C1A1FF cell line

Contributor:

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Organism:

Mus musculus (H-2K^b-tsA58 'Immortomouse': mouse described by Jat *et* al. Proc Natl Acad Sci U S A 88:5096-5100 - PMID:1711218)

Tissue:

Metanephros, from 11.5dpc embryos

Growth Properties:

Adherent

Original production method:

Complete instructions can be found in Tai G, Hohenstein P, Davies J, *Meth Mol Biol* (in press). E11.5 kidneys were isolated from embryos of H-2Kb tsA58 transgenic mice ('immortomice') by manual dissection in DMEM (Sigma D5546). They were incubated 5-10 min in 2U/ml dispase at room temperature (about 14°C: this is Scotland!), and the ureteric bud was gently pulled away from the MM, like a hand from a glove. Tissues were rinsed in DMEM, then 8-10 buds or mesenchymes were crudely chopped up and placed in 1x Trypsin-EDTA for 10 mins at 37°C, then transferred to 'Immortal medium' (1:1 DMEM-F12 (Sigma D8437) with 10% heat-inactivated fetal bovine serum (Invitrogen), IFN-γ 100U/ml (ProSpecBio cyt-358), 1% ITS supplement (Sigma I2521 – ie a final 1 in 100 dilution of the manufacturer's stock: this supplement contains 1 mg/ml insulin, 0.55 mg/ml human transferrin, and 0.5 µg/ml sodium selenite), glutamine, penicillin and streptomycin (these last three coming from a single stock solution; Invitrogen 10378016) and 1x antioxidants (Sigma A1345). After 10 mins they were triturated through a yellow tip until they were a single-celled suspension. They were plated on Matrigel-coated dishes in Immortal medium with 10µM Y-27632 (Sigma Y0503) and incubated at 33°C for 48h. The medium was replaced with plain Immortal medium and the cells were incubated for a further 72h at 33°C, given new medium and incubated for 72h more at the same temperature. Cloning rings were used to isolate clones, which here harvested and used to seed new wells in the usual way, incubation still being done at 33°C.

Propagation:

Maintain at 33°C in DMEM-F12 medium (Sigma D8437) with 10% FCS (Invitrogen 10108165), 20 U/ml gamma interferon and 1x ITS (insulin, transferrin, selenium) supplement (Sigma I3146), 1x antioxidant supplement, (Sigma A1345), 1x penicillin-streptomycin-glutamine mix (Invitrogen 10378016). Passage using Trypsin-EDTA, diluting cells about 1:3. Change medium every 3 days. For cell freezing, freeze slowly in 90% growth medium, 10% DMSO.

Characterization:

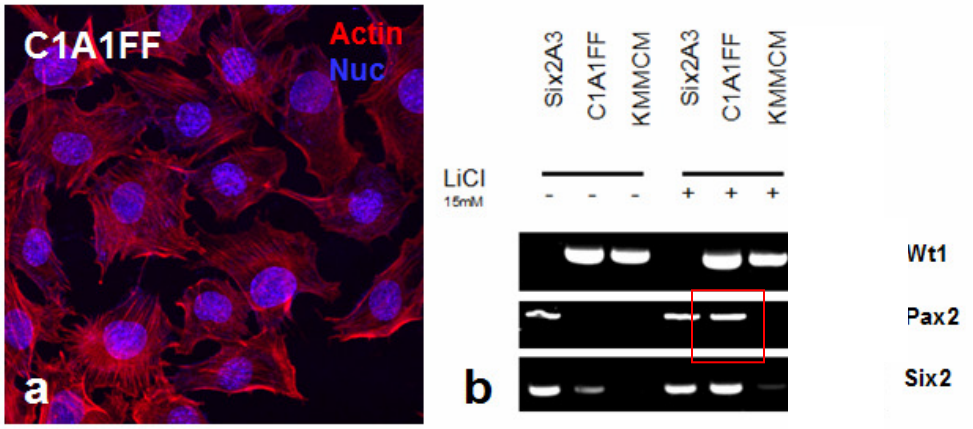
RT-PCR (this cell line is in the red box)

Cell lines	OSR1	SIX2	Pax2	WT1	Megalin	nphs2	Hoxb7	Cbd28k	SV40T
Six2A3	+	+	+	+	-	-	-	-	+
KMMCM	+	+	-	+	-	-	-	-	+
C1A1FF	+	+	-	+	-	-	-	-	+
C1B6EM	+	+	-	+	-	-	-	-	+
KMML1	+	+	-	+	-	-	-	-	+
C1B2EM	+	+	+	-	-	-	-	-	+
Six5N5	+	+	+	-	-	-	-	-	+
Six5N6	+	+	+	-	-	-	-	-	+
Six4C6	-	-	+	-	+	-	-	-	+

OSR1(odd-skipped related 1), Nphs2 (nephrin), Cbd28k (Calbindin 28KD)

Submitter's interpretation: cell line C1A1FF appears to represent the Pax2-low fraction of the cap mesenchyme.

contd…



Lithium induces Pax2 gene expression in the C1A1FF immortalized cell line: LiCl was applied at 15mM where indicated, and expression of Pax2 was detected by RT-PCR.

Reference: (detailed method):

Tai G, Davies JA (2011) Making immortalized cell lines from embryonic mouse kidney, to use as an alternative to in vivo experiments in renal research. *Meth Mol Biol* (in press at time of submission).

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