[Cell Cycle 6:6, 638-642, 15 March 2007]; ©2007 Landes Bioscience

Extra View

Reactivation of *Snail* Genes in Renal Fibrosis and Carcinomas

A Process of Reversed Embryogenesis?

Agnès Boutet[1]
Miguel A. Esteban[2]
Patrick H. Maxwell[2]
M. Angela Nieto[1,*]

[1]Instituto de Neurociencias de Alicante; CSIC-UMH; Sant Joan d'Alacant, Spain

[2]Imperial College London; Hammersmith Campus; London, UK

*Correspondence to: M. Angela Nieto; Instituto de Neurociencias de Alicante; CSIC-UMH; Apartado 18; Sant Joan d'Alacant 03550 Spain; Tel.: +34.96.591.92.43; Fax: +34.96.591.95.61; Email: anieto@umh.es

Original manuscript submitted: 02/08/07
Revised manuscript submitted: 02/15/07
Manuscript accepted:02/19/07

Previouisly published online as a *Cell Cycle* E-publication:
http://www.landesbioscience.com/journals/cc/abstract.php?id=4022

KEY WORDS

Snail, renal fibrosis, renal cell carcinoma, epithelial-mesenchymal transitions, tumor progression, epithelial homeostasis

ABSTRACT

While the activity of *Snail* genes is required during embryonic development for the formation of different tissues and organs, they must be repressed in the adult in order to maintain epithelial integrity and homeostasis. Indeed, pathological activation of Snail in epithelial tumors induces malignancy and the recurrence of tumors. Here we show that in dedifferentiated areas of human renal carcinomas, *Snail* undergoes a process of reactivation. In addition to tumor progression, renal fibrosis is also linked to the activity of *Snail* genes and indeed, reactivation of Snail in the adult kidney is sufficient to induce fibrosis. Thus, *Snail* genes illustrate a paradigm whereby reactivation of crucial embryonic genes in adult tissues provokes the onset of devastating diseases.

INTRODUCTION

Developmental plasticity can often be regarded as the counterpoint of adult homeostasis. Indeed, during embryonic development, numerous phenotypic transitions take place to ensure the correct formation of different tissues and organs. In particular, epithelial to mesenchymal transitions (EMTs) are crucial for the development of many tissues, including the mesoderm and the neural crest.[1] *Snail* genes are fundamental for the induction of the different EMT events that occur during embryonic development.[2] Indeed, Snail induces the conversion to mesenchyme by regulating a plethora of downstream genes, repressing epithelial markers, activating mesenchymal markers, and producing the reorganization of molecules involved in cell shape changes and in promoting cell motility and invasion (reviewed in ref. 2). Interestingly, the majority of the mesenchymal cells produced during early embryonic development subsequently undergo the reverse process (MET) to generate terminally differentiated adult epithelia. This is certainly the case in the kidney, which develops after a series of METs accompanied by the inactivation of *Snail* genes as described recently.[3] Epithelial homeostasis can be disrupted by reinitiating EMT in the adult leading to the development of epithelial tumors and organ fibrosis.[4,5] Since reactivation of *Snail* genes is sufficient to induce tumor progression and fibrosis[2,3,6] (Fig. 1), inhibiting Snail expression may be a promising strategy to prevent or reverse these pathological conditions commonly associated with ageing and/or degenerative diseases.

REPRESSION OF *SNAIL* GENES DURING RENAL ONTOGENESIS

Two independent mesodermal populations are involved in the formation of the mammalian kidney: the intermediate mesoderm and the metanephric mesenchyme. Renal tubular epithelial cells and collecting duct cells differentiate from these mesodermal populations through MET.[7] Our analysis of *Snail* gene expression during kidney ontogenesis indicated that *Snail1* and *Snail2 a*re present in both these mesodermal populations and that they are down-regulated just prior to epithelial differentiation.[3] Differentiation occurs upon the up-regulation of HNF-1β, which in turn activates the kidney-specific adhesion molecule, Cadherin-16 (ksp-cadherin). Indeed, both Snail proteins are direct repressors of *HNF-1*β transcription and thus, they indirectly prevent the expression of Cadherin-16[3] and renal epithelial differentiation.

