

## BRIEF COMMUNICATION

# Identification of multinucleated cells in human kidney cortex: A way for tissue repairing?

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## Abstract

The presence of multinucleated cells has never been demonstrated in renal tissue, although, polyploid cells were recently observed in the tubules of normal and pathological human kidney. Therefore, the aim of the present study is to identify and quantify, by electron microscopy, multinucleated cells in the cortical tissue of normal human kidney i.e., in the three compartments of renal tubule: the proximal tubule (PT), the distal tubule (DT), and the collecting duct (CD), as well as, in the glomerulus (podocytes). The percentage of the multinucleated cells observed was 5% (95%CI: 3.6%–6.7%) in renal cortical tubules with distribution in each tubular compartment of 6% in PT, 4% in DT and 3% in CD with no statistically significant difference in the distribution of multinucleated cells according to tubular compartments. Four percent of analysed podocytes (in total 149 podocytes) were multinucleated (95%CI: 1.5%–8.6%). In conclusion, multinucleated cells were identified and quantified in functionally normal kidneys, as previously demonstrated in other organs such as the liver.

## KEYWORDS

electron microscopy, kidney, multinucleate cells, podocytes, quantitative analysis

Giovanni Tossetta and Sonia Fantone equally contributed as first authors.

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## 1 | INTRODUCTION

Tubular multinucleated cells (Seaman, 1986) as well as bi- and multinucleated podocytes (Bonsib & Horvath, 1999; Chandra et al., 2010; Muhldorfer et al., 2018; Nagata et al., 1995) were identified in pathological human kidneys. Our unpublished electron microscopy observations, obtained analysing diagnostic kidney biopsies from 1985 to date at our electron microscopy unit (Electron Microscopy Unit, Università Politecnica delle Marche) suggested the presence of multinucleated cells in the nephron, i.e. in glomeruli, in proximal (PT) and distal (DT) tubules, including collecting ducts (CD), of patients affected by glomerulonephritis. Since only mononucleated polyploid tubular cells were detected in healthy kidneys (Lazzeri et al., 2018), we used standard morphological techniques, including light and electron microscopy, to identify and quantify multinucleated cells in the renal cortex of normal human kidney. We considered glomeruli, PTs, DTs and CDs separately. Multinucleated cells are polyploid cells that arise from cell cycle-dependent mechanisms (such as endocycle and endomitosis) or from cell cycle-independent mechanisms (such as cell fusion) (Dörnen et al., 2020; Edgar et al., 2014). At present, it is suggested that polyploid cells could play a pivotal role in maintaining tissue function performance as shown by differentiated parenchymal cells that survive to organ failure (Gjelsvik et al., 2019; Lee et al., 2009; Ovrebo & Edgar, 2018; Shu et al., 2018). Although present, the real function of polyploid cells in tissue regeneration is not sufficiently demonstrated (Ovrebo & Edgar, 2018). We hypothesized that multinucleated cells could be considered resident cells in the kidney with physiological normal function, perhaps to assure renal tissue homeostasis as observed in the liver (Diril et al., 2012; Miyaoka et al., 2012).

## 2 | MATERIALS AND METHODS

### 2.1 | Tissue collection

Four normal-looking human tissue samples, with no macroscopic and microscopic cancer foci or ischaemic lesions, as confirmed by the analysis of frozen sections by a pathologist (Section of Pathological Anatomy, Università Politecnica delle Marche, School of Medicine, United Hospitals, Ancona, Italy), were immediately collected after surgical resection of the neoplastic lesion (Division of Urology, Università Politecnica delle Marche). All samples were fixed and accurately prepared for electron microscopy analyses, following standard methods. Table 1 summarizes the characteristics of the patients. Normal renal function with creatinine values between 0.8 and 1.0 mg/dl was recorded at the time of sample harvesting in all the patients. Normal-looking renal tissue was obtained at the bench right after partial nephrectomy, distancing at least 10 mm from the renal tumour. This approach was performed in accordance with the ethical standards as reported in the manuscript.

TABLE 1 Characteristics of tissue samples studied (Lorenzi et al., 2020)

Cases	Gender	Age	Tumour histopathology
Case 1	Female	46	Clear cell carcinoma
Case 2	Male	60	Clear cell carcinoma
Case 3	Male	60	Oncocytoma
Case 4	Male	75	Clear cell carcinoma

### 2.2 | Transmission electron microscopy

Renal specimens were collected and processed as previously described (Cangiotti et al., 2018). Briefly, for transmission electron microscopy, four kidney and one liver (positive control) specimens were fixed in 2% glutaraldehyde/2% paraformaldehyde in 0.1 M phosphate buffer for 3 h at 4°C, postfixed in 1% osmium tetroxide in the same buffer solution, dehydrated in graded alcohols, and embedded in an Epon-Araldite mixture. Semithin sections (2 µm) were obtained from each specimen with a MICROM HM 355 microtome (Zeiss) and stained with toluidine blue. Thin sections were cut by an MTX ultramicrotome (RMC), stained with lead citrate, and examined with a CM10 transmission electron microscope (Philips, Eindhoven, The Netherlands).

