

Mechanisms of Renal Cell Repair and Regeneration After  
Acute Renal Failure

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## Renal Cell Repair and Regeneration

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ARF, acute renal failure; RPTC, renal proximal tubular cell(s); BUN, blood urea nitrogen;

DCVC, S-(1,2-dichlorovinyl)-L-cysteine; AscP, L-ascorbic acid-2-phosphate; ECM,

extracellular matrix; BM, basement membrane

## Abstract

In many cases, acute renal failure (ARF) is the result of proximal tubular cell injury and death and can arise in a variety of clinical situations, especially following renal ischemia and drug or toxicant exposure. Though much research has focused on the cellular events leading to ARF, less emphasis has been placed on the mechanisms of renal cell repair and regeneration though ARF is reversed in over half of those who acquire it. Studies using *in vivo* and *in vitro* models have demonstrated the importance of proliferation, migration, and repair of physiological functions of injured renal proximal tubular cells (RPTC) in the reversal of ARF. Growth factors have been shown to produce migration and proliferation of injured RPTC, though the specific mechanisms through which growth factors promote renal regeneration *in vivo* are unclear. Recently, interactions between integrins and extracellular matrix proteins such as collagen IV were shown to promote the repair of physiological functions in injured RPTC. Specifically, collagen IV synthesis and deposition following cellular injury restored integrin polarity and promoted repair of mitochondrial function and active Na<sup>+</sup> transport. Further, exogenous collagen IV, but not collagen I, fibronectin, or laminin, promoted the repair of physiological functions without stimulating proliferation. These findings suggest the importance of establishing and/or maintaining collagen IV-integrin interactions in the stimulation of repair of physiological functions following sublethal cellular injury. Further, the pathway that stimulates repair is distinct from that of proliferation and migration and may be a viable target for pharmacological intervention.

## Introduction

Most cases of acute renal failure (ARF) result from renal ischemia or acute drug or toxicant exposure, affecting up to 5% of all long-term hospital patients. Despite the advent of dialysis and increasing knowledge regarding the causes and effects of ARF, nearly half of those who develop the disease do not survive, a trend that has not changed for several decades (Thadhani, 1996; Molitoris *et al.*, 2000). A vast majority of research in the field of ARF has focused on the determination of events and factors that cause renal proximal tubular cell (RPTC) injury and death leading to the development of ARF. Unfortunately, the development of therapeutic strategies that are efficacious in humans with ARF has proven problematic. This suggests that the development of more successful therapies requires approaching the problem from a different vantage point (Molitoris *et al.*, 2000). The regenerative capacity of the kidney is well documented, and more than half of non-critically ill patients who acquire ARF survive (Abbate and Remuzzi, 1996; Toback *et al.*, 1993; Liano and Pascual, 1998). The responses of surviving RPTC are thought to be crucial to the restoration of renal function following ARF. Consequently, understanding RPTC repair and regeneration mechanisms may uncover new therapeutic targets that promote renal recovery and decrease the severity of ARF. This review will examine the experimental approaches used and recent advances in the study of the mechanisms of renal regeneration following ischemia or toxicant injury.

### RPTC Injury, Repair and Regeneration

As a result of acute chemical insult or ischemia, RPTC injury is characterized by mitochondrial dysfunction, ATP depletion, impaired solute and ion transport, loss of cell polarity and cytoskeletal disruption, and a variety of other effects (Bush *et al.*, 2000; Fish and Molitoris,

1994; Kays and Schnellmann, 1995; Molitoris *et al.*, 1997; Molitoris *et al.*, 1989). If severe, impairment of these functions leads to RPTC death and loss, events that compromise renal efficacy and efficiency and can lead to ARF. In patients and animals that survive ischemia- or chemical-induced ARF, injured RPTC that do not die or detach from the BM are thought to contribute to the regeneration of the tubular epithelium and the restoration of overall renal function. The prevailing theory is that surviving cells repair inhibited physiological functions and re-polarize and/or dedifferentiate, proliferate, migrate to denuded areas, differentiate, and restore nephron structure and function (Toback, 1992; Abbate and Remuzzi, 1996; Molitoris and Marrs, 1999).

