The Mitogen-activated Protein (MAP) Kinases p38 and Extracellular Signal-regulated Kinase (ERK) Are Involved in Hepatocyte-mediated Phenotypic Switching in Prostate Cancer Cells*

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Background: Epithelial mesenchymal phenotypic switching enables cancers to seed and survive in metastatic sites. The greatest challenge for the seeding of cancer in metastatic sites is integration into the ectopic microenvironment despite the lack of an orthotopic supportive environment and presence of pro-death signals concomitant with a localized “foreign-body” inflammatory response. In this metastatic location, many carcinoma cells display a reversion of the epithelial-to-mesenchymal transition that marks dissemination in the primary tumor mass. This mesenchymal to epithelial reverting transition (MErT) is thought to help seeding and colonization by protecting against cell death. We have previously shown that hepatocyte coculture induces the re-expression of E-cadherin allowing, respectively, for hepatocyte-mediated MErT and tumor cell survival.

Results: Both p38 and ERK1/2 MAP kinases need to be inhibited to allow for an epithelial reversion but activated to provide survival advantage in the face of chemotherapy. Distinct p38/ERK signaling outcomes are involved in hepatocytes-mediated MErT and tumor cells survival.

Conclusion: The greatest challenge for the seeding of cancer in metastatic sites is integration into the ectopic microenvironment despite the lack of an orthotopic supportive environment and presence of pro-death signals concomitant with a localized “foreign-body” inflammatory response. In this metastatic location, many carcinoma cells display a reversion of the epithelial-to-mesenchymal transition that marks dissemination in the primary tumor mass. This mesenchymal to epithelial reverting transition (MErT) is thought to help seeding and colonization by protecting against cell death. We have previously shown that hepatocyte coculture induces the re-expression of E-cadherin allowing, respectively, for hepatocyte-mediated MErT and tumor cell survival.

Significance: Metastasis is the main cause of death in most cancers. It has been suggested that this complex metastatic cascade could be conceptually organized and simplified into two major phases: (i) physical translocation of a cancer cell from the primary tumor to the microenvironment of a distant tissue and then (ii) colonization (1). Therefore, understanding the many molecules and processes leading to successful colonization may lead to effective therapies for patients with already established metastases. It is established that successful dissemination results from a series of phenotypic switches that are regulated by the microenvironment (2, 3). Cancer cells down-regulate E-cadherin to allow for translocation in a process known as epithelial-to-mesenchymal transition (EMT) (1). However, survival and colonization in the distant metastatic site, where there is a lack of orthotopic supportive environment and the presence of pro-death signals, requires a reversion of the phenotype, a mesenchymal-to-epithelial reverting transition (MERt) to reside among ectopic tissue epithelial cells. This is strongly supported by the accumulating evidence that show re-express E-cadherin in metastatic tumors (4–7). Re-expression of E-cadherin is the crucial step in MERt.

Due to the key role of E-cadherin in human carcinoma progression, much effort has been devoted to understanding how E-cadherin is regulated during cancer progression, especially in EMTs. Various signaling molecules and transcription factors regulate the expression of E-cadherin (8). Accumulating evidence suggests that growth factor-induced EMT is the result of transcriptional reprogramming and chromatin remodeling (9, 10). It has been shown that abrogated epidermal growth factor receptor (EGFR) activity recovers E-cadherin expression in prostate cancer cells (11, 12). Moreover, by co-culturing the primary hepatocytes and carcinoma cells to recreate a key

The abbreviations used are: EMT, epithelial-to-mesenchymal transition; MErT, mesenchymal-to-epithelial reverting transition; EGFR, EGFR receptor; RFP, red fluorescence protein; ca-, constitutively active; CPT, camptothecin; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; MAP, mitogen-activated protein; PCa, prostate cancer.

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interaction extant in the liver microenvironment, it has been demonstrated that primary hepatocytes elicit E-cadherin cell surface expression in prostate carcinoma cells concomitant with down-regulation of EGFR signaling (12). These hepatocyte-induced E-cadherin re-expression in prostate and breast cancer cells increases the chemoresistant (13).

