Extracellular Proteolysis and Cancer: Meeting Summary and Future Directions

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Abstract

The contribution of extracellular proteolysis to processes of tumor invasion and metastasis has been recognized for more than 20 years. However, after millions of dollars and untold hours of investment in preclinical research and the development of broad range inhibitors of MMPs, clinical trials of late-stage tumor patients show no indication that this approach will be successful. In the basic science arena, there have been stunning advancements that illustrate novel biological activities for proteases and that suggest they are key regulators of many physiological and pathological processes. The Proteases and Cancer: Biology and Therapeutics Workshop (held in Bethesda, MD, November 20–22, 2002) was organized by the Division of Cancer Biology, National Cancer Institute (NCI) to identify research areas and directions that will accelerate understanding protease biology and enhance clinical translation. The overall consensus was that protease biology represents fertile ground for advances that will be clinically useful but perhaps not for the reasons or purpose originally thought. Protease-related technologies show particular promise for the detection, prognosis, and prevention of cancer, and for therapeutic purposes in defined situations. Promising areas for further research are identified, and specific recommendations for the development of a consortium to coordinate the efforts of the protease community are made.

Workshop Synopsis

The introductory session of the meeting was designed to provide an overview of the normal physiological role of extracellular proteases, the preclinical data that supported a role for extracellular proteolysis in cancer, and an evaluation of the clinical trials with MMP inhibitors (L. Matrisian, moderator, C. Alexander, scribe; Refs. 1–6 for general reviews). R. Khokha (University of Toronto) stressed the diversity of enzymes involved in pericellular proteolysis, which could be well over 200 distinct gene products, and the diversity of their functions in normal physiology. Substrate cleavage is linked to the activation of key signaling pathways and results in alterations in cellular proliferation, apoptosis, differentiation and migration, thus challenging the general assumption that extracellular proteolysis is always “bad.” L. Matrisian (Vanderbilt University) used research investigating the function of specific MMPs as an example of how complex the contribution of proteases to tumor progression can be. Sophisticated genetic approaches to manipulate MMP levels generated some support for the concept that these enzymes are bulldozers that pave the way for tumor cell to cross a basement membrane, but more often indicate roles in tumor growth, angiogenesis, and the ability of metastatic cells to establish and grow in ectopic sites. G. Sledge (Indiana University) reviewed the results of the MMP inhibitor clinical trials that failed to support the efficacy of MMP inhibitors in prolonging survival, reducing time to progression, or reducing tumor burden in late-stage cancers. He pointed out the unexpected side effects of joint pain and immobility that limited the dosing of these compounds, and indicated the aggressive nature of the cancers chosen for clinical trials to facilitate the rapid collection of data. The concept that proteases are highly regulated and reactive to the signaling pathways operational during neoplastic progression indicates that the application of information regarding these enzymes can be important at more than one level: their expression can be useful for cancer detection, diagnosis, or prognosis, and at least some of these enzymes play a causal role in tumor progression and represent valid therapeutic targets. Proteases often represent a host response to the tumor and can play a critical role in mediating the communication between a tumor and its microenvironment.

The potential for proteases to be applied to the detection, diagnosis, and prognosis of cancer was addressed in the second session, chaired by B. Sloane (U. Mahmood, scribe). T. Meade (Northwestern University) demonstrated the exciting use of protease-sensitive magnetic resonance imaging contrast agents to determine the functional activity of proteases in vivo (7). An optical imaging approach to detect proteolytic activity was presented by U. Mahmood (Massachusetts General Hospital) and was demonstrated to be useful for the detection of tumors as well as for monitoring the efficacy of proteolytic inhibition in a mouse model (8). P. Nelson (Fred Hutchinson Cancer Research Institute) reviewed the well-established use of the serine protease prostate-specific antigen to detect and follow the treatment of prostate cancer and described the power of evaluating other serum serine proteases to enhance the sensitivity of the approach (9). J. Duffy (St. Vincents University Hospital, Dublin, Ireland) presented extensive studies that validate the use of the serine protease uPA and plasminogen activator inhibitor-1 as prognostic indicators in breast and other cancers (10). Plasminogen activator inhibitor-1 and uPA have also been reported to be predictive of response to chemotherapy, suggesting that they have utility in the individual management of patients with breast cancer (11). E. Diamandis (Mount Sinai Hospital, Toronto Canada) described studies indicating that circulating levels of steroid-regulated enzymes in the kallikrein family of serine proteases can indicate the presence of ovarian and prostate cancer (12). Thus, there are both well-established and novel applications for proteases in the detection, diagnosis, and prognosis of cancer.

