Published in final edited form as:

Methods Enzymol. 2008 ; 439: 39–51. doi:10.1016/S0076-6879(07)00404-1.

A method to generate genetically defined tumors in pigs

Stacey J. Adam¹ and Christopher M. Counter^{1,*}

¹ *Department of Pharmacology and Molecular Cancer Biology, Duke University, Durham, NC 27701.*

Abstract

As a biomedical model, pigs offer many advantages, and hence have been utilized extensively for toxicology, Crohn's disease, diabetes, and organ transplantation, as well as many other research areas. However, the advantages of porcine models, particularly its large size and similarity to humans, were not previously exploited to any large degree for cancer research. One reason for this lack of porcine cancer models was the inability to genetically induce cancer in pigs. Here we describe a rapid, reproducible, and genetically malleable method to induce large tumors in pigs.

Introduction

There is a need to develop large animal models of cancer, especially for studies that depend upon large tumors which cannot be modeled in rodents (Rangarajan and Weinberg, 2003). Spontaneous tumors arising in companion animals offer one solution, although typically assembling large cohorts of animals with the appropriate cancer is difficult and can be prohibitively expensive, as well as there is no genetic control over the tumors in this approach (Dewhirst, 2000). It would therefore be of value to be able to genetically induce tumors in a large animal.

Pigs are just such an animal. Pigs are large mammals that grow in similar size and weight to humans. Their anatomy is also similar to humans, and genetically pigs share key sequence homology to humans in xenobiotic receptors, which are divergent in mice, that are responsible for modulating the metabolism of drugs (Swanson, et al., 2004). Also, like humans, cancer is rare in pigs. Cancers resembling human childhood cancers occur in young pigs (Anderson and Jarrett, 1968), whereas more age-related cancers occur in adult pigs (Brown and Johnson, 1970). Moreover, due to their size, physiologically relevant sized tumors could be generated in pigs for development of experimental therapeutics. Thus, there would be value in developing a method to gene induced cancer in pigs.

It had been demonstrated enforced expression of transgenes that mimic genetic changes occurring in many types of human cancers could drive normal primary human cells to a tumorigenic state. Specifically, ectopic expression of hTERT, p53^{DD} (a dominant negative truncation mutant of p53), cyclin D1, CDK4^{R24C} (an activated version of cyclin-dependent kinase 4 mutant), c-Myc^{T58A} (a stabilized version of the oncogene c-Myc), and H-Ras^{G12V} (a constitutively active form of Ras GTPase) are together sufficient to drive a variety of human cells to form tumors when explanted into immunocompromised mice (Kendall, et al., 2005). These transgenes disrupt five essential cell processes involved in tumorigenesis, including telomere maintenance, cell cycle check point abrogation, anti-growth signals insensitivity, mitogenic growth signal self-sufficiency, and promotion of angiogenesis (Hanahan and Weinberg, 2000). Taking advantage of this approach, we reasoned porcine cells could similarly be converted to tumorigenic state by expression of these transgenes, and moreover, could be

*Corresponding author. Phone: (919) 684-9890 e-mail: E-mail: count004@mc.duke.edu.

engrafted into immunosuppressed pigs to form tumors, much like mice are used to grow tumors from tumor cell lines. Indeed, this has been the case for a number of porcine cell types. Here we describe how such a genetically defined large animal model of cancer can be generated.

Materials and Methods

Overview

The generation of porcine cells transformed employing hTERT, p53^{DD}, cyclin D1, CDK4^{R24C}, c-Myc^{T58A}, and H-Ras^{G12V} and the use of these cells to create tumors in host pigs will be described. First, establishment of primary porcine dermal fibroblasts will be detailed. Second, generation of amphotrophic retroviruses containing the aforementioned genes will be explained. Finally, transformation of primary porcine cell strains with these viruses, detection of aberrant helper virus production, verification of appropriate transgene expression, injection of transformed cells into isogenic host swine, and immunosuppression therapy will be described.

