Extracellular Signal-regulated Kinases 1/2 Control Claudin-2 Expression in Madin-Darby Canine Kidney Strain I and II Cells*

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The tight junction of the epithelial cell determines the characteristics of paracellular permeability across epithelium. Recent work points toward the claudin family of tight junction proteins as leading candidates for the molecular components that regulate paracellular permeability properties in epithelial tissues. Madin-Darby canine kidney (MDCK) strain I and II cells are models for the study of tight junctions and based on transepithelial electrical resistance (TER) contain “tight” and “leaky” tight junctions, respectively. Overexpression studies suggest that tight junction leakiness in these two strains of MDCK cells is conferred by expression of the tight junction protein claudin-2. Extracellular signal-regulated kinase (ERK) 1/2 activation by hepatocyte growth factor treatment of MDCK strain II cells inhibited claudin-2 expression and transiently increased TER. This process was blocked by the ERK 1/2 inhibitor U0126. Transfection of constitutively active mitogen-activated protein kinase/extracellular signal-regulated kinase kitnase kinase into MDCK strain II cells also inhibited claudin-2 expression and increased TER. MDCK strain I cells have higher levels of active ERK 1/2 than do MDCK strain II cells. U0126 treatment of MDCK strain I cells decreased active ERK 1/2 levels, induced expression of claudin-2 protein, and decreased TER by ~20-fold. U0126 treatment also induced claudin-2 expression and decreased TER in a high resistance mouse cortical collecting duct cell line (94D). These data show for the first time that the ERK 1/2 signaling pathway negatively controls claudin-2 expression in mammalian renal epithelial cells and provide evidence for regulation of tight junction paracellular transport by alterations in claudin composition within tight junction complexes.

Epithelial tissues serve as selective permeability barriers, separating fluid compartments with very different chemical compositions. Specific substrates may cross the epithelial cell layers by two mechanisms, the transcellular route and the paracellular route (movement between adjacent cells). Transcellular transport is catalyzed by carrier-mediated proteins on the apical and basolateral membrane surfaces and represents a very important focus of research in epithelial cell physiology. Much less is known about the mechanisms regulating paracellular transport. The tight junctions in renal epithelium (as well as other mammalian epithelial organs) mediate this barrier role and seal cells together in a way that impedes the paracellular leakage of small molecules. Tight junctions are located at the most apical aspect of the lateral membranes of epithelial cells and function as the primary barrier to the diffusion of small molecules through the paracellular pathway (1, 2). In renal tubules, the paracellular pathway is now recognized to play an important role in vectorial transport with some selectivity for transported ions such as magnesium, calcium, sodium, and chloride (3–7).

The Madin-Darby canine kidney (MDCK)1 cell line has been widely used as an in vitro model of simple epithelium to study various issues in epithelial cell biology including the formation and regulation of tight junctions (8). Two strains of MDCK cells have been established, MDCK strain I and II cells. These two established strains of MDCK cells show functional and biochemical differences including a marked disparity in transcellular electrical resistance (TER) possibly related to their derivation from different tubular segments in the kidney (9, 10). MDCK strain I cells show much higher (10- to 100-fold) TER than MDCK strain II cells (11). MDCK strain I cells thus represent a “tight” epithelium whereas MDCK strain II cells are thought to represent a “leaky” epithelium. The differences between TER in MDCK strain I and II cells appear to be caused by claudin-2 expression with only MDCK strain II cells expressing claudin-2. Both MDCK strain I and II cells express tight junction proteins claudin-1, claudin-3, and claudin-4 as well as ZO-1 and occludin (12). Importantly, MDCK strain I cells have been converted to a leaky epithelium through the introduction of exogenous claudin-2 cDNA into MDCK strain I cells (12).

