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Regeneration of the Kidney - Viewed from ES Cell

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1. Introduction

Embryonic stem (ES) cells are derived from inner cell mass of blastocyst stage of fertilized eggs and remain undifferentiated in the presence of leukemia inhibitory factor (LIF) (Evans M.J & Kaufman M.H. (1981), Martin G.R. (1981).). Depletion of LIF leads to ES cell to form embryoid body (EB) that can differentiate into several different lineage cells such as endodermal, mesodermal and ectodermal lineage cells. Until now, ES cells have been induced to differentiate in vitro into several cell types such as hematopoietic cell (Nakano T.,et al. (1994), Nishikawa S.I.,et al. (1998), Palacios R.,et al. (1995).), pancreatic cell (Soria B.,et al. (2000).), neuronal cell (Brustle O.,et al. (1999), Scholer H.R.,et al. (1991).) and cardiomyocyte (Boheler K.R.,et al. (2002), Doetschman T.C.,et al. (1985), Klug M.G.,et al. (1996).).
To be sure that ES cells are pluripotent, but mere transplantation of non-differentiated ES cells leads to teratomas as Yamamoto reported the teratoma formation after the transplantation of undifferentiated ES cells into retroperitoneum of nude mice (Yamamoto M.,et al. (2006).). This is one of the main obstacles that limit the clinical application of ES cell transplantation therapy. Therefore, it is essential to differentiate ES cells to some degree before transplantation. But the information as to the directed differentiation of ES cells to kidney lineages are limited although spontaneous differentiation of ES cells to various cells is observed. We have gathered pieces of information of the developmental biology and the differentiation conditions of several stem/progenitor cells to matured kidney. In this review, we focus on a dream of the generation of kidney from ES cells or stem/progenitor cells.

2. Developmental biology of the kidney

Mammalian kidney traces three successive stages during the development: pronephros, mesonephros and metanephros. In mammals, pronephros, a rudimentary and temporary structure consisting of one nephron, retrogrades at the first stage of nephrogenesis. Next, mesonephros arises as Wolffian duct from the intermediate mesoderm, elongates along the oral-caudal axis, and forms immature glomerular and tubular structures consisting of several dozens of nephrons. However, both of the structures at this stage are not functional and then retrograde.

Metanephros develops from the caudal part of Wolffian duct and finally produces functional glomeruli and tubules as follows: A single ureteric bud emerges from the caudal part of the retrograding mesonephros and starts to branch. Immature mesenchymal cells condense around the ureteric buds to form the metanephric blastema. Cells around an each tip of the branching ureteric buds and another type of cells surrounding such cells are termed the metanephric mesenchyme and stromal cells, respectively. The former metanephric mesenchymal cells differentiate through mesenchymal-to-epithelial transformation (MET) into various types of epithelial cells including visceral glomerular epithelial cells (podocytes), parietal glomerular epithelial cells, proximal tubular epithelial cells, and distal tubular epithelial cells, and develop into a cylindrical epithelial structure at an each tip of the ureteric buds. At an end of the differentiating epithelial structures, podocytes and parietal glomerular epithelial cells interact with mesangial cells and fenestrated endothelial cells, and form globular networks of capillaries (glomeruli). At the other end, the epithelial structures connect in a one-to-one manner to collecting ducts each of which is derived from one branch of the ureteric buds. This whole structure is called the nephron, a unit to produce urine. The final number of nephrons depends both on the number of branching of the ureteric buds and on the differentiation potentials of the metanephric mesenchyme independently. In human, one kidney has one million nephrons. The interaction in a reciprocal and inductive manner between the metanephric mesenchyme and the ureteric bud plays an important role in the nephrogenesis. A mouse or rat embryonic kidney further develops when placed in culture, but is not functional because vascular networks are not integrated. In contrast, rat kidney rudiments at early stage of the development, which contain the metanephric mesenchyme and the ureteric bud, can form functional nephrons when it is transplanted in the omentum of other living animals (Hammerman M.R. (2004).). These facts indicate that a set of the metanephric mesenchyme and the ureteric bud has the perfect intrinsic program to regulate immature cells, to recruit and integrate vascular components, and to form functional nephrons.

