogene. We then extended these studies to the transformation of primary, diploid rat embryo cells. Although NIH 3T3 cells when transfected with single oncogenes such as H-*ras* transform at high frequency in seemingly a single step, primary rodent cells are recalcitrant to transformation by this means (Land *et al.*, 1983a; Ruley, 1983). Primary diploid rat cells can be transformed at a low frequency by the H-*ras* gene transfected with a dominant selectable marker (Pozzatti *et al.*, 1986; Spandidos and Wilkie, 1984). When *ras* is transfected with another oncogene such as *myc* or *myb*, the early region of adenovirus E1A or middle T of polyoma virus, transformation frequency is increased by 100- to 1000-fold (Land *et al.*, 1983b). Using rat embryo cells transformed either by H-*ras* plus a dominant selectable marker or by H-*ras* with the co-operating oncogene *myc*, we found that these transformed primary cells were highly metastatic in both the experimental and the spontaneous metastasis assay (Pozzatti *et al.*, 1986). These experiments indicated that the ability of H-*ras* to induce metastatic behavior was generalizable to primary diploid cells and that in fact H-*ras* is able to reliably induce metastatic potential in a wide variety of cells.

We have also studied the effect of *ras* on another parameter of tumor progression, that of resistance to the killing effects of ionizing radiation. Experiments of Sklar (Sklar, 1988) have indicated that NIH 3T3 cells transformed by H-*ras* have increased resistance to radiation compared with NIH 3T3 cells themselves. We found that rat embryo cells transformed by *ras* alone show mild increases in radioresistance while those immortalized with *myc* are identical to the parent cell. Together however, *ras* and *myc* yield transformed rat embryo cells which have high degrees of resistance to radiation (McKenna *et al.*, 1989a, and Table 2.1). These results further suggest that oncogenes may have substantial effects upon the phenotypes associated with tumor progression such as metastasis or radiation resistance.

In an attempt to uncover potential additional genetic events which might also contribute to the development of these phenotypic changes in transformed cells, we initiated studies of the karyotypes of the transformed diploid cells. Although chromosomal aberrations are frequently seen in human solid tumors, these transformed rat embryo cells had minimal karyotypic changes (Muschel *et al.*, 1986; McKenna *et al.*, 1988). However, among the cell lines which had been established through transformation by H-*ras* plus the cooperating oncogene *myc* four out of seven lines we have studied were found to have a deletion of the short (p) arm of