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Toward the renal vesicle: Ultrastructural investigation of the cap mesenchyme splitting process in the developing kidney

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Abstract

Background: A complex sequence of morphogenetic events leads to the development of the adult mouse kidney. In the present study, we investigated the morphological events that characterize the early stages of the mesenchymal-to-epithelial transition of cap mesenchymal cells, analyzing in depth the relationship between cap mesenchymal induction and ureteric bud (UB) branching.

Design and methods: Normal kidneys of newborn non-obese diabetic (NOD) mice were excised and prepared for light and electron microscopic examination.

Results: Nephrogenesis was evident in the outer portion of the renal cortex of all examined samples. This process was mainly due to the interaction of two primordial derivatives, the ureteric bud and the metanephric mesenchyme. Early renal developmental stages were initially characterized by the formation of a continuous layer of condensed mesenchymal cells around the tips of the ureteric buds. These caps of mesenchymal cells affected the epithelial cells of the underlying ureteric bud, possibly inducing their growth and branching.

Conclusions: The present study provides morphological evidence of the reciprocal induction between the ureteric bud and the metanephric mesenchyme showing that the ureteric buds convert mesenchyme to epithelium that in turn stimulates the growth and the branching of the ureteric bud.

Keywords

Electron microscopy, nephrogenesis, kidney development, ureteric bud, cap mesenchyme

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Introduction

Nephrogenesis is mainly characterized by the interaction of two primordial derivatives, the ureteric bud (UB) and the metanephric mesenchyme. The complex sequence of morphogenetic events that occur in mouse and in several other animal species leads to the development of the adult kidney and is induced mainly by specific signals from the UB to surrounding metanephric mesenchyme. These signals supposedly determine the condensation process of mesenchymal cells around the tips of the UB, forming most of the cap mesenchymal aggregates that characterize the outer portion of the renal cortex in the developing kidney.[1] At this stage of development, the mouse kidney, which seems to be in the grip of chaotic events, actually is under the control of specific events, among these hypoxia probably plays a major role.[2] Cap mesenchymal aggregates undergo differentiation and intense proliferation that lead to the formation of spherical cysts, the so-called renal vesicles, through the mesenchyme-to-epithelium transition process.[3] Between these stages several morphogenetic events occur that are controlled by a complex interaction of specific intercellular signals involved in the regulation of protein synthesis, cell proliferation, cell motility, and apoptosis.[4] However, although the molecular mechanisms involved in the conversion of the surrounding mesenchyme to epithelial cells are not completely understood, a new hypothesis is emerging. The classic notion of mesenchymal and epithelial constituents, as separate and unrelated components has recently been replaced by the more dynamic idea of renal primordial constituents that can change their identity from mesenchymal-to-epithelial and vice versa.[5] Recent studies from our group suggest the existence of multiple stem cell niches scattered in human developing kidney.[6,7]

The aim of the present study was to determine by means of light and electron microscopy some of the early morphological events that lead to the differentiation of the cap mesenchyme and formation of the renal vesicle, with special attention to the relationships between cap mesenchymal differentiation and UB branching.

Materials and methods

Five newborns non-obese diabetic (NOD) mice were obtained from a local colony housed in a pathogen-free environment in the animal care facility of the University of Cagliari. The animals were euthanized according to the guidelines for the Care and Use of Laboratory Animals (NIH) and the European Communities Council Directive for the use of animals in scientific experiments. Authorization n. 120/2019-PR. Issued by the Italian Ministry of Health according to the law D.lgs 26/2014 (for the use of experimental animals). The excised kidneys were cut into small pieces and fixed in a mixture of 3% formaldehyde and 0.1% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2, then rinsed and stored in 0.1 M cacodylate buffer at 4°C. As described in previous works,[7–9] samples were dehydrated in a cold methanol in ascending concentrations and infiltrated with LR gold resin, transferred to gelatin capsules or to flat polyethylene molds filled with fresh resin and placed in polymerization chamber under UV light (365 nm) at −20°C. For light microscopic observations, 1 µ-thick sections stained with toluidine blue were observed and photographed in a Leica 2000 microscope. For electron microscopy, ultrathin sections were stained with uranyl acetate and bismuth subnitrate and observed and photographed in a JEOL 100 S transmission electron microscope (TEM).