Cuppage, *et al.* (1972) made the important observation that rats treated with mercuric chloride underwent ARF that was followed by functional and morphological repair of the kidney over time. Using <sup>3</sup>H-thymidine incorporation into DNA as a marker of cell proliferation, they demonstrated nephron repair driven by regenerating cells that originated in areas of tubular necrosis. Houghton *et al.*, (1976), who studied the pathological effects of the nephrotoxic aminoglycoside antibiotic gentamicin in rats found that after 10 days of gentamicin exposure, animals exhibited signs of ARF (increases in BUN and serum creatinine levels) that were accompanied by proximal tubular cell necrosis and desquamation, cellular detachment, and organelle disruption. Over several days, cellular debris was cleared from the nephrons as numerous regenerating cells began to reline the tubules where cells had been lost. This regeneration was accompanied by a decrease in the signs of ARF, and gentamicin-exposed kidneys visually were comparable to controls several weeks after the insult. More recently, Wallin *et al.* (1992) observed an early proliferative response in the S<sub>2</sub> and S<sub>3</sub> segments of the proximal tubule of rats treated with the nephrotoxicant S-(1,2-dichlorovinyl)-L-cysteine (DCVC).

These studies established that a proliferative response occurs in the surviving cells to regenerate the damaged tubules following injury. Further, the proliferation and regeneration is associated with the return of normal renal proximal tubular morphology and function.

For previously quiescent RPTC of the injured nephron to carry out tubular regeneration, upregulation of genes and protein synthesis, and entry into the cell cycle is required for the repair, migration, and proliferation involved in the process. Several investigators examined the production and localization of growth factors as well as their ability to stimulate cell growth and proliferation in regenerating proximal tubules of animals that were chemically injured or subjected to ischemia-reperfusion injury. For example, Kawaida *et al.* (1994) demonstrated the positive effects of hepatocyte growth factor to prevent ARF and to promote renal regeneration in mice following acute cisplatin toxicity. Using S-(1,1,2,2-tetrafluoroethyl)-L-cysteine to produce nephrotoxic damage in rats, Ichimura *et al.* (1995) demonstrated both paracrine and autocrine effects of fibroblast growth factor-1 to promote proximal tubular regeneration. The precise mechanisms by which growth factors or other mitogens direct this type of renal proximal tubular repair and regeneration remain largely unknown. While the major limitation in this field traditionally was the lack of good model systems, the development of “knock-out” and “over-expression” models, including “conditional knockouts”, have begun to contribute to our understanding of renal proximal tubular repair and regeneration. An example is the work by Haq *et al.* (1998), showing that interleukin-1 receptor-null mice, though equally susceptible to ischemia-reperfusion injury, exhibit quicker return of renal function following ischemia. These results suggest that interleukin-1 plays a negative role in the repair of renal function. Similarly, Terzi, *et al* (1997), demonstrated renal regeneration following transient unilateral ischemia in mice bearing a null mutation for vimentin, a marker of cellular dedifferentiation previously

thought necessary for regeneration in the kidney. These types of studies are valuable for their ability to assess the relative contribution of single gene products to ARF and the repair and regenerative process. However, elucidating the role(s) of individual cell types, growth factors, other cellular macromolecules, or combinations thereof in renal repair and regeneration is still not practical at this time in whole animals (Molitoris *et al.*, 2000).

Like all adherent cell types, RPTC require adhesion to the basement membrane for normal function and to avoid anoikis, or apoptosis due to loss of cell adhesion. Anchorage of RPTC to the basement membrane is mediated by cellular integrins, heterodimeric transmembrane receptors that adhere to extracellular matrix (ECM) proteins. Disruption of integrin function can result in the diminished cell-ECM interactions and RPTC detachment, leading to the formation of cell aggregates in the tubular lumen, glomerular filtrate back-leak, and loss of renal tubular function. Changes in the expression and localization of integrins and other plasma membrane proteins have been documented in epithelial cell types following renal tubular cell injury *in vitro* and *in vivo* (Walker, 1994; Zuk *et al.*, 1998; Breuss *et al.*, 1995; Wang *et al.*, 1996; French-Constant *et al.*, 1989; Fukijawa *et al.*, 1981; Goke *et al.*, 1996). For example, Spiegel *et al.* (1989), using a rat model of renal clamp ischemia, demonstrated ischemia-induced loss of plasma membrane polarity measured as loss of transport function and redistribution of the Na<sup>+</sup>/K<sup>+</sup>-ATPase to the apical membrane. Gailit *et al.* (1993) observed the appearance of  $\alpha_3$  integrins on the apical membrane of monkey BSC-1 cells following sublethal oxidative injury. This was accompanied by the disruption of focal contacts, contributing to a decrease in cell adhesion to collagen IV and other ECM substrates. Zuk *et al.* (1998) demonstrated a role for  $\beta_1$  integrins in ischemia-reperfusion injury in which  $\beta_1$  integrins were redistributed to lateral membrane segments, decreasing their concentration in the basal domain, and possibly

