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MEK1 is Required for Invasive Growth of mIMCD3 Renal Cells in a 3D Collagen Matrix

MEK1 – niezbędny czynnik wzrostu komórek nerkowych mIMCD3 w 3D macierzy kolagenu

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Abstract

Background. Branching morphogenesis is an essential process during kidney development. This process controls epithelialization of the metanephric mesenchyme during the induction of branching structures and in this way determines the number of nephrons. Various growth factors, such as HGF, have been implicated in branching morphogenesis in renal cells, including mIMCD3 (murine inner medullary collecting duct) and MDCK cells. Growth factors which induce branching morphogenesis in renal cells also activate the MEK1/ERK pathway.

Objectives. The authors analyzed the potential role that this pathway might play in branching morphogenesis.

Results. The authors show that PD98059, a specific inhibitor of MEK1, inhibits branching of mIMCD3 cells. Adenoviral-driven expression of the activated form of MEK1, Ad-MEK1-DD, in mIMCD3 cells, results in the induction of branching structures, whereas non-infected IMCD3 cells exhibit little or no branching. Furthermore, the authors show that Stat3 is not activated in branching mIMCD3 cells.

Conclusions. Presented data show that activation of the MEK1/ERK pathway is necessary for the induction of branching in mIMCD3 renal cells, independent of Stat3 activation (**Dent. Med. Probl. 2004, 41, 3, 403–411**).

Key words: kidney development, branching morphogenesis in renal cells, MEK1/ERK pathway.

Streszczenie

Wprowadzenie. Morfogeneza nefronów jest zasadniczym procesem podczas rozwoju nerki. Proces ten kontroluje epitelizacja mezenchymy metanerkowej podczas powstawania rozgałęzień, co determinuje liczbę nefronów. Różne czynniki wzrostu, takie jak HGF, mają wpływ na morfogenezę rozgałęzień w komórkach nerkowych, w tym mIMCD3 (mysich wewnętrznych rdzeniowych kanalików zbiorczych) i komórek MDCK. Czynniki wzrostu, które indukują powstawanie rozgałęzień w komórkach nerkowych, aktywują również drogę MEK1/ERK.

Cel pracy. Analiza oddziaływania szlaku MEK1/ERK na powstawanie rozgałęzień w komórkach nerkowych.

Wyniki. Wykazano, że PD98059 (swoisty inhibitor MEK1) hamuje rozgałęzianie się komórek mIMCD3. Wirusowa stymulacja ekspresji aktywnych form MEK1 w komórkach mIMCD3 nasilała powstawanie struktur rozgałęziających się, podczas gdy niezakażone przez wirusy komórki mIMCD3 wykazywały nieznaczne rozgałęzienia lub ich brak. Stwierdzono, że Stat3 nie jest aktywowany w rozgałęzieniach komórek mIMCD3.

Wnioski. Przeprowadzone badania dowiodły, że aktywacja szlaku MEK1/ERK jest niezbędna do zapoczątkowania rozgałęzień komórek nerkowych mIMCD3, niezależnie od aktywacji Stat3 (**Dent. Med. Probl. 2004, 41, 3, 403–411**).

Słowa kluczowe: rozwój nerki, morfogeneza rozgałęzień komórek nerkowych, szlak MEK1/ERK.

Kidney development results from the reciprocal interactions of two primordial mesodermal derivatives, the ureteric bud (UB) and metanephric mesenchyme (MM). During mouse embryonic days 10.5 to 11.5, the UB emanates from the Wolffian

duct, invades the MM and undergoes a series of dichotomous branching. Interestingly, many cells in the metanephric mesenchyme which ultimately take part in branching morphogenesis express the c-met receptor, suggesting a potential role for HGF and

other growth factors in this process. Mesenchymal cells at the tip of the growing bud begin to condense and undergo epithelial differentiation, forming spherical cysts called renal vesicles. Cells within this vesicle are patterned, as cells close to the ureteric bud express the adhesion molecule E-cadherin, and those more distal, express K-cadherin. The renal vesicles then undergo several invagination and elongation events to generate comma-shaped and eventually S-shaped bodies. S-shaped bodies are made up of precursor cells with distinguishing molecular markers suggestive of proximal-distal segmentation along the tubular epithelium. Cells adjacent to the ureteric bud, which express E-cadherin, are destined to form the distal tubule and collecting duct. Cells that are distally located, express the transcription factor, WT1, and develop into the glomerular epithelium. Between these two regions are the proximal tubule precursor cells that express K-cadherin. Mesenchymal cells that do not undergo epithelial differentiation form interstitial mesenchyme or undergo apoptosis. Ultimately, the number of nephrons is determined by the level of branching of the UB and the ability of the terminal branchpoints to induce renal vesicles, and the many branches of the UB form the collecting duct system [1–3].

