Adjuvant Therapies of Cancer

Edited by
G. Mathé G. Bonadonna S. Salmon

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With 108 Figures and 146 Tables

Springer-Verlag
Berlin Heidelberg New York 1982

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Sponsored by the Swiss League against Cancer

ISBN 3-540-10949-8 Springer-Verlag Berlin Heidelberg New York
ISBN 0-387-10949-8 Springer-Verlag New York Heidelberg Berlin

Library of Congress Cataloging in Publication Data. Main entry under title: Adjuvant therapies of cancer. (Recent results in cancer research; 80) Bibliography: p. Includes index. 1. Cancer–Treatment. I. Mathé, Georges, 1922. II. Bonadonna, G., 1934. III. Salmon, S. E. (Sydney E.), 1936. IV. Series. [DNLM: 1. Adjuvants, Immunologic–Therapeutic use. 2. Adjuvants, Pharmaceutic–Therapeutic use. 3. Neoplasms–Drug therapy. W1 RE106P v. 80/QZ 267 A235] RC261.R35 vol. 80 [RC270.8] 616.99'4s 81-9289 [616.99'406] AACR2.

Typesetting and printing: Carl Ritter GmbH & Co. KG, Wiesbaden
Binding: J. Schäffer OHG, Grünstadt

2125/3140–543210

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Summary

The metastasizing capacity of spontaneous metastases of several murine tumors of different histology, origin, and disseminative pattern was investigated to test the hypothesis that metastases originate from various subpopulations within the primary tumor. Overall, tumor cells from individual metastases did not show greater metastasizing capacity than the cells of the original tumor, although a degree of heterogeneity was seen. Differences in the immunologic profile among metastases of the same tumor were also observed. The possible therapeutic implications of these findings are discussed.

Introduction

Oncologists need not be reminded that the real targets of adjuvant chemotherapy are tumor metastases. Although it can easily be advanced that the formation of metastases is the root of clinical malignancy, our ignorance of metastasis formation, the final result of a complex series of events in which an array of host and tumor factors interact, is still substantial. Yet, to devise more effective and intelligent therapeutic strategies aimed at the prevention and/or treatment of metastatic disease, a more in-depth understanding is needed as to the pathogenesis of invasion, dissemination, and peripheral colonization, and of the properties of metastatic cells vis à vis those of the primary tumor.

The Metastatic Potential of Metastatic Cells

Studies from various groups have recently provided evidence that clones from primary murine neoplasms can differ markedly in various biologic characteristics including their capacity to form secondary tumors [5, 11]. Tumor cell lines could be obtained possessing increased metastasizing capacity or which were capable of homing

* This work was supported by PHS grant R01-CA-12764 from the NCI, USA, and by contract 78.0279.96 from Progetto Finalizzato Controllo Crescita Neoplastica, CNR, Rome, Italy

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selectively at certain anatomic sites [1, 11, 14]. With this background, the contention has been advanced that the formation of secondary tumors is not the result of the random survival of cells which have disseminated from the primary tumor, but that they derive from the establishment and growth of a specialized subpopulation of cells preexisting within the primary tumor. These cells would be endowed with specific and still largely undefined properties allowing them to more effectively complete a highly complex and very selective process [4, 11]. With regard to the latter point, less than 0.1% neoplastic cells from a primary tumor which have reached the circulation may survive arrest in peripheral organs and, still less, establish and grow into an acutal metastasis. If proven true, the hypothesis of the existence of cell subpopulations with intrinsically greater metastasizing ability could obviously have important implications. It was, therefore, of interest to investigate the metastasizing capacity of cells from spontaneous experimental metastases. In fact, if metastatic cells are the survivors of a highly selective process as the hypothesis implies, it would be expected that metastatic cells are better equipped to undergo the demanding process of metastasization.

In a first series of studies, individual pulmonary metastases of a murine fibrosarcoma (mFS6) were investigated for their capacity to spontaneously metastasize after one s.c. passage in syngeneic animals (protocol A) or immediately after their isolation from the lung (protocol B). Since results obtained with the two protocols were similar, the data from protocol A are shown. Details of experimental conditions can be found elsewhere [7]. Table 1 shows that although all cell lines had comparable growth rates, they were heterogenous in their metastasizing ability; some lines (M4 and M7) being more metastatic, others (M8 and M9) being less metastatic than the primary cells. The majority of cell lines were, however, as metastatic as the primary mFS6 tumor.

Since the B16 melanoma was the tumor first employed to show that clones from the primary tumor can markedly differ in their capacity to give artificial metastases upon i.v. cell inoculation [3], it was of interest to examine the metastatic potential of single spontaneous metastases. Following protocol A, it was found that nine of ten lines from individual pulmonary nodules were as metastatic as the primary tumor in terms of both number of lung lesions and their weight, whereas line B8 had a significantly reduced metastatic capacity (Table 1).

