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New Pathway Links From Cancer-Progression Determinants to Gene Expression of Matrix Metalloproteinases in Breast Cancer Cells

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AP-2α, interleukin-4 (IL-4), E-cadherin, fibulin 1D, $p16^{INK4\alpha}$, PTEN, RKIP, and S100A4 are determinants (suppressors, except for S100A4) of cancer cell invasiveness and other traits of cancer progression, which are located upstream of matrix metalloproteinases (MMPs) in cell signaling pathways. We will refer to them as upstream cancer-progression determinants (UCPDs, for brevity). MMP-1, MMP-2, MMP-9, MMP-11, MMP-13, MMP-14, MMP-16, and MMP-19 are enhancers of cancer cell invasiveness and other traits of cancer progression, in MDA-MB-231 breast cancer cells. We are interested in pathway links from UCPDs to gene expression of cancer cell MMPs in MDA-MB-231 cells. To test models about these links, wild-type copies of UCPDs were transiently overexpressed and then MMP mRNAs were measured by reverse transcription real-time PCR. The present results show that each of eight UCPDs is linked to the gene expression of a unique set of MMPs. This indicates that the effects are sequence-specific and that each UCPD reaches these MMP expressions through different sets of signaling pathways. We have detected 20 new pathway links, 11 are downregulatory and nine are upregulatory; 15 are new links in any cell, and five are new links in breast cancer. In seven links, three cancer-progression suppressing UCPDs unexpectedly enhance the gene expression of five cancer-progression promoting MMPs.

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Cancer traits tend to depend on cell signaling pathways rather than a specific member of each pathway (Vogelstein and Kinzler, 2004; Wang et al., 2007). For restorative genetic and epigenetic therapy of a given type of metastatic cancer, it is a high priority to identify the highest number of cell signaling pathways which determine the progression of that cancer because they are potential targets for therapy. This overview information is needed to design alternative therapies, as the metastatic cancer becomes resistant to standard therapies. Much is unknown about these signaling pathways, particularly in MDA-MB-231 cells. We chose to work with these cells because they are well characterized to be human, metastatic, invasive, cancer cells (Neve et al., 2006).

Several cancer cell matrix metalloproteinases (MMPs), such as MMP-1, MMP-2, MMP-9, MMP-11, MMP-13, MMP-14, MMP-16, and MMP-19, enhance MDA-MB-231 cancer cell invasiveness (Ramos-DeSimone et al., 1999; Jiang et al., 2005, 2006; Wyatt et al., 2005; Merrell et al., 2006; Muñoz-Nájar et al., 2006; Hegedüs et al., 2008). Activator protein-2α (AP-2α), E-cadherin, fibulin 1D, interleukin 4 (IL-4), KAI1, $p16^{INK4\alpha}$, phosphatase and tensin homolog (PTEN), and raf kinase inhibitor protein (RKIP) suppress, and S100A4 enhances, cancer cell invasiveness and other cancer progression traits, in MDA-MB-231 breast cancer cells and cells from other types of cancers (Uchiyama et al., 1996; Chintala et al., 1997; Qing et al., 1997; Hayashido et al., 1998; Twal et al., 2001; Adachi et al., 2002; Fu et al., 2003; Wong and Gumbiner, 2003; Zheng et al., 2003; Schuierer et al., 2004; Sumigama et al., 2004; Hjelmeland et al., 2005; Saleem et al., 2006; Chen et al., 2007;). They are located upstream of MMPs in cell signaling pathways. The term "upstream cancer-progression determinants" (UCPDs, for brevity) will be used for them in this text.

A very large number of proteins determine invasiveness and other cancer progression traits (Uchiyama et al., 1996; Twal et al., 2001; Wong and Gumbiner, 2003; Zheng et al., 2003; Schuierer et al., 2004; Sumigama et al., 2004; Saleem et al., 2006). The progression of different types of cancers depends on different profiles of cell signaling pathways (Walsh and King, 2007; Cybulski et al., 2008). In order to begin the identification of a cell signaling pathway between two factors, it is essential to have detected first a pathway link between them. The present study of some UPCDs and gene expression of some cancer progression-promoting MMPs showed 20 pathway links in MDA-MB-231 cells. We have found that different types of cancers have distinct pathway links from these UCPDs to the gene expression of these MMPs (manuscript in preparation). Thus, the cumulative number of cell signaling pathways involved in the progression of various types of cancers is expected to be high. Identification of signaling pathway is labor intensive. Therefore, strategically it makes sense to obtain first an overview of the pathway links. This overview is important to prioritize the pathway links whose signaling pathway should be identified first regarding potential therapeutic value.

Therapy does not require identification of all of the signaling pathways now. Thus, it is reasonable to focus first on mRNA measurements to detect pathway links because it is faster, less expensive, more sensitive and more quantitative to measure gene expression by reverse transcription by real-time PCR than to measure protein levels (Bakalova et al., 2005; Pérez-Ruiz et al., 2007). In addition, we have found that some cancer cell proteins whose mRNA levels are very low, are important for invasiveness (Hegedüs et al., 2008).

The goals of the present study were to find out: (a) whether there are any pathway links from any of these UCPDs to the

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gene expression of any of these MMPs in MDA-MB-231 cells; (b) if so, whether each UCPD is linked to the same or a unique set of MMP gene expressions; and (c) whether the links are up- or down-regulatory. We found several connections from UCPDs to gene expression of various downstream MMPs. The results show that several UCPDs belong to signaling pathways which up- or down-regulate the gene expression of unique profiles of cancer cell MMPs. Surprisingly, some of cancer progression inhibitors stimulated the gene expression of some cancer progression enhancers.