contributing to the exfoliation of RPTC following reperfusion injury in rats.  $\beta_1$  integrin distribution subsequently repolarized to the basal membrane domain, and the return of membrane polarity accompanied the return of renal function. In another study, integrin antagonism with GRGD peptides following oxidant injury was shown to inhibit regeneration in rat RPTC (Wijesekera *et al.* (1997). Collectively, these studies demonstrate that the loss of ECM-integrin interactions and plasma membrane polarity after ischemia contributes to RPTC injury and ARF and that repolarization of integrins and the  $\text{Na}^+/\text{K}^+$ -ATPase may play an important role in RPTC repair.

A potential role of ECM proteins in the mechanisms of renal tubular regeneration has been demonstrated. Zuk *et al.* (1998) observed damage to the basement membrane using antibodies to collagen IV and laminin in rats exposed to ischemia reperfusion injury, suggesting that degradation of the basement membrane may contribute to injury. Studies by Walker (1994) examined changes in renal fibronectin and laminin protein levels in rats subjected to bilateral ischemia-reperfusion injury. While fibronectin levels were increased in the renal cortex immediately following reperfusion and remained elevated for days, laminin levels initially decreased then rose to levels higher than in control animals following reperfusion. Basile *et al.* (1998) also used renal ischemia in rats to examine the expression of certain genes in the proximal tubule following injury. They found that collagen IV and fibronectin mRNAs were upregulated soon after injury and for a period of weeks, and these increases were localized to the regenerating cells of the proximal tubule. These studies suggest that ECM proteins are altered following injury, that the regulation of ECM proteins plays an important role the progression of tubular injury, and that cell adhesion to specific ECM proteins may promote the repair process.



### ***In Vitro* Models For Studying RPTC Repair**

Despite the various drawbacks inherent to *in vitro* models, human and animal immortalized renal epithelial cell lines and primary cultures of RPTC have provided useful models for research into the mechanisms of proximal tubular cell injury and death associated with ARF (Boogaard *et al.*, 1990; Racusen, 1994; Molitoris *et al.*, 2000). In addition, immortalized renal epithelial cell lines are good for over-expression and knockout experiments. For example, using an antisense expression vector in cultures of immortalized normal rat kidney (NRK) epithelial cells, Providence *et al.* (2000) demonstrated that decreased plasminogen activator inhibitor type I (PAI-1) expression inhibits repair. Further, transfection of a PAI-1 sense vector into a repair-deficient NRK cell line not only increased PAI-1 expression, but also restored the ability to repair. Given the value of RPTC lines for studying mechanisms of repair and regeneration, many cell lines still suffer from loss of differentiated functions, appearance of other normally absent functions, altered cellular metabolism, and immortalization itself, which may alter basal and induced gene transcription as they relate to regeneration.

Ideally, *in vitro* model systems for studying mechanisms of regeneration retain the highest degree of *in vivo*-like morphology, metabolism, and behavior, including the ability to be affected by ischemic or toxic insults and in return to respond to those insults (repair and regeneration) as RPTC are capable of doing in animals. To date, the closest representations of the ideal *in vitro* model system are primary cultures of RPTC. Over the years researchers have prepared primary cultures of rat, mouse, rabbit, dog, and human RPTC using a variety of isolation methods with varying degrees of success (Boogaard, 1990). More recently, a number of improvements have been made in primary cultures of RPTC from numerous species, including humans, that result in greater retention of the renal physiology and differentiated functions found

*in vivo* (Kruidering *et al.*, 1996; Gstraunthaler *et al.*, 1999; Sens *et al.*, 1999). For example, rabbit RPTC grown with gentle orbital shaking under hormonally-defined conditions, including physiological concentrations of L-ascorbic acid-2-phosphate and the absence of glucose, proliferate and grow to confluence, become quiescent, and exhibit metabolic capacity and transport functions that are very similar to that of RPTC *in vivo* (Nowak and Schnellmann, 1995; Nowak and Schnellmann, 1996). Compared to cultures grown in stagnant, high glucose conditions that promote hypoxia and glycolysis, rabbit RPTC grown under improved conditions consume lactate, are gluconeogenic, and are non-glycolytic. They exhibit increased levels of active Na<sup>+</sup> transport, Na<sup>+</sup>-coupled glucose transport, and brush border enzyme activity; and consume oxygen at levels equal to that in freshly isolated renal proximal tubules (Nowak and Schnellmann, 1995; Nowak and Schnellmann, 1996). These enhancements have made the rabbit RPTC model and other primary cultures very relevant and useful *in vitro* systems for studying RPTC injury and repair.