Snail1 and *Snail2* arose from the duplication of an ancestral *Snail* gene[8] and as shown both in vitro and in vivo, they may be functionally equivalent.[9,10] Accordingly, the overlapping expression of the two family members in the nephrogenic mesenchyme[3] may explain why mice carrying a loss-of-function mutation of *Snail2* do not display significant defects in kidney development.[11,12] In these mice Snail1 is probably sufficient for the

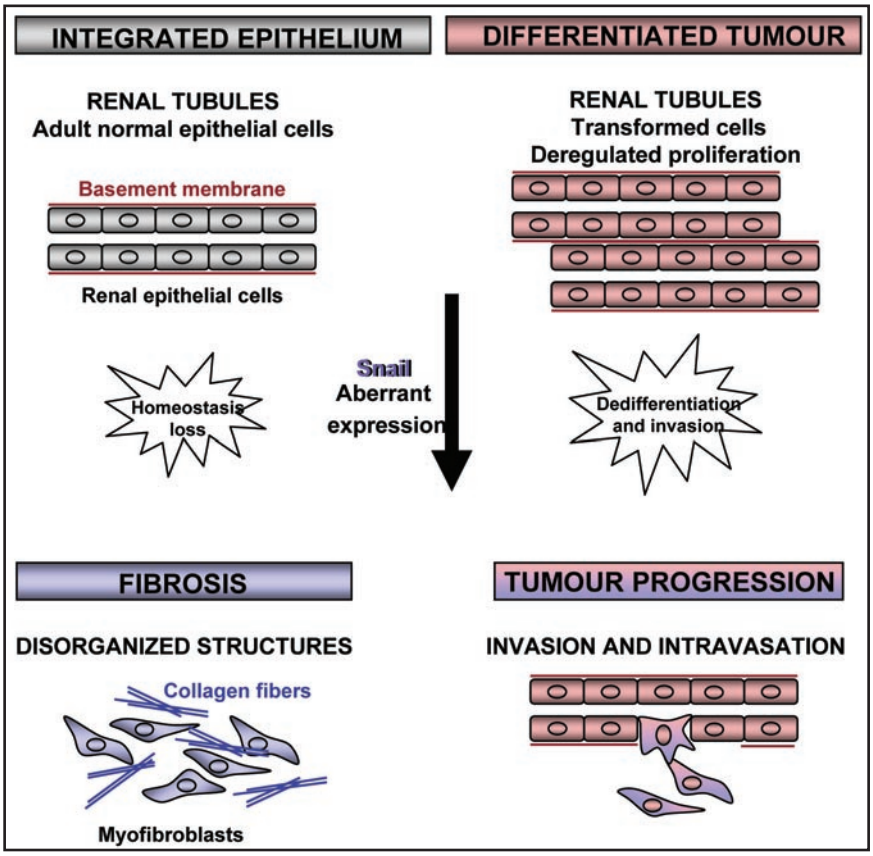

Figure 1. Differential responses of adult epithelial cells to Snail activation. While *Snail* genes are silenced in normal adult tissues, their activation (shown in blue) induces fibrosis in normal epithelial cells (grey) and tumor progression in transformed carcinoma cells (pink).

correct spatio-temporal localization of HNF-1β and Cadherin-16 during the differentiation of renal epithelia.

Given that *Snail* genes are downregulated during the MET involved in renal development, it is tempting to speculate that inducers of MET repress Snail. In *Xenopus laevis*, antisense morpholino injection or pharmacological inactivation of FGF8 prevents the pronephric mesenchymal primordium from undergoing the MET required to form a polarized pronephric tubule.[13] This is consistent with the severe renal hypoplasia and deficiencies in nephron formation induced by the conditional inactivation of FGF8 in the mouse.[14] In addition, BMP7 also plays an important role in the modulation of MET.[15,16] BMP7 is secreted by the bud tip cells and the condensed mesenchyme[17] but not by the metanephric undifferentiated mesenchyme where *Snail* genes are specifically expressed. This suggests that BMP7 may participate in the downregulation of *Snail* genes concomitant with renal mesenchyme differentiation. On the basis of these data, it will be interesting to analyze whether FGF8 and BMP7 might function as Snail repressors during kidney morphogenesis.

