Histol Histopathol (1999) 14: 665-671

http://www.ehu.es/histol-histopathol

Histology and Histopathology

Invited Review

Regulation of tumor cell invasion by extracellular matrix

D.L. Crowe and C.F. Shuler

Center for Craniofacial Molecular Biology, University of Southern California, Los Angeles, CA, USA

Summary. The ability of malignant tumor cells to invade normal surrounding tissue contributes in large part to the significant morbidity and mortality of these cancers. The process of invasion involves adherence of the tumor cells to the extracellular matrix (ECM), degradation of matrix components, and movement of the cell body. Attachment to ECM molecules is mediated by the integrin family of extracellular matrix receptors. Integrins are a large family of heterodimeric proteins which transduce a variety of signals from the ECM. Ligand occupancy is critical for activation of integrin signaling. This signaling may occur via several different pathways. One of the best characterized of these pathways is the mitogen activated protein kinase (MAPK) cascade. This serial phosphorylation of substrate proteins terminates in activation of transcription factors which regulate expression of target genes. Many of these genes are critical for extracellular matrix degradation or cell migration. Among these are the matrix metalloproteinases (MMPs), a large family of ECM-degrading enzymes. Regulatory elements in the promoters of MMPs have been characterized, providing insight into how MMP expression is controlled. This review focuses on mechanisms by which the ECM regulates tumor cell invasion through integrin signaling via the MAPK pathway using MMP expression as the model.

Key words: Extracellular matrix, Integrins, Mitogen activated protein kinase, ets transcription factors, Matrix metalloproteinase

Introduction

The ability of tumor cells to invade surrounding tissue and vital structures is one of the most important features of the malignant phenotype. Degradation of the basement membrane and invasion of underlying connective tissue have long been the histologic criteria for diagnosis of carcinoma (for review see Liotta, 1986; Lohi et al., 1998). In order to accomplish this transformation, potential tumor cells must alter their phenotype and histologic appearance. Invading tumor cells must secrete proteolytic enzymes to degrade basement membranes (for review see Kohn and Liotta, 1995). The invading cell must then extend long cytoplasmic processes (lamellopodia) which attach to specific proteins of the extracellular matrix (ECM). This attachment is necessary for the cell to generate traction in order to translocate the cell body (Bretscher, 1996). Further migration requires invading tumors to repeat this process which at the cellular level involves organizing complex focal adhesive contacts with the ECM, reorganization of the cytoskeleton, and bulk flow of cytoplasm and plasma membrane in the direction of movement (Damsky and Werb, 1992). Additionally, genes encoding the proteolytic enzymes necessary for connective tissue degradation must be precisely regulated to avoid autodigestion (Sato et al., 1994; Witty et al., 1994). This review will examine how ECM attachment generates cytoplasmic signaling resulting in activation of a family of target genes crucial to the invasive phenotype, the matrix metalloproteinases (MMPs). The first section reviews ECM attachment by integrin receptors and recruitment of signaling molecules to the cytoplasmic domain of these proteins. Next propagation of these signals by the mitogen activated protein kinase (MAPK) pathway will be considered. Finally, MAPK activation of transcription factors which regulate MMP expression will be examined.

Offprint requests to: Dr. David L. Crowe, Center for Craniofacial Molecular Biology, University of Southern California, 2250 Alcazar Street, Los Angeles, CA, USA. Fax: 323-442-2981

The integrin family of ECM receptors

Integrins are a large family of cell surface receptors that mediate ECM attachment in almost all cell types. Attachment to ECM is involved in complex biologic processes such as embryogenesis, carcinogenesis, apoptosis, cell division, differentiation, and inflammation (for review see Clark and Brugge, 1995). Functional integrins are composed of α and ß subunits. Each subunit consists of extracellular, transmembrane, and cytoplasmic domains. Currently 16 α and 8 ß subunits have been characterized (Clark and Brugge, 1995). The α subunits have relative molecular masses of 120-180 kD while the ß subunits range from 90-110 kD. Alternative splicing creates additional subunit variation

(Hynes, 1992). Several subunits including α_3, α_6, β_1, β_3, and β_4 possess alternatively spliced cytoplasmic domains (van Kuppevelt et al., 1989; Altruda et al., 1990; Suzuki and Naitoh, 1990; Hogervorst et al., 1991; Tamura et al., 1991). The α and β subunits heterodimerize to create more than 20 receptors.

The ligand binding specificity of integrin heterodimers has been elucidated (for review see Hynes, 1992). Individual integrins may bind to more than one ECM ligand. These molecules include type I collagen, type IV collagen, laminin, fibronectin, and vitronectin. Some integrins can also bind to soluble ligands such as fibrinogen or to other receptors such intercellular adhesion molecules on adjacent cells. Integrin heterodimers bind to specific peptide recognition sequences in ECM molecules. One of these sequences is Arg-Gly-Asp (RGD) present in fibronectin and vitronectin. RGD is recognized by $\alpha_5\beta_1$, $\alpha_{IIb}\beta_3$, and $\alpha_v\beta$ integrins. $\alpha_{IIb}\beta_3$ also recognizes the sequence KQAGDV in fibrinogen. Other sequences recognized by integrins include DGEA ($\alpha_2\beta_1$) and EILDV ($\alpha_4\beta_1$). Thus, structural similarities exist between integrins which recognize a specific peptide sequence.