3. Toward the regeneration of the kidney from ES cells

3.1 Differentiation of rodent ES cells into renal lineage

The kidney is a highly terminated and complex organ. It needs to be formed so that the adjacent placement in three dimensions of several components of kidney cells are critical and only work when the placement is perfect unlike hematopoietic cells or insulin-secreting cells working as a cell itself. In this section, we focus on the differentiation trials of murine ES cells into renal lineage. Steenhard B.M. et al showed an integration of ES cells in metanephric kidney organ culture (Steenhard B.M.,et al. (2005).). The authors microinjected murine ROSA26 ES cells that express ubiquitously β-galactosidase when cultured in the presence of LIF to suppress differentiation, into E12 or E13 metanephroi and kept them on organ culture system. ES cell-derived, β-galactosidase-positive cells were found in epithelial structure resembling tubules and, on rare occasions, in structures resembling glomerular tufts. By the analysis of electron microscopy, the ES cell-derived tubules were surrounded by basement membrane and had epithelial microvilli and junctional complexes. Subset of these epithelial tubules expressed markers of *Lotus tetragonolobus* (LTA) and α_1 Na^+/K^+ ATPase. By infecting cytomegalovirus-promoter green fluorescent protein (GFP) adenovirus to ES cells before injection, injected ES cells were traced by GFP from 18h to 48h in cultured kidney, indicating that injected ES cells were definitely alive and functioning in the cultured kidney.

Kobayashi T. et al reported that Wnt4-transformed mouse ES cells differentiated into renal tubular cell in vitro and in vivo. The authors stably transformed Wnt4 cDNA to mouse ES cells (Wnt4-ES cells) and incubated them by hanging drop culture method (Kobayashi T.,et al. (2005).). Hepatocyte growth factor (HGF) and activin-A enhanced the expression of aquaporin (AQP)2 in Wnt4-ES cells and induced tubular-like structure formation in three dimensional culture. The authors confirmed that the addition of anti-Wnt4 antibody in the culture system downregulated the frequency of tubular-like structure formation. When Wnt4-ES cells were transplanted in the renal cortex of four-week-old mouse, they also formed tubular-like structure in teratoma. The authors showed that embryoid bodies from the Wnt4-ES cells had an ability to differentiate into renal tubular cells. The most significant point is that Wnt4 expressing ES cells can be integrated into tubular cells in the adolescent (4 week old) kidney microenvironment which would be less suitable for ES cells to differentiate than in the microenvironment of E12 or E13 of kidney adopted by Steenhard. Wnt4, HGF and activin-A may be promoting factors for the differentiation of ES cells to renal tubular cells.

Kim D.et al reported that mouse ES cells can differentiate into renal epithelial cells with the combination of retinoic acid, activin-A and Bone morphogenetic protein (Bmp)-7 (Kim D. & Dressler G.R. (2005).). The authors showed that cultured ES cells can be induced to express markers specific for the intermediate mesoderm with the combination of these three nephrigenic growth factors. They also showed that treated ES cells can contribute to tubular epithelial cells with almost 100% efficacy by injecting into developmental kidney rudiments. The injected Rosa 26 ES cells were traced by lac-Z staining and the tubular cells were confirmed morphologically by immunohistochemical staining with LTA and laminin. The transplantation or injection of cells entails a contentious problem of cell fusion although the authors insist that the occurrence of cell fusion in their experiment is very rare. Investigation of karyotype would be ideal for the detailed analysis.