INVOLVEMENT OF *SNAIL* GENES IN HUMAN RENAL CARCINOMA

EMT is recognized as a crucial event during tumor progression and it has been correlated with aberrant *Snail* expression in carcinomas of different origins (reviewed in ref. 2). Snail activation correlates with the loss of E-Cadherin, which is associated with poor prognosis.[18] Since this issue has not been addressed in renal cancer, we wondered whether pathological activation of Snail might also occur in renal carcinomas. Hence, we analyzed the expression of Snail in biopsies from patients with kidney tumors that also contained normal adjacent tissue. As expected,[3] no *Snail1* or *Snail2* expression was observed in the normal adult kidney tissue, however, activation of *Snail2* (Fig. 2) but not *Snail1* (not shown) was detected within the tumor. *Snail2* expression was observed in de-differentiated areas that had lost *E-Cadherin* and *Cadherin-16* expression (Fig. 2). The loss of both these Cadherins was concordant with our recent finding that Snail not only represses E-cadherin expression but also that of Cadherin-16, albeit through different mechanisms.[3] It is noteworthy that *Snail2* rather than *Snail1* is activated in the tumor. However, this may not be surprising if we take into account the ontogeny of this renal tissue in which *Snail2* is more prominently expressed than *Snail1*, and that both genes may be functionally equivalent. Thus, as in breast tumors,[19,20] Snail is likely to influence the progress and potential for recurrence of renal carcinoma and the reactivation of its expression probably accounts for the loss of E-Cadherin and Cadherin-16 during tumor evolution.[21-24]

We observed large areas in the tumor where *E-Cadherin* and *Cadherin-16* had been lost. To gain further insight into the changes that occur during tumor development, we analyzed the expression of these two Cadherins in the normal tissue adjacent to the tumor. To identify nephron segments, we combined our analysis with PNA and LTA lectin staining as markers of the distal and proximal renal tubules, respectively.[25,26] As described previously, the collecting ducts and the distal tubules (PNA labeled) expressed both *E-Cadherin* and *Cadherin-16*,[24,27] whereas *E-Cadherin* was absent from the proximal renal tubules.[28] While Cadherin-16 expression has previously only been observed in distal tubules in humans,[24] we also detected *Cadherin-16* expression in both human and mouse proximal tubules (Fig. 3). This data is consistent with that obtained in the rabbit[29] and from mice expressing a *Cadherin-16* reporter gene.[30]

Since E-cadherin is not expressed in the proximal tubules, the repression of E-Cadherin in undifferentiated areas of the tumor by Snail is consistent with the proposal that E-cadherin-expressing distal tubules[28] may also participate in clear cell renal carcinomas, as well as the proximal tubules.[31] Furthermore, since proximal and distal tubules express Cadherin-16 and Snail downregulates *Cadherin-16* expression,[3] Snail reactivation would induce the loss of cadherin and EMT in the proximal tubules. Thus, aberrant Snail expression may play a significant role in the loss of epithelial characteristics and the dedifferentiation associated with renal carcinoma progression.

INHIBITING SNAIL1 ACTIVATION IN THE ADULT KIDNEY: A NEW STRATEGY TO TREAT RENAL FIBROSIS?

Fibrosis is instrumental in the progressive loss of kidney function that occurs in many conditions including glomerulonephritis, diabetes, urinary tract obstruction and chronic rejection of transplanted kidneys. The progression of chronic kidney disease (CKD) is irreversible and currently, the only treatment available is dialysis or