Claudin-2 appears to confer increased permeability in renal epithelial cells. In the kidney, claudin-2 expression is restricted to leaky epithelial cells in the proximal tubule and thin descending limb of Henle and is absent in the remaining distal nephron which is considered to be a tight epithelium (13–15). Exogenous claudin-2 expression in MDCK-C7 cells (a twin to MDCK strain I cells) also induces cation-selective channels in the tight junctions (16). We previously showed that hepatocyte growth factor (HGF) alters the polarity and abrogates contact inhibition of mitosis in MDCK cells (17–19). We then identified the early HGF-

1 The abbreviations used are: MDCK, Madin-Darby canine kidney; TER, transcellular electrical resistance; HGF, hepatocyte growth factor; ERK, extracellular signal-regulated kinase; MEK, mitogen-activated protein kinase/extracellular signal-regulated kinase kinase.

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induced genomic responses of polarized MDCK strain II cell monolayers using a newly available canine DNA microarray, which we described and validated (20). Of the 12,473 gene sequences on the canine microarray, the microarray identified 117 genes not previously known to be involved in the HGF pathway, at least in the context of MDCK strain II cells grown in monolayer. One of the most dramatic changes in gene expression in MDCK strain II cells following 24 h of HGF stimulation was a 23.8-fold reduction in claudin-2 gene expression (20). We also recently showed that HGF increased phospho-ERK 1/2 (active ERK 1/2) levels in MDCK strain II cells (19). We hypothesized that MDCK strain I cells might have undetectable levels of claudin-2 because of relatively higher levels of active ERK 1/2. Here we demonstrate that the ERK 1/2 signaling pathway is a negative regulator of claudin-2 expression in dog and mouse renal epithelial cells with concomitant effects on tight junction permeability and renal epithelial function.

EXPERIMENTAL PROCEDURES

Cell Culture and Transepithelial Electrical Resistance—Renal epithelial cell monolayers were grown as described previously (17−19, 21). The plasmids encoding constitutively active MEK1 (S218E/S222E) and dominant negative MEK1 (S218A/S222A) were kindly provided by Dr. Kun-Liang Guan (University of Michigan, Ann Arbor, MI) (22) and have in the past been successfully transfected into MDCK cells (23). For transient transfections of constitutively active MEK1 and dominant negative MEK1, MDCK strain II cells were plated at confluence on Transwell filters. 14 h after plating, cells were transfected with 0.5 μg of plasmid encoding constitutively active MEK1 or dominant negative MEK1 using Lipofectamine Plus according to the manufacturer’s instructions. 6 h later, the medium was replaced with fresh medium. TER was measured and cell lysates were prepared 24 h after transfection. 94D mouse cortical collecting duct cell line was developed and maintained as previously described (24). All renal cell lines were cultured on 12-mm diameter, 0.4-μm pore size filters, and electrical resistance was measured with the EVOM electrical resistance system (World Precision Instruments, New Haven, CT). All TER experiments were performed at least three times. Representative data are present under “Results.” The TER results are expressed as the measured resistance in ohms multiplied by the area of the filter (1 cm²) ± S.D.

Real-time PCR—Fifteen micrograms of total RNA collected from MDCK strain I cells grown for 4 days on Transwell filters and exposed to 1 and 3 h of 0.0128 (10 μM) were converted to first-strand cDNA. cDNA and the TaqMan primer/probe system, designed to specifically recognize claudin-2 cDNA, were used in conjunction with the 7700 PRISM Sequence Detection Instrument (both Applied Biosystems) as described in the Applied Biosystems technical manual. When the reaction product amplification exceeded the threshold value (denoted by the solid line in Fig. 6), the corresponding cycle number was termed CT (arrows). Fold change between conditions was calculated through an exponential function of the observed difference in CT as described previously (25). The values were normalized to a control mRNA, the 18S ribosome, and all real-time PCR studies were performed in triplicate.

