Organoid models of childhood kidney tumours

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Paediatric kidney tumours comprise many different subtypes, each being heterogeneous in their cellular as well as genetic composition. Advances in the past decade in 3D culture models create new opportunities for the generation of preclinical models capturing this phenotypic and genetic heterogeneity, potentially enabling the generation of patient-tailored therapies.

> “Advances in 3D culture technologies … hold promise for the development of representative models of paediatric kidney tumours”

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Paediatric kidney tumours comprise ~7% of all childhood cancers and consist of distinct subtypes that differ in histology and prognosis (National Cancer Institute information on Wilms tumour and other childhood kidney tumours). The most common subtype is Wilms tumour, followed by clear cell sarcoma of the kidney (CCSK), malignant rhabdoid tumour of the kidney (MRTK), renal cell carcinoma (RCC) and congenital mesoblastic nephroma (CMN). These tumours are treated with surgery combined with chemotherapy and/or radiotherapy. These regimens have considerable early and late adverse effects, emphasizing the need for targeted therapies. The development of such therapies strongly depends on the availability of preclinical research models that recapitulate key aspects of the different kidney tumour subtypes.

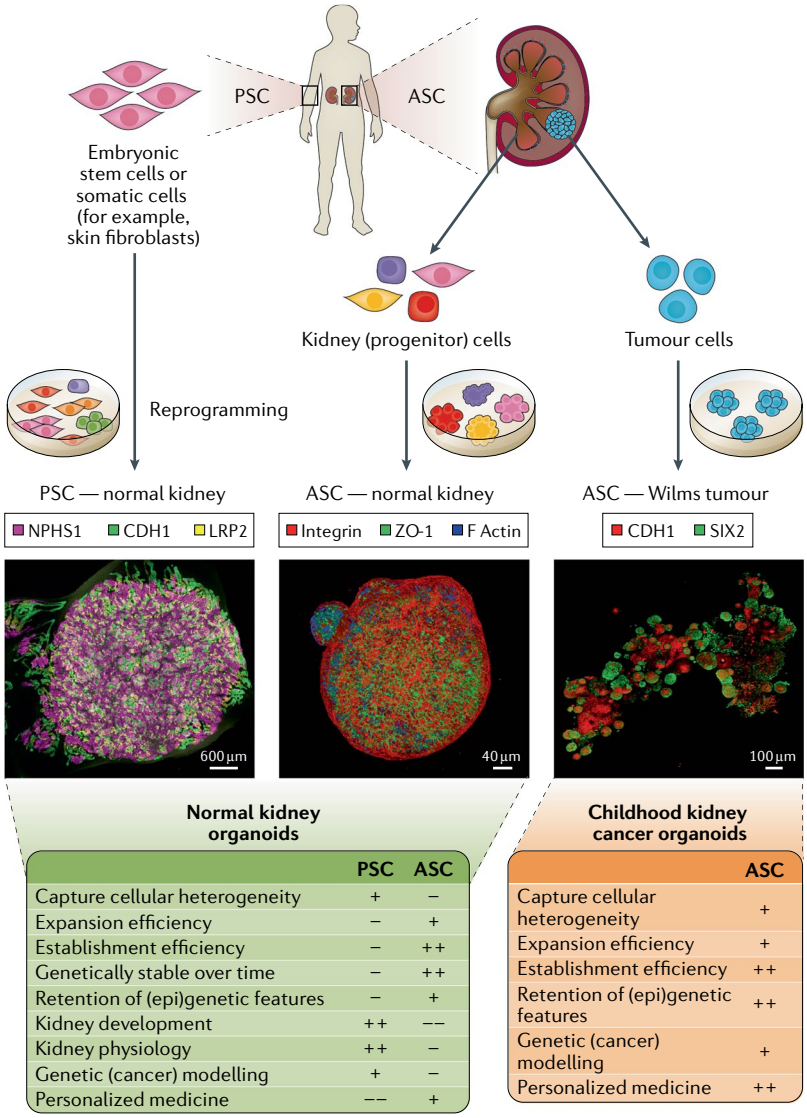
Classical preclinical cancer models include cell lines, genetically engineered mouse models and patient-derived xenografts. However, these models are scarce in paediatric kidney cancer (and for some subtypes even lacking) and typically do not capture the cellular and genetic heterogeneity of native tumour tissues. Thus, many therapies that demonstrate efficacy in models fail in patients. Advances in 3D culture technologies, such as organoids, hold promise for the development of representative models of paediatric kidney tumours and further progress in paediatric kidney cancer research. Organoids can be derived from pluripotent stem cells (PSCs) or organ-restricted adult stem cells (ASCs).

