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Acyl-CoA binding protein (ACBP) is highly expressed in the developing human kidney

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Abstract. – OBJECTIVE: Acyl-CoA-binding protein (ACBP), also known as diazepam binding inhibitor (DBI), is a small phylogenetically conserved protein. This ancestral peptide is multifunctional, performing intracellular activities as ACBP protein or extracellular roles as DBI. Several studies showed its endless facets, including a relevant activity as appetite stimulator and as anabolic factor. High levels of ACBP have been described in erythrocytes, liver, kidney, and gut cells. The aim of this study was to analyze, at immunohistochemical level, the expression of ACBP in fetal human tissues during development, focusing on the developing kidney.

MATERIALS AND METHODS: Immunohistochemistry for ACBP was performed on 30 human fetal kidneys, from 15 fetuses of gestational age ranging from 13 to 19 weeks. At autopsy, all kidney samples were 10% formalin-fixed, routinely processed and paraffin-embedded. Five micron-thick paraffin sections were stained with Hematoxylin and Eosin and PAS stain for a morphological examination.

RESULTS: ACBP was detected in all 30 kidneys analyzed in this study. No significant changes in ACBP expression were observed at different gestational ages. Immunostaining for ACBP was restricted to the epithelium covering the renal pelvis, the papillae, the collecting tubules, and the proximal and distal tubules. On the other hand, medullary regions and in the metanephric mesenchymal stem/progenitor cells did not show any reactivity for ACBP.

CONCLUSIONS: According to our findings, ACBP should be considered as a new player in the complex field of human nephrogenesis, given that it was detected in all fetal kidneys immunostained. Its preferential localization in the renal structures derived from the Wolf duct, such as pelvis epithelium and collecting ducts, suggests a major role for ACBP in the induction of the metanephric mesenchymal cells toward the differentiation into glomerular structures. ACBP expression in proximal and distal tubules, two structures originating from the metanephric mesenchyme, indicates a further role of this protein in nephron development. In conclusion, ACBP should be added to the multiple molecules involved in human nephrogenesis.

Key Words:
Acyl-CoA binding protein, Immunohistochemical study, Developing kidney.

Introduction

Acyl-CoA binding protein (ACBP) is a small (about 10 KDa) protein acting as an intracellular acyl-CoA transporter[1]. It is an ancestral phylogenetically conserved protein, present in plants, fungi and animals although it appears to be absent in prokaryotes except few bacteria[2-4]. It has been proposed that ACBP plays a pivotal role in the intracellular trafficking and utilization of long-chain fatty acyl-CoA esters. Indeed, ACBP has been isolated from bovine liver specimens,

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where it participates to long chain fatty acid synthesis[5], in porcine intestinal cells, regulating insulin release[6], and in bovine adrenal glands, where it acts as a regulator of the intracellular transport of cholesterol[7]. Depletion of acyl-CoA binding protein in yeast results in aberrant organelle morphology including fragmentation of endosomes and vacuoles, multi-layered membranes, and accumulation of vesicles of variable sizes[3]. The levels of very-long-chain fatty acids, long-chain bases and ceramide are severely affected by ACBP depletion, suggesting that ACBP, rather than playing a general role, might accomplish specific tasks in the cellular lipid metabolism[3]. ACBP is also known as diazepam binding inhibitor (DBI), because it was described in 1983 as a brain peptide with high affinity for benzodiazepam Gamma-AminoButyric Acid (GABA) receptors[8], even though this ability was not confirmed by Faergeman et al[3].

Besides serving as essential substrate for β-oxidation and synthesis of triacylglycerols, ACBP is increasingly recognized as an important regulator of enzyme activities and gene transcription[3].