The transmembrane regions of most integrin subunits consist of a single hydrophobic segment of less than 50 amino acids. Both subunits contain extensive disulfide bonds (Calvete et al., 1991). Divalent cations are required for receptor function (Kirchhofer et al., 1991). The integrin ligand binding domain is proximal to these cation binding sites extracellularly (Smith and Cheresh, 1990). Point mutations in the ligand binding domain ablates the association with specific ECM molecules (Arnaout et al., 1990). Both α and β subunits contribute to ligand binding and subunit switching may change this specificity.

Receptor clustering and ligand occupancy are critical for integrin signaling. This signal transduction is accomplished by the integrin cytoplasmic domains (Sastry and Horwitz, 1993). The cytoplasmic domains have no known ezymatic activity. Instead, they couple cytoskeletal and signaling proteins into focal adhesions. The α subunits have divergent amino acid sequences, whereas the β subunits exhibit some homology. The β cytoplasmic domains are required for targeting integrins to focal adhesions, while the α subunits provide ligand specificity (LaFlamme et al., 1994). Integrins link the ECM to cytoskeletal proteins such as actin. Binding proteins for actin such as talin, vinculin, tensin, and α-actinin have been localized within focal adhesions (Otey et al., 1993). Talin and α-actinin bind directly to the β subunit. These structural proteins organize the signaling complex leading to integrin induced changes in gene expression.

The integrin signaling complex

Integrin signaling is accomplished by protein phosphorylation (Clark et al., 1994). Phosphorylation on tyrosine residues is a common response to integrin binding to ECM ligands in many cell types (Hynes, 1992; Juliano and Haskill, 1993; Arroyo et al., 1994). Among the proteins which localize to integrin signaling complexes are those with modular domains homologous to the Src tyrosine kinase (Cohen et al., 1995; Pawson, 1995). These domains, termed SH2 and SH3, mediate protein-protein association. SH2 domains bind to proteins containing specific phosphotyrosine motifs while SH3 domains interact with proline rich sequences on target proteins. These adaptor proteins allow recruitment of additional signaling molecules to the focal adhesion.

Many kinases have been implicated in integrin signaling. One of these, focal adhesion kinase (FAK), is believed to have a central role in both signaling and coupling the active complex to the cytoskeleton (Fig. 1). Inhibition of FAK signaling has been shown to decrease cell motility and proliferation (Gilmore and Romer, 1996; Richardson and Parsons, 1996). Similar phenotypes have been described in cells from FAK deficient mice (Ilic et al., 1995). FAK is itself phosphorylated on tyrosine and its activity is enhanced by integrin signaling (Schaller and Parsons, 1994). The cytoplasmic domain of the integrin β subunit is required for FAK phosphorylation (Lukashev et al., 1994). FAK targeting to focal adhesions requires specific sequence elements in the protein. A C terminal focal adhesion targeting (FAT) sequence is necessary for localizing FAK to focal adhesions (Hildebrand et al., 1993). The cytoskeletal protein paxillin can associate with the FAK C terminus separate from the FAT sequence. The N terminus of FAK can bind to the cytoplasmic domain of the integrin β subunit. FAK likely uses all of these domains in order to localize to the focal adhesion. FAK phosphorylation may also serve as docking sites for SH2 containing proteins. Thus FAK serves as a central mediator for many integrin

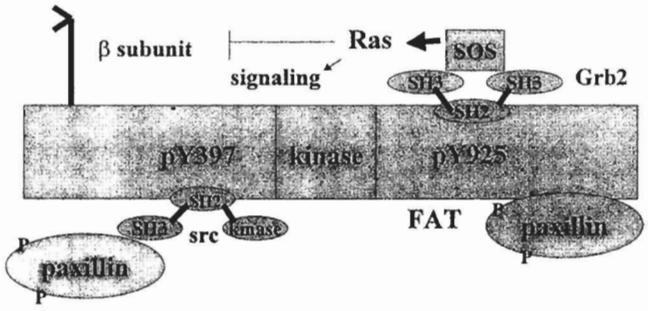

Fig. 1. The integrin signaling complex. Upon activation, the integrin cytoplasmic domain (here the β subunit) recruits a variety of downstream signaling molecules. Central to this interaction is focal adhesion kinase (FAK), which upon association with the integrin cytoplasmic domain is phosphorylated by members of the src tyrosine kinase family. Phosphorylated FAK then serves to recruit adaptor molecules containing SH2 and SH3 domains such as GRB2. The SH3 domains of these adaptors can interact with guanine nucleotide exchange factors such as Sos which transfers GTP to Ras to initiate the MAPK signaling pathway. Paxillin links the signaling complex to the structural proteins in the focal adhesion.

signaling proteins.

