Published in final edited form as:

J Urol. 2010 March ; 183(3): 1193–1200. doi:10.1016/j.juro.2009.11.006.

Human Amniotic Fluid as a Potential New Source of Organ Specific Precursor Cells for Future Regenerative Medicine Applications

Stefano Da Sacco, Sargis Sedrakyan, Francesco Boldrin, Stefano Giuliani, PierPaolo Parnigotto, Rezvan Habibian, David Warburton, Roger E. De Filippo*, and Laura Perin*[†]
Division of Urology, Laboratory for Organ Regenerative Research and Cell Therapeutics, Saban Research Institute, Children's Hospital Los Angeles and Developmental Biology Program, Keck School of Medicine, University of Southern California, Los Angeles, California

Abstract

Purpose—Human amniotic fluid contains multiple cell types, including pluripotent and committed progenitor cells, and fully differentiated cells. We characterized various cell populations in amniotic fluid.

Materials and Methods—Optimum culture techniques for multiple cell line passages with minimal morphological change were established. Cell line analysis and characterization were done with reverse transcriptase and real-time polymerase chain reaction. Immunoseparation was done to distinguish native progenitor cell lines and their various subpopulations.

Results—Endodermal and mesodermal marker expression was greatest in samples of early gestational age while ectodermal markers showed a constant rate across all samples. Pluripotent and mesenchymal cells were always present but hematopoietic cell markers were expressed only in older samples. Specific markers for lung, kidney, liver and heart progenitor cells were increasingly expressed after 18 weeks of gestation. We specifically focused on a CD24+OB-cadherin+ population that could identify uninduced metanephric mesenchyma-like cells, which in vivo are nephron precursors. The CD24+OB-cadherin+ cell line was isolated and subjected to further immunoseparation to select 5 distinct amniotic fluid kidney progenitor cell subpopulations based on E-cadherin, podocalyxin, nephrin, TRKA and PDGFRA expression, respectively.

Conclusions—These subpopulations may represent different precursor cell lineages committed to specific renal cell fates. Committed progenitor cells in amniotic fluid may provide an important and novel resource of useful cells for regenerative medicine purposes.

Keywords

kidney; amniotic fluid; stem cells; gene expression; cell lineage

Amniotic fluid fills the amniotic cavity, providing a supportive medium for the developing fetus. Amniocentesis is a common, safe, reliable screening tool for the human fetus. AF volume and composition change during pregnancy and reflect fetal physiology. AF is

© 2010 by American Urological Association Education and Research, Inc.

[†] Correspondence: Developmental Biology and Regenerative Medicine Program, Saban Research Institute, Children's Hospital Los Angeles, 4661 Sunset Blvd., Mailstop 35, Los Angeles, California 90027 (telephone: 323-361-8334; FAX: 323-361-3613; lperin@chla.usc.edu).

*Equal study contribution.

Supplementary material for this article can be obtained at <http://www.childrenshospitala.org/AmnioticFluid>.

principally composed of fetal urine and lung exudates with a minor contribution from the amnion.¹ The recent discovery that AF contains novel stem cells (AFS) characterized by the expression of *c-kit* (stem-cell factor receptor) suggests that AFS may be useful for regenerative medicine. De Coppi et al noted that *c-kit*⁺ cells isolated from AF have the potential to differentiate into all 3 germ layers.² Our group reported that AFS can integrate into kidney³ and lung.⁴ However, *c-kit*⁺ cells derived from AF comprise less than 1% of the entire AF cell population. Characterization of the remaining cells in AF is incomplete, including their possible role as putative stem cells or progenitors capable of differentiation into mature, functional cell types.

We further characterized the AF cell population from samples obtained between 15 and 20 weeks of gestation (the most common time points for amniocentesis), focusing on progenitor cells of all 3 germ layers and on cells committed to specific organs/tissues. From AF we isolated a cell subpopulation with characteristics of tubular and glomerular precursor kidney progenitor cells.

Subpopulations of AF progenitor cells with renal characteristics could be a useful tool for therapy for various kidney diseases due to their commitment toward kidney cell types. Isolation of tubular and glomerular progenitors, particularly podocyte progenitors, may herald a novel approach to kidney regeneration compared to using pluripotential undifferentiated cells.

