

Meeting Report: Proteases, Extracellular Matrix, and Cancer: An AACR Special Conference in Cancer Research¹

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PROTEASES, ECM, AND CANCER

Our view of the ECM³ and its role in cancer has substantially evolved over the last decade. It is now apparent that the ECM is much more than a physical barrier against tumor invasion. It is an integral part of the tumor microenvironment and extensively modified by proteases during malignant progression. We have begun to appreciate that these proteases do more than just degrade the ECM and allow cells to invade and metastasize. Their role in earlier stages of malignant transformation and angiogenesis has become increasingly apparent. Almost every day we discover new substrates for these proteases and realize that some very important ones are not ECM proteins but growth factors or cytokines and their binding proteins. We now understand that proteases can bind to cell surface receptors where they cross-talk with other receptors and activate signaling pathways that regulate cell survival. We have appreciated that they can modify the ECM to make it less or more hospitable to malignant cells. Imaging technologies have also opened new horizons by allowing us to examine the activity of these proteases *in vivo*. At the same time, the role of many ECM-degrading proteases in tumor invasion and metastasis has been reexamined, and their function as potential novel targets for therapeutic intervention has been questioned. The ECM and proteases that degrade its components were the subject of a recent special AACR conference that was held at the Westin Resort in Hilton Head Island in October 2002 and chaired by Ruth J. Muschel of the University of Pennsylvania (Philadelphia, PA) and Yves A. DeClerck of Children's Hospital Los Angeles and the University of Southern California. This conference was the third of a series of conferences focused on extracellular proteases in cancer initiated in 1996.

ARCHITECTURAL BASIS FOR TUMOR CELL PLASTICITY

The keynote address was delivered by Dr. Mina Bissell from the Lawrence Berkeley National Laboratory (Berkeley, CA) who pioneered work demonstrating the capacity of the ECM to regulate the

malignant phenotype. Using mammary gland development as a model for cancer progression, Dr. Bissell has demonstrated that ECM modification could alter acinar formation and branching and thus cancer progression, *e.g.*, modification of ECM by MMP-3 acts as a carcinogen because of its ability to induce EMT and genetic instability in mammary epithelial cells. Dr. Bissell emphasized that cell–cell and cell–ECM interactions are essential organizers that define the nature of the tissue context and play a crucial role in regulating homeostasis and tissue specificity and that loss of tissue structure alters the cellular phenotype that accompanies the development of malignancy. Thus, the cellular and tissue context itself confer additional and crucial information that is necessary for mutated genes to exert their influence. This provides an explanation for the observation that many cancer susceptibility genes show a high degree of tissue specificity in their association with neoplastic transformation.

MATRIX RECEPTORS AND SIGNALING PATHWAYS IN CANCER

One group of talks focused on the contribution that matrix receptors, and the resultant signaling pathways, have toward cancer progression, with presentations by Drs. Arthur Mercurio (Beth Israel Deaconess Medical Center, Boston, MA), Benjamin Geiger (Weizmann Institute of Science, Rehovot, Israel), Martin Hemler (Dana-Farber Cancer Institute, Boston, MA), Martin Schwartz (The Scripps Research Institute, La Jolla, CA), and Debbie Mustacich (Arizona Cancer Center, Tucson, AZ).

Dr. Mercurio presented evidence indicating that induction of cell motility and autonomous cell survival are two consequences of EMT which facilitate progression to invasive carcinoma. Inflammatory stromal cells promote tumorigenesis by releasing factors, such as TNF- α , that stimulate EMT through activation of p38 MAPK. As a result, the $\alpha 6 \beta 4$ integrin, which is localized to the basal surface of epithelial and well-differentiated cells, translocates to actin protrusions that characterize invasive carcinoma cells. Dr. Mercurio highlighted the importance of VEGF as an autocrine growth factor in this context. VEGF, which has been considered solely as an angiogenic factor, additionally appears to stimulate both migration and survival of tumor cells, albeit by distinct signaling pathways. This autocrine signaling of migration and invasion occurs via Gi-signaling, and it involves the ability of VEGF to regulate the expression of CXCR4, a Gi-linked chemokine receptor. Interestingly, $\alpha 6 \beta 4$ integrin induces VEGF translation in carcinoma cells by a mechanism that involves regulation of the phosphatidylinositol 3'-kinase/Akt/mTor pathway and translation initiation factor eIF-4E activity, thus linking this ECM receptor to VEGF and cell migration, invasion, and survival.