Moreover, ACBP has been recently implicated in the regulation of autophagy, the mechanism throughout which portions of the cytoplasm are sequestered within autophagosomes, digested in secondary lysosomes and converted into nutrients for adaptative stress responses[9]. The possible link between autophagy and ACBP is connected with the ability of the autophagosomes to induce release of ACBP out of cells when starvation induces autophagy, allowing ACBP to move on the cell surface receptors and so stimulating feeding behaviours[10]. Recently, ACBP has been characterized as well as a relevant appetite stimulator factor, carrying an evolutionary ancient role in appetite control[11]. In other words, ACBP might act as an anabolic factor, balancing and mitigating the catabolic effects due to nutrient deprivation[12,13].

Despite of the different amount detected, ACBP expression is ubiquitously expressed, but in different amounts, in all the tissues analyzed from early stages of embryogenesis[14]. A study[15] carried out in rat evidenced high ACBP levels in red blood cells, followed by liver, kidney, heart, intestine and muscle cells. Moreover, ACBP is expressed in selected neuronal populations of the rat brain[16]. In humans, ACBP has been detected in the brain, spinal cord and in peripheral tissues[17] as well as in brain tumors[18], in hepatocellular carcinoma[19] and in ovarian cancer[20]. A reference database for gene expression profiling in human tissues showed that ACBP is primarily expressed in the hypothalamus[21].

Given the scarcity of immunohistochemical studies regarding ACBP expression in human tissues during fetal development, the aim of this study was to analyze ACPB expression in the human kidney during the intrauterine life.

Materials and Methods

Ethical approval has been granted by Ethics Experimentation Committee of the University of Cagliari (Protocol number: PG/2020/10914 EMI-FU). All protocols, in this context, have been carried out in full conformity with the rules and guidelines expected for this kind of trial.

A total of 30 kidneys were utilized for the immunohistochemical study, from 15 fetuses of gestational age ranging from 13 to 19 weeks. All kidneys were retrieved from the files of the Department of Pathology of the University Hospital San Giovanni di Dio of the University of Cagliari. Kidneys were 10% formalin-fixed, routinely processed and paraffin-embedded. Five micron-thick paraffin sections were stained with Hematoxylin and eosin and Periodic Acid-Schiff (PAS) stain for a morphological examination.

Immunohistochemistry

The streptavidin-biotin-peroxidase detection system was employed for immunohistochemistry. In brief, paraffin sections were mounted on aminopropyl-triethoxysilane-coated glass slides, then, deparaffinized in xylene (2 X 5 min) and rehydrated in graded solutions of ethanol (100, 90 and 70%, 3 min each).

In order to block endogenous peroxidase, sections were immersed in 3% hydrogen peroxide for 10 min, and then, left in running water for 2 min. For antigen retrieval, sections underwent microwave treatment and then they were incubated with the primary antibody against ACBP [Source: ABCAM, (Cambridge, UK) (AMab16806); Clone: 27C9; Working dilution: 1:600; Incubation time: 32']. Sections were incubated with the secondary antibody, biotinylated rabbit anti-mouse IgG (1:50 dilution in phosphate buffered-saline, PBS; Roche "Basel, Switzerland") for 30 min and washed in PBS (3 X 5 min). The streptavidin-biotin-horseradish peroxidase complex (Roche, Ba-

silea, Switzerland) was then added to the sections (1:500 dilution in Tris-buffered saline) for 30 min, followed by a wash in PBS (3 min).

Sections were counterstained with Mayer hematoxylin, rehydrated in graded ethanol rinses, cleared with xylene and mounted in a mixture of distyrene, a plasticizer, and xylene (DPX).

Five normal fetal human livers were used as positive control. For negative controls, the primary antibody was replaced by PBS. In order to ensure reproducibility, immunostained sections were scored separately by two pathologists. No significant interobserver variability was found.

The pattern of immunostaining of ACBP was recorded as one of the following: nuclear, paranuclear (Golgian-like), cytoplasmic diffuse, cytoplasmic vesicular, membranous. The intensity of immunoreactivity was also scored as either negative, mild, and strong.