The study of branching morphogenesis in *in vitro* cultures has been facilitated by the availability of renal epithelial cell lines, such as murine inner medullary collecting duct cells (mIMCD3). These cells can undergo branching tubulogenesis when cultured in threedimensional collagen gels [4–6]. Cells cultured in collagen have allowed for the study of the role of various extracellular matrix (ECM) components and growth factors in tubulogenesis. Behaviour in *in vitro* cell culture is reflective of results seen with embryonic kidney organ cultures [2].

Growth factors, such as HGF, EGF, and IGF-I, which induce branching morphogenesis in mIMCD3 and MDCK cells [4], activate the MEK/ERK pathway [7–10]. The authors examined whether activation of any of these pathways is necessary for tubulogenesis. The authors examined the effects of a specific MEK1 inhibitor, PD98059 [11–12], as well as the constitutively active form of MEK1 on branching tubulogenesis in mIMCD3 cells on the processes of branching morphogenesis.

Material and Methods

3D Collagen Gel Assay

The 3D collagen gel assay was performed as previously described by Santos et al. [4]. Briefly, collagen solution mixture was prepared, consisting

of 80% Type I rat tail collagen (Collaborative Biomedical Products), 10% 10X DMEM/F12 media (Gibco), and 10% ddH₂O, and the pH was adjusted to 7.5. Cells were resuspended at 50 000 cells/ml collagen solution, and 100 µl was added per well of a 96 well plate. Plates were placed in a 37°C incubator for 15 minutes and then 100 µl of DMEM/F12 + 10% fetal bovine serum (FBS) was added. After 8 days in collagen, mIMCD3 cells were stained with MTT (Sigma), a vital dye, at 1 mg/ml PBS for 1 hour at 37°C. Collagenase (Worthington Biomedical Corp.) (1 mg/ml PBS) was added and the stained cells sedimented to the bottom of the well. The cells were photographed and the MTT was extracted with isoamyl alcohol and read at 590 nm.

Infection of Cells with Adenovirus Expressing MEK1-DD

Adenovirus expressing MEK1-DD was prepared as described in Choukroun et al. [13]. mIMCD3 and MDCK cells were infected at a multiplicity of infection (m.o.i.) of 125 with adenovirus expressing MEK1-DD, for 2 days at 37°C in complete medium. All cells were harvested and assayed for branching tubulogenesis using 3D collagen gels in the presence of 10% or 1% complete medium.

Determining Cell Number of Cells Grown in 3D Collagen Assays

Cells were grown in 96 well plates in collagen. Wells were treated with collagenase (5 mg/ml PBS; Sigma) for 1 hour at 2, 4, and 6 days. Cell number was determined using a hemocytometer.

DAPI Staining of mIMCD3 Cells Grown in 3D Collagen Gels

4,6-diamidino-2-phenylindole (DAPI) was added to cells grown in collagen at a concentration of 2 µg/ml PBS overnight at 4°C. Staining was visualized using fluorescence microscopy.

Collagen Sandwich Technique (CST)

Collagen solution was prepared as described above. The bottom of 6-well plates or 10 cm plates were coated with collagen solution and excess

solution was removed. Plates were placed in a 37°C incubator for 15 minutes. Cells were resuspended at 50 000 cells/ml DMEM/F12 + 10% FBS and added to the precoated plates – at a volume of 3 ml/well for a 6-well plate or 10 ml/10 cm plate. The plates were placed in a 37°C incubator for 1 hour. Afterwards, another coat of collagen was added and the excess solution drained. Plates were placed in a 37°C incubator for 15 minutes, then the appropriate amount of complete medium was added and cells were incubated for 4 days. In some experiments, HGF at a concentration of 40 ng/ml was added to MDCK cells. Cells were harvested by the addition of collagenase (Sigma) at 5 mg/ml PBS for 5 minutes at 37°C. The cells were spun down at 1500 rpm in a tabletop centrifuge, the supernatant was discarded, and the pellets were washed once in PBS. Pellets were frozen in liquid nitrogen and placed at –80°C.

Immunocytochemistry

mIMCD3 cells in collagen, using CST, were washed with PBS (0.8 g NaCl, 0.02 g KCl, 0.144 g Na₂HPO₄, 0.024 g KH₂PO₄, pH 7.4) and blocked in 3% bovine serum albumin, 2% FCS, 0.01% NP40 for 1 hour at room temperature. The cells were then incubated with anti-Stat3 antibodies (diluted 1 : 100 in fresh pre-block solution) overnight at 4°C. After washing with PBS containing 0.01% NP40, anti-rabbit secondary antibodies (Cy3, 1 : 500 diluted in fresh pre-block) were added for 1 hour at room temperature. MDCK cells were grown in collagen, on the 6th day HGF (40 ng/ml) was added and on the 7th day cells were fixed and stained for total Stat3. Subsequent image analysis was conducted using Adobe Photoshop® 4.0.