RESULTS

ERK 1/2 Activation by Hepatocyte Growth Factor Inhibits Claudin-2 Expression in MDCK Strain II Cells—When polarized monolayers of MDCK strain II cells are treated with HGF for 24 h, claudin-2 gene expression decreases 23.8-fold and claudin-2 protein levels drop below the level of detection by Western blot (20). We examined the effect of HGF treatment on the expression of claudin-2 and other tight junction associated proteins expressed in MDCK strain II cells. MDCK II cell monolayers were exposed to HGF (100 ng/ml) for 0, 1, 3, 6, 12, and 24 h. Following HGF treatment, cell lysates were prepared using radioimmune precipitation assay buffer. Cell lysates were analyzed by Western blot analysis using specific antibodies against known tight junction proteins (occludin, ZO-1, and claudins 1−4, 7) in MDCK II cells (Fig. 1A). HGF treatment of MDCK cells dramatically decreased the levels of claudin-2 protein. The decrease in claudin-2 expression was detectable after 6 h of HGF treatment. There was also a slight decrease in claudin-1 levels after 24 h HGF treatment. Occludin, ZO-1, and claudin-3, claudin-4, and claudin-7 protein expression were not changed by 24 h HGF treatment.

Tight junction-associated proteins in control and HGF treated MDCK II monolayers were also evaluated using immunofluorescent microscopy. MDCK II monolayers were treated with fresh medium containing recombinant HGF (100 ng/ml) or fresh medium alone for 24 h and were then labeled for individual tight junction proteins (Fig. 1B). The morphology of the tight junction is altered by HGF treatment. As previously reported, HGF treatment changes cell morphology of MDCK II cells; these changes also include alterations of cell polarity and pseudostratification (17, 26). After 24 h of HGF treatment, junctional labeling of claudin-2 was qualitatively decreased. Junctional labeling of claudin-1, claudin-3, claudin-4, and claudin-7, as well as ZO-1 and occludin were still observed following HGF treatment.

We previously showed that HGF treatment of MDCK strain II cells induced ERK 1/2 phosphorylation by a pathway that is blocked by the ERK 1/2 inhibitors U0126 and PD98059 and that U0126 is a significantly more potent inhibitor of ERK 1/2 phosphorylation in MDCK II cells than PD98059 (19). The role of HGF-induced ERK 1/2 phosphorylation in inhibition of claudin-2 expression in MDCK strain II cells was tested using U0126 (10 μM). At this concentration ERK 1/2 phosphorylation was shown to be effectively inhibited (19). Western blot analysis demonstrated that U0126 inhibition of ERK 1/2 phosphorylation reversed the HGF-mediated down-regulation of claudin-2 expression in MDCK strain II cells (Fig. 1C). Occludin expression was not influenced by HGF and/or U0126 treatment (Fig. 1C).
**FIG. 1. HGF treatment of MDCK strain II cells inhibits claudin-2 expression.** A, Western blot analysis of claudins, occludin, and ZO-1 expression in lysates of MDCK cell monolayers treated with HGF (100 ng/ml) for the indicated time points. B, immunofluorescent labeling of claudins, occludin, and ZO-1 in control and 24 h-treated MDCK cell monolayers. *Space bar = 20 μm.* C, U0126 blocks HGF-induced inhibition of claudin-2 in MDCK strain II cells. MDCK II cells were exposed to medium or medium containing HGF for 24 h in the presence and absence of U0126 (10 μM). Cell lysates were prepared and analyzed for claudin-2 and occludin expression by Western blot. HGF and/or U0126 treatment did not influence occludin expression.

**U0126 Inhibits the HGF-mediated Increase in TER in MDCK II Cells**—Next we examined the functional effect of U0126 treatment on the HGF-mediated increase in TER. Recombinant HGF, in the absence and presence of 10 μM U0126, was added to MDCK strain II cells. In the presence of U0126, no HGF-mediated increase in TER was seen (Fig. 2). These data...
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**Fig. 2.** HGF treatment of MDCK strain II monolayers increases TER by a process that is blocked by U0126. MDCK II monolayers were exposed to medium or medium containing HGF (100 ng/ml) in the presence and absence of U0126 (10 μM). TER was measured at the indicated time points.

demonstrate that HGF is able to increase TER and that U0126 inhibits this process. The HGF-induced increase in TER was transient, returning to baseline after 48 h HGF treatment with continued suppression of claudin-2 expression (data not shown and Ref. 26).

