

REVIEW

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Choosing the right cell line for renal cell cancer research

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Abstract

Cell lines are still a tool of choice for many fields of biomedical research, including oncology. Although cancer is a very complex disease, many discoveries have been made using monocultures of established cell lines. Therefore, the proper use of in vitro models is crucial to enhance our understanding of cancer. Therapeutics against renal cell cancer (RCC) are also screened with the use of cell lines. Multiple RCC in vitro cultures are available, allowing in vivo heterogeneity in the laboratory, but at the same time, these can be a source of errors. In this review, we tried to sum up the data on the RCC cell lines used currently. An increasing amount of data on RCC shed new light on the molecular background of the disease; however, it revealed how much still needs to be done. As new types of RCC are being distinguished, novel cell lines and the re-exploration of old ones seems to be indispensable to create effective in vitro tools for drug screening and more.

Keywords: Renal cell cancer, Cell lines, Clear cell RCC, Papillary RCC, In vitro RCC

Background

Cell line-based research had a major impact on the development of cancer treatment allowing an innumerable amount of effective drugs to be introduced into practice [1]. Cancer cell lines, especially those in NCI-60 panel, are the first target of preclinical drug screening that enables the quick elimination of ineffective compounds from further preclinical and clinical testing, requiring laboratory animals and patients [2]. Since 1955, the US National Cancer Institute (NCI) has provided screening support to cancer researchers worldwide. Until 1985, the NCI screening program and the selection of compounds for further preclinical and clinical development under NCI auspices had relied predominantly on the in vivo L1210 and P388 murine leukemias and certain other transplantable tumor models. In June of 1984, the author presented to the NCI Division of Cancer Treatment's Board of Scientific Counselors (BSC) a preliminary concept of a disease-oriented in vitro primary anticancer drug screen as a potential replacement for the P388 in vivo primary screen; after the development of the 60 cell lines panel, it was formally launched in 1990 and is

embodied in the present-day screen [3]. The ultimate goal of this disease-oriented screen is to facilitate the discovery of new compounds with potential cell line-specific and/or subpanel-specific antitumor activity [4]. The 60 cell lines of the National Cancer Institute Anticancer Drug Screen (NCI-60) constitute the most extensively characterized in vitro cancer cell model. They have been tested for sensitivity to more than 100,000 potential chemotherapy agents and have been profiled extensively at the DNA, RNA, protein, functional, and pharmacologic levels. Cell lines as a tool in biomedical research have both advantages and disadvantages in comparison with primary cultures and laboratory animals. First, they provide large numbers of cells available for testing, while primary cultures typically have a limited lifespan and require regular access to donors [5]. Importantly, cell lines are a crucial tool in implementing the 3Rs principle of animal research - Replacement, Reduction and Refinement [6] reducing the number of laboratory animals used during primary drug screening. This provides ease and speed of inventions. At the same time established cell lines represent a simplification of natural phenomena, as they are deprived of multilateral relations between different cell populations, microenvironment and responses of the host [7]. This can be partially overcome by culturing cells in complex three-

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dimensional systems or co-cultures, which better mimics natural surroundings in the host, retaining the ease of work and controlled conditions [8, 9]. However, further testing in more relevant biological models is indispensable to proceed to clinical trials but cell line research will still underlie most of them [10, 11].

Renal cell cancer (RCC) research has greatly benefited from cell line studies. Cell biology studies enable an understanding of RCC biology and translational studies [12–15]. To analyze homogeneous populations of cancer cells and feasibly identify genetic changes (mutations, gene expression) cell lines derived from tumor tissues (nephrectomy and metastasectomy specimens) from patients with renal cell carcinomas are being established [16]. The reported efficacy rate in establishing new cell lines is 75% from fresh and from 35% frozen specimens [16]. In a large study covering 498 successive attempts to establish RCC cell lines, 63 were successful (12%) [17]. The development of new drugs requires an appropriate model for testing; therefore the analysis of the rationale for choosing appropriate cell lines for RCC research is an objective of this review. We focus on popular RCC cell lines, their properties, and usefulness but also note the issues that may be vital for RCC cell line-based research.

RCC subtypes

Cancer sample (nephrectomy, metastasectomy) derived cell lines are used as *in vitro* RCC models, and it is important to remember that cell lines are in fact genetic models of their parent tumor histology [18]. Different clinical characteristics and treatment susceptibility are apparent between histotypes of RCC. Cancer cell lines *in vitro* preserve the unique genetic aberrations of parent tumor from which they were derived, and in long-term culture they acquire additional specific alterations [18]. In culture, cells no longer have easily identifiable morphological characteristics used in the histological classification of tumor specimens. As with primary RCC tumors, the mutation status of cancer including *VLH*, *cMET* and *TP53* and a general marker immunohistochemistry profile may serve to define the histotype of RCC cell lines (Table 1). Molecular and cell biology researchers using *in vitro* cell culture as experimental models need to recognize that, like primary cancers, the models used to study diseases genetics, biomarkers, and drug activity/resistance must also be stratified. RCC cell line-based studies are often hampered by a lack of proper annotation of RCC lines. Disease-specific studies need to incorporate cellular and clinical contexts [19, 20]. Surprisingly, many basic pre-clinical RCC studies employing functional research on “renal cancer/renal carcinoma/renal

adenocarcinoma/renal cell cancer” cell lines do not analyze the background of the investigated model and analyze different subtypes of RCC together, including wild-type cell lines and those harboring mutations (i.e. VHL) and cell lines of different histotypes [21–23]. The conclusions of such projects may be difficult to interpret, and the value of potential therapeutic targets is rather questionable, as is the true relevance to a particular RCC. Confirming established histotype-specificity markers for RCC cell lines should become the standard in planning and executing experiments on renal carcinoma [23, 24].