EGFR activates several signaling cascades such as mitogen-activated protein kinases (MAPKs), PI3K-AKT, and JAK pathways. All of these kinase pathways are dysregulated in human tumors. The heterogeneity of the cellular models and the different experimental approaches result in the conflicting results of the roles of MAPK families in the genesis of EMTs. There is compelling evidence that ERKs and PI3K drive EMTs in many experimental systems (14–16). Inhibition of ERK MAP kinase was able to completely restore E-cadherin cell-cell junctions in Ras-transformed breast epithelial cells (17). However, the role of SAPKs is less studied, although some reports indicate that JNK is an EMT inducer (18). p38 appears to promote EMT during development and in tumors (19–21) but maintains E-cadherin expression in human primary mesothelial cells (22). However, the role of these kinases in disseminated cancer cells colonization in distant sites is unclear. Herein, we demonstrate that p38 and ERK1/2, but not JNK, JAK, or PI3K activation, are inhibited in prostate cancer cells in a hepatocyte microenvironment and result in the re-expression of E-cadherin. When challenged with chemotherapy and cell death-inducing cytokine, E-cadherin triggered p38 and ERK1/2 activity in PCa cells, which results in increased cells survival.

**EXPERIMENTAL PROCEDURES**

**Reagents and Antibodies**—Cell culture reagents were obtained from Invitrogen. Human primary hepatocyte maintenance medium HMM was purchase from Lonza (Mapleton, IL). p38α, phospho-p38 MAPK (Thr-180/Tyr-182), p44/42 MAPK, phospho-p44/42 MAPK (Thr-202/Tyr-204), phospho-JNK (Thr-183/Tyrs-185), MEK1, MKK6, phospho-AKT(Ser-473), AKT, phosphor-GSK-3β (Ser-9), phospho-JAK2 (Tyr-1007/1008), JAK2, phospho-stat3 (Tyr-705), connexin 43, and immunoblot E-cadherin(24E10) monoclonal antibodies were purchased from Cell Signaling Technology (Danvers, MA). E-cadherin and ZO-1 antibodies for immunofluorescence were purchased from Santa Cruz Biotechnology (Dallas, TX). Inosine, cytosine, uracil, guanine, and thymine deaminase inhibitor were purchased from Sigma-Aldrich. Control and E-cadherin shRNA expression plasmids, p38α, and ERK1 and ERK2 siRNA pools were purchased from Santa Cruz Biotechnology (Dallas, TX). Kinase inhibitors PD153035, SB203580, PD98059, JNK inhibitor II, LY294002, JAK inhibitor I, and AKT inhibitor IV were purchased from EMD Millipore (Billerica, MA).

**Cell Lines and Cell Culture**—American Type Culture Collection cell lines DU145, PC3 prostate cancer cells, MDA-MB-231 breast cancer cell lines, and NCI-H1299 and A549 lung cancer cell lines were cultured in media recommended by supplier. DU145-red fluorescence protein (RFP) and PC3-RFP were selected in growth medium with 1000 μg/ml G418. Polyclones of DU145-shctl-RFP and DU145-shEcad-RFP were selected in growth medium supplemented with 0.5 μg/ml puromycin and 1000 μg/ml G418. Human fibroblast HS-68 cells were cultured in DMEM with 1× nonessential amino acids, 1× sodium pyruvate, 2 mM l-glutamine, 1× streptomycin/penicillin (Invitrogen).

**Quantitative Real-time PCR**—Total RNA was extracted from cells by TRIzol (Invitrogen), and cDNA was reverse-transcribed by QuantiTect (Qiagen, Germantown, MD). SYBR Green RT-PCR (Agilent Technologies, Santa Clara, CA) was performed with following primers: E-cadherin forward (5′-GTCTGAGGTCTCCTGG-3′) and reverse (5′-AGGCCAGGTAGACCCAG-3′) and GAPDH forward (5′-GAGTTACGGATTTGTGCGT-3′) and reverse (5′-TTGATTTTGGAGGGACT-TGG-3′). GAPDH was used for control and normalization.