Western Blot Analysis

Western blot analysis was performed as previously described, with some modifications [14]. mIMCD3 cells were lysed in potassium phosphate buffer (10 mM KPO₄, pH 7.05/ 1 mM EDTA/ 5 mM EGTA/10 mM magnesium chloride/ 50 mM β -glycerophosphate/1 mM sodium vanadate/1 mM DTT/0.5% NP 40/0.1% Brij-35). Lysates were clarified at 14000 g for 10 minutes. The protein concentration in the supernatant was determined by the Bradford assay (Bio-Rad, 5000–006). Phosphorylated Stat3 was determined by immunoprecipitating Stat3 from 100 μ g total cell lysate using anti-Stat3 antibody (1 : 100; NEB) and separating the precipitate on a 7.5% SDS-PAGE gel. Western analysis was performed using anti-phosphoStat3 (Ser727) antibodies (1 : 1000; NEB) and anti-Stat3

antibodies (1 : 1000; NEB). Membranes were then incubated with anti-rabbit-horseradish peroxidase-conjugated antibodies (Amersham) at a dilution of 1 : 2000. Membranes were subjected to electrochemiluminescence (NEN) and exposed to X-OMAT AR film (Kodak).

Results

PD98059, an Inhibitor of MEK1, Inhibits Branching in mIMCD3 Cells

Treatment of mIMCD3 cells grown in collagen gels with 50 μ M PD98059 inhibits branching morphogenesis (Fig. 1). The authors developed a technique to quantitate the relative branching volume of mIMCD3 cells when grown in Type I collagen. Figure 1 shows that inhibition of MEK1 with 50 μ M PD98059 leads to > 90% inhibition of branching tubulogenesis in mIMCD3 cells. To test if the observed inhibition was due to toxicity of the drug, the authors exposed mIMCD3 cells to 50 μ M PD98059 for 48 hours and then removed the drug. Within 7 days, the cells resume branching tubulogenesis (Fig. 2). Thus the inhibition observed with PD98059 was not due to cellular toxicity, but to inhibition of branching tubulogenesis. Cell number did not considerably change in PD98059-treated cells 7 days after plating when compared to the number of cells originally plated (data not shown). The authors have seen similar results using cortical thick ascending cells (data not shown). Therefore, the MEK/ERK pathway is essential for branching tubulogenesis of mIMCD3 cells in 3D collagen gel assays.

Constitutively Active MEK1, MEK1-DD, Induces Branching in mIMCD3 Cells

These data implicate MEK1 in branching morphogenesis in mIMCD3 cells. To corroborate the role for activated ERK, activated form of MEK1, MEK1-DD, was expressed in mIMCD3 cells. MEK1-DD contains two regulatory serines which were changed to aspartates to mimic phosphorylation making the kinase constitutively active [14–17]. Infection of cells with an adenovirus that expresses MEK1-DD (AdMEK1-DD) induced branching at a low serum concentration (1% FBS) at which mIMCD3 cells ordinarily exhibit little branching. Two days after infection, mIMCD3 cells were plated in collagen gels and incubated in

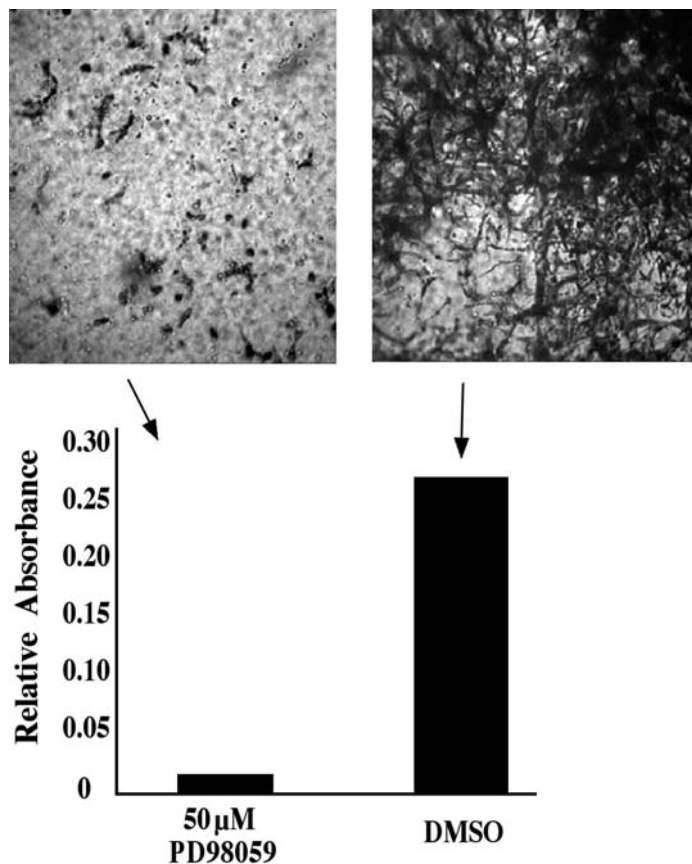


Fig. 1. Treatment of mIMCD3 cells with the MEK1 inhibitor, PD98059, results in a reduction in branching morphogenesis. mIMCD3 cells were grown in collagen Type I collagen for 8 days in the presence or absence of 50 μ M PD98059, MTT stained, harvested, and relative branching volume was assessed as described in Material and Methods section. Inhibition of MEK1 in mIMCD3 cells grown in collagen results in a reduction in branching. Values are the means \pm SD ($n = 3$) and are representative of three similar experiments