To examine further whether activation of ERK leads to inhibition of claudin-2 expression and higher TER values, constitutively active MEK was expressed in MDCK strain II cells (Fig. 3). As expected, the expression of constitutively active MEK resulted in ERK activation, inhibition of claudin-2 expression, and elevated TER values. Expression of dominant negative MEK in MDCK strain II cells did not change claudin-2 expression or TER values.

Taken collectively, these data provide evidence supporting the hypothesis that ERK 1/2 activation in leaky renal epithelia inhibits claudin-2 expression in the tight junction and inhibits paracellular permeability. HGF treatment of high resistance MDCK strain I cells over a 24-h period did not alter TER (data not shown).

**MDCK Strain I Cells Express Relatively Higher Levels of Phospho-ERK 1/2 and Lower Levels of Claudin-2 Than MDCK Strain II Cells**—We compared relative amounts of claudin-2, phospho-ERK 1/2, and ERK 1/2 in confluent monolayers of polarized MDCK strain I and II cells grown on permeable supports. MDCK strain I cells possessed much higher levels of phospho-ERK 1/2 than did strain II cells and did not express claudin-2 (Fig. 4). Because MDCK strain II cells have relatively low baseline levels of phospho-ERK 1/2, high levels of claudin-2, and inhibition of claudin-2 expression following ERK phosphorylation by HGF treatment, we had hypothesized that MDCK strain I cells might have low (undetectable) levels of claudin-2 because of relatively higher levels of phospho-ERK 1/2 under basal conditions.

**U0126 Treatment of MDCK Strain I Cells Inhibits ERK Phosphorylation and Induces Rapid Expression of Endogenous Claudin-2 Protein**—To test if low levels of claudin-2 in MDCK strain I cells were dependent on relatively higher levels of active ERK 1/2, we examined the effect of U0126 treatment on phospho-ERK 1/2 and claudin-2 expression. U0126 at a concentration of 10 μM completely inhibited HGF-induced ERK 1/2 phosphorylation in MDCK strain II cells (19). Western blot analysis of MDCK strain I cells showed no claudin-2 at baseline with a dramatic increase in claudin-2 protein expression observed as early as 15 min after treatment with U0126 (10 μM) (Fig. 5A). An equally dramatic decrease in phospho-ERK 1/2 expression was seen over the same time course following U0126 treatment (Fig. 5A). Western blot also showed that U0126 reduced claudin-3 and claudin-7 to below basal levels. Immunofluorescence microscopy demonstrated that newly expressed claudin-2 co-localized with the tight junction protein ZO-1 in MDCK strain I cells treated with U0126 (120 min) (Fig. 5B). Accordingly, after 15 min of U0126 treatment, the MDCK strain I cell monolayers demonstrated a dramatic decrease in TER (Fig. 5C). With U0126 treatment periods extending to 24 h, the TER dropped to the range of 200 ohms per cm². This TER reading observed in strain I cells treated with U0126 for 24 h was nearly identical to that observed in MDCK strain II cells under basal conditions. The data are consistent with the previous report that claudin-2 is capable of making MDCK strain I cells relatively leaky (12). Treatment of MDCK strain II cells with U0126 alone did not affect claudin-2 expression or TER in the cells (Figs. 1C and 2). Table I summarizes the baseline characteristics of MDCK strain I and II cells with respect to TER, ERK 1/2 activity, and claudin-2 expression and the change in these parameters under conditions of ERK 1/2 activation by HGF and inhibition by U0126.