Members of the Src family of tyrosine kinases associate with integrin signaling proteins through binding of their SH3 domains to paxillin (Weng et al., 1993). These kinases may also associate via binding of their SH2 domains to phosphotyrosine residues (Okamura and Resh, 1994). Paxillin, which is a substrate for tyrosine kinases, may be involved in formation and regulation of integrin signaling complexes. Paxillin binds to vinculin and is phosphorylated on tyrosine in adherent cells by FAK or other kinases (Pavalko and Otey, 1994). Inhibition of cell spreading by expression of a dominant negative FAK can be rescued by Src (Richardson et al., 1997). Although many Src family proteins contain catalytic domains with kinase activity, some molecules are composed entirely of SH2 and SH3 domains (Cohen et al., 1995). One example is the growth factor receptor bound protein 2 (Grb2), which links tyrosine kinases to activators of the Ras signaling pathway (van der Geer et al., 1994). One activator of the Ras pathway is mSOS1, a guanine nucleotide exchange factor (GNEF) that functions by converting Ras to its active GTP bound state. In adherent cells, Grb2 and mSOS1 bind to FAK. The SH2 domain of Grb2 binds to a phosphotyrosine sequence in the FAK C terminus (Schlaepfer et al., 1994), thus directly linking the integrin signaling complex to Ras and the mitogen activated protein kinase (MAPK) pathway. It should be noted however that FAK independent mechanisms of MAPK activation may exist (Lin et al., 1997).

The mitogen activated protein kinase (MAPK) pathway

The link between integrin signaling and the MAPK pathway has been the subject of intense investigation in recent years (Juliano and Haskill, 1993; Lin et al., 1997). Mechanical stressing of integrin receptors induced phosphorylation of MAPKs (Schmidt et al., 1998). Binding to laminin triggered MAPK activation in macrophages via the integrin α_6 subunit (Wei et al., 1998). The integrin $ß_4$ subunit has been shown to recruit the adaptor proteins Shc and Grb2 to its phosphorylated cytoplasmic domain (Mainiero et al., 1995). Ligation of the $\alpha_6ß_4$ integrin caused tyrosine phosphorylation of Shc, recruitment of Grb2, activation of Ras, and stimulation of MAPKs in keratinocytes (Mainiero et al., 1997). This activation stimulated the serum response promoter element of c-fos and was inhibited by dominant negative mutants of Ras and Shc. Ras activation was also necessary for MAPK regulation in NIH 3T3 cells (Clark and Hynes, 1996). This effect may be both cell type and integrin dependent given that other studies have shown that integrin mediated activation of MAPKs was independent of Ras (Chen et al., 1996). MAPK activation may result in a negative feedback loop in that signaling via this pathway inhibited further integrin activation (Hughes et al., 1997).

The mechanisms by which the MAPK cascade transmits extracellular signals has been the subject of intense investigation for many years (for review see Blenis, 1993; Crews and Erikson, 1993; Davis, 1993; Cobb and Goldsmith, 1995). As their name implies, MAPK phosphorylation has an impact on diverse cellular processes. As stated above, activated GTP bound Ras binds the upstream protein kinases Raf-1 and B-Raf, targeting them to the cell membrane and increasing their kinase activity (Fig 2). The MAPK kinases 1 and 2, known as MKK or MEK, are phosphorylated and activated by Raf (Ahn et al., 1991; Seger et al., 1991; Campbell et al., 1995). MEKs phosphorylate the extracellular signal regulated kinases ERK1 and 2. The current model for MAPK signaling relies on this three kinase module (Neiman et al., 1993).

MEK phosphorylates MAPKs on tyrosine and threonine residues. In ERK2, these amino acids are tyr-185 and thr-183 (Payne et al., 1991). Tyrosine phosphorylation precedes that of threonine, although either residue can be phosphorylated alone (Robbins et al., 1993). ERK2 consists of an N terminal domain and a larger C terminus connected by a linker region (Zhang et al., 1994). The ATP binding site is located between the domains, while substrates bind on the surface. Phosphorylation of ERK2 causes a conformational change in the protein which aligns the catalytic residues (Cobb and Goldsmith, 1995). In its unphosphorylated state, the tyrosine and threonine residues block access to the catalytic site. The side chain of the tyrosine residue occupies the substrate binding site. Phosphorylation displaces this amino acid and activates the catalytic site. The activity of ERK2 containing a T183E mutation is dramatically increased following tyrosine phosphorylation (Robbins et al., 1993). The activated ERKs are capable of phosphorylating downstream transcription factors which regulate target gene expression.

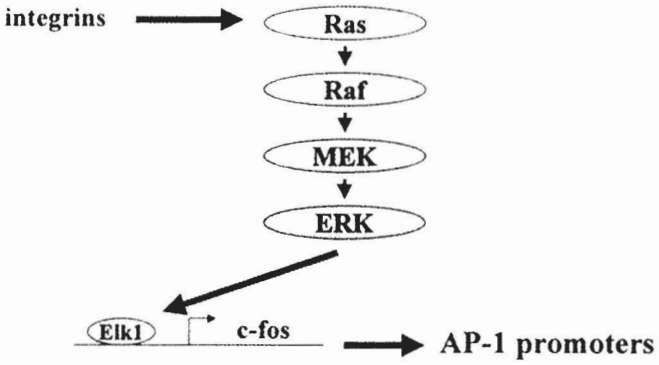

Fig. 2. The mitogen activated protein kinase pathway. Ras, the most upstream effector in the cascade, activates Raf (a mitogen activated protein kinase kinase kinase, MAPKKK). Raf in turn phosphorylates MEK (a MAPKK) which then activates the extracellular regulated kinase (ERK/MAPK). ERK then phosphorylates members of the ets transcription factor family such as ets-1, ets-2, Elk-1 and PEA3. The c-fos promoter contains recognition sequences for these factors. Induction of c-fos expression may then activate genes containing AP-1 regulatory elements.

