Journal of Cellular Biochemistry 87:253–257 (2002)

MEETING REPORT

UICC Study Group on Basic and Clinical Cancer Research: Mechanisms of Metastasis

Max M. Burger*

Novartis Science Board, Novartis Intl. AG, CH-4002 Basel, Switzerland

The purpose of these study group meetings, which are organized by the Tumor Biology Program of the International Union Against Cancer (UICC), is to establish a basis for possible clinical applications founded on molecular concepts. For this purpose, generally a few clinicians, pathologists, and epidemiologists are invited together with a core group of cell and molecular biologists. The meetings are of a particularly informal nature, to foster the exchange of ideas rather than to discuss data. It is for this reason that no book is published as a follow-up but rather a brief report is published as this one. More detailed data can be requested from the participants directly. Their addresses are provided at the end of this report.

The first meeting of these UICC Study Groups was held in Annapolis in 1983 and was devoted to "Cancer Metastasis." Nineteen years later we thought that it is high time to revisit this particular topic. Although most meetings in between were targeted largely to oncogenes, tumor suppressor genes signaling and the cell cycle, some of these were, similar to metastasis, directed to tissue and whole animal topics like angiogenesis [Burger and Folkman, 1994; Burger, 2000a] and animal models [Burger, 2000b].

Many of the most intriguing questions which were raised at the first meeting within the series of these UICC meetings were expected to be answered about 20 years later. Some questions have been answered and others are yet to be answered. What is surprising is the fact that downright biological concepts are still prevailing among the research topic as well as the enigmas to be resolved; more so than in the primary tumor world of oncogenes and tumor suppressor genes. However, the key increments in information on the metastasis process over the last 20–30 years also derive from biology and pathobiology primarily. Thus, the pronounced heterogeneity of metastatic versus primary tumor cells has not only consequences for the plethora of mechanisms involved in metastasis but also for the difficulty to come to simple concepts about treatment of metastatic tumors.

A tumor cell has to overcome many steps from the primary tumor until it successfully establishes a metastasis. The same type of tumor may have developed entirely different capabilities to overcome different steps in different patients. This then strengthens the concept that the midrange future will require careful diagnostic clarification of an individual patient's tumor cell deficiencies and a corresponding tailor-made therapeutical approach.

For the time being some of the immediate topics which metastasis research deals with, are the mechanisms of lymphatic versus vascular escape of viable tumor cells, the mechanisms of survival in the blood and lymph system, the chemotactic movement and its organ specificity as well as a second look at adhesion to the tumor cells' microenvironment all along its way from primary site to growth in the target organ. Many a dogma had recently to be revised or refined and that applies to primary tumor research as well as to the process of metastasis.

The meeting was held at Woods Hole, Massachusetts from June 6–9, 2002.

Grant sponsor: Novartis, Inc.

*Correspondence to: Max M. Burger, MD, PhD, Novartis Science Board, Novartis Intl. AG, CH-4002 Basel, Switzerland. E-mail: max.burger@group.novartis.com

Received 11 July 2002; Accepted 17 July 2002

DOI 10.1002/jcb.10367

Metastasis Depending on Angiogenesis and the Maintenance of the Vascular System

Dr. I.J. Fidler opened the meeting with a general survey of metastasis as a phenomenon and of mechanisms leading to metastasis. The continuous growth of metastases depends on induction and maintenance of vasculature. Tumor cells growing in a specific organ microenvironment express ligands that regulate expression of receptors on the tumor cells and tumor-associated endothelial cells. Protein tyrosine kinase inhibitors inhibit phosphorylation of specific growth factor receptors. Coupled with chemotherapy these produce apoptosis in tumor-associated endothelial cells and hence destruction of organ-specific-metastases. He then exemplified these general thoughts by showing that downregulation of the EGF-receptor signaling pathways with a novel EGF-R tyrosine kinase inhibitor (PKI 166) did not only inhibit growth and metastasis of human pancreatic carcinoma cells implanted into pancreas of nude mice, but also inhibited angiogenesis of endothelial cells and resorption of bone when human PC-3M prostate cancer cells were implanted into bones of nude mice. Blockade of EGF-R signaling can therefore provide an attractive approach to therapy of osteolytic bone cancer metastases.