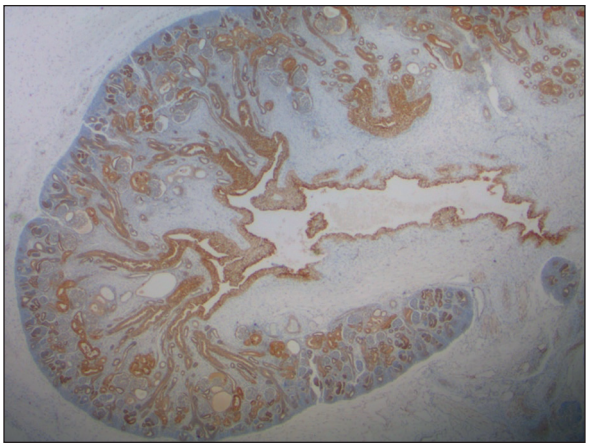

Figure 1. A panoramic view of a fetal kidney (14 weeks of gestation) immunostained for ACBP. Reactivity is mainly observed in the epithelial cells of the renal pelvis and along the collecting tubules. No reactivity is observed in the medulla. In the cortex, ACBP is mainly expressed in large tubules. No immunostaining is detected in the metanephric mesenchyme, located in close proximity to the renal capsule (original magnification ×15).

Results

All 30 kidneys analyzed were characterized by a relevant immunoreactivity for ACBP. No significant changes regarding immunoreactivity for ACBP were detected among the fetal kidneys at different gestational ages (13-19 weeks) analyzed.

At low power, the expression of ACBP appeared restricted to some kidney compartments, whereas others were devoid of it. The highest reactivity was observed in the epithelium covering the renal pelvis, the papillae, in the collecting tubules and in the proximal and distal tubules (Figure 1). No significant immunostaining was found in the medullary regions and in the metanephric mesenchymal cells located in close proximity to the renal capsule.

At higher power, ACBP expression in the nephrogenic zone appeared very selective, with intensively reactive compartments adjacent to negative structures. The highest levels of ACBP expression were found in the proximal tubules, in the different stages of nephron development. Regarding the pattern of immunostaining, ACBP appeared highly expressed at the cell membrane, diffuse in the cytoplasm and inside the nucleus. Moreover, a strong ACBP immunoreactivity was detected in the brush border of the epithelial cells of the proximal tubules. A mild cytoplasmic staining was observed in the ureteric bud tips, in the collecting tubules and in distal tubules. No reactivity was found in the subcapsular stem/progenitor cells, in the interstitial cells and in the glomeruli (Figure 2). The analysis of the most differentiated proximal tubules revealed the occurrence of ACBP secretion into the lumen by epithelial cells, where the top ACBP expression were localized at the brush border (Figure 3). The study of developing nephrons in the early stages further confirmed the strong variability of ACBP expression in adjacent structures. The

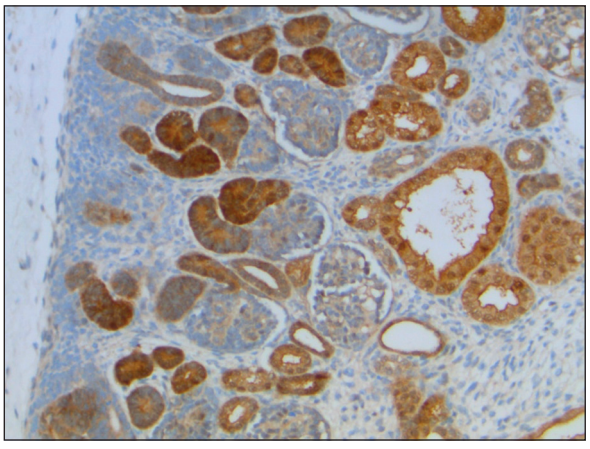

Figure 2. At higher power, ACBP is unevenly expressed in the cortical zone. Absent in the capsule and in the undifferentiated metanephric mesenchyme (on the left), ACBP is highly expressed in comma-shaped and in S-shaped tubules of developing nephrons. Absent inside glomeruli and in the interstitial cells, ACBP is strongly expressed at cytoplasmic and nuclear levels in the more mature proximal tubules (PT) (original magnification ×25).