Dr. Geiger reviewed his recent work studying molecular events associated with the assembly of cell-matrix adhesions. Using microscope-based fluorescence resonance energy transfer, he revealed molecular interactions between specific FA molecules, tagged with appropriate fluorescent proteins, in live spreading cells. Accumulation

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³ The abbreviations used are: ECM, extracellular matrix; MMP, matrix metalloproteinase; TNF, tumor necrosis factor; MAPK, mitogen-activated protein kinase; EMT, epithelial-mesenchymal transition; FA, focal adhesion; FAK, focal adhesion kinase; MT1-MMP, membrane-type 1 matrix metalloproteinase; THP, triple-helical peptide; uPA, urinary/urokinase-like plasminogen activator; uPAR, urokinase-like plasminogen activator receptor; EGFR, epidermal growth factor receptor; uPARAP, uPAR-associated protein; RECK, reversion-inducing cysteine-rich protein with a kazal motif; VEGF, vascular endothelial growth factor; MT-SP, membrane-type serine protease; AMF, autocrine motility factor; PAI-1, plasminogen activator inhibitor 1; DQ-collagen, quenched fluorescent derivative of type IV collagen; ERK, extracellular signal-regulated kinase; MMPI, matrix metalloproteinase inhibitor; PDGFR, platelet-derived growth factor receptor.

of a pericellular hyaluronan coat during spreading is followed by the assembly of small, actin-associated adhesions that grow and merge, eventually forming a complex network of FA-anchored actin bundles. During cell migration, ECM adhesion to integrin-mediated contacts, known as focal complexes, form early under the leading edge of motile endothelial cells. Different FA molecules are then sequentially recruited into these newly formed complexes in lamellipodial protrusions that regulate the assembly of these adhesion sites and their transition into *bona fide* FA. Dr. Geiger provided evidence that tyrosine phosphorylation of adhesion sites is primarily responsible for FA signaling and turnover.

Dr. Hemler discussed the role of tetraspanins in tumor cell metastasis. This family of cell surface transmembrane proteins contains ≥ 30 members, which appear to function by organizing into specific protein- and lipid-containing microdomains. The expression of CD82/KAI1 and CD9, two members of the tetraspanin family, correlates with reduced metastasis and enhanced survival in many cancers. Conversely, the expression of other tetraspanins like CD151 correlates with enhanced cell motility and invasion, increased metastasis, and poor survival in patients. CD9 interacts with transmembrane immunoglobulin superfamily proteins EWI-2 and EWI-F, and CD151 is tightly associated with $\alpha 3\beta 1$ integrin. Dr. Hemler presented evidence suggesting that the migratory/invasive phenotypes of tumor cells are regulated by CD9-EWI-2 and CD151- $\alpha 3\beta 1$ complexes. CD151 has three domains required for its promigratory function: (a) one mediates direct contact with the $\alpha 3$ subunit of the $\alpha 3\beta 1$ integrin; (b) another domain, within the short intracellular COOH-terminal cytoplasmic tail, is needed for connecting to the cytoskeleton; and (c) a third domain consists of palmitoylation sites that influence the transition of epithelial cells to a fibroblastic migratory phenotype. Intervention at any of these three CD151 sites could potentially have an antimetastatic effect on tumor cells.

Dr. Schwartz discussed how survival is regulated by integrin signals. He showed that decreased cell-ECM adhesion leads to resistance to apoptosis induced by DNA damage. This effect is mediated in part by decreased levels of p19Arf and p53 tumor suppressors, as well as by the tyrosine kinase c-Abl. Activation of c-Abl by DNA damage requires integrin-mediated cell adhesion. Using a c-Abl inhibitor and c-Abl-null fibroblasts, he discussed experiments indicating that the c-Abl pathway is independent of p53 and accounts for $\sim 50\%$ of cell death. Thus, activating integrin pathways in invasive or metastatic cancers may be an effective adjunct therapy to enhance killing by DNA damage.

Dr. Mustachic used a panel of breast cancer cell lines that vary with respect to invasiveness to demonstrate the presence of a proteolytically cleaved form of $\alpha 6(\alpha 6p)$ integrin subunit in the more invasive cell lines of her panel. $\alpha 6p$ was not detected in the weakly invasive and noninvasive breast cancer cell lines MCF7 and MCF10A. These data suggest that integrin cleavage and release from the cell surface by proteases can be an important mechanism promoting cell migration.

PROTEOLYTIC MODIFICATION OF THE ECM AND CANCER CELL SURVIVAL

Drs. Yves DeClerck, Peter Brooks (New York University School of Medicine, New York, NY), Vito Quaranta (The Scripps Research Institute), Gregg Fields (Florida Atlantic University, Boca Raton, FL), and Lisa Coussens (University of California, San Francisco, CA) contributed to a session describing the functional consequences of proteolytic modification of ECM.

Dr. DeClerck presented data indicating the importance of the physical state of collagen in regulating cell cycle progression in melanoma cells. He demonstrated that contact between melanoma cells and

collagen can drive cells into arrest or proliferation depending on the fibrillar organization of the collagen. On contact with fibrillar cross-linked collagen, cells are arrested in the G_1 phase, whereas on contact with nonfibrillar collagen, cells enter the cell cycle. This effect is not caused by differences in adhesion, morphology, or stress induced by the different forms of collagen. Adhesion to fibrillar collagen results in an increase in p27^{KIP-1} protein and cell cycle arrest. Although increased p27^{KIP-1} protein levels were associated with cell cycle arrest, short interfering RNA experiments indicated that preventing p27^{KIP-1} increase did not block cell cycle arrest, suggesting the involvement of other factors. Thus, degradation of fibrillar collagen by MMPs produced by melanoma cells may represent a mechanism to escape growth inhibitory signals.

