

Case Report

Capillary Endothelia from Two ADPKD Patients are Polyploidy

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Submitted: 10 March 2016

Accepted: 31 April 2016

Published: 25 April 2016

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Abstract

Bilateral renal cyst formation is the main feature of autosomal dominant polycystic kidney disease (ADPKD). We and other laboratories have previously shown that cyst-lining epithelia of kidneys from ADPKD patients are characterized by polyploidy. In this report, we show that endothelia from the renal capillary beds of two ADPKD patients are also polyploidy. Spectral karyotyping study further confirms our flow cytometry analyses. We suggest that polyploidy may be used as a potential cellular marker in ADPKD.

INTRODUCTION

Autosomal dominant polycystic kidney disease (ADPKD) is the most common life-threatening hereditary genetic disease. Although kidneys are the major sites of clinical disease, the prevalence of extra renal manifestations in ADPKD is very high. These extra renal manifestations include cyst formation in other ductal organs and various cardiovascular abnormalities [1-3]. Polycystic kidney has also been associated with polyploidy [4-7]. At the molecular level, vascular endothelia from ADPKD have been associated with dysfunctional primary cilia [8]. Aberrant cell proliferation has been reported in cells with defective cilia [9,10]. We recently showed that cystic renal epithelia from patients are polyploidy, probably through abnormal regulation of chromosomal passenger complex. Abnormality of this protein complex has also been associated with aneurysm formation in vascular endothelia of mouse polycystic models [11,12]. However, the endothelia of renal capillary beds from ADPKD patients have never been previously studied.

CASE REPORT

Human kidney samples from three subjects were taken; two were from ADPKD patients (54-years-old female and 49-years-old male), and the other was from a non-ADPKD subject. The nephrectomy was done in both ADPKD patients, because these kidneys had reached the end-stage renal failure and were further complicated by infections. In general, ADPKD kidney has a grossly distorted architecture characterized by fluid-filled cysts (Figure 1). We isolated endothelia from the renal capillary beds. First branches of capillaries from interlobar arteries were pooled. Due to the nature of primary cells from the capillary beds, we were not able to purify these isolated endothelial cells. Successful cell

isolation typically provides homogenous cell morphology based on the side scatter from the flow cytometry. Capillaries were then briefly rinsed with phosphate buffered saline (pH 7.4). To dissociate the endothelial cells, capillaries were subsequently incubated with trypsin for 20-30 minutes at 37°C. Because capillaries contain mainly endothelia, the isolation typically provides relatively pure endothelial cells. Endothelial cells from the samples were then processed for flow cytometry and spectral karyotyping analysis to investigate cellular polyploidy. Propidium iodide (PI) and 5-bromodeoxyuridine (BrdU) staining were used to analyze cellular polyploidy from the samples. Generally, PI was used for DNA content quantification, while BrdU was used as a marker for cell division. Our flow cytometry analyses showed abnormal polyploidy peaks in both ADPKD samples but not in non-ADPKD sample (Figure 2). Cells with >2N represent

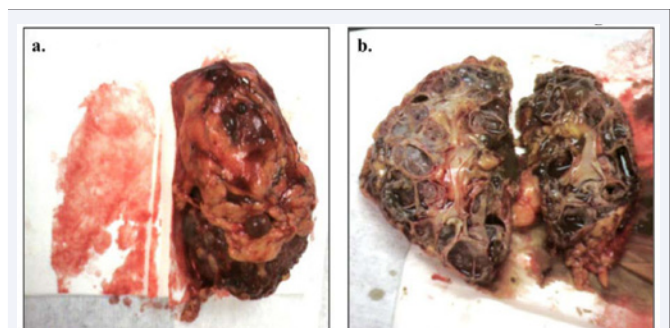


Figure 1 Autosomal dominant polycystic kidney a: Polycystic kidney is characterized by the grossly distorted and enlarged kidney. b: A polycystic kidney was cut in half, showing the presence of numerous fluid-filled cysts. Fluid accumulation in the cysts contributes to the heavy weight and large size of an ADPKD kidney.

polyploidy and have higher DNA content. In non-ADPKD, cells with >2N represent about 0.8%, while in ADPKD patients # 1 and 2 the polyploidy cells constitute of 16.5% and 8.2%, respectively. Interestingly, further analysis of PI and BrdU showed that the abnormal polyploidy cells retain their ability to undergo cell division. This result indicates that endothelia from ADPKD patients are associated with polyploidy and that those polyploidy cells could undergo cell division. Spectral karyotyping (SKY) was also used to confirm our flow cytometry results. Chromosomes from a single endothelium were differentially labeled with probes containing a mixture of fluorescent dyes (Rhodamine, Texas Red, Cy5, FITC and Cy5.5), as describe previously [13]. Unfortunately, we were not successful to karyotype endothelial sample from the second ADPKD patient. From the first ADPKD patient, a single ADPKD endothelium was analyzed, and it showed cellular tetraploidy (Figure 3).