Dr. M.J.C. Hendrix presented data demonstrating the plasticity of aggressive melanoma cells that express multiple phenotypic markers, similar to embryonic cells. Vasculogenic mimicry is one example of plasticity in which highly aggressive, but not poorly aggressive, melanoma cells express endothelial-specific genes (such as VE-cadherin). The aggressive melanoma cells are able to participate in the neovascularization of ischemic muscle, and they secrete and modify their extracellular environment; that can induce melanoma cells to express a vasculogenic phenotype. These results highlight the importance of the microenvironment in influencing the expression and fate of tumor cells.

Compared with our understanding of a tumor's vascular system, our understanding of its lymphatic system is minimal. Dr. R.K. Jain's conclusions, that lymphatics are absent or not functional within tumors and that peritumoral lymphatics are a poor prognostic factor, has a practical value. He pointed out that VEGF-C and D increase the surface area of lymphatics in the tumor margin and in the peritumor normal tissue, and thus facilitates lymphatic metastasis. He triggered an interesting discussion when proposing that judicious application and not overdosing of anti-angiogenic therapy can "normalize" the abnormal vasculature of tumors, and thus augment the delivery and response to cytotoxic therapies while a complete deletion of the vascular supply may be counterproductive during chemotherapy since the drugs may not reach the tumor.

Chemokines and Cell Motility

Dr. A. Zlotnik introduced the rapidly expanding concept that organ-specific metastasis may be due to a fit of high ligand concentration in the target organ and high receptor presence in the "homing" tumor cell. The generality of this principle like many other valid ones will have to be confirmed. The chemokine superfamily includes 46 ligands and 18 receptors. Some ligands are species specific. He concluded that tumor cells express a non-random, specific pattern of chemokine receptors, and that the ligands of these receptors are expressed in organs that represent common metastatic destinations. Dr. Zlotnik convincingly showed that an anti-CXCR4 antibody blocks metastasis in a mouse model of breast cancer.

Microenvironmental hypoxia and genetic changes such as the functional inactivation of the von Hippel-Lindau (VHL) tumor suppressor trigger the activation of a hypoxia-inducible transcription program which is critical for tumor growth. Dr. W. Krek has identified a series of novel hypoxia-inducible genes, among them the gene encoding the chemokine receptor CXCR4 that promotes cell migration in response to its natural ligand SDF-1. These findings uncovered an unexpected link between microenvironmental hypoxia/VHL inactivation and the processes of cell migration and invasion/metastasis involving SDF1/CXCR4 signaling.

Dr. J. Condeelis discussed the chemotaxis of carcinoma cells toward gradients of EGF which occurs in the primary tumors of rats and mice in the "breast" (mammary fatpad). Sources of EGF are associated with blood vessels. One of these sources has been identified as tumor associated macrophages that congregate around vessels. There appears to be an obligatory paracrine loop for invasion and intravasation by carcinoma cells of the primary tumor involving carcinoma cell secreted CSF-1 and macrophage secreted

EGF. He showed that invasive carcinoma cells in these mammary tumor models upregulate expression of EGF-R and molecules involved in activation of Arp2/3 complex and Arp2/3 complex itself. His future work will focus on a molecular definition of chemotaxis in carcinoma cells to EGF.

Dr. M.E. Hemler reviewed the large family of tetraspanins (30 distinct members) which are transmembrane proteins that are abundantly expressed on nearly all human cells. Many reports have suggested that tetraspanins CD9 and CD82 may act as tumor metastasis suppressors, whereas CD151 appears to promote tumor cell metastasis. New studies now begin to suggest that CD151 in particular, and tetraspanins in general, may regulate cellular mechanical force transduction through certain integrins, thus resulting in altered cell motility and morphology. Such results could help to explain the role of tetraspanins during metastasis.