**Co-culture**—Primary human hepatocytes, obtained from excess pathologic specimens, were isolated and plated at 8 × 10^5 cells per well in 6-well plates coated with 1% rat tail collagen in distilled H_2O (BD Biosciences) and allowed to attach overnight. The next day 2 × 10^5 RFP-labeled cancer cells were seeded onto hepatocyte monolayers. The co-cultured cells were maintained with hepatocyte maintenance media (Lonza). For fibroblast cocultures, the fibroblast monolayer was initially plated at 1 × 10^5 cells per well in 6-well plates and seeded with 2 × 10^4 the following day. Medium was replenished daily.

**Cell Death Resistance Assay**—On day 5 of co-culture cells were treated with camptothecin (CPT) or/and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) for 24 h, and the absolute living cell numbers were accessed by flow cytometry. Annexin V-488 (Invitrogen) was used to identify apoptotic cells, and absolute counting beads (Invitrogen) were used to count absolute viable cell numbers.

**Immunoblot and Immunofluorescence**—Hepatocytes and cancer cells were seeded on a coverslip precoated with 1% collagen and then fixed on day 5 with 4% formaldehyde followed by permeabilization with 0.1% Triton X-100. After blocking with 2% BSA, the cells were incubated with primary antibody at 4 °C overnight and then with Alexa Fluor® 488/647-conjugated secondary antibodies at room temperature for 1 h. DAPI was applied to stain the nucleus. For the Western blot, cells were lysed with radioimmune precipitation assay buffer with phosphate inhibitor mixture II, III (Sigma) and protease inhibitor mixture (BD Biosciences).

**Protein Chace Assays**—DU145 cancer cells were seeded at 3 × 10^5 cells/well in 6-well plates and treated with or without 10 μM SB203580 or 50 μM PD98059 for 24 h after the addition 100 ng/ml actinomycin D or 100 μM cycloheximide for the indicated times. Cells were lysed with radioimmune precipitation assay buffer supplemented with a pan-kinase inhibitor mixture. 20 μg of protein was used to assess protein levels by immunoblotting.
RESULTS

Hepatocyte-educated Prostate Cancer Cells Re-express E-cadherin and Other Epithelial Markers—We have reported that hepatocyte co-culture can lead to up-regulation or re-expression of E-cadherin even in highly aggressive carcinoma cells (12, 13). Herein, we show that this phenotypic switch extends to other epithelial markers of cell-cell cohesion including ZO-1 and connexin 43 (Fig. 1a). Most of single DU145 and PC3 cells developed a colony after 5 days of co-culture with hepatocytes. Microscopic observation of these prostate cancer cells in the presence of hepatocytes, but not fibroblasts or other cancer cells, revealed a change in cell morphology from elongated spindle-like shape to smaller cuboidal with reversion to an epithelial phenotype (Fig. 1b), indicative of a cell phenotypic change from mesenchymal to epithelial.

Interestingly, different stages of converted PCa cells were found in hepatocytes microenvironment. The first stage was spindle-like with low E-cadherin expression levels, which was similar with parental PCa cells; the second stage was spindle-like with high E-cadherin in cytoplasm, and no E-cadherin was found on the membrane for the spindle-like PCa cells; the third stage was cuboidal-like with E-cadherin in the perinuclear, and the final stage was cuboidal-like with E-cadherin on the rim of cells. DU145 cells were found in the last two stages (Fig. 1c), and PC3 cells were found in the all four stages (Fig. 1d) due to their different morphology. On the 3rd day, 24.1% PC3 colonies developed to a cuboidal-like phenotype, and only 1% of colonies found E-cadherin expressing on the cell membrane, but on the 5th day, 37.5% PC3 colonies were cuboidal cells and 17.5% were in colonies with E-cadherin on the cell membrane (Fig. 1e). The percentage of DU145 colonies with E-cadherin on the cell membrane was increased from 3.7% (day 3) to 57.5% (day 5) (Fig. 1f), which indicated hepatocytes educate PCa cells to develop an E-cadherin-dependent MErT in addition to simply re-expressing E-cadherin.