PSC-derived kidney organoids

Wilms tumours, CMN, CCSK and MRTK usually occur in infants and very young children (<5 years of age) and are, therefore, considered embryonal tumours that result from a differentiation block during embryonic development. A detailed understanding of which developmental pathways are impaired in kidney tumorigenesis could provide new therapeutic targets. PSC-derived organoid models potentially enable identifying such pathways, as they recapitulate organ development in vitro. PSCs can be derived from embryonic stem cells (ESCs) or from forced dedifferentiation of committed cells (induced PSCs (iPSCs)) through the expression of specific pluripotency factors in somatic cells. PSCs can then be differentiated into essentially all cell types of the body through their strictly timed exposure to specific growth factor cocktails. Takasato et al.[1] published a detailed protocol for the generation of kidney organoids from iPSCs, which results in the progenitors required for the development of nephrons, the functional units of the kidney. These organoids contained nephrons segmented into glomeruli as well as all tubule compartments, from proximal tubule to collecting duct, an endothelial network and renal interstitium. In a subsequent study, Low et al.[2] described a protocol for differentiation of iPSCs into segmentally patterned kidney organoids including an intrinsic vascular network that developed from a specific subpopulation of the nephron progenitor cells. These organoids were functional when orthotopically xenografted into mice. Kidney organoids derived from iPSCs provide the means to study kidney development and renal physiology. Thus, they might also enable studying the initiating steps of paediatric kidney tumorigenesis by, for example, introducing recurrent tumour-driving mutations in early nephron progenitor cells. Indeed, human iPSCs were used to study tumorigenesis of extrarenal rhabdoid tumours. Through neural induction of *SMARCB1*-deficient human iPSCs, Terada et al.[3] demonstrated that *SMARCB1* loss induces an ESC-like signature. When these cells were transplanted into the mouse brain, highly aggressive brain tumours with rhabdoid histology developed.

ASC-derived kidney organoids

ASCs can be expanded as organoids by providing a cocktail of stem cell niche factors mimicking the native environment of the respective ASC pool. After being embedded in an extracellular matrix, they typically grow as polarized structures resembling the organ they were derived from[4]. ASC-derived organoids can be



Normal kidney organoids

	PSC	ASC
Capture cellular heterogeneity	+	–
Expansion efficiency	–	+
Establishment efficiency	–	++
Genetically stable over time	–	++
Retention of (epi)genetic features	–	+
Kidney development	++	––
Kidney physiology	++	–
Genetic (cancer) modelling	+	–
Personalized medicine	––	+

Childhood kidney cancer organoids

	ASC
Capture cellular heterogeneity	+
Expansion efficiency	+
Establishment efficiency	++
Retention of (epi)genetic features	++
Genetic (cancer) modelling	+
Personalized medicine	++

Fig. 1 | **Kidney organoids derived from pluripotent and adult stem cells.** Organoids from pluripotent stem cells (PSCs) can be derived from embryonic stem cells as well as by forced dedifferentiation of somatic cells. Organoids from adult stem cells (ASCs) are grown from tissue-resident stem cell populations, found both in healthy and tumour tissue. Organoids established with each approach have advantages and disadvantages as research models. Left: 3D immunofluorescence image of an induced-PSC-derived organoid showing distal tubules (green), proximal tubules (yellow) and podocytes (magenta). Middle: 3D immunofluorescence image of an ASC-derived kidney organoid showing basolateral integrin (red), subapical tight junction protein (green) and filamentous actin (blue). Right: 3D immunofluorescence image of a Wilms tumour organoid showing epithelial cells (red) and blastemal cells (green). Respective features were judged as very suitable (+ +), suitable (+), not very suitable (–) or unsuitable (– –). Left image courtesy of M. Takasato, RIKEN Center for Biosystems Dynamics Research, Japan. Middle image courtesy of H. Clevers, Princess Máxima Center for Pediatric Oncology, Netherlands.