Dr. Brooks has identified a proteolytic COOH-terminal fragment of collagen that inhibits tumor progression. Further analysis narrowed the activity of this fragment to a nine amino acid domain called Pexstatin. In the chick chorioallantoic membrane assay, a synthetic peptide of these nine amino acids inhibits angiogenesis and tumor growth. It also induces inhibition of tumor growth in nude mice. Dr. Brooks attributed the effects of Pexstatin to its ability to accelerate tissue inhibitor of MMP-free MMP-2 processing by inducing autocatalytic degradation and inactivation of the protease.

Dr. Quaranta focused on a proteolytic fragment of another protein of the ECM, laminin-5. He described how the γ chain of laminin-5 (an $\alpha\beta\gamma$ heterotrimer) is cleaved at a Gly⁴¹³-Asp⁴¹⁴ site by MT1-MMP, MMP-14, resulting in fragments $\gamma 2^1$ lacking domain V and IV (D_V and D_{IV}) from the NH_2 terminus. A subsequent cleavage of $\gamma 2^1$ by MMP-2 generates a $\gamma 2x$ and D_{III} fragment. Among those multiple fragments, fragment D_{III} has an epidermal growth factor-like structure, stimulates cell migration, and promotes cell survival by binding to the EGFR. Dr. Quaranta discussed the biological relevance of laminin-5 and its breakdown products by highlighting their role in tumor vascular mimicry and mammary gland remodeling.

Dr. Fields has constructed synthetic THPs that correspond to specific regions within collagen IV and used these peptides as ligands to stimulate distinct signaling pathways that induce MMP expression. Focusing on the ligands for $\alpha 2\beta 1$ ($\alpha 1[IV]$ 402–413 THP) and CD44/CSPG ($\alpha 1[IV]$ 1263–1277 THP), he showed that both could induce MMP-1 expression, although to different levels and at different times. Only $\alpha 1[IV]$ 402–413 THP induced MMP-13 and MMP-14 expression, whereas only $\alpha 1[IV]$ 1263–1277 THP up-regulated MMP-8. These effects required the triple-helical conformation of the peptides, because loss of the triple-helical structure quenched the response, as did a single glycosylation of $\alpha 1[IV]$ 1263–1277 THP. The THP ligands provide valuable tools to dissect how individual cell-matrix receptors contribute to tumor progression.

Dr. Coussens further emphasized the important regulatory function of interstitial collagen in cancer progression. Intercrossing transgenic mice that have a mutated $\alpha 1$ type I collagen gene that renders the collagen resistant to degradation by collagenase with transgenic mice that develop *de novo* squamous cell carcinoma, she demonstrated that defective collagen remodeling attenuates malignant progression and tumor formation. She also showed that collagenase-resistant mice develop less lymph node metastasis and have decreased cell proliferation and vascular permeability.

RECEPTORS CROSS-TALK

A session focusing on the involvement of receptor cross-talk during cancer included presentations by Drs. Sharon Stack (Northwestern University School of Medicine, Chicago, IL), Harold Chapman (University of California), Liliana Ossowski (Mount Sinai School of Medicine, New York, NY), Lars Kjoller (Rigshospitalet, Copenhagen,

Denmark), and Darren Seals (Van Adel Research Institute, Grand Rapids, MI).

Dr. Stack showed that in oral squamous cell carcinoma, multivalent aggregation of $\alpha 3\beta 1$ integrins up-regulates both uPA and MMP-9 expression. She demonstrated that integrin-induced uPA expression requires ERK1/2 activation and is accompanied by a dramatic redistribution of the uPAR to sites of clustered integrins. Conversely, in E-cadherin-dependent cell–cell adhesion, formation of new cell–cell contacts results in suppression of uPA and MMP-9 expression. In this case, both phosphatidylinositol 3'-kinase-dependent AKT phosphorylation and EGFR-dependent mitogen-activated protein/ERK kinase/ERK activation are increased. The data presented provided evidence for a novel bi-directional communication between proteases and cadherins that suggests preferential engagement of cell–matrix *versus* cell–cell adhesion receptor. This engagement results in differential activation of signaling pathways that positively or negatively modulate proteinase expression.

Dr. Chapman focused his remarks on interactions between integrins and proteases in cell adhesion and migration. He showed that the receptor for uPA, uPAR, mediates disruption of E-cadherin/actin complexes via an integrin-dependent pathway as uPAR preferentially associates with $\alpha 3\beta 1$. uPAR association with $\alpha 3$ requires a preserved His²⁴⁵ residue present in $\alpha 3$ and $\alpha 6$ but not $\alpha 5$ and αv integrin subunits. uPAR overexpression in cells results in loss of E-cadherin. Transforming growth factor- β up-regulates uPAR independently of $\alpha 3\beta 1$ but by doing so down-regulates E-cadherin and promotes cell–cell dissociation and cell scatter.