Adhesion to the Vascular Tree and to the Tumor Cell's Microenvironment

Dr. R. Hynes introduced this topic which has long been too mechanistically treated while selectivity of binding and signaling has now brought new refined insights. Acquisition of selectin ligands by human carcinoma cells is a sign of poor prognosis, suggesting that tumor cells may use selectins in their spread. He has investigated this hypothesis using selectin-deficient mice and found that metastatic spread is indeed reduced by the absence of selectins. Surprisingly, in different assays, he found that growth of primary implanted tumors is suppressed by the absence of selectins and the evidence indicates that some bone marrow-derived selectin-dependent host cells, other than lymphocytes, contribute to this anti-tumor response.

The exclusively mechanical adhesion aspect prevailed over a long time for the role of E-cadherin in the metastasis process. E-cadherin is known to act as a tumor suppressor in colorectal tumor cells by antagonizing beta-catenin signaling in the nucleus. Dr. B. Gumbiner concluded, however, that only a small fraction of cytosolic beta-catenin can interact with TCF transcription factors or cadherins. He proposed that E-cadherin acts as a tumor cell invasion suppressor independently of its role in cell adhesion or in regulation of beta-catenin signaling. Based on the observation that beads coupled with functionally active E-cadherin inhibited the growth of sparsely seeded cells, he concluded that E-cadherin mediates contact inhibition of cell growth directly and independently of other cell–cell contacts like adhesion mediated alteration in juxtacrine signaling via other receptors in cell–cell contacts.

Except for a few reports, metastases in a peripheral organ were generally thought to occur in the target organ parenchyme after penetrating and leaving the vessel. Dr. R. Muschel's direct observation of pulmonary vasculature in isolated lungs has revealed that circulating tumor cells attach to the vessels and then proliferate within the blood vessels. Non-metastatic tumor cells also attach, but then they die by apoptosis. These studies then describe a model for metastasis of attachment, survival, and proliferation with survival as the rate limiting step within the target organ vessel.

By probing the heterogeneity of the vascular endothelium Dr. E. Ruoslahti has opened up entirely new avenues of targeting specific drugs to where they are useful and not where they lead to toxic side effects. His laboratory uses in vivo selection of peptide libraries displayed on phage to identify peptides that selectively recognize the vasculature of individual tissues or tumors. The newest generation of these homing peptides have striking properties: they take a payload such as a fluorescein molecule into tumor cells and tumor endothelial cells. One of these peptides selectively recognizes the lymphatic vasculature in certain tumors but does not bind to tumor blood vessels or lymphatics in normal tissues. Thus, tumor lymphatics, like their blood vessels, are specialized and may provide new opportunities for targeting of therapies to tumors.

Can the Metastatic Potential of a Primary Tumor be Predicted?

Earlier efforts to predict the metastatic potential of colon carcinoma, particularly by I.J. Fidler, have shown that lower E-cadherin, higher proteases, and other specific in situ expressions had prognostic value. Dr. T.R. Golub presented his genomewide expression profiles in leukemias, lymphomas, brain, lung, and prostate cancers. He compared expression profiles of primary human tumors to that of metastatic lesions derived from different patients, thereby

creating a metastasis "signature." The metastasis signature was already present in a subset of primary tumors, and these patients had a higher likelihood of developing metastases than those patients lacking the signature. These data suggest that some tumors are preconfigured to metastasize, arguing against the model of selection of rare metastatic variants within a largely non-metastatic primary tumor.

Cell Cycle and Tumor Suppressors

Three presentations did not directly deal with the metastatic process but with derangements in the cell cycle which contribute to growth aberrations already in the primary tumor. Dr. D. Livingston found that Rb is constitutively concentrated in two nuclear subfractions—the soluble fraction and the chromatin fraction. Entry of Rb into the chromatin fraction is a protein phosphatase 2A-dependant process. Once in the chromatin fraction, Rb can, after S-phase DNA damage, be translocated to certain replication origins where it suppresses unwarranted initiating activity. PP2A inhibition by SV40 small t-Antigen prevented post-damage Rb/origin binding.