Ryc. 1. Zastosowanie PD98059 (inhibitora MEK1) na komórki mIMCD3 powoduje redukcję rozgałęzień komórek nerkowych. Komórki mIMCD3 były hodowane w kolagenie typu 1 przez 8 dni w obecności lub bez 50 μ M PD98059, barwienie MTT, zbieranie bakterii i ocenę zakresu rozgałęzień opisano w części Materiał i metody. Hamowanie MEK1 w komórkach mIMCD3 hodowanych w kolagenie powodowało redukcję w rozgałęzianiu. Wartościami są średnie \pm odchylenie standardowe z trzech podobnych eksperymentów

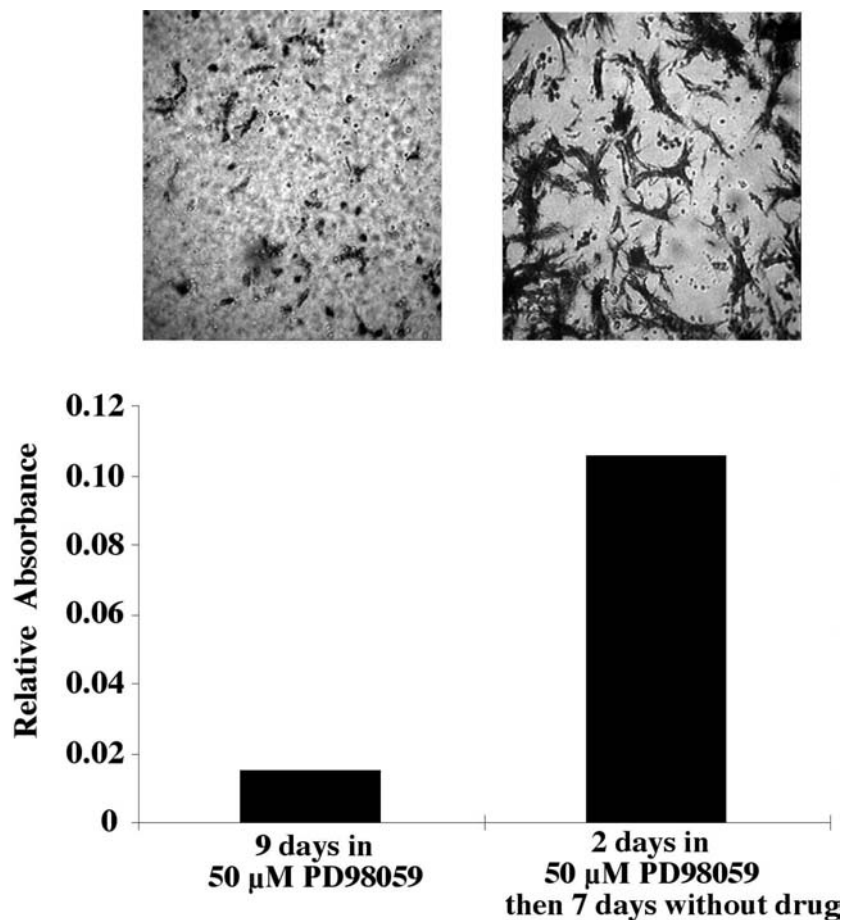


Fig. 2. Inhibition of branching morphogenesis in mIMCD3 cells by PD98059 is not due to toxicity by the drug. mIMCD3 cells were treated with 50 μ M PD98059 and after 2 days, PD98059 was removed from some wells. Removal of the MEK1 inhibitor resulted in the reinstatement of branching in mIMCD3 cells. Cells that were treated for 9 days with PD98059 did not exhibit any branching but still stained with MTT and therefore were still viable. Values are the means \pm SD ($n = 3$) and are representative of three similar experiments

Ryc. 2. Hamowanie powstawania rozgałęzień w komórkach mIMCD3 przez PD98059 nie było spowodowane toksycznością środka. Komórki te inkubowano z 50 μ M PD98059 i 2 dni później środek był usuwany. Usuwanie inhibitora MEK1 powodowało ponowne rozgałęzianie się komórek mIMCD3. Komórki, które były inkubowane przez 9 dni z PD98059 nie ukazywały rozgałęzień, lecz ciągle barwiły się MTT, co potwierdzało ich żywotność. Wartościami są średnie \pm odchylenie standardowe z 3 podobnych eksperymentów