Dr. Ossowski focused her presentation on tumor dormancy. She showed that human head and neck carcinoma Hep3 cells maintain their tumorigenic and metastatic properties when serially passaged *in vivo* but lose these properties when cultured *in vitro*. At the same time, the expression of uPAR is diminished. Dr. Ossowski showed that uPAR physically interacts and activates $\alpha 5\beta 1$ and initiates a signaling cascade that culminates in a strong and persistent ERK activation. Activation of $\alpha 5\beta 1$ by overexpressed uPAR leads to FAK phosphorylation and also to the recruitment of EGFR into the integrin complex and its independent activation in the absence of epidermal growth factor. Thus, Dr. Ossowski showed that EGFR is the mediator of signals initiated by the uPA/uPAR/fibronectin/ $\alpha 5\beta 1$ complex responsible for persistent levels of ERK activity and *in vivo* growth of Hep3 cancer cells. By lowering the level of uPAR expression, the positive signaling pathway to ERK is deactivated, whereas p38MAPK is activated, and cells enter dormancy. These data add to the data of Drs. Stack and Chapman to point to a critical role for a protease receptor like uPAR, in controlling cell migration and proliferation independently of its proteolytic-promoting activity.

Dr. Kjøller provided further evidence supporting a role for uPAR in the regulation of cell motility. Expression of human uPAR in a wide range of cell types induces reorganization of the actin cytoskeleton, as well as increased cell motility through activation of the small GTPase Rac. uPAR induces multiple rapidly advancing protrusions that each resemble the leading edge of migrating cells. Additionally, cytoskeletal changes require uPAR binding to vitronectin but appear independent of uPA binding or its association with the uPAR-associated protein (endo180), a member of the macrophage mannose receptor family.

Dr. Seals presented data on the localization of the adapter protein Fish, a Src tyrosine kinase substrate adapter protein, in invasive carcinoma cells. Fish is primarily distributed in the cytosol of normal mouse fibroblasts but translocates to specialized cell surface structures called podosomes or invadopodia in Src-transformed fibroblasts. Podosomes are F-actin-enriched structures seen in Src-transformed cells and in some highly invasive carcinomas. Fish is highly expressed

in certain invasive breast carcinomas where it can colocalize with Src and ADAMs (a disintegrin and metalloproteinase) at podosomes in both Src-transformed fibroblasts and Hs 578T breast carcinoma cells.

MEMBRANE-ASSOCIATED PROTEASES AND REMODELING OF THE PERICELLULAR SPACE

The importance of membrane-associated proteases in physiological and pathological conditions is becoming widely known. Several speakers specifically addressed this aspect. Drs. Motoharu Seiki (The University of Tokyo, Tokyo, Japan), Marc Lafleur (St. Vincent's Institute of Medical Research, Fitzroy, Victoria, Australia), and Jian Cao (State University of New York, Stony Brook, NY) discussed MT1-MMP; Dr. Makato Noda (Kyoto University School of Medicine, Kyoto, Japan) talked about a newly identified membrane-anchored MMP RECK; Drs. Charles Craik (University of California) and Thomas Bugge (National Institute of Dental and Craniofacial Research, Bethesda, MD) discussed membrane-associated serine proteases.

Dr. Seiki demonstrated the importance of MT1-MMP regulation for normal cell migration. He developed a series of mutant MT1-MMPs that were either constitutively active or inactive for MMP-2 activation and did not support cell migration. These mutagenesis studies highlighted the necessary colocalization of MT1-MMP with CD44, via the MT1-MMP PEX domain, which allows MT1-MMP to associate with the actin cytoskeleton and be localized to the leading front of migrating cells, promoting motility. At the leading front, MT1-MMP not only promotes the activation of MMP-2 but also regulates adhesion to hyaluronic acid by shedding its receptor CD44. Dr. Seiki showed that MT1-MMP's internalization is another critical regulatory step for migration and that its disruption impairs this process.

Dr. Lafleur discussed how collagen I stimulates pro-MMP-2 activation in an MT1-MMP-dependent manner. This stimulatory effect is dependent on the COOH-terminal hemopexin domain of MT1-MMP and involves a post-translational regulatory step but does not require the transmembrane domain or cytoplasmic tail of MT1-MMP. Dr. Lafleur showed that exposure of cells to collagen I results in a signal via a tyrosine kinase pathway. Discoidin domain receptors rather than integrin $\alpha 1$ may be the signaling receptor because genistein reduced pro-MMP-2 activation, whereas blocking antibodies to $\alpha 1$ had no effect.

Dr. Cao presented data identifying the domains of MT1-MMP that are critical for cancer cell invasion and migration. Using a number of MT1-MMP mutants transfected into Cos-1 cells and cultured on FITC-labeled fibronectin cross-linked gelatin film, he demonstrated that the propeptide and catalytic domains of MT1-MMP are required for substrate degradation, whereas the hemopexin domain is essential for MT1-MMP-mediated cell migration. Dr. Cao showed that MT1-MMP-dependent signaling for cell migration includes the small GTPase, Rac1.