The Ets family of transcription factors

One of the major targets of MAPK phosphorylation is the Ets family of transcription factors. The ets oncogene was discovered as a fusion protein with gag and myb expressed by the avian erythroblastosis virus (Nunn et al., 1983). Ets family proteins are characterized by a homologous 84 amino acid sequence known as the ets domain (Seth et al., 1992). The most divergent ets domains (Ets1 and PU1) are 35% identical. Ets1 also has alternatively spliced isoforms (Koizumi et al., 1990). The sequence similarity of the ets domains allows the Ets proteins to be grouped into subfamilies. The ets domain is found in the C terminus of Ets1 and 2, near the middle of Elf1, and towards the N terminus of Elk1 and SAP1 (Wasylyk et al., 1993). Other family members in various species include EtsB, vEts, Fli1, Erg, Ets3, Ets4, Ets6, GABP, DELG, PEA3, ELKX, and E74A.

Ets proteins can bind DNA containing the sequence C/A GGA A/T. Ets1 directly contacts this motif and 10 bp of DNA immediately surrounding the sequence (Nye et al., 1992). These flanking sequences are variable and may determine which Ets proteins will bind. Different Ets proteins vary in their selectivity for a particular motif (Rao and Reddy, 1992). The ets domain itself is sufficient for DNA binding (Gegonne et al., 1992). Ets family members lack features of other transcription factors such as homeodomains, helix-turn-helix, zinc fingers, leucine zippers, or rel domains. Ets1 has a putative nuclear localization sequence (GKRKNKPK; Boulukos et al., 1989). Some Ets proteins bind DNA as monomers (Nye et al., 1992), but others bind in conjunction with other factors such as SRF (Hipskind et al., 1991).

A variety of growth modulation signals affects activation of Ets proteins. Mitogenic signals via the MAPK pathway alter the phosphorylation status of Ets1 and Ets2 (Wasylyk et al., 1993; O'Hagan et al., 1996). The Ets protein PEA3 is phosphorylated both by ERK and the stress activated protein kinase/jun N terminal kinase (SAPK/JNK; O'Hagan et al., 1996). Ets1 and Ets2 were shown to be targets of Ras signaling by phosphorylation of conserved threonine residues (Yang et al., 1996). Phosphorylation of Ets1 and Ets2 increased DNA binding in vitro. Ets1 is hyperphosphorylated during mitosis (Fleischman et al., 1993), indicating that Ets proteins are regulated by mitogenic signals.

Ets proteins regulate a variety of cellular processes, including development, differentiation, migration, and tumorigenesis (Wasylyk et al., 1993). The role of MAPK signaling and Ets proteins in regulation of tumor cell invasion has been intensively studied in recent years. For tumor cells to invade basement membranes, they secrete a variety of proteolytic enzymes which degrade specific ECM molecules. Increased expression and secretion of a large group of these enzymes, the matrix metalloproteinases (MMPs), have been demonstrated in many tumor cells (for review see Matrisian, 1992; Davies et al., 1993; Lohi et al., 1998). Members of this group include MMP-1 (interstitial collagenase), MMP-2 and 9 (gelatinases A and B), MMP-3 (stromelysin), and MMP-12 (metalloelastase). The domain structure of the MMPs include a predomain that targets the enzymes for secretion, a prodomain which is cleaved upon activation, and a catalytic domain which binds zinc ions required for activity (Matrisian, 1992).

One of the most important MMP regulatory pathways in both normal and tumor cells is MAPK activation of Ets transcription factors. The promoters of many MMPs have multiple binding sites for Ets proteins (Sato and Seiki, 1993; Tremble et al., 1995; Fig. 3). Stimulation of collagenase activity by adhesion to ECM