Materials and Methods

Cells

MDA-MB-231 metastatic human breast cancer cells were obtained from the U.S. National Cancer Institute, and were grown in DMEM supplemented with 10% fetal bovine serum and maintained at 37°C in a humidified 10% CO_2 incubator.

Plasmids

The following plasmids were used: pCMV-PTEN-WT for PTEN, provided by Donald Tindall; pCEP4mp16 for $p16^{INK4\alpha}$, from Laura Taylor; pcDNA-(+)-ssRKIP for RKIP, from Evan Keller; pSG5-neo-AP-2α for AP-2α, from Michael Tainsky; pCMV-KAI1 for KAI1, from J.Carl Barrett; pcDHuIL-4 for (IL-4), from Nancy Magnuson; hEcad/pcDNA3 for E-cadherin, from Barry Gumbiner; pSV2neo.S100A4 for S100A4, from Barry Davies; pcDNANeo-fibulin-1D for fibulin 1D, from W. Scott Argraves; pEGFP-C1 (Becton Dickson, Franklin Lakes, NJ) for green florescent protein (eGFP); and pcDNA3.1 (Invitrogen, Carlsbad CA) was the empty vector control (Yokota et al., 1986; Buettner et al., 1993; Davies et al., 1994; Dong et al., 1995; Gottardi et al., 2001; Huang et al., 2001; Twal et al., 2001; Fu et al., 2003).

Transient transfections

Samples of 1×10^6 MDA-MB-231 cells were electroporated with a mixture of 1 μg of peGFP (green fluorescent protein) DNA and 1 μg of either plasmid wild-type cDNA of one of the factors indicated in Figures 1–3, or with empty pcDNA3 vector, using an Amaxa nucleofector set at X-13. The cells were examined under fluorescence microscopy to assess transfection efficiency (eGFP expression) 2 days after transfection, and they were harvested 4 days after transfection. All cell cultures whose mRNAs were measured were at least 70% eGFP-positive.

RNA isolation, reverse transcription and real-time PCR

Whole cell RNA was extracted with Trizol (Invitrogen) and digested with RNase–free DNase (0.1 U/μg of RNA) in the presence of RNase inhibitor (0.5 U/μg of RNA). MMP-7 and MMP-11 mRNAs were reverse transcribed with avian myeloblastosis virus reverse transcriptase (14 U/μg of RNA), primed with oligo(dT), in the presence of RNase inhibitor (0.5 U/μg of RNA). Reverse transcription of other MMP mRNAs was done with Moloney murine leukemia virus reverse transcriptase (20 U/μg of RNA). Cell mRNA levels were measured by quantitative real-time PCR (qRT-PCR) of cDNA in the presence of SYBR Green I. The reverse transcription specific reverse primers and the PCR primers used were as indicated before (Hegedüs et al., 2008). All cell MMP mRNA levels were normalized by the GAPDH mRNA level in each sample. The MMP mRNAs analyzed in this study are (their common names and accession numbers are in parenthesis): MMP-1 (collagenase-1; NM_002421); MMP-2 (gelatinase A; NM_004530); MMP-9 (gelatinase B; NM_004994); MMP-11 (stromelysin-3; NM_005940); MMP-13 (collagenase-3; NM_002427); MMP-14 (MT1-MMP, MT-MMP1; NM_004995); 6,347-base long MMP-16 variant 1 mRNA (MT3-MMP, MT-MMP3; MMP-16-1 in this text; NM_005941) that generates a membrane-inserted MMP-16; 1,800-base long MMP-16 variant 2 mRNA (NM_022564) which produces a secreted MMP-16 (MMP-16-2 in this text); and MMP-19 (RASI-1, MMP-18; NM_002429).

Results

We wished to test three models in MDA-MB-231 cells. First, overexpression of cancer progression-suppressing UCPDs might or might not affect the gene expression of cancer progression-promoting, cancer cell MMPs. Second, each UCPD may affect the gene expression of either the same or different profile of MMPs. Third, the result of each effective cancer progression-suppressing UCPD may or may not be only downregulation of MMP gene expression. Gene expression was