Results

At the light microscopic level, nephrogenesis was observed in the outer portion of the renal cortex, where scattered, isolated mesenchymal cells were intermingled with several cap mesenchymal aggregates (Figure 1(d)). Cap mesenchymal aggregates were of variable size and morphology, forming a spectrum from isolated mesenchymal nodules to continuous layers of condensed mesenchymal cells embracing the UB tips.

At the electron microscopic level, we examined the ultrastructure of the metanephric condensation process and the relationship between metanephric mesenchyme and UB (Figure 1(a)–(c)). Ultrastructurally, the cellular constituents of the mesenchymal aggregates exhibited peculiar morphological features. Variations in cellular shape were frequently observed in different mesenchymal aggregates. In general, the cells were small, with skimpy cytoplasm containing few cellular organelles; the nuclei usually were large and contained pleomorphic nucleoli (Figure 1(a)–(c)). There were obvious intercellular spaces in specific areas of the different mesenchymal aggregates (Figure 1). These spaces were related to the sequential morphological changes that characterize the profound ultrastructural reorganization that cap mesenchymal aggregates undergo, together with the branching UB, before renal vesicle formation. The "cap mesenchymal splitting process" appeared initially as the formation of a continuous layer of condensed mesenchymal cells (Figures 1(a) and 2(a)), that underwent a progressive enlargement at one of its extremities, forming a spherical nodule (Figures 1(b) and 2(b)). At the same time, the presence of condensed mesenchymal cells affected the epithelial cells of the adjacent UB, possibly influencing the growth and branching of the UB (Figures 1(c) and 2(c)). The two processes, that is, cap mesenchyme enlargement and bud branching, proceed in unison until the cap mesenchyme split into two parts by the expansion of the adjacent UB tips (Figures 1(d) and 2(d)).

Discussion

The complex sequence of morpho-molecular events taking place during kidney development is not well understood