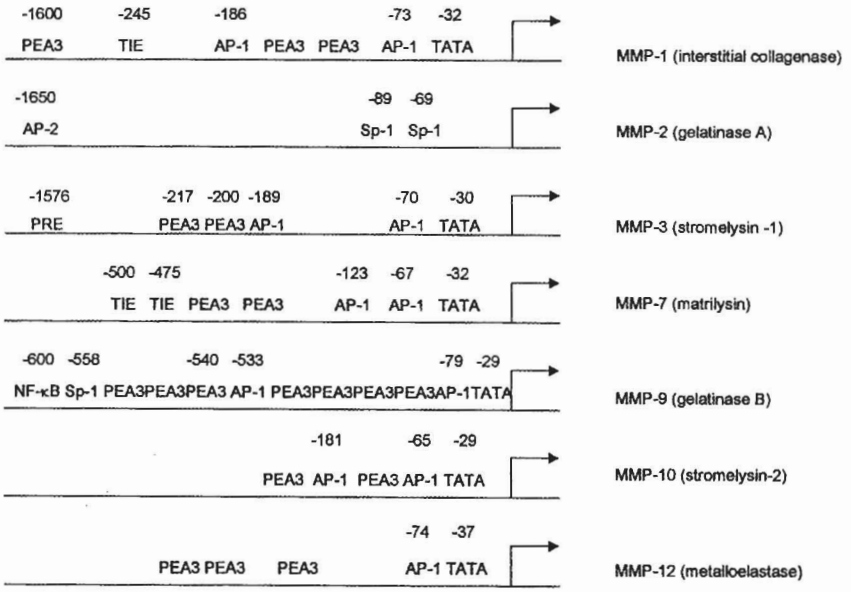

Fig. 3. Comparison of transcriptional regulatory elements in human MMP promoters. The presence of many different recognition sequences illustrates the complex regulation of these genes by cis acting factors and their upstream activators. AP-1: activator protein 1; PEA3: polyomavirus enhancer activation protein 3; TATA: binding site for general transcription factors; Sp-1 site; AP-2: activator protein 2; NF-κB: nuclear factor kappa B; TIE: TGFß inhibitory element; PRE: PDGF regulatory element.

molecules requires tyrosine kinase activity (Sudbeck et al., 1994). Integrin signaling via the MAPK pathway has been shown to activate MMP expression and secretion (Tremble et al., 1995; Shibata et al., 1998). Ras signaling is required for MMP activation in many model systems (Silberman et al., 1997; Himelstein et al., 1997; Shibata et al., 1998). In addition to ERK activation, MMPs are also regulated by the SAPK/JNK branch of the MAPK pathway (Gum et al., 1997). These studies illustrate the importance of integrin mediated MAPK signaling in regulation of MMP activity in invasive cells.

Many experimental and clinical challenges remain in understanding the interaction between ECM attachment and tumor cell invasion. Increased understanding of how integrin signals transmitted by the MAPK pathway affect MMP gene transcription will provide insight into the invasive phenotype of tumor cells. Further elucidation of these regulatory pathways will be necessary in order to improve therapeutic intervention and reduce the morbidity and mortality associated with tumor cell invasion and metastasis.

Acknowledgements. We gratefully acknowledge the members of the laboratory for many helpful discussions. We apologize to authors whose excellent work we were unable to include in this review. Research in the authors' laboratories is supported by National Institutes of Health Grants DE10966 and DE09165.

References

Ahn N.G., Seger R., Bratlien R.L., Diltz C.D., Tonks N.K., and Krebs E.G. (1991). Multiple components in an epidermal growth factor stimulated protein kinase cascade. J. Biol. Chem. 266, 4220-4227.

Altruda F., Cervella P., Tarone G., Botta C., Balzac F., Stefanuto G. and Silengo L. (1990). A human integrin β_1 subunit with a unique cytoplasmic domain generated by alternative mRNA processing. Gene 95, 261-266.

Arnaout M.A., Dana N., Gupta S.K., Tenen D.G. and Fathallah D.M. (1990). Point mutations impairing cell surface expression of the common ß subunit (CD18) in a patient with leukocyte adhesion molecule (Leu-CAM) deficiency. J. Clin. Invest. 85, 977-981.

Arroyo A.G., Campanero M.R., Sanchez-Mateos P., Zapata J.M., Ursa M.A., del Pozo M.A. and Sanchez-Madrid F. (1994). Induction of tyrosine phosphorylation during ICAM-3 and LFA-1 mediated intercellular adhesion and its regulation by the CD45 tyrosine phosphatase. J. Cell Biol. 126, 1277-1286.

Blenis J. (1993). Signal transduction via the MAP kinases: proceed at your own RSK. Proc. Natl. Acad. Sci. USA 90, 5889-5892.

Boulukos K.E., Pognonec P., Rabault B., Begue A. and Ghysdael J. (1989). Definition of an Ets1 protein domain required for nuclear localization in cells and DNA binding activity in vitro. Mol. Cell. Biol. 9, 5718-5721.

Bretscher M.S. (1996). Getting membrane flow and the cytoskeleton to cooperate in moving cells. Cell 87, 601-606.

Calvete J.J., Henschen A. and Gonzalez-Rodriguez J. (1991). Assignment of disulfide bonds in human platelet GPIIIa. A disulfide pattern for the beta subunits of the integrin family. Biochem. J. 274, 63-71.

Campbell J.S., Seger R., Graves J.D., Graves L.M., Jensen A.M. and Krebs E.G. (1995). The MAP kinase cascade. Recent Prog. Horm. Res. 50, 131-159.

Chen Q., Lin T.H., Der C.J. and Juliano R.L. (1996). Integrin mediated activation of mitogen activated protein (MAP) or extracellular signal related kinase kinase (MEK) and kinase is independent of Ras. J. Biol. Chem. 271, 18122-18127.

Clark E.A. and Brugge J.S. (1995). Integrins and signal transduction pathways: the road taken. Science 268, 233-239.