**De Novo Protein and RNA Synthesis Are Required for U0126-induced Claudin-2 Accumulation in MDCK Strain I Cells**—To gain more information about the mechanism for U0126-induced claudin-2 expression in MDCK strain I cells, we tested the effect of actinomycin D and cycloheximide on this process. When added to the culture medium to block new RNA or protein synthesis, respectively, actinomycin D (2.5 μg/ml) inhibits incorporation of [3H]uridine into RNA by 99%, and cycloheximide (1 μg/ml) blocks incorporation of [3H]leucine into protein by 85% over the course of 6 h in MDCK cells (27). MDCK strain I monolayers were pretreated with vehicle (control), cycloheximide (1 μg/ml), or actinomycin D (2.5 μg/ml) for 3 h prior to treatment with U0126 for 1 h. Fig. 5D illustrates that U0126 induced expression of claudin-2 and that actinomycin D completely inhibited and cycloheximide partially inhibited this process. These data suggest that both gene transcription and protein synthesis are involved in the U0126 mediated changes in claudin-2 expression in MDCK strain I cells, and rule out a purely post-translational mechanism, which we had considered given the rapidity of the increase in claudin-2 expression (i.e. within 15 min of U0126 treatment).

**Real-time PCR Targeting Claudin-2 in MDCK Strain I Cells**—To determine whether the U0126 stimulated increase in claudin-2 protein in MDCK strain I cells was occurring at the level of gene transcription, real-time PCR analysis of RNA isolated from control MDCK strain I cells and cells treated with U0126 for 1 and 3 h was performed. These time points were chosen with the intention of demonstrating increased claudin-2 mRNA levels following U0126 treatment. The RNA was converted to cDNA and amplified using the TaqMan primer/probe fluorescence assay on a 7700 PRISM instrument (Applied Biosystems). The primer and probe were designed to specifically recognize claudin-2 cDNA. Minimal or no claudin-2 mRNA could be detected in the control MDCK strain I cells. A 12-fold increase in claudin-2 mRNA expression was observed in cells treated with U0126 for 1 h compared with 3 h with U0126 (Fig. 6). These data provide evidence that U0126 stimulation of MDCK strain I cells causes a rapid increase in claudin-2 gene transcription. Our previous work using a canine microarray showed that 24-h HGF treatment of MDCK strain II cells caused a 24-fold reduction in claudin-2 mRNA (20).
For a summary of the data presented to this point please see Table I, which lists the baseline characteristics of MDCK strain I and II cells with respect to TER, ERK 1/2 activity, and claudin-2 expression, and the change in these parameters under conditions of ERK 1/2 activation by HGF and inhibition by U0126. Actinomycin D’s complete inhibition of U0126 induced claudin-2 expression in combination with the real-time PCR data provide evidence that gene transcription plays a central role in this process. 

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A High Resistance Mouse Cortical Collecting Duct Cell Line Expresses Claudin-2 in Response to U0126 Treatment—To investigate if the data reported above were specific to MDCK cells or more generalized, the effect of U0126 on a high resistance mouse renal collecting duct cell line was studied. We previously generated a cortical collecting duct cell line from orpk mutant mice (24). These cells have been rescued by transfection of the defective orpk gene and are referred to as 94D cells. These cells form polarized monolayers with TER in the 4,000 ohms per cm² range. Because of their high TER values, we predicted that the 94D cells would express little or no claudin-2 and would respond to U0126 by producing claudin-2. 94D cell monolayers were grown on permeable supports for 4 days and treated with U0126 for different time periods. Cell lysates were prepared and subjected to Western blot analysis for phospho-ERK 1/2, total ERK, and claudin-2 expression (Fig. 7A). 94D cell lysates expressed phospho-ERK 1/2, and the phospho-ERK 1/2 levels were inhibited by U0126. At baseline, claudin-2 protein was not detected in the 94D cell lysates; however, a dramatic increase in claudin-2 protein expression was detected within 15–30 min following U0126 treatment. Claudin-1 levels were not changed by U0126 treatment. The effects following U0126 treatment on TER in 94D cells were then examined. In

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the absence of U0126 treatment the 94D cells exhibited relatively high TER of approximately 4,000 ohms per cm². Following U0126 treatment the TER rapidly dropped in a time-dependent fashion (Fig. 7B). After 120 min of U0126 treatment, the 94D cells demonstrated TER values that were similar to those observed in MDCK strain II cells. These data are consistent with findings in MDCK strain I cells and provide evidence that ERK 1/2 regulation of claudin-2 in renal epithelia crosses mammalian species.