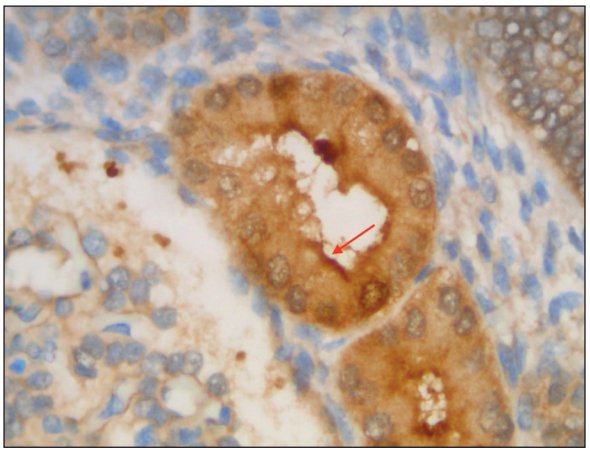

Figure 3. High power view of a proximal tubule shows strong cytoplasmic and nuclear immunostaining for ACBP in epithelial cells. The highest levels of immunoreactivity are observed along the brush border (*arrow*). ACBP appears to be also secreted into the lumen (original magnification ×40).

strong expression of the protein in the proximal tubules contrasted with the very mild, or absent immunostaining in the glomerulus and with a mild reactivity in the epithelium of the distal tubules. Regarding the Bowman capsule, the majority of parietal epithelial cells did not show any reactivity for ACBP, whose expression was restricted to capsular epithelial cells of the urinary pole (Figure 4). No staining was observed in capsular epithelial cells of the other regions of the Bowman capsule. ACBP expression in the very early stages of nephron development, at the S-shaped stage, revealed a strong expression in the proximal developing tubule and in the distal developing tubule, but confirmed the very mild or absent immunoreactivity in the precursors of the Bowman capsular cells and in the podocyte precursors (Figure 5). A strong reactivity for ACBP allowed the identification of a fundamental process occurring in the nephrogenic zone: the fusion between the developing distal tubules and the ureteric bud tips, granting the passage of the glomerular fluid into the collecting tubules. ACBP appeared strongly expressed in the cells giving rise to this fusion, showing a cytoplasmic diffuse staining pattern, which contrasted with the mild expression of ACBP in the collecting tubules (Figure 6). A mild level of reactivity for ACBP was detected in the cells of the ureteric bud tips, the cells deputed to induce epithelial-mesenchymal transition in metanephric mesenchymal precursors, leading to the nephron origin (Figure 7). At high power, a strong membranous pattern of reactivity was found in the epithelial layer of the pelvis and papillae, as well as in the epithelium of the developing collecting tubules (Figure 8). In these cells, ACBP was mainly expressed along the cell membrane: the highest levels of reactivity were observed at the apical pole of the epithelial cells.

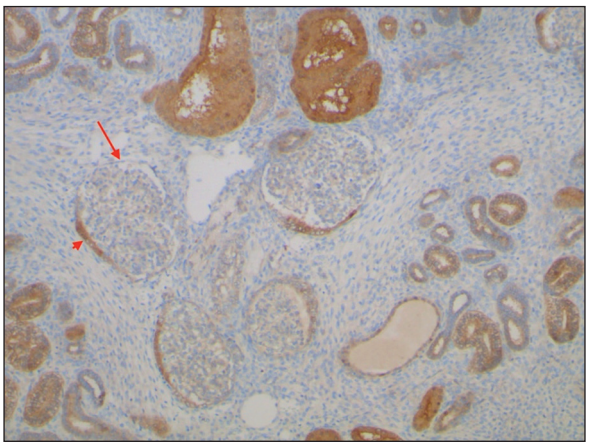

Figure 4. The selective reactivity for ACBP allows the easy detection of the proximal tubule (*strongly positive*) emerging from the developing glomerulus (negative). Note the absence of immunostaining in the capsular epithelial cells of the Bowman capsule (*arrows*) contrasting with the strong immunoreactivity in the capsular cells at the urinary pole (*arrowheads*). A mild focal cytoplasmic expression is found in a distal tubule (DT) (original magnification ×20).