We have previously shown that E-cadherin expression in prostate cancer metastases is inversely correlated with the size of metastasis (13), suggesting a metastable nature to the reversion toward an epithelial phenotype. To explore the relationship between the colony size and E-cadherin expression patterns in vitro, the cell number per colony with different E-cadherin expression levels was determined. For DU145 cells, the colonies with E-cadherin on the cell membrane had a smaller size than in the cytoplasm (Fig. 1g); for spindle-like PC3 cells, most E-cadherin low-expressed cells were single or two-cell colonies, but E-cadherin high expression colonies had the bigger size; for cuboidal PC3, E-cadherin expression levels was inversely correlated with the size of colonies, which was similar with DU145 (Fig. 1h). This suggests both a metastable nature of the phenotypic reversion and cell distance limit to the hepatocyte-induced changes.

Hepatocytes Suppress p38 and ERK1/2 Activation in DU145 Cells Result in E-cadherin Re-expression—Based on the above, we looked at what intermediary signaling in the cell could account for the changes in phenotype. Abrogation of EGFR signaling in prostate cancer cells can lead to up-regulation of E-cadherin (11), and co-culture with hepatocytes leads to suppression of the EGFR autocrine signaling in these cells indicative of the role of EGFR signaling cascade(s) might be involved in hepatocyte-regulated E-cadherin re-expression in PCa cells. Negligible p-JNK, moderate p-p38, p-JAK2, and high p-ERK1/2 and p-AKT levels were found in parental DU145 cells. The presence of hepatocytes with the DU145 cells resulted in decreased levels of all mentioned above (Fig. 2, a–d). To look at which pathway(s) plays a dominant role in E-cadherin regulation in DU145 cancer cells, selective kinase inhibitors were applied, and relevant E-cadherin expression was detected. All inhibitors efficiently abrogated their targets (Fig. 2i). Inhibition of p38 or ERK MAPK activity increased E-cadherin expression at both the transcriptional and protein level, and although inhibition of PI3K or AKT decreased E-cadherin, inhibition of JAK did not affect E-cadherin (Fig. 2, e and f). Moreover, the lung cancer cell A549 responded similarly to DU145 to the kinase inhibitors (Fig. 2j). Knockdown of p38a or EKR1/2 expression by siRNA produced a similar up-regulation of E-cadherin (Fig. 2g).

To probe how p38 and ERK regulate E-cadherin expression, actinomycin was applied to block de novo RNA synthesis. SB203580 and PD98059 could not enhance E-cadherin expression in DU145 anymore. However, cycloheximide was applied to block new proteins synthesis; neither SB203580 nor PD98059 prevented E-cadherin degradation compared with control (Fig. 2h). These data suggested p38 and ERK regulate E-cadherin through RNA synthesis control and not at the protein level.

Constitutively Active MEK1 or MEK6 Abrogates Hepatocyte-induced E-cadherin Re-expression in DU145 Cells—Because hepatocytes enhanced E-cadherin expression in DU145 cells and this was phenocopied by suppressing p38 and ERK1/2 activity, demonstrating sufficiency, we queried whether abrogation of these was required for re-expression of E-cadherin, or whether activation of these intermediary signalers could overcome the hepatocyte influence. We introduced constitutively active MEK6 (caMEK6, activates p38 constitutively) and MEK1 (caMEK1, activates ERK1/2 constitutively) into DU145 cells. CaMEK6-expressing DU145 cells showed a notable increase in p38 phosphorylation levels in comparison to empty vector (EV)-expressing cells (Fig. 3a) as well as caMEK1-activated ERK1/2 (Fig. 3b). We next applied these cells co-cultured with hepatocytes. As expected, constitutively active MEK1 or MEK6 abrogated, at least partially, hepatocyte-induced E-cadherin re-expression in DU145 cells (Fig. 3, c and d). Thus each intermediary kinase was both sufficient and required.