Materials and Methods

Human Total AF Cell Population Expansion

A total of 28 discarded human AF samples (Genzyme®) with normal male karyotype and fetal ultrasound were collected by amniocentesis between 15 and 20 weeks of gestation. Cells were expanded in tissue culture dishes (BD-Falcon, Franklin Lakes, New Jersey) with 3 types of culture medium, including 1) Chang's medium, composed of α -MEM, 20% Chang-B and 2% Chang-C (Irvine Scientific®), L-glutamine, 20% ES fetal bovine serum and 1% antibiotic, 2) Amniomax™-II as provided, and 3) Dulbecco's MEM with 10% fetal bovine serum and 1% antibiotic (Gibco®/Invitrogen™). Cells were trypsinized using trypsin 0.25% ethylenediaminetetraacetic acid (Gibco/Invitrogen) and cultured at 37°C in 5% CO₂ for 50 passages.

Mesenchymal, Hematopoietic and Solid Organ Precursors

RT and real-time PCR—The 28 human AF samples were stratified by gestational age, cultured for 4 to 5 passages and assigned to be assayed by RT-PCR or real-time PCR. A total of 16 AF samples were analyzed by RT-PCR for an extensive panel of markers from all 3 germ layers, including mesenchymal and hematopoietic precursors, and early progenitor cells from specific solid organs. Total RNA was isolated using the RNeasy™ Mini Kit as described on the data sheet. RNA (1 μ g) was reverse transcribed and amplified in the presence of specific primers (Operon, Huntsville, Alabama) according to standard amplification conditions. A total of 12 AF samples were analyzed by real-time PCR to quantitate expression of the mentioned specific markers. Real-time PCR was done using a LightCycler® 480 and LightCycler TaqMan® Master Mix. A total of 35 cycles were done under standard conditions.

Western blot analysis—Total cell lysates were prepared using the Nuclear Extract Kit (Active Motif®) according to manufacturer instructions. Protein concentration was measured with ultraviolet-visible spectroscopy. Proteins were probed with various antibodies at 1:1,000 concentration. Peroxide conjugation of secondary antibodies (Sigma-

Aldrich®) was done and signal detected for 1 minute using echochemiluminescence detection reagent on Biomax® Light Film.

Renal Precursor Cell Types, and CD24+OB-Cadherin+ (MMC) and Kidney Progenitor (AKPC) Cells

CD24+OB-cadherin+ cell and AKPC immunoseparation—Immunoselection was done with MS columns (Miltenyi Biotec, Bergisch Gladbach, Germany) for CD24 according to manufacturer instructions. After CD24+ cell expansion second selection was done for OB-cadherin. CD24+OB-cadherin+ cells were replated for subsequent expansion. Further immunoselection to identify subpopulations of renal progenitors from the CD24+OB-cadherin+ population was performed as described for human nephrin, TRKA, PDG-FRA, E-cadherin and podocalyxin. This final subpopulation selection was done after 18 passages. Cells were reseeded under the conditions described.

CD24+OB-cadherin+ and AKPC RT and real-time PCR—The CD24+OB-cadherin+ population and the 5 subpopulations were analyzed by RT-PCR for markers of cells committed to kidney development to compare gene expression patterns, and by real-time PCR for *GDNF*, *WT-1*, *LIM-1*, *PAX-2*, nephrin, *OCT-4*, *TRKA*, *PDGFRA*, E-cadherin, *ZO-1*, podocalyxin and occludin.

Statistical Analysis

Real-time PCR results are shown as the normalized ratio $\pm$ SD, as calculated by LightCycler 480 relative quantification software (figs. 1 to 4).

Results

Human Total AF Cell Culture

Total cell population morphology was heterogeneous with a preponderance of fibroblastoid shapes (fig. 5, A). Total AF cell population expansion was possible for up to 10 passages using Dulbecco's MEM, after which cells ceased to grow. Amniomax II and Chang medium allowed expansion for more than 50 passages but only Chang medium maintained original cell morphology. Thus, we used Chang medium in all experiments.

Mesenchymal, Hematopoietic and Solid Organ Precursors

Human AF cell population RT-PCR—AF cell samples were stratified by week of gestation (15 to 20 weeks) and analyzed using RT-PCR for markers in all 3 germ layers, including pluripotent, mesenchymal, hematopoietic and early organ progenitor cells. Expression of genes characteristic of endodermal and mesodermal germ layers decreased with time, while ectodermal markers were consistently expressed (fig. 5, B). Pluripotent markers were expressed at all gestational ages analyzed at less than 19 weeks. While the mesenchymal marker CD90 was expressed at all time points, the hematopoietic marker CD34 was absent in early gestation samples but appeared after 18 weeks of gestation (fig. 5, B). Early progenitor markers from different organs were expressed in 18-week and in older samples (fig. 5, C).

