Published in: Cells Tissues Organs (2007), vol. 185, iss. 1-3, pp. 61-65
Status: Postprint (Author's version)

β-Catenin and ZO-1: Shuttle Molecules Involved in Tumor Invasion-Associated Epithelial-Mesenchymal Transition Processes

Myriam Polette[a] Mélanie Mestdagt[b] Sandrine Bindels[b] Béatrice Nawrocki-Raby[a] Walter Hunziker[c] Jean-Michel Foidart[b] Philippe Birembaut[3] Christine Gilles[b]

[a]INSERM UMRS 514, Laboratory of Histology, IFR 53, CHU Maison Blanche, Reims, France; [b]Laboratory of Developmental and Tumor Biology, University of Liège, CHU Sart-Tilman, B23, Liège, Belgium; [c]Institute of Molecular and Cell Biology, Epithelial Cell Biology Laboratory, Singapore, Singapore

Abstract

The cytoplasmic/nuclear relocalization of β-catenin and ZO-1 from the adherens and tight junctions are common processes of the epithelial-mesenchymal transition (EMT) associated with tumor invasion. Data are now accumulating to demonstrate that these molecules, which shuttle between the plasma membrane and the nucleus or the cytosol, are involved in signaling pathways,and contribute to the regulation of genes such as vimentin or matrix metalloproteinase-14 which are turned on during EMT.

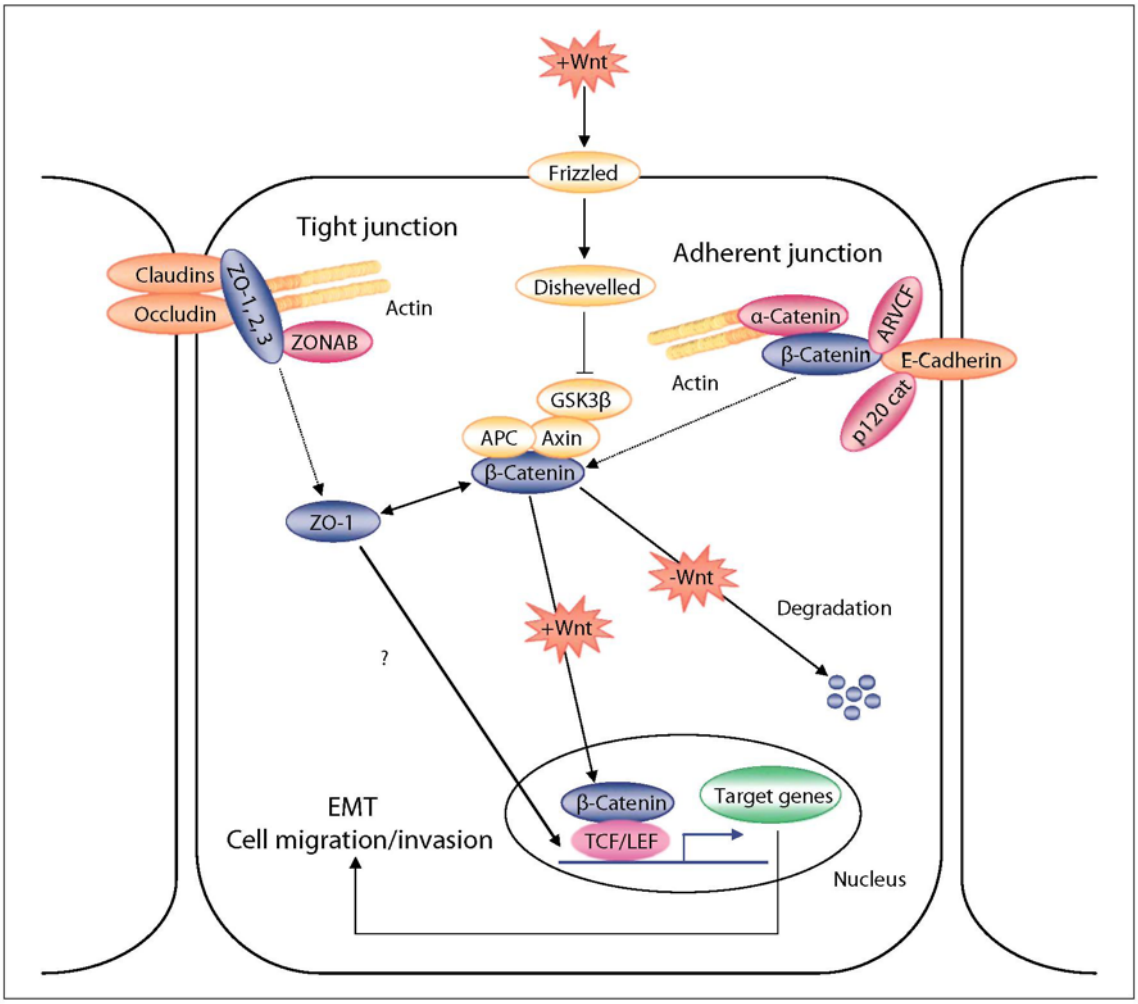
Keywords: β-Catenin · ZO-1 · Vimentin · Matrix metalloproteinase · Epithelial-mesenchymal transition