Vigneau C. et al used mosue ES cell line with GFP knocked into the functional brachyury locus as well as lacZ in the ROSA26 locus (LacZ/brachyury/GFP) in selection and lineage tracing (Vigneau C.,et al. (2007).). The authors firstly optimized the culture conditions using activin-A to give rise to maximal numbers of renal progenitors identified by expression of the combination of renal markers, cadherin11, WT1, Pax2 and Wnt4. Then, they next enriched LacZ/brachyury/GFP$^+$ cells by FACS and injected these cells into embryonic kidney explants in organ culture. Five days after the injection, β-galactosidase positive cells were incorporated into blastema of the nephrogenic zone. Even after the single injection into developing live newborn mouse kidneys, the LacZ/brachyury/GFP$^+$ cells were stably integrated into proximal tubules with normal morphology and normal polarization of alkaline phosphatase and AQP1 for 7 months without teratoma formation. These results suggest that differentiation of ES cells with activin-A and selection for brachyury expression lead to the isolation and purification of renal proximal tubular progenitor cells.

Bruce S.J. et al reported that ES cells can be differentiated into renal lineage using BMP4 in vitro (Bruce S.J.,et al. (2007).). With their induction culture system, the authors confirmed that gene expressions of WT1, Wnt4, GDNF, Nephrin, mineral corticoid receptor, podocalyxin, Wnt11, Pod1 or AQP1 were upregulated at later stage.

Ross E.A. et al reported a study of ES cells showing an importance of scaffolds (Ross E.A.,et al. (2009).). The authors firstly decellularized intact rat kidney in a fashion that retained the matrix and secondly seeded them with pluriopotent rat ES cells either through the artery or through the ureter. The transplanted cells populated and proliferated within the

glomerular, vascular and tubular structures. The cells lost their original embryonic appearance and expressed differentiated immunohistochemical markers. But the cells that lost the contact with basement membrane matrix became apoptotic. This study suggests the importance of extracellular matrix for the kidney regeneration to occur as a scaffold.

Morizane R. et al reported that differentiation of murine ES ells and iPS cells into renal lineage *in vitro* (Morizane R.,et al. (2009).). By hanging drop of murine ES cells, renal inducing factors were screened. GDNF and BMP7 enhanced the expression of Pax2 and WT1, markers of metanephric mesenchyme. Activin enhanced the expression of Pax-2 and KSP, suggesting activin enhanced the differentiation of ES cells into not only to mesoderm but also to tubular cells. According to their analysis, it is difficult to promote ES cells to differentiate into podocyte, because no inducer was found to enhance the expression of nephrin. To be sure, their analysis is based on *in vitro* marker analysis only and morphogenic or functional analyses are lacking, but several differentiation markers are investigated to compare the differentiation of ES cells and iPS cells. The information is useful to understand the effects of cytokines or growth factors for the designated differentiation of ES cells.

Sall1, a multi-zinc finger transcription factor which is expressed at metanephric mesenchyme, is involved in mouse kidney organogenesis because Sall1-deficient mouse show kidney agenesis or severe dysgenesis (Nishinakamura R.,et al. (2001).). It is highly expressed in ES cells and, most recently, it was reported to regulate ES cell differentiation in accordance with Nanog (Karantzali E.,et al. (2010).). Sall1 inhibits the ectodermal and mesodermal differentiation. More specifically, the induction of the gastrulation markers T brachyury, Goosecoid, and Dkk1 and the neuroectodermal markers Otx2 and Hand1 was inhibited by Sall1 overexpression during embryoid differentiation. In consideration of the fact that kidney is derived from intermediate mesoderm, switching off at an appropriate timing the signal Sall1 once positive at an early stage of development would be important for the kidney organogenesis.

Luis M. et al reported a method to isolate renal stem cells from embryonic kidney not from ES cells (Lusis M.,et al. (2010).). Metanephric mesenchyme of the developing kidney gives rise to various types of epithelial cells including visceral glomerular epithelial cells (podocytes), parietal glomerular epithelial cells, proximal tubular epithelial cells, and distal tubular epithelial cells. It is regarded as progenitor population pool of the developing kidney. But isolated metanephric mesenchyme does not self renew and requires immortalization for survival in culture. The authors developed a method of isolation and sustained culture of long-term repopulating, clonal progenitors from mouse embryonic kidney (E12.5) as free floating nephrospheres just as neurosphere often observed in neural stem cell culture system. These cells showed clonal self renewal for more than twenty passages when cultured with bFGF and thrombin, showed broad mesodermal multipotency, but held the expression of renal transcription factors such as Wt1, Sall1, Eya1, Six1, Six2, Osr1 and Hoxa11. These cells' capacity to contribute to developing embryonic kidney was limited, and nephrospheres did not display in vitro renal epithelial capacity. Holding mesodermal multipotency but renal transcription markers means that nephrosphere would be a developmentally transient state between pluripotent ES cells and renal progenitors. The nephrospheres can be cultured from Sall1-positive and –negative fraction of embryonic kidney, which suggests that they were derived from metanephric mesenchyme as a whole. This renal stem cell population could be cultured from only embryonic kidney, not from postnatal kidney.

