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REVIEW-HYPOTHESIS

Hypothesis: Chronic Progressive Nephropathy in Rodents as a Disease Caused by an Expanding Somatic Mutant Clone

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Abstract—Chronic progressive nephropathy is a common noninfectious disease in aging (mice, rats) and non-aging (naked mole rat) rodents, sometimes resulting in death. The etiology and pathogenesis of the disease remain mysterious. For instance, it remains unclear what is the immediate cause of the disease and where exactly in the kidneys, glomerular or tubulointerstitial compartment, do primary and secondary changes occur. Here, I propose a potential scenario for development of progressive nephropathy that is based on an assumption that the disease is caused by occurrence and spread of mutant cellular clones from tubular epithelium secreting proinflammatory and prosclerotic cytokines. The hypothesis considers some features of the disease that have never been discussed earlier. According to the proposed concept, a clone of mutant cells secretes cytokines inducing chronic inflammation, proliferation of fibroblasts, and active collagen production that eventually results in sclerosis and thickening of tubular basement membranes. Sclerosis of interstitium and thickening of tubular basement membranes cause narrowing of some parts of the nephron, especially collecting ducts, which hinders passage of the urine, elevates tubular hydrostatic pressure, and impairs filtration and reabsorption in the kidneys. High hydrostatic pressure and reabsorption-induced elevated concentration of macromolecular substances in the primary urine result in development of large cysts and glomerular hyalinosis followed by renal failure. Based on this, it might be concluded that chronic progressive nephropathy in rodents represents a special type of tubulointerstitial dysplasia (or "non-tumorous neoplasia") in kidneys with secondary glomerular disorder at late stage of the disease. The concept for development of the disease proposed here may be of special importance from the viewpoint of toxicological pathology and gerontology, particularly for analysis of pathological features resulting in death of non-aging animals (naked mole rats).

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CHRONIC PROGRESSIVE NEPHROPATHY IN AGING AND NON-AGING RODENTS

Chronic progressive nephropathy in rodents (CPNR) is the most common spontaneous kidney injury in aging laboratory mice and rats, generally being considered as one of the most typical non-tumor diseases in these animals [1-5]. Animal strains do not suffer from this disease equally; moreover, in males, the disease develops at remarkably higher rate than in females [5]. Regarding the influence of CPNR on lifespan of laboratory rodents, some authors suggest that this disease is fatal only to rats (that develop symptoms of chronic renal failure), but not to mice; this issue needs to be properly investigated [4]. Apart from mice and rats, similar disease was described in gerbils, whereas a segmented nephrosclerosis may be considered as its analog (only partial) in guinea pigs [4, 6]. Glomerulonephropathy (arteriolar nephrosclerosis) that significantly differs from CPNR is a lead age-related renal pathology in hamsters [7, 8].

Recently published data about CPNR occurring in naked mole rat as a non-aging species are extremely intriguing [9]. This disease was found in more than 50% of adult animals during their postmortem examination. So far, it is impossible to tell what place CPNR holds among causes of death in naked mole rats (primarily, due to a principal insufficiency of a postmortem histopathological examination only to resolve correctly the question about fatal character of diseases [10]); however, lethal outcome for nephropathy developed in this species is considered highly possible even by the authors of the paper cited above [9]. To summarize, it should be mentioned that CPNR is one of the most important non-neoplastic diseases for gerontology, toxicological and veteri-

Abbreviations: CPNR, chronic progressive nephropathy in rodents; PDGF-BB, platelet-derived growth factor-BB composed of two β-chains.

nary pathology due to its incidence in aged rodents of different species and potential role it may play as a cause of death in non-aging animals. Altogether, it makes analysis of CPNR pathogenesis highly complicated. On the other hand, it is equally important from the viewpoint of theory about a programmed body death as well as possibility to abolish it [11-13].