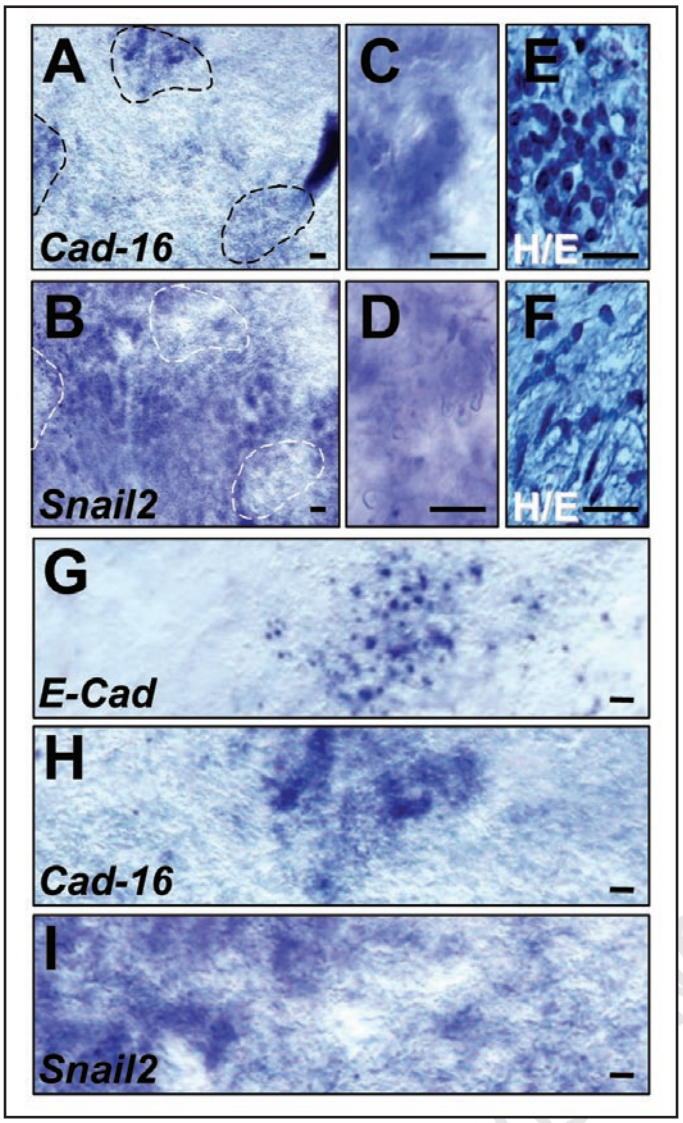

Figure 2. *Snail2* is pathologically activated in the de-differentiated areas of human renal carcinoma. Adjacent sections from human renal cell carcinoma were hybridized for *Snail2*, *E-Cadherin* or *Cadherin-16*. (A and B) *Snail2* expression is induced in areas lacking *Cadherin-16*, these genes showing an overall complementary pattern. (C and D) High power views of *Cadherin-16* and *Snail2* expression and histological (hematoxylin and eosin) staining in adjacent sections (E and F). Note the de-differentiated phenotype of *Snail2*-expressing cells. (G, H and I) Another area of the tumor showing how *Snail2* expression and the loss of both *E-Cadherin* and *Cadherin-16* expression coincide. Renal tumors were obtained during nephrectomy and approx 1 cm^3 specimens were snap frozen in liquid nitrogen-cooled isopentane. Approval to analyze the tissue was obtained from the Research Ethics Committee of Guy's and St Thomas's Hospitals. The specimens were processed for ISH and histology as described.[3] Scale bars indicate 25μm.