Abbreviations used in this paper	
EMT	epithelial-mesenchymal transition
MMP	matrix metalloproteinase
TCF/LEF	T cell factor/lymphoid enhancer factor
ZO-1	zonula occludens protein-1

The diminution of cell-cell adhesion is instrumental in the loss of epithelial features occurring in epithelial-mesenchymal transition (EMT) processes associated with the metastatic conversion of epithelial tumor cells. At the molecular level, this involves the reorganization of cell-cell adhesion complexes including adherens and tight junctions [Reichert et al., 2000; Ikenouchi et al., 2003; Thiery, 2003; Huber et al., 2005]. A reorganization of E-cadherin/catenins complexes has indeed been largely involved in EMT processes associated with epithelial cell migration in both physiological and pathological conditions. Such a reorganization also involves a relocalization of β-catenin, which in normal conditions participates in the linking of E-cadherin to the actin cytoskeleton [Wheelock and Johnson, 2003]. During EMT, β-catenin dissociates from the E-cadherin/catenin cell membrane complexes, accumulates in the cytoplasm and translocates into the nucleus where it acts as a transcriptional coactivator through its binding with the members of the T cell factor/lymphoid enhancer factor (TCF/LEF) transcription factor family [Harris and Peifer, 2005; Brembeck et al., 2006] (fig. 1). An increasing number of genes have been identified as targets of the β-catenin/ TCF/LEF pathway. These include members of the matrix metalloproteinases (MMP), chemokines or cytoskeletal protein families, some of them having major implications in tumor progression [Brabletz et al., 1999; Marchenko et al., 2001; Levy et al., 2002; Takahashi et al., 2002; Gilles et al., 2003; Mestdagt et al., 2006]. Even though the implication of tight junction molecules in EMT is less well documented, a parallelism can nevertheless be depicted between the reorganization of adherens junctions and tight junctions occurring during EMT (fig. 1). Indeed, similar to adherens junctions, tight junctions comprise transmembrane molecules (occludin, claudins or junctional adhesion molecules/JAM) linked to the actin cytoskeleton through cytoplasmic linker molecules such as zonula occludens proteins (ZO) [Gonzalez-Mariscal et al., 2003]. A delocalization of ZO-1 from the cell membrane has been reported during epithelial cell migration and the presence of ZO-1 in the nuclei of migratory cells has also been observed [Gottardi et al., 1996]. Also, an interaction between ZO-1 and the transcriptional repressor ZONAB (ZO-1-associated nucleic acid-binding protein) has been demonstrated at the tight junctions. This results in the regulation of ZONAB nuclear levels and its subsequent ability to regulate gene expression [Balda and Matter, 2000; Balda et al., 2003]. Although the nuclear localization of ZO-1 remains controversial, both β-catenin and ZO-1 appear to be shuttle molecules. Depending upon the state of differentiation and migration of epithelial cells, β-catenin and ZO-1 may be found in different subcellular compartments and act as signaling proteins, regulating gene transcription. Although such shuttling properties have been assigned to several other adherens or tight junction molecular components [Balda and Matter, 2003], we will focus this summary on β-catenin and ZO-1 in tumor EMT systems.