3.2 Differentiation of human ES cells into renal lineage

As to the human cells, there are only a few reports of differentiation of ES cells to renal lineage. The concomitant use of retinoic acid, activin-A, and BMP-4 or BMP-7 induced the directed differentiation of human ES cells to renal precursors when analyzed by differentiation marker (Batchelder C.A.,et al. (2009).). As to the culture substrate, gelatin was reported to most closely recapitulate the directed developmental pattern of renal gene expression. Human ES cells also have an ability to differentiate into WT-1 and renin-expressing cells with the combination of several growth factors including either nerve growth factors (NGF) or HGF (Schuldiner M.,et al. (2000).). Tarantal A.F. et al reported an imaging study of human ES cells (Tarantal A.F.,et al. (2011).). The authors radiolabeled human ES cells expressing firefly luciferase, transplanted them to fetal monkey kidney at early second trimester under ultrasound guidance, and traced them by non-invasive imaging (positron emission tomography (PET) and bioluminescence imaging (BLI)). Transplanted cells were imaged in vivo and identified at the site of injection. Lin et al reported an isolation method by flow cytometer of putative renal progenitor fraction of human ES cells (Lin S.A.,et al. (2010).). The authors reduced serum concentration and feeder cell density to differentiate human ES cells in vitro for 14 days. Then they fractioned the differentiated ES cells based on the expression marker CD24, podocalyxin and GCTM2. These cells upregulated the renal transcription factor, PAX2, LHX1 and WT1 compared to unfractionated ES cells. Immunohistochemical analysis also confirmed these cells coexpressed nuclear factor WT1 and PAX2 protein. Microarray analysis showed that the most differentially up-regulated genes in the fraction were associated with kidney development compared to other fractions.

3.3 Lessons from animal cap study

The differentiation conditions of murine or human ES cells to renal lineage are similar to the developmental biology in amphibians. In amphibian eggs, the ectodermal cell mass of mid-blastula embryos, what is celled “animal cap”, is similar to ES and iPS cells in mammals. When animal caps are treated with the combination of retinoic acid and activin-A, they differentiated into pronephric tubules (Osafune K.,et al. (2002).). Using this in vitro system to generate pronephros, the marker gene expression for pronephric tubules were observed (Brennan H.C.,et al. (1999).) and the molecular mechanism underlying pronephros development was investigated. These differentiation-inducing factors, retinoic acid and activin-A have been applied to directed differentiation trials of ES cells to renal lineage.

3.4 ES cells and kidney stem/progenitor cells

In consideration of these previous reports as to the differentiation of ES cells to renal lineage, the information is limited and we have to learn the microenvironment from signaling during the embryonic development as an early stage of differentiation and from transdifferentiation or conversion condition from other precursor and/or stem cells to the renal lineage as a late stage of development. In general, stem/progenitor cells and the proper microenvironment are essential for the repair or regeneration of damaged tissue. The same is true to the ES cell for the directed differentiation. A suitable microenvironment includes the presence of local cytokines as well as of extracellular matrix which can function as a scaffold and give rise to cytokines or growth factors around the stem/progenitor cell. Micro RNA coated with microvesicle which is discussed later would be added.