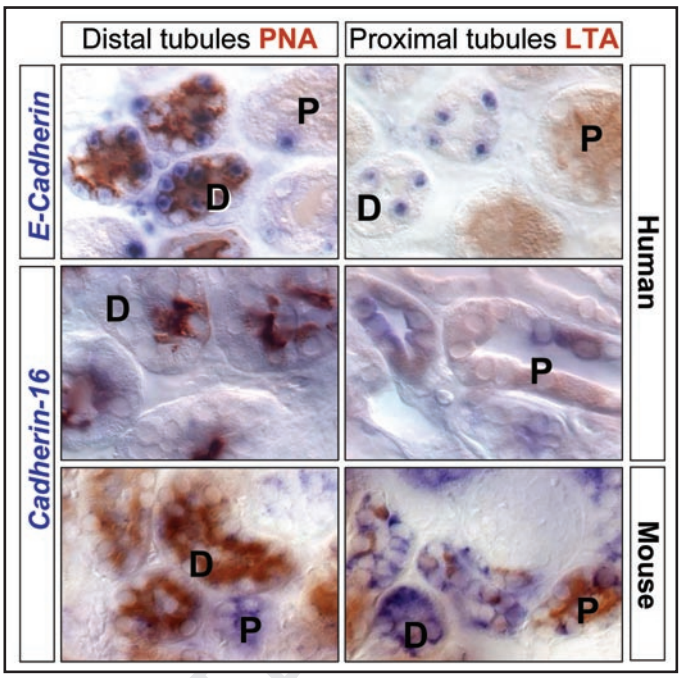

Figure 3. Cadherin expression in the adult human and mouse kidney. Kidneys dissected from paraformaldehyde-perfused mice or obtained from patients at nephrectomy were hybridized with *E-Cadherin* or *Cadherin-16* probes as previously described,[3] (both are shown in blue). Subsequently, the sections were subjected to immunohistochemistry for PNA and LTA lectins as markers of distal and proximal tubules, respectively (brown). *E-Cadherin* is specifically expressed in the distal tubule while *Cadherin-16* expression is readily apparent in both the distal and proximal renal tubules. D, distal; P, proximal.

transplantation. Fibrotic lesions are initiated by the accumulation of fibroblasts which in turn deposit excess extracellular matrix molecules, particularly collagen fibers. This fibrotic tissue is comprised of both activated resident fibroblasts and epithelial tubular cells that have undergone EMT.[32,33] We have recently shown that activation of Snail1 in transgenic mice results in large scale EMT in the kidney, reproducing all the features of renal fibrosis. Moreover, we demonstrated that fibrosis in the human kidney is accompanied by the aberrant activation of Snail.[3] In the mouse, Snail not only provoked the loss of epithelial characteristics in kidney tubular and collecting duct cells, but it also induced the expression of vimentin, smooth muscle actin and the deposition of collagen fibers (Fig. 4). Strikingly, cysts were readily observed in these fibrotic kidneys. Given that Snail directly represses *HNF-1*β[3] (Fig. 4), our findings are compatible with the development of cystic renal disease in mice and humans with defects in HNF-1β.[34,35]

It is worth mentioning that Snail may also play an important role in other fibrotic diseases (see discussion in ref. 3). Although a causal role for Snail has not yet been demonstrated in mesothelial fibrosis, it is interesting that Snail is activated during the EMT observed in mesothelial cells of patients subjected to peritoneal dialysis.[36]

Elevated levels of TGF-β are commonly seen in patients with renal fibrosis.[37] Indeed, evidence from mouse models suggests that an increase in TGF-β participates in the renal fibrosis that is induced after unilateral ureteral obstruction (UUO). Accordingly, mice lacking Smad3, a signaling molecule downstream of TGF-β receptors, are protected against the induction of renal fibrosis.[38] Moreover, systemic injection of BMP7, an endogenous TGF-β antagonist, can revert the fibrotic lesions developed after UUO[39] providing a very promising strategy to treat renal fibrosis. Finally, a synthetic vitamin D analogue (paricalcitol), also attenuates UUO-induced renal fibrosis by suppressing the expression of TGF-β and of its type I receptor[40] (Fig. 4). TGF-β is a well known and potent inducer of Snail[2] and interestingly, the expression of *Snail* usually observed in UUO-induced renal fibrosis is absent in Smad3 null mice.[38] Furthermore, paricalcitol inhibits the TGF-β-mediated induction of Snail in vitro[40] and it also increases the levels of the vitamin