Published in: Cells Tissues Organs (2007), vol. 185, iss. 1-3, pp. 61-65
Status: Postprint (Author's version)

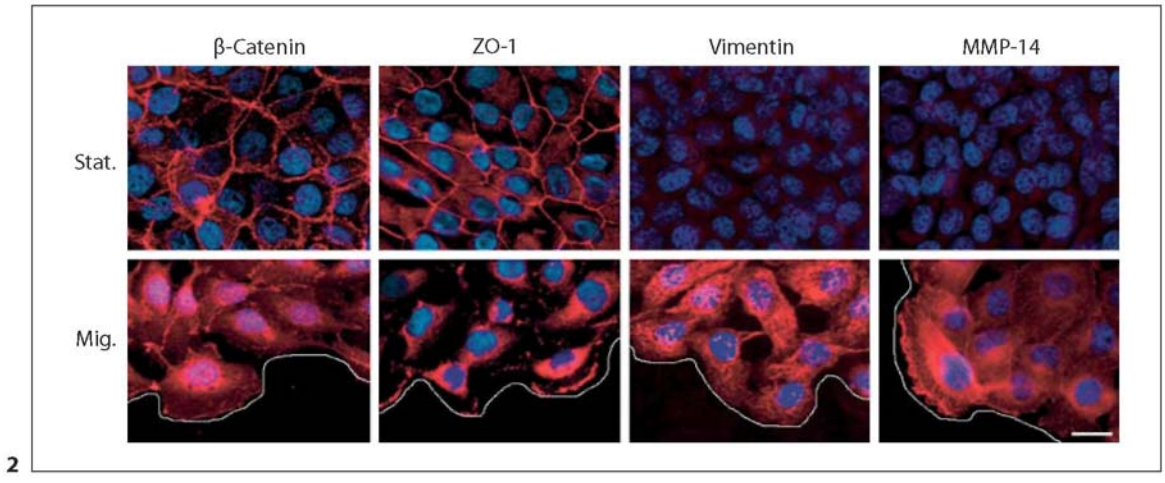
Fig. 1: *Schematic representation of β-catenin and ZO-1 subcellular relocalization during EMT processes. In differentiated normal epithelial cells, both β-catenin and ZO-1 mostly localize at the cell-cell adhesion membrane complexes. During EMT, both β-catenin and ZO-1 relocalize from these membrane complexes, accumulate in the cytoplasm and eventually translocate to the nucleus. The activation of the Wnt pathway, inhibiting the degradation of the cytoplasmic β-catenin through the proteasome system, plays an important regulatory role in this scheme.*