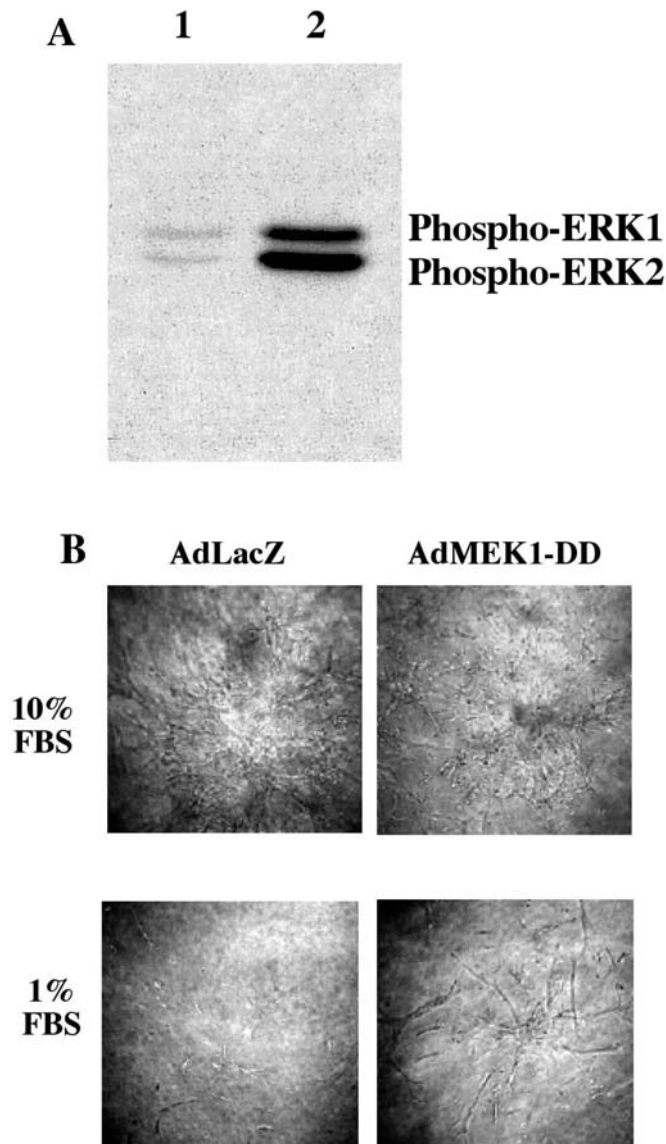


Fig. 3. Expression of constitutively active MEK1, MEK1-DD, results in increased multinucleated, branching structures. mIMCD3 cells were infected with adenovirus expressing MEK1-DD (Ad-MEK1-DD) or adenovirus expressing LacZ (Ad-LacZ). **A** – Western blot analysis was performed on lysates (30 μ g per lane) from mIMCD3 infected with Ad-LacZ (lane 1) and with Ad-MEK1-DD (lane 2). Phospho-specific ERK1/2 antibodies (New England Biolabs) were used in immunoblotting. Results are representative of four independent experiments. Infection with MEK1-DD results in an increase in the phosphorylation of endogenous ERK1/2.

B – mIMCD3 cells infected with Ad-LacZ and with Ad-MEK1-DD were plated in Type I collagen and grown in 10% or 1% FBS. Wells containing Ad-MEK1-DD-infected cells grown in 1% serum showed a greater number of long, branch-like structures compared to wells that contained Ad-LacZ-infected cells

Ryc. 3. Ekspresja MEK1, MEK1-DD powodowała nasilanie powstawania wielojądrowych struktur rozgałęzionych. Komórki mIMCD3 były zakażane adenowirusem, wywołującym ekspresję MEK1-DD (AdMEK1-DD) lub LacZ (Ad-LacZ). **A** – Analizę immunoblotingu przeprowadzono na produktach rozpadu komórek mIMCD3 zakażonych Ad-LacZ (prążek 1) i AdMEK1-DD (prążek 2). W badaniu zastosowano swoiste przeciwciała dla ERK1/2. Wyniki były reprezentatywne w 4 niezależnych eksperymentach. Zakażenie MEK1-DD powodowało wzrost fosforylacji endogennego ERK1/2.