Real-time PCR—Four samples per time point (15 to 16, 17 to 18 and 19 to 20 weeks) were analyzed. Some markers, such as brachyury, *TAL-1*, nephrin and *TRKA*, were not expressed in 1 or more samples. Goosecoid and *PDX-1* were not found in any sample analyzed. The epithelial marker E-cadherin increased 15-fold at 17 to 18 weeks of gestation. *NCAM* and *FGF5* did not change significantly with time (fig. 1). The mesodermal marker brachyury was expressed at 15 to 16 weeks in only 1 sample. *TAL-1* appeared to decrease

with time but *FLK1* increased 4-fold (fig. 1). The endodermal marker *CXCR-4* increased 3.5-fold between 15 to 16 and 19 to 20 weeks while *SOX-17* and *AFP* tended to decrease (fig. 1). The pluripotency marker *OCT-4* did not change over the range investigated but *c-kit* increased 3-fold at 17 to 18 weeks only to disappear in older samples (fig. 2, B). The hematopoietic marker *CD34* decreased after 17 to 18 weeks. The mesenchymal marker *CD90* increased 2-fold by 17 to 18 weeks (fig. 2, A). Progenitor markers, excluding *PDX-1* with no expression, generally increased with gestational age. The early cardiac marker *NKX2.5* was increased 6-fold at 19 to 20 weeks (fig. 3, A). The lung/thyroid marker *NKX2.1* doubled at 17 to 18 weeks and was 2.5-fold at 19 to 20 weeks. *CEBPG* increased 5-fold at 17 to 18 weeks vs that at 15 to 16 and 19 to 20 weeks of gestation (fig. 3, C).

Western blot analysis—Samples were analyzed by Western blotting for the described markers. Data confirmed RT-PCR results (data not shown).

Renal Precursor Cell Types, and CD24+OB-Cadherin+ (MMC) and Kidney Progenitor (AKPC) Cells

RT and real-time PCR—On RT-PCR *LIM-1*, aquaporin-1, *ZO-1* and occludin were found in early and late AF samples. *CD24*, OB-cadherin, *PAX-2*, *GDNF* and nephrin were mostly expressed by 18 weeks of gestation (fig. 4, A). On real-time PCR *CD24* and OB-cadherin doubled at 17 to 18 weeks. *CD24* remained unchanged between 17 to 18 and 19 to 20 weeks while OB-cadherin decreased to the previous level (fig. 4, B). *PAX-2* increased slightly between 15 to 16 and 19 to 20 weeks but *LIM-1* did not change (fig. 4, B). One sample per period was positive for nephrin, which increased with time to double at 19 to 20 weeks of gestation (fig. 4, B). *ZO-1* and aquaporin-1 did not change significantly with time. Occludin was increased 8-fold at 19 to 20 weeks (fig. 4, B). *GDNF* and *PDGFRA* were found in only some samples. No conclusive analysis could be done of the partial data retrieved but graphs are shown as additional information.

CD24+OB-cadherin+ cell and AKPC immunoseparation—CD24+OB-cadherin + cells had more uniform morphology than the total AF cell population (fig. 6, A). Long cell processes typical of podocytes cultured in vitro were more apparent in CD24+OB-cadherin+ cells. After expansion for 4 passages subpopulations expressing podocalyxin, TRKA, nephrin, PDGFRA and E-cadherin were obtained by immunoseparation.

Further RT-PCR—The CD24+OB-cadherin + population and the 5 derived subpopulations were characterized by RT-PCR for kidney and pluripotency markers. Renal marker expression differed in these populations (fig 6, B). The CD24+OB-cadherin+E-cadherin+ population expressed E-cadherin and *GDNF*, and was mildly positive for *nephrin*. CD24+OB-cadherin+ nephrin+ cells were clearly positive for *nephrin*. CD24+OB-cadherin+PDGFRA+ cells were positive for *ZO-1* and *PDGFRA*. CD24+OB-cadherin+TRKA+ cells expressed *TRKA*, *ZO-1* and a low level of *PDGFRA*. CD24+OB-cadherin+podocalyxin+ cells were positive for *GDNF* and slightly positive for *nephrin* (fig. 6, C).