The PTEN tumor supressor regulates cell-cycle progression, at least in part, through regulation of PI3K signaling induced relocalization of forkhead transcription factors. Transcriptional profiling and functional data from Dr. W.R. Seller's laboratory reveal that forkhead regulates cell-cycle progression and suppresses tumor formation through IRS-independent transcriptional repression of D-type cyclins. These data suggest that different functional outputs downstream of FKHR, may be enacted through distinct transcriptional methods.

Dr. B.R. Zetter discussed a novel role for the protein antizyme in regulating the cell cycle in prostate cancer cells. Antizyme is upregulated in response to high local polyamine concentrations in the prostate. Elevated antizyme levels result in cell cycle arrest due to selective antizyme-induced degradation of specific cell cycle proteins including cyclin D1 and cdk4. Loss of antizyme during tumor progression may consequently lead to increased prostate cancer cell proliferation. There is additional evidence that downregulation of antizyme may also be important in the progression of oral and gastric cancer as well and insofar as it is not only an early event, may well have a role also in later events like metastasis.

ACKNOWLEDGMENTS

This meeting was sponsored by Novartis, Inc., Basel, Switzerland. The International Union against Cancer and the participants express their gratitude for the unselfish support and low-key presence of the sponsor.

REFERENCES

Burger MM, Folkman J. 1994. UICC study group on basic and clinical cancer research: Tumor angiogenesis. Meeting, Woods Hole, MA, September 18–21, 1993. Int J Cancer 56:311–313.

Burger MM. 2000a. UICC study group on basic and clinical cancer research: Angiogenesis revisited. Meeting, Woods Hole, MA, June 16–18, 2000. Int J Cancer 88: 835–837.

Burger MM. 2000b. UICC study group on basic and clinical cancer research: Animal models for the natural history of cancer. Meeting, Woods Hole, MA, June 21–23, 1999. Int J Cancer 85:303–305.

APPENDIX

LIST OF THE PARTICIPANTS

Benjamin, Thomas L., Department of Pathology, Harvard Medical School, 200 Longwood Avenue, Boston, MA 02115; Fax: +1-617-277 5291, E-mail: thomas_benjamin@hms.harvard.edu

Burger, Max M., Novartis International AG, Novartis Science Board, WKL-125.13.02 CH-4002 Basel, Switzerland; Fax: +41-61-696 7693, E-mail: max.burger@group.novartis.com

Chène, Patrick, Novartis Pharma AG, WKL-125.4.42, P.O.Box, CH-4002 Basel, Switzerland; Fax: +41-61-696 3835, E-mail: patrick.chene@pharma.novartis.com

Condeelis, John, Department of Anatomy and Structural Biology, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461; Fax: +1-718-430 8996, E-mail: condeeli@aecom.yu.edu

Fidler, Isaiah J., Department of Cell Biology, M. D. Anderson Cancer Center, University of Texas, 1515 Holcombe Boulevard, Box HMB 173, Houston, TX 77030-4009; Fax: +1-713-792 8747, E-mail: ifidler@mdanderson.org

Golub, Todd R., Whitehead Institute, Center for Genome Research, 320 Charles Street, Cambridge, MA 02141-2023; Fax: +1-617-632 4903, E-mail: golub@genome.wi.mit.edu

Gumbiner, Barry, Department of Cell Biochemie and Biophysics, Memorial Sloan-

Kettering Cancer Center, 1275 York Ave, Box 564, New York, NY 10021; Fax: +1-212-717 3047, E-mail: b-gumbiner@ski.mskcc.org

Hemler, Martin E., Department of Cancer Immunology and AIDS, Dana-Farber Cancer Institute, 44 Binney Street, Boston, MA 02115; Fax: +1-617-632 2662, E-mail: martin-hemler@dfci.harvard.edu

Hendrix, Mary J.C., Department of Anatomy and Cell Biology, The Holden Comprehensive Cancer Center, University of Iowa, 1-100 Bowen Science Build., Newton Rd, Iowa City, IA 52242-1109; Fax: +1-563-355 7770, E-mail: mary-hendrix@uiowa.edu

