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Xenograftic tumor models in mice for cancer research, a technical review

Review Article

Dietmar Jacob[1], John Davis[1,2], Bingliang Fang[1,2]

[1]Departments of Thoracic and Cardiovascular Surgery, The University of Texas M. D. Anderson Cancer Center, and [2]Program in Gene Therapy and Virology, The University of Texas Graduate School of Biomedical Sciences, Houston, TX 77030, USA.

***Correspondence**: Bingliang Fang, Department of Thoracic and Cardiovascular Surgery, Unit 445, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Blvd., Houston, TX 77030, USA. Phone: (713) 794-4039. Fax: (713) 794-4669. E-mail: Bfang@mdanderson.org.

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Abbreviations: Dulbecco's modified Eagle's medium, (DMEM); natural killer, (NK); phosphate-buffered saline, (PBS); Surgical orthotopic implantation, (SOI)

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Summary

The transplantation and growth of human tumor xenografts in immunodeficient mice is frequently used in preclinical studies to test the efficacy of various anticancer agents. Subcutaneous models are most commonly used because of the simple methods of implanting tumor cells and monitoring tumor growth. A major criticism of subcutaneous models, however, is the unrealistic growing condition of the local skin environment. Alternatively, direct intravascular or intraorgan injections can be used to produce metastatic or orthotopic tumor models under more realistic and clinically applicable conditions. Despite the apparent widespread use of these models, detailed technical information on establishing these tumor models is difficult to find in the literature. Therefore, the purpose of this article is to provide the technical details for establishing useful tumor models in mice.

I. Introduction

The transplantation and growth of tumor xenografts in immunodeficient mice is frequently used in preclinical studies to test the cytotoxic potential of various anticancer agents. The most common tumor models are grown from cells injected subcutaneously, intraperitoneally, intravascularly, and directly into an organ, such as the spleen, liver, and pancreas (Bruns et al, 1999, 2002; Schumacher et al, 1999; Kagawa et al, 2001; Yoon et al, 2001; Gu et al, 2002; Huang et al, 2002; Stapfer et al, 2003). Surgical orthotopic implantation (SOI) has been used to transplant small, established tumor fragments of pancreatic, colon, lung, stomach, prostate, ovarian, renal, and bladder cancers into organs of choice (Fu et al, 1991; Fu et al, 1992; Wang et al, 1992; Fu and Hoffman, 1993; Furukawa et al, 1993; An et al, 1999; Sun et al, 1999; Bouvet et al, 2000; Rashidi et al, 2000; Lee et al, 2001). These implantations normally are derived from previously inoculated subcutaneous tumor grafts.

Subcutaneous models are the most commonly used, because of the simple inoculation procedure, the lack of a need for anesthetics, the consistency and reproducibility of tumor growth, and the easy accessibility of the tumor for treatment and measurement. A major criticism of the subcutaneous model, however, is the unrealistic growing conditions of the local skin environment. It is conjectured that, for this reason, subcutaneous tumors rarely develop metastasize (Fidler, 1994; Killion et al, 1999), frequently show an unnatural, increased responsiveness to treatments (Wilmanns et al, 1993), and express different cell surface molecules from those normally found in their tissue of origin (Killion et al, 1999). These factors, among others, limit the applicability of subcutaneous data in applied or pre-clinical cancer research.

In contrast, direct intravascular or intraorgan injections can produce metastatic or orthotopic tumor models. Orthotopic tumor models may also have an increased metastatic potential, which would provide more predictive and clinically applicable means of assessing responses to treatment (Welch, 1996). Despite the widespread use of these models, however, detailed technical information on establishing these tumor models is difficult to find in the literature. Therefore, the purpose of this article is to provide technical details for

establishing some common tumor models in mice. There are several factors and procedures that have shown to be integral parts to the success of the model. Even slight modifications to the conditions and/or reagents can lead to failure, as will be pointed out. We also recommend the excellent review article of Danny R Welch (1996) for further information about metastasis and animal work.