PATHOLOGICAL ANATOMY, ETIOLOGY, AND PATHOGENESIS OF CPNR: WHAT WE KNOW ABOUT THEM

Generally, pathological changes occurring in mice and rats during CPNR are well known [1-5]. Enlarged pale-yellow kidneys with small multiple pits on the renal surface and small cysts in the medulla are found during necropsy of ill animals. During microscopic examination, dilated parts of the kidney tubular system in some nephrons are found together with regenerative, dystrophic, and atrophic changes in tubular epithelium as well as irregular thickening of their basement membranes, interstitial fibrosis, lymphocyte and macrophage infiltration (chronic productive inflammation) and, importantly, "proteinaceous cysts" formed by extremely dilated renal tubules filled by oxyphilic components of primary urine and cellular debris. During CPNR, glomerular lesions are presented as expanded Bowman's space, thickening of glomerular loops, and increased cellularity inside them. At the final step, glomerular hyalinosis emerges together with signs of chronic renal failure such as anemia, hypertrophy of parathyroid glands, ascites (polyserositis), calcinosis in different tissues (only in rats, but not in mice), and impaired heart functioning associated with blood electrolyte imbalance.

In contrast to the morphological picture of the disease, knowledge regarding causes and mechanism for development of CPNR are extremely scarce and controversial, despite this disease being documented in virtually each study of geroprotectors and chronic toxicity of drugs performed on laboratory mice and rats. It is believed that CPNR is a multi-factorial disease having a complex pathogenesis [4, 5]. Nonetheless, it was assumed that excessive intake of dietary proteins, defects of basement membranes and hypertrophy of renal glomeruli, as well as sharply enhanced filtration in the kidneys are potential mechanisms leading to CPNR [4, 5, 14]. Changes occurring in renal interstitium can sometimes be taken into consideration in terms of disease pathogenesis as well [15, 16]. However, it seems that two issues, primarily related to mechanism of the disease development, have not yet been discussed.

First, early changes occurring at the onset of CPNR both in mice and rats are presented as manifestation of abnormal basophilic cytoplasm in tubular epithelial cells (all other morphological parameters of the cells remain normal) [1, 5]. Later on, thickening of basement membranes was found to occur in these areas. Remarkably, such changes do not cover entire kidneys, but were only revealed as separate tubular lesions within renal medulla. Although such events are sometimes called "degenerative", in reality it is accepted that sharp basophilic reaction of the cytoplasm is related to an increased amount of rRNA and enhanced protein synthesis [5]. This implies that the described changes are not degenerative, but rather have hyperfunctional nature. Moreover, it is known that altered epithelium has a positive immunohistochemical reaction with antibodies against vimentin and highly produce some cytokines including PDGF-BB [15] (the latter is discussed below), which do not agree with the viewpoint that these changes are a classical kind of degeneration [17].

Second, pathological changes during CPNR are extremely similar to those occurring upon chronic pyelonephritis in humans known as "thyroid kidney" [18]. During pyelonephritis, bacterial infection causes deep injury and inflammation in renal interstitium followed by fibroblast collagen hyperproduction and sclerosis. A marked interstitial sclerosis results in obstructions of nephrons at the level of collecting ducts and, subsequently, impaired urine movement along renal tubules and their dilation with development of cysts. The cysts contain eosinophilic material enriched with macromolecular compounds, as interstitial sclerosis hinders reabsorption of components from the primary urine. Cysts containing such "colloid-like material" highly resemble thyroid follicles, so that, generally, a histology sample from a patient with this type of chronic pyelonephritis looks akin to a section of thyroid gland (hence, giving the name "thyroid kidney"). Exactly the same picture is observed during CPNR as well. A "thyroid kidney" is typical for chronic pyelonephritis and other diseases accompanied by damage of renal interstitium (analgesic nephropathy, hydronephrosis, renal tuberculosis), but not diseases with primary glomerular lesions known as glomerulonephritis [17, 18].

Both comments mentioned above suggest that obstruction of nephrons most likely represents the main part of CPNR pathogenesis (rather than primary glomerular lesion) caused by an excessive connective (sclerotic) tissue formation in the interstitium and thickening of tubular basement membranes being somehow connected to occurrence of abnormal synthetic activity in the tubular epithelium. Altogether, this leads to a natural question: what underlies abnormal synthetic activity exhibited by renal epithelium?

CLONAL EXPANSION AS A SCENARIO FOR PATHOGENESIS OF SPONTANEOUS PROGRESSIVE NEPHROPATHY

Based on the above, the following probable sequence of events occurring during development of CPNR can be

proposed. First of all, it seems possible that an occurrence of a clone of precursor cells of tubular epithelium endowed with the ability to secrete proinflammatory and prosclerotic cytokines due to mutation or epigenetic changes of relevant genes might be a first event in CPNR pathogenesis. Based on this hypothesis, a basophilic reaction of cytoplasm in altered tubular epithelium and abundant rRNAs are exactly related to abnormally high production of protein factors (cytokines).