Dr. Noda focused on the structure and function of the RECK protein. This glycosylphosphatidyl inositol-anchored *N*-glycosylated protein is widely distributed in normal tissues but rarely detected in cancer-derived cells. RECK negatively regulates at least three MMP family members: (a) MMP-2; (b) MMP-9; and (c) MT1-MMP. Accordingly, the absence of RECK in cancer cells results in hyperactivation of these MMPs. Homozygous deletion of RECK in mice is embryonic lethal at about E10.5, with defects in angiogenesis. When expression is forced in tumors, RECK causes a suppression of invasion and tumor metastasis and has a negative effect on vessel sprouting in the tissue surrounding neoplastic cells.

Dr. Craik described a new family of type II MT-SPs that contributes to epithelial cancers and could potentially be a diagnostic marker.

Ecotin, a class-specific inhibitor of serine proteases, was used to isolate one of these proteases, MT-SP1, from tumor tissues. MT-SP1 is mainly expressed in the gastrointestinal tract of normal tissues and frequently found in breast, prostate, and colon cancers. Additionally, MT-SP1 is found in the lumen of prostate cancers and subsequently in the urine of patients with late stage prostate cancer and may therefore be a useful diagnostic marker. Tetrapeptide positional scanning, synthetic combinatorial library screening, and substrate bacteriophage display were then used to profile the catalytic domain of MT-SP1 and identify potential substrates, which include hepatocyte growth factor, uPA, and protease-activated receptor 2.

Dr. Bugge showed that epidermal expression of MT-SP1 regulates stratum corneum formation and hair follicle development in mice. Mice with a null mutation in the *MT-SP1* gene uniformly die within 48 h of birth, because of a lack of epidermal water barrier function and uncontrolled dehydration. Loss of this proteinase seriously compromises hair canal genesis and creates extensive hair follicle abortion. His data show that epidermal MT-SP1 plays a key role in both follicular and interfollicular epidermal development. The mice also have an abnormal thymic development with increased apoptosis in CD4- and CD8-positive lymphocytes. Preliminary data suggest that prokeratinocyte growth factor may be a potential substrate for MT-SP1.

CELL MOTILITY

A critical feature of cancer is tissue invasion, which, in addition to ECM degradation, requires cell migration through the surrounding ECM. A session devoted to cell motility included talks by Drs. Abraham Raz (Karmanos Cancer Institute, Detroit, MI), J. Thomas Parsons (University of Virginia Health Systems, Charlottesville, VA), Peter Friedl (University of Wuerzburg, Wuerzburg, Germany), Susanne Mueller (Georgetown University School of Medicine, Washington, DC), and Rebecca Riggins (University of Virginia Health Systems).

Dr. Raz addressed the role of an AMF, a tumor-associated CXXC cytokine, with a CEGC motif that is a potent stimulator of cell motility. This protein is homologous to phosphohexose isomerase. He showed that AMF acts as the ligand for a M_r 78,000 glycoprotein AMF receptor. AMF stimulation of human melanoma cells induces stress-fiber formation, up-regulation, and activation of RhoA and Rac1 expression in the absence of changes in Cdc42. Both c-Jun NH₂-terminal kinase 1 and 2 are simultaneously activated by AMF, supporting the hypothesis that they are involved in the signaling pathway of RhoA. In bladder cancer, there is an increase in AMF expression and a concomitant decrease in E-cadherin expression as the cancer progresses from T1 to metastatic stages.

Dr. Parsons reported that prostate cell lines exhibiting increased metastatic potential have increased FAK expression, overall tyrosine phosphorylation, and autophosphorylation of tyrosine 397. Phosphorylation of Tyr³⁹⁷ creates a high affinity-binding site for the nonreceptor tyrosine kinase, Src. Accordingly, inhibition of the FAK/Src signal transduction pathway significantly inhibits migration. Using dominant negative forms of FAK and FAK short interfering RNA, he demonstrated that suppression of FAK expression or activity induces a block in cell growth and inhibits cell motility.

Dr. Friedl examined tumor cell migration in a dynamic three-dimensional collagen matrix model, based on time-lapse videomicroscopy and confocal backscatter imaging. He showed that MV3 melanoma and HT1080 fibrosarcoma cells move through the collagen by developing spindle-shaped processes and adopt what he described as a mesenchymal single cell migration pattern. Inhibition of the proteolytic activity of these cells by pharmacological compounds blocking MMPs, serine proteases, and cathepsins simultaneously or inhibition

of β 1 integrin slowed migration but surprisingly did not completely block it. He showed that under these conditions, cells adopted a default motility pattern consisting in the formation of amoeboid movements and associated with a loss of integrin clustering and the dissociation of MT1-MMP from its interaction with collagen fibers leading to its accumulation in the cytoplasm. He speculated that this amoeboid movement might represent a functional escape mechanism for migrating tumor cells exposed to protease inhibitors. These intriguing data challenge the prevailing concept that proteolytic degradation of the ECM is necessary for tumor cell invasion.