B – Komórki mIMCD3 zakażone Ad-LacZ i AdMEK1-DD były hodowane w kolagenie typu I w 10% lub 1% FBS. Dołki zwiększające komórki Ad-MEK1-DD hodowane w 1% surowicy miały większą liczbę długich, rozgałęzionych struktur w porównaniu z dołkami zawierającymi komórki Ad-LacZ

the presence of 1% and 10% FBS. The state of ERK1/2 phosphorylation was analyzed in the remaining cells. Figure 3a shows that cells infected with AdMEK1-DD showed an increase in ERK1/2 phosphorylation compared to AdLacZ-infected cells. AdMEK1-DD infected mIMCD3 cells plated in collagen gels, they show an increase in branching at low serum concentrations compared to AdLacZ-infected cells grown in similar serum concentrations as visualized by light microscopy (Fig. 3b). The increase in branching seen in MEK1-DD-infected cells grown in 1% serum correlates with a 2.5-fold increase in cell number (Fig. 4). DAPI staining reveals an increase in longer, multinucleated branching structures in MEK1-DD-infected cells (Fig. 5), with an average number of labelled nuclei/tubule of 21.3 ± 3.7 ($n = 6$) versus 9.3 ± 0.8 ($n = 6$) for AdLacZ-infected control cells.

Stat3 is not Required in Branching mIMCD3 Cells

Boccaccio et al. have suggested that HGF induces MDCK to undergo branching tubulogenesis by activating the STAT pathway MEK1 [18]. The STAT proteins are transcriptional factors that were originally shown to be activated by phosphorylation by cytoplasmic Jak kinases in response to cytokines and interferons [19, 20]. When phosphorylated, these factors dimerize and translocate into the nucleus, where they bind to specific promoter sequences (SIE; *sis* inducible element), leading to transcriptional activation of specific genes MEK1 [18]. It is now known that receptor tyrosine kinases, such as HGF and EPO receptors, can also phosphorylate and activate STATs [18, 19]. Since it has been shown that activation of Stat 3, an ERK1/2 substrate [21], results in the induction

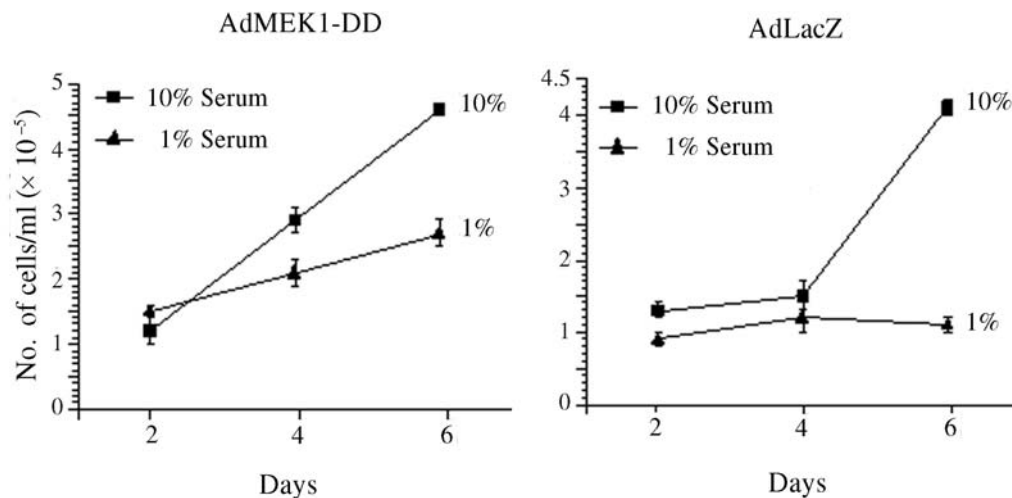


Fig. 4. mIMCD3 cells infected with MEK1-DD exhibit an increase in proliferation. Cells were grown in collagen in 96 well plates and cell number was determined at 2, 4, and 6 days using a hemocytometer. Values are the means \pm SD ($n = 3$) and are representative of three similar experiments. Cells grown in the presence of 10% FBS (■) and 1% FBS (▲). No PD98059 was added throughout the experiment

Ryc. 4. Komórki mIMCD3 zakażone MEK1-DD wykazywały wzrost proliferacji. Hodowano je w kolagenie w 96 dołkowych płytkach, a ich liczba była określana 2., 4. i 6. dnia za pomocą hemocytometru. Wartościami są średnie \pm odchylenie standardowe z 3 podobnych eksperymentów. Komórki hodowano w obecności 10% FBS (■) i 1% FBS (▲)