3.5 Lessons from the repair of kidney by non-ES cells

In thinking of the natural development of ES cells into cells of the kidney, ES cells would develop into component cells of the kidney via renal stem/progenitor cells (pathway II in fig 1). Or, ES cells may develop into component cells of the kidney without going through renal stem/progenitor cells (pathway I in fig 1). But renal stem/progenitor cells can give rise to all component cells of the organ, developing ES cells into renal stem/progenitor cells would be the most efficient way to create component cells of the kidney. We have focused on the renal stem/progenitor cell for the regeneration or repair, and ES cell is one of the promising candidates apart from the ethical problem. Let us first overview the repair of kidney by the stem/progenitor cell other than ES cells, and then try to find out the clue to manipulate the ES cells to the renal lineage.

As an extra-renal source of the kidney stem/progenitor cell, bone marrow cells or other cells (Wang Y.,et al. (2004).) have been investigated. In the early 2000s, pluripotent bone marrow-derived stem cells were thought to contribute to kidney repair. Bone marrow comprises several cell types such as endothelial progenitor cells, hematopoietic stem cells (HSCs), and mesenchymal stromal cells (MSCs) which is often referred to as mesenchymal stem cells. Bone marrow-derived stem cells appeared to be able to replace damaged renal tissue by replacing mesangial cells (Imasawa T., et al. (2001), Ito T.,et al. (2001).), tubular epithelia cells (Poulsom R.,et al. (2001).), endothelial cells (Rookmaaker M.B.,et al. (2003).) and even podocytes (Prodromidi E.I., et al. (2006), Sugimoto H.,et al. (2006).). But these researches were based on the transplantation method and the kidney specific marker analysis. Therefore, an event of cell fusion appears to represent a part of the engraftment of the recipient cells by the donor cells which was interpreted as transdifferentiation in the publication of that era. In a strict meaning, the transdifferentiation is conversion from one cell lineage to another different lineage with conversion of cellular functions and markers and maintenance of a normal karyotype. In studies of these periods, the concept of transdifferentiation was loosely defined and clear transdifferentiation was not demonstrated in many of the studies. In thinking of the ES cell differentiation via extrarenal cells into renal lineage (pathway III or IV in Fig 1), these *in vivo* transplantation studies seem helpful, however, they should be interpreted with caution. *In vitro* differentiation condition from extrarenal source into renal component cells (Suzuki A.,et al. (2004).) may be helpful in manipulating ES cells into renal lineage. As to the cell fusion, Bonde S. et al recently reported an interesting research. When bone marrow cells are cocultured with ES cells, the phenomenon of cell fusion occurs. Almost all the hybrid cells had lost CD45 expression. In short, after cell fusion, the ES cells can reprogram the cellular contents of somatic cells (bone marrow cells in this research) to adopt an ES cell phenotype.

Bone marrow-derived pluripotent MSCs, but not HSCs have an ability to engraft to tubular epithelial cells in an experimental kidney injury model and to help to recover the lost renal function (Morigi M.,et al. (2004).). It has recently been reported that kidney-derived MSC contribute to vasculogenesis, angiogenesis, and endothelial repair (Chen J.,et al. (2008).). This finding supports the possibility that MSC residing in kidney can participate in kidney repair or regeneration.

But recent studies argue against the direct differentiation of bone marrow-derived stem cells or MSC into kidney. The relatively small frequency of engraftment of bone marrow-derived cells (3-22%) means that most of the kidney component cells are composed of kidney resident cells. Moreover the functional improvement in the recipient transplanted with stem/progenitor cells is not due to the existence of transplanted cell itself in the kidney. For

example, after ischemia-reperfusion injury, recipients transplanted with MSC keep improved renal function in spite of the small percentage of engraftment by the donor cells. This may be due to the various cytokines secreted from transplanted MSC that organize an appropriate microenvironment for the kidney repair in autocrine and paracrine fashion. To be sure, MSC secretes various cytokines such as VEGF, HGF and IGF-1 (Togel F.,et al. (2005).). Injection of bone marrow-derived MSC into peritoneal cavity attenuated the cisplatin-induced renal failure without the engraftment of MSC in the kidney. The conditioned media from culture of these stromal cells induced migration and proliferation of kidney-derived epithelial cells and significantly diminished cisplatin-induced proximal tubule cell death (Bi B.,et al. (2007).). In considering the cell administration pathway, the finding from these two experiments indicates that it is the humoral factors, not cellular factors that contribute to the kidney repair or regeneration by MSC.