### **Mechanisms of Renal Proximal Tubular Cell Regeneration**

Migration, proliferation, and repair of physiological functions are the three crucial processes that must be achieved for RPTC structural and functional regeneration to be complete *in vivo* and *in vitro*. Following acute cellular injury and loss, it is believed that an initial migratory response into the denuded area occurs in the remaining sublethally injured cells followed by a proliferative response to replace lost cells. Finally, regenerating RPTC differentiate and resume normal functions. While a common theory regarding RPTC regeneration after ischemic or chemical injury involves the roles of autocrine, paracrine, and/or endocrine growth factors that promote cell proliferation and differentiation (Wagener *et al.*,

1995; Hammerman *et al.*, 1994), the molecular mechanisms of action of growth factors in the regenerative process remain elusive (Hammerman *et al.*, 2000).

**Migration.** The migration of RPTC to denuded areas within the nephron following injury is key for the structural and functional recovery of the nephron. The mechanical scrape technique is a popular method for simulating RPTC loss. In this model, confluent, quiescent monolayers of RPTC are scraped to remove a tract of cells, leaving a cell-free space for the remaining cells to migrate. Several key studies have used this technique to measure migration using *in vitro* models of RPTC. For example, Kartha and Toback (1992) observed the migration of monkey kidney epithelial (BSC-1) cells into mechanically denuded areas of the monolayer, an effect that was increased by the addition of adenine nucleotides. Using the same model system, Pawar *et al.* (1995) examined migration and related gene expression following mechanical injury to the monolayer. Several important genes including immediate-early genes as well as *c-myc*, HSP-70, and urokinase-type plasminogen activator were upregulated hours after monolayer wounding. These results provide some initial insight into the identity of gene products that might drive migration. Using mechanical injury of primary cultures of rabbit RPTC, Counts *et al.* (1995) measured the effects of various growth factors and nephrotoxicants on the ability of RPTC to migrate into the denuded area. They found that RPTC recovered approximately 77% of the scraped area over 7 days with no treatment. Epidermal growth factor (EGF; 10 ng/ml) stimulated complete monolayer regeneration through migration and proliferation while transforming growth factor  $\beta_1$  (TGF- $\beta_1$ ; 0.2 ng/ml) inhibited migration though cell number was restored. Additional experiments were conducted to determine whether known nephrotoxicants including mercuric chloride (1-20  $\mu$ M), fumonisin B<sub>1</sub> (0.1-2  $\mu$ M), and DCVC (5-20  $\mu$ M) acted in part by inhibiting the migratory repair response. In general, the nephrotoxicants only inhibited

migration when they exhibited overall cytotoxicity. These studies suggest that the migration response observed after injury is critical to the repair response and is resistant to inhibition.

**Proliferation.** In addition to migration, the replacement of lost RPTC following toxicant injury or ischemia requires the proliferation of surrounding cells that survive the insult. The specific factors driving the proliferative response are unknown although several studies have studied proliferation of cells in the proximal tubules of chemically injured animals (Cuppige *et al.*, 1972; Kovacs *et al.*, 1982; Wallin *et al.*, 1992). Using primary cultures of rat RPTC, Wallin *et al.* (1992) observed a proliferative response in dedifferentiated, vimentin expressing cells that closely resembled the *in vivo* proliferative response in the rat. In the rabbit RPTC model, Kays and Schnellmann (1995) induced extensive chemical injury (77% cell death and loss) using brief exposures to the oxidant *tert*-butyl hydroperoxide (TBHP; 0.8 mM) or the nephrotoxicant DCVC (0.4 mM). Over time, injured RPTC migrated and reached confluency through migration and spreading, but not proliferation. However, exogenous epidermal growth factor (EGF; 1 ng/ml) promoted proliferation and complete regeneration of the monolayer whereas exogenous insulin-like growth factor-1 (IGF-1; 100 ng/ml) produced only a modest increase in proliferation. Using the same model, Nowak *et al.* (1997) found that exogenous TGF- $\beta_1$ ; (0.2 ng/ml) inhibited EGF-stimulated regeneration following injury through the potentiation of cell death and monolayer degeneration. In addition, they observed increases in endogenous TGF- $\beta_1$  production by TBHP-injured RPTC, suggesting that autocrine production of TGF- $\beta_1$  inhibits monolayer regeneration. These studies demonstrate the influence of exogenous growth factors on RPTC proliferation following injury and that autocrine actions of growth factors can have both positive and negative effects on RPTC regeneration.