Major improvements in the pathologic classification of RCC have been reported over last 30 years. The first, known only as renal cell carcinoma, was in the 1960s divided into clear cell and granular histotypes. Currently five traditional and well-defined subtypes of RCC are known: conventional clear cell RCC, papillary (types 1 and 2) RCC, chromophobe RCC, carcinoma of the collecting ducts of Bellini, and unclassified RCC and these subtypes represent the majority of RCC cases diagnosed [25]. Clear cell RCC (ccRCC) is the most common subtype of renal cancer and accounts for approximately 70–75% of cases, so it cannot be assumed that all RCC-derived cell lines represent ccRCC. Papillary RCC (pRCC) is the second most common subtype of RCC and is diagnosed in approximately 10–16% of cases; pRCC is therefore expected among cell lines already in research. In the case of cell lines established in the 1970s or 1980s, histology (based on specific mutations and genetic changes) should be verified before any conclusions of the studies using cell lines are drawn. This particularly applies to new drug developments that are most often histotype specific [26].

In particular, pRCC was characterized in the 1980s as tumors containing more than 75% of papillary structures and not bearing 3p chromosomal loss on the contrary to ccRCC. Later [27], it was found that two different subtypes of papillary tumors may be distinguished (referred as to pRCC Types 1 and 2). Genomic characterization of types 1 and 2 papillary tumors is still incomplete. Inherited forms of types 1 and 2 tumors are referred as to hereditary papillary renal cell and hereditary leiomyomatosis and RCC (HLRCC), respectively. Germline met proto-oncogene (*MET*) and fumarate hydratase (*FH*) alterations are the hallmark of these cancer syndromes, but are infrequent in sporadic cases [28, 29]. It also needs to be underlined that the RCC subtype of clear cell papillary renal cell carcinoma [30] has mixed characteristics of both clear cell and papillary RCC, but possibly some of cell lines may represent this phenotype.

Chromophobe RCC (chRCC) is the third most common RCC subtype, and it was described for the first time in the mid-1980s. Additionally, rare histologic RCC subtypes were discovered in the 1990s and 2000s and

Table 1 Differentiation of RCC subtypes

Marker	Clear cell RCC	Papillary RCC Type 1	Papillary RCC Type 2	Chromophobe RCC	Oncocytoma	Xp11.2 translocation RCC
VHL mutation	+ (~90%)	-	-	-	-	-
cMET mutation	-	+	-	-	-	-
TP53 mutations	-	-	-	+	-	-
Other mutations	PBRM1 (~50%), BAP1 (~15%), SETD2 (~15%)	NRF2, CUL3	FH	-	Mitochondrial complex I genes	translocations of Xp11.2 (TFE3) or 6p21 (TFEB)
CK8	+/-	+	+	+/-	+	ND
CAIX	+	+/-	+	-	-	+
CAM 5.2	+	+	+	+	+	-
CD10	+	+	+	+/-	+/-	+
CD15	+	+	+	-	+	ND
CK18	+	+	+	+	+	ND
EMA	+	+	+	+	+	-
GST-alpha	+	-	-	-	-	ND
PAX2	+	+	+/-	-	+	+/-
PAX8	+	+	+	+	-	+
RCC Ma	+	+	+/-	-	-	+
VIM	+	+	+	-	-	+
AMACR	-	+	+	-	-	+
CD117	-	-	-	+	+	ND
CK7	-	+/-	-/+	+/-	-	+/-
CK19	-	-	-	-	-	+/-
CK20	-	-	-	-	-	-
c-KIT	-	+/-	+/-	+	+	ND
CLDN7/8	-	-	-	+	+	ND
E-cadherin	-	+	+/-	+	-	+
EpCAM	-	-	-/+	+	+/-	ND
Ksp-cad	-	-	-	-	+	+
PVALB	-	-	-	+	+/-	ND
TFE3	-	-	-	-	-	+
SMA	-	-	-	-	+	ND

Legend: *AMACR* α-methylacyl coenzyme A racemase, *CAIX* carbonic anhydrase IX, *CK7* cytokeratin 7, *CLDN7/8* claudin 7/8, *EMA* epithelial membrane antigen, *GST-alpha* glutathione S-transferase alpha, *EpCAM* epithelial cell adhesion molecule, *Ksp-cad* kidney-specific cadherin, *PVALB* parvalbumin, *RCC Ma* renal cell carcinoma marker, *SMA* smooth muscle action, *TFE3* Transcription factor E3 - transcription factor binding to IGHM enhancer 3, *PAX2/8* paired box gene 2/8, *VIM* vimentin, *ND* no reported data

include collecting duct carcinoma, medullary RCC, translocation RCC, and mucinous tubular and spindle-cell RCC. Recently, even more new subtypes have been described- hybrid oncocytic chromophobe tumor, mucinous tubular and spindle cell carcinoma, multilocular cystic clear cell RCC of low malignant potential, carcinoma associated with neuroblastoma, and renal medullary carcinoma [25, 31, 32]. Nevertheless, one should also not forget RCC types that were approved in 2013 by the International Society of Urological Pathology (ISUP)

Vancouver Consensus Statement including five more epithelial tumor subtypes: the microphthalmia (MiT) family translocation RCCs (Xp11 translocation RCC), tubulocystic RCC, acquired cystic disease-associated, RCC clear cell tubulopapillary RCC, and hereditary leiomyomatosis–RCC syndrome-associated tumors. Next, three RCC subtypes were given provisional status- thyroid-like follicular carcinoma of kidney, succinate dehydrogenase B deficiency-associated RCC, and anaplastic lymphoma kinase translocation RCC [31, 32].