Hepatocytes Render DU145 Resistant to Death Induced in an E-cadherin-dependent Manner—E-cadherin re-expression in the liver microenvironment increases the chemoresistance of breast and prostate cancer cells after treatment with chemotherapeutic agents such as staurosporine and camptothecin (13). TRAIL is a member of the tumor necrosis factor family that preferentially kills tumor cells. Chemotherapeutic agents enhance TRAIL-induced apoptosis in PCA cells (24, 25). On the 5th day of co-culture, cells were treated with drugs for 24 h, and
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a) DU145-RFP only
   DU145-RFP +Heps
   PC3-RFP

b) PCa-RFP cells+
   Heps
   fibroblast
   PCa cells

C) Only
   DU145-RFP +Heps

D) #1 Only
   #2
   #3
   #4

E) DU145
   cuboidal, E-cad in cytoplasm
   cuboidal, E-cad on membrane

F) PC3
   spindle, E-cad low
   spindle, E-cad high
   cuboidal, E-cad in cytoplasm
   cuboidal, E-cad on membrane

G) DU145
   cell number/cell
   E-cad in cytoplasm
   E-cad on membrane
   P < 0.01

H) PC3
   cell number/cell
   spindle E-cad low
   spindle E-cad high
   cuboidal E-cad in cytoplasm
   cuboidal E-cad on membrane
   P < 0.05
   P < 0.001
then surviving DU145 cells were assessed by flow cytometry; annexin-V was applied to label apoptotic cells, and the absolute living cell number was calculated. Reverted DU145 cells became more resistant to killing after the treatment of TRAIL (Fig. 4a) or co-treatment of TRAIL and CPT (Fig. 4b). Down-regulation of E-cadherin by stably introducing shRNA abrogated this resistance to death, but no difference was noted in cell survival between parental control and E-cadherin KD DU145 cells in the face of the same cell death challenge (Fig. 4c). This is not due to simply being in G0 or mitotic arrest, as the E-cadherin knockdown cells presented a similar proliferation rate compared with control cells when co-cultured with hepatocytes (Fig. 4d). This indicates this cell death resistance was proliferation-independent.

**Select MAPK Effectors Are Required for Chemoresistance in DU145 Cells**—To investigate the molecular mechanism of E-cadherin-related cell survival, the various MAP kinases were selectively inhibited in the co-culture cells, and then the cells were treated with CPT. Inhibition of p38 and ERK1/2 activities (Fig. 5c) but not JNK and PI3K (data not shown) abrogated DU145 cells survival advantage. This observation was also confirmed with PD153035-induced DU145 cell survival. After 48 h of PD153035 treatment to induce E-cadherin re-expression, DU145 cells displayed chemoresistance to CPT in a manner dependent on intact p38 and ERK (Fig. 5b). To eliminate that the kinase inhibitor per se altered cell survival, these inhibitors were applied to the parental DU145 cells followed by challenge with CPT. P38, JNK, and PI3K inhibitors did not alter cell survival, but ERK inhibitor improved cell survival from cell death (Fig. 5a). This effect was shown to be due to limiting cell proliferation, as a counting of viable cell numbers before and after inhibitors treatment demonstrated that the ERK inhibitor limited the increase in cell number (data not shown). These data suggested p38 and ERK1/2 played roles in the PCa cell survival via E-cadherin. Additionally, high levels of ERK1/2 and p38 phosphorylation were detected in DU145 cells treated with CPT and TRAIL. All challenges stimulated ERK1/2 and p38 phosphorylated in control cells but not E-cadherin shRNA expression cells (Fig. 5d).

**DISCUSSION**

In patients with advanced cancer, widespread manifestation of distant metastases is a major cause of cancer-related deaths. Despite this important clinical problem, little is known about the mediators that promote tumor outgrowth in the metastatic organ. The role of the MErT in cancer metastasis is controversial (2, 8). Most likely this is due to cellular heterogeneity and the complex multistep process of cancer development and progression and its likely reversion back to a mesenchymal phenotype when metastatic nodules grow out (7). Thus, it is hard to capture MErT in vivo and in vitro.