II. Preparation of tumor cells

The success of a tumor model begins with the initial cell culture. Before cells are harvested, any contaminants, such as yeast or *mycoplasma*, must be verifiably excluded by observation or PCR analysis. The medium and serum chosen are extremely important to the health and implantability of the cells. For example, we maintain the cell lines in Dulbecco's modified Eagle's medium (DMEM) with a high glucose level, or in RPMI 1640 medium. Both medias are supplemented with 10% (vol/vol) heat-inactivated fetal bovine serum, 1% glutamine, and 1% penicillin and streptomycin (Gibco-BRL, Life Technologies, Inc., Grand Island, NY). Cells are cultured at 37°C in a humidified incubator containing 5% CO_2. We recommend a high-quality serum for developing metastatic models (Gibco). In nearly all the models we describe here, lower-quality serum can threaten the success of the model; therefore, a more consistent, albeit expensive product should be used. The effect of different serums is remarkable; indeed, many of our models have shown to depend heavily on this factor.

Cells should be 70%-80% confluent before being harvested. They should also be in an actively growing stage to ensure that they possess the viability, strength, and morphologic properties needed to become implanted. Two to three hours before the cells are harvested, the medium should be completely changed. Doing so has demonstrated a distinct increase in the chances of success by helping to remove excess wastes, dead, or dying cells that may corrupt the concentration of viable cells for injection. Cells are removed from the plate using trypsin-EDTA (Gibco). The trypsinization of the cells should involve the most minimal exposure necessary to produce individual, free-floating cells. Subsequently, new medium with serum is added to the trypsinized cells, usually in a 10:1 or 5:1 media-to-trypsin ratio. The cells are centrifuged immediately at or below 1500 rpm for 2 to 5 minutes and are washed twice with phosphate-buffered saline (PBS) to eliminate trypsin, serum, and excess debris, and then stored immediately on ice. At this point, the cells are counted using a hemocytometer.

During the harvesting of cells for a metastasis model, it is important to obtain an accurate concentration of viable cells. Counterstaining the dead cells with trypan blue, or other cell stain will provide a distinction between living and dead cells and thus confirm the percentage of viable cells. Specifically, it is important to obtain a final solution containing more than 90% viable cells because less than 0.1% of injected cells will produce macroscopic metastasis (Fidler, 1970, 1973). The harvesting process can be rigorous, which can be harsh on some cell types. Therefore, care must be taken not to make the harvesting process too harsh for the cells to survive.

We suspend the harvested cells in PBS; however, unsupplemented DMEM is also an option. The mixture volume is adjusted to the proper concentration immediately prior to injection. It is preferable that all cells be used within 1 hour of harvesting. We have tested cells that have been kept on ice in PBS for more than 3 hours and have found that 60%-70% have retained their original concentration/viability as shown by trypan blue exclusion (data not shown). However, if an experimental procedure is projected to last longer than 1 to 2 hours, we recommend that the cells be harvested in groups, rather than all at once, to provide a continual source of fresh cells.

III. Preparation of mice

Animal experiments are carried out in accordance with the *Guidelines for the Care and Use of Laboratory Animals* (National Institutes of Health publication number 85-23) and the institutional guidelines of The University of Texas M. D. Anderson Cancer Center. All experiments described in this article were done on female, athymic nude mice (nu/nu). Animals should be as young as possible (4-6 weeks), because they have a lower natural killer (NK) cell activity (Hanna et al, 1982; Welch, 1996), and there is an inverse correlation between NK cell activity and the formation of metastases (Hanna, 1982, 1985; Welch, 1996). In addition, age-dependent hemodynamic parameters such as vessel diameter and endothelium thickness (Arguello et al, 1991; Taniguchi, 1992) can affect tumorigenesis and also the likelihood of metastasis. Experiments should not be performed for 3 to 5 days after the arrival of the animals to reduce stress upon the animals. Some investigators have shown stress can reduce the occurrence of metastases (Kodama et al, 1992; Stone and Bovbjerg, 1994). If necessary, 2 to 5 days before tumor inoculation, the mice can undergo whole-body irradiation with no more than 3.5 Gy by short exposure to Cs^{137}, performed in a Nasatron (US Nuclear, Burbank, CA). This will abrogate residual immune response in nude mice and improve the rate of success in establishing xenograftic tumor models. If there is hair or anything else on the animal that would interfere with the experiment, it must be removed well before the injection and in a manner not to overly disturb the mice.