I. DERIVATION OF CELL LINE FROM SWINE EAR NOTCHES

Materials

70 % Ethanol
 Betadine swabs
 50-ml conical tubes
 Hanks' Balanced Salts (HBSS) (Sigma H-6136, 1 L, 9.8 g/L)
 Penicillin-Streptomycin 100x Stock (Gibco catalog number 15140–122)
 Fungizone 100x Stock (Gibco catalog number 15290–018)
 Sterile gauze
 Sterile forceps
 Sterile earnotchers
 Phosphate Buffered Saline, pH 7.4, 1x (Gibco catalog number 10010–023)
 Cell Dissociation Buffer, enzyme free, PBS-based (Gibco catalog number 13151–014)
 Blendzyme II (Roche catalog number 1988425)
 10-cm tissue culture dishes
 Sterile scalpels (or razor blades)
 D-MEM, 1X, liquid (Gibco catalog number 12491–015)
 F-10 Nutrient Mixture (Ham), 1X, liquid (Gibco catalog number 11550–043)
 Fetal Calf Serum (FCS) (Gibco catalog number 16000–044)

Methods

Ear Notch Collection—NOTE: Solutions should be made AT LEAST 12 hours before ear notch collection.

1. Filter deionized water through Millipore filter, making at least 3 liters.
2. Create HBSS + 8x Penicillin-Streptomycin + 1x Fungizone (HBSS++)

3. Scrub pigs ears with 70% EtOH, followed by Betadine, and again with 70% EtOH.
4. Punch ears, using sterile earnotchers.
5. Using sterile forceps aseptically drop ear notches into sterile 50 ml conical tube containing 25 ml of HBSS++.

Processing Ear Notches

1. Create Stock and Working solutions of Blendzyme II, using a dilution buffer of Cell Dissociation Buffer.

Stock solution of 14 U/ml

Working solution of 0.14 U/ml (Dilution Factor = 1:200, For 4 samples, combine 250 μ l of prepared enzyme and 49.75 ml of straight PBS.)

NOTE: Antibiotics and fungicides inhibit enzymes.

2. Mix 50% DMEM/50% F-10 Nutrient Mixture Solution + 10% FCS in a sterile 500-ml bottle.
3. Place ear notches into clean 50-ml conical tube. Add 5 ml of 100X Penicillin-Streptomycin.
4. Agitate samples and then incubate at room temperature for 10 minutes.
5. Pour tube contents into 10-cm tissue culture dish and dice samples using a sterile scalpel (or razor blades).
6. Collect large sample pieces and place in clean 50-ml conical tubes. Rinse 10-cm dish with 10 ml of HBSS++, pouring liquid into same 50-ml conical tube. Centrifuge samples at 1000g for 5 minutes.
7. Aspirate supernatant into waste bottle containing 10% bleach. Wash sample with 50 ml of Cell Dissociation Buffer. Centrifuge samples at 1000g for 5 minutes.
8. Aspirate supernatant into waste bottle containing 10% bleach. Wash sample with 50 ml of PBS. Centrifuge samples at 1000g for 5 min.
9. While samples spin, prepare Blendzyme II in clean 50-ml conical tube. (See Step 1.)
10. Add 12 ml of Blendzyme II working solution to each sample. Incubate for 4–6 hours at 37°C (depending on tissue) to digest. (DO NOT leave samples in enzyme solution overnight.)
11. Agitate samples and add 5 ml DMEM/F10 media, QNS volume to 50 ml with HBSS++. Centrifuge at 800g for 5 minutes.
12. Aspirate supernatant into waste bottle containing 10% bleach. Add 50 ml of HBSS++ to sample. Centrifuge at 800g for 5 minutes.
13. Repeat step #12 twice.
14. Aspirate supernatant into waste container. Add 10 ml of DMEM/F10 media, containing 4x Penicillin-Streptomycin and 1x Fungizone to each sample.
15. Agitate samples and let large tissue pieces settle.
16. Separate two phases into two 10-cm tissue culture dishes. One 10-cm dish should contain tissue pieces and the second should contain dispersed cells in liquid media.
17. Incubate at 37°C. DO NOT disturb 10-cm dishes for at least 24 hours.