3.6 Humoral factors important for the kidney repair or regeneration

Then the next question is "what are the humoral factors important for the kidney repair or regeneration?" Many factors have been proposed as candidates. One candidate is various cytokines secreted by MSC. There is a report indicating that administration of MSC to a rat model of ischemia-reperfusion-induced acute renal failure improved renal function, whereas administration of syngeneic fibroblasts did not. MSC expressed more growth factors VEGF, HGF and IGF-1 than fibroblasts (Togel F., et al (2005)). Some of these factors are known to modulate kidney function or repair. For example, VEGF attenuates glomerular inflammation and accelerates glomerular capillary repair (Shimizu A.,et al. (2004).). HGF, an angiogenic growth factor, prevents epithelial cell death and enhances regeneration and remodeling of injured or fibrotic renal tissue (Matsumoto K. & Nakamura T. (2001).). The effect of these growth factors or cytokines is evaluated as individual effect. If these factors are mixed and administered at a time, or if the administration timing is sequential, the effect might be different and complicated. Understanding the microenvironment in which the stem/progenitor cells is put in time and space in terms of cytokine expression would be firstly vital. This is the point from protein expression level.

Another humoral candidate factor would be RNA in microvesicle. This is a newly proved communication system. Microvesicles derived from MSC activate the proliferation of surviving renal tubular cells after injury by transferring mRNA (Bruno S.,et al. (2009).). Thus, MCS can repair the damaged tubuli by secreting microvesicles which function in a paracrine fashion. This is a communication tool in nucleotide level whose regulation system is not well known. Moreover, a microRNA are reported to mediate transforming growth factor-β1 autoregulation in renal glomerular mesangial cells (Kato M.,et al. (in press).). Ho J. et al reported that the loss of microRNAs in nephron progenitors resulted in a premature depletion of the population during kidney development, and as a consequence, a marked decrease in nephron number, which was accompanied by the increased apoptosis and expression of the pro-apoptotic protein Bim (Ho J.,et al. (in press).). Expression profiling in the embryonic kidney revealed several microRNAs (mmu-miR-10a, mmu-miR-17-5p and mmu-miR-106b) that are expressed in nephron progenitors and that are thought to target Bim transcript. MicroRNA-mediated regulation of Bim expression affects nephron survival. The importance of RNA is not restricted to only nephrogenesis. Recently, non-coding RNA has been emerging as key regulators of embryogenesis in general. Transcription factors OCT4 and NR2F2 and microRNA miR-302 are linked in the regulatory circuitry that critically regulate both pluripotency and differentiation in human ES cells (Rosa A. &

Brivanlou A.H. (2011).). The understanding of the microenvironment in terms of nucleotide level, microRNA during embryogenesis including nephrogenensis is important and the research has just begun.

Then, the last question would be "what are the cellular targets of these humoral factors?" The answer would be definitely the renal stem/progenitor cells including ES cells. Many researchers have undertaken this challenging theme to get the clearly defined renal stem/progenitor cells. Based on the characteristics of slow cycling property of stem/progenitor cells, the renal stem cell has been reported to exist as a label-retaining cell in renal papilla (Oliver J.A.,et al. (2004).) or tubular epithelial cells (Maeshima A.,et al. (2003).). Bowman's capsule (Sagrinati C.,et al. (2006).) and the S3 segment of the proximal tubules (Kitamura S.,et al. (2005).) have also been reported to contain renal stem/progenitor cells. A fraction of $CD133^+$ $CD24^+$ cells within the population of parietal epithelial cells are reported to engraft to tubular cells (Sagrinati C. (2006).). As markers of renal stem/progenitor cells are not clear, side population (SP) phenotype was adopted as a tool to investigate stem/progenitor cells in the kidney. Under our experimental conditions, kidney-derived side population cells did not engraft to kidney component cells (Iwatani H.,et al. (2004).), while other researchers reported the cells differentiated into multiple lineages *in vitro* and the transplantation of these cells *in vivo* ameliorated the impaired renal function without apparent integration into kidney component cells (Challen G.A.,et al. (2006).). Induction of ES cells into the above mentioned candidates of kidney stem/progenitor cells might be a target of the future study (pathway II-a in Fig 1).