We successfully induced MErT in prostate cancer cells via co-culturing with human hepatocytes, which elicited E-cadherin and other epithelial cell markers such as ZO-1 and connexin 43 re-expressing on the cell membrane. We found that this PCA cell phenotypic conversion appeared to be a process of education by hepatocytes (Fig. 1, c-f) in that there were intermediary forms of morphological shapes with E-cadherin being mainly expressed in intracellular vesicles. It could be that the low E-cadherin expressing spindle-like PCa cells with high motility, prevented clustering. It appears that over time these E-cadherin low, spindle-like, high motility PCa cells converted to E-cadherin high, cuboidal, low motility. E-cadherin expression level in PCa cells was inversely correlated with size of colony (Fig. 1, g and h), which was consistent with a previous study (7). Our study highlights the role of the cancer cell extrinsic microenvironment prevailing in the metastatic organ as a major promoter of survival and outgrowth of disseminated tumor cells by induction of MErT.

To intervene in this phenotypic plasticity, the molecular triggers need to be discerned. Although we had reported earlier that for prostate cancers, disruption of autocrine EGFR signaling was sufficient for E-cadherin re-expression (11, 12), the intracellular pathways have not been defined. EGFR activates several signaling cascades such as MAPKs, PI3K-AKT, and JAK pathways in cancers. ERK and AKT are the essential downstream effectors of EGFR pathway. Moreover, EGFR also activates the JAK and PKC pathway, with PKC further activating p38 and JNK (23). Herein, we queried whether the various MAP kinase pathways may be involved. Pharmacologic inhibitors suggested roles for the ERK and p38 pathways, with p38 being the most prominent, suppression of which allowed for E-cadherin up-regulation and MErT. The counter to this was achieved by constituutive activation of p38 using a MEK6 construct that prevented E-cadherin up-regulation. Although MEK6 activates all p38 isoforms (26), and both p38α and p38β are sensitive to SB203580 (27), we used siRNA to define p38α as the key isoform. There are four genes that encode p38 MAPKs: MAPK14 (that encodes p38α), MAPK11 (that encodes p38β), MAPK12 (that encodes p38γ), and MAPK13 (that encodes p38δ). p38α and p38β are closely related proteins that could have overlapping functions. Whereas p38α is highly abundant in most cell types, p38β seems to be expressed at very low levels and is highly expressed in prostate cancer cells. Therefore, we focused on p38α and p38β and their roles in MErT.

![FIGURE 1. DU145 and PC3 PCa cells undergo mesenchymal to epithelial reverting transition in a hepatocyte microenvironment.](image-url) Prostate cancer cell lines were cocultured with primary human hepatocytes for up to 5 days before examination. a, the expression of ZO-1 (left panel) and connexin 43 (right panel) in the human prostate cancer cell line DU145 were examined by immunofluorescence with mouse monoclonal antibody against ZO-1 or rabbit monoclonal antibody against connexin 43. Representative images are shown for DU145 cells alone (upper panel) co-cultured with hepatocytes (lower panel) are shown. b, phase contrast and fluorescent images of red fluorescence-labeled PCa cells DU145 (upper panels) and PC3 (lower panels) cocultured with hepatocytes (left panels), fibroblasts (middle panels), and parental PCa cells (right panels) after 5 days. c (DU145) and d (PC3), immunofluorescence for E-cadherin (green) in RFP-labeled DU145 cells alone (left panel) or co-cultured with hepatocytes (middle and right panels); E-cadherin alone is shown in the upper panels. e, quantification of E-cadherin localization in DU145 cells with hepatocytes on day 3 (n = 12) and with (n = 26) or without (n = 153) hepatocytes on day 5, f, quantification of cell morphology (spindle-like and cuboidal) as E-cadherin expression levels (low and high) and localization (in cytoplasm and on membrane) in PC3 cells with hepatocytes on day 3 (n = 36) and with (n = 83) or without (n = 81) hepatocytes on day 5. g and h, quantification of cell number per colony for DU145 (g) and PC3 (h) cocultured with hepatocytes on day 5. Data are presented as the mean ± S.D. using Student’s t test. All images shown are representative of at least three separate experiments.
low levels, and its contribution to p38 MAPK signaling is not clear. p38γ and p38δ are only expressed in specific tissues (29, 30). Most of the published literature, including our study on p38 MAPKs, refers to p38α. Consistent with our implications of p38 being involved in tumor progression, several negative regulators of p38 MAPK signaling have been found to be overexpressed in human tumors and cancer cell lines (31, 32), supporting a tumor suppressor function of p38α. However,
increased levels of phosphorylated p38α have been correlated with malignancy in various cancers (33–36); this could lead to a divergent phenotype. Unlike the unsettled literature on the role of p38 signaling, ERK1/2 MAPKs are widely accepted as the tumor promotores.