Further real-time PCR—The CD24+OB-cadherin+ population and the 5 subpopulations were analyzed by real-time PCR for specific kidney markers. The E-cadherin+ subpopulation showed 2-fold increased E-cadherin, 7-fold increased occludin and 9-fold increased *LIM-1* expression. *OCT-4* was increased 12-fold. The PDGFRA+ subpopulation showed a 2-fold increase in *PDGFRA*. The TrKA+ subpopulation expressed comparable levels of *LIM-1*, *PAX-2*, *OCT-4*, E-cadherin, *PDGFRA* and *TRKA* while occludin increased about 2-fold. The nephrin+ selection showed an 11-fold increase in *GDNF*, *LIM-1* and *OCT-4*, a 4-fold increase in *PAX-2* and a 2-fold increase in nephrin as well as a 4-fold increase in occludin and a 7-fold increase in *ZO-1*. *WT-1* was expressed but podocalyxin

was not. The podocalyxin + population showed decreased *LIM-1*, *PAX-2*, *GDNF* and *OCT-4* while podocalyxin increased. *WT-1* was also noted.

Discussion

In recent years stem and progenitor cells have emerged as a promising regenerative medicine tool. Their characteristics of self-renewal and pluripotency suggest that stem cells may be useful to repair injured tissue and reconstruct damaged organs.⁵ AF recently raised the interest of scientists as an alternative source of pluripotent cells. Due to its contact with the developing fetus AF contains abundant suspended cells, which have long been used for diagnostic purposes. The presence of mature cells in AF, such as smooth muscle cells, osteoblasts and lung epithelial cells, has long been known.⁶ More recently previously described stem cells were identified.² These AFSs are easily obtained, largely avoid the ethical concerns associated with embryonic stem cell use and propagate in culture without difficulty, maintaining their pluripotential capacity. However, they comprise no more than 1% of the total AF cellular population.

Our main objective was to better characterize the remaining cells in AF, focusing on cells belonging to the renal lineage. We identified cells expressing markers of all 3 germ layers, mesenchymal and hematopoietic, progenitors of different organs and particularly cells with specific renal characteristics, including podocyte precursors, epithelial tubular cells and mesangial cells.

Human embryo development follows a precise timetable during gestation. We hypothesized that cells in the AF are committed to various organs at different gestational time points. In the time range investigated younger samples expressed more frequently and at higher levels markers related to mesodermal and endodermal germ layers while in older samples these markers decreased in frequency and quantity due to AF cell differentiation with time and the different degrees of maturation of cells detaching from the fetus. The expression of organ specific markers also increased with gestational age, presumably due to organogenesis maturation. Since most AF volume derives from fetal urine,¹ it is reasonable to assume that kidney progenitor cells are a major constituent of AF. The expression of renal markers such as *PAX-2*, *LIM-1*, nephrin, *PDGFRA*, *TRKA*, E-cadherin, *CD24* and OB-cadherin showed a clear increase by the end of week 17 of gestation (fig. 4, A).

After noting that kidney progenitors were present in the total AF cell population we selected a specific AF population based on in vivo studies⁷ showing CD24 and OB-cadherin co-expression in the developing kidney, particularly in MM, which together with the ureteral bud gives rise to the mature kidney. We called this population MM-like cells (CD24+OB-cadherin +) (fig. 7). When MM is induced by the ureteral bud, the expression of *GDNF*, *LIM-1*, *PAX-2*, *BMP-2* and other genes becomes evident, as also expressed in our CD24+OB-cadherin+ population. Subsequently with kidney maturation gene expression varies and begins to be restricted to specific cell lineages. Each cell lineage acquires characteristic traits that are driven by specific gene expression and indicated by surface markers such as E-cadherin (mesenchymal-to-epithelial transition cells),⁸ nephrin (podocytes),⁸ podocalyxin (mature podocytes),⁹ *TRKA* (stromogenic cortical mesenchymal cells)¹⁰ and *PDGFRA* (mesangial cells).¹⁰ Thus, these surface markers were used to perform additional immunoselection from the initially isolated CD24+OB-cadherin+ population.

Genes and proteins such as *GDNF*, *WT-1*, *LIM-1* and *PAX-2* in the 5 CD24+OB-cadherin+ subpopulations determine the fate of renal cell types. We also investigated *OCT-4* expression to determine whether subselected cells still had multidifferentiation potential. During kidney development organ specific precursors go through different stages of

differentiation to attain the mature state. During this maturation process pluripotent genes are not turned off suddenly and all maturity specific genes are not suddenly turned on. In the intermediate state co-expression of the 2 gene types occurs. Real-time PCR confirmed a specific temporal pattern of renal marker expression in each of the 5 subpopulations derived from CD24+OB-cadherin + cells (fig. 8).