Ms. Riggins presented data on the adapter proteins Cas and a guanine nucleotide exchange factor (AND-34/BCAR3). She showed that AND-34/BCAR3 synergistically increases Cas-mediated activation of Src and phosphorylation of several Src substrates. The AND-34 SH-2 domain was critical for promoting Cas-dependent Src activity, as well as cell spreading and localization of AND-34/BCAR3 to lamellipodia. The guanine nucleotide exchange factor domain of AND-34 contributes to the enhancement of Src activity, possibly via the small GTPase Rap1. Cas-dependent increase in Src kinase activity correlated with an increase in cell migration, which was further enhanced by AND-34 in an SH2 domain-dependent manner.

Dr. Mueller continued the theme of the role of Src in invasiveness of breast cancer cells by providing evidence that overexpression of constitutively activated c-Src dramatically increases the formation of invadopodia with concomitant ECM invasion in MDA-MB-231 cells. Activated c-Src-expressing cells show elevated levels of MMP-9 secretion, whereas overexpression of kinase inactive c-Src suppresses MMP-9 secretion, as well as the formation of invadopodia with concomitant ECM invasion in MDA-MB-231 cells. She concluded that c-Src is critical for invasion by MDA-MB-231 cells.

ECM, PROTEASES, AND ANGIOGENESIS

Angiogenesis has been identified as a major target for therapeutic interventions aimed at inhibiting cancer progression. Talks on this subject were given by Drs. Jean-Michel Foidart (University of Liege, Liege, Belgium), Mary Hendrix (University of Iowa College of Medicine, Iowa City, IA), Raghu Kalluri (Beth Israel Deaconess Medical Center), Emmanuelle Liaudet-Coopman (Institut National de la Santé et de la Recherche Médicale, Paris, France), and Hanspeter Gerber (Genentech, San Francisco, CA).

Dr. Foidart focused on the importance of PAI-1 and plasminogen in angiogenesis and tumor growth. He showed that PAI-1 and plasminogen knockout mice do not develop tumors when injected with cancer cells, unlike wild-type mice or uPA, tissue-type PA, and uPAR-deficient mice. Adenovirus-delivered PAI-1 in PAI-1-deficient mice restores their ability to support tumor growth. Using PAI-1 mutants deficient in either the antiproteolytic activity or the vitronectin binding activity of PAI-1, he demonstrated that it is the interaction of PAI-1 with PA rather than with vitronectin that is crucial for this effect. This observation also underlines the important contributory role of host cells in regulating tumor growth and provides an explanation for the apparently contradictory elevation of PAI-1 in more advanced stages of cancer.

Dr. Hendrix showed data demonstrating that aggressive and non-aggressive melanoma cell lines substantially differ in their vasculature. In aggressive (uveal) melanoma, tumor cells form part of the vascular endothelium (vascular mimicry). This can be recapitulated in tissue culture if aggressive cells are plated in three-dimensional collagen gels, where they form a vasculogenic network, whereas non-aggressive cell lines do not. RNA microarray analysis of melanoma cells in these conditions highlighted differences in gene expression, with typical endothelial markers, including VE-cadherin, and epithelial cell

kinase/EphA2 being expressed only by the aggressive cell lines. The ability of tumor cells to behave like endothelium requires MMP activity. The ECM can also influence the phenotype of these cells because nonaggressive melanoma cells switch to the aggressive phenotype when plated on a collagen matrix that had aggressive cells cultured on it previously. These data demonstrate the remarkable influence of the microenvironment on the transdifferentiation of melanoma cells.

Dr. Kalluri focused his talk on proteolytic fragments of the ECM that exhibit antiangiogenic properties. One fragment, Tumstatin, a M_r 28,000 cleavage product of collagen IV with antiangiogenic activity, was further characterized. Using Lewis lung carcinoma in mice as a model, he showed that Tumstatin specifically localizes to the tumor when injected *i.v.* and inhibits tumor growth. Consistently, tumors implanted in collagen IV knockout mice grow faster than controls, and the addition of Tumstatin prevents growth stimulation. Tumstatin is generated by MMPs like MMP-9, which is localized to tumor endothelial cells, where it binds to $\alpha v\beta 3$ at an RGD-independent site.

Dr. Liaudet-Coopman showed in 3Y1-Ad12 human breast cancer cells that cathepsin D stimulates cancer cell proliferation and angiogenesis independent of its catalytic activity, because both wild-type and catalytically active mutants were active. In contrast, cathepsin also inhibits apoptosis but only if catalytically active.

Dr. Hanspeter Gerber presented work identifying ANGPTL3, a liver-specific secreted factor consisting of an NH_2 -terminal domain and the $COOH$ -terminal fibrinogen-like domain of angiopoietin as the first member of the angiopoietin-like family of secreted factors that can bind to $\alpha v\beta 3$ and possibly regulate angiogenesis. Binding of ANGPTL3 to $\alpha v\beta 3$ induces haptotactic endothelial cell adhesion and migration and stimulates the phosphorylation of AKT, MAPK, and FAK.