Clark E.A. and Hynes R.O. (1996). Ras activation is necessary for integrin mediated activation of extracellular signal regulated kinase 2 and cytosolic phospholipase A2 but not for cytoskeletal organization. J. Biol. Chem. 271, 14814-14818.

Clark E.A., Shattil S.J. and Brugge J.S. (1994). Regulation of protein tyrosine kinases in platelets. Trends Biochem. Sci. 19, 464-469.

Cobb M.H. and Goldsmith E.J. (1995). How MAP kinases are regulated. J. Biol. Chem. 270, 14843-14846.

Cohen G.B., Ren R. and Baltimore D. (1995). Modular binding domains in signal transduction proteins. Cell 80, 237-248.

Crews C.M. and Erikson R.L. (1993). Extracellular signals and reversible protein phosphorylation: what to Mek of it all. Cell 74, 215-217.

Damsky C.H. and Werb Z. (1992). Signal transduction by integrin receptors for extracellular matrix: cooperation and processing of extracellular information. Curr. Opin. Cell Biol. 4, 772-781.

Davies B., Miles D.W., Happerfield L.C., Naylor M.S., Bobrow L.G., Rubens R.D. and Balkwill F.R. (1993). Activity of type IV collagenases in benign and malignant breast disease. Br. J. Cancer 67, 1126-1131.

Davis R.J. (1993). The mitogen activated protein kinase signal transduction pathway. J. Biol. Chem. 268, 14553-14556.

Fleischman L.F., Pilaro A.M., Murakami K., Kondoh A., Fisher R.J. and Papas T.S. (1993). c-Ets-1 protein is hyperphosphorylated during mitosis. Oncogene 8, 771-780

Gegonne A., Punyammalee B., Rabault B., Bosselut R., Seneca S., Crabeel M. and Ghysdael J. (1992). Analysis of the DNA binding and transcriptional activation properties of the Ets1 oncoprotein. New Biol. 4, 512-519.

Gilmore A.P. and Romer L.H. (1996). Inhibition of focal adhesion kinase (FAK) signaling in focal adhesions decreases cell motility and proliferation. Mol. Biol. Cell 7, 1209-1224.

Gum R., Wang H., Lengyel E., Juarez J. and Boyd D. (1997). Regulation of 92 kDa type IV collagenase expression by the jun amino terminal kinase and the extracellular signal regulated kinase dependent signaling cascades. Oncogene 14, 1481-1493.

Hildebrand J., Schaller M.D. and Parsons J.T. (1993). Identification of sequences required for the efficient localization of the focal adhesion kinase $pp125^{FAK}$ to cellular focal adhesions. J. Cell Biol. 123, 993-1005.

Himelstein B.P., Lee E.J., Sato H., Seiki M. and Muschel R.J. (1997). Transcriptional activation of the matrix metalloproteinase 9 gene in an H-ras and v-myc transformed rat embryo cell line. Oncogene 14, 1995-1998.

Hipskind R.A., Rao V.N., Mueller C.G.F., Reddy E.S.P. and Nordheim A. (1991). Ets related protein Elk1 is homologous to the c-fos regulatory factor p62TCF. Nature 354, 531-534.

Hogervorst F., Kuikman I., von dem Borne A.E.G.K. and Sonnenberg A. (1990). Cloning and sequence analysis of beta-4 cDNA: an integrin subunit that contains a unique 118 kD cytoplasmic domain. EMBO J.

9, 765-770.

Hughes P.E., Renshaw M.W., Pfaff M., Forsyth J., Keivens V.M., Schwartz M.A. and Ginsberg M.H. (1997). Suppression of integrin activation: a novel function of a ras/raf initiated MAP kinase pathway. Cell 88, 521-530.

Hynes R.O. (1992). Integrins: versatility, modulation and signaling in cell adhesion. Cell 69, 11-25.

Ilic D., Furuta Y., Kanazawa S., Takeda N., Sobue K., Nakatsuji N., Nomura S., Fujimoto J., Okada M., Yamamoto T. and Aizawa S. (1995). Reduced cell motility and enhanced focal adhesion contact formation in cells from FAK deficient mice. Nature 377, 539-544.

Juliano R.L. and Haskill S. (1993). Signal transduction from the extracellular matrix. J. Cell Biol. 120, 577-585.

Kirchhofer D., Grzesiak J. and Pierschbacher M.D. (1991). Calcium as a potential physiologic regulator of integrin mediated cell adhesion. J. Biol. Chem. 266, 4471-4477.

Kohn E.C. and Liotta L.A. (1995). Molecular insights into cancer invasion: strategies for prevention and intervention. Cancer Res. 55, 1856-1862.

Koizumi S., Fisher R.J., Fujiwara S., Jorcyk C., Bhat N.K., Seth A. and Papas T.S. (1990). Isoforms of the human ets-1 protein: generation by alternative splicing and differential phosphorylation. Oncogene 5, 675-681.

LaFlamme S., Thomas S., Yamada S.S. and Yamada K. (1994). Single subunit chimeric integrins as mimics and inhibitors of endogenous integrin function in receptor localization, cell spreading and migration and matrix assembly. J. Cell Biol. 126, 1287-1298.