An interesting result was revealed by WT-1 expression, which was expressed in vivo in the metanephros in the proximal part of the S-shaped body and then exclusively in mature podocytes. Of all isolated subpopulations only nephrin+ and podocalyxin+ cells expressed *WT-1*, indicating that they are indeed podocyte precursor cells.¹¹ Genes such as *PAX-2* or *LIM-1* were not expressed in podocalyxin+ selected cells but were evident in nephrin+ cells, suggesting that nephrin+ cells may represent a more immature podocyte lineage than podocalyxin expressing cells. This concept was also supported by the finding that podocalyxin+ cells did not express *OCT-4* but nephrin+ cells expressed *OCT-4* at high levels. Cultured podocalyxin+ cells showed the typical morphology of cultured human podocytes with primary processes similar to those described by Vogelmann et al (fig. 9).¹² In the other populations *WT-1* was not expressed, showing no commitment to podocyte differentiation. The expression pattern of PDG-FRA+ cells with *PAX-2* expression and absent *LIM-1* suggested commitment to the nephrogenic lineage but not current mesenchymal-to-epithelial transition cells. *OCT-4* was highly expressed, a characteristic shared with the TRKA+ population. The TRKA+ population was highly positive for *PAX-2*, *LIM-1* and *TRKA*. *TRKA*, which is expressed only in developing kidney stromogenic cortical mesenchymal cells,⁹ suggests TRKA+ cell commitment. E-cadherin+ cells did not express *PAX-2* but rather showed *LIM-1* and E-cadherin expression, suggesting incomplete commitment to the nephrogenic differentiation pattern.¹²

Conclusions

Apart from the 1% of cells with pluripotent characteristics the composition of the other 99% of AF cells is diverse with a large subpopulation showing commitment to defined germ lines or tissue end points ranging from unspecified progenitors to organ specific progenitors and mature differentiated cell types. We identified a MM-like population in AF from which specific subpopulations could be successfully separated and grown in culture for several passages. The presence and successful identification of specific renal progenitors, in particular podocyte precursors, in human AF may represent a valuable new source of cells for regenerative therapies that are potentially applicable to a broad range of renal diseases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Dr. K. V. Lemley provided support and helpful discussion, and G. Carraro and G. Turcatel provided technical support.

Supported by the California Institute of Regenerative Medicine and American Urological Association Foundation.

References

1. Underwood MA, Gilbert WM, Sherman MP. Amniotic fluid: not just fetal urine anymore. *J Perinatol*. 2005; 25:341. [PubMed: 15861199]
2. De Coppi P, Bartsch G Jr, Siddiqui MM. Isolation of amniotic stem cell lines with potential for therapy. *Nat Biotechnol*. 2007; 25:100. [PubMed: 17206138]

3. Perin L, Giuliani S, Jin D. Renal differentiation of amniotic fluid stem cells. *Cell Prolif.* 2007; 40:936. [PubMed: 18021180]
4. Warburton D, Perin L, DeFilippo R. Stem/progenitor cells in lung development, injury repair, and regeneration. *Proc Am Thorac Soc.* 2008; 5:703. [PubMed: 18684721]
5. Stocum DL. Stem cells in regenerative biology and medicine. *Wound Repair Regen.* 2001; 9:429. [PubMed: 11896986]
6. Tsangaris G, Weitzdörfer R, Pollak D. The amniotic fluid cell proteome. *Electrophoresis.* 2005; 26:1168. [PubMed: 15706572]
7. Challen GA, Bertoncello I, Deane JA. Kidney side population reveals multilineage potential and renal functional capacity but also cellular heterogeneity. *J Am Soc Nephrol.* 2006; 17:1896. [PubMed: 16707564]
8. Oliver J, Al-Awqati Q. Development of vascular elements during renal organogenesis. *Kidney Int.* 2000; 57:2167. [PubMed: 10792639]
9. Pavenstadt H, Kriz W, Kretzler M. Cell biology of the glomerular podocyte. *Physiol Rev.* 2003; 83:253. [PubMed: 12506131]
10. Horster MF, Braun GS, Huber SM. Embryonic renal epithelia: induction, nephrogenesis, and cell differentiation. *Physiol Rev.* 1999; 79:1157. [PubMed: 10508232]
11. Barisoni L, Mundel P. Podocyte biology and the emerging understanding of podocyte diseases. *Am J Nephrol.* 2003; 23:353. [PubMed: 12915777]
12. Vogelmann SU, Nelson WJ, Myers BD. Urinary excretion of viable podocytes in health and renal disease. *Am J Physiol Renal Physiol.* 2003; 285:F40. [PubMed: 12631553]

Abbreviations and Acronyms

AF	amniotic fluid
AFS	AF stem cell
AKPC	AF kidney progenitor cell
MEM	modified Eagle's medium
MM	metanephric mesenchyma
MMC	MM cell
PCR	polymerase chain reaction
RT	reverse transcriptase

Figure 1.