**DISCUSSION**

MDCK strain I and II cells, which express tight and leaky tight junctions, respectively, provide an excellent model system to investigate the determinants of tight junction permeability (11). Using this model system, we demonstrated that claudin-2 expression correlates with TER in MDCK cells. Stimulation of claudin-2 expression in canine MDCK strain I and mouse 94D cells decreased the TER value to that seen in MDCK strain II cells, which abundantly express claudin-2. The data presented here show for the first time that MDCK strain I cells can produce endogenous claudin-2 following inhibition of ERK 1/2 activity. Conversely, endogenous claudin-2 expression in MDCK strain II cells is inhibited by ERK 1/2 activation. Collectively, the data presented here are congruent with the results of transfection studies demonstrating that claudin-2 overexpression confers a leaky tight junction phenotype in MDCK.
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cells (12, 16). We further demonstrate for the first time that differential ERK 1/2 activity accounts for the differences in claudin-2 expression in renal epithelial cells. These data provide evidence that tight junctions are dynamic organelles that can rapidly be converted from tight to leaky and vice versa by biological signaling cascades.

The results described here are consistent with another study showing that epidermal growth factor stimulation (which also activates ERK 1/2) inhibits claudin-2 expression (28). The present data, however, are not congruent with those of Kinugasa et al. (29), who reported that claudins regulate intestinal tight junction barrier through interleukin-17 dependent regulation of the ERK pathway. These investigators provided evidence that ERK signal transduction pathway activation increased the expression of claudin-2 in T84 intestinal epithelial cells using the ERK inhibitor PD98059. The differences in ERK regulation of claudin-2 expression in the present study and that of the Kinugasa study may be caused by tissue-specific differences (renal versus intestinal).

The induction of claudin-2 expression in MDCK strain I and 94D cells following ERK 1/2 inhibition is temporally associated with dramatic changes in TER. We cannot exclude the possibility that U0126-induced decreases in claudin-3 and claudin-7 expression also influence TER in MDCK strain I cells; however, Furuse et al. (12) reported a lack of claudin-3 expression in MDCK strain I cells and found that expression of claudin-3 in the same cells did not influence TER. Li et al. (30) have recently reported that claudin-7 localizes to the basolateral surface of the aldosterone sensitive regions of the distal nephron. Another study reported that claudin-7 mRNA is found exclusively in the proximal segment of the nephron (15).

Claudin-2 appears to confer relatively increased permeability in renal epithelial cells. In the kidney, claudin-2 expression is restricted to leaky epithelium in the proximal tubule and thin descending limb of Henle and is absent in the remaining distal nephron which is considered to be a tight epithelium (13–15). A recent abstract has reported that disruption of claudin-2 gene converts the proximal tubule from a leaky to a tight epithelium (31). It is not known if claudin-2 expression in the kidney is a static or dynamic process. The findings presented here suggest that claudin-2 expression in the kidney may be a dynamic process and paracellular permeability in the different nephron segments can be modulated by biological agents that modulate the ERK 1/2 signaling pathway.

Other claudin molecules also appear to be important in paracellular transport. In addition to decreasing TER, exogenous claudin-2 expression in MDCK-C7 cells (a twin to MDCK strain I cells) also induces cation-selective channels in the tight junctions (16). Claudin-1 over-expression in MDCK cells increases TER by 4-fold and reduces the paracellular flux of dextran molecules (32). Claudin-1 deficient mice die within 1 day of birth because of transdermal water loss (33). Human claudin-16 mutations cause familial hypercalciuric hypomagnesemia. The claudin-16 mutation causes a defect in resorption of magnesium in the thick ascending limb of the renal tubule and renal magnesium wasting (4). Another study has shown that overexpression of claudin-8 in MDCK cells strengthens the paracellular barrier to mono- and divalent cations, but not anions or neutral solutes (3).