### 2.3 | Cell counting

For the quantitative analysis of multinucleated cells, we examined two ultrathin sections for each sample (from a total of 8 sections). The total tubular mono- and multinucleated cells and their percentage were derived (Table 2). In addition, the percentage of mono- and multinucleated cells for each tubular compartment of the cortex (PT, DT and CD) was calculated (Table 3). Moreover, 19 glomeruli were examined for the quantitative analysis of the mono- and multinucleated podocytes. Then, the total number of podocytes, the mononucleated and multinucleated podocytes number, and therefore the percentage of multinucleated podocytes were calculated (Table 2).

### 2.4 | Statistical analysis

The percentage of multinucleated cells in cortical tubules and podocytes was estimated by means of 95% confidence interval (95%CI). The comparison of multinucleated cells among tubular compartments (PT, DT and CD) of the renal cortex was performed using chi-square test. R statistical package 4.0.2 (R Core Team, 2020. A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>) was used for the analysis and the statistical significance was assessed by considering a level of probability of 5% ( $p = 0.05$ ).

**TABLE 2** Percentage of multinucleate cells in podocytes and tubules of renal cortex

Multinucleate cells in tubules of cortex	
Number of cells analysed	835
Number of multinucleate cells	42
% of multinucleate cells	5%
Multinucleate cells in Podocytes	
Number of glomeruli analysed	19
Number of podocytes studied	149
Number of mononucleate podocytes	143
Number of multinucleate podocytes	6
% of multinucleate podocytes	4%

**TABLE 3** Distribution of the multinucleate cells in each tubular compartment of the renal cortex

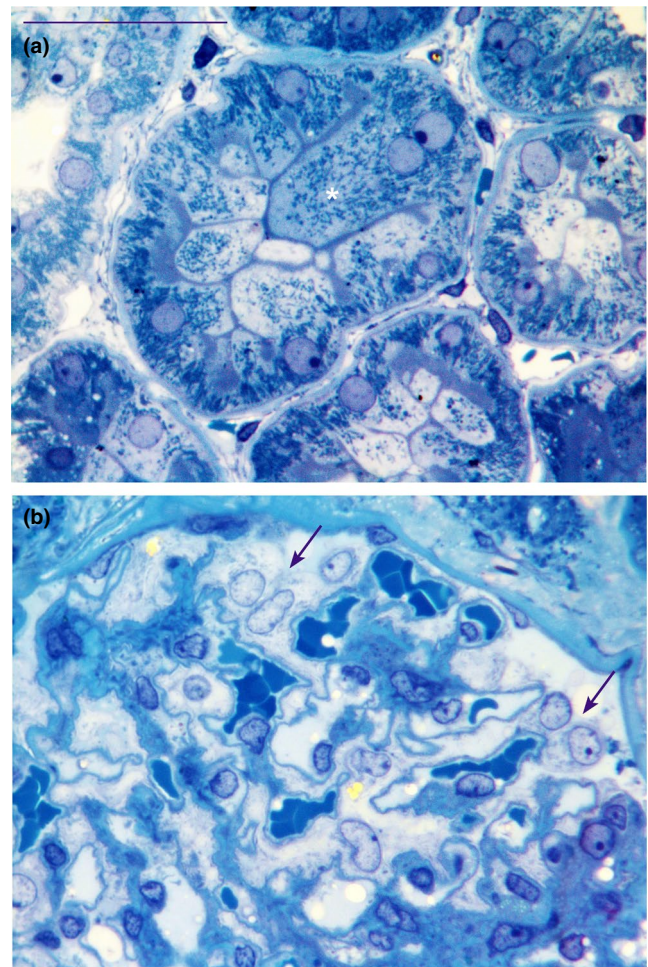
Cell type, n (%)	PT	DT	CD	<i>p</i> <sup>a</sup>
MoC	447 (93.9)	216 (96.0)	130 (97.0)	0.247
MuC	29 (6.1)	9 (4.0)	4 (3.0)	
Total	476	225	134	

Abbreviations: CD, collecting ducts; DT, distal tubule; MoC, mononucleate cells; MuC, multinucleate cells; PT, proximal tubule.

<sup>a</sup>Chi-squared test.

