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Subcapsular transplantation of tissue in the kidney

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Abstract

There are multiple sites used for engraftment of primary human cells and tissues. Leukemias as usually best engrafted intravenously in adult mice (tail vein) or in newborn mice (superficial temporal vein or in the heart ventricle) (Pearson et al. 2008). Leukemic cells have also been engrafted directly into the bone marrow cavity of adult mice. Some solid tumors such as colon tumors grow well following subcutaneous engraftment. Matrigel™ is often used to provide artificial basement membrane. In certain studies, i.e. human bladder cancer, the autochthonous site is employed. Engraftment of a PDX bladder cancer was successful following injection of single cancer cells directly in the bladder wall of NSG mice (Lin et al. 2012). Human ductal breast carcinomas grow most efficiency when transplanted into the mammary ducts of female NSG mice (Valdez et al. 2011) while they fail to grow or grow only poorly when transplanted subcutaneously or under the renal capsule. Human prostrate tumor initiating cells isolated from primary prostate cancer grow well when implanted subcutaneously in a matrigel™ matrix in male NSG mice supplemented with testosterone implants (Goldstein et al. 2010). In contrast, injection of serous ovarian cancer cells into the peritoneal cavity, kidney capsule, ovarian bursa or mammary fat pad resulted in growth and detection most rapidly and reliably in the mammary fat pad and peritoneal cavity (Bankert et al. 2011; Stewart et al. 2011). These data suggest that the ability to grow solid tumors is dependent on the site of implantation, and that simple subcutaneous implantation of tumors may lead to false negatives as to the ability these tumors to grow in NSG mice. These different outcomes depending on the tumor type and site of implantation may be due to vascularization issues, organ or tissue-specific microenvironmental factors, or species-specific factors that facilitate growth of specific human tumors. The kidney is especially suited for the transplantation of normal as well as malignant cells and tissues as it is easily accessible and transplanted tissues are well contained under the renal capsule in a highly vascularized site. The retroperitoneal location of the kidney with its separation from other organs is advantageous for imaging and biopsy. This protocol describes the surgical procedure for implantation of tissues under the kidney capsule.

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MATERIALS

Supplies

Betadine, 10% solution

Isopropyl alcohol, 70%

Cotton tipped applicators

Drape for sterile surgical field

Gelfoam® hemostatic agent

Puralube ophthalmic ointment

Saline, 0.9% sterile

Carprofen

Scale

Surgical gloves

Surgical mask

Suture, absorbable, 5-0

3 French silicone catheter with a 2 French rounded tip (Norfolkaccess Technologies)

25g blunt tip needle

1cc syringe

Equipment

HEPA-filtered laminar flow hood

Isoflurane anesthesia machine or injectable general anesthetic

Stereomicroscope

Fiber-optic illuminator

Autoclave

Circulating water blanket and pump or other warming device

Fur Clipper, #40 blade

Wound clip, Autoclip® 9mm kit

Surgical instruments (Fine ScienceTools):

(1) Extra fine Graefe forceps, straight, 1×2 teeth (11153-10)

(1) Fine scissors, sharp (14060-09)

(2) Graefe forceps, curved (11051-10)

(1) Blunt probe (10160-13)

(1) Moria iris forceps, curved (11373-12)

(1) Vannas spring scissors, 3mm cutting edge (15000-00)

(1) Olsen-Hegar needle holders with scissors (12002-12)

METHOD

This procedure describes the subcapsular transplantation of tissue on the left kidney. The left kidney is easier to access for surgical procedures because of a more caudal location in the abdomen relative to the right kidney. If bilateral transplantation is required the same procedure is used on the right kidney.

Aseptic technique is essential for any survival surgery; it requires that all surgical instruments and supplies are sterile. For immunocompromised recipients, such as the NSG mouse, surgery should be conducted in a HEPA-filtered laminar flow hood to prevent microbial contamination of the surgical site.

Procedure for removal of fur and preparation of skin

1. The mouse is anesthetized and assessed for a surgical plane of anesthesia by the absence of movement to a firm toe pinch.
2. Fur is removed, using clippers, from the left flank in an area bounded cranial-caudal by the last rib and iliac crest, and dorsal-ventral by the spine and lower third of the abdominal wall.
3. Loose fur is removed with adhesive tape, dry gauze or gauze slightly dampened with ethanol.
4. Ophthalmic ointment is placed on the eyes to prevent drying of the cornea and carprofen (5mg/kg) is administered subcutaneously.
5. The skin is disinfected with surgical iodine and 70% ethanol using sterile swabs. Application of 70% ethanol starts in the center of the proposed incision site and works outward in ever widening circles to cover the entire clipped area. Using a new sterile swab, surgical iodine is applied in the same manner. Repeat 70% ethanol and surgical iodine scrub one additional time.

Description of the surgical procedure

1. The mouse is placed in right lateral recumbency and a drape positioned over the surgical site.
2. Straight forceps and fine scissors are used to make a 6-9mm skin incision parallel and ventral to the spine and midway between the last rib and the iliac crest. (For tissue transplantation under the capsule of the right kidney the incision is made immediately caudal to the last rib.)
3. A similar incision is made in the underlying abdominal wall.

4. The kidney is externalized by placing curved forceps under the caudal pole and gently lifting the caudal pole through the abdominal incision. Reposition the forceps under the cranial pole and gently lift through the incision. Rotate the kidney slightly such that it is held in place by the abdominal wall. Do not grasp the kidney or renal vessels with the forceps.

5. A small incision is made with spring scissors in the capsule over the caudal-lateral aspect of the kidney. The size of the incision depends on the size of the transplanted tissue. Generally, the size of the tissue that is to be transplanted should not exceed ~1/5 of the size of the kidney.

6. The kidney should be kept moist with warm sterile saline.

7. A shallow subcapsular pocket is made with a blunt probe and the transplant tissue is placed into the pocket with iris forceps. The tissue is advanced toward the cranial pole by placing the forceps on the capsule surface, caudal to the tissue, and gently moving the forceps cranially.

   Alternatively, cells to be injected are drawn up in a 1cc syringe and delivered through a blunted 25g needle attached to a silicone catheter. The catheter is advanced through the capsule incision to the cranial pole of the kidney and the cells discharged slowly from the catheter.

   See Troubleshooting

8. The incision in the capsule is not routinely closed. However, to prevent backflow of injected cells Gelfoam® is placed over the capsule incision.

9. The kidney is returned to the abdomen.

10. The incision in the abdominal wall is closed with 5-0 absorbable suture with a swaged needle. The skin incision is closed with a wound clip.

11. The mouse is placed in a clean cage and recovered on a circulating warm water pad until ambulatory.

12. The mouse should be checked daily for normal wound healing until the wound clip is removed in 5-7 days.

TROUBLESHOOTING

Problem (step 7): Injury to the kidney beneath the capsule will result in bleeding. If clotting is extensive the capsule will become distended and this may compromise the security of the transplanted tissue. Gelfoam® should be placed over the capsule incision to help retain the transplanted tissue under the capsule. If bleeding is excessive the mouse should be euthanized.

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