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Cell Proliferation and Renal Carcinogenesis

by Brian G. Short

Enhanced cell proliferation occurs at several stages of renal tumorigenesis. Initiation by genotoxic nephrocarcinogens such as dimethylnitrosamine (DMN) is likely a result of DNA damage coupled with an initial burst of DNA synthesis associated with the cytotoxic effects of the compound. The level of initiation by DMN can be further enhanced by unilateral nephrectomy or hydronephrosis, which induces a brief burst of cell proliferation followed by tumorigenesis in the contralateral kidney. The role of sustained cell proliferation in renal tumor development is less well understood. The most compelling evidence comes from studies with nongenotoxic renal carcinogens such as unleaded gasoline and *d*-limonene, which induce α_{2u}-globulin (αG) nephropathy and renal epithelial tumors exclusively in male rats. Sustained increases in cell proliferation in these studies depend on the presence of a chemical–αG complex in phagolysosomes of P_2 proximal tubule cells, which results in cytotoxicity and compensatory hyperplasia only in male F344 rats, but not female F344 rats or αG deficient male NBR rats. Furthermore, initiation-promotion experiments demonstrated a strong correlation between the dose-response of cell proliferation and the incidence of preneoplastic and neoplastic lesions. Clearly, similar correlative studies with a number of other renal carcinogens and noncarcinogens are warranted before general conclusions can be made. Cell proliferation is excessively elevated in tubules affected by chronic progressive nephropathy, but the significance of the lesion to renal carcinogenesis is unclear. Elucidating mechanisms of renal cell proliferation are necessary for our understanding of cause and effect relationships. An exciting recent finding is altered expression of transforming growth factor-α in hereditary rat renal cell carcinoma. This animal model may be useful for studying detailed histochemical relationships between altered growth factors and cell proliferation in various stages of renal carcinogenesis.

Introduction

Limited knowledge exists for evaluating the role of renal cell proliferation in renal carcinogenesis. Compounds from diverse chemical classes that induce renal tumors in different species of experimental animals have been reviewed (*1,2*) The broad morphological array of tumor types (e.g., nephroblastoma, renal mesenchymal tumors, renal epithelial tumors, and renal transitional tumors) reflect the marked heterogeneity of cell types of the kidney. Renal epithelial cell tumors comprises the vast majority of induced tumors, often with specific sites of origin within various segments of proximal and distal tubules and collecting ducts. For these reasons, evaluation of cell proliferation should be conducted within specific subpopulations of the nephron. Information on toxicokinetics, metabolism, genotoxicity, cytotoxicity, repair, and gene expression induced by a particular chemical may also be necessary to properly assess the relationship between cell proliferation and renal cancer.

Renal Cell Proliferation in Initiation

Cell proliferation is required for the conversion of DNA lesions to mutations. Sustained elevations in cell proliferation may also yield mutations secondary to errors in DNA synthesis and/or endogenous DNA damage. Thus, an agent can increase the probability of DNA damage by either directly altering the DNA or by increasing the number of times DNA replicates. In the kidney, the evidence for cell proliferation enhancing the rate of initiation has been evaluated in a limited number of experimental studies. In the case of mesenchymal and cortical epithelial renal neoplasms induced by dimethylnitrosamine (DMN), a correlation exists between the ability of this renal carcinogen to cause toxic injury and to stimulate a pulse of early proliferation in the same cell populations that give rise to tumors (*3*). Thus, the DNA damage induced by DMN in the resident cortical fibrocyte and proximal tubule epithelial cells is fixed by the proliferative stimulus of DMN.

Department of Toxicology–U.S., SmithKline Beecham Pharmaceuticals, UE0364, King of Prussia, PA 19406.

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The level of initiation by DMN can be further enhanced by unilateral nephrectomy or hydronephrosis, which induces a brief burst of cell proliferation followed by tumorigenesis in the contralateral kidney (*4*). These experiments are similar in principle to those in the liver, in which hepatocyte initiation is enhanced by partial hepatectomy. However, mechanistic studies in the liver, which demonstrated that initiation is highest when DNA injury has been induced immediately preceding the S phase of DNA synthesis (*5*), have not been conducted in the kidney. Studies in the liver have demonstrated that proliferation induced by mitogenic, as opposed to cytotoxic, agents does not enhance hepatocyte initiation (*6,7*). These investigators have suggested that initiated cells formed after exposure to mitogenic agents are prone to apoptosis, which eliminates initiated cells. Similar mitogenic agents for the kidney, such as lead nitrate, induce apoptosis after withdrawal of the mitogenic stimulus, (*8*) but the effect of mitogenic agents on renal initiation is unknown.