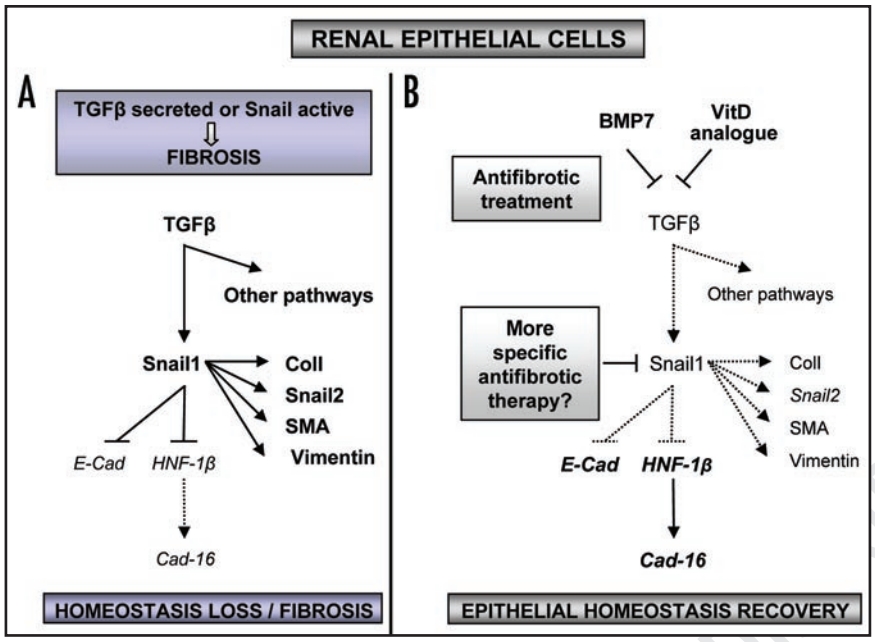

Figure 4. A: TGF-β secretion or Snail activation induces fibrosis in the adult kidney. B: Inhibition of TGF-β signaling by BMP7[39] or vitamin D analogues (paricalcitol)[40] are promising strategies to attenuate renal fibrosis. Since Snail induces renal fibrosis[3] and seems to transduce the deleterious effects of TGF-β, direct inhibition of Snail activation may provide a specific way to prevent or treat such diseases. The active pathways and targets are shown in bold.

D receptor (VDR),[40] the expression of which Snail is known to repress.[41] Thus, both BMP7 and paricalcitol may indirectly inhibit Snail expression by abrogating TGF-β signaling and in conjunction, the available data suggest that it is Snail that transduces the deleterious effects of TGF-β during renal fibrosis. Taking into account that TGF-β triggers complex signaling cascades, some of which may be beneficial, inhibiting Snail may prove to be a more specific way to treat renal disease than inhibiting the TGF-β pathways (Fig. 4).

SNAIL IN ADULT RENAL PATHOLOGIES: THE REACTIVATION OF DEVELOPMENTAL PROGRAMMES UNVEILS A CERTAIN DEGREE OF CELLULAR PLASTICITY

Besides their involvement in renal fibrosis and the progression of carcinomas, Snail reactivation in renal disease may have more extensive consequences as it was recently shown to repress nephrin expression.[42] Nephrin is another cell adhesion molecule, which is critical for maintaining the integrity of podocytes and preventing the loss of plasma proteins by glomerular filtration. Hence, it is tempting to speculate that Snail activation occurs in nephrotic syndromes.

Regardless of the disease, the reactivation of Snail in adult tissues can be regarded as a return to the embryonic phenotype since Snail appears to fulfill the same function in the adult as in the embryo: conferring epithelial cells with the ability to migrate and invade adjacent tissues. This activity is essential during embryonic development as it allows the precursors of different tissues to migrate to their final destinations.[2] However, during tumor progression Snail expression enables malignant cells to colonize distant territories and form metastases.[2] This is achieved by the aberrant activation of normal Snail proteins, rather than through the appearance of mutations in the *Snail* coding sequence. In renal fibrosis, the Snail-induced conversion of terminally differentiated tubular epithelial cells into "activated fibroblasts" that can migrate into the interstitial space and deposit extracellular matrix unveils a degree of cellular plasticity in the adult kidney. This unforeseen plasticity may provide a therapeutic opportunity to restore the normal epithelial phenotype by inactivating Snail.