NOTE: You may place pieces directly onto 10-cm dish with no excess media and incubate for several hours at 37°C. (This helps with attachment.) Then flood flask with ~5 ml media, very gently, taking care not to dislodge adhered tissue pieces.

II. CREATION OF 6 GENE TRANSFORMED CELLS

Virus Creation and Cell Infection

IMPORTANT: All virus work detailed in this manuscript should be performed within a certified BSL-3 laboratory facility!

Materials

293 T cells (ATCC catalog number CRL-11268 [HEK 293T/17])
 Serum-Free media (α MEM or DMEM) (Gibco catalog numbers 12561–056, 11995–065)
 Porcine dermal fibroblasts established from ear notches (or other primary porcine cells)
 FuGene-6 (Roche catalog number 1814443)
 pCL-10A1 (viral packaging plasmid) (Imgenex catalog number 10047P)
 pBabe-hTERT+p53^{DD} (Adam, et al., 2007)
 pBabe-cyclin D1+CDK4^{R24C} (Adam, et al., 2007)
 pBabe-c-Myc^{T58A}+H-Ras^{G12V} (Adam, et al., 2007)
 10-cm tissue culture dishes
 0.45- μ m Acrodisc w/HT tufttyn membrane (VWR catalog number 28144–007)
 10-ml syringe
 15-ml conical tubes
 Sterile 1.5-ml microcentrifuge tubes
 Filter sterilized 800 μ g/ml stock of polybrene (hexadimethrine bromide) (Sigma catalog number H9268) in 1x phosphate-buffered saline (PBS)

Methods—Day 1. *Morning*. Seed 293T cells to 30% confluency onto 10cm dish in DMEM (or α MEM) supplemented with 10% fetal calf serum (FCS) (no antibiotics).

Day 2. *Morning*. 1st Cell Transfection. Combine in laminar flow hood in microcentrifuge tube: 184 μ l serum-free, antibiotic free media, 12 μ l Fugene-6, 3 μ g pCL-10A1, and 3 μ g pBabe plasmid (pBabe-hTERT+p53^{DD}, pBabe-cyclin D1+CDK4^{R24C}, or pBabe-c-Myc^{T58A}+H-Ras^{G12V}), making sure to add the serum-free media first and then Fugene-6. Tap the side of the tube gently to mix. Then add each plasmid DNA to the mixture and tap the side of the tube gently to mix. Incubate mixture at room temperature for 15–45 minutes. Finally, add the mixture drop wise to the aforementioned 293T cells from Day 1 and incubate overnight at 37°C.

Day 3. *Morning*. 2nd Cell Transfection. Repeat transfection protocol from Day 2. Once the transfection mix is added to the cells, incubate for at least 8 hours.

Evening. Media change cells with 6 ml media, ideally within the type of media in which primary porcine cells will be cultured.

NOTE: Virus collection protocol has two options. First, collect a 24-hour virus and then place 6 ml back on cells for another 24 hours and collect a 48-hour virus, freezing the 24-hour virus at -80°C for later use and using the 48-hour virus to infect cells. Second, the first 6 ml of media can be place on cells and left it for 48 hours, collected, and used to infect cells.

Day 4. *Evening.* Collect 24-hour virus if desired (see NOTE above). Aspirate media from virus plate with 10-ml syringe, attach $0.45\ \mu\text{m}$ filter to syringe, and filter the virus into a 15-ml conical tube. Add polybrene to the tube and pipette to mix ($800\ \mu\text{g}/\mu\text{l}$ stock solution of polybrene, desired concentration of $4\ \mu\text{g}/\mu\text{l}$, $5\ \mu\text{l}$ per 1 ml of media). Place the 15-ml conical tube in -80°C to store virus. Split primary porcine cells to be infected to 25–30% confluency.