To distinguish RCC subtypes, genetic analysis may be employed. The von Hippel-Lindau (VHL) gene is known to be most often mutated in renal cell carcinoma of clear cell type (ccRCC) in up to 90% of sporadic ccRCC cases [33] and multiple surprising and contradictory reports on the VHL gene status in common RCC cell lines have been published (Additional file 1: Table S1). Moreover recently three other tumor suppressor genes PBRM1 (mutated in ~50%), BAP1 (~15%), and SETD2 (~15%) were defined as specific for the ccRCC subtype. PBRM1, also known as BAF180 or Polybromo, is a member of the PBAF SWI/SNF chromatin remodeling complex. *VHL*, *PBRM1*, *BAP1* and *SETD2* are allocated on chromosome 3p. BAP 1 is a BRCA1- associated protein-1 (ubiquitin carboxy-terminal hydrolase). Mutations in *BAP1* and *PBRM1* in ccRCC tend to be mutually exclusive [34].

This in vivo heterogeneity of RCC should be mimicked in vitro; a wide panel of cell lines with different characteristics is needed to provide us with a tool for both basic and applied research.

RCC cell lines used in research

The number of available RCC cell lines is impressive: more than 20 cell lines are widely used- deposited in cell banks- and dozens of others were established and used for research in selected laboratories (Fig. 1) [16]. The most popular RCC cell lines are delivered by ATCC and other certified cell banks (Additional file 1: Table S1). Most cell lines were established between the mid-1970s till the late 1980s, when subtypes of RCC including clear cell, papillary, or chromophobe RCC were not yet distinguished; therefore, all the subtypes may be represented among RCC cell lines. Many researchers currently refer generally to RCC or renal carcinoma when describing their laboratory model. Although this is true, it regrettably narrows conclusions that can be drawn from their research, thus limiting the translational potential of in

vitro studies. However, a growing amount of data can help to categorize cell lines established before 1995 into correct RCC subtypes as the cell lines genetic profile is analyzed and cells lines are characterized for markers of particular RCC subtypes with multiple methods including immunohistochemistry (IHC), gene sequencing, and xenografted tumors histology analysis [35, 36]. The proper initial molecular characterization of cell lines is indispensable to provide later in vitro tools to study genetic and cellular events underlying carcinogenesis, disease progression, and/or drug activity [23, 37–39]. Often, information on original patients- cell line donors- is fragmentary and more precise characteristics of established cell lines come with time, thanks to cell biology and genetic studies [28, 33]. Most recently, interesting cell lines representing novel RCC subtypes have been established, including NCCFH1 for hereditary papillary renal cell carcinoma type 2 [40] or the S-TFE cell line for Xp11 translocation renal cell carcinoma [41]. In Additional file 1: Table S1, we collected various information available for over 60 cell lines- including the source of starting material, basic genomic data, and database tools that might be useful in designing RCC cell line-based experiments.

ACHN cell line

The ACHN cell line represents an uncertain RCC histotype (Fig. 1). It was established from pleural effusion and models metastatic disease. In early xenograft studies, tumors were described as poorly differentiated clear cell [42] however, a more recent genomic comparison suggests papillary characteristics of ACHN [43]. Moreover, this cell line harbors a *c-met* polymorphism that is specific for papillary RCC [44]. Chromosome aberrations in ACHN cells also resemble those of papillary tumors [45]. Yet, gene expression analysis revealed similarities to clear cell tumors, especially when concerning the *MYC* pathway [29]. No mutations in VHL and HIF-1 α

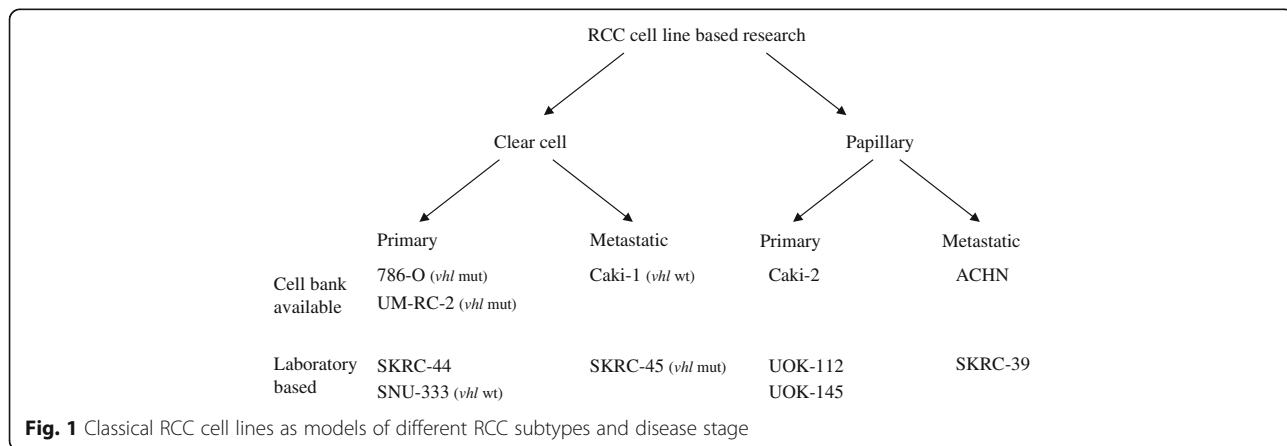


Fig. 1 Classical RCC cell lines as models of different RCC subtypes and disease stage

mRNA in ACHN cell line, could confirm non-clear cell histology [46].

A-498 cell line

A-498 is a “classical” RCC cell line belonging to the NCI-60 panel and is therefore widely used in cancer research. Mutated *vhl* [46, 47] would suggest a clear cell subtype; however, a study by [48] detected no mutation. In addition, [49] claimed that A-498 is of papillary origin, as xenografts exhibited such histology. To some extent this observation was confirmed by chromosome 8 and *MYC* analysis [29]. Nonetheless, this issue was not significantly recognized by scientific community and most researchers use it as a model of ccRCC [47, 50, 51] with rare exceptions [52].