IV. Preparation of the injection

The incidence of tumor implantation and/or metastasis is proportional to the amount of viable cells injected (Wood et al, 1954; Fidler, 1973). However, an increase in the viable cell number does not automatically lead to more metastases or larger local tumor volume (Tarin et al, 1984). To establish tumors in vivo, it is much more important to give a consistent, homogeneous mixture of viable cells, rather than an injection consisting of clumped, unevenly mixed, and/or unviable cells.

For the preparation of the tumor cells for injection, it is important to draw the cells into a syringe without a needle. Using a needle causes a strong, negative pressure to form, which can cause considerable cell damage, lysis, and death. The most practical injection method is to use a 1-cc (1-ml) syringe and a 27- or 30-gauge needle. Because

some cells are larger and/or more sensitive to the size and pressure constraints of a 30-gauge needle, a significant proportion of cells may be lysed or damaged during injection. It is, therefore, a good idea to test the cells prior to injection by first pushing them through the required syringe and needle gauge and then performing a trypan blue exclusion test on a hemocytometer to compare the cell viability before and after passing through a needle. In most cases, we have found that more than 90% of cells survive a 30-gauge needle injection.

The gauge of the needle must be chosen on the bases of the location of the injection. A simple intraperitoneal or subcutaneous injection can be performed with a 23-, or 25-gauge needle that is strong enough to penetrate the skin efficiently and wide or blunt enough so as not to penetrate unintended organs. However, the gauge should not be too large, since the injected volume may leak out of the injection site.

Finally, it is absolutely necessary that the cells be mixed frequently, if not continuously, in the syringe prior to the injection. Cells suspended in PBS or in medium will quickly succumb to gravity and begin to adhere to each other, forming clumps of cells that hinder their distribution and thus the metastatic potential. This could also inadvertently kill the mouse during the injection. In fact, it is typical for intravascular, intrasplenic, and intrahepatic injections to cause 10 to 20% or more of injected mice to spontaneously develop pulmonary failure or go into respiratory arrest as a result of emboli, strokes, or other blockages in the circulatory system caused directly by poorly mixed, highly concentrated, or hastily injected volumes of cells. Hence, it is imperative that the cells be evenly mixed and homogenous, and not be so highly concentrated that the cells will not separate and flow in the blood stream. The cells must also be warmed, by hand or other means, to room temperature or warmer, to minimize hypothermia. An ideal injection volume for each model is described later on, however, a general rule of thumb is that when cells are being introduced into an organ or the venous system, the volume should be large enough to be measurable and consistent, though small enough to cause no damage to the tissue or hemodynamic parameters of the organ.