Induced cell proliferation may yield mutations directly by fixing endogenous DNA damage. This hypothesis has no direct experimental evidence in the kidney, but the idea deserves support because it may explain how nongenotoxic carcinogens increase the number of spontaneously initiated cells and thus contribute to the carcinogenicity of these agents.

Renal Cell Proliferation in Promotion and Progression

Increased rates of cell proliferation may be important in the promotion and progression phases of carcinogenesis by increasing the clonal expansion of initiated cells (*9*). Cell proliferation is essential for tumor formation, as cancer is a proliferative disease by definition (*10*). Correlations between increased cell proliferation and tumor development in the target organ are usually based on incomplete data because proliferation rates are collected at a single, early time point. Studies of renal cell proliferation at multiple time points are lacking. Furthermore, most studies have not identified the target renal cell population for tumorigenesis. Therefore, relatively few examples exist that adequately support the correlation between renal cell proliferation and tumor promotion.

α_{2u}-Globulin Inducing Agents

Many chemicals have been identified that induce α_{2u}-globulin (αG) nephropathy and renal cancer exclusively in male F344 rats (Table 1), and these chemicals have been reviewed elsewhere (*11,12*). Increases in renal cell proliferation associated with αG nephropathy have been demonstrated in male rats after acute exposure to pentachloroethane and perchloroethylene (*14*) and 1,4-dichlorobenzene (*23,28*). Sustained increases in cell proliferation associated with αG nephropathy have been demonstrated in male rats after acute or chronic exposure to unleaded gasoline [UG (*29,30*)] or d–limonene [dL(*31*)]. Five- to 11-fold increases in P_2 cell proliferation observed after 6 or 12 months of exposure to UG or dL were strongly correlated with the presence of chemical-αG complex in phagolysosomes of P_2 proximal tubule cells of male rats. Furthermore, the absence of αG nephropathy and P_2 cell proliferation in female F344 rats exposed to UG or in αG-deficient male NBR rats exposed to dL demonstrated the requirement of this protein for protein droplet nephropathy and cell proliferation. In addition, initiation-promotion experiments of UG and dL demonstrated a strong correlation between the presence of increased cell proliferation and promotion of preneoplastic and neoplastic lesions (*31,32*). These experiments with UG and dL provide a convincing case for a causal relationship between cell proliferation and renal carcinogenesis in male rats.

Table 1. Chemicals reported to cause male rat-specific α_{2u}-globulin nephropathy and renal carcinogenesis.

Chemical	References
Pentachloroethane	(*13,14*)
Unleaded gasoline	(*15,16*)
JP-5 jet fuel	(*17,18*)
Isophorone	(*19,20*)
Perchloroethylene	(*14,21*)
1,4-Dichlorobenzene	(*22,23*)
Dimethylmethylphosphonate	(*24*)
Hexachloroethane	(*25*)
d-Limonene	(*26,27*)

Cell proliferation is increased in P_3 cells and proximal tubular cells within foci of chronic progressive nephrosis (CPN) from rats exposed to UG (*30*). Although the mechanism for increased cell proliferation of P_3 and CPN tubules is unknown, the cause is most likely secondary to cytotoxicity observed in P_2 segments. In any event, these cells types may also be at risk for developing renal tumors.

The magnitude and duration of increases in renal cell labeling index necessary for kidney tumor formation are unknown, but these few examples with α-inducing agents suggest that P_2 cell labeling indexes of 5-fold or greater for at least 6 months are needed. Twenty-one-month recovery studies after 3 months of exposure to decaline or JP-8 were not associated with a tumor response, suggesting that a longer exposure period may be required for tumor formation (*17,33*). Perchloroethylene causes αG nephropathy at high gavage or inhalation concentrations, but a 28–day inhalation exposure study of male rats to 400 ppm, a concentration associated with kidney tumors, did not cause αG nephropathy in these rats (*14,34*). Recently, the pharmaceutical agent, 1-(aminomethyl)cyclohexaneacetic acid, was shown to induce αG nephropathy but not renal tumors in a 2-year bioassay in rats (35). Male rats exposed to this chemical may have had insufficient renal tubular injury and regeneration to effectively promote tumor formation. Clearly, data on magnitude and duration of cell proliferation following chronic exposure to these and other αG-inducing agents are required to enhance our understanding of the relationships between αG nephropathy, cell proliferation, and renal cancer in male rats. Although increased cell proliferation appears to be a necessary event for the formation of renal tumors caused by αG-inducing agents, other contributing mechanisms may also be present in some cases. For example, genotoxicity of perchloroethylene through the glutathione conjugation/β-lyase pathway in the rat may contribute to the development of kidney tumors caused by this αG-inducing agent (*34*).