### 3 | RESULTS AND DISCUSSION

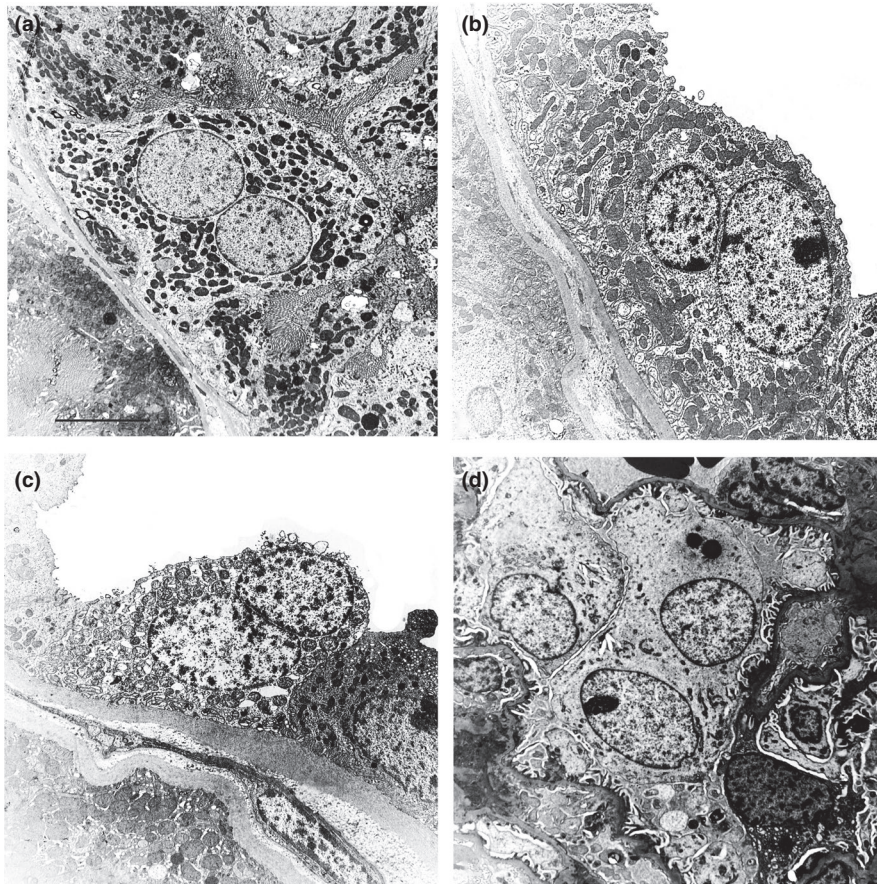
In this study, we demonstrated for the first time that multinucleated cells are present in the cortical tubules and in the podocytes of normal human renal tissues, as previously demonstrated in pathological tissues (Bonsib & Horvath, 1999; Chandra et al., 2010; Muhldorfer et al., 2018; Nagata et al., 1995; Seaman, 1986). Kidney semithin sections showed evidence of multinucleated tubular cells (Figure 1a) while no mitoses were observed. These multinucleated cells, frequently hypertrophic, showed euchromatin in the nucleus, a hypertrophic nucleolus, and sometimes up to five nuclei (see Figure S1). These nuclei were positioned either far from each other or were wedged with one other (the concave part of a nucleus adapted to the convexity of the other nucleus). In addition, ultrastructural investigation allowed a clear identification of the cortical tubular multinucleated cells in the different compartments of nephron (PT, DT and CD) (Figure 2a–c). Ninety-nine PTs, 31 DTs and 15 CDs and a total of 835 cells were analysed. Four hundred and seventy-six, 225 and 134 cells were analysed in PT, DT and CD, respectively. The total number of the multinucleated cells was 42 (5%; 95%CI: 3.6%–6.7%) (Table 2). The average percentage of the multinucleate cells was 5.70%, 4.50%, 4.90% and 5.30% for each of the four distinct patient samples analysed. The distribution of multinucleated cells in each tubular compartment of the renal cortex was: 6% in PT, 4% in DT and 3% in CD. No significant differences were found in the distribution of multinucleated cells according to