V. Anesthesia

Because of ease of handling and speed of use, we anesthetized our mice using an intraperitoneal injection of a mixture of ketamine-HCl and xylazine (150 mg/Kg and 10mg/kg, respectively, IP). For simple and short procedures, isoflurane inhalation may be a more suitable anesthetic for some investigators. However, in our experience, inconsistent dosing, random animal movements, and spontaneous periods of consciousness indicate that isoflurane may not be a suitable surgical anesthetic for small animals. In fairness, for human application, nebulized or inhaled anesthetics are of great success when delivered directly into the lungs via tracheal intubations. But this procedure is nearly impossible in large group animal studies where ventilation equipment and time limitations prohibit lengthy intubation procedures. Typicaly for mice, isoflurane delivery is done in an open air environment, forcing the investigator to constantly manipulate the subject into positions allowing anesthetic inhalation, however not necessarily conducive to surgery. Without constant anesthetic inhalation, the subject will quickly regain responsiveness, especially to pain, and could endanger itself and the outcome of the experiment. Typically, we did not enjoy isoflurane in any application, due to its weakness as an anesthetic, its requirement for outside equipment (air supply, intubation procedures, nebulizer, etc.), and its overall poor performance in our experience. However, it may also be observed that for heavy anesthetics such as ketamine/xylazine, in combination with blood and temperature loss, an invasive surgery/injection procedure may be harmful, if not fatal, to a mouse that cannot quickly regain consciousness and reestablish a normal breathing and heart rate. In these cases, a reduced dose of general anesthetic, in addition to light isoflurane inhalation, might be suitable. In any case, it is highly recommended to become comfortable with the anesthetic of choice, including its dose, duration, and consistency. Regardless of the method, inappropriate anesthetic preparation and procedure will result in high mortality rates, inconsistent surgical method, and poor tumor results.

VI. Procedures

We strongly recommend that all animal experiments be done with a qualified and knowledgeable assistant or co-investigator, and according to the advice of institutional veterinarians. For some procedures, such as hepatic portal vein injections, it is nearly impossible to perform the injection without the help of someone to open the incision, expose the vein, and apply pressure to stop bleeding. An assistant can also dramatically increase the speed, reproducibility, and consistency of large experiments, thus reducing costs, failure, frustration, and the need to repeat an experiment.

In general, all experiments should be performed with equal care and attention. All injection or incision sites should be properly cleaned and sterilized with ethanol and/or iodine solutions. Animals will quickly lose body heat during the procedure, therefore, a suitable heat source, such as a lamp or heating pad must always be used, but especially with anesthetized mice. If the mouse shows a mild twitch response or some other abnormal movement, it should be anesthetized further. Incisions should be made so that they will be easy to close and heal quickly. At the same time, initial incisions should be as large as needed so that the underlying anatomy can be visibly understood. After several subsequent procedures are performed and the investigator is more comfortable, the incision size and position can be optimized. A larger-than-normal skin incision can normally be closed easily with sutures or staples with little harm to the animal. However, a severed or fatally perforated hepatic portal vein, torn spleen, or lacerated liver lobe resulting from poor access or visualization is irreparable**.** Before the incision is closed, the areas surrounding the injection site should be washed with a generous amount of warm PBS to dissipate any cells that may have spilled or leaked, hopefully preventing the development of unintended

tumors in the region of the injection. We use a wound-clip stapler (MikRon Autoclip; Becton Dickinson, Sparks, MD) to close all wounds, however suturing can be done when necessary. The mouse should still be under the full effects of the anesthesia during closing, otherwise, the underlying organs will be involuntarily pressed out of the abdomen or into the staples or sutures, causing incarcerations. Most wounds can be closed with two to four staples, and the staples can be removed after 5 to 7 days.

Care should be taken during surgery to avoid cutting large vessels to help prevent considerable blood loss. Major veins that must be cut can be cauterized, and a subcutaneous injection of 500 µl of PBS can be given to compensate for fluid loss and maintain blood pressure. The temperature, breathing, and heart rates of animals should be monitored closely. To reestablish or maintain a steady breathing rate, a simple maneuver in animals having difficulty breathing is to gently turn them over and back for 10-20 seconds or to rotate their position every few minutes (normally on their side or stomach). The actual procedure time should be minimized to ensure that the mice are not overly anesthetized, the treatment agents and especially cells are not damaged while waiting to be injected, but the procedure should never be hastened.