786-O cell line

786-O, established as one of the first RCC cell lines, has many characteristics of ccRCC and is used most commonly in RCC-focused research (Fig. 1). The 786-O RCC line is defective in *VHL* expression, as it harbors mutated *VHL* [53, 54] with altered HIF and VEGF (Vascular endothelial growth factor) pathways [46] and gives rise to clear cell tumors in nude mice [55, 56]. In this RCC model the vast majority (122/160) of genes induced by hypoxia in wt-*VHL* transfected 786-O (*VHL*⁺) cells are not significantly up-regulated in *VHL* mutated 786-O cells, confirming that the loss of *VHL* is not equivalent to hypoxic exposure and that in RCC, the *VHL* tumor suppressor has a distinct role from its activity in the hypoxia-inducible pathway [57]. Interestingly, side populations (SPs) of higher tumorigenicity were observed in this cell line, proving its usefulness in cancer stem cell studies [58, 59]. Surface receptors also confirm the ccRCC phenotype of 786-O cells, as these cells are positive for CD10 [60] and vimentin [61]. These cells produce high levels of VEGF [46] as well, which is characteristic of ccRCC. This cell line can be used to model bone metastasis in RCC- 786-O cells injected into nude mice, both directly to the tibia or to the cardiac ventricle, cause bone destruction and vascularization [62, 63]. A subline derived from such metastatic tumors can be cultured in vitro in 3D systems that retain bone metastasis characteristics [64].

Caki-1 cell line

Caki-1 is a widespread model line of metastatic ccRCC (Fig. 1). While harboring wild-type *vhl*, it was shown to produce tumors of clear cell histology in nude mice, both by the total population and SP cells [65]. High VEGF production [66] (especially in hypoxic conditions [56]) in those cells is also a hallmark of ccRCC. Interestingly, this cell line was also proposed as a model system of proximal tubule epithelium, as in culture, cells can

form a polarized layer with morphological, physiological and biochemical characteristics of functional, well-differentiated kidney tissue [67].

Caki-2 cell line

Caki-2 was established from a primary tumor of the kidney. This cell line was primarily defined as the ccRCC cell line (Fig. 1) that expresses wild-type pVHL but does not express HIF-2 α . However, a low expression of HIF-1 α is detected in this cell line for unknown reasons [46]. The recent evaluation of tumors formed by Caki-2 in nude mouse in orthotopic and sub-cutaneous implantations revealed cystic papillary tumors with microvilli and microfilaments, few mitochondria, lysosomes or lipid droplets, and multilamellar bodies [68–70]. Although the Caki-2 cell line has been treated as a model for primary ccRCC, a growing amount of data suggests that it is a cell line of papillary RCC. These cells express high levels of MET and LRRK2 [71] and harbor chromosome 8 aberrations [29] characteristic of papillary RCC. Interestingly, reports on *VHL* gene status of this cell line are inconsistent— some researchers detected no mutations [46], while others [47, 48] reported mutations in α -domain of VHL, which may imply the clear cell histology of Caki-2. Such misleading observations have led to non-uniform use of this cell line—it is now widely used both in clear cell RCC [66, 72, 73] and papillary RCC [69–71, 74] research. Finally, it is generally described as clear cell RCC by most cell banks, which may lead to misinterpretation.

769-P cell line

The 769-P cell line, established along with 786-O by [75], harbors mutated *vhl* and secretes high levels of VEGF, suggesting a ccRCC phenotype (Additional file 1: Table S1 for ref). These cells contain a SP of higher tumorigenicity that can create tumors even after serial passage in nude mice retaining their original histological characteristics [76]. However, the validity of this cell line in xenograft studies is limited; injected subcutaneously [56] or *i.v.* [55], they failed to form tumors in some models.

RCC4 cell line

Another interesting cell line is RCC4, a *vhl* mutant [77, 78] derived from a primary tumor widely used as a model for VHL-dependant mechanisms, with a commercially available counterpart cell line with restored wild-type gene [79]. It is used [80–83] for both in vitro and in vivo experiments, as RCC4 cells are tumorigenic in nude mice. Unfortunately, no data on the original patient can be found; supposedly the cells were obtained by Prof. C.H.C.M. Buys, Department of

Medical Genetics, University of Groningen, Groningen, the Netherlands.

SMKT-R cell lines

SMKT-R1, SMKT-R2, SMKT-R3 and SMKT-R4 are cell lines established in Sapporo Medical College from primary lesions of RCC from xenotransplantable tumors at passage 2 or 1 in nude mice [84]. *Vhl* is mutated in cell lines SMKT-R2 and SMKT-R3 [48], and in SMKT-R2 and SMKT-R3 HIF- α proteins are expressed [85]. SMKT-R3 original tumors are characterized as papillary type and granular cell subtype, and the level of secreted VEGF is lower than in SMKT-R2 line [86] confirming the non-ccRCC histology of this cell line. Research on the SMKT-R cell lines confirms that RCC cell lines can retain the histology of the original tumor after in vitro culture and passage in nude mice.

Memorial Sloan Kettering Cancer Centre cell line collection

The metastatic dissemination of cancer may be partially recapitulated in vitro with analysis of multiple metastatic loci and metastasis-derived cell lines. This in vivo heterogeneity of tumors can be mimicked in vitro with use of a panel of cell lines in the place of multiple biopsy derived samples [87]. The Memorial Sloan Kettering Cancer Centre provides an RCC repository with over 30 primary tumor-derived and 15 metastatic tumor-derived cell lines (SK-RC panel) collected between 1972 and 1987 [17]. The MSKCC panel covers cell lines obtained from tumors that developed in the most common RCC metastases loci, including the adrenal glands (SK-RC-45), lymph nodes (SK-RC-18, SK-RC-26b), lungs (SK-RC-26a SK-RC-31, SK-RC-38 SK-RC-54), bones (SK-RC-42, SK-RC-46), soft tissue (SK-RC-17, SK-RC-39), and the brain (SK-RC-9, SK-RC-13). This collection consists of samples with different features; however, the molecular characterization of particular cell lines is still incomplete (Additional file 1: Table S1). The SK-RC-45 line was used to study immune responses in RCC and the induction of T cell apoptosis [88, 89], while SK-RC-42 was shown to contain cancer stem cells ("CSCs") [90]. These cell lines express either HIF-2 α only (SKRC-21, SKRC-17) or both HIF-1 α and HIF-2 α (SKRC-7, SKRC-10, SKRC-52) [91].