Lin T.H., Aplin A.E., Shen Y., Chen Q., Schaller M., Romer L., Aukhil I. and Juliano R.L. (1997). Integrin mediated activation of MAP kinase is independent of FAK: evidence for dual integrin signaling pathways in fibroblasts. J. Cell Biol. 136, 1385-1395.

Lin T.H., Chen Q., Howe A. and Juliano R.L. (1997). Cell anchorage permits efficient signal transduction between ras and its downstream kinases. J. Biol. Chem. 272, 8849-8852.

Liotta L.A. (1986). Tumor invasion and metastases - role of the extracellular matrix. Cancer Res. 46, 1-7.

Lohi J., Leivo I., Oivula J., Lehto V.P. and Virtanen I. (1998). Extracellular matrix in renal cell carcinomas. Histol. Histopathol. 13, 785-796.

Lukashev M.E., Sheppard D. and Pytela R. (1994). Disruption of integrin function and induction of tyrosine phosphorylation by the autonomously expressed ß1 integrin cytoplasmic domain. J. Biol. Chem. 269, 18311-18314.

Mainiero F., Murgia C., Wary K.K., Curatola A.M., Pepe A., Blumemberg M., Westwick J.K., Der C.J. and Giancotti F.G. (1997). The coupling of $\alpha_6\beta_4$ integrin to ras-MAP kinase pathways mediated by Shc controls keratinocyte proliferation. EMBO J. 16, 2365-2375.

Mainiero F., Pepe A., Wary K.K., Spinardi L., Mohammadi M., Schlessinger J. and Giancotti F.G. (1995). Signal transduction by the $\alpha_6\beta_4$ integrin: distinct β_4 subunit sites mediate recruitment of Shc/Grb2 and association with the cytoskeleton of hemidesmosomes. EMBO J. 14, 4470-4481.

Matrisian L.M. (1992). The matrix degrading metalloproteinases. BioEssays 14, 455-463.

Neiman A.M., Stevenson B.J., Xu H.P., Sprague G.F., Herskowitz I., Wigler M. and Marcus S. (1993). Functional homologues of protein kinases required for sexual differentiation in *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae* suggests a conserved signal transduction module in eucaryotic organisms. Mol. Biol. Cell 4, 107-120.

Nunn M.F., Seeburg P.M., Moscovici C. and Duesberg P.H. (1983). Tripartite structure of the avian erythroblastosis virus E26 transforming gene. Nature 306, 391-395.

Nye J.A., Petersen J.M., Gunther C.V., Jonsen M.D. and Graves B.J. (1992). Interaction of murine ets1 with GGA binding sites establishes the ets domain as a new DNA binding motif. Genes Dev. 6, 975-990.

O'Hagan R.C., Tozer R.G., Symons M., McCormick F. and Hassell J.A. (1996). The activity of the Ets transcription factor PEA3 is regulated by two distinct MAPK cascades. Oncogene 13, 1323-1333.

Okamura H. and Resh M.D. (1994). Differential binding of pp60c-src and pp60v-src to cytoskeleton is mediated by SH2 and catalytic domains. Oncogene 9, 2293-2303.

Otey C.A., Vasquez G.B., Burridge K. and Erickson B.W. (1993). Mapping of the alpha actinin binding site within the ß1 integrin cytoplasmic domain. J. Biol. Chem. 268, 21193-21197.

Pavalko F.M. and Otey C.A. (1994). Role of adhesion molecule cytoplasmic domains in mediating interactions with the cytoskeleton. Proc. Soc. Exp. Biol. Med. 205, 282-293.

Pawson T. (1995). Protein modules and signaling networks. Nature 373, 573-580.

Payne D.M., Rossomando A.J., Martino P., Erickson A.K., Her J.H., Shananowitz J., Hunt D.F., Weber M.J. and Sturgill T.W. (1991). Identification of the required phosphorylation sites in pp42 mitogen activated protein kinase (MAP kinase). EMBO J. 10, 885-892.

Rao V.N. and Reddy E.S.P. (1992). A divergent ets regulated protein, elk1, recognizes similar c-ets-1 proto-oncogene target sequences and acts as a transcriptional activator. Oncogene 7, 65-70.

Richardson A., Malik R.K., Hildebrand J.D. and Parsons J.T. (1997). Inhibition of cell spreading by expression of the C terminal domain of focal adhesion kinase (FAK) is rescued by coexpression of Src or catalytically inactive FAK: a role for paxillin tyrosine phosphorylation. Mol. Cell. Biol. 17, 6906-6914.

Richardson A. and Parsons J.T. (1996). A mechanism for regulation of the adhesion associated protein tyrosine kinase pp125FAK. Nature 380, 538-540.

Robbins D.J., Zhen E., Owaki H., Vanderbilt C., Ebert D., Geppert T.D. and Cobb M.H. (1993). Regulation and properties of extracellular signal regulated protein kinases 1 and 2 in vitro. J. Biol. Chem. 268, 5097-5106.

Sastry S.K. and Horwitz A.F. (1993). Integrin cytoplasmic domains: mediators of cytoskeletal linkages and extra- and intracellular initiated transmembrane signaling. Curr. Opin. Cell Biol. 5, 819-831.