A similar pattern was seen when another murine neoplasm of still different histologic origin and metastases from organs other than the lung were examined. Upon i.m., s.c., or i.p. transplantation the M5076/73 ovarian carcinoma gives secondary tumors only in subdiaphragmatic organs, lung nodules not being observed after i.v. injection [10]. Also, in this condition, the majority of cells lines from individual metastases were not hypermetastatic compared to the primary tumor (Table 2), and equivalent results were seen when protocol B was adopted. This confirms, in this model, the comparability of the two experimental approaches previously observed for the mFS6 tumor. The representative results of Table 2 additionally show that, in the majority, cell lines obtained from single metastasis which originated in a given organ did not appear to preferentially show secondary localizations in organ where they had originally settled. Again, comparable results as regards both the metastatic potential of spontaneous metastases, as well as the general absence of a preferential localization in the site of origin, were seen when the colon 26 carcinoma was studied. Since tumors of relatively long transplantation history had been used for our studies, it was of interest to also investigate a neoplasm of recent origin. For this purpose a second passage, which spontaneously metastasized, methylcholanthrene-induced sarcoma of C57B1/6 mice (MN/MCA 1) was used. Here also a degree of heterogeneity in metastatic potential

Table 1. Spontaneous metastasizing capacity of tumor cell lines derived from individual lung secondary tumors of the mFS6 sarcoma and B16 melanoma

Tumor line	MST (range)[a]	Mice with metastases/ total	Metastases number (± SE)[b]	Metastases weight (mg ± SE)[b]
Primary mFS6[c]	33 (25–50)	17/32	3.3 ± 0.3	18.2 ± 5.4
Cell line from metastasis				
no. M1	38 (25–49)	4/8	5.2 ± 3.2	50.1 ± 0.9
M2	32 (28–55)	6/15	3.2 ± 1.2	7.8 ± 7.0
M3	36 (25–48)	10/16	8.7 ± 3.0	48.9 ± 33.2
M4	36 (30–49)	13/14[e]	16.7 ± 3.6[e]	122.5 ± 38.5[e]
M5	33 (25–41)	10/15	8.7 ± 1.8	45.7 ± 20.0
M6	31 (27–42)	10/15	7.8 ± 2.9	11.3 ± 4.0
M7	44 (33–52)	15/15[e]	13.8 ± 2.6[e]	170.2 ± 12.7[e]
M8	35 (26–27)	1/16[e]	1.0	0.5
M9	38 (30–51)	0/15[e]	–	–
Primary B16[d]	28 (24–33)	11/12	7.11 ± 2.2	4.0 ± 1.1
Cell line from metastasis				
no. B1	28 (24–35)	4/7	7.5 ± 2.5	14.3 ± 3.8
B2	30 (28–37)	4/7	1.7 ± 0.4	2.6 ± 1.7
B3	32 (28–36)	2/6	4.0 ± 1.0	5.7 ± 1.5
B4	31	6/8	8.1 ± 3.0	7.3 ± 3.3
B5	27 (23–32)	2/6	2.5 ± 0.5	1.3 ± 0.9
B6	27 (24–38)	6/9	8.8 ± 3.3	5.8 ± 1.7
B7	32 (29–42)	5/8	3.2 ± 1.1	1.6 ± 0.1
B8	28 (24–37)	1/7[e]	10 ± 0	8.8 ± 0
B9	32 (23–37)	4/5	11.5 ± 4.6	8.7 ± 2.9
B10	35 (26–42)	7/8	10.3 ± 3.9	9.9 ± 3.1

[a] Median survival time in days with range
[b] Number and weight of lung metastases/mouse
[c] Tumor cells (10^4) injected i.m. in C57Bl/6 mice. Metastases counted at death
[d] Tumor cells (10^5) injected i.m. in C57Bl/6 mice. Metastases counted at death
[e] Compared to primary tumor, $P < 0.01$

was seen (Table 3), since the L2 metastatic line was hypermetastatic and lines L5 and L7 were hypometastatic (indeed, not metastasizing) compared to the primary tumor. However, again the majority (10 of 14) of cell lines from metastases produced metastases comparable to the primary in terms of incidence of animals with secondaries, as well as number and weight of lesions per lung.

From the data presented and obtained using a series of murine tumors of different histology, pattern of dissemination, and transplantation history, it appears that cells from spontaneous metastases do not generally possess an enhanced metastasizing capacity in respect to the primary tumor from which they had originated. Although a heterogeneity is clearly recognizable both among metastases and compared to the primary with both hyper- and hypometastatic cells, our findings do not appear to support the general contention that metastases arise from the progeny of cells with