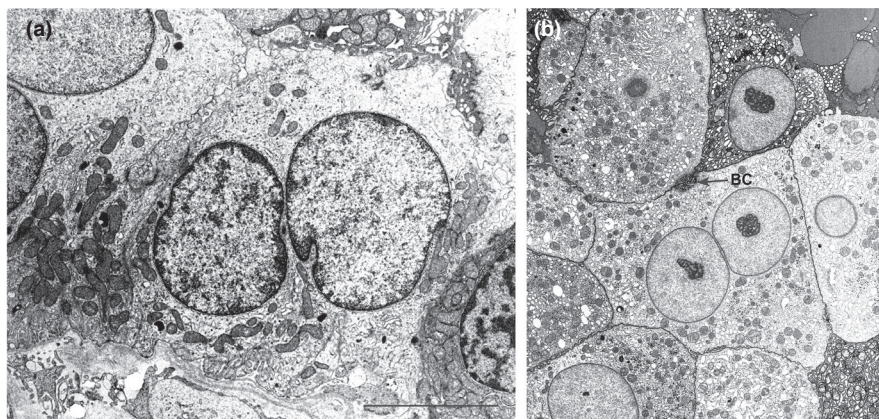
**FIGURE 1** Semithin sections stained with toluidine blue of normal human renal cortical tissue that show in (a) a binucleate hypertrophic cell (white asterisk) in the proximal tubule and in (b) two binucleate podocytes (arrows) in the glomerulus. Scale bar: a and b = 40 μm [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

tubular compartments (Table 3). Consistently, we observed rare binucleated cells also in the macula densa (Figure 3a) by ultrathin serial sections of normal human kidney tissue (unpublished data).

We confirmed the presence of multinucleated podocytes (Figures 1b and 2d) even if some nuclei showed irregular profile (Muhldorfer et al., 2018). One-hundred and forty-nine podocytes were analysed and six were multinucleated (4%; 95%CI: 1.5%–8.6%) (Table 2). We suggest that these multinucleated cells can be considered as resident parenchymal cells that together with scattered tubular cells (Angelotti et al., 2012; Hansson et al., 2014; Kang et al., 2016; Lazzeri et al., 2018, 2019; Lindgren et al., 2011; Lorenzi et al., 2020; Rinkevich et al., 2014; Smeets et al., 2013) have the capacity to maintain renal tissue homeostasis. The presence of multinucleated cells has been previously demonstrated in the liver (Figure 3b) (Kudryavtsev et al., 1993; Wang et al., 2017) and polyploidy was demonstrated in accordance to the different grades of hepatocyte maturity, i.e. progenitors were diploid, while differentiated hepatocytes become polyploid via endoreplication (Wang et al., 2015). Polyploid differentiated hepatocytes drive the recovery of



**FIGURE 2** Normal human renal tissue. Ultrastructure of binucleate cells identified in proximal tubule (a), distal tubule (b), collecting duct (c) and in a podocyte of the glomerulus (d). Scale bar: a = 6.5  $\mu\text{m}$ ; b = 5  $\mu\text{m}$ ; c = 4.5  $\mu\text{m}$ ; d = 6.5  $\mu\text{m}$



**FIGURE 3** Ultrastructure of a binucleate cell contained in the macula densa (a) of normal human renal tissue. A binucleate hepatocyte of normal human liver (b) is shown as control. BC, bile canaliculus. Scale bar: a and b = 3  $\mu\text{m}$

liver function, while the diploid hepatocyte progenitors drive the restoration of the liver mass by proliferation and differentiation of new hepatocytes (Lazzeri et al., 2019). In addition, recent studies have demonstrated an increased percentage of polyploid cells in the damaged kidney compared to the healthy one (Lazzeri et al., 2018, 2019).

The limit of this study is due to the use of normal-looking human renal samples taken from kidneys affected by benign and malignant

lesions. However, all patients had a normal renal function at the time of sampling; the samples were obtained from tissue distancing at least 10 mm from the lesion; these tissues had a normal histological and ultrastructural morphology.

In conclusion, multinucleated cells were identified and quantified in the cortical tissue of functionally normal kidneys. Further studies will be needed to identify the function of these cells in the physiology of the kidney; nevertheless, we hypothesize that these cells

could be involved in renal tissue homeostasis as previously demonstrated in other organs such as the liver (Orr-Weaver, 2015).

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## CONFLICT OF INTEREST

The authors report no relationships with companies that may have a financial interest in the information contained in the manuscript.

## AUTHOR CONTRIBUTIONS

S.F., G.T., E.S., L.G.: performed the experiments, analysed and interpreted the data. A.B.G.: provided the tissue samples and critically revised the manuscript. D.M.: critically revised the final version of the manuscript. M.M.: conceptualized and supervised the study, analysed and interpreted the data, and wrote the manuscript. All authors approved the final version of the manuscript.

## ETHICAL STANDARDS

All procedures performed in this study involving human subjects were in accordance with the ethical standards of the Università Politecnica delle Marche and with the 1964 Helsinki declaration and its later amendments on comparable ethical standards. Written informed consent was obtained from all the subjects included in this study.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding authors upon reasonable request.

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