Sato H. and Seiki M. (1993). Regulatory mechanism of 92 kD type IV collagenase gene expression which is associated with invasiveness of tumor cells. Oncogene 8, 395-405.

Sato H., Takino T., Yasunori O., Cao J., Shinagawa E.Y. and Seiki M. (1994). A matrix metalloproteinase expressed on the surface of invasive tumor cells. Nature 370, 61-65.

Schlaepfer D.D., Hanks S.K., Hunter T. and van der Geer P. (1994). Integrin mediated signal transduction linked to Ras pathway by GRB2 binding to focal adhesion kinase. Nature 372, 786-791.

Schmidt C., Pommerenke H., Durr F., Nebe B. and Rychly J. (1998). Mechanical stressing of integrin receptors induces enhanced tyrosine phosphorylation of cytoskeletally anchored proteins. J. Biol. Chem. 273, 5081-5085.

Seger R., Ahn N.G., Boulton T.G., Yancopoulos G.D., Panayotatos N., Radziejewska E., Ericsson L., Bratlien R.L., Cobb M.H. and Krebs

E.G. (1991). Microtubule associated protein 2 kinases ERK1 and ERK2 undergo autophosphorylation on both tyrosine and threonine residues: implications for their mechanism of activation. Proc. Natl. Acad. Sci. USA 88, 6142-6146.

Seth A., Ascione R., Fisher R.J., Mavrothalassitis G.J., Bhat N.K. and Papas T.S. (1992). The ets gene family. Cell Growth Differ. 3, 327-334.

Shaller M.D. and Parsons J.T. (1994). Focal adhesion kinase and associated proteins. Curr. Opin. Cell Biol. 6, 705-710.

Shibata K., Kikkawa F., Nawa A., Thant A.A., Naruse K., Mizutani S. and Hamaguchi M. (1998). Both focal adhesion kinase and c-Ras are required for the enhanced matrix metalloproteinase 9 secretion by fibronectin in ovarian cancer cells. Cancer Res. 58, 900-903.

Silberman S., Janulis M. and Schultz R.M. (1997). Characterization of downstream ras signals that induce alternative protease dependent invasive phenotypes. J. Biol. Chem. 272, 5927-5935.

Smith J.W. and Cheresh D.A. (1990). Integrin a Vß3-ligand interaction. J. Biol. Chem. 265, 2168-2172.

Sudbeck B.D., Parks W.C., Welgus H.G. and Pentland A.P. (1994). Collagen stimulated induction of keratinocyte collagenase is mediated via tyrosine kinase and protein kinase C activities. J. Biol. Chem. 269, 30022-30029.

Suzuki S. and Naitoh Y. (1990). Amino acid sequence of a novel integrin ß4 subunit and primary expression of the mRNA in epithelial cells. EMBO J. 9, 757-763.

Tamura R.N., Cooper H.M., Collo G. and Quaranta V. (1991). Cell type specific integrin variants with alternative α chain cytoplasmic domains. Proc. Natl. Acad. Sci. USA 88, 10183-10187.

Tremble P., Damsky C.H. and Werb Z. (1995). Components of the nuclear signaling cascade that regulate collagenase gene expression in response to integrin derived signals. J. Cell Biol. 129, 1707-1720.

Van der Geer P., Hunter T. and Lindberg R.A. (1994). Receptor protein tyrosine kinases and their signal transduction pathways. Annu. Rev. Cell Biol. 10, 251-337.

Van Kuppevelt T.H.M.S.M., Languino L.R., Gailit J.O., Suzuki S. and Ruoslahti E. (1989). An alternative cytoplasmic domain of the integrin ß3 subunit. Proc. Natl. Acad. Sci. USA 86, 5415-5418.

Wasylyk B., Hahn S.L. and Giovane A. (1993). The Ets family of transcription factors. Eur. J. Biochem. 211, 7-18.

Wei J., Shaw L.M. and Mercurio A.M. (1998). Regulation of mitogen activated protein kinase activation by the cytoplasmic domain of the α6 integrin subunit. J. Biol. Chem. 273, 5903-5907.

Weng Z.G., Taylor J.A., Turner C.E., Brugge J.S. and Seidel-Dugan C. (1993). Detection of Src homology 3 binding proteins including paxillin in normal and v-src transformed Balb/c 3T3 cells. J. Biol. Chem. 268, 14956-14963.

Witty J.P., McDonnell S., Newell K.J., Cannon P., Navre M., Tressler R.J. and Matrisian L.M. (1994). Modulation of matrilysin levels in colon carcinoma cell lines affects tumorigenicity in vivo. Cancer Res. 54, 4805-4812.

Yang B.S., Hauser C.A., Henkel G., Colman M.S., Van Beveren C., Stacey K.J., Hume D.A., Maki R.A. and Ostrowski M.C. (1996). Ras mediated phosphorylation of a conserved threonine residue enhances the transactivation activities of c-ets1 and c-ets2. Mol. Cell. Biol. 16, 538-547.

Zhang F., Strand A., Robbins D., Cobb M.H. and Goldsmith E.J. (1994). Atomic structure of the MAP kinase ERK2 at 2.3Å resolution. Nature 367, 704-710.

Accepted October 28, 1998