References

1. Hay ED. The mesenchymal cell, its role in the embryo, and the remarkable signaling mechanisms that create it. Dev Dyn 2005; 233:706-20.
2. Barrallo-Gimeno A, Nieto MA. The *Snail* genes as inducers of cell movement and survival: Implications in development and cancer. Development 2005; 132:3151-61.
3. Boutet A, De Frutos CA, Maxwell PH, Mayol MJ, Romero J, Nieto MA. Snail activation disrupts tissue homeostasis and induces fibrosis in the adult kidney. EMBO J 2006; 25:5603-13.
4. Thiery JP, Sleeman JP. Complex networks orchestrate epithelial-mesenchymal transitions. Nat Rev Mol Cell Biol 2006; 7:131-42.
5. Liu Y. Epithelial to mesenchymal transition in renal fibrogenesis: Pathological significance, molecular mechanism, and therapeutic intervention. J Am Soc Nephrol 2004; 15:1-12.
6. Nieto MA. The Snail superfamily of zinc-finger transcription factors. Nat Rev Mol Cell Biol 2002; 3:155-66.
7. Dressler GR. Development of the excretory system: In mouse development. In: Rossant J, Tam PPL, eds. Academic Press, 2002.
8. Manzanares M, Locascio A, Nieto MA. The increasing complexity of the *Snail* gene superfamily in metazoan evolution. Trends Genet 2001; 17:178-81.
9. Bolos V, Peinado H, Perez-Moreno MA, Fraga MF, Esteller M, Cano A. The transcription factor Slug represses E-cadherin expression and induces epithelial to mesenchymal transitions: A comparison with Snail and E47 repressors. J Cell Sci 2003; 116:499-511.
10. del Barrio MG, Nieto MA. Overexpression of Snail family members highlights their ability to promote chick neural crest formation. Development 2002; 129:1583-93.
11. Jiang R, Lan Y, Norton CR, Sundberg JP, Gridley T. The *Slug* gene is not essential for mesoderm or neural crest development in mice. Dev Biol 1998; 198:277-85.
12. Oram KF, Carver EA, Gridley T. *Slug* expression during organogenesis in mice. Anat Rec A Discov Mol Cell Evol Biol 2003; 271:189-91.
13. Urban AE, Zhou X, Ungos JM, Raible DW, Altmann CR, Vize PD. FGF is essential for both condensation and mesenchymal-epithelial transition stages of pronephric kidney tubule development. Dev Biol 2006; 297:103-17.
14. Perantoni AO, Timofeeva O, Naillat F, Richman C, PajniUnderwood S, Wilson C, Vainio S, Dove LF, Lewandoski M. Inactivation of FGF8 in early mesoderm reveals an essential role in kidney development. Development 2005; 132:3859-71.
15. Simon M, Maresh JG, Harris SE, Hernandez JD, Arar M, Olson MS, Abboud HE. Expression of bone morphogenetic protein-7 mRNA in normal and ischemic adult rat kidney. Am J Physiol 1999; 276:F382-9.
16. Roxburgh SA, Murphy M, Pollock CA, Brazil DP. Recapitulation of embryological programmes in renal fibrosis—The importance of epithelial cell plasticity and developmental genes. Nephron Physiol 2006; 103:139-48.