Sodium Barbital

Sodium barbital (NaBB), a renal tumor promoter and weak renal carcinogen, induced chronic nephropathy resembling CPN and sustained, 5- to 10-fold increases in renal cell proliferation over a 2- to 52-week exposure period (*36*). Cell proliferation studies of the renal tumor-promoting activity of NaBB after initiation with streptozotocin (STZ) demonstrated amelioration of the NaBB-induced nephropathy and a decreased proliferative response after STZ treatment (*37*). However, STZ initiation did not abolish the renal tumor-promoting effect of this compound, implying a lack of correlation between cell proliferation and renal-promoting effect. The authors acknowledged that specific cell types were not counted separately and that the proliferative rate of renal cells seemed to be a more accurate quantitative index of the severity of nephropathy. The authors suggested that tumor promoters may target initiated cells for mitogenesis rather than noninitiated parenchymal cells, which undergo hyperplasia as a reparative response to cytotoxicity. These initiated cells may have different growth control mechanisms than noninitiated cells and their response to mitogens or toxins may differ as for hepatocytes. Studies such as this indicate the importance of attempting to identify initiated (target) cells within the renal tubule that respond to the mitogenic or other effects of tumor promoters. It is suggested that quantification of labeled cells within areas resembling CPN should be separated from more normal-appearing renal cells because the contribution of CPN tubules to renal cancer is yet to be defined.

Miscellaneous Chemicals, Hormones, and Drugs

Lead acetate, a renal carcinogen and tumor promoter in rats, induces sustained, 15-fold increases in cell proliferation in proximal tubule cells (*38–40*). Estrogen-induced kidney tumors in hamsters may be caused by both mitogenic effects and formation of reactive estrogen metabolites (*41*). Characterization of early kidney lesions in diethylstilbestrol-induced tumors in hamsters implicate the primitive interstitial cell, which differentiates into malignant tubules (*42*). Cell proliferation studies of estrogenic compounds may also be complicated by the fact that different cell types are affected by various estrogenic compounds, such as diethylstilbestrol, and ethinyl estradiol.

Sustained cytotoxicity, preneoplastic lesions, and renal cell tumors are observed with a number of agents, including antibiotics, analgesics, metal compounds, mycotoxins, tumor promoters, and other agents (*1,2,43,44*). Although it is reasonable to suggest that cell proliferation may be associated with the tumorigenic effects of these agents, the data are lacking. Renal toxicity and hyperplasia has also been observed without a carcinogenic effect following chronic exposure to several chemicals, including chloromethane, hydrochlorothiazide, α-methyldopa, toluene, and mercuric chloride (*44,45*). Cell proliferation studies of renal carcinogens as well as noncarcinogens are warranted to clarify the role of sustained proliferation and renal tumorigenesis.

Developing a Data Set on Cytotoxicity, Cell Proliferation, and Renal Cell Tumors

Several key ingredients of cell proliferation studies are necessary to establish relationships of cytotoxicity to renal tumors (Table 2). Examination of cell proliferation at bioassay doses and at multiple time points is critical. Cell proliferation data on doses where tumor information is not available are of limited use. Likewise, cell proliferation data obtained after acute dosing may be misleading for determining correlations with tumorigenicity. Continuous labeling with [^{3}H]thymidine or 2-bromodeoxyuridine via osmotic pumps to identify S-phase cells is recommended for most studies as this technique is more sensitive than pulse administration of label. Microscopic identification of the site of cytotoxicity and the cell type of preneoplastic and neoplastic lesions is important for determining the target cell population. Perfusion, rather than immersion fixation of the kidney and thin (2–3 μm) sections may be needed to adequately characterize cytotoxicity and preneoplastic lesions. In addition, preneoplastic and neoplastic lesions should be examined in

Table 2. Developing a data set on cytotoxicity, cell proliferation, and renal cell tumors.