It is known that precursor cells of tubular epithelium are located in the renal interstitium, where they migrate towards the tubules followed by incorporation into the epithelium [19, 20]. In case a mutant clone emerges among precursor cells, then the cells descended from it (while migrating through interstitium) along with unaltered clones might be incorporated into the different nephrons and form local foci of epithelium with abnormal activity due to local propagation of mutant epithelial cells. In this case, a mosaic pattern for distribution of basophilic foci in diseased kidneys must be expected, which is actually observed. Thus, a clonal expansion of mutant epithelial cells is characterized as a random process resembling gene drift.

Probability of occurring somatic mutations increases with age of animals, and, it is not surprising that CPNR is considered as a typical age-related disease. Also, clonal expansion depends on time factor and determines disease severity. Altered cells produce cytokines that result in activation of fibroblasts and attraction of inflammatory effector cells. Indeed, the factors produced by basophilic tubular epithelial cells such as PDGF-BB are inducers for myofibroblast differentiation and development of sclerosis [15]. It is considered that excessive collagen production in the interstitium and components of basement membrane in the tubules result from these cytokines. Attracted inflammatory cells such as macrophages form foci of chronic productive inflammation and induce local damage of interstitium due to released reactive oxygen species and lysosomal enzymes, which enhance activation of fibroblasts and exacerbate sclerosis [16]. Renal interstitial sclerosis and thickening of basement membranes result in narrowing the lumen in some areas of nephron, especially at the level of collecting ducts, which hinders passage of the urine (until complete urinary obstruction), increases hydrostatic pressure in the tubules, results in their extension and development of cysts, as well as decreases filtration and reabsorption until complete cessation. Increased hydrostatic pressure in the proximal part of nephrons and high concentration of non-reabsorbed macromolecular substances in primary urine cause formation of precipitates, which infiltrate glomeruli (glomerular hyalinosis). Thus, renal glomeruli turn out to be secondarily involved in the pathological process during CPNR, whereas tubules and interstitium are the compartments where development of the disease begins.

SOME CONSEQUENCES OF CLONAL EXPANSION HYPOTHESIS FOR CPNR

If an assumption about mutations in genes involved in signaling pathways regulating cytokine secretion and the scenario described for CPNR pathogenesis are correct, then this disease must be classified as type of renal tubulointerstitial dysplasia, or even a non-tumorous neoplasia [21]. It is quite remarkable that CPNR is a facultative precancer condition akin to many other dysplasias such as myeloproliferative syndrome. In particular, an increased probability for development of kidney cancer in rats with CPNR was showed by J. Seely et al. [14]. This fact is explained without any problem by the proposed hypothesis that seems strange from the viewpoint of other concepts, e.g. when glomerular changes are considered as the main part of pathogenesis of the disease. Pretty much the same can be said about basophilic, eosinophilic, and other foci in altered hepatocytes in liver from mice and rats often associated with genotoxic impacts and tumor development [1, 22]. Moreover, based on the proposed concept a prediction can be formulated stating that different drugs or other interventions decreasing mutagenesis in renal cells may also prevent development of CPNR and result in decreasing age-related mortality rate in animals, and, inversely, carcinogens and mutagens should facilitate to development of the disease. By the way, SkQ1 antioxidant that targets mitochondria might be able to lower incidence rate and severity of CPNR not only due to ameliorating genotoxic effects from reactive oxygen species [23], but also due to its capacity to inhibit development of proinflammatory activation of vascular endothelium [24] and fibrosis [25]. Unfortunately, no data have yet been published to address this issue. Another consequence of a mutation concept states that the level of somatic mutagenesis in kidney cells from animals predisposed to CPNR should be much higher than in species and strains that do not develop such pathology or have it at low level.

In summary, it should be said that a mutation-clonal idea for CPNR pathogenesis proposed here might be very useful for correct interpretation of study results aimed at fighting against aging, particularly, pathological processes in naked mole rat as well as mechanism of action for geroprotectors. No doubt, another high priority issue is to identify what genes and signaling pathways might become disturbed and result in development of CPNR in laboratory animals.

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