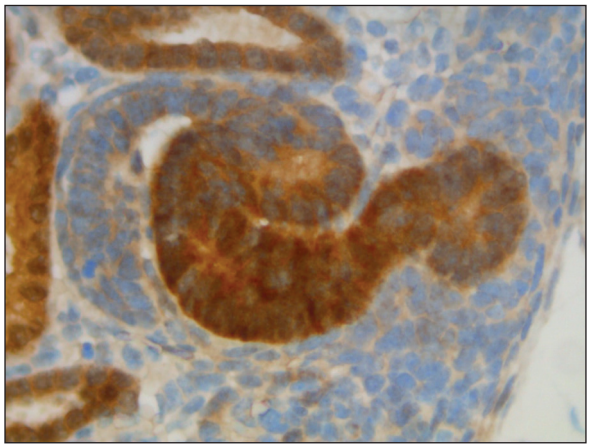

Figure 5. A nephron in its very early stage of development. ACBP is not detected in the podocyte precursors nor in the developing Bowman capsular cells. A strong cytoplasmic reactivity is detected in the proximal and distal portion of the S-shaped body, giving rise to the proximal tubule (PT) and to the distal tubule (DT). No significant reactivity for ACBP is found in the surrounding spindle-shaped metanephric mesenchymal cells (original magnification ×63).

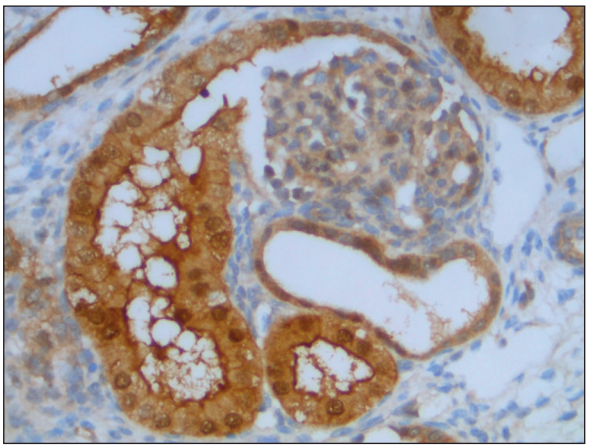

Figure 6. Strong diffuse cytoplasmic expression of ACBP in the distal tubule emerging from a developing nephron (*on the right*). The ACBP-reactive distal tubule is going to fuse with a collecting tubule (CT) originating from the ureteric bud. ACBP-positive globules are also evidenced inside the lumen of the distal tubule (original magnification ×40).

Discussion

Kidney development is mainly based on differential cell type-specific expression of a vast number of genes and gene products[22,23]. While the multiple critical genes and molecular pathways involved in human nephrogenesis have been elucidated[24], a wide immunohistochemical analysis focused on the expression of the multiple gene products within the specific cells and anatomic structures involved in nephrogenesis epithelial-mesenchymal transition in metanephric mesenchymal precursors, leading to[25,27,28]. Accomplishing this objective might provide important new insights toward a better comprehension of the fundamental developmental mechanisms involved in human nephrogenesis. These main mechanisms embrace branching morphogenesis, intimal interactions between ureteric bud tips and the metanephric mesenchyme, mesenchymal-epithelial transition, and multiple segmentation events[23,29]. Previous studies from our group revealed the expression pattern of multiple gene products in the developing human kidney. Examples include Thymosin beta-10[30], Thymosin beta-4[31], Wilms Tumor Suppressor (WT1)[32], mucin 1, cell surface associated (MUC1)[33,34], proteolytic enzyme (CD10)[35], galectin-3[36], and CD44[37]. In this study, we introduce a new actor in the complex scenario of human nephrogenesis, showing that ACBP is highly expressed in the developing human kidney.