***Repair of Physiological Functions.*** The reabsorption of water, solutes, and xenobiotics from the tubular lumen by RPTC relies heavily on the maintenance of ion gradients between the intracellular and extracellular environments. Therefore, impairment of transport and metabolic functions in RPTC is a primary contributor to renal dysfunction leading to ARF. To examine the repair of physiological functions requires the induction of sublethal injury. Nowak *et al.* (1998) treated primary cultures of rabbit RPTC with TBHP (0.2 mM) for a brief period to allow limited cell death and detachment to occur. After 4 hours, 24% of the cells were lethally injured and died and/or detached from the culture plate, leaving the remaining 76% of cells sublethally injured. Measurement of a variety of physiological functions revealed that mitochondrial function, Na<sup>+</sup>/K<sup>+</sup>-ATPase activity, and Na<sup>+</sup>-coupled glucose transport were all decreased approximately 80%. In addition net lactate consumption was decreased and glutamine consumption increased, altering the metabolic profile of RPTC. RPTC initially migrated and then proliferated such that four days after injury by TBHP, RPTC reached monolayer confluence. However, repair of RPTC functions and lactate consumption to control levels did not occur until day six after injury. These findings are summarized in figure 1 and illustrate an important hierarchy of responses following cell injury. The initial and robust response is the migration of RPTC into the denuded area. This response is followed by proliferation, if possible, to replace lost RPTC. Finally, the return of differentiated functions occurs after regeneration of the monolayer.

In a subsequent set of experiments, Nowak *et al.* (1999) showed that sublethal injury by the nephrotoxicant DCVC (0.2 mM) produced an irreversible inhibition of physiological functions in rabbit RPTC; that is, sublethally injured RPTC migrated but exhibited impaired physiological functions for the length of time measured and did not proliferate. In many ways RPTC treated with DCVC exhibited a “suspended state” of existence, deriving enough energy to

remain viable but lacking the ability to repair or proliferate. The addition of EGF (10 ng/ml) during the sublethally injured state (24 hr after DCVC exposure and thereafter), did promote repair of physiological functions with the exception of Na<sup>+</sup>-coupled glucose transport. These studies demonstrate the differing effects of chemicals on RPTC, the sensitivity of key RPTC physiological functions to chemical injury, and that changes in cellular metabolism may accompany cell injury.

Ascorbic acid is a cofactor in collagen biosynthesis, and the majority of collagen found in the rabbit renal proximal tubule basement membrane consists of globular collagen IV (Gibbs *et al.*, 1999). Since ECM proteins are known to play a key role in cell polarity, migration, and proliferation, Nowak *et al.* (2000) and Nony *et al.* (2001) examined repair of RPTC physiological functions and proliferation following DCVC (0.2 mM) injury in the presence of a pharmacological concentration of L-ascorbic acid-2-phosphate (AscP; 0.5 mM). Pharmacological concentrations of AscP promoted the repair and proliferation of DCVC-injured RPTC six days after injury. Examination of collagen synthesis and deposition revealed that DCVC inhibited collagen IV deposition, but not synthesis, and that AscP restored collagen IV deposition in DCVC treated RPTC. These results suggested an association between the ability of injured RPTC to deposit collagen IV and to proliferate and repair physiological functions. To further explore this association, Nony *et al.* (2001) tested the efficacy of exogenous ECM proteins to promote repair and proliferation in DCVC-injured RPTC. When added to the culture media of DCVC-injured RPTC, collagen IV (50 µg/ml) promoted the repair of physiological functions, but not proliferation. In contrast, fibronectin, laminin, and collagen I were ineffective at promoting repair or proliferation. These data reveal a specific role for collagen IV in the stimulation of repair of RPTC physiological functions but not cell proliferation.