Because of their implication in gene regulation, we and others have investigated the potential roles of β-catenin and ZO-1 in upregulation of the mesenchymal gene expressions which is observed during the EMT processes associated with epithelial tumor cell invasion. Among the mesenchymal genes considered as good markers of EMT, there is vimentin. Vimentin is a type III intermediate filament which, in normal conditions in adults, is expressed by cells of mesenchymal origin [Steinert and Roop, 1988]. Accumulating evidence has now clearly shown that vimentin can also be expressed by migratory/invasive epithelial cells, and is functionally implicated in cell migration [Sommers et al., 1989, 1992, 1994b; Gilles et al., 1994b, 1996a, 1999, 2003; Hendrix et al., 1997; Singh et al., 2003]. Besides vimentin, several studies have also shown that MMP genes are turned on during EMT changes [Gilles et al., 2004]. MMPs are a family of proteases which cannot only degrade almost all ECM components, but also other key substrates such as cell-cell adhesion molecules, chemokines and cell surface receptors [Egeblad and Werb, 2002]. The consensus view is that many MMPs are, in general, mostly produced by stromal cells surrounding tumor cell clusters [Gilles et al., 2004; Polette et al., 2004]. However, expression of 'stromal' MMPs now appears as one of the major attributes that epithelial cells acquire after undergoing EMT processes [Gilles et al., 2004; Polette et al., 2004]. Among the MMP family, MMP-14 (MT1-MMP) and MMP-3 have been more particularly examined in relationship with EMT [Lochter et al., 1997; Pulyaeva et al., 1997; Sternlicht et al., 1999; Gilles et al., 2004]. If MMP-3 has been mostly studied as a potential inducer of EMT in mouse tumor cells, MMP-14, on which this summary is focused, has rather been looked at as a target gene of EMT events.

In summarizing data describing the expression and localization of β-catenin, ZO-1, vimentin and MMP-14 in different breast, cervical and lung cell lines displaying different degrees of invasiveness, a clear-cut correlation can be drawn. Indeed, noninvasive cell lines do not express vimentin or MMP-14 and mostly express β-catenin and ZO-1 at the membrane, whereas invasive cell lines express high levels of vimentin and MMP-14 and display a rather diffuse cytoplasmic and/or nuclear staining of β-catenin and ZO-1 [Sommers et al., 1989, 1992, 1194a; Thompson et al., 1992; Gilles et al., 1994a, b, 1996a, b; Pulyaeva et al., 1997; Polette et al., 1998]. These correlations have also been made using in vitro cell systems in which EMT can be induced. This is shown in figure 2, in a two-dimensional migration assay, using MCF10A human mammary epithelial cells. In this assay, one can observe that the migratory subpopulation undergoes an EMT characterized by the expression of vimentin and MMP-14 and a nuclear and/or cytoplasmic relocalization of β-catenin and ZO-1 [Gilles et al., 1999, 2001, 2003; Bindels et al., 2006]. In contrast, stationary cells neither express vimentin nor MMP-14, but show a typical epithelial honeycomb staining of β-catenin and ZO-1. In addition to these correlative data, we also showed that vimentin can be regulated by the β-catenin/TCF pathway in human breast tumor cells [Gilles et al., 2003]. Similarly, as also described for other MMPs, MMP-14 has been reported to be a target gene of the β-catenin/TCF pathway [Takahashi et al., 2002]. Regarding ZO-1, the transfection of the N-terminal fragment of ZO-1, which is unable to localize to the cell membrane, has been reported to induce EMT changes in MDCK cells characterized by an increased vimentin expression and enhanced invasiveness [Reichert et al., 2000]. We have also recently reported that transfection of ZO-1 siRNA downregulates MMP-14 mRNA and protein, subsequently decreasing the in vitro invasive abilities of human breast tumor cells [Polette et al., 2005]. Furthermore, both studies have demonstrated that an induction of the β-catenin/TCF/LEF pathway occurs following ZO-1 transfection, showing evidence of a cross talk between both pathways [Reichert et al., 2000; Polette et al., 2005].

Fig. 2: *Differential expression and localization of β-catenin, ZO-1, vimentin and MMP-14 in migratory versus stationary MCF10A human breast cells. MCF10A cells were seeded in growth medium inside a glass ring. Twenty-four hours after plating, the glass ring was removed and the cells at the periphery of the outgrowth initiated a directed migration from the confluent area initially delimited by the ring. Video microscopy measurements clearly identified a migratory (Mig.) subpopulation at the periphery of the outgrowth and a stationary subpopulation (Stat.) in the area initially delimited by the ring [Gilles et al., 1999]. In this assay, the migratory subpopulation clearly turned on vimentin and MMP-14 expression and displayed a rather cytoplasmic and/or nuclear staining for β-catenin and ZO-1. In contrast, the stationary subpopulation did neither express vimentin nor MMP-14 and displayed a membrane staining for both β-catenin and ZO-1. The white line schematically represents the migration front. β-Catenin, ZO-1, vimentin and MMP-14 immunostainings are in red. DAPI nuclear staining is in blue. Bar = 12 μm.*