Laboratory specific RCC cell line collections

Thirty cell lines from primary tumors as well as four lines from metastatic tissues taken from 31 patients were established in the National Cancer Institute in Bethesda (UOK 101–131 cell lines). Sixteen of the cell lines were derived from tumors composed predominantly of clear cell RCC, three were granular cell RCCs, and one

papillary type (UOK112). The remaining tumors were of mixed types including clear and granular cells, clear cell + granular and sarcomatoid cells (UOK 105, 117, 119, 123 and 127), or clear cell and papillary (OUK 120). Cell line phenotypes, including morphology, in vitro growth characterization, and tumorigenicity in nude mice were determined for these cell lines [16]. Another seven RCC cell lines were established in the Korean Cell Line Bank of Cancer Research Center and Cancer Research Institute. In particular, five cell lines were derived from clear cell RCC (SNU-228, -267, -328, -349, and -1272), one from granular RCC (SNU-482), and one from mixed clear and granular RCC (SNU-333). The mutational status of cell lines was confirmed for von Hippel-Lindau (VHL), p53, TGF-beta type II receptor (TGF-betaRII), hMSH2, and hMLH1 genes [92]. More recently, Chinese-origin cell lines named NRCC from the primary ccRCC and MRCC from the metastatic ccRCC were established from the primary tumor of a 49-year-old male ccRCC patient and the metastatic tumor of a 62-year-old male with ccRCC. The morphology of cell lines along with the doubling times, colony formation rates, invasion assay, anchorage-independent growth, cytogenetic characteristics, and expression of CD105, CD133, CD44, CD24, CD56, CD99, and CD74 markers as well as N-cadherin, E-cadherin, and vimentin were described and have shown that NRCC cells displayed more epithelial characteristics, while MRCC cells are mesenchymal-like [93].

RCC cancer stem cell cultures

Specific subpopulations of cancer cells are available for culture. In particular cancer stem cells referred to as tumor-initiating cells are currently becoming available. In particular, donor specific kidney cancer cells derived from primary tumors cultured in stem cell-promoting media are enriched in CSCs (Promab cat. No. CF100107, Celprogen cat. No. 36117-44). The role of CSCs in RCC has been reviewed elsewhere [94], as they are potential treatment targets [95]. They are putatively tumor-initiating cells that promote disease development and progression and may be distinguished using different approaches. CSCs can be discriminated based on their unique features; in different cancers, in comparison to other tumor cells, they have elevated aldehyde dehydrogenase (ALDH) activity, increased glycolysis and glycine/serine metabolism or low concentrations of reactive oxygen species and ATP, the ability to form spheres, and a reduced level of proliferation. Such functional characteristics can be used to selectively isolate subpopulations of cancer cells [96–99]. Moreover, due to the increased action of ABC transporters, CSCs are more resistant to drug treatment, which enables the separation of CSCs from other tumor cells based on the increased efflux of ABC-dependent dyes (Rhodamine123 or Hoechst33342)

as a dye-negative side population (SP) [98, 100]. In RCC, CD105 and CD133 surface markers are also suspected to identify cells with stem properties [101, 102]. CSCs express genes typical for multi/pluri-potent cells; Oct4, Sox-2, Nanog, and Bmi-1 [98, 103] and are shown to have increased tumorigenicity and clonogenicity. In stable cell cultures of RCC cells, cancer stem cells have been identified, which primarily reflects the unexpected heterogeneity of cell culture in vitro. In 769-P, ACHN, Caki-1, SMKT-R2, and SMKT-R3 cell lines, the SP of Hoechst33342 negative cells was shown to express CSC properties [76, 104]. The ACHN line CSC subpopulation is Hoechst33342-negative and ALDH-positive [98], while Caki-2 CSCs are also ALDH-positive and form spheres [99]. The 786-O cell line is also shown to bear the CSC subpopulation, as confirmed by Rh123 fluorescent intensity based isolation [105]. At the same time, SK-RC-42 cells cultured in spheres have features of CSCs [90]. However, methods in CSCs research are not yet uniform, and some contradictory data complicate the explanation of their role in RCC. In particular, [105] showed that Rh123^{low} cells show stronger CSC properties than the Rh123^{high} population that could be suspected to group stem cells.

Normal renal proximal tubule cells

Primary cultures of normal human epithelial cells of proximal origin derived from the renal cortex have been shown to present very homogeneous morphology in vitro [106]. If normal tissue originating from the same donor is not available, RPTEC cells are used as the control cell line for the comparative analysis of cancer and normal renal cells [57]. RPTEC cells are human renal proximal tubule epithelial cells that are derived from normal or diseased (e. g. diabetic) donors [107]. As primary cells, they have a limited lifespan, but usually can reach at least eight passages maintaining typical epithelial, cobblestone morphology and the expression of epithelial and renal markers like E-Cadherin, CK, or ZO-1 [108, 109]. These cells are also available as immortalized lines: HK-2 and RPTEC/hTERT. The former was transduced with human papilloma virus (HPV 16) E6/E7 genes [110] while the latter ectopically expresses the catalytic subunit of telomerase (TERT) [111]. These modifications enable the cells to be cultured continuously constituting a convenient control for RCC cell lines. Many studies show that the cells are genomically stable and most functional characteristics remain typical for RPTEC [111]. RPTECs are used to model basic kidney functions and renal diseases. Nephrotoxicity [112], the efflux of drugs [113], responses to environmental toxicants [114] or renin-angiotensin system (RAS) signaling [115] can be studied with the use of these cells. In RPTECs, the steady-state amount of VHL protein is

strictly regulated by the cell density, and the cellular VHL content is more than 100-fold higher in dense cultures than in sparse cultures [116].