3.7 Epigenetic factors important for the kidney repair or regeneration

A proliferative potential of the kidney is much lower than that of epithelial cells of the intestine. The characteristics of kidney are also borne out by the incidence of malignant tumors, as the kidney is less likely to be the origin of a tumor than is the gastrointestinal tract. The less proliferative potential of the kidney may be due to gene programming of its constituent cells. More precisely, partial and programmed gene inactivation of genomic DNA that is controlled in time and space, may be involved in the regulation of kidney fate. Different gene expressions in different organs for all the same genome: this mysterious but important fact cannot be solved without the concept of organ specific gene activation or inactivation. The system of epigenetic regulation could explain the concept. Actually, the epigenetic regulations are thought to be important for developmental biology. In terms of the renal progenitor cells, de Groh E.D. et al reported an interesting investigation (de Groh E.D.,et al. (2010).). The authors found that the treatment with 4-(phenylthio)butanoic acid (PTBA), which functions as histone deacetylase (HDAC) inhibitor expanded the renal progenitor cells in zebrafish. The PTBA-mediated expansion of renal progenitor cells was involved in retinoic acid signaling. Finally, the authors suggest a mechanistic link between renal progenitor cells, HDAC and the retinoic acid signaling pathway, although the precise target of HDAC is not clarified.

Aiden A.P. et al reported a comparison of genome-wide chromatin profiles of Wilms tumors, ES cells and normal kidney (Aiden A.P.,et al. (2010).). Wilms tumor is a pediatric cancer, characterized by a triphasic histology that includes undifferentiated blastemal components and varying amounts of epithelial and stromal elements (Rivera M.N. & Haber D.A. (2005).). The tumor can be associated with developmental abnormalities of nephrogenic rest and thought to be closely related to kidney organogenesis. Wilms tumor exhibited active chromatin domains previously observed in ES cells in terms of epigenetic

state. In the tumor cells, the domains often correspond to the genes that are critical for renal development and are expressed in renal stem cells. Wilms tumor cells also expressed "embryonic" chromatin regulators and maintained stem cell-like p16 silencing. It is suggested that Wilms tumor cells share transcriptional and epigenetic characteristics with normal renal stem cells As the developmental biology of the kidney is closely related to epigenetic state, the investigation of the mechanism of the epigenetic regulation of pluripotent stem cells would be highlighted.

In terms of the current difficulty in making many homogenous, high quality iPS cells available due to the lack of quality checking system, ES cell would be the best target for the development of the induction or differentiation conditions for pluripotent stem cells to the renal lineage. ES cell study will strongly lead iPS cell study and make the iPS cell study more matured.

