Characterisation of Crandell-Rees Feline Kidney (CRFK) cells as mesenchymal in phenotype

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The Crandell Rees Feline Kidney Cell (CRFK) is an immortalised cell line derived from the feline kidney that is utilised for the growth of certain vaccinal viruses. Confusion exists as to whether CRFK are epithelial or mesenchymal in phenotype. The aim of this study was to characterise CRFK cells via immunofluorescence, enzyme cytochemistry, western blotting, RT-qPCR for S100A4 and comparison to primary feline proximal tubular epithelial cells (FPTEC) and feline cortical fibroblasts (FCF). CRFK cells were of fusiform morphology and appeared similar to FCF. CRFK expressed the mesenchymal intermediate filament (IF) protein vimentin together with two cell adhesion molecules associated with feline fibroblasts (CD29 and CD44), and lacked expression of the epithelial IF cytokeratin, myogenic IF desmin and endothelial marker von Willebrand factor (vWF). In addition, CRFK did not demonstrate brush border enzyme activity typical of FPTEC. S100A4 gene expression, implicated in both neoplastic transformation and epithelial to mesenchymal transition, was highly upregulated in CRFK in comparison to the primary feline renal cells. CRFK appear phenotypically similar to fibroblasts, rather than tubular epithelial cells, and may have undergone neoplastic transformation or epithelial-to-mesenchymal transition after extensive passaging. This finding may have potential implications for future research utilising this cell line.
Fig. 1. CRFK cells exhibit fusiform morphology (A) and appear more similar to primary feline fibroblasts (B) than feline proximal tubular cells (C). CRFK were positive for the mesenchymal markers vimentin (D), CD29 (E), and CD44 (F), and negative for the epithelial marker cytokeratin AE1/AE3 (G), myogenic marker desmin (H) and endothelial marker vWF (I). Both mouse IgG (G) and rabbit IgG (H) isotype controls were negative. CRFK cells did not demonstrate either ALP (L) or GGT (M) activity (FPTEC positive control inset). Greyscale photomicrographs were collected using a DMIRB inverted microscope with samples illuminated using an EBQ100 light source and an AxioCam ICm1 monochrome camera controlled through Axiovision software version 4.8.2. Immunofluorescence images were collected using a DM4000B upright microscope with samples illuminated using an EBQ100 light source and filter cubes A4 and L5 (all from Leica Microsystems) and an AxioCam MRm monochrome camera controlled through Axiovision software version 4.8.2 (Carl Zeiss Ltd). Cell nuclei were stained with DAPI (blue). Colour photomicrographs for the enzyme activity experiments were collected using an Axiovert 135 inverted microscope (Carl Zeiss Ltd., Cambridge, UK) and an Infinity 3-3UR colour camera (Lumenera, Ottawa, ON, Canada) controlled through Image Pro Insight software version 9.1.4 (Media Cybernetics, Rockville, MD, USA). Images are representative of three experiments. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
Expression of S100A4 was quantified by RT-qPCR and normalised to GAPDH/RPS7 using previously published primers (Lawson et al., 2018b). Data are expressed as mean fold change relative to untreated control and statistical significance was evaluated by one-way analysis of variance (ANOVA) with post-hoc Dunnet’s test. All experiments were carried out in triplicate.

CRFK cells were of fusiform morphology, with cells demonstrating a multipolar or bipolar cell shape at lower densities (Fig. 1A). Cells initially formed monolayers, but did not exhibit contact inhibition at confluence. CRFK cultures appeared similar to that of FCF (Fig. 1B), and were distinctly different to the “cobblestone” monolayers formed by FPTEC (Fig. 1C). CRFK were positive for vimentin, CD44 and CD29 by immunocytochemistry (Fig. 1D, E, F), and negative for cytokeratin AE1/AE3, desmin and vWF (Fig. 1G, H, I). CRFK were also positive for immunocytochemistry (Fig. 1D, E, F), and negative for cytokeratin AE1/AE3 by the case of the primary cells or experiments undertaken on 3 batches of CRFK (Yang et al., 2012; Zhou et al., 2005). S100A4 expression has also been associated with epithelial-to-mesenchymal transition, a process by which epithelial cells dedifferentiate and gain mesenchymal characteristics (Okada et al., 1997). S100A4 expression was highly upregulated in CRFK cells, suggesting that CRFK may have originally been epithelial, but have undergone extensive phenotypic and genotypic drift after long-term culture, resulting in a form of neoplastic transformation enabling indefinite proliferation. There are, however, other possible explanations for the discrepancy in the reportedly original epithelial phenotype and the current mesenchymal phenotype. Original cultures were established with no attempt at cell selection, and it is possible that long-term culture selected for faster growing, fibroblastic cells, which overgrew the original epithelial cell population. It is also possible that contamination of the CRFK cell line has occurred at some point, as numerous cell lines have been found to be cross-contaminated with faster growing cell types (Buehring et al., 2004). Further information on the status of CRFK could be gained from DNA profiling or examining expression of additional marker proteins, but such work was beyond the scope of the current study.

In summary, CRFK appear phenotypically similar to fibroblasts and characterisation was not supportive of the originally reported epithelial phenotype. The findings of this study raise concerns over the use of CRFK as an in vitro model for the study of the feline renal tubular epithelium. This study also provides supporting information for future investigation of the link between CRFK lysates in feline vaccines and the development of CKD.

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Declaration of competing interest

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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