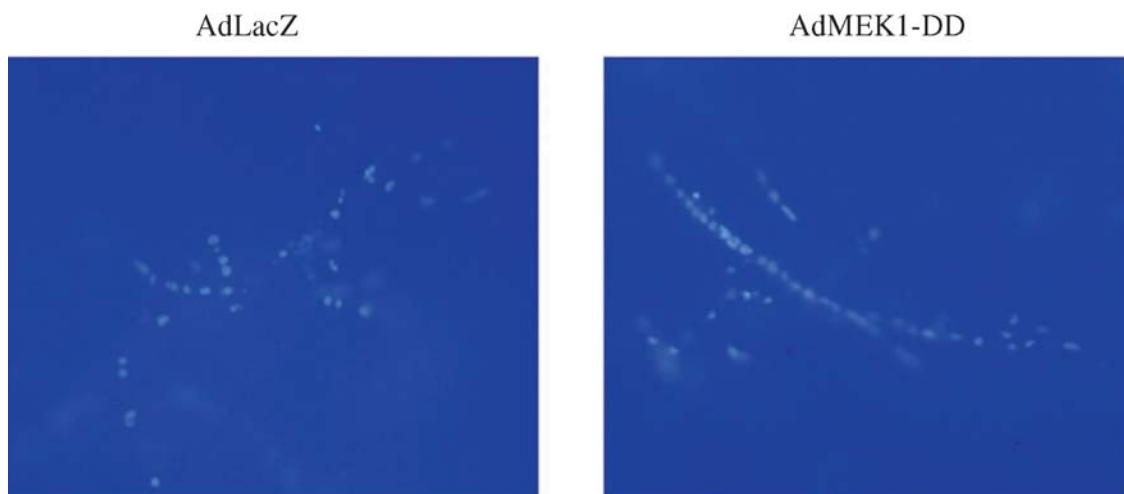


Fig. 5. mIMCD3 cells infected with MEK1-DD exhibit an induction of multinucleated, branching structures. DAPI (2 μ g/ml PBS) was added to cells grown in collagen for 8 days in 1% FBS. Staining was visualized using fluorescence microscopy

Ryc. 5. Komórki mIMCD3 zakażone MEK1-DD wykazują indukcję wielojądrowych struktur rozgałęzionych. DAPI (2 μ g/ml PBS) dodano do hodowli komórek w kolagenie przez 8 dni w 1% FBS. Barwienie było wykazane przy użyciu mikroskopu fluorescencyjnego

of epithelial tubules in MDCK cells, it is possible that Stat3 may play a similar role in branching morphogenesis in mIMCD3 cells.

In order to discern the phosphorylation status of Stat3 in IMCD3 cells, the authors grew IMCD3 cells using a modified collagen gel assay, the collagen sandwich technique or CST (see Methods for details). Cells were grown for one week using CST and then the authors treated gels with collagenase, as described in Methods. Western blot analysis shows that IMCD3 cells grown in colla-

gen gels do not exhibit Stat3 phosphorylation on Ser727 (Fig. 6) when compared to cells grown on 2 dimensions. Immunofluorescence analysis of branching mIMCD3 with anti-Stat3 antibodies revealed staining mainly in the cytosol (Fig. 7). These data suggest that Stat3 does not play a role in branching mIMCD3 cells. The authors used MDCK cells treated with HGF (40 ng/ml) as a control for Stat3 translocation into the nucleus (Fig. 7). While HGF-induced branching of MDCK cells was dependent on Stat3 activation, Boccaccio

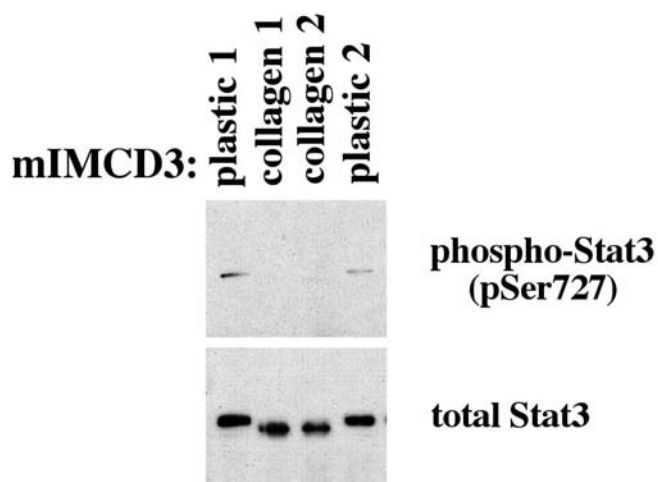


Fig. 6. Decreased Stat3 phosphorylation in mIMCD3 cells grown in collagen. Anti-Stat3 antibodies were used to immunoprecipitate Stat3 from lysate from mIMCD3 cells grown on plastic and in collagen. Western blot analysis was performed using phospho-Stat3 (Ser 727). The blot was stripped and reprobed with antibodies to total Stat3

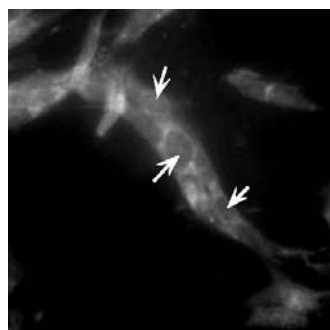
Ryc. 6. Spadek fosforylacji Stat3 w komórkach mIMCD3 hodowanych w kolagenie. Zastosowano przeciwciała Stat3 w celu immunoprecypitacji Stat3 z produktów rozpadu komórek mIMCD3 hodowanych na plastiku i w kolagenie. Analizę immunoblotingu przeprowadzono, używając fosfo-Stat3/Ser727/

et al. did not observe similar activation in EGF-induced branching in MDCK cells MEK1 [18]. This observation and presented results suggest that branching in mIMCD3 cells occurs via a Stat3-independent pathway.

