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UCH-L1 induces podocyte hypertrophy in membranous nephropathy by protein accumulation

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ABSTRACT

Podocytes are terminally differentiated cells of the glomerular filtration barrier that react with hypertrophy in the course of injury such as in membranous nephropathy (MGN). The neuronal deubiquitinase ubiquitin C-terminal hydrolase L1 (UCH-L1) is expressed and activated in podocytes of human and rodent MGN. UCH-L1 regulates the mono-ubiquitin pool and induces accumulation of poly-ubiquitinated proteins in affected podocytes. Here, we investigated the role of UCH-L1 in podocyte hypertrophy and in the homeostasis of the hypertrophy associated "model protein" $p27^{Kip1}$. A better understanding of the basic mechanisms leading to podocyte hypertrophy is crucial for the development of specific therapies in MGN. In human and rat MGN, hypertrophic podocytes exhibited a simultaneous up-regulation of UCH-L1 and of cytoplasmic $p27^{Kip1}$ content. Functionally, inhibition of UCH-L1 activity and knockdown or inhibition of UCH-L1 attenuated podocyte hypertrophy by decreasing the total protein content in isolated glomeruli and in cultured podocytes. In contrast, UCH-L1 levels and activity increased podocyte hypertrophy and total protein content in culture, specifically of cytoplasmic $p27^{Kip1}$. UCH-L1 enhanced cytoplasmic $p27^{Kip1}$ levels by nuclear export and decreased poly-ubiquitination and proteasomal degradation of $p27^{Kip1}$. In parallel, UCH-L1 increased podocyte turnover, migration and cytoskeletal rearrangement, which are associated with known oncogenic functions of cytoplasmic $p27^{Kip1}$ in cancer. We propose that UCH-L1 induces podocyte hypertrophy in MGN by increasing the total protein content through altered degradation and accumulation of proteins such as $p27^{Kip1}$ in the cytoplasm of podocytes. Modification of both UCH-L1 activity and levels could be a new therapeutic avenue to podocyte hypertrophy in MGN.

1. Introduction

Glomerular podocytes are highly specialized cells with a complex cytoarchitecture. Their most prominent features are interdigitated foot-processes with filtration slits in between. These are bridged by the slit-diaphragm, which plays a major role in establishing the selective permeability of the glomerular filtration barrier [1]. Podocytes are terminally differentiated [2] and respond to injury with foot-process retraction, hypertrophy and loss, which ultimately results in proteinuria. Podocyte hypertrophy occurs in human membranous nephropathy (MGN) and in the corresponding rat model of passive Heymann nephritis (PHN) [3]. Podocyte hypertrophy is considered as a maladaptive attempt to cover denuded areas of the glomerular basement membrane [4]. The mechanisms of podocyte hypertrophy in MGN are poorly defined.

We recently described the de novo expression of ubiquitin C-terminal hydrolase-L1 (UCH-L1) in podocytes in human [5] and rodent membranous glomerulonephritis [6] and suggested that UCH-L1 is involved in the accumulation and aggregation of poly-ubiquitinated proteins in injured podocytes [6]. UCH-L1 represents one member of the ubiquitin carboxy-terminal hydrolase (UCH) family [7]. UCH enzymes belong to the de-ubiquitinating enzymes of the ubiquitin-proteasome pathway. Besides de-ubiquitinating enzymes, this pathway comprises enzymes that ubiquitinate target proteins and the 26S proteasome complex, which selectively degrades poly-ubiquitinated proteins. De-

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ubiquitinating enzymes are thought to counteract the effects of ubiquitin conjugation by removing the poly-ubiquitin chain from conjugated proteins prior to their degradation by the proteasome [8,9]. Biochemically, UCH-L1 plays a role in proteasomal protein degradation via two opposing enzymatic activities. Firstly, UCH-L1 hydrolyses ubiquitin pro-proteins and small ubiquitin-adducts, which results in the generation of free monomeric ubiquitin, the active component of the ubiquitin-dependent proteolytic system [10,11]. Secondly, UCH-L1 has been shown to have a dimerization dependent ubiquitin–ubiquitin ligase activity [12]. Recent publications have demonstrated that some biological effects of UCH-L1 are based on its ability to bind to and thus stabilize mono-ubiquitin [13]. Little is known regarding UCH-L1 specific substrates or biological activity in vivo.