The intimate relationship between collagens and integrins led to the hypothesis that collagen binding integrins were involved in the repair process. Nony and Schnellmann (2001) did not observe changes in total plasma membrane expression of the collagen binding integrin subunits  $\alpha_1$ ,  $\alpha_2$ , or  $\beta_1$  (collagen IV is thought to be bound by the integrins  $\alpha_1\beta_1$  and  $\alpha_2\beta_1$ ) following DCVC exposure, suggesting that the plasma membrane expression of collagen-binding integrins is unaltered in DCVC-injured RPTC. However, there was a decrease in the intensity of integrin subunit  $\alpha_1$  fluorescent staining at the basal membrane on day one following DCVC exposure (figure 2, left panels A-D). By day six after injury, only RPTC cultured in pharmacological concentrations of AscP or exogenous collagen IV (50  $\mu\text{g/ml}$ ) had restored integrin localization to the basal membrane (figure 2, left panels G, H). With respect to the apical membrane, integrin subunit  $\alpha_1$  was partially redistributed to the apical membrane in sublethally injured RPTC on day one after injury, demonstrating loss of integrin-ECM binding and cellular disorientation (figure 2, right panels A-D). On day six, only RPTC cultured in the presence of pharmacological concentrations of AscP or exogenous collagen IV exhibited a complete disappearance of integrin  $\alpha_1$  from the apical membrane (figure 2, E-H). The same effects on basal and apical localization were seen for integrin subunits  $\alpha_2$  and  $\beta_1$ . These results reveal that the injury-associated appearance of collagen-binding integrins on the apical membrane results not from altered integrin levels but from redistribution of integrin receptors throughout the plasma membrane after the loss of integrin-ECM interactions. In addition, the return of integrin polarity accompanies the repair of physiological functions, verifying previous studies suggesting that cell polarity and repair are closely related (Molitoris, 1991). This leads to the concept that integrin ligation to collagen IV elicits signal transduction events in injured RPTC that stimulate cell survival and are crucial to the repair of physiological functions.

### Future Studies in Repair and Regeneration

The specific signals generated through integrins upon binding to collagen IV are unknown in the RPTC model but need to be elucidated to understand repair and to manipulate the repair process pharmacologically for clinical use. The integrin- and focal adhesion-associated adaptor proteins paxillin, Grb2, p130<sup>cas</sup>, and Shc as well as focal adhesion kinase (FAK), Src, Crk, Csk, and PI-3 kinase all interact at sites of integrin-ECM binding to regulate the activity of MAP kinases in response to ECM- and growth factor-derived signals. The regulation of each of these proteins has been studied extensively under normal conditions. However, the key to RPTC repair following chemical-injury may lie in the altered regulation of one or more of these proteins in response to collagen IV-integrin-specific signaling. Alternatively, the loss of contact between integrins and collagen IV may result in the inhibition of a normally positive signal that in turn prevents survival and repair.

Closely related to the regulation of collagen-integrin interactions are the matrix metalloproteinases (MMPs) that drive proteolytic processes crucial to renal basement membrane remodeling and migration in development and in disease states (Kanwar *et al.*, 1999; Lenz *et al.*, 2000). In addition to their roles in development, MMPs have well-characterized roles in diabetic nephropathy (decreased MMP expression) and in inflammatory glomerulonephritis (increased MMP expression) (Lenz *et al.*, 2000). In non-renal models, the role of MMPs in the regenerative process has received some attention. For example, in a model of liver ischemia/reperfusion, Cursio *et al.* (2002) reported time-dependent changes in the expression of MMPs and tissue inhibitors of MMPs (TIMPs) suggesting that some MMPs may play a role in injury while others participate in the repair process. To our knowledge, however, the role of MMPs in RPTC repair



and regeneration following toxicant or ischemia-induced injury has not been studied making this a ripe area for research.

Another area of interest in renal cell repair and regeneration involves the cross-talk between ECM and growth factor receptors (Sieg *et al.*, 2000; Renshaw *et al.*, 1999). Recent studies show that interactions between FAK and PYK2, a G-protein-coupled receptor kinase, lead to the activation of mitogenic signaling cascades (Litvak *et al.*, 2000). In addition, Rho GTPases have been intimately linked with integrins in the regulation of cell adhesion and the resulting signal transduction events (Schwartz and Shattil, 2000). Rho family GTPases also have been linked to growth factor mediated stress fiber and focal adhesion assembly (Ridley and Hall, 1992), and inhibition of Rho GTPase activity is involved in actin depolymerization and tight junction dysfunction in injured renal tubular epithelia (Gopalakrishnan *et al.*, 1998). The role of ECM- and growth factor-derived signals in repair and regeneration of injured tubular epithelial cells is more likely through the integration of complementary cascades rather than mutual exclusion. As the mechanisms of growth factor- and ECM-directed repair and regeneration become clearer, the development of new therapeutic strategies for ARF will probably take advantage of both pathways.