VII. Subcutaneous injection

The purpose of tail vein injections is to produce metastasis-like nodules throughout the lung. These are also useful for the administration of drugs or treatments. Typically, an anesthetic is not necessary. Materials needed include the necessary syringes and needles, in addition to a heat source to dilate the vessels of the mouse. A restriction device is also needed to securely position the mouse so that its tail is towards the injector with little or no bend in the animal's central line of symmetry. Once the tail is warmed and the veins are visibly dilated, the tail should be held firmly to prevent the mouse from moving and straighten the tail vein. Cleaning the tail with warm water or xylene can further dilate the vessels, in addition to improving visibility. The injection should be carefully made using a 25 to 27 gauge needle directly into the lateral tail vein. Start with placing the injection as distally as possible and move proximal in subsequent injection attempts so that, if any failed attempts result in damaged vasculature, there is adequate tail vein remaining for further attempts. Typically, the needle should be easy to insert with little to no back pressure and should show a visible presence in the vein by pushing out the present blood . The injection should then be given slowly and with as little motion as possible. In experiments in which multiple injections are to be performed over several days, it is obviously imperative that the mouse's tail vein be carefully injected so as to preserve it for future injections. Normally, for the injection of drugs, virus, or other substances, a volume of 50 to 300 µl of an isotonic solution will have minimal effect on the mouse. However, this volume should be minimized to the best ratio of dose to volume to ensure that the injection does not significantly alter the hemodynamic characteristics of the mouse. For cell injections, depending on the cell line, it is not advisable to inject more than two million cells in a volume of more than 200 µl, to minimize emboli formation and maintain proper hemodynamics. Typically, a single mouse can be stabilized and injected in less than 1 minute. On average, 5% of the mice may die as a result of emboli or adverse reactions to the injection.

Problems: Embolism with lung failure and tumor growth at the site of the injection.

IX. Portal vein injection

For portal vein injection, we anesthetized mice in groups of 3 to 5. Even for an experienced investigator with an assistant and without any complications, it will likely take 5 minutes to anesthetize, open, inject, close, and recover each mouse. In some cases, a bad injection, difficult clot, or deceptively conscious mouse can draw out the procedure. These considerations must be taken into account when choosing this model because such long procedures can endanger the viability of the injected cells or agents, the consistent quality of the injection technique, and the instruments involved. Under even optimal circumstances, 10% to 25% of the mice may die during surgery, simply as a result of the necessary strength of a general anesthetic, the invasiveness of the procedure, the loss of blood, the drop in temperature, or other surgical complications, in addition to problems with the injectables, such as clumping, concentration, or injection speed.

The mouse is placed on its back with its head away from the operator. If the mouse shows a mild twitch or other abnormal movement, it should be anesthetized further and/or secured with tape or fine needles (30 gauge) through its skin. A transverse incision should be made directly beneath the xyphoid process (~2.5 cm long and 0.5 cm off center) towards the animal's left side. The intestine loop should be moved away from the lower liver lobe and the pancreas isolated, which should be attached to the splenic vein; the larger portal vein should then become visible. Be extremely careful not to make contact with, stretch, or otherwise damage any of the major vessels in the abdomen. An assistant sitting directly across or cornering the operator can provide invaluable assistance in drawing open the wound, holding apart the liver lobes and intestines, and putting a very mild amount of tension on the portal vein to ease the process of injection. The portal vein has adequate pressure to show a large, visible diameter, and when the vein is stretched lightly to straighten it, it is a fairly pressureless injection. However, any sort of sudden lateral movement, inadvertent pressure, or a twitch in the local tissue can easily cause a 30-gauge needle to puncture or tear the vessel, depending on the tension and position of the needle. These sorts of incidents typically cause the inevitable death of an animal.

The injection should be performed slowly and without excessive pressure. In a successful injection, there should be little or no leakage from the injection site, a positive flow of injectable into the vein, and an immediately visible browning or yellowing of the liver lobes, due to light infarction and blood being substituted for injected fluid, lasting upwards of 30 seconds. The injection volume should not exceed 100 µl. In our experience, a tumor injection of more than one million

cells of any line consistently resulted in the eventual, if not immediate death of 50% or more of the mice due to respiratory failure and arrest. A total of 500,000 cells appears to be the most stable and survivable number, and is more than adequate to produce widespread tumor growth. After a successful injection, very carefully remove the needle from the vessel, being careful to avoid any sort of horizontal or vertical motion, which