Hofmann, Francesco, Novartis Pharma AG, Department of Oncology, WKL125.3.08, CH-4002 Basel, Switzerland; Fax: +41-61-696 3389, E-mail: francesco.hofmann@pharma.novartis.com

Hynes, Richard, Center for Cancer Research, MIT, Bldg. E17, Rm 227, 77 Massachusetts Avenue, Cambridge, MA 02139-4307; Fax: +1-617-253 8357, E-mail: rohynes@mit.edu

Jain, Rakesh K., Department of Radiation Oncology, Harvard Medical School, Massachusetts General Hospital, Boston, MA 02114; Fax: +1-617-726 1819, E-mail: jain@steele.mgh.harvard.edu

Krek, Wilhelm, Friedrich Miescher Institut, Maulbeerstrasse 66, P.O.Box 2543, CH-4002 Basel, Switzerland; Fax: +41-61-697 3976, E-mail: wilhelm.krek@fmi.ch

Lassota, Peter, Novartis Pharmaceuticals Corporation, 556 Morris Ave, Summit, NJ 07901; Fax: +1-908-277 5752, E-mail: peter.lassota@pharma.novartis.com

Livingston, David, Dana-Farber Cancer Institute, 44 Binney Street, Boston, MA 02115-6084; Fax: +1-617-632 4381, E-mail: david_livingston@dfci.harvard.edu

Matter, Alex, Novartis Pharma AG, Head of TA Oncology, WKL-136.P.15, P.O.Box, CH-4002 Basel, Switzerland; Fax: +41-61-696 7826, E-mail: alex.matter@pharma.novartis.com

Muschel, Ruth, University of Pennsylvania, Department of Pathology, 269-A J Morgan Bldg., 36th St. & Hamilton Walk, Philadelphia, PA 19104-6082; Fax: +1-215-573 4243, E-mail: muschel@xrt.upenn.edu

Pardee, Arthur B., Dana-Farber Cancer Institute, Department of Pharmacology, Division of Cell Growth and Regulation, 44 Binney Street D810A, Boston, MA 02115; Fax: +1-632 4680, E-mail: pardee@mbcrr.harvard.edu

Ruetz, Stephan, Novartis Pharma AG, WKL-125.3.01, P.O.Box, CH-4002 Basel, Switzerland; Fax: +41-61-696 3835, E-mail: stephan.ruetz@pharma.novartis.com

Ruoslahti, Erkki, The Burnham Institute, 10901 N. Torrey Pines Rd., La Jolla, CA 92037; Fax: +1-858-646 3198, E-mail: ruoslahti@burnham-inst.org

Sellers, William R., Dana-Farber Cancer Institute, 44 Binney Street, Boston, MA 02115; Fax: +1-617-632 5417, E-mail: william_sellers@dfci.harvard.edu

Stein, Gary S., Department of Cell Biology, University of Massachusetts Medical Center, 55 Lake Avenue North, Worcester, MA 01655; Fax: +1-508-856 6800, E-mail: gary.stein@umassmed.edu

Wong, Sunny, Department of Cell Biology, Center for Cancer Research, MIT, 77 Massachusetts Avenue, Cambridge, MA 02139-4307; E-mail: syw5@mit.edu

Wood, Alexander, Novartis Pharmaceuticals Corporation, 556 Morris Avenue, Summit, NJ 07901; Fax: +1-908-277 5752, E-mail: alexander.wood@pharma.novartis.com

Zetter, Bruce R., Surgery Research Laboratory, Children's Hospital, Harvard Medical School, 300 Longwood Ave, Boston, MA 02115-5737; Fax: +1-617 355 7043, E-mail: bruce.zetter@tch.harvard.edu

Zlotnik, Albert, Eos Biotechnology, Inc., 225A Gateway Blvd., South San Francisco, CA 94080; Fax: +1-650-583 3881, E-mail: azlotnik@eosbiotech.com