As for the other previous immunohistochemical markers analyzed in the fetal kidney, ACBP expression pattern was also characterized by differences within the multiple cells and anatomical structures cooperating in human nephrogenesis. The highest ACBP expression was detected in the proximal tubules, in the distal extreme of the S-shaped body during the process of fusion with the collecting tubules, in Bowman capsular cells at the urinary pole, in the collector tubules and

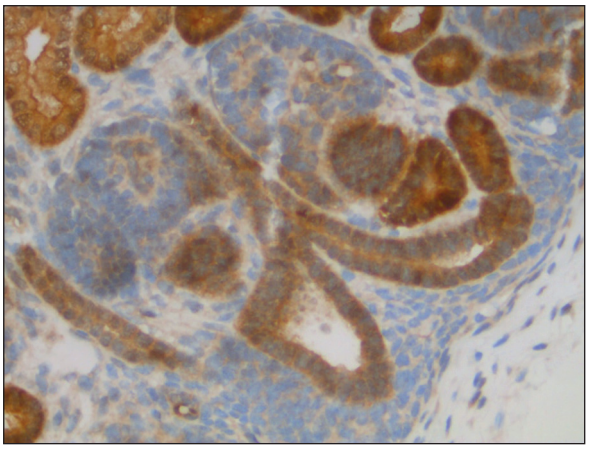

Figure 7. The ypsilon-shaped structure represents two ureteric bud tips originating from the renal hilum and extending towards the subcapsular nephrogenic zone. The highest levels of immunoreactivity for ACBP are observed in the renal vesicles (RV) and in the distal tubules originating from the developing nephrons (original magnification ×40).

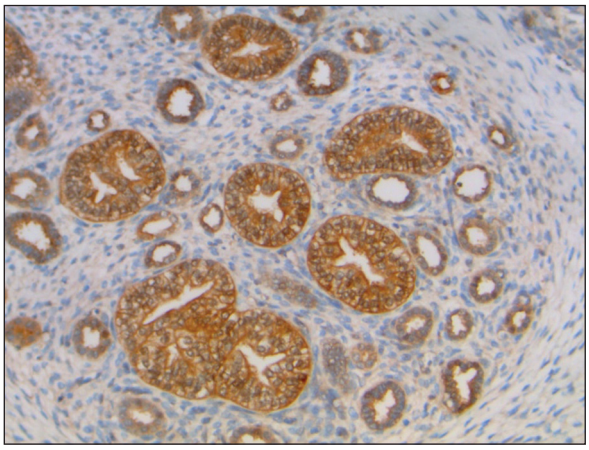

Figure 8. The highest levels of ACBP expression are detected in the renal pelvis and in the epithelial cells of the collecting ducts emerging from it. In these structures, ACBP immunostaining shows a membranous pattern, with reinforcement at the luminal pole of the cells. Moreover, a strong expression of ACBP is observed at nuclear level. Note the absence of reactivity in the interstitial cells (original magnification ×20).

in the transitional epithelium of the renal pelvis. On the other hand, ACBP expression was weak or even absent in the renal capsule, in the stem/progenitors of the metanephric mesenchyme and in the glomeruli. Moreover, ACBP immunostaining was negative in the medulla, including Henle loops and medullary interstitial cells.

These data taken together, indicate a strict association between ACBP expression and a high cellular metabolic activity. Focusing on the kidney cells characterized by the highest immunoreactivity for ACBP, we may point out different examples. The epithelial cells of the proximal tubules, which are responsible for most of sodium, chloride, and bicarbonate reabsorption, are also those that firstly showed the highest immunoreactivity for ACBP[38]. Since this process occurs against the electrochemical gradient, it is maintained by the basolateral Na (+)-K (+)-ATPase, the primary energy-requiring transport step in the process of reabsorption from the glomerular fluid[39]. In short, the proximal tubule has one of the highest metabolic rates among kidney structures. This functional specificity is confirmed, at ultrastructural level, by the high number of mitochondria, that are necessary to sustain the high levels of ATP needed for oxidative phosphorylation to support high electrolyte transport activity in renal proximal tubules[40]. The finding of a strong immunoreactivity for ACBP in proximal tubule cells represent a link between ACBP expression and a high metabolic cell rate.