The third session focused on the therapeutic applications of protease inhibitors (G. Sledge, moderator, L. Gorden, scribe). E. Hawk (Division of Cancer Prevention, NCI) presented data suggesting that MMP inhibitors may be effective in benign tumors and dispelled the notion that prevention trials are by necessity long term and extremely expensive. He outlined a plan for a short Phase II prevention trial that could build rationale for developing Phase III trials (13). V. Giranda (Abbott Laboratories) described the experience with uPA inhibitors, indicating that although there was extensive rationale for the therapeutic application of uPA inhibitors, animal models revealed inconsistent results, and several uPA inhibitors failed to demonstrate sufficient efficacy for further development (14). C. Carron (Pharmacia and Upjohn) reviewed the development of a cancer marker targeting the urokinase plasminogen activator system that is in a Phase II clinical trial. A. Wood (NCI, scribe) concluded that the protease/breast cancer therapeutics Workshop was a success.
The development of a semiselective MMP inhibitor, presenting the case that detailed knowledge of the target enzyme and the enzyme responsible for toxicity is essential to the development of an effective therapeutic (15). A protease-dependent “prodrug” approach to therapy was presented by T. Bugge (National Institute of Dental and Craniofacial Research, NIH). His laboratory generated a uPA-activated anthrax toxin that displayed broad and potent tumoricidal activity, with minor toxicities to lymphoid tissues (16). The new prodrug strategy is highly versatile and can be used to improve the therapeutic index of immunotoxins already in clinical use for the treatment of cancer. There are considerable challenges for traditional drug-development efforts associated with the apparent requirement to target multiple protease systems, while avoiding significant toxicities, for the treatment of cancer.

The normal function of proteases was addressed in the fourth session (M. Hendrix, moderator, L. Iruela-Arispe, scribe). S. Rafii (Cornell University) identified membrane-bound Kit-ligand as a substrate for MMP-9 (17). The release of soluble, bioavailable Kit-ligand from the bone marrow stromal cells is essential for the mobilization of hematopoietic and endothelial stem cells. Dr. Rafii indicated that the toxicity associated with inhibiting MMP-9 could be controlled by diminishing myelosuppression and controlling infection with antibiotics. C. Blobel (Memorial Sloan-Kettering Cancer Center) addressed the importance of protein ectodomain shedding as a component of signaling pathways. Ligands in the EGF receptor require release to elicit paracrine signals. Members of the ADAMs family of proteases, a subfamily of metalloproteinases with disintegrin domains, play a critical role in this function, and elegant studies with ADAM-null mice are beginning to define specific roles for specific ADAM family members (18). M. Hung (M. D. Anderson Cancer Center) extended this observation in a later session, demonstrating that EGF receptors are also a signaling event, with the surprising result that the full length of EGF receptors get translocated to the nucleus and function as a transcriptional factor (19). It is clear that the physiological substrates for proteases and their biological consequences are highly complex, and that knowledge of these activities in physiological and pathological settings is essential.

At the cultural role for proteases in tumor invasion and metastasis was the focus of the fifth session (V. Quaranta, moderator, C. Jorczyk, scribe). P. Friedl (University Wuerzburg, Germany) surprised everyone by using two-photon microscopy in a living mouse to demonstrate that tumor cells can move through the extracellular matrix in the absence of all proteolytic activity, and do so by adopting amoeboid patterns of movement rather than directed motility that involves proteolysis (20). B. Sloane (Wayne State University) provided visual evidence that pericellular proteolysis correlates with tumor cell invasion, but that normal fibroblasts collaborate with tumor cells in producing proteases and the most intense proteolytic activity occurs at sites of tumor-stromal cell interaction (21). R. Muschel (University of Pennsylvania) used an ex vivo model of lung function to demonstrate that MMP-9 influences the ability of tumor cells to attach and survive in the lung vasculature (22), and that it is this effect rather than an effect on early extravasation that eventually determines the metastatic potential of the cells (23). V. Quaranta (Scripps Research Institute) demonstrated the complexity of the role of matrix proteolytic cleavage in cell migration. Native laminin-5 promotes cell migration; cleavage of the molecule with serine proteases can result in static adhesion, but subsequent cleavage by MT1-MMP results in enhanced motility (24). The plasticity of highly aggressive tumor cells expressing multiple cellular phenotypes was presented by M. Hendrix (University of Iowa), who demonstrated that vascuogenic mimicry by these cells may act in a coordinated manner with angiogenesis for the perfusion of tumors (25). In general, it became clear that the application of sophisticated new technologies has revealed that our previous concepts of the role of tumor proteases in motility, invasion, angiogenesis, and metastasis must be greatly expanded and significantly modified.