expanded long term while remaining genetically and phenotypically stable. We and others have established ASC-derived organoids from adult human kidney tissue[5,6]. These cultures, termed tubuloids, primarily contain tubular epithelial cells from proximal and distal nephron segments. These studies show that the tubular cells express functional transporter proteins, such as P-glycoprotein, and that tubuloids can be used to study nephrotoxicity and model infectious diseases (for example, BK-virus-mediated infection). As ASC-derived organoids mainly comprise epithelial cells and recapitulate tissue repair rather than kidney development (early (mesenchymal) nephrogenic progenitors are absent), they seem not very suitable for genetic modelling of embryonal kidney tumour development.

One major advantage of ASC-derived organoids compared with iPSC-derived organoids is that they can be grown from primary tumour tissue with high efficiency. Organoid models of many different adult cancers have been shown to reiterate the phenotypic and genetic heterogeneity of the tumour tissue they were derived from[7]. We have established the first collection of organoid models from a large spectrum of different paediatric kidney cancer subtypes, including Wilms tumours, RCC, CMN and MRTK[6,8]. These models are generated by enzymatic digestion of tumour tissue pieces and subsequent plating in ASC-based culture conditions[6], which enables long-term propagation of primary tumour tissue. Importantly, the established models generally retain the phenotypic, genetic, epigenetic and transcriptomic characteristics of their respective tumour type. For instance, Wilms tumours usually present with a tri-phasic histology of blastemal, epithelial and stromal cells. The cellular composition of the tumour correlates with prognosis: tumours with a high percentage of blastemal cells after pre-operative chemotherapy represent a high-risk group. Thus, a representative preclinical cell culture model for Wilms tumours should consist of the different tumour elements. Wilms tumour organoid cultures largely capture the cellular heterogeneity of primary tumour tissue, with epithelial, stromal and blastemal-like tumour cells[8]. Wegert et al.[9] describe the generation of 3D spheroids composed of blastemal Wilms tumour cells, supporting another method to grow ASC-derived cultures from high-risk cases. The high establishment efficiency of tumour organoid cultures from primary patient material, their compatibility with (high-throughput) drug screening[8] and their demonstrated predictive value of patient drug responses[7] create opportunities for the development of more targeted, and perhaps even patient-tailored, therapies. Notably, expansion of healthy tissue, including kidney tissue, as organoids enables toxicity testing in drug screening by selecting drugs that specifically kill tumour organoids while leaving healthy cells unharmed. This approach could also be used in a patient-matched fashion.

Outlook

PSC-derived and ASC-derived organoid models have revolutionized adult cancer research. The recent developments of organoid technology in paediatric kidney cancer hold great promise and will aid in efficient translation of research findings from bench-to-bedside. However, some important aspects need consideration (FIG. 1). For instance, Wilms tumours in particular are highly heterogeneous in their cellular composition and, although patient-tumour-derived ASC organoids largely retain this heterogeneity, capturing the complexity of a Wilms tumour can possibly only be achieved by growing organoids from multiple different regions of the tumour. Moreover, the efficiency of establishing iPSC tumour models from primary material remains low and is

influenced by differences in the genetic background of tumours[2]. Some tumours are, therefore, resistant to the reprogramming protocol, which introduces a bias towards, as well as over-representation or under-representation of, specific tumour subtypes[10]. Furthermore, iPSCs usually retain the epigenetic status of the parental cells (for example, skin fibroblasts) and the reprogramming protocol can induce genetic instability[10], which can compromise the resemblance of the generated model to the epigenetic and genetic characteristics of the patient tumour. Lastly, organoid cultures consist of pure tumour cell populations without their native microenvironment, which encompasses infiltrated stromal cells, immune cells and vascularization. This tumour microenvironment is known to have an important role in drug sensitivity and efficacy. Organoid co-cultures with stromal cells, immune cells and even vascularization have been reported for adult normal and tumour kidney tissues and efforts are currently ongoing to generate more representative preclinical tumour models. This approach would make organoid models more accurate avatars of patient tumours.

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Competing interests
The authors declare no competing interests.

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