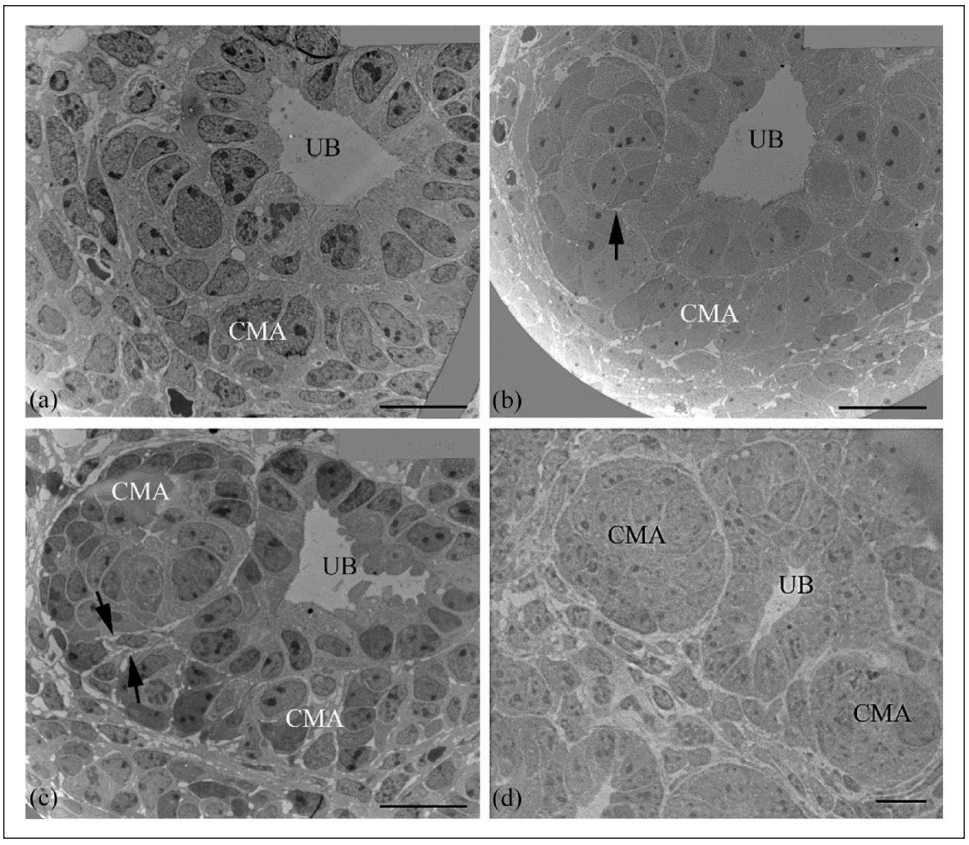

Figure 1. Sequential morphological events that characterize the "cap mesenchymal splitting process." (a–c) Electron micrographs of the outer portion of the mouse renal cortex. (a) Initially, mesenchymal cells condense forming a continuous cap mesenchymal aggregate (CMA) around the ureteric bud tip (UB). (b) The cap mesenchymal aggregate starts to enlarge at one of its extremities, forming a spherical nodule. (c) The condensed mesenchymal cells seem to affect the epithelial cells of the adjacent ureteric bud, inducing branching of the ureteric bud (UB). Note the presence of intercellular spaces in specific areas of the different mesenchymal aggregates (arrows). (d) Light micrograph of the outer portion of the mouse renal cortex. The cap mesenchyme (CMA) is finally split into two parts by the expansion and growth of the ureteric bud tip (UB). Bars = 10 µm.

yet. Most of studies on nephrogenesis have produced a huge amount of data describing the main steps of kidney development[10–14] and, others new potential biological events, like milk mesenchymal stem cells, could be involved in post-natal kidney remodeling.[15,16] Authors have usually subdivided nephrogenesis into five main stages: (1) primary UB; (2) cap mesenchyme formation; (3) mesenchymal epithelial-transition; (4) glomerulogenesis; (5) interstitial cell differentiation.[11,17,18] However, significant gaps in the knowledge of the molecular and morphological mechanisms involved in nephrogenesis were left. Recently we have focused more in depth on the early events of mouse nephrogenesis, that, starting from the cap mesenchymal induction, lead to the renal vesicle formation ending with nephron development. We have found out that, between these two extremes, additional and significant morphological events take place.[11] The cap mesenchymal induction process has been shown to give rise to specific mesenchymal aggregates that we defined the "pine-cone bodies," due to their peculiar architectural organization.[19] In the present study, we further investigated the morphological events that characterize the early stages of the mesenchymal-to-epithelial transition of cap mesenchymal cells. In particular we analyzed the relationship between cap mesenchymal induction and UB branching. Our results show a tight dependency of the two processes. Cap mesenchymal induction is initiated by the growing UB, determining the differentiation and proliferation process of the mesenchymal cells that start to condense around the tips of the UB. This process is initially characterized by the formation of a continuous layer of condensed mesenchymal cells that enlarges at one of its extremities, forming a spherical nodule. At the same time, the presence of condensed mesenchymal cells seems to affect the epithelial cells of the adjacent UB, which undergo a burst of proliferation, determining the growth and the branching of the UB. The two processes, that is, cap mesenchyme enlargement and bud branching, proceed in unison until each cap mesenchymal aggregate is split into two parts by the expansion of the UB tips (Figure 2).