MODELS TO STUDY CELL-ECM INTERACTION

Models to study cell-ECM and cell-cell interactions have been important in testing novel hypotheses or challenging old ones. Several presentations were devoted to this issue, including presentations by Drs. Ruth Muschel (University of Pennsylvania), James Quigley (The Scripps Research Institute), and Wen-Tien Chen (State University of New York).

Using vital fluorescent microscopy of isolated perfused lungs as a model to study the fate of tumor cells into the circulation, Dr. Muschel showed that tumor cells injected into the circulation rapidly attach to the pulmonary endothelium. This attachment is inhibited by blocking antibodies to $\alpha 3$ or $\beta 1$ integrin subunits, as does genetic deficiency of either the $\alpha 3$ or $\beta 1$ integrin subunit. After attachment, metastatic tumor cells proliferate, but unexpectedly, this proliferation first occurs within vascular channels, and extravasation rarely occurs. The $\alpha 3\beta 1$ integrin, which is commonly expressed in tumor cells, is thus an important mediator of the early arrest of tumor cells in the pulmonary vasculature and a contributor to metastasis that may be a therapeutic target. In contrast, extravasation might be a poor target for therapy.

Dr. Quigley discussed models that allow for examination of the relationship between proteases and angiogenesis. Using a modified *in vivo* angiogenesis model in the developing chick embryo and an *ex vivo* vessel outgrowth model made of explanted mouse aorta grown in three-dimensional collagen gels, he showed that MMPs like MMP-2 and MT1-MMP are involved in growth factor-induced angiogenesis. The formation of three-dimensional vascular tubes in this model was also highly dependent on the expression of a member of type II transmembrane serine proteases, TMPRSS2 (epitheliasin), which is expressed by the tube-forming microvascular endothelial cells.

Dr. Chen presented a novel technique that allows the single-step

isolation of carcinoma cells circulating into blood. This technique is based on the principle that only tumor cells, and not normal blood cells, bind to a fibrous collagenous matrix scaffold coated with blood-borne adhesion molecules, such as plasma fibronectin, laminin, and vitronectin, collectively called cell-adhesion matrix. These metastatic cancer cells express markers for both epithelial and endothelial cells, have the capability to degrade and ingest fluorescent collagen fragments, and show low rates of apoptosis, similar to invasive breast ductal carcinoma cells.

IMAGING ECM REMODELING

New imaging technologies have rapidly developed over the last few years providing novel tools to examine ECM remodeling and protease activity *in vitro*, *ex vivo*, and *in vivo*. These technologies were addressed in presentations by Drs. Bonnie Sloane (Wayne State University School of Medicine, Detroit, MI), Matthew Bogyo (Celera, South San Francisco, CA), Ching Tung (Massachusetts General Hospital, Charlestown, MA), Caroline Scatena (Xenogen Corp., Alameda, CA), and Linda Meade-Tollin (Arizona Cancer Center).

Dr. Sloane used confocal microscopy to examine the degradation of a DQ-collagen by several human cancer cell lines. The generation of degradation products of DQ-collagen was observed intracellularly, suggesting that intracellular proteolysis is important for tumor cell invasion. Tumor spheroids degrade DQ-collagen IV intracellularly and pericellularly, with most of the degradation occurring pericellularly and focally on the tumor cell surface. Intracellular degradation is in a cathepsin B-containing vesicular compartment. Dr. Sloane showed that proteases associated with the tumor cell surface are derived from tumor or stromal cells, and the addition of fibroblast to tumor cells significantly increases pericellular proteolysis in cocultures. Thus, therapeutic approaches aimed at reducing tumor-associated proteolysis will need to target stromal and tumor cells, and to target the interactions between stromal and tumor cells may be important.

Dr. Bogyo has developed a series of small molecule activity-based probes that can be used to tag members of the papain family of lysosomal cysteine proteases. The probes allow the monitoring of protease expression and obtaining activity profiles both *in vitro* and *in vivo*. In a multistage mouse model of human pancreatic cancer (RIP-Tag), he showed that *in vivo* administration of a fluorescent affinity probe followed by histological and biochemical analysis allows the following, in time and space, of the proteolytic activity during tumor development. He observed a reduction in tumor burden and protease activity in animals treated with inhibitors of cysteine proteases. Dr. Bogyo also used these activity-based probes to determine protease activity profiles in distinct cellular populations within the tumor.

Dr. Tung described how probes consisting of quenched near-IR fluorochromes conjugated to specific peptide substrates grafted to a polylysine backbone can be used to monitor proteolytic activity in tumor xenografts *in vivo* and demonstrate the efficacy of specific inhibitors of MMPs. Using probes with an MMP-2-specific sequence, he showed detectable activity *in vitro* in HT1080 tumor-bearing mice and inhibition of this activity when mice were treated with the MMPI AG3340. As new protease-specific probes are developed, this methodology will provide valuable biological end points to monitor the therapeutic efficacy of protease inhibitors *in vivo*.