In summary, recent improvements in experimental models and characterization of the interactions between RPTC and the ECM have broken new ground in the search for detailed mechanisms of repair and regeneration in injured RPTC. The current understanding of renal repair and regeneration following acute toxicant exposure or ischemia is summarized in figure 3. One recent and important finding in this field is that stimulation of collagen IV deposition and subsequent cellular binding to collagen IV is sufficient to stimulate repair of injured RPTC. Equally important is that collagen IV can stimulate repair, but not regeneration following

sublethal injury, suggesting a specific role for collagen IV in promoting the return of cell polarity and repair of physiological functions. Based on these findings, an effective therapy for ARF might stimulate the signals derived from both growth factors and integrin-ECM interactions to promote RPTC regeneration and repair.

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## Figure Legends

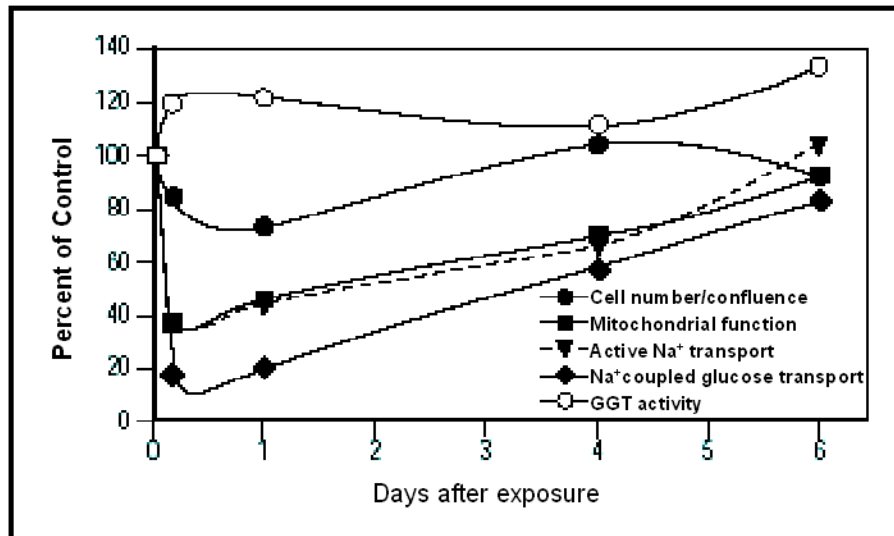
**Fig. 1.** Inhibition and repair of renal proximal tubule cellular functions after exposure to the model oxidant t-butylhydroperoxide. Approximately 24% cell loss and marked inhibition of mitochondrial function, active Na<sup>+</sup> transport, and Na<sup>+</sup>-coupled glucose transport occurred 24 hours after oxidant exposure. The activity of the brush border membrane enzyme  $\gamma$ -glutamyl transferase (GGT) was not affected by oxidant exposure. Cell proliferation and migration or spreading was complete by day 4, whereas active Na<sup>+</sup> transport and Na<sup>+</sup>-coupled glucose transport did not return to control levels until day 6. (Figure taken from Schnellmann and Kelly, 1999).

**Fig. 2.** Membrane localization of integrin subunit  $\alpha_1$  in RPTC sublethally-injured by DCVC and cultured in the absence or presence of pharmacological concentrations of AscP (500  $\mu$ M) or exogenous collagen IV (50  $\mu$ g/ml). On days 1 (left panels A-D) and 6 (right panels E-H), plasma membrane localization of integrin  $\alpha_1$  at the basal (left half) or apical (right half) domains was analyzed by confocal microscopy. A, control RPTC; E, subconfluent control RPTC; B and F, DCVC-injured RPTC; C and G, DCVC-injured RPTC cultured in the presence of pharmacological concentrations of AscP; D and H, DCVC-injured RPTC cultured in the presence of exogenous collagen IV. Shown are representative confocal photomicrographs from 3-4 separate experiments (magnification=400X). (Data from Nony and Schnellmann, 2001)