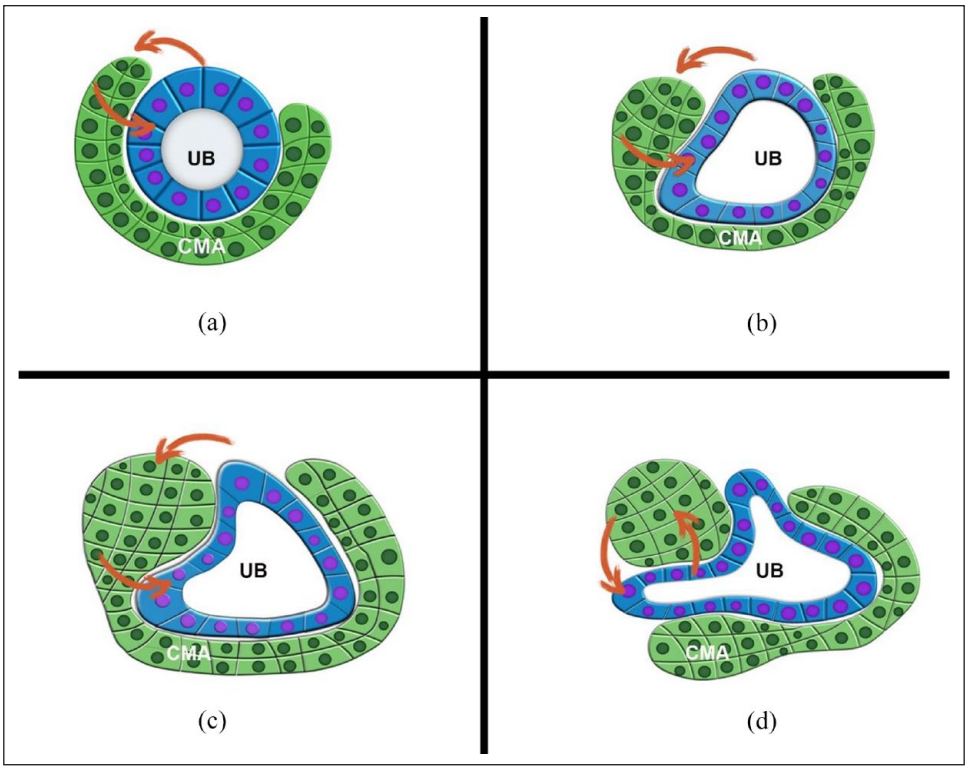

Figure 2. Schematic representation of the sequential morphological steps that characterize the "cap mesenchymal splitting process" in the developing mouse renal cortex.
CMA: cap mesenchymal aggregates; UB: ureteric bud.

These fascinating phenomena suggest that nephrogenesis is fundamentally based on the reciprocal induction and tight interaction between the UB and the metanephric mesenchyme. Each UB tip converts metanephric mesenchymal cells to epithelial cells and, in turn, cap mesenchyme stimulates growth and branching of the UB tips, as demonstrated in this study by the presence of intense mitotic events among the epithelial constituents of the UB. Previous studies have shown that different genes products are needed to control kidney organogenesis.[14,20–23] Among these factors, Thymosin Beta 4 (TB4) has been detected in the fetal kidney and mighty regulate the main morphological changes that occur during the early events of nephrogenesis.[24] Most of them regulate the main morphological changes that occur during the early events of nephrogenesis. In particular, cell motility and cell proliferation represent specific target processes that are under the control of definite gene products. Early studies have reported the role of multiple signaling genes that regulate UB origin and growth.[25] In mouse, a member of the receptor tyrosine kinase superfamily (RET) that was found to be is expressed in the branching UB, has been supposed to regulate signals that induce proliferation of the UB epithelium.[26,27] Moreover, the glial cell line-derived neurotrophic factor (GDFN) seems to interact with RET in the same signaling pathway involved in the regulation of bud growing and branching.[28] Several other regulatory genes have been reported to act as inducers of metanephric mesenchyme[29,30]; however, other specific genes involved in the molecular mechanism that regulate cell motility and adhesion need to be discovered. It's well known that development and growth of the differentiating tissues require changes in cellular shape and motility.[31] Our data show, at ultrastructural level, a sequential rearrangement of the renal cellular constituents that change shape and move continuously, migrating toward specific kidney areas in order to give rise to different aggregates. The presence of evident extracellular spaces, that we observed among the cap mesenchymal cells adjacent to the branching UB region, seems to confirm this hypothesis. The cap mesenchymal splitting process here described could depend on the differential expression of specific adhesion molecules in the mesenchymal cells that are condensing around the tip of the UB. Previous analyses have investigated the expression of E-cadherin and K-cadherin in the cellular constituents forming renal vesicles. They have shown how the expression of these adhesion molecules may depend on the distance from the UB,[32] and therefore upon signaling generated by the adjacent UB epithelial cells. In turn, the conversion of mesenchyme to epithelia could trigger the branching process of the UB, by activating the proliferation and growth of their cellular constituents. Further evidences, in line with our findings, have been provided by

previous experiments in vitro of UB cell lines, that were found to express mesenchymal growth proteins as well as receptors for factors produced by the metanephric mesenchyme.[33–35] Molecular technologies expanded the number of diagnostic approaches in clinical protocols and detection of important nucleic acids sequences present in different types of cancer and new molecular intraoperative test are now available.[36–38]