Finally, the specific role of proteases in metastasis to the bone was addressed in the sixth session (S. Mohla, moderator, S. Sheng, scribe). R. Poole (McGill University, Canada) started by giving an excellent overview of what is known about the role of proteases in cartilage function, their potential as targets for the treatment of arthritis, and tools developed to monitor them (26). S. Sheng (Wayne State University) demonstrated that maspin, a novel serine protease inhibitor, inhibits prostate metastasis to the bone (27). M. Cher (Wayne State University) used a model of metastasis of human tumor cells to human bone in a mouse host to demonstrate that inhibition of MMP activity could reduce metastatic cell growth and bone remodeling (28). E. Thompson (University of Melbourne, Australia) also demonstrated that MMP inhibitors were effective in reducing breast cancer metastasis to the bone (29) and suggested that the observed differential in efficacy between different synthetic MMP inhibitors held promise for a better understanding of MMP-specificity in the process, and the potential for specific therapy. Although many questions remain to be answered, there was considerable excitement over the potential for protease inhibition to be effective in the control of bone metastasis.

Conclusions of the Workshop

The conclusions of the Workshop were framed in the context of the issues, barriers, and solutions to the application of protease biology to cancer. A summary of the most pertinent points raised during the discussion periods is provided in Table 1.

The overall consensus was that protease biology still represents fertile ground for advances that will be clinically useful, but perhaps not for the reasons or purpose originally thought. The plethora of different enzymes that contribute to matrix degradation presents a considerable challenge from a therapeutic viewpoint, yet represents an opportunity from the perspective of tumor detection, diagnosis, and prognosis. The remarkable tissue- and stage-specificity of some of these enzymes could be effectively exploited for this purpose, as illustrated by the success of the tissue-specific serine proteinase prostate-specific antigen for the detection and monitoring of prostate cancer. The catalytic nature of these enzymes presents an opportunity for highly sensitive new methods to detect small, early-stage tumors. Finally, the specificity and diversity of tumor-specific expression also offers an opportunity to generate prodrugs that are activated by proteolytic activity and represent a “smart bomb” approach to tumor elimination.

From a therapeutic perspective, the idea that total elimination of matrix degradation will prevent tumor metastasis appears to be impractical and has been unable to withstand the test of experimentation. Complete inhibition of matrix degradation requires the targeting of many enzymes from many different classes, increasing the risk of unacceptable side effects. Even with elimination of matrix degradation, there is experimental evidence that tumor cells may be resilient enough to find alternative methods to accomplish important cellular processes such as motility. Nevertheless, at least one therapeutic opportunity for the inhibition of matrix degradation in advanced cancers may remain. The bone microenvironment appears to be unusually sensitive to manipulations of the extracellular matrix, and there are indications that protease inhibition is effective in the control of bone metastasis and can reduce bone damage.

The identification of “sheddase” activity as a major biological activity of extracellular proteinases indicates that proteolysis can be...
Solutions and use of selective inhibitors. Equally important is understanding and the specific tumor phenotype affected, is critical to the design going to the clinic. Identification of the specific enzymes responsible, importance of a strong scientific knowledge base before proceed-

some exciting opportunities for specific interventions in pathways mediated by proteolysis is in its infancy but appears to provide generally perceived. Molecular understanding of the signaling activ-

ation as a chemopreventive approach has not been adequately explored, pathway inhibitors should be considered. The use of protease inhibitors can be designed to have either broad or narrow specificities.

Clinical trials with MMP inhibitors failed to show clinical benefit.
- The target MMP was not always validated.
- Performed with advanced-stage cancers.
- Arthralgia was a dose-limiting toxicity.
- The ability of the drugs to inhibit tumor MMP activity was not determined.

Clinical trials were not adequately designed to distinguish between failure of the drugs and failure of the hypothesis.