Dr. Scatena introduced an application of the IVIS imaging system to detect micrometastatic lesions *in vivo* in animals injected with tumor cells stably transfected with constitutive promoters linked to luciferase. Using tumor cells transfected with the MT1-MMP promoter-driving luciferase gene, she was able to track these cells *in vitro* and *in vivo* and examine their metastatic fate. TNF- α , which increases

MT1-MMP expression *in vitro*, was also shown to increase luciferase expression and tumor cell detection *in vivo*.

Dr. Meade-Collin presented a flexible *in vitro* model to investigate MMP-integrin interactions during migration of microvascular endothelial cells. Using time lapse differential interference contrast microscopy, she showed the association of MMP-2, MMP-9, $\alpha v \beta 3$, and $\alpha v \beta 5$ at the surface of endothelial cells forming capillary-like networks. This model could be used to screen for angiogenic inhibitors.

THERAPEUTIC OPPORTUNITIES

The final session of the conference focused on how our knowledge gained on the role of proteases and the ECM in cancer should be applied to test potential novel anticancer strategies. Presentations in this session were given by Dr. Lynn Matrisian (Vanderbilt University, Nashville, TN), who reviewed the recent experience with MMPs in clinical trials, and Drs. Claude Libert (Ghent University, Ghent, Belgium), Gabriele Bergers (University of California), Achim Krüger (Technical University Munich, Munich, Germany), and Curtis Braun (Twinstrand Therapeutics, Inc., Burnaby, British Columbia, Canada).

Dr. Matrisian reviewed the recent experience with MMPs in the clinical setting. She pointed out that although MMPs have shown promise in preclinical models, clinical trials in cancer patients have been unexpectedly disappointing, to the point that very few clinical trials are still ongoing. Some of these inhibitors have been toxic, and others were associated with an acceleration rather than an inhibition of cancer progression. However, several important lessons that will be valuable for many drugs targeting specific proteins must be learned. In particular, we need better preclinical models that closely reflect the complex interaction between tumor cells and the microenvironment. We need to have specific biological end points to monitor the activity of the agents *in vivo*, and we need to test these agents much earlier during cancer progression rather than in patients with end-stage diseases that are resistant to most chemotherapeutic agents. This will require a close collaboration between biologist, pharmacologists, clinicians, and imaging scientists.

Dr. Libert presented a new role for MMPs in tumor therapy by demonstrating that MMPs can reduce the cytotoxicity of TNF and IFN- γ . Administration of TNF or IFN- γ in tumor-bearing mice has a dramatic effect on tumor regression but kills the host because of severe liver cytotoxicity associated with increases in liver necrosis

and apoptosis and a massive increase in MMP activity. The administration of MMPs reduced toxicity in the host while maintaining the antitumor effect. Using MMP knockout mice, he then observed that this protective response is attributable to inhibition of MMP-2.

Dr. Bergers described her work identifying a population of pericytes in pancreatic islet carcinomas in RIP-Tag transgenic mice, of which only a subpopulation express PDGFR. Pancreatic tumor cells do not express PDGFRs or ligands, whereas the endothelial cells express platelet-derived growth factor, and pericytes along the microvasculature express PDGFR. She showed that inhibition of PDGFR signaling causes pericyte detachment from endothelial cells and vessel disruption, thus identifying pericytes as a potential new target for antiangiogenic therapy. PDGFR inhibitors like Su6668 or STI 571 (Gleevec) synergize with VEGF receptor inhibitors (Su5416), resulting in a greater inhibition of angiogenesis.

Dr. Kruger analyzed a panel of specific inhibitors for their inhibition activity on metastasis. He demonstrated that the more successful inhibitors of metastasis were also the better inhibitors of Factor Xa, suggesting the importance of Factor Xa in metastasis.

Dr. Braun presented data showing that MMPs can be useful to activate new synthetic prodrugs at the site of tumors, thereby localizing their cytotoxicity. He has developed a prodrug TST220 that is specifically activated by MMPs and a prodrug TST314 that is activated by urokinase. These prodrugs are more cytotoxic toward tumor cell lines as compared with Cos-1 (control) cells. These prodrugs could thus be tailored to be activated by cancer-associated proteases.

SUMMARY

In summary, this conference succeeded in bringing together researchers from different disciplines with a common research interest focused on the study of tumor cells and their matrix environment. At the end of this conference, it became apparent that much effort is needed to achieve a better understanding of the complex and dynamic interactions between tumor cells, proteases, and ECM proteins. The ECM and proteases remain an attractive therapeutic target for cancer treatment and control, but to perform clinical trials in this field will require a deeper knowledge of their mechanisms of action, the availability of end points to monitor the activity of target-based cytostatic agents, and the opportunity to test their activity in earlier stages of cancer progression.