The inhibition of claudin-2 expression in MDCK strain II cells induced only a modest increase in TER. Despite the inhibition of claudin-2 expression, the TER increase in MDCK strain II cells never approached TER values seen in claudin-2 deficient MDCK strain I cells. We suspect that this modest change in TER of MDCK strain II cells with inhibition of claudin-2 expression is related to the other complex biological

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**TABLE I**

| Characteristic                  | MDCK strain I     | MDCK strain II    |
|--------------------------------|-------------------|-------------------|
| Baseline TER                   | ~4000 Ω          | ~100 Ω           |
| Base-line claudin-2 protein    | None              | Abundant          |
| Base-line ERK 1/2 activity     | High              | Low               |
| Effect of ERK 1/2 activation   | No change         | ↓↓↓               |
| on claudin-2 protein           |                   |                   |
| Effect of ERK 1/2 activation   | No change         | Transient increase |
| on TER                         |                   |                   |
| Effect of ERK 1/2 inhibition   | ↑↑↑               | No change         |
| on claudin-2 protein           |                   |                   |
| Effect of ERK 1/2 inhibition   | ↓↓↓               | No change         |
| on TER                         |                   |                   |

**Fig. 6.** Real-time PCR targeting claudin-2 in MDCK type I cells. RNA was harvested from MDCK type I cells grown on filters for 4 days and exposed to 0 h of U0126 (Control, final tracings), 1 h of U0126 (first tracings), or 3 h of U0126 (second tracings). The RNA was converted to cDNA and amplified using the TaqMan primer/probe fluorescence assay on a 7700 PRISM instrument (Applied Biosystems). The primer and probe were designed to specifically recognize claudin-2 cDNA. When the reaction product amplification exceeds the threshold value (denoted by the black line above the 0 reaction product line), the corresponding cycle number is termed C<sub>T</sub> (arrows). Fold change between conditions is calculated through an exponential function of the observed difference in C<sub>T</sub>. There was minimal or no claudin-2 mRNA detected with 0 h of U0126. The fold decrease from 1 h of U0126 treatment to 3 h of U0126 treatment was 12.1-fold. Six replicates were recorded for each condition, and all data were normalized to a control mRNA, the 18 S ribosome.
effects of HGF. These effects include inhibition of E-cadherin-mediated adhesion and initiation of cell dedifferentiation and proliferation (17–19, 21). Moreover, more specific activation of ERK 1/2 by transient transfection of dominant negative MEK1 results in a more dramatic increase in TER as well as inhibition of claudin-2 expression.

A major question resulting from this work is why are there differences in basal ERK 1/2 activities in MDCK strain I and II cells? Moreover, the present results suggest that the distal segments of the nephron (which lack claudin-2) might have increased levels of basal ERK 1/2 activity. As predicted from the present findings, a recent investigation has documented that in normal human kidney, active ERK 1/2 is largely restricted to the cytoplasm of the collecting duct (distal nephron segment) (34). These findings lead to speculation that tight junctions in vivo might be regulated in part by the ERK 1/2 signaling pathway. We have recently reported that cell contact in MDCK strain II cells inhibits ERK 1/2 (19). Perhaps the mechanism controlling this process is absent in MDCK strain I and 94D cells? We are currently investigating the regulation of ERK 1/2 activity in MDCK strain I cells.

In summary, these data demonstrate that the ERK 1/2 signaling pathway is a negative regulator of claudin-2 expression in renal epithelial cells. MDCK strain I cells have relatively high levels of active ERK 1/2, undetectable levels of claudin-2, and high TER in contrast to MDCK strain II cells which have low levels of active ERK 1/2, high levels of claudin-2, and low TER. Inhibition of ERK 1/2 by U0126 induces claudin-2 expression in MDCK strain I cells and is associated with a dramatic decrease in TER. Activation of ERK 1/2 in MDCK II cells inhibits claudin-2 expression. Importantly, this phenomenon was not limited to MDCK cells as the effect of ERK 1/2 inhibition was also observed in a high resistance mouse cortical collecting duct cell line. These results provide evidence that
tight junction permeability and paracellular transport can be rapidly changed through a cell signaling pathway that alters the composition of claudins within the tight junction complex.

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