The other structure in which ACBP appeared constantly hyper-expressed is the distal part of the S-shaped body going toward the fusion with the nearest collecting duct. This event is a crucial one in the complex process of formation of a functional renal network during nephrogenesis[23]. Once the renal vesicle is formed and the new nephron arises from the metanephric mesenchyme, the interconnection of two epithelial tubes is required. These two structures incorporate: 1) the distal part of the new nephron, which will give rise to the distal tubule, and 2) the collecting tubule, which originates from the branching ureteric epithelium. How this connection occurs, however, is incompletely understood[41]. Even in this case, we may speculate that such a complex process should necessitate a high metabolic activity, given the complexity of a fusion process between two tubular structures.

Another structure carrying a strong reactivity for ACBP in our study was the epithelium of the renal pelvis and that of the collecting tubules, both originating from the ureteric bud. In the early stages of kidney development, the primary ureteric bud, emerging from the Wolffian duct, progressively invades the metanephric mesenchyme and, through a process of branching morphogenesis, the branching tips join the mesenchymal precursors located in close proximity to the renal capsule[23,42]. As a consequence of this dynamic process, collecting tubules should be considered as a very active component in this phase of kidney development, being involved in the invasion of the metanephric mesenchyme. The high expression of ACBP at the cell membrane of the proliferating epithelial cells of the collecting tubules probably underlay the high metabolic rate of this renal compartment. This hypothesis may be reinforced by the report of high glycogen deposits in the cytoplasm of collecting duct epithelium during nephrogenesis, easily revealed by the PAS stain[43].

On the opposite side of the spectrum, the glomeruli displayed weak or absent immunostaining for ACBP. In our study, this observation was detected both in the early stage of nephron development and in more mature glomeruli. In the former, ACBP was absent in podocyte precursors as well as in parietal capsular cell precursors (Figure 5); in the latter, no significant reactivity for ACBP was detected in podocytes, in mesangial cells, in glomerular capillaries and in parietal epithelial cells (Figure 2). In a previous immunohistochemical study carried out on fetal human kidneys, CD10 was identified as a useful marker for the identification of podocytes and parietal epithelial cells in the glomeruli of all the different steps of their differentiation[35].

The other renal compartment in which very low levels of ACBP was poorly detected is the nephrogenic zone, including the multipotent spindle mesenchymal stem cells and the roundish cap mesenchymal cells surrounding the ureteric bud tips. In previous studies, undifferentiated stem/progenitors were characterized by immunoreactivity for WT1[32], whereas the cap mesenchyme showed immunoreactivity for B-cell lymphoma 2 (Bcl2) and paired box 2 (PAX2)[26]. According with the hypothesis that weak ACBP expression might parallel a low metabolic rate, a recent immunohistochemical study, focused on the development of the vascular tree in the developing kidney and revealed a low vascularization of the nephrogenic zone. This study also suggested a major role of hypoxia in the maintenance of nephrogenesis[44]. Given that oxygen supply is a fundamental factor for cell metabolism, and that stem/progenitors frequently adopt a quiescent state to preserve key

functional features[45], the poor ACBP expression in the renal stem cell compartment might reflect a low metabolic state of this zone.

Conclusions

Our study showed that ACBP plays a major role in human nephrogenesis. According to with its patchy expression, ACBP influence might be restricted to the development of peculiar structures, involving proximal and distal tubules, and developing collecting tubules. The high ACBP expression in cells participating to the fusion between the distal part of a new nephron and a collecting duct, a crucial advent in nephrogenesis, indicates a major role for ACBP in nephrogenesis. For these reasons, further studies are necessary to better analyze ACBP expression in all stages of kidney development, as well as in postnatal kidneys, in health and in pathological conditions.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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