Total kidney isolates are also available (e. g. Applied StemCell, cat. no. ASE-5186); after the dissociation of a healthy renal sample, all cells are frozen which allows various cell types to be cultured without direct access to donors.

Another widely used non-cancer renal cell line is the HEK293 cell line. Generated in 1977 by the viral transformation of human embryonal kidney cells, [117] HEK293 cell line is a widely used expression tool [118]. Although studies show that these cells have many features of neuronal cells [119, 120], they are still used as a model for kidney physiology [121, 122]. Therefore, they may be used as convenient controls in RCC in vitro research as well, but caution is needed for interpretation [73, 123].

RCC cell lines specific for bone metastasis-oriented research

RCC cell lines have also been developed as tools to study specific phenomena in RCC. In particular, a model to study the biology of the bone metastasis of renal cell carcinoma has been established. This cell line induced osteolytic lesions in nude mice after injecting into the tibia. RBM1 cell line cells expressed high levels of cytokines involved in osteoclast activation and bone resorption- parathyroid hormone-related peptide, interleukin-6, and macrophage colony-stimulating factor. Moreover, cells were confirmed to express EGFR and c-MET [124]. SK-RC-42 and SK-RC-46 also represent bone metastasis-derived cell lines [17], as well as the CRBM-1990 cell line [125], while ACHN and 786-O cells transplanted into the left ventricle establish bone metastases [126, 127]. If studied after injection, bone metastasis-derived 786-O cells (Bo-786-O) compared to parental 786-O cells or cells that localized in the liver or lymph nodes had significantly overexpressed cadherin-11, but not CXCR4, HIF-1 α , VEGF, angiopoietin-1, Tie2, c-MET, PTHrP, IL-6 or RANKL [128]. Sunitinib prevents the growth of ACHN cells in a bone metastatic model. The number of osteoclasts in sunitinib-treated ACHN-bearing mice is significantly lower than that in non-treated mice [126].

Other RCC cell lines

RCC subtypes less frequent than clear cell and papillary are even more difficult to study in vitro as specimens are obtained rarely. Recently, several cell lines were developed to model HLRCC; a rare genetic disorder that manifests by cutaneous and uterine leiomyomas and kidney tumors. Metastatic NCCFH1, UOK262, and primary

UOK268 cell lines are FH deficient and can serve as models for hereditary papillary type 2 RCC [40, 129, 130]. An in vitro model for Xp11.2 translocation carcinoma has recently been established as well. S-TFE cell line is tumorigenic in nude mice and has fused TFE3 and ASPL genes [41].

Large scale molecular data and RCC cell lines

Some RCC cell lines, mostly present in NCI-60, were used in whole genome analyses. Mutational, copy variant, and expression analyses available for cells lines are provided as links to databases in Additional file 1: Table S1. The Cancer Genome Atlas (“TGCA”) provided much valuable information on RCC characteristics that can now be used in cell line-based research. TGCA analyses identified *vhl*, *pbrm1*, *bap1*, *setd2*, *kdm5c*, *pten* and *mtor* as the most frequently mutated genes in ccRCC [131]. *vhl* mutant cell lines are easily accessible; 786-O, 769-P, RCC4 (with reintroduced *vhl* control cell line available), are the most widely studied models for VHL role in RCC. Apart from gene mutation, *vhl* promoter region methylation often occurs in ccRCC samples which effects in no protein expression. Such a phenomenon was reported for SK-RC-54, 769-P, and A-704 [132].

Classical cell lines were recently verified for PBRM1 expression status and confirmed ACHN- positive, 786-O- positive, and A-704- negative. The ACHN cell line expresses the protein, but harbors a heterozygous nonsense mutation, while 786-O, 769-P, Caki-1, and A-498 express wild-type PBRM1 [133, 134]. The PBRM1 mutation is also reported in Caki-2 and A-704 lines; loss-of-function gene mutations in A-704 and the deletion in exon 17 of the PBRM1 gene in Caki-2 [135] results in no protein expression [133, 134, 136]. Mutations have also been detected in OS-RC-2 and RCC-ER (see Additional file 1: Table S1 for ref) cell lines; however, the former was reported to express PBRM1 protein [133]. The strong and moderate expression of PBRM1 was also confirmed in the next 16 cell lines, including normal human embryonic renal cell lines (HEK 293 and 293T) and human renal proximal tubular epithelial cells (HK2) as well as cancer cell lines- 769-P, A-498, KC12, Caki-1, SW156, and SLR21-26 [133]. The knockdown of PBRM1 in cells with wild-type gene increased the proliferation, migration and colony formation abilities [137], supporting this gene’s important role in RCC progression, as the loss of PBRM1 was correlated with a worse disease outcome in patients [133].

BAP1 mutants are available as UM-RC-6, 769-P, and SN12C cell lines [138]; however, 769-P cells still produce the protein [139]. The reintroduction of the wild-type gene reduced cell proliferation and sensitized cells to treatment, and it was proposed that BAP1 is a tumor

suppressor, as gene loss is associated with patients with higher-grade RCC [139]. SETD2 mutations have also been detected in A-498, A-704, Caki-1, and RCC-ER [140–142], PTEN mutations in 786-O, and OS-RC-2, while no mutated RCC cell lines could be found for KDM5C. An mTOR mutation was found for SNU349 and RCC-ER cell lines only (see Additional file 1: Table S1 for ref). Studies on SETD2-defective RCC cells proved that the mutation of this gene affects DNA repair and may correlate with in vivo disease progression [143].

Chromosome alterations are also common in ccRCC with the loss of the 3p chromosome (containing *vhl*, *pbrm1*, *bap1*, and *setd2*), 14q loss (*hif1a*), or 5q gain being the most frequent [131]. However, the chromosome analysis of RCC cell lines is not readily available; a 5q gain was observed in most RCC cell lines tested by [144] apart from A-498. Chromosome 3p loss was reported in several UOK RCC cell lines: UOK108, 121, 125, and 127 [145]. Simultaneous losses in 3p and 14q were observed in 769-P, 786-O, A-704, and Caki-1 [146, 147].