Day 5. *Evening.* Collect 48-hour virus and filter virus into a 15-ml conical tube. As described in Day 4 procedure. Replace media on the primary porcine cells to be infected with the pBabe-hTERT+p53^{DD} 48-hour virus media. Place the 15-ml conical tubes of pBabe-cyclin D1+CDK4^{R24C}, or pBabe-c-Myc^{T58A}+H-Ras^{G12V} in -80°C to store virus until required.

NOTE: Frozen virus appears to work a well as fresh virus.

Day 6. After 24 hours of infection with the virus, media change cells with their fresh, non-viral media.

Day 7. After 24 hours in fresh media, to allow the cells to rest, infect cells with 48-hour pBabe-cyclin D1+CDK4^{R24C} virus. Let virus sit for 24 hours.

Day 8. Repeat procedure from Day 6.

Day 9. After 24 hours in fresh media, to allow the cells to rest, infect cells with 48-hour pBabec-Myc^{T58A}+H-Ras^{G12V} virus. Let virus sit for 24 hours.

Day 10. Repeat procedure from Day 6. Over the next few days, allow the plate to grow to 100% confluency.

Horizontal Spread Assay (for cells containing NO selection markers)

Materials

0.45- μm Acrodisc w/HT tufttyn membrane (VWR catalog number 28144–007)

10-ml syringe

15-ml conical tubes

Filter sterilized $800\ \mu\text{g}/\text{ml}$ stock of polybrene (hexadimethrine bromide) (Sigma catalog number H9268) in 1x phosphate-buffered saline (PBS)

293 T cells (ATCC catalog number CRL-11268 [HEK 293T/17])

Cell Line Containing puromycin resistance marker (Any line verified as non-virus producing will work. Cells containing other selection markers can be used, but puromycin is usually fastest.) 10-cm tissue culture dishes

Methods—Day 1. Plate (or split) cells that have been infected with desired virus (es) to ~50% confluency to be tested for helper virus.

NOTE: Final viral infection of the cells must be at least 48 hours prior to beginning this protocol.

Day 2. Plate (or split) cells stably expressing the desired selection marker (i.e.-puromycin) to ~50% confluency.

Day 3. Remove media from virally infected cells plated on Day 1 via syringe. Filter the media from the plate through a 0.45- μ m filter that attaches to the syringe into a 15-ml conical tube. Add polybrene to the tube and pipette to mix (800 μ g/ μ l stock solution of polybrene, desired concentration of 4 μ g/ μ l, 5 μ l per 1 ml of media). Aspirate media off of cells stably expressing the desired selection marker from Day 2. Use the polybrene/filtered media to replace the media on the desired selection marker cells from Day 2.

Day 4: Plate (or split) 293T cells to ~50% confluency.

Day 5: After 48 hours, remove media that had been placed on the cells stably expressing the desired selection marker during Day 3 via syringe and filter through a 0.45- μ m filter attached to the syringe into a 15-ml conical tube. Add polybrene (as described on Day 3). Aspirate media off 293T cells from Day 4. Use the polybrene/filtered media to replace the media on the selection marker negative cells from Day 4.

Day 7: After 48 hours, aspirate media off of the selection marker negative cells from Day 5. Replace with 10 ml of media containing appropriate growth selection agent at the appropriate concentration.

i.e. — DMEM + 10% FBS + Puromycin 1 μ g/ml for transformed cells, such as 293 T cells

Day 8: Monitor cells for death over the next few days to weeks.

NOTE: If no helper virus is being produced by original cells, then the entire population of selection marker negative cells should die in a relatively short amount of time.

Reverse-Transcriptase PCR Verification of Transgene Expression

Materials

RNAzol B (Tel-Test)

Sterile 1.5-ml microcentrifuge tubes (must be RNase-free)

Sterile Cell Scrappers

Chloroform (Stored at 4°C)

75% EtOH (made with DEPC treated H₂O)

1 mM EDTA, pH 7

Transformed Cell Lines for testing

Omniscript Kit (Qiagen catalog number 205113)

RNase Out (Invitrogen catalog number 10777-019)

Oligo dT (Invitrogen catalog number 18418-012)

Red Taq polymerase and 5x buffer (Sigma catalog number D4309)