Use bioassay doses at multiple time points to evaluate cytotoxicity and renal cell proliferation
Determine the site of cytotoxicity and cell type of preneoplastic and neoplastic lesions by light microscopy
Evaluate cell proliferation using a technique to identify S-phase cells
Evaluate differential cytotoxicity and cell replication in lesion and nonlesion tissue, including separation of chronic progressive nephropathy from lesion and nonlesion tissue

as many sections per kidney as possible (at least 4 sections/kidney). Established classification schemes for preneoplastic and neoplastic lesions should be used to maintain consistency of terminology. A generalized and more widely used classification, which incorporate early lesions, has recently been proposed and may improve the current database on renal lesions (46). It is advisable to separate epithelial from interstitial cells, as well as proximal from distal tubular epithelial cells in quantitation. Identification of P_1, P_2 and P_3 proximal tubule cells may be critical in certain cases, such as α_{2u}G-inducing agents. Tubules affected by CPN should be counted as a separate entity because their relevance to tumorigenesis is unknown.

Additional Information Needed for Modeling Renal Cancer

Simply measuring cell proliferation is inadequate for judging whether a chemical will ultimately increase the risk of cancer. The ultimate goal for such investigations in renal cancer should be two-stage growth modeling, similar to approaches developed for the liver and urinary bladder (*47*). Because the most relevant proliferation is that which occurs in the stem cell population, future cell proliferation studies in the kidney should be directed toward collecting these data within preneoplastic lesions, using recently developed biochemical markers of these lesions (*48,49*). Second, the total number of stem cell divisions, not only proliferation rate, must be ascertained. Thus, we need to include data on the number of normal cells and stem cells within the kidney. Third, we should include cell death rates, which means we need to begin to identify and count apoptotic renal cells.

Mechanisms of Renal Cell Proliferation

Studies of oncogenes, tumor-suppressor genes, and growth factors are essential for our understanding of the relationships between cell proliferation and renal cancer. The Eker rat, a model for hereditary renal cell carcinoma, has altered expression of transforming growth factor-α in early tumor development, but no expression is observed in highly replicating CPN foci (*49*). Cell proliferation studies in carrier and noncarrier rats have demonstrated increased cell proliferation rates in atypical tubules, atypical hyperplasias, and renal cell tumors (50). Labeling indexes in normal and CPN foci did not differ between carrier and noncarrier rats. This animal model may prove useful for investigations into the genetic basis for spontaneous and carcinogen-induced renal cell cancer, including relationships between growth-factor expression and cell proliferation in lesion and nonlesion tissue.

Investigating the nephrogenic repair response may be valuable for clarifying our understanding of renal cancer. Nephrotoxic and nephrocarcinogenic haloalkenes, which form toxic and mutagenic intermediates by the cysteine conjugate β-lyase pathway, cause necrosis of P_3 proximal tubule epithelial cells. This event leads to a dramatic site-specific increase in cell proliferation, followed by a decrease in differentiation (dedifferentiation), and an increase in markers more characteristic of embryonic kidney (*51*). Furthermore, proliferation but not dedifferentiation occurs in undamaged P_2 proximal tubule cells. Proliferation and dedifferentiation of P_3 proximal tubule cells may be important in transformation to a malignant phenotype (*52*).

Investigations on kinematics, or the movement of proliferating cells, within the nephron have identified two kinetic compartments: a progenitor compartment composed of proximal and distal convoluted epithelia and a part of thick, straight tubules, and a quiescent compartment composed of the remaining nephron within the papilla (*53*). Cells in the cortical regions of the nephron stream toward the papilla at a rate of 1.1 locations/day and are eliminated. The importance of kinematics in nephrotoxicity and renal carcinogenesis is unknown, but may be important for understanding the repair response of the nephron.

Conclusions

Relationships between sustained renal cell proliferation and carcinogenic potential have been established for several chemicals, but general conclusions must await data from correlative studies with other chemicals, including studies with noncarcinogens as well as carcinogens. Cell proliferation rates as well as total cell numbers should be quantitated in lesion and nonlesion tissue. Counting and classifying preneoplastic and neoplastic lesions must be thorough and standardized. Underlying mechanisms of renal cell proliferation must be understood before cause and effect relationships are drawn.

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