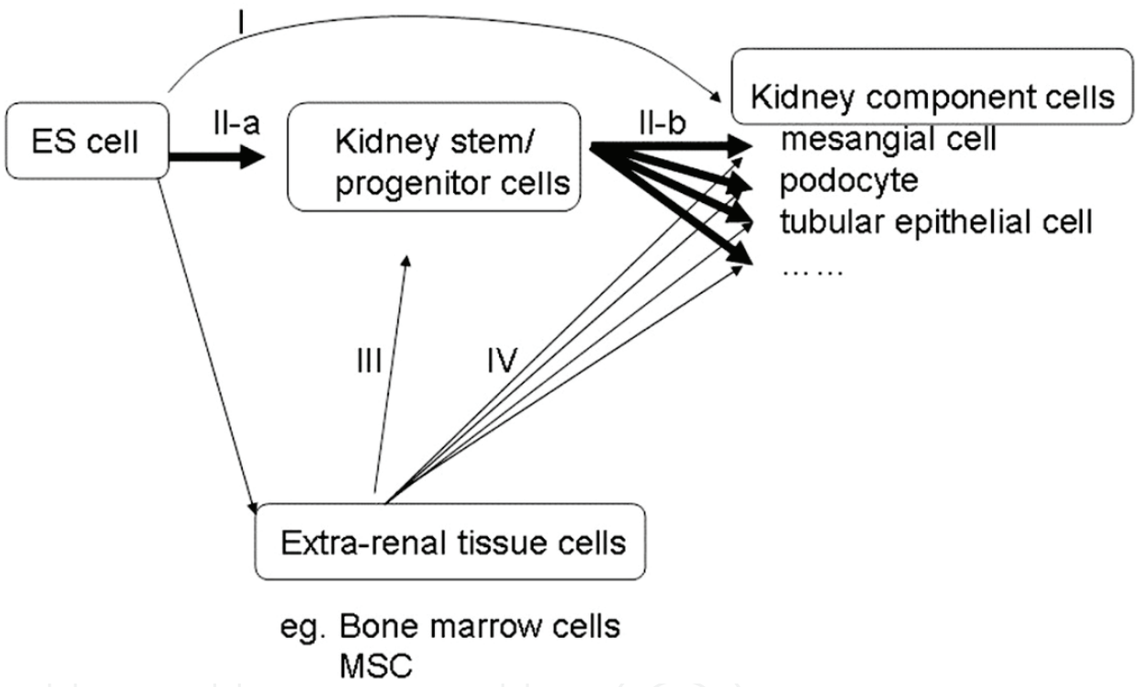

Fig. 1. Possible pathways for ES cells to differentiate into renal lineage
There may be several pathways for ES cells to differentiate into renal lineage.
ES cells would develop into component cells of the kidney via renal stem/progenitor cells (pathway II-a and II-b). Or, ES cells may develop into component cells of the kidney without going through renal stem/progenitor cells (pathway I), although the differentiation condition into kidney from ES cell is not clarified yet. Thick arrow (pathway II-a and II-b) would be the putative mainstream of ES cell differentiation. Previous reports of pathway IV may be helpful in understanding pathway II-b.

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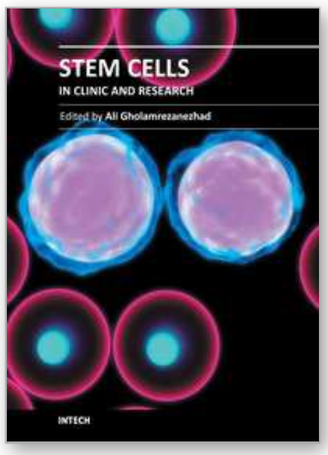

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Based on our current understanding of cell biology and strong supporting evidence from previous experiences, different types of human stem cell populations are capable of undergoing differentiation or trans-differentiation into functionally and biologically active cells for use in therapeutic purposes. So far, progress regarding the use of both in vitro and in vivo regenerative medicine models already offers hope for the application of different types of stem cells as a powerful new therapeutic option to treat different diseases that were previously considered to be untreatable. Remarkable achievements in cell biology resulting in the isolation and characterization of various stem cells and progenitor cells has increased the expectation for the development of a new approach to the treatment of genetic and developmental human diseases. Due to the fact that currently stem cells and umbilical cord banks are so strictly defined and available, it seems that this mission is investigationally more practical than in the past. On the other hand, studies performed on stem cells, targeting their conversion into functionally mature tissue, are not necessarily seeking to result in the clinical application of the differentiated cells; In fact, still one of the important goals of these studies is to get acquainted with the natural process of development of mature cells from their immature progenitors during the embryonic period onwards, which can produce valuable results as knowledge of the developmental processes during embryogenesis. For example, the cellular and molecular mechanisms leading to mature and adult cells developmental abnormalities are relatively unknown. This lack of understanding stems from the lack of a good model system to study cell development and differentiation. Hence, the knowledge reached through these studies can prove to be a breakthrough in preventing developmental disorders. Meanwhile, many researchers conduct these studies to understand the molecular and cellular basis of cancer development. The fact that cancer is one of the leading causes of death throughout the world, highlights the importance of these researches in the fields of biology and medicine.

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