2.5 mM dNTPs (Diluted from Invitrogen 100 mM dNTP mix catalog number 10297-117)
ddH₂O (PCR grade)

Gene specific primers (See Methods for sequence)

SeaKem LE Agar (Fisher Sciences catalog number BMA 50004)

Dimethylsulfoxide (DMSO)

Methods

RNA Extraction

1. Isolate RNA from one 10-cm confluent tissue culture dish, as described by the manufacturer, using RNazol B reagent (Tel-test, Inc.).
2. Aliquot 3 µl of RNA from Step 1 into 97 µl of RNase-free deionized water to measure RNA concentration via spectrophotometer, using standard procedures (Maniatus).

Reverse Transcription Reaction and PCR Amplification

1. Reverse transcribe (RT) 9 µg of RNA from each sample using the Qiagen Omniscript Kit, as described by the manufactures. RT reaction mix as follows:

10 × buffer RT	6.0 µl
5 mM dNTP's	6.0 µl
RNase Out	0.75 µl
Omni RT	3.0 µl
Oligo dT	1.5 µl
RNA (9 µg)	varies according to RNA concentration
<u>RNase free dH₂O</u>	<u>varies according to RNA amount</u>
Final volume	60.0 µl

NOTES: A master mix can be made for everything except the RNase free water and RNA.

2. PCR amplify cDNA products of above RT reactions for each sample to be tested, as follows:

ddH ₂ O (PCR grade H ₂ O)	33.5 µl
Red Taq buffer	5.0 µl
2.5 mM dNTPs	2.0 µl
20 µM forward primer	2.0 µl
20 µM reverse primer	2.0 µl
cDNA (from above RT reaction)	5.0 µl
Red Taq polymerase	1.0 µl
DMSO	3.5 µl

NOTE: Make master mix including everything EXCEPT primers and cDNA for number of reactions that you need to do (over-estimate by ~2 reactions). For each cell line needing to be tested you will do 7 reactions. (β-tubulin, hTERT, p53^{DD}, CD1, CDK4^{R24C}, Myc^{T58A}, HRas^{G12V})

3. Aliquot 45.0 µl of master mix into each PCR tube.
4. Add 2.0 µl of appropriate 20 µM forward primer for each reaction.

β-Tubulin F - 5'- TCAGAAATACGTGCCCAGGG - 3'

hTERT - 5'- TGGCTGTGCCACCAAGCATT -3'

p53^{DD} - 5'- GCTCACTCCAGCTACCTGAA -3'

cyclin D1 - 5' - AACATGGACCCCAAGGCC -3'
 CDK4^{R24C} - 5' - GGTGGTACCTGAGATGGA -3'
 c-Myc^{T58A} - 5' - ACGAGCACAAGCTCACC -3'
 H-Ras^{G12V} - 5' - GCACGCACTGTGGAATCT -3'

5. Add 2.0 µl of appropriate 20 µM reverse primer for each reaction.
 β-Tubulin R - 5' - GAAGGGGACCATGTTCACA - 3'
 hTERT - 5' - TTTCCACACCTGGTTGC -3'
 p53^{DD} - 5' - ATGCCTTGCAAAATGGCG -3'
 cyclin D1 - 5' - TTCTGCCTGCTGGGGAG -3'
 CDK4^{R24C} - 5' - TAGCTTGCCAAACCTACAGG -3'
 c-Myc^{T58A} - 5' - TTTCCACACCTGGTTGC -3'
 H-Ras^{G12V} - 5' - TAGCTTGCCAAACCTACAGG -3'
6. Add 5.0 µl of appropriate cDNA from each reverse transcription reaction for each cell line.
7. Cap reactions and vortex for 30 seconds. Centrifuge tubes 20–30 seconds to ensure all reactions mix is in the bottom of each tube.
8. Using the following cycles amplify DNA:
 1 cycle – 94.0°C – 5 min
 25 cycles – 94.0 °C – 30 sec.
 55.0 °C – 1 min.
 72.0 °C – 30 sec.
 1 cycle – 72.0 °C – 10 min.
 Hold – 4.0 °C
9. Resolve 25 µl of reaction products on 2% agarose gels to confirm PCR amplification of transgenes.