DISCUSSION

We here report that endothelia from renal vascular of ADPKD patients are polyploidy. Those cells could potentially have abnormal cell division that may lead to vascular dysfunction. We previously reported that vascular endothelia from ADPKD patients are less sensitive to fluid-shear stress [8]. This report may thus associate the mechano-dysfunction and polyploidy in ADPKD endothelia. Independent studies have shown centrosomal over-amplification in polycystic kidneys from mice and human [4-7]. The abnormal centrosomal amplification was suggested to occur early in cystic kidney disease. Consequently, abnormal cell division and chromosomal segregation resulted from centrosomal over-duplication lead to polyploidy and genomic instability. Polyploidy has also been observed in vascular endothelial of polycystic kidney mouse models [11,12]. At least in the mice, it

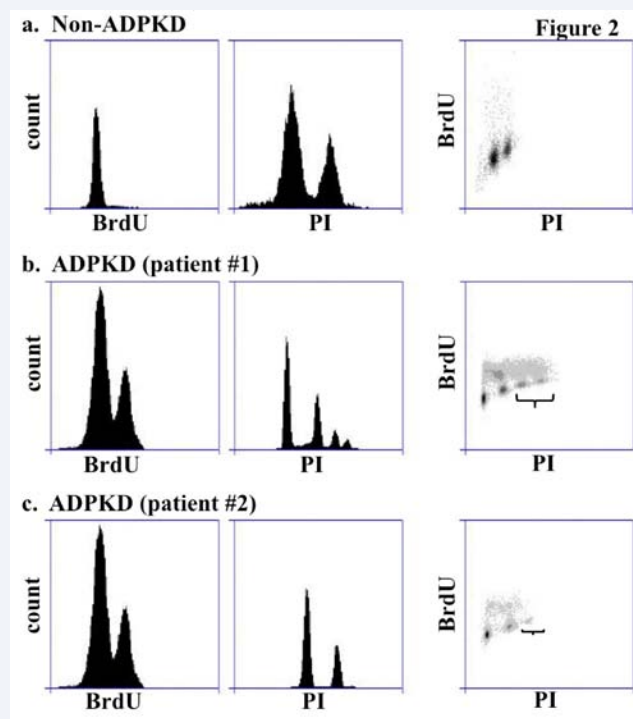


Figure 2 Flow cytometry analysis: Vascular endothelia were isolated from the renal capillary beds of non-ADPKD (a) and two independent ADPKD (b, c) kidneys. Cells were then labeled with propidium iodide (PI) and 5-bromodeoxyuridine (BrdU). PI and BrdU staining were analyzed individually and interdependently. Brackets indicate the presence of polyploidy cells.

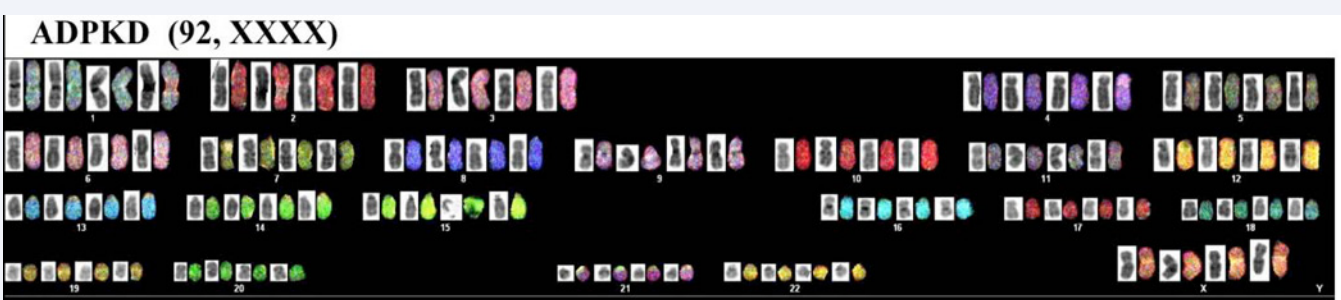


Figure 3 Spectral Karyotype analysis Chromosomes from an endothelium were isolated and processed for labeling with fluorescence probes specific for each individual chromosome. A tetraploidy with a total of 92 chromosomes was observed in the sample obtained from a female ADPKD patient.

was postulated that polyploidy in endothelial cells could result in aneurysm formation, probably through abnormal chromosomal passenger complex. Our current study reinforces the previous findings that vascular endothelia and renal epithelia from polycystic mouse models and ADPKD patients are polyploidy.

CONCLUSION

Flow cytometry analyses using PI and BrdU indicate that ADPKD endothelia have higher DNA content and polyploidy. Analysis with spectral karyotyping further confirmed this result showing abnormal chromosomal number and tetraploidy. Our study is preliminary in nature, and a greater number of patients are needed for a future study. If confirmed in a larger scale study, this result might have an impact on the pathogenic event leading to the development of vascular abnormalities in ADPKD. We propose that polyploidy can be a broad cellular feature not only in the renal epithelia but also in vascular endothelia and that polyploidy may be used as a cellular marker to understand disease progression in ADPKD.

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Cite this article

Kathem SH, AbouAlaiwi WA, Zi X, Nauli SM (2016) Capillary Endothelia from Two ADPKD Patients are Polyploidy. *Ann Clin Cytol Pathol* 2(2): 1022.