Renal cell hypertrophy is thought to be in part mediated by cell cycle arrest, either at the G_1/S phase resulting in increased cell size, and protein and RNA contents [14] or in the G_2 phase subsequent to DNA replication resulting in bi- or multinucleated cells [4,15]. Cell cycle progression is regulated by the accumulation of cyclins, which bind to and activate different cyclin-dependent kinases, which in turn are regulated by cyclin-dependent kinase inhibitors. The cyclin-dependent kinase inhibitor $p27^{Kip1}$ is a major regulator of podocyte hypertrophy in vitro in response to hyperglycemia [16] and stretch [17] and in vivo in a model of type 2 diabetes [18]. $P27^{Kip1}$ levels increase in passive Heymann nephritis [19]. Therefore $p27^{Kip1}$ was suggested to function as a regulatory switch of podocyte hypertrophy by inhibition of cell cycle progression through the G_1/S phase. Multiple posttranscriptional mechanisms regulate $p27^{Kip1}$, such as translation [20] or proteasomal degradation [21,22].

UCH-L1 has been described as a tumor oncogene [23–25] and as a tumor suppressor gene influencing p53 and $p27^{Kip1}$ [26]. UCH-L1 interacts with $p27^{Kip1}$ through the generation of a heteromeric complex with the Jun activation domain-binding protein 1 (JAB-1) in ovaries [27] and lung cancer cells [28]. Nuclear translocation of both UCH-L1 and JAB-1 coincides with reduced nuclear levels of $p27^{Kip1}$, proposing that UCH-L1 may contribute to $p27^{Kip1}$ degradation via its interaction and nuclear translocation with JAB-1 [28] in tumor cells.

The aim of this study was to analyze whether UCH-L1 induces podocyte hypertrophy by protein stabilization of proteins like/such as $p27^{Kip1}$ in human and rodent membranous nephropathy and in cultured podocytes.

2. Results

2.1. In human MGN UCH-L1 is upregulated and $p27^{Kip1}$ localizes to the cytoplasm in hypertrophic podocytes

In human membranous nephropathy (MGN), swollen podocytes with increased cell body area impose along the glomerular filtration barrier by light microscopy (Fig. 1A). Recently we demonstrated that UCH-L1 is strongly expressed in podocytes in patients with MGN [5]. Immunohistochemistry showed that UCH-L1 was preferentially expressed in hypertrophic podocytes in MGN (Fig. 1B, a). Podocyte hypertrophy is thought to be mediated by $p27^{Kip1}$. We therefore analyzed whether $p27^{Kip1}$ expression was changed in MGN in comparison to control. Stainings against $p27^{Kip1}$ demonstrated an increased expression of $p27^{Kip1}$ in the cytoplasm of podocytes in MGN (Fig. 1C). Confocal analyses showed that both UCH-L1 and $p27^{Kip1}$ were expressed in the same hypertrophic podocytes (Fig. 1D). Both proteins co-localized in the nuclei and in the cytoplasm, suggesting that UCH-L1 might induce podocyte hypertrophy through altered $p27^{Kip1}$ homeostasis in injured podocytes. We therefore analyzed, whether $p27^{Kip1}$ was regulated on the transcriptional or post-translational level in MGN. Quantitative PCR (Fig. 1E) against $p27^{Kip1}$ in micro-dissected glomeruli from patients with MGN in comparison to living donors demonstrated that transcripts of $p27^{Kip1}$ did not change significantly.