In this contest, our study shows the presence of additional events that trigger the epithelialization of the metanephric mesenchyme and provides further morphological evidence of the reciprocal induction between UB and cap mesenchyme. However, several important questions still remain open, including which genetic and post-translational factors regulate the reciprocal induction process between UB and metanephric mesenchyme. For instance, some of these factors might be studied by electron immunohistochemical analyses of the developing renal tissues and by developing of an in vitro "ad hoc model" of induction of UB and mesenchymal cell lines. A better knowledge of these basic mechanisms by using in vitro and animal models will provide critical data for human nephrogenesis allowing, in the future, the development of a renal regenerative medicine that could be applied starting from the perinatal period, aimed at preventing chronic renal failure in adulthood.[39]

Declaration of conflicting interests

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References

1. Faa G, Gerosa C, Fanni D, et al. Marked interindividual variability in renal maturation of preterm infants: lessons from autopsy. *J Matern Fetal Neonatal Med* 2010; 23: 129–133.
2. Gerosa C, Fanni D, Faa A, et al. Low vascularization of the nephrogenic zone of the fetal kidney suggests a major role for hypoxia in human nephrogenesis. *Int Urol Nephrol* 2017; 49: 1621–1625.
3. Fanos V, Gerosa C, Loddo C, et al. State of the art on kidney development: how nephron endowment at birth can shape our susceptibility to renal dysfunction later in life. *Am J Perinatol* 2019; 36: S33–S36.
4. Faa G, Fanni D, Gerosa C, et al. Kidney development and susceptibility to develop kidney disease in adulthood. *Jpn J Med* 2018; 1: 217–221.
5. Faa G, Gerosa C, Fanni D, et al. Kidney embryogenesis: how to look at old things with new eyes. In: Fanos V, Chevalier R, Faa G, et al. (eds) *Developmental nephrology: from embryology to metabolomics*. Hygeia Press, 2011, pp.23–45.
6. Fanni D, Sanna A, Gerosa C, et al. Each niche has an actor: multiple stem cell niches in the preterm kidney. *Ital J Pediatr* 2015; 41: 78.
7. Gerosa C, Fanos V, Puddu M, et al. Not all renal stem cell niches are the same: anatomy of an evolution. *J Pediatr Neonatal Individ Med* 2016; 5: e050225.
8. Coni P, Piras M, Mateddu A, et al. Thymosin β4 cytoplasmic/nuclear translocation as a new marker of cellular stress. A caco2 case study. *RSC Adv* 2020; 10: 12680–12688.
9. Pichiri G, Coni P, Nemolato S, et al. Cellular trafficking of thymosin beta-4 in HEPG2 cells following serum starvation. *PLoS One* 2013; 8: e67999.
10. Piludu M, Piras M, Pichiri G, et al. Thymosin beta 4 may translocate from the cytoplasm in to the nucleus in HepG2 cells following serum starvation. An ultrastructural study. *PLoS One* 2015; 10: 10–14.
11. Gerosa C, Fanos V, Fanni D, et al. Toward nephrogenesis in the pig kidney: the composite tubulo-glomerular nodule. *J Matern Fetal Neonatal Med* 2011; 24: 52–54.
12. Horster MF, Braun GS and Huber SM. Embryonic renal epithelia: induction, nephrogenesis, and cell differentiation. *Physiol Rev* 1999; 79: 1157–1191.
13. Rosenblum ND. Developmental biology of the human kidney. *Semin Fetal Neonatal Med* 2008; 13: 125–132.
14. Faa G, Gerosa C, Fanni D, et al. CD10 in the developing human kidney: immunoreactivity and possible role in renal embryogenesis. *J Matern Fetal Neonatal Med* 2012; 25: 904–911.
15. Reali A, Puddu M, Pintus MC, et al. Multipotent stem cells of mother's milk. *J Pediatr Neonatal Individ Med* 2016; 5(1): e050103.
16. Pichiri G, Lanzano D, Piras M, et al. Human breast milk stem cells: a new challenge for perinatologists. *J Pediatr Neonatal Individ Med* 2016; 5(1): e050120.
17. Fanni D, Gerosa C, Vinci L, et al. Interstitial stromal progenitors during kidney development: here, there and everywhere. *J Matern Fetal Neonatal Med* 2016; 29: 3815–3820.
18. Wang P, Pereira FA, Beasley D, et al. Presenilins are required for the formation of comma- and S-shaped bodies during nephrogenesis. *Development* 2003; 130: 5019–5029.
19. Piludu M, Fanos V, Congiu T, et al. The pine-cone body: an intermediate structure between the cap mesenchyme and the renal vesicle in the developing nod mouse kidney revealed by an ultrastructural study. *J Matern Fetal Neonatal Med* 2012; 25: 72–75.
20. Poleev A, Fickenscher H, Mundlos S, et al. PAX8, a human paired box gene: isolation and expression in developing thyroid, kidney and Wilms' tumors. *Development* 1992; 116: 611–623.
21. Carroll TJ, Park J-S, Hayashi S, et al. Wnt9b plays a central role in the regulation of mesenchymal to epithelial transitions underlying organogenesis of the mammalian urogenital system. *Dev Cell* 2005; 9: 283–292.
22. Dressler GR. Epigenetics, development, and the kidney. *J Am Soc Nephrol* 2008; 19: 2060–2067.
23. Fanni D, Fanos V, Monga G, et al. MUC1 in mesenchymal-to-epithelial transition during human nephrogenesis: changing the fate of renal progenitor/stem cells? *J Matern Fetal Neonatal Med* 2011; 24: 63–66.