It has been shown that inhibition of AKT activity restores E-cadherin expression in KB and KOSCC-25B oral squamous cell carcinoma cells (39), which is in contrast with our findings in DU145 prostate cancer cells. We speculated that this may be due to different carcinoma cell lines controlling E-cadherin levels via diverse mechanisms. In OSCC cells the E-cadherin promoter is hypermethylated, but in the prostate lines E-cadherin levels are reduced at the protein and transcriptional levels. To confirm our speculation, other cancer cells, breast carcinoma MAD-MB-231 cells and lung adenocarcinoma H1299 cells, were treated with AKT inhibitor for 48 h, which restored E-cadherin mRNA expression levels by 2.8- and 5.2-fold, respectively (data not shown).

In our study both p38 and ERK1/2 displayed biphasic functions. First, PCA cells with high basal MAPKs levels underwent MEiT either by inhibiting ERK1/2 and p38 activities or by hepatocyte co-culture that suppressed such activities (Fig. 2). Constitutively active MEK1 or MEK6 abrogates hepatocytes-induced E-cadherin re-expression in DU145 cells. Second, MEK1/2 activity is decreased in DU145 cells cocultured with hepatocytes, which results in E-cadherin re-expression. We speculate that this may be the result of the MEK1/2-dependent inhibition of AKT activity, which is known to stimulate protein expression (40).

To further investigate the role of AKT, we used a combination of inhibitors, and showed that the re-expression of E-cadherin is dependent on AKT inhibition. We demonstrated that the expression of E-cadherin is not restored by AKT activation, which suggests that AKT is involved in the regulation of E-cadherin expression.

Finally, we examined the effect of AKT inhibition on cell proliferation and apoptosis. We found that AKT inhibition leads to a decrease in cell proliferation and an increase in apoptosis. These results support the idea that AKT inhibition is a promising strategy for the treatment of prostate cancer.
the epithelial PCa cells activate ERK1/2 and p38 secondary to E-cadherin binding. This provides resistance to chemotherapeutics and death factors (Fig. 5e). Of further interest, exposure of the PCa cells to either CPT or TRAIL increased the levels of phosphorylated ERK1/2 and p38 (Fig. 5e), suggesting a compensatory change to protect the cells. This can be modeled by invoking specific temporospatial signaling cascades for each aspect. However, the main implication for designing approaches to limit MErT is that it is critically important to carefully consider the tumor stage before attempting to modulate p38/H9251 or ERK1/2 activity for cancer therapy.

One caveat is noted to the cell survival aspect of this signaling network. Suppression of cellular proliferation and/or metabolism suppression also could provide survival advantage (8, 37). Our finding showed the similar relative cell numbers of control and E-cadherin shRNA introduced PCa cells co-culturing with hepatocytes (Fig. 4d). Although these studies do not conclusively demonstrate that the epithelial PCa cells have the same proliferation rate as the mesenchymal cells, it implies that the survival advantage arises from the intrinsic survival signals but not proliferation suppression or cell quiescence.

In summary, the data herein paint a picture of varied and stage-specific activation of select MAP kinases during tumor progression. First, high levels of ERK1/2 and p38 activity assist in establishing a mesenchymal phenotype required for dissemination from the prostate. Upon metastatic seeding, this is suppressed to allow for survival in a hostile environment, but separate signaling cascades that use these same intermediaries provide at least some of the survival signals. Last, it is possible, if not likely, that during the later reversion back to an aggressive mesenchymal phenotype, the PCa again up-regulates ERK1/2 and p38 to drive this outgrowth.

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