17. Perantoni AO. Renal development: Perspectives on a Wnt-dependent process. Semin Cell Dev Biol 2003; 14:201-8.
18. Perl AK, Wilgenbus P, Dahl U, Semb H, Christofori G. A causal role for E-cadherin in the transition from adenoma to carcinoma. Nature 1998; 392:190-3.
19. Blanco MJ, Moreno-Bueno G, Sarrio D, Locascio A, Cano A, Palacios J, Nieto MA. Correlation of *Snail* expression with histological grade and lymph node status in breast carcinomas. Oncogene 2002; 21:3241-6.
20. Moody SE, Perez D, Pan TC, Sarkisian CJ, Portocarrero CP, Sterner CJ, Notorfrancesco KL, Cardiff RD, Chodosh LA. The transcriptional repressor *Snail* promotes mammary tumor recurrence. Cancer Cell 2005; 8:197-209.
21. Katagiri A, Watanabe R, Tomita Y. E-cadherin expression in renal cell cancer and its significance in metastasis and survival. Br J Cancer 1995; 71:376-79.
22. Mazal PR, et al. Expression of kidney-specific cadherin distinguishes chromophobe renal cell carcinoma from renal oncocytoma. Hum Pathol 2005; 36:22-28.
23. Shen SS, Krishna B, Chirala R, Amato RJ, Truong LD. Kidney-specific cadherin, a specific marker for the distal portion of the nephron and related renal neoplasms. Mod Pathol 2005; 18:933-40.
24. Thedieck C, Kuczyk M, Klingel K, Steiert I, Muller CA, Klein G. Expression of Ksp-cadherin during kidney development and in renal cell carcinoma. Br J Cancer 2005; 92:2010-7.
25. Silva FG, Nadasdy T, Laszik Z. Immunohistochemical and lectin dissection of the human nephron in health and disease. Arch Pathol Lab Med 1993; 117:1233-9.
26. Laitinen L, Virtanen I, Saxen L. Changes in the glycosylation pattern during embryonic development of mouse kidney as revealed with lectin conjugates. J Histochem Cytochem 1987; 35:55-65.
27. Cho EA, Patterson LT, Brookhiser WT, Mah S, Kintner C, Dressler GR. Differential expression and function of cadherin-6 during renal epithelium development. Development 1998; 125:803-12.
28. Esteban MA, Tran MG, Harten SK, Hill P, Castellanos MC, Chandra A, Raval R, O'brien TS, Maxwell PH. Regulation of E-cadherin expression by VHL and hypoxia-inducible factor. Cancer Res 2006; 66:3567-75.
29. Thomson RB, Aronson PS. Immunolocalization of Ksp-cadherin in the adult and developing rabbit kidney. Am J Physiol 1999; 277:F146-56.
30. Shao X, Somlo S, Igarashi P. Epithelial-specific Cre/lox recombination in the developing kidney and genitourinary tract. J Am Soc Nephrol 2002; 13:1837-46.
31. Grone HJ, Weber K, Helmchen U, Osborn M. Villin: A marker of brush border differentiation and cellular origin in human renal cell carcinoma. Am J Pathol 1986; 124:294-302.
32. Strutz F, Okada H, Lo CW, Danoff T, Carone RL, Tomaszewski JE, Neilson EG. Identification and characterization of a fibroblast marker: FSP1. J Cell Biol 1995; 130:393-405.
33. Iwano M, Plieth D, Danoff TM, Xue C, Okada H, Neilson EG. Evidence that fibroblasts derive from epithelium during tissue fibrosis. J Clin Invest 2002; 110:341-50.
34. Hiesberger T, Bai Y, Shao X, McNally BT, Sinclair AM, Tian X, Somlo S, Igarashi P. Mutation of hepatocyte nuclear factor-1beta inhibits *Pkhd1* gene expression and produces renal cysts in mice. J Clin Invest 2004; 113:814-25.
35. Gresh L, Fischer E, Reimann A, Tanguy M, Garbay S, Shao X, Hiesberger T, Fiette L, Igarashi P, Yaniv M, Pontoglio M. A transcriptional network in polycystic kidney disease. EMBO J 2004; 23:1657-68.
36. Yanez-Mo M, Lara-Pezzi E, Selgas R, Ramirez-Huesca M, Dominguez-Jimenez C, Jimenez-Heffernan JA, Aguilera A, Sanchez-Tomero JA, Bajo MA, Alvarez V, Castro MA, del Peso G, Cirujeda A, Gamallo C, Sanchez-Madrid F, Lopez-Cabrera M. Peritoneal dialysis and epithelial-to-mesenchymal transition of mesothelial cells. N Engl J Med 2003; 348:403-13.
37. Liu Y. Renal fibrosis: New insights into the pathogenesis and therapeutics. Kidney Int 2006; 69:213-7.
38. Sato M, Muragaki Y, Saika S, Roberts AB, Ooshima A. Targeted disruption of TGF-beta1/Smad3 signaling protects against renal tubulointerstitial fibrosis induced by unilateral ureteral obstruction. J Clin Invest 2003; 112:1486-94.
39. Zeisberg M, Hanai J, Sugimoto H, Mammoto T, Charytan D, Strutz F, Kalluri R. BMP-7 counteracts TGF-beta1-induced epithelial-to-mesenchymal transition and reverses chronic renal injury. Nat Med 2003; 9:964-8.
40. Tan X, Li Y, Liu Y. Paricalcitol attenuates renal interstitial fibrosis in obstructive nephropathy. J Am Soc Nephrol 2006; 17:3382-93.
41. Palmer HG, Larriba MJ, Garcia JM, Ordonez-Moran P, Pena C, Peiro S, Puig I, Rodriguez R, de la Fuente R, Bernad A, Pollan M, Bonilla F, Gamallo C, de Herreros AG, Munoz A. The transcription factor SNAIL represses vitamin D receptor expression and responsiveness in human colon cancer. Nat Med 2004; 10:917-9.
42. Matsui I, Ito T, Kurihara H, Imai E, Ogihara T, Hori M. Snail, a transcriptional regulator, represses nephrin expression in glomerular epithelial cells of nephrotic rats. Lab Invest 2007; 87:273-83.