Real-time PCR for ectodermal (A), endodermal (B) and mesodermal (C) markers in human total AF cell population reveals that E-cadherin (ectoderm), FLK-1 (mesoderm) and CXCR-4 (endoderm) increased, ectodermal markers FGF-5 and NCAM were constant with time, and endodermal and mesodermal markers decreased in older samples.

Figure 2.

Real-time PCR for pluripotency, hematopoietic and mesenchymal markers in human total AF cell population. Hematopoietic marker CD34 dramatically decreased at 19 to 20 weeks but mesenchymal cell marker CD90 was highly expressed in all samples (A). OCT-4 was stable but c-kit increased between 17 and 18 weeks, and disappeared at 19 to 20 weeks (B).

Figure 3.

Real-time PCR for progenitor markers in human total AF population. Early cardiac marker NKX2.5 (A), and marker of lung and thyroid differentiation TTF-1 (B) increased at 19 to 20 weeks. CEBPG increased between 17 and 18 weeks but returned to level comparable to that at 15 to 16 weeks (C).

Figure 4. RT and real-time PCR for kidney markers in human total AF population (A and B). Uninduced MM markers CD24 and OB-cadherin were increased at 17 to 18 weeks. OB-cadherin then decreased to previous level but CD24 maintained high expression. PAX-2 showed trend toward increase with time, as did podocyte marker nephrin, but LIM-1 was constant. GDNF was detected in only 2 samples but showed strong increase at 19 to 20 weeks. PDGFRA did not significantly vary with time. TRKA decreased at 19 to 20 weeks. Occludin increased at 19 to 20 weeks. AQP-1 and ZO-1 were constant with time.

Figure 5. Expression of markers for 3 germ layers and progenitor cells by gestational weeks on RT-PCR in human total AF cells. Population contained prevalence of fibroblastoid shapes (A). In Chang's medium morphology was unchanged up to 50 passages in culture. Reduced from $\times 10$. Endodermal and mesodermal markers decreased in older samples but ectodermal and pluripotency markers were constant with time (B). By 17 weeks several progenitor markers were increased (C). Colored areas include time of significant expression.

Figure 6. Morphology and characterization of MMCs and RT-PCR of CD24+OB-cadherin+ derived subpopulations (AKPCs). MMC selection showed peculiar morphology (A). Reduced from $\times 10$. RT-PCR of MMCs vs CD24-OB-cadherin negative selection (B). Expression of renal markers occludin, nephrin, LIM-1, PAX-2, GDNF and ZO-1 was positive only in MMC population but aquaporin-1 (*Aqp1*) was present in each population. AKPC E-cadherin+ cells expressed GDNF, E-cadherin (*Cad*) and nephrin (*Nep*). Nephrin+ population was positive for nephrin and slightly for ZO-1. TRKA AKPCs were positive for TrKA. PDGFRA+ cells expressed PDGFRA and ZO-1. Podocalyxin+ AKPCs strongly expressed GDNF and ZO-1. *B-Act*, β -actin.

Figure 7.
Cell lineages identified in AF

	WT-1	SYNPO	PODXL	ZO-1	GDNF	LIM-1	PAX-2	NPHS1	OCT-4	ECAD	TRKA	PDGFRA	OCLN
AKPC TrkA+	-	+	-	-	-	+/-	+	-	+/-	+	+	+	+
AKPC PDGFRA+	-	-	-	-	-	-	+/-	-	+/-	+/-	-	++	+/-
AKPC Nephlin+	+	+	-	+	++	++	++	++	++	+	-	+/-	+
AKPC E-Cadherin+	-	-	-	-	-	++	+/-	-	++	++	-	-	++
AKPC Podocalyxin+	++	++	++	-	+/-	-	-	+	-	-	-	-	-

Figure 8.

Genes expressed in AKPC subpopulations on real-time PCR

Figure 9. Morphology of AKPC podocalyxin+ cells cultured with Chang's medium shows primary processes elongating from cell body. Reduced from $\times 10$.