In conclusion, during EMT, the loss of epithelial features can directly regulate the expression of mesenchymal genes. β-Catenin and ZO-1, which are a structural component of cell-cell adhesion complexes in differentiated epithelial cells, could each play a key role in such regulations through their ability to shuttle from the membrane to the cytosol or nucleus and act as signaling molecules.

Acknowledgments

This work was supported by grants from the CGRI-FNRS-INSERM coopération, the Lions Club of Soissons, the Fonds National de la Recherche Scientifique (FNRS, Belgium), the Fédération Belge Contre le Cancer and the Agency for Science, Technology and Research, Singapore.

References

Balda, M.S., K. Matter (2000) The tight junction protein ZO-1 and an interacting transcription factor regulate ErbB-2 expression. EMBO J *19:* 2024-2033.

Balda, M.S., M.D. Garrett, K. Matter (2003) The ZO-1-associated Y-box factor ZONAB regulates epithelial cell proliferation and cell density. J Cell Biol *160:* 423-432.

Balda, M.S., K. Matter (2003) Epithelial cell adhesion and the regulation of gene expression. Trends Cell Biol *13:* 310-318.

Bindels, S., M. Mestdagt, C. Vandewalle, N. Jacobs, L. Volders, A. Noel, F. van Roy, G. Berx, J.M. Foidart, C. Gilles (2006) Regulation of vimentin by SIP1 in human epithelial breast tumor cells. Oncogene *25:* 4975-4985.

Brabletz, T., A. Jung, S. Dag, F. Hlubek, T. Kirchner (1999) Beta-catenin regulates the expression of the matrix metalloproteinase-7 in human colorectal cancer. Am J Pathol *155:* 1033-1038.

Brembeck, F.H., M. Rosario, W. Birchmeier (2006) Balancing cell adhesion and Wnt signaling, the key role of beta-catenin. Curr Opin Genet Dev *16:* 51-59.

Egeblad, M., Z. Werb (2002) New functions for the matrix metalloproteinases in cancer progression. Nat Rev Cancer *2:* 161-174.

Gilles, C, J. Piette, W. Peter, N.E. Fusenig, J.M. Foidart (1994a) Differentiation ability and oncogenic potential of HPV-33 - and HPV-33+ ras-transfected keratinocytes. Int J Cancer *58:* 847-854.

Gilles, C, M. Polette, J. Piette, P. Birembaut, J.M. Foidart (1994b) Epithelial-to-mesenchymal transition in HPV-33-transfected cervical keratinocytes is associated with increased invasiveness and expression of gelatinase A. Int J Cancer *59:* 661-666.

Gilles, C, M. Polette, J. Piette, A.C. Delvigne, E.W. Thompson, J.M. Foidart, P. Birembaut (1996a) Vimentin expression in cervical carcinomas: association with invasive and migratory potential. J Pathol *180:* 175-180.

Gilles, C, M. Polette, J. Piette, C. Munaut, E.W. Thompson, P. Birembaut, J.M. Foidart (1996b) High level of MT-MMP expression is associated with invasiveness of cervical cancer cells. Int J Cancer *65:* 209-213.

Gilles, C, M. Polette, J.M. Zahm, J.M. Tournier, L. Volders, J.M. Foidart, P. Birembaut (1999) Vimentin contributes to human mammary epithelial cell migration. J Cell Sci *112:*4615 -4625.

Gilles, C, M. Polette, C. Coraux, J.M. Tournier, G Meneguzzi, C. Munaut, L. Volders, P. Rousselle, P. Birembaut, J.M. Foidart (2001) Contribution of MT1-MMP and of human laminin-5 gamma2 chain degradation to mammary epithelial cell migration. J Cell Sci *114:* 2967-2976.

Gilles, C, M. Polette, M. Mestdagt, B. Nawrocki-Raby, P. Ruggeri, P. Birembaut, J.M. Foidart (2003) Transactivation of vimentin by beta-catenin in human breast cancer cells. Cancer Res *63:* 2658-2664.

Gilles, C, D. Newgreen, H. Sato, E.W. Thompson (2004) Matrix metalloproteases and epithelial-to-mesenchymal transition: implications for carcinoma metastasis; in Savagner, P. (ed): Rise and Fall of Epithelial Phenotype. Georgetown, Eurekah.com and Kluwer Academic/Plenum Publishers, chap 2.

Gonzalez-Mariscal, L., A. Betanzos, P. Nava, B.E. Jaramillo (2003) Tight junction proteins. Prog Biophys Mol Biol *81:* 1-44.

Gottardi, C.J., M. Arpin, A.S. Fanning, D. Louvard (1996) The junction-associated protein, zonula occludens-1, localizes to the nucleus before the maturation and during the remodeling of cell-cell contacts. Proc Natl Acad Sci USA *93:* 10779-10784.

Harris, T.J., M. Peifer (2005) Decisions, decisions: beta-catenin chooses between adhesion and transcription. Trends Cell Biol *15:* 234-237.

Hendrix, M.J., E.A. Seftor, R.E. Seftor, K.T. Trevor (1997) Experimental co-expression of vimentin and keratin intermediate filaments in human breast cancer cells results in phenotypic interconversion and increased invasive behavior. Am J Pathol *150:* 483-495.

Huber, M.A., N. Kraut, H. Beug (2005) Molecular requirements for epithelial-mesenchymal transition during tumor progression. Curr Opin Cell Biol *17:* 548-558.

Ikenouchi, J., M. Matsuda, M. Furuse, S. Tsukita (2003) Regulation of tight junctions during the epithelium-mesenchyme transition: direct repression of the gene expression of claudins/occludin by Snail. J Cell Sci *116:* 1959-1967.

Levy, L., C. Neuveut, C.A. Renard, P. Charneau, S. Branchereau, F. Gauthier, J.T. Van Nhieu, D. Cherqui, A.F. Petit-Bertron, D. Mathieu, M.A. Buendia (2002) Transcriptional activation of interleukin-8 by beta-catenin-Tcf4. J Biol Chem *277:* 42386-42393.

Lochter, A., S. Galosy, J. Muschler, N. Freedman, Z. Werb, M.J. Bissell (1997) Matrix metallo-proteinase stromelysin-1 triggers a cascade of molecular alterations that leads to stable epithelial-to-mesenchymal conversion and a premalignant phenotype in mammary epithelial cells. J

Cell Biol *139:* 1861-1872.

Marchenko, G.N., B.I. Ratnikov, D.V. Rozanov, A. Godzik, E.I. Deryugina, A.Y. Strongin (2001) Characterization of matrix metallo-proteinase-26, a novel metalloproteinase widely expressed in cancer cells of epithelial origin. Biochem J *356:* 705-718.

Mestdagt, M., M. Polette, G Buttice, A. Noel, A. Ueda, J.M. Foidart, C. Gilles (2006) Transactivation of MCP-1/CCL2 by beta-catenin/ TCF-4 in human breast cancer cells. Int J Cancer *118:* 35-42.

Polette, M., C. Gilles, S. de Bentzmann, D. Gruenert, J.M. Tournier, P. Birembaut (1998) Association of fibroblastoid features with the invasive phenotype in human bronchial cancer cell lines. Clin Exp Metastasis *16:* 105-112.

Polette, M., B. Nawrocki-Raby, C. Gilles, C. Clavel, P. Birembaut (2004) Tumour invasion and matrix metalloproteinases. Crit Rev Oncol Hematol *49:*179-186.

Polette, M., C. Gilles, B. Nawrocki-Raby, J. Lohi, W. Hunziker, J.M. Foidart, P. Birembaut (2005) Membrane-type 1 matrix metalloproteinase expression is regulated by zonula occludens-1 in human breast cancer cells. Cancer Res *65:* 7691-7698.

Pulyaeva, H., J. Bueno, M. Polette, P. Birembaut, H. Sato, M. Seiki, E.W. Thompson (1997) MT1-MMP correlates with MMP-2 activation potential seen after epithelial to mesenchymal transition in human breast carcinoma cells. Clin Exp Metastasis *15:* 111-120.

Reichert, M., T. Muller, W. Hunziker (2000) The PDZ domains of zonula occludens-1 induce an epithelial to mesenchymal transition of Madin-Darby canine kidney I cells. Evidence for a role of beta-catenin/Tcf/Lef signaling. J Biol Chem *275:*9492-9500.

Singh, S., S. Sadacharan, S. Su, A. Belldegrun, S. Persad, G Singh (2003) Overexpression of vimentin: role in the invasive phenotype in an androgen-independent model of prostate cancer. Cancer Res *63:* 2306-2311.

Sommers, C.L., D. Walker-Jones, S.E. Heckford, P. Worland, E. Valverius, R. Clark, F. McCormick, M. Stampfer, S. Abularach, E.P. Gelmann (1989) Vimentin rather than keratin expression in some hormone-independent breast cancer cell lines and in oncogene-transformed mammary epithelial cells. Cancer Res *49:* 4258-4263.

Sommers, C.L., S.E. Heckford, J.M. Skerker, P. Worland, J.A. Torri, E.W. Thompson, S.W. Byers, E.P. Gelmann (1992) Loss of epithelial markers and acquisition of vimentin expression in adriamycin- and vinblastine-resistant human breast cancer cell lines. Cancer Res *52:* 5190-5197.

Sommers, C.L., S.W. Byers, E.W. Thompson, J.A. Torri, E.P. Gelmann (1994a) Differentiation state and invasiveness of human breast cancer cell lines. Breast Cancer Res Treat *31:* 325-335.

Sommers, C.L., J.M. Skerker, S.A. Chrysogelos, M. Bosseler, E.P. Gelmann (1994b) Regulation of vimentin gene transcription in human breast cancer cell lines. Cell Growth Differ *5:*839-846.

Steinert, P.M., D.R. Roop (1988) Molecular and cellular biology of intermediate filaments. Annu Rev Biochem *57:* 593-625.

Sternlicht, M.D., A. Lochter, C.J. Sympson, B. Huey, J.P. Rougier, J.W. Gray, D. Pinkel, M.J. Bissell, Z. Werb (1999) The stromal proteinase MMP3/stromelysin-l promotes mammary carcinogenesis. Cell *98:* 137-146.

Takahashi, M., T. Tsunoda, M. Seiki, Y. Naka-mura, Y. Furukawa (2002) Identification of membrane-type matrix metalloproteinase-1 as a target of the beta-catenin/Tcf4 complex in human colorectal cancers. Oncogene *21:* 5861-5867.

Thiery, J.P. (2003) Epithelial-mesenchymal transitions in development and pathologies. Curr Opin Cell Biol *15:* 740-746.

Thompson, E.W., S. Paik, N. Brunner, C.L. Sommers, G Zugmaier, R. Clarke, T.B. Shima, J. Torri, S. Donahue, M.E. Lippman (1992) Association of increased basement membrane invasiveness with absence of estrogen receptor and expression of vimentin in human breast cancer cell lines. J Cell Physiol *150:* 534-544.

Wheelock, M.J., K.R. Johnson (2003) Cadherins as modulators of cellular phenotype. Annu Rev Cell Dev Biol *19:* 207-235.