III. INJECTING CELLS INTO PIGS

Tumorigenic Growth Assay in Pigs

Materials

15-cm tissue culture dishes
 50% D-MEM/50% F-10 Nutrient Mix Media + 10% FCS, 1x (Gibco catalog numbers 12491–015, 11550–043, 16000–044)
 0.05% Trypsin (Gibco catalog number 25300–054)
 Phosphate Buffered Saline (PBS), pH 7.4, 1x (Gibco catalog number 10010–023)
 Dulbecco's Phosphate Buffered Saline with MgCl₂ and CaCl₂ (Sigma catalog number D8662)
 Cell Dissociation Buffer (Gibco catalog number 13151–014)
 50-ml conical tubes

3-ml syringe

21-gauge needle

Transformed porcine cells to be injected

Telazol-Ketamine-Xylazine (TKX) anesthesia (by veterinary prescription)

Methods—IMPORTANT: Transformed porcine cells should be checked for bacterial, fungal, and mycoplasma infection before injection. Bacterial and fungal infection can be ruled out by culturing cells without Penicillin-Streptomycin and Fungizone in the culture media for 4–5 days. Mycoplasma should be tested for using the Gen-Probe nucleic acid hybridization procedure (Fisher Scientific catalog number GP-1591) or similar assay.

1. Transfer media from 15 cm tissue culture dishes into 50 ml tubes. Centrifuge at 4°C at 1000g for 5 minutes.

NOTE: This protocol assumes that volume in twenty 15-cm dishes will contain enough cells for injection. Cells have a tendency to no longer adhere to the tissue culture dish; however they are still living and dividing. If needed, spin down all media.

2. Discard supernatant.
3. Add ~8 ml of Cell Dissociation Buffer to each 15-cm dish. Ensure full coverage of bottom of flask.
4. Let stand for ~ 5 minutes. Tap side of dish to dislodge cells. Transfer liquid containing cells to 50 ml conical tubes. Centrifuge at 4°C at 1000g for 5 minutes.

NOTE: If Cell Dissociation Buffer does not cause cells to dislodge from tissue culture dish, remove buffer and add to 50 mL conical tube. Add 2 mL of trypsin to each dish.

5. Repeat steps #3–4 until all cells are removed from dishes and pelleted in tubes.
6. Resuspend each pellet in 6 ml PBS and consolidate all cell suspensions into one 50 ml conical tube per cell line. Centrifuge at 4°C at 1000g for 5 minutes.
7. Wash with PBS with MgCl₂ and CaCl₂. Centrifuge at 4°C at 1000g for 5 minutes. Discard supernatant.
8. Repeat step 7 a minimum of 3 times.
9. Remove 50 µl of cell suspension and place in 1.5-ml microcentrifuge tube. Add 50 µl of Trypan Blue to tube and mix.
10. Remove 10 µl of cell suspension/Trypan Blue mixture and plate to a hemacytometer. Count cells.
11. Add 1×10^8 cells into each of two 50-ml conical tubes. Centrifuge at 4°C at 1000g for 5 minutes.
12. Aspirate PBS and resuspend each pellet in 1 ml PBS and place in a microcentrifuge tube.
13. Inject TKX anesthesia intramuscularly into the flank of each pig to be injected based on the weight of the pig (1 ml/50–75 lbs). Wait 5–10 minutes for TKX to take effect and monitor pig's sedation according to institution IACUC approval.
14. Draw the each cell suspension into a sterile 3-ml syringe. Attach 21-gauge needle to syringe. Inject cell suspension subcutaneously behind the ear of the pig (one cell suspension for each ear).

NOTE: Cells suspensions can also be injected into the mammary fat pad of the isogenic host pig.

10. Over the following 15–30 days, track tumor progression by measuring the large and small diameters and extrapolating tumor volume using the equation:

$$\text{Eq. - Tumor Volume} = (\text{Small diameter})^2 + (\text{Large diameter}/2)$$

IMPORTANT: All animal work *must* be done by a trained person under and approved IACUC protocol.