In the case of pRCC, TCGA indicated *met*, *setd2*, *nf2*, *kdm6a*, *smarcb1* and *fat1*, *bap1*, *pbrm1*, *stag2*, *nfe2l2*, *tp53* genes being the most frequently mutated among patient samples [148]. The UOK112 cell line derived from pRCC patient [16] was studied in the context of HGF/MET signaling; however, no data on *met* status could be found [149]. Interestingly, Caki-1 harbors a mutation in *met* (COSMIC database). As mentioned above, ACHN cells have a MET polymorphism (by some also referred to as a mutation [150]), but protein was detected and can be phosphorylated [151]. This cell line was also shown to contain an NF2 mutation, together with SN12C, while Caki-1, A-704, 769-P, TK10, 786-O, A-498, and OS-RC-2 were confirmed to be wild-type for NF2 [142]. A KDM6A mutation was reported for the SN12C cell line only (COSMIC database), but no alterations in the methylation of gene promoter was observed in 5 other cell lines (786-O, 769-P, A-498, ACHN, and Caki-1) by [152]. A FAT1 mutation was found in Caki-1, OS-RC-2, SN12C, RCC-FG2, and TK10 (COSMIC database) but no functional analysis of this gene in in vitro RCC was reported.

Chromosome aberrations present in pRCC include chromosomes 7 and 17 gains and 9p loss [148]. In the case of cell lines, Caki-2 and ACHN cells show a gain in genes located on chromosomes 7 and 17 and 9p loss, which may be an additional clue confirming the papillary origin of these cells [146, 153].

TP53 tumor-suppressor mutations, present in 50% of tumor cases in general, are less frequent in RCC (around 20% cases [154]), but confirmed in 786-O, A-498 (COSMIC and CCLE databases), SN12C, TK10 [155] and reported as wild-type in ACHN, Caki-1, and Caki-2 [156]. Varied expression of the protein is visible in cell lines,

which corresponds with *in vivo* data, as p53 over-expression occurs in later stages of the disease [157] and to some extent correlates with poor prognosis [158]. A relatively high expression of p53 was observed in ACHN, Caki-2, UOK121, and UM-RC-6 and low in A-498 (although mutated) [159, 160]. Other genes, established by TCGA studies to be often mutated in RCC, have been less frequently studied with the use of RCC cell lines, and no detailed information on gene status could be found.

RCC cell lines in xenograft studies

The clinical translatability of cell line experiments relies on valid animal models. They provide complex platforms for studying oncogenesis and the effectiveness of therapeutic approaches and, enable the verification of cell lines' tumorigenicity. While tumorigenic cells can be implanted into the same species (allografts) or another species (xenografts), we will focus on immunocompromised experimental animals that are injected with human cell lines. The wide application of this approach in oncology studies followed the discovery of two groups of immunodeficient animals: "nude" mice [161] and later severe combined immunodeficient (severe combined immunodeficiency; SCID) mice [162].

Athymic nude mice are hairless, an effect of the *Foxn1^{nu}* (Forkhead box protein N1) mutation, but more importantly they lack a thymus and are T-cell deficient but produce functional B-cells [163]. This is not the case with SCID mice that have a single nucleotide polymorphism (*Prkdc^{scid}*) within the DNA-dependent protein kinase of the catalytic polypeptide *Prkdc* gene. This mutation affects both T and B lymphocytes [162]. SCID rodents display less pronounced immunoreactivity than athymic nude mice to implanted cancer cells that results in greater receptivity to tumor xenotransplantation [164]. Recently, several promising transgenic models have become available, including humanized NSG mice [165]. Still, most of the available data on xenografted RCC cell lines comes from the athymic nude mice model.

The nature of the outcome of animal experiments strictly relies on the place and route of cell inoculation. Ectopic tumor xenograft models employ subcutaneous (s.c), intraperitoneal (i.p.), intravenous (i.v.) or intramuscular (i.m.) implantation of tumor cells. While advantages are the approach's simplicity and reproducibility, an obvious disadvantage of this approach is the non-physiologic growth location. An alternative approach, the orthotopic xenograft model, involves the implantation of a tumor into the originating tissue site of the cancer in rodents. In the case of RCC, the widely accepted implantation site is the renal subcapsule [166, 167]. It is believed that

orthotopic tumor implantation more closely simulates the microenvironment of the original tumor [168]. However, orthotopic xenograft models have some additional disadvantages that could be crucial in RCC research: they are more technically challenging and may have highly variable tumor take rates and growth rates; they could also result in significant animal morbidity due to the surgical implantation of tumor cells [168]. Importantly, the monitoring of tumor growth is also more challenging in orthotopic models. All these difficulties have led to subcutaneous ectopic implantation being the most widely used approach in RCC cell lines animal research.

Ectopic xenograft models of athymic nude mice with various genetic backgrounds have been extensively used for studying established RCC lines, including 769-P, 786-O, Caki-1, SK-RC-38, SK-RC-42, SK-RC-44, SK-RC-45, SK-RC-46, and others [17, 55, 169–173]. As summarized in Additional file 1: Table S1, most of the cell lines described in this article were proven to be tumorigenic in nude mice. The implanted tumors normally become palpable within 5 days and reach a volume of 100 mm³ in 2 weeks [169–171]. Implanted established cell lines xenografted into nude mice preserve essentially the same histology as the primary tumors [174]. In addition, orthotopic xenotransplantation of Caki-2 into nude mouse produces tumors that closely resemble histology of human RCC.