2.2. In experimental MGN both UCH-L1 and $p27^{Kip1}$ are upregulated in hypertrophic podocytes

Similar to human MGN, the well-established rat model of MGN called passive Heymann nephritis (PHN) also induced swollen podocytes (Fig. 2A, c, d) with increased cell body area by light microscopy (Fig. 2B). Hypertrophy, which is defined as an increase of cell size in conjunction with increased protein content and stable gDNA content, was further quantified by measuring protein to gDNA ratio in glomerular lysates after RNAse treatment. The protein to gDNA ratio was significantly increased in nephritic glomeruli (Fig. 2C), indicating glomerular cellular hypertrophy. Immunohistochemical stainings demonstrated that UCH-L1 was strongly and preferentially expressed in hypertrophied podocytes in PHN (Fig. 2D, b). Again, as in MGN, UCH-L1 expression correlated with $p27^{Kip1}$ expression in hypertrophic podocytes in PHN. Furthermore, $p27^{Kip1}$ expression was pronounced in the cytoplasm of podocytes in PHN (Fig. 2E). Quantification of $p27^{Kip1}$ content in isolated glomeruli exhibited elevated $p27^{Kip1}$ protein levels in PHN by WB (Fig. 2F) and by ELISA (Fig. 2G) 18 days after disease induction, albeit, glomerular $p27^{Kip1}$ transcript levels were not increased on day 18 after disease induction (Fig. 2H). Double-stainings demonstrated UCH-L1 and $p27^{Kip1}$ co-expression in hypertrophied podocytes in PHN (Fig. 2I). Cytoplasmic $p27^{Kip1}$ staining was prominent in podocytes of PHN rats, whereas mostly a nuclear $p27^{Kip1}$ signal was observed in podocytes of control rats.

Taken together, these data suggest that in human and rodent MGN UCH-L1 expression was increased in hypertrophic podocytes and correlated with increased $p27^{Kip1}$ protein expression and cytoplasmic localization.

2.3. Podocyte hypertrophy and glomerular protein content are regulated by UCH-L1 in experimental MGN

We therefore asked whether inhibition of UCH-L1 hydrolase function in PHN reduced the morphologic and biochemical parameters of podocyte hypertrophy. PHN and control rats were treated for 14 days with LDN57444, a selective inhibitor of UCH-L1 hydrolysis function [6]. Light-microscopic evaluation demonstrated a decreased swelling of podocytes in PHN rats treated with LDN57444 (Fig. 3A, d). Quantification of podocyte cell body area on PAS stainings demonstrated a significant reduction in podocyte area following UCH-L1 inhibition in PHN rats (Fig. 3B). Supporting, the biochemical parameter of hypertrophy (protein/gDNA ratio) was decreased in LDN57444 treated compared to untreated PHN glomeruli (Fig. 3C). The extent of subepithelial immune deposits and local complement activation was similar in PHN rats compared to PHN+LDN rats (Supplementary Fig. 1).

2.4. UCH-L1 over-expression increases podocyte hypertrophy by accumulation of protein

Since UCH-L1 inhibition reduced total glomerular protein content and podocyte hypertrophy in PHN rats, we evaluated whether UCH-L1 over-expression in cultured podocytes caused hypertrophy through increased protein levels. We established an inducible over-expression system using the doxycycline sensitive tet-on system, in order to control for the extent of UCH-L1 protein increase in podocytes (Fig. 4). Differentiated podocytes exhibited a concentration (Fig. 4A) and time-dependent (Fig. 4B) over-expression of UCH-L1 in response to doxycycline. Control podocytes (tet−, which lack the UCH-L1 insert in the pRetroX-Tight plasmid) exhibited no increased UCH-L1 expression upon doxycycline stimulation (Fig. 4C). For the following experiments UCH-L1 induction was performed with 5 ng/ml doxycycline for 72 h, conditions that induced a moderate and robust UCH-L1 over-expression.

The cell diameter was measured in non-adherent podocytes in an automated cell counter. Over-expression of UCH-L1 significantly increased the cell diameter in comparison to un-induced control