Immunosuppression Regime

Materials

Azathioprine pills (by veterinary prescription)
 Prednisone pills (by veterinary prescription)
 Cyclosporine A liquid (by veterinary prescription)
 Standard pig chow
 Chocolate Syrup
 Chocolate corn puff cereal (generic cocoa puffs)
 Feed bowl
 Rubber Gloves

Methods—The immunosuppression regime will be administered on a twice daily beginning one week prior to injection of the transformed cells. Before immunosuppression regime begins, the pigs are conditioned to eat the chocolate corn puff cereal and chocolate syrup that the drugs will be administered in.

Morning Dosing

1. Take azathioprine and prednisone pills (enough for 2 mg/kg and 4 mg/kg, respectively) out of bottle and place in small dish. Crush pills into small pieces. Cover with chocolate syrup and mix with a handful of normal pig food. (Mixture should be thick enough to form into a small ball about ~3–4 inches in diameter.)
2. Add 1 ml of cyclosporine A (1 ml/50–75 lbs) to the ball and mix in enough chocolate corn cereal into the ball to ensure full incorporation of cyclosporine A into the ball (total ~20 ml).

NOTE: Ensure food, chocolate syrup, and chocolate corn cereal is added to disguise the taste to prevent the pigs from developing an aversion to the chocolate.

3. Feed food ball mixture to pig in their designated food bowl. Make sure that the pig consumes the entire food/medicine ball.
4. Place pig back in cage and give one cup of normal feed mixed with chocolate corn cereal to reward the pig.

Evening Dosing

5. Feed as above with food balls made from mixing 1 ml of cyclosporine A (1 ml/50–75 lbs), food, chocolate corn cereal, and chocolate syrup into the ball to ensure full incorporation of cyclosporine A into the ball (total ~20 ml).

Concluding Remarks

The described procedure provides a rapid and reproducible method to generate genetically malleable tumors in a large mammal. Other primary porcine cell lines have already been transformed (Adam, et al., 2007), utilizing the six gene transformation method, and the model could be expanded still further, potentially, to any cell type that can be cultured. Similarly, the genetic make-up of these cells could also be altered to suit the specific needs of the cancer studies. In summary, the porcine tumorigenesis model opens many new avenues of research that before were limited by the lack of an affordable, genetically malleable large animal model.

References

- Adam SJ, Rund LA, Kuzmuk KN, Zachary JF, Schook LB, Counter CM. Genetic induction of tumorigenesis in swine. *Oncogene* 2007;26:1038–45. [PubMed: 16964292]
- Anderson LJ, Jarrett WF. Lymphosarcoma (leukemia) in cattle, sheep and pigs in Great Britain. *Cancer* 1968;22:398–405. [PubMed: 5694959]
- Brown DG, Johnson DF. Diseases of aged swine. *J Am Vet Med Assoc* 1970;157:1914–8. [PubMed: 5530385]
- Dewhirst, MW.; Thrall, D.; Macewen, E. Spontaneous Pet Animal Cancers.. In: Teicher, BA., editor. *Tumor Models in Cancer Research*. Humana Press; Totowa, NJ: 2000. p. 565-589.
- Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell* 2000;100:57–70. [PubMed: 10647931]
- Kendall SD, Linardic CM, Adam SJ, Counter CM. A network of genetic events sufficient to convert normal human cells to a tumorigenic state. *Cancer Res* 2005;65:9824–8. [PubMed: 16267004]
- Rangarajan A, Weinberg RA. Opinion: Comparative biology of mouse versus human cells: modelling human cancer in mice. *Nat Rev Cancer* 2003;3:952–9. [PubMed: 14737125]
- Swanson KS, Mazur MJ, Vashisht K, Rund LA, Beever JE, Counter CM, Schook LB. Genomics and clinical medicine: rationale for creating and effectively evaluating animal models. *Exp Biol Med* (Maywood) 2004;229:866–75. [PubMed: 15388881]