Generally, xenografted tumors are considered as not producing metastasis in most cases. Sharkey and Fogh [175] studied 106 malignant human tumor lines and observed metastasis in only approximately 1%. Such factors as tumor size and growth rate and age and sex of the host mouse appear unrelated to metastasis [167]. However, the incidence of metastasis is increased in SCID rodents [176]. Moreover, the route of injection into nude mice affects the metastasis incidence. Naito et al. [167] indicated that even in highly metastatic cells, *i.v.* injection did not yield significant metastasis, but the injection of cells orthotopically into the renal subcapsule resulted in extensive metastasis to the lungs and in all peritoneal organs. However, Strube et al. [127] were successful in generating remarkable metastasis to the bone by inoculating human 786-O cells into the left cardiac ventricle of athymic nude mice. Caki-1, A-498, and 786-O injected intravenously produced metastasis very rarely in nude mice as well as in NOD SCID [177].

Finally, some cell lines fail to be tumorigenic in nude mouse; 769-P, SK-RC-7, TK 10, TK 164 UM-RC-6, or UOK108 [17, 145, 172, 178, 179]. Tumorigenicity in animal models depends on the intrinsic capability of the tumor line employed. However, these results should be interpreted with some caution, as potential failure could also rely on the specific strain or age of animals [180].

Still, precisely designed and controlled xenograft experiments remain a powerful and useful tool in RCC research.

Future of RCC cell line based research

Despite the undisputed profits that translational medicine has gained from research conducted on cell lines, several concerns have arisen regarding the extent to which cell line results can be trusted [181–183]. Despite its limitations, as a cell line does not strictly resemble an *in vivo* tumor [184], the proper description of widely used cell lines is indispensable. Currently, new RCC cell lines are still being established. The isolation of cells from normal and tumor samples can be performed with various protocols [107, 185]. These can be used either for primary cultures [186] or to establish new cell lines [125]. Unfortunately, newly established cell lines rarely receive recognition in the RCC community. Most stick to widely known cell lines, such as: 786-O, 769-P, Caki-1, or ACHN. At the same time, the interpretation of research on some of these cell lines seems to be challenging and may require re-interpretation. As described above, data on particular cell lines, like most scientific information [187], grows cumulatively, and at times we need to redefine primary assumptions (e. g., cancer type) and consequently interpreted phenomena. For instance, if the Caki-2 cell line is indeed a model of papillary RCC, reviewing past studies may shed new light on the molecular background of this particular cancer subtype. As papillary RCC research is rather underrepresented [188], this would be of value for scientists and patients.

Cancer is a complex disease shaped by changes in cell functions, but also intrinsic signaling inside the tumor and extrinsic interactions with other cells of the host as well as various components of the local microenvironment. Such a multiplex disorder is very difficult to interpret, and cell line research does not always reflect cancer diversity. The limitations of the validity of cell line cultures apply also to RCC. To increase the usefulness of cell lines for RCC discovery, complex *in vitro* models have been designed. It was shown that samples from RCC patients cultured as 3D organoids create structures that closely mimic *in vivo* tumor, serving as a useful model for personalized drug screening [186]. 3D cultures for cancer studies is a hot topic that has been reviewed recently in different aspects [189, 190]. Such structures can also be created by cell lines; 3D cultures have been shown to be a better model of *in vivo* mechanisms in cancer than standard 2D techniques [191]. The use of established cell lines would allow high throughput platforms to be created that are useful for effective drug screening [190, 192]. Such methods are of need in RCC research; however, to create a valid *in vitro* screening tool properly characterized cell lines are indispensable.

Conclusions

When conducting research using established cell lines, one should carefully study the data on their establishment and subsequently available characteristics. Thorough data on original patients has not been provided consistently in prior research; however, modern molecular analysis helps to characterize cell line features. Certain RCC specimens are underrepresented, such as: papillary and chromophobe RCC, metastatic ccRCC (especially tissues that are most often affected *in vivo*; lungs, bones), and early stage tumors. When establishing new cell lines, it is essential to follow the best available guidelines, e. g. reviewed elsewhere [193]. Thorough data on original patients' tissue in terms of histological and molecular characteristics should be collected, and subsequent cell line stability should be monitored. A panel of well-characterized RCC cell lines that reflect *in vivo* heterogeneity in terms of different subtypes, grades, and drug resistance would arm us with a screening tool to test new therapy strategies and understand the molecular background of RCC subtypes.

Take-home message

- Molecular profiling of RCC cell lines is not always available, which may limit the clinical translation of *in vitro* research; back checking of model cell lines for typical RCC features is needed
- research on numerous cell lines with relation to healthy tissues can increase the clinical value of RCC cell line research; familiarity with their features is indispensable to draw accurate conclusions
- Additional RCC cell lines and culture models are needed to mirror *in vivo* heterogeneity
- When establishing new RCC cell lines thorough characterization of OP data and subsequent culture are indispensable to create a useful *in vitro* tool

Additional file

Additional file 1: Characteristics of renal cell cancer cell lines including their origin, histology, culture conditions, and molecular characteristics. (DOCX 355 kb)

Abbreviations

ALDH: Aldehyde dehydrogenase; ccRCC: Clear cell renal cell carcinoma; FH: Fumarate hydratase; Foxn1: Forkhead box protein N1; MET: Met proto-oncogene; PDGFR: Platelet-derived growth factor receptors; pRCC: Papillary renal cell carcinoma; RAS: Renin-angiotensin system; RCC: Renal cell carcinoma; RPTEC: Renal proximal tubule cells; SCID: Severe combined immunodeficiency; TERT: Telomerase; VEGF: Vascular endothelial growth factor; VHL: Von Hippel-Lindau

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Authors' contributions

Conceptualizing and refining problem - AMC, CS; Creating review design and best-evidence synthesis - AMC; Collection of literature data - KKB, AMC, MF; Data evaluation and selection - KKB, AMC, MF; Interpreting analyses - KKB, AMC, MF, CP; Drafting manuscript KKB, AMC, MF; Editing manuscript AMC, KKB. All authors read and approved the final manuscript.

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The authors of this manuscript have no competing interests that influence the results and discussion of this paper, that is no significant competing financial, professional or personal interests that might have influenced the performance or presentation of the work described in this manuscript.

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