Barriers
- The number of extracellular proteases is in the hundreds.
- Positive correlations between protease expression and disease outcome do not necessarily indicate a causative role.
- Endogenous protease inhibitors can have other activities in addition to protease inhibition.
- Identification of physiological substrates is technically difficult and expensive.
- The ability and incentive for an individual laboratory to focus on more than one protease family is limited and results in narrow knowledge in specific areas.
- Inhibition of proteases that regulate essential normal physiological processes will have adverse side effects.
- There are technical and economic barriers to the development of in vivo imaging strategies for proteolytic activity for every protease.
- Both target (“bad”) and nontarget (“good”) proteases must be identified: those whose inhibition results in therapeutic effects and those that produce unwanted side effects.
- Tumors are incredibly plastic.
- They can devise alternative approaches to critical processes such as angiogenesis.
- Proteolytic inhibition can result in the exposure of a default pathway that allows processes such as motility to occur in the absence of proteolysis.
- There is a lack of reproducible and validated animal models that predict clinical response.
- Balancing the design of synthetic inhibitors to achieve an appropriate degree of specificity to maximize efficacy and minimize toxicity is difficult.
- Agents to be considered for long-term treatments must show the absence of significant short- and long-term side effects.
- Quality-of-life issues are rarely considered as clinical trial end points.
- There are regulatory and corporate barriers to the use of combination therapies.
- The failure of initial MMP inhibitor clinical trials discourages additional pharmaceutical investments.
- There are barriers to implementing the use of biomarkers in clinical practice even in light of clear demonstration of their utility.

Solutions
- Use profiling approaches to define the expression and activity of the “proteome” in normal tissues and specific tumor types and stages.
- Create interactive networks of investigators with different expertise focused on protease expression and function in a specific tumor/organ system.
- Build on the exciting developments in nuclear and optical imaging to devise methods to noninvasively image proteolytic activity and its inhibition in vivo.
- Develop and validate standard model systems for in vitro and in vivo studies.
- Devis animal model systems that more faithfully recapitulate the human situation.
- Consider that metastasis to bone may have special protease requirements that can be exploited.
- Devis strategies to allow effective academic and industrial partnerships permitting access to a range of pharmacological protease inhibitors.
- Clearly identify the effects of therapeutic protease inhibitors on key cell signaling pathways at an early stage of their development.
- Use the power of genetic and pharmacological inhibition of specific proteases to define the roles of individual proteases in normal physiology and pathological conditions.
- Use semiselective protease inhibitors at doses above \( K_i \) for efficacy and below \( K_i \) for toxicity.
- Include biomarker assessment in large clinical trials.
- Use well-designed Phase II trials to guide decisions on Phase III trials.
- Focus therapeutic trials on early- or minimal-stage disease.
- Develop rational strategies for the testing of combinations of therapeutics.
- Initiate prevention trials with high-risk patients.

viewed as a regulator of signal transduction pathways. Numerous opportunities present themselves when viewed from this perspective. It appears clear that early stages of tumor development are more sensitive to protease inhibition than are advanced stages. It is likely that this represents a dependence on paracrine activities in early tumors, which often involve a proteolysis step, whereas advanced cancers have acquired independent, autocrine activities. Proteolytic inhibition may, therefore, be much more effective in the intervention of early-stage cancers, and rational combinations with other targeted pathway inhibitors should be considered. The use of protease inhibition as a chemopreventive approach has not been adequately explored, and does not have to be as time-intensive or cost-prohibitive as generally perceived. Molecular understanding of the signaling activities mediated by proteolysis is in its infancy but appears to provide some exciting opportunities for specific interventions in pathways critical for tumor development and growth.

The clinical development of MMP inhibitors demonstrated the importance of a strong scientific knowledge base before proceeding to the clinic. Identification of the specific enzymes responsible, and the specific tumor phenotype affected, is critical to the design and use of selective inhibitors. Equally important is understanding the role of specific proteases in normal processes to avoid unac-

ceptable side effects of protease inhibition. Toxicity may be min-

imized with information on the tissue specificity of the effect. The need to design assays to determine whether the target protease is effectively inhibited in the target tissue is apparent, and advances in optical imaging have provided some encouraging results in this arena. There is a clear need to identify clinical end points for cytostatic agents, such as protease inhibitors, as opposed to cyto-

xic agents, and these need to be applied to phase II trials. It is likely that protease inhibitors will be most effective in combination with other therapeutic agents, and improved strategies for testing combinations of agents and overcoming regulatory and corporate barriers is required.