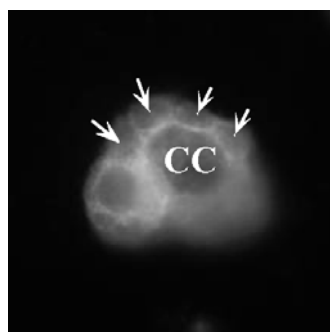
Discussion

Presented data show that the MEK1/ERK pathway is essential for branching observed in IMCD3 cells grown in Type I collagen gels. Treatment of mIMCD3 cells with PD98059, a MEK1 inhibitor, resulted in a reduction in branching morphogenesis. This effect is not due to toxicity resulting from treatment with the drug. Activation of ERK by infection of cells with MEK1-DD induced branching within 2 days. Induction of the MEK1/ERK pathway in mIMCD3 cells resulted in an increase in mitogenesis, which paralleled an increase in multinucleated, branching cords.

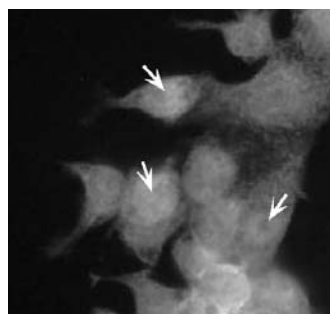
The downstream effectors of MEK1 activation important for branching are yet unknown. Similarly, the authors did not address whether MEK1 activation alone is sufficient for tubulogenesis, a process that follows branching in HGF-treated MDCK cells. Bocaccio et al. [18] have proposed that HGF-induced tubulogenesis is depen-



mIMCD3 cells grown in collagen for 5 days



MDCK cells grown in collagen for 5 days



MDCK cells grown in collagen for 5 days and treated with 40 ng/ml HGF for 24 hours

Fig. 7. Stat3 does not translocate into the nucleus of branching mIMCD3 grown in collagen.

Immunofluorescence was performed using antibodies to total Stat3 on branching mIMCD3 cells grown in collagen. MDCK cells in the absence or presence of 40 ng/ml HGF were used as a positive control for translocation. Stat3 stays primarily in the cytosol on branching mIMCD3 cells. White arrows identify nuclei (not all nuclei are marked); CC refers to cystic cavity

Ryc. 7. Stat3 nie przemieścił się do jądra rozgałęziających się komórek mIMCD3 hodowanych w kolagenie. Immunofluorescencję wykonano z użyciem przeciwciał dla całkowitego Stat3 na rozgałęziających się komórkach mIMCD3 hodowanych w kolagenie. Komórki MDCK były użyte jako kontrola (w celu translokacji w obecności lub bez 40 ng/ml hepatocytowego czynnika wzrostu (HGF)). Stat3 wykazywano pierwotnie w cytozolu rozgałęziających się komórek mIMCD3. Białe strzałki wskazują jądra (nie wszystkie jądra zaznaczono); CC oznacza aktywność torbielowatą

dent on STAT pathway activation, and that this process might occur independently of MEK1. Bocaccio et al. [18] suggest that HGF-induced branching occurs through sequential steps, including the activation of scatter via a PI3-kinase dependent manner, followed by an increase in pro-

liferation and branching via MEK1/ERK. Whether these steps are sequential or parallel events remains to be determined. Presented data support the hypothesis that these pathways are parallel since infection of MDCK cells with Ad-MEK1-DD results in increased branching, independent of the addition of HGF and PI3-kinase activation (paper in preparation). It is conceivable that a major role of MEK1 activation is to direct branching cellular outgrowths which extend through the extracellular matrix, and are subsequently stimulated to form tubules. The authors have shown that branching of mIMCD3 cells appears independent of Stat3 activation, suggesting at least two distinct pathways exist for branching morphogenesis in renal cells.

In conclusion, the authors have shown that MEK1 is necessary and sufficient for the induction of branching in mIMCD3. These findings suggest a critical role of the MEK1/ERK pathway in nephrogenesis, which involves the conversion of cystic structures into branching tubules. Further, these data have broad implications in many renal pathologies in which cyst generation plays a central role, such as in polycystic kidney disease and medullary sponge kidney disease. These findings which implicate the MEK/ERK pathway, if validated using *in vivo* disease models, also suggest important gene targets in the treatment and prevention of cystic kidney diseases.

Footnotes. MEK, MAP kinase/ERK kinase; ERK – Extracellular signal-regulated kinase; PI 3-kinase, phosphatidylinositol kinase; mIMCD3 – murine inner medullary collecting duct cells; MDCK – Madin-Darby canine kidney cells; HGF – hepatocyte growth factor.

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