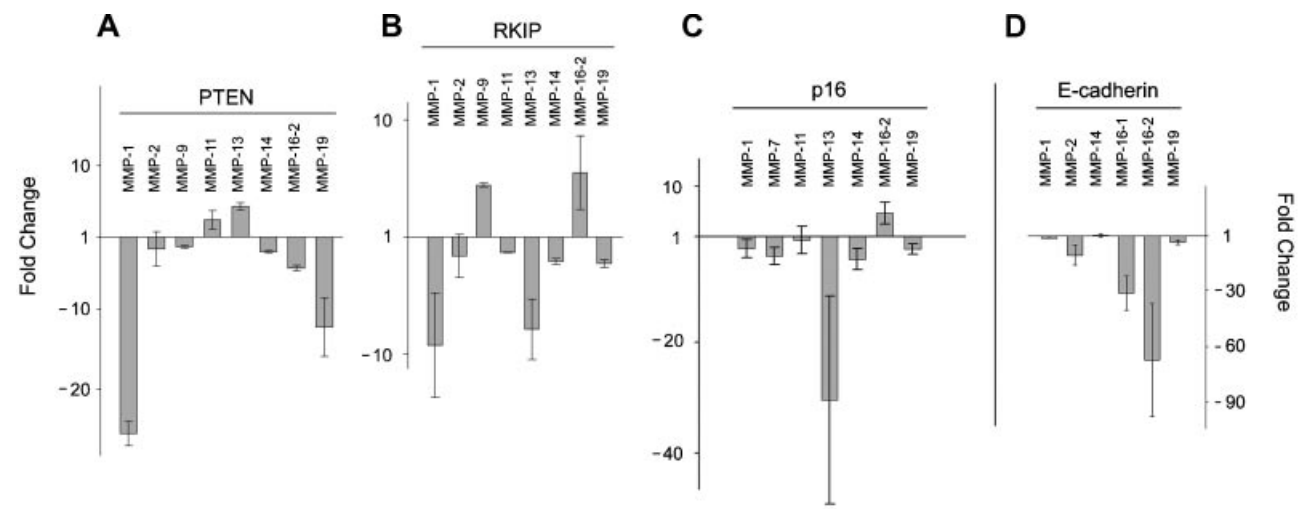


Fig. 1. Effects of PTEN, RKIP, $p16^{INK4\alpha}$ or E-cadherin overexpression on the gene expression of various MMPs in transfected MDA-MB-231 cells. Bars represent averages of measurements by RT-PCR of different transfections. Positive numbers represent increases in MMP:GAPDH ratios relative to vector-only transfected cells and negative numbers represent decreases in MMP:GAPDH ratios relative to vector-only transfectants, in linear scales. All of this set of MMPs were analyzed from each transfection, but only the conclusive, reproducible results are shown.

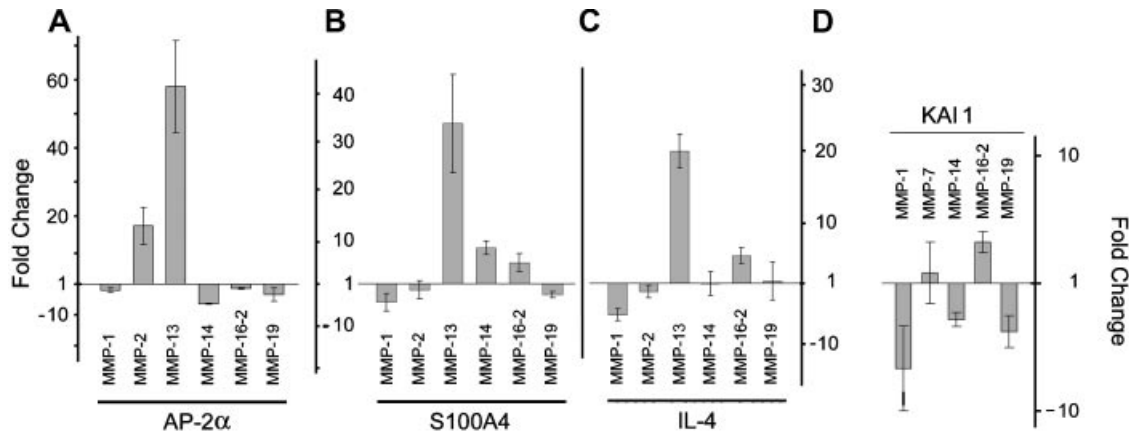


Fig. 2. Effects of AP-2α, S100A4, IL-4, or KAI1 overexpression on the gene expression of various MMPs in MDA-MB-231 cells. Bars and positive and negative numbers represent the same as in Figure 1.

assayed by measuring mRNA rather than protein levels because reverse transcription qRT-PCR is about ten orders of magnitude more sensitive than immunoblotting (Bakalova et al., 2005; Pérez-Ruiz et al., 2007).

PTEN decreases MMP-1 promoter transcription in fibroblasts (Oh et al., 2006). In order to test its effects on MMP gene expression in a metastatic breast cancer cell, MDA-MB-231 cells were transfected to overexpress the PTEN gene or empty pcDNA vector as a control. Four days later cells were harvested and RNA was isolated and then analyzed by qRT-PCR to measure the levels of various MMP mRNAs in the control and PTEN-transfected cells. MMP-1 and MMP-19 mRNAs decreased in PTEN-overexpressing cells relative to control cells (Fig. 1A). These effects were sequence-specific because PTEN overexpression did not substantially alter MMP-2, MMP-9, MMP-13, MMP-14, or MMP-16-2 mRNA levels (Fig. 1A).

RKIP decreases MMP-13 gene expression in chondrocytes (Xu et al., 2007). RKIP overexpression reduced MDA-MB-231 cell MMP-1 and MMP-13 mRNA levels, increased MMP-9 gene expression, and had negligible effect on MMP-2, MMP-11, MMP-14, MMP-16-2, or MMP-19 mRNA levels (Fig. 1B). We do not know of any effect of $p16^{INK4\alpha}$ on gene expression of any MMP in any cell. $p16^{INK4\alpha}$ overexpression lowered MMP-13 gene expression in MDA-MB-231 cells (Fig. 1C). Cleaved E-cadherin upregulates MMP-2 gene expression in lung cancer cells (Nawrocki-Raby et al., 2003). E-cadherin overexpression decreased MDA-MB-231 cell MMP-2, MMP-16-1 and MMP-16-2 gene expression, without substantial change in MMP-1, MMP-14, or MMP-19 mRNA (Fig. 1D).

AP-2α stimulates MMP-2 gene expression in endothelial cells (Park et al., 2007). AP-2α overexpression induced a decrease in MDA-MB-231 cell MMP-14 mRNA, had no appreciable effect on the expression of MMP-1, MMP-16-2 or MMP-19 (suggesting sequence specificity) and, unexpectedly, increased MMP-2 and MMP-13 mRNAs (Fig. 2A). S100A4 promotes the expression of MMP-13 in fibroblasts (Senolt et al., 2006). S100A4 overexpression in MDA-MB-231 cells increased MMP-13 and MMP-14 mRNA, and did not substantially alter the expression of MMP-1, MMP-2, MMP-16-2, or MMP-19 (Fig. 2B). IL-4 suppresses gene expression of MMP-1 in fibroblasts (Leonardi et al., 2003) and of MMP-13 in chondrocytes (Tardif et al., 1999). IL-4 overexpression decreased MDA-MB-231 cell MMP-1 mRNA, increased MMP-16-2 mRNA, had no appreciable effect on MMP-2, MMP-14 or MMP-19 gene expression and, unexpectedly, increased MMP-13 mRNA (Fig. 2C). KAI1 overexpression did not have any discernable effect on the mRNA level of any of the MMPs tested in MDA-MB-231 cells (Fig. 2D).