24. Nemolato S, Cabras T, Fanari MU, et al. Immunoreactivity of thymosin beta 4 in human foetal and adult genitourinary tract. *Eur J Histochem* 2010; 54: e43.
25. Costantini F. Renal branching morphogenesis: concepts, questions, and recent advances. *Differentiation* 2006; 74: 402–421.
26. Schuchardt A, D'Agati V, Pachnis V, et al. Renal agenesis and hypodysplasia in ret-k-mutant mice result from defects in ureteric bud development. *Development* 1996; 122: 1919–1929.
27. Vega QC, Worby CA, Lechner MS, et al. Glial cell line-derived neurotrophic factor activates the receptor tyrosine kinase RET and promotes kidney morphogenesis. *Proc Natl Acad Sci USA* 1996; 93: 10657–10661.
28. Trupp M, Arenas E, Fainzilber M, et al. Functional receptor for GDNF encoded by the c-ret proto-oncogene. *Nature* 1996; 381: 785–789.
29. Faa G, Gerosa C, Fanni D, et al. Morphogenesis and molecular mechanisms involved in human kidney development. *J Cell Physiol* 2012; 227: 1257–1268.
30. Poladia DP, Kish K, Kutay B, et al. Role of fibroblast growth factor receptors 1 and 2 in the metanephric mesenchyme. *Dev Biol* 2006; 291: 325–339.
31. Ben-Ze'ev A. The role of changes in cell shape and contacts in the regulation of cytoskeleton expression during differentiation. *J Cell Sci* 1987; 8: 293–312.
32. Lechner MS and Dressler GR. The molecular basis of embryonic kidney development. *Mech Dev* 1997; 62: 105–120.
33. Barasch J, Pressler L, Connor J, et al. A ureteric bud cell line induces nephrogenesis in two steps by two distinct signals. *Am J Physiol Renal Physiol* 1996; 271: F50–F61.
34. Barasch J, Qiao J, McWilliams G, et al. Ureteric bud cells secrete multiple factors, including bFGF, which rescue renal progenitors from apoptosis. *Am J Physiol Renal Physiol* 1997; 273: F757–F767.
35. Sakurai H, Barros EJ, Tsukamoto T, et al. An *in vitro* tubulogenesis system using cell lines derived from the embryonic kidney shows dependence on multiple soluble growth factors. *Proc Natl Acad Sci USA* 1997; 94: 6279–6284.
36. Gerosa C, Faa G, Fanni D, et al. Fetal programming of COVID-19: May the barker hypothesis explain the susceptibility of a subset of young adults to develop severe disease? *Eur Rev Med Pharmacol Sci* 2021; 25: 5876–5884.
37. Coni P, Mateddu A, Kuqi L, et al. LncRNA colon cancer-associated transcript 1 (CCAT1) in ovarian cancer. *Eur Rev Med Pharmacol Sci* 2018; 22: 1525–1527.
38. Medas F, Coni P, Podda, F, et al. Evaluation of accuracy of one-step nucleic acid amplification (OSNA) in diagnosis of lymph node metastases of papillary thyroid carcinoma: Diagnostic study. *Ann Med Surg* 2019; 46: 17–22.
39. Faa G, Sanna A, Gerosa C, et al. Renal physiological regenerative medicine to prevent chronic renal failure: should we start at birth? *Clin Chim Acta* 2015; 444: 156–162.