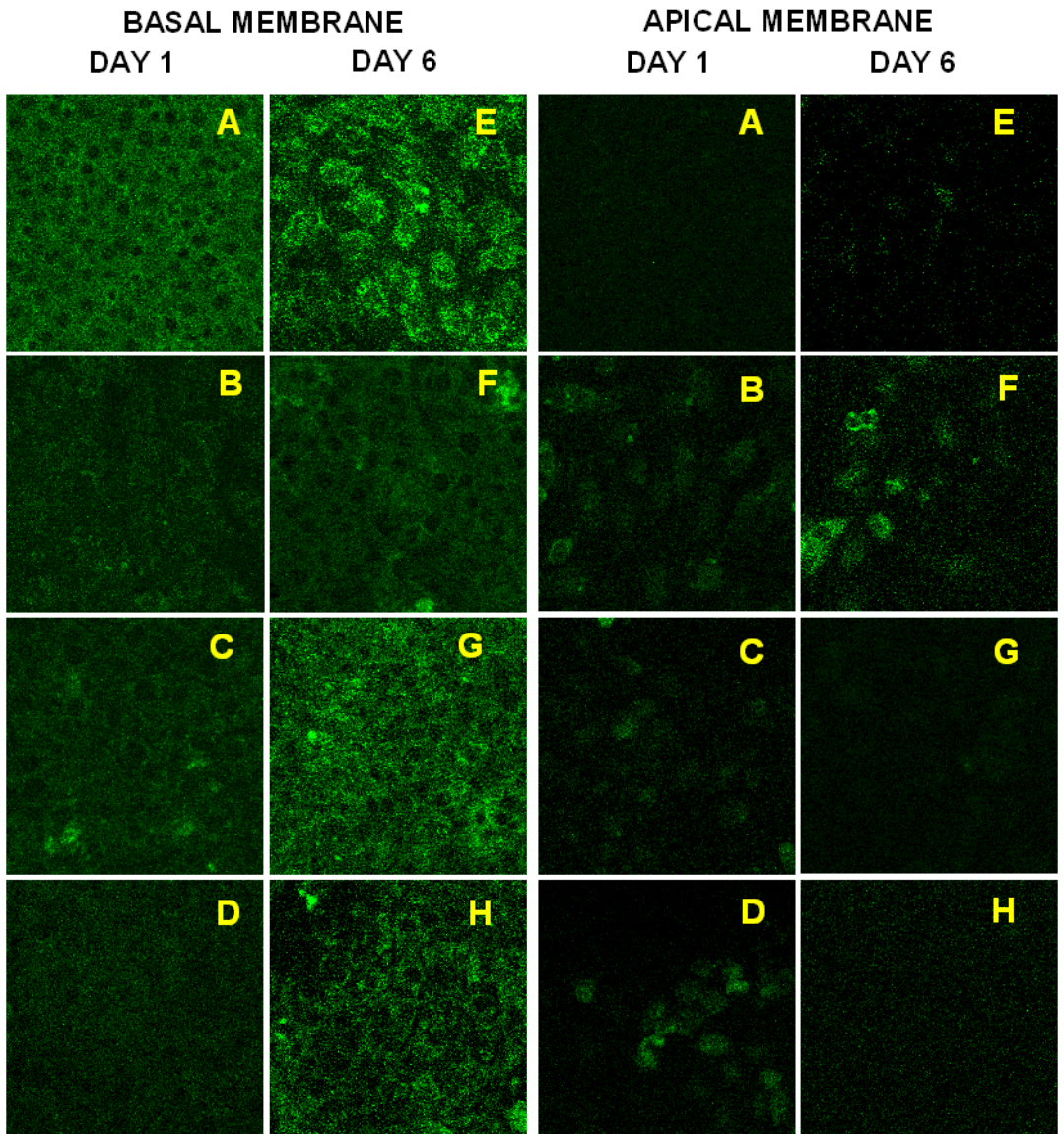
**Fig. 3.** Repair and regeneration of RPTC following acute sublethal toxicant injury. Sublethally injured RPTC either repair physiological functions and restore normal tubular

JPET/2002/35022 PiP

function or dedifferentiate, migrate, and/or proliferate to replace lost cells, then differentiate and resume normal function. The processes of repair and regeneration work in concert to ensure relining of the damaged nephron and restoration of renal function.

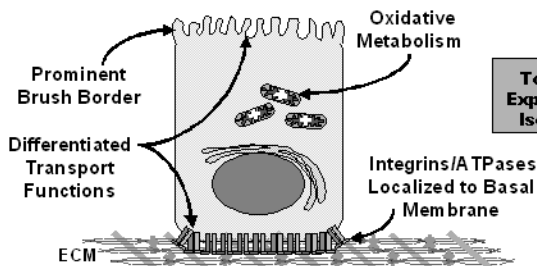




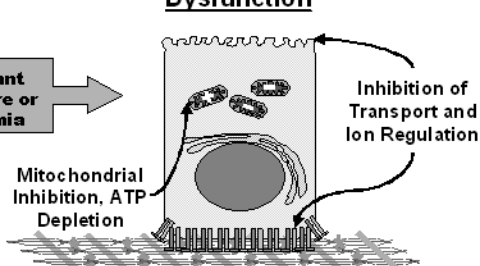


**FIGURE 2**

## Quiescent RPTC



## Physiological Dysfunction



Toxicant Exposure or Ischemia

## Sublethal Injury