Recommendations

The base of information regarding the normal roles for proteases and their contribution to tumorigenesis must be enhanced. Much of this information can be obtained under investigator-initiated funding mechanisms, and support for fundamental biochemistry, cellular, molecular, and developmental biology studies must continue. However,
real advances are likely to be accelerated by a more focused and cohesive effort encompassing multidisciplinary approaches. The creation of a consortium-style funding mechanism is recommended with a focus on addressing the following needs:

- a mechanism to reliably determine the expression pattern of all proteases and endogenous protease inhibitors in normal and neoplastic tissues, and in tumor and stromal compartments. This includes a broad, comprehensive approach to identify expression patterns, as well as a detailed analysis of the cell type responsible for expression;
- a standardized and systematic evaluation of effects of key proteases on basic cellular processes (i.e., proliferation, and so forth) in selected physiological systems;
- in vivo imaging approaches for proteolytic activity and inhibition;
- a systematic evaluation of the role of specific proteases in early, mid, and late stages of tumor development and progression;
- guidelines for the standardization of key assays and model systems so a data bank of results that can be directly compared can be obtained;
- a curated database that allows the compilation of all data (including negative results) on substrates and biological activities of specific proteases;
- a centralized bank of critical reagents, including nucleotide probes, antibodies, protease-deficient mice, and small molecule inhibitors that can be easily disseminated to investigators for investigational work; and

- a clinical correlates component that is responsible for identifying opportunities to interface with pharmaceutical and government-sponsored clinical trials, and has resources to enable correlative study implementation.

For several of these goals, collaborative efforts with existing structures, such as the NCI Mouse Models of Human Cancer Consortium, Molecular Signatures of Cancer Cells, In Vivo Cellular and Molecular Imaging Program, and Early Detection Research Network, as well as partnership with the pharmaceutical and biotechnology industries, are likely to be beneficial and highly synergistic.

Summation

We are in the midst of an explosion of information that dramatically changes our perceptions of the role of extracellular proteolysis in physiological and pathological processes. The excitement in the basic science arena contrasts with the discouragement in the pharmaceutical and clinical arena regarding the therapeutic potential of protease inhibition. Specific issues and barriers to the clinical application of knowledge of protease biology have been explored, and potential solutions to the hurdles are proposed. There are clear opportunities for the future, in particular in early detection, prognosis, and prevention of cancer, with some therapeutic opportunities in the prodrug approach, bone metastasis, and as a modulator of signal transduction pathways. What is readily apparent is that the complexity of the biology and the substantial barriers to clinical translation dictates that future endeavors include a concerted, coordinated effort from investigators with diverse but complementary perspectives and expertise.

Appendix

The “Proteases in Cancer: Biology and Therapeutics Workshop” was sponsored by the Division of Cancer Biology, NCI, and was held at the Pook’s Hill Marriott, Bethesda MD, Nov. 20–22, 2002. In attendance were Shakeel Ahmad, Caroline Alexander, Grace Ault, Donald G. Blair, Carl P. Blobel, Diane Bronzert, Thomas Bugge, Christopher Carron, Michael L. Cher, Sarah L. Dallas, Phillip J. Daschner, Elefterios P. Diamandis, Emmanuelle Di Tomaso, Michael J. Duffy, Peter Friedl, Rafael Fridman, Paramita M. Ghosh, Supurna Ghosh, Vincent L. Giranda, Lee Gorden, Ernest Hawk, Mary J.C. Hendrix, Mien-Chie Hung, Luisa Iruela-Arispe, Cheryl L. Jorcyk, Sharron X. Lin, Rama Khokha, A. Craig Lockhart, Carol L. MacLeod, Umar Mahmoon, Lynn M. Matrisian, Susan A. McCarthy, Thomas J. Meade, Suresh Mohan, Rahul J. Muschel, Peter S. Nicholas, Emmanuelle Di Tomaso, Lily Perry, A Robin Poole, Syed Muasaddaq Quadri, Vito Quaranta, Shahin Rafii, Qing-Xiang Amy Song, Neeraja Sathyamoorthy, Shijie Sheng, Christine Siemon, Dinah Singer, George W. Sledge, Jr., Bonnie F. Sloane, John Sogn, Jenny Strasburger, Erik (Rik) W. Thompson, Lily Wu, and Mary M. Zutter.

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