We are not aware of any known effect of fibulin 1D on gene expression of any MMP in any cell. Fibulin 1D overexpression in MDA-MB-231 cells decreased MMP-1 and MMP-13 mRNA levels, had little effect on MMP-2, MMP-14 or MMP-16-2 and, surprisingly, not only increased MMP-11 gene expression levels but did it by nearly three orders of magnitude (Fig. 3).

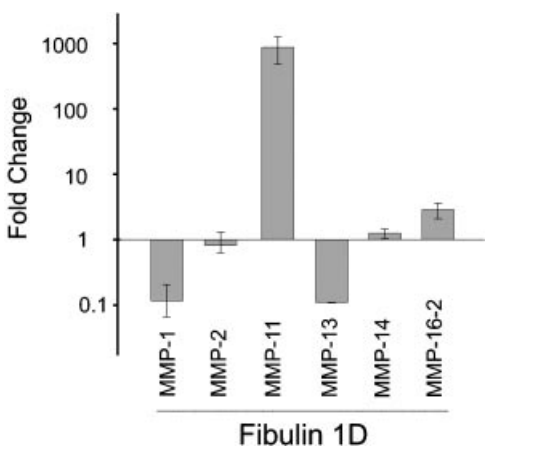


Fig. 3. Effects of fibulin 1D overexpression on the gene expression of various MMPs in MDA-MB-231 cells. Bars and positive and negative numbers represent the same as in Figure 1, except that the scale is logarithmic.

Discussion

It is well documented that the proteins studied in the present work are important in cancer progression. For example, MMPs play roles in cancer invasion, metastasis, growth regulation, immune evasion, apoptosis, and angiogenesis (Egeblad and Werb, 2002; Polette et al., 2004; Deryugina and Quigley, 2006). Cancer cell MMP-1, MMP-2, MMP-9, MMP-11, MMP-13,

MMP-14, MMP-16, and MMP-19 stimulate MDA-MB-231 cancer cell invasiveness (Ramos-DeSimone et al., 1999; Jiang et al., 2005, 2006; Wyatt et al., 2005; Merrell et al., 2006; Muñoz-Nájar et al., 2006; Hegedüs et al., 2008). AP-2α, E-cadherin, fibulin 1D, IL-4, KAI1, $p16^{INK4\alpha}$, PTEN and RKIP inhibit, and S100A4 promotes, cancer cell invasiveness and other cancer progression traits, in MDA-MB-231 breast cancer cells, and other types of cancers (Frixen et al., 1991; Dong et al., 1995; Uchiyama et al., 1996; Chintala et al., 1997; Qing et al., 1997; Hayashido et al., 1998; Tamura et al., 1999; Uozumi et al., 2000; Koul et al., 2001; Twal et al., 2001; Yang et al., 2001; Adachi et al., 2002; Fu et al., 2003; Lee et al., 2003; Wong and Gumbiner, 2003; Xu and Yu, 2003; Zheng et al., 2003; Gildea et al., 2004; Lu et al., 2004; Schuierer et al., 2004; Sumigama et al., 2004; Wu et al., 2004; Zhou et al., 2004; Cai et al., 2005; Hjelmeland et al., 2005; Jee et al., 2006; Saleem et al., 2006; Sridhar and Miranti, 2006; Chen et al., 2007; Schwartz et al., 2007). These UCPDs have been connected to various cell signaling pathways (Fig. 4). The present work showing that their pathway connections extend to MMP gene expression suggests how they might affect cancer progression.

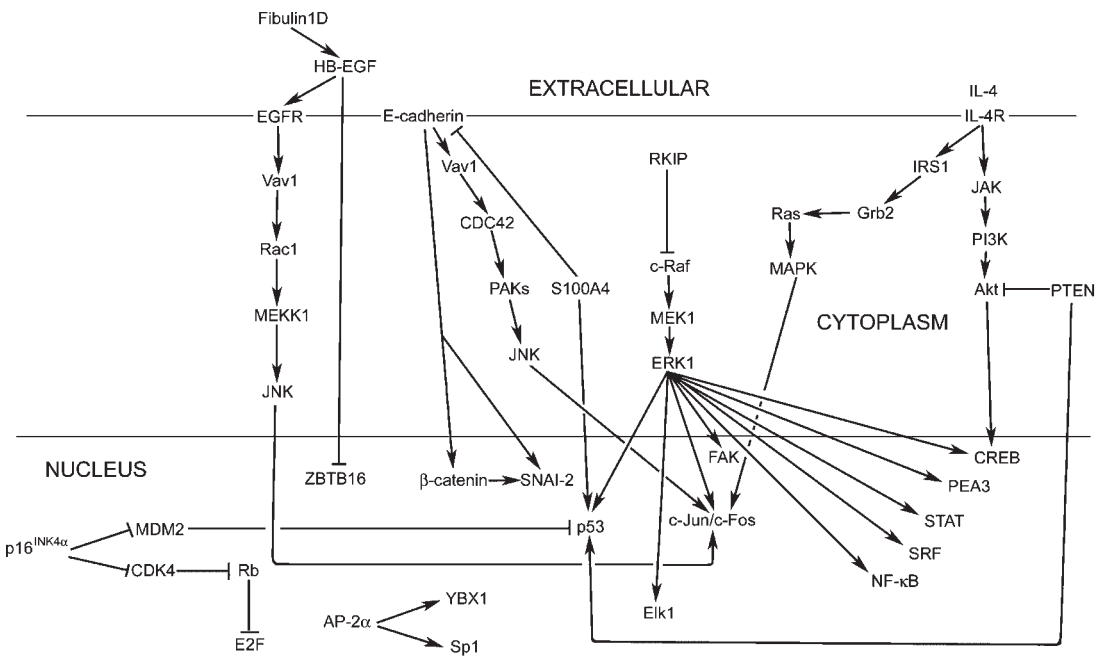
However, before the present work, only a few pathway links had been detected from these UCPDs to the expression of these MMP genes, namely PTEN to MMP-1, RKIP to MMP-13, E-cadherin to MMP-2, AP-2α to MMP-2, S100A4 to MMP-13, and IL-4 to MMP-1 and MMP-13, and only in either fibroblasts, chondrocytes, endothelial cells or lung cancer cells (Tardif et al., 1999; Leonardi et al., 2003; Nawrocki-Raby et al. 2003; Oh et al., 2006; Senolt et al., 2006; Park et al., 2007; Xu et al., 2007).

In contrast, the present results show that eight UCPDs (AP-2α, S100A4, IL-4, E-cadherin, fibulin 1D, $p16^{INK4\alpha}$, PTEN and RKIP) are part of signaling pathways which up- or down-regulate the gene expression of nine cancer cell MMPs (MMP-1, MMP-2, MMP-9, MMP-11, MMP-13, MMP-14, MMP-16, and MMP-19; Table 1). Each UCPD is linked to the gene expression of a unique set of MMPs (Table 1). This indicates that the effects are sequence-specific and that each UCPD reaches these MMP expressions through different sets of signaling pathways.

We have detected 20 new pathway links, 11 are downregulatory and nine are upregulatory; 15 are new links in any cell, and five are new links in breast cancer (Table 1). In six pathway links, three cancer-progression suppressing UCPDs (AP-2α, fibulin 1D and RKIP) unexpectedly enhanced, rather than suppress, the gene expression of five cancer-progression promoting, cancer cell MMPs (MMP-2, MMP-9, MMP-11, MMP-13, and MMP-16-2; Table 1). This suggests that some of these cell signaling pathways may have unanticipated features. This is the first report of any effect on MMP-16-2 gene expression by any factor in any cell.

All pathway links to MMP-1 gene expression were downregulatory; a mixture of downregulatory and upregulatory pathway links was observed for the expression of most of the other MMPs tested (Table 1). Suppressions of MMP gene expression were the only effects seen from E-cadherin and PTEN; the effects of most of the other UCPDs tested was a mixture of stimulations and inhibitions of MMP gene expression (Table 1). All of the UCPDs tested were linked to MMP-13 gene expression (Table 1).

Cell signaling pathways, rather than a specific member of each pathway, determine a given cancer trait (Vogelstein and Kinzler, 2004; Wang et al., 2007). It is important to identify many of the cell signaling pathways which determine cancer progression traits because they are potential therapy targets. Identification of each signaling pathway is time consuming and requires first the detection of its pathway link. To detect pathway links, measuring gene expression, as opposed to protein levels or activity, has the advantages of sensitivity, quantitation and cost. In addition, some cancer cell proteins whose mRNA levels are very low, are important for invasiveness (Hegedüs et al., 2008). The present study shows that working with transfected cancer cells in culture and



Arrowheads show activation. Tips ending with short perpendicular lines show inactivation.

Fig. 4. Cell signaling pathways linking UCPDs and other proteins discussed in this study.

TABLE 1. Effect of overexpression of the indicated UCPDs on gene expression of various MMPs in MDA-MB-231 cells

	Measured MMP mRNA																	
	1		2		9		11		13		14		16-1		16-2		19	
Overexpressed UCPD	Effect	F.E.[1]	Effect	F.E.[1]	Effect	F.E.[1]	Effect	F.E.[1]	Effect	F.E.[1]	Effect	F.E.[1]	Effect	F.E.[1]	Effect	F.E.[1]	Effect	F.E.[1]
AP-2α	●		Δ	2					Δ	1	▼	1			●		●	
E-cadherin	●		▼	3							●		▼	1	▼	1		
Fibulin 1D	▼	1	●				Δ	1	▼	1	●				●			
Interleukin 4	▼	2	●						Δ	1	●				Δ	1	●	
p16	●								▼	1	●				●		●	
PTEN	▼	2	●		●		●				●						▼	1
RKIP	▼	1	●		Δ	1	●		▼	2	●						●	
S100A4	●		●						Δ	2	Δ	1					●	

The symbols represent an increase (Δ), a decrease (▼), or no substantial change (●) in MMP gene expression relative to vector-only transfectants.
[1]F.E. (first evidence): to our knowledge, this is the first evidence of such an effect in any cell ("1"), in any cancer cell ("2") or in any breast cancer cell ("3").

focusing on the measurement of mRNA levels has the additional advantage of easily detecting a large number of pathway links.

The effects of the tested UCPDs on MMP gene expression could be mediated by any of the transcription factors or other downstream proteins which they are known to regulate, such as: (a) RKIP through p53, c-Jun/c-Fos, NF-κB, SRF, STAT, PEA3, or CREB; (b) PTEN through Akt or p53; (c) fibulin 1D through ZBTB16 or JNK; (d) E-cadherin through β-catenin or JNK; (e) IL-4 through Akt or MAPK; (f) AP-2α through Sp1 or YBX1 or directly on MMP gene expression as a transcription factor; (g) $p16^{INK4\alpha}$ through p53 or Rb; and (h) S100A4 through JNK or p53 (Fig. 4).

Cancer progression depends on many factors and signaling pathways (Uchiyama et al., 1996; Twal et al., 2001; Wong and Gumbiner, 2003; Zheng et al., 2003; Schuierer et al., 2004; Sumigama et al., 2004; Saleem et al., 2006). Our present results show many pathway links from some UCPDs to the gene expression of some cancer-progression enhancing MMPs. We have also found that different types of cancers have different pathway links from these UCPDs to the gene expression of these MMPs (manuscript in preparation). In view of this complexity, one of the next challenges will be to identify pathway links between other cancer-progression determinants and then to prioritize which of these links should first be studied further, in order to identify the signaling pathways involved.

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Literature Cited

Adachi Y, Chandrasekar N, Kin Y, Lakka SS, Mohanam S, Yanamandra N, Mohan PM, Fuller GN, Fang B, Fueyo J, Dinh DH, Olivero WC, Tamiya T, Ohmoto T, Kyritsis AP, Rao JS. 2002. Suppression of glioma invasion and growth by adenovirus-mediated delivery of a bicistronic construct containing antisense uPAR and sense p16 gene sequences. Oncogene 21:87–95.

Bakalova R, Zhelev Z, Ohba H, Baba Y. 2005. Quantum dot-based western blot technology for ultrasensitive detection of tracer proteins. J Am Chem Soc 127:9328–9329.

Buettner R, Kannan P, Imhof A, Bauer R, Yim SO, Glockshuber R, Van Dyke MW, Tainsky MA. 1993. An alternatively spliced mRNA from the AP-2 gene encodes a negative regulator of transcriptional activation by AP-2. Mol Cell Biol 13:4174–4185.

Cai XM, Tao BB, Wang LY, Liang YL, Jin JW, Yang Y, Hu YL, Zha XL. 2005. Protein phosphatase activity of PTEN inhibited the invasion of glioma cells with epidermal growth factor receptor mutation type III expression. Int J Cancer 117:905–912.

Chen PS, Wang MY, Wu SN, Su JL, Hong CC, Chuang SE, Chen MW, Hua KT, Wu YL, Cha ST, Babu MS, Chen CN, Lee PH, Chang KJ, Kuo ML. 2007. CTGF enhances the motility of breast cancer cells via an integrin-{alpha}vbeta3-ERK1/2-dependent S100A4-upregulated pathway. J Cell Sci 120:2053–2065.

Chintala SK, Fueyo J, Gomez-Manzano C, Venkaiah B, Bjerkvig R, Yung WK, Sawaya R, Kyritsis AP, Rao JS. 1997. Adenovirus-mediated p16/CDKN2 gene transfer suppresses glioma invasion in vitro. Oncogene 15:2049–2057.

Cybulski C, Masojc B, Oszutowska D, Jaworowska E, Grodzki T, Waloszczyk P, Serwatowski P, Pankowski J, Huzarski T, Byrski T, Gorski B, Jakubowska A, Debniak T, Wokolorczyk D, Gronwald J, Tarnowska C, Lubinski J, Narod SA. 2008. Constitutional CHEK2 mutations are associated with a decreased risk of lung and laryngeal cancers. Carcinogenesis 29:762–765.

Davies BR, Barraclough R, Rudland PS. 1994. Induction of metastatic ability in a stably diploid benign rat mammary epithelial cell line by transfection with DNA from human malignant breast carcinoma cell lines. Cancer Res 54:2785–2793.

Deryugina EI, Quigley JP. 2006. Matrix metalloproteinases and tumor metastasis. Cancer Metastasis Rev 25:9–34.

Dong JT, Lamb PW, Rinker-Schaeffer CW, Vukanovic J, Ichikawa T, Isaacs JT, Barrett JC. 1995. KAI1, a metastasis suppressor gene for prostate cancer on human chromosome 11p11.2. Science 268:884–886.

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

Frixen UH, Behrens J, Sachs M, Eberle G, Voss B, Warda A, Lochner D, Birchmeier W. 1991. E-cadherin-mediated cell-cell adhesion prevents invasiveness of human carcinoma cells. J Cell Biol 113:173–185.

Fu Z, Smith PC, Zhang L, Rubin MA, Dunn RL, Yao Z, Keller ET. 2003. Effects of raf kinase inhibitor protein expression on suppression of prostate cancer metastasis. J Natl Cancer Inst 95:878–889.

Gildea JJ, Herlevsen M, Harding MA, Gulding KM, Moskaluk CA, Frierson HF, Theodorescu D. 2004. PTEN can inhibit in vitro organotypic and in vivo orthotopic invasion of human bladder cancer cells even in the absence of its lipid phosphatase activity. Oncogene 23:6788–6797.

Gottardi CJ, Wong E, Gumbiner BM. 2001. E-cadherin suppresses cellular transformation by inhibiting beta-catenin signaling in an adhesion-independent manner. J Cell Biol 153:1049–1060.

Hayashido Y, Lucas A, Rougeot C, Godyna S, Argraves WS, Rochefort H. 1998. Estradiol and fibulin-1 inhibit motility of human ovarian- and breast-cancer cells induced by fibronectin. Int J Cancer 75:654–658.

Hegedüs L, Cho H, Xie X, Eliceiri G. 2008. Additional MDA-MB-231 breast cancer cell matrix metalloproteinases promote invasiveness. J Cell Physiol 216:480–485.

Hjelmeland AB, Hjelmeland MD, Shi Q, Hart JL, Bigner DD, Wang XF, Kontos CD, Rich JN. 2005. Loss of phosphatase and tensin homologue increases transforming growth factor beta-mediated invasion with enhanced SMAD3 transcriptional activity. Cancer Res 65:11276–11281.

Huang H, Cheville JC, Pan Y, Roche PC, Schmidt LJ, Tindall DJ. 2001. PTEN induces chemosensitivity in PTEN-mutated prostate cancer cells by suppression of Bcl-2 expression. J Biol Chem 276:38830–38836.

Jee BK, Park KM, Surendran S, Lee WK, Han CW, Kim YS, Lim Y. 2006. KAI1/CD82 suppresses tumor invasion by MMP9 inactivation via TIMP1 up-regulation in the H1299 human lung carcinoma cell line. Biochem Biophys Res Commun 342:655–661.

Jiang WG, Davies G, Martin TA, Parr C, Watkins G, Mason MD, Mokbel K, Mansel RE. 2005. Targeting matrilysin and its impact on tumor growth in vivo: The potential implications in breast cancer therapy. Clin Cancer Res 11:6012–6019.

Jiang WG, Davies G, Martin TA, Parr C, Watkins G, Mason MD, Mansel RE. 2006. Expression of membrane type-1 matrix metalloproteinase, MT1-MMP in human breast cancer and its impact on invasiveness of breast cancer cells. Int J Mol Med 17:583–590.

Koul D, Parthasarathy R, Shen R, Davies MA, Jasser SA, Chintala SK, Rao JS, Sun Y, Benvenisite EN, Liu TJ, Yung WK. 2001. Suppression of matrix metalloproteinase-2 gene expression and invasion in human glioma cells by MMAC/PTEN. Oncogene 20:6669–6678.

Lee JH, Seo YW, Park SR, Kim YJ, Kim KK. 2003. Expression of a splice variant of KAI1, a tumor metastasis suppressor gene, influences tumor invasion and progression. Cancer Res 63:7147–7255.

Leonardi A, Cortivo R, Fregona I, Plebani M, Secchi AG, Abatangelo G. 2003. Effects of Th2 cytokines on expression of collagen, MMP-1, and TIMP-1 in conjunctival fibroblasts. Invest Ophthalmol Vis Sci 44:183–189.

Lu W, Zhou X, Hong B, Liu J, Yue Z. 2004. Suppression of invasion in human U87 glioma cells by adenovirus-mediated co-transfer of TIMP-2 and PTEN gene. Cancer Lett 214:205–213.

Merrell MA, Ilvesaro JM, Lehtonen N, Sorsa T, Gehrs B, Rosenthal E, Chen D, Shackley B, Harris KW, Selander KS. 2006. Toll-like receptor 9 agonists promote cellular invasion by increasing matrix metalloproteinase activity. Mol Cancer Res 4:437–447.

Muñoz-Nájar UM, Neurath KM, Vumbaca F, Claffey KP. 2006. Hypoxia stimulates breast carcinoma cell invasion through MT1-MMP and MMP-2 activation. Oncogene 25:2379–2392.

Nawrocki-Raby B, Gilles C, Polette M, Bruyneel E, Laronze JY, Bonnet N, Foidart JM, Mareel M, Birembaut P. 2003. Upregulation of MMPs by soluble E-cadherin in human lung tumor cells. Int J Cancer 105:790–795.

Neve RM, Chin K, Fridlyand J, Yeh J, Baehner FL, Fevr T, Clark L, Bayani N, Coppe JP, Tong F, Speed T, Spellman PT, DeVries S, Lapuk A, Wang NJ, Kuo WL, Stilwell JL, Pinkel D,

Albertson DG, Waldman FM, McCormick F, Dickson RB, Johnson MD, Lippman M, Ethier S, Gazdar A, Gray JW. 2006. A collection of breast cancer cell lines for the study of functionally distinct cancer subtypes. Cancer Cell 10:515–527.

Oh J-H, Kim A, Park J-M, Kim S-H, Chung A-S. 2006. Ultraviolet B-induced matrix metalloproteinase-1 and -3 secretions are mediated via PTEN/Akt pathway in human dermal fibroblasts. J Cell Physiol 209:775–785.

Park MJ, Kwak HJ, Lee HC, Yoo DH, Park IC, Kim MS, Lee SH, Rhee CH, Hong SI. 2007. Nerve growth factor induces endothelial cell invasion and cord formation by promoting matrix metalloproteinase-2 expression through the phosphatidylinositol 3-kinase/Akt signaling pathway and AP-2 transcription factor. J Biol Chem 282:30485–30496.

Pérez-Ruiz M, Collao X, Navarro-Mari JM, Tenorio A. 2007. Reverse transcription, real-time PCR assay for detection of Toscana virus. J Clin Virol 39:276–281.

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

Qing J, Maher VM, Tran H, Argraves WS, Dunstan RW, McCormick JJ. 1997. Suppression of anchorage-independent growth and matrigel invasion and delayed tumor formation by elevated expression of fibulin-1D in human fibrosarcoma-derived cell lines. Oncogene 15:2159–2168.

Ramos-DeSimone N, Hahn-Dantona E, Sipley J, Nagase H, French DL, Quigley JP. 1999. Activation of matrix metalloproteinase-9 (MMP-9) via a converging plasmin/stromelysin-1 cascade enhances tumor cell invasion. J Biol Chem 274:13066–13076.

Saleem M, Kweon MH, Johnson JJ, Adhami VM, Elcheva I, Khan N, Bin HB, Bhat KM, Sarfaraz S, Reagan-Shaw S, Spiegelman VS, Setaluri V, Mukhtar H. 2006. S100A4 accelerates tumorigenesis and invasion of human prostate cancer through the transcriptional regulation of matrix metalloproteinase 9. Proc Natl Acad Sci 103:14825–14830.

Schuierer MM, Bataille F, Hagan S, Kolch W, Bosserhoff AK. 2004. Reduction in raf kinase inhibitor protein expression is associated with increased ras-extracellular signal-regulated kinase signaling in melanoma cell lines. Cancer Res 64:5186–5192.

Schwartz B, Melnikova VO, Tellez C, Mourad-Zeidan A, Blehm K, Zhao YJ, McCarty M, Adam L, Bar-Eli M. 2007. Loss of AP-2alpha results in deregulation of E-cadherin and MMP-9 and an increase in tumorigenicity of colon cancer cells in vivo. Oncogene 26:4049–4058.

Senolt L, Grigorian M, Lukanidin E, Simmen B, Michel BA, Pavelka K, Gay RE, Gay S, Neidhart M. 2006. S100A4 is expressed at site of invasion in rheumatoid arthritis synovium and modulates production of matrix metalloproteinases. Ann Rheum Dis 65:1645–1648.

Sridhar SC, Miranti CK. 2006. Tetraspanin KAI1/CD82 suppresses invasion by inhibiting integrin-dependent crosstalk with c-Met receptor and Src kinases. Oncogene 25:2367–2378.

Sumigama S, Ito T, Kajiyama H, Shibata K, Tamakoshi K, Kikkawa F, Williams T, Tainsky MA, Nomura S, Mizutani S. 2004. Suppression of invasion and peritoneal carcinomatosis of ovarian cancer cells by overexpression of AP-2alpha. Oncogene 23:5496–5504.

Tamura M, Gu J, Takino T, Yamada KM. 1999. Tumor suppressor PTEN inhibition of cell invasion, migration, and growth: Differential involvement of focal adhesion kinase and p130Cas. Cancer Res 59:442–449.

Tardif G, Pelletier J-P, Dupuis M, Geng C, Cloutier J-M, Martel-Pelletier J. 1999. Collagenase 3 production by human osteoarthritic chondrocytes in response to growth factors and cytokines is a function of the physiologic state of the cells. Arthritis Rheum 42:1147–1158.

Twal WO, Czirok A, Hegedus B, Knaak C, Chintalapudi MR, Okagawa H, Sugi Y, Argraves WS. 2001. Fibulin-1 suppression of fibronectin-regulated cell adhesion and motility. J Cell Sci 114:4587–4598.

Uchiyama A, Essner R, Doi F, Nguyen T, Ramming KP, Nakamura T, Morton DL, Hoon DSB. 1996. Interleukin 4 inhibits hepatocyte growth factor-induced invasion and migration of colon carcinomas. J Cell Biochem 62:443–453.

Uozumi M, Murao S, Katayama N, Kitazawa S, Amatsu M, Maeda S. 2000. Induction of S100A4 gene expression inhibits in vitro invasiveness of human squamous cell carcinoma, KOSC-3 cells. Cancer Lett 149:135–141.

Vogelstein B, Kinzler KW. 2004. Cancer genes and the pathways they control. Nat Med 10:789–799.

Walsh T, King MC. 2007. Ten genes for inherited breast cancer. Cancer Cell 11:103–105.

Wang W, Eddy R, Condeelis J. 2007. The cofilin pathway in breast cancer invasion and metastasis. Nat Rev Cancer 7:429–440.

Wong AS, Gumbiner BM. 2003. Adhesion-independent mechanism for suppression of tumor cell invasion by E-cadherin. J Cell Biol 161:1191–1203.

Wu X, Obata T, Khan Q, Highshaw RA, De Vere WR, Sweeney C. 2004. The phosphatidylinositol-3 kinase pathway regulates bladder cancer cell invasion. BJU Int 93:143–150.

Wyatt CA, Geoghegan JC, Brinckerhoff CE. 2005. Short hairpin RNA-mediated inhibition of matrix metalloproteinase-1 in MDA-231 cells: Effects on matrix destruction and tumor growth. Cancer Res 65:11101–11108.

Xu Y, Yu Q. 2003. E-cadherin negatively regulates CD44-hyaluronan interaction and CD44-mediated tumor invasion and branching morphogenesis. J Biol Chem 278:8661–8668.

Xu L, Peng H, Glasson S, Lee PL, Hu K, Ijiri K, Olsen BR, Goldring MB, Li Y. 2007. Increased expression of the collagen receptor discoidin domain receptor 2 in articular cartilage as a key event in the pathogenesis of osteoarthritis. Arthritis Rheum 56:2663–2673.

Yang X, Wei LL, Tang C, Slack R, Mueller S, Lippman ME. 2001. Overexpression of KAI1 suppresses in vitro invasiveness and in vivo metastasis in breast cancer cells. Cancer Res 61:5284–5288.

Yokota T, Otsuka T, Mosmann T, Banchereau J, DeFrance T, Blanchard D, Vries JED, Lee F, Arai KI. 1986. Isolation and characterization of a human interleukin cDNA clone, homologous to mouse B-cell stimulatory factor 1, that expresses B-cell- and T-cell-stimulating activities. Proc Natl Acad Sci 83:5894–5898.

Zheng HC, Sun JM, Li XH, Yang XF, Zhang YC, Xin Y. 2003. Role of PTEN and MMP-7 expression in growth, invasion, metastasis and angiogenesis of gastric carcinoma. Pathol Int 53:659–666.

Zhou B, Liu L, Reddivari M, Zhang XA. 2004. The palmitoylation of metastasis suppressor KAI1/CD82 is important for its motility- and invasiveness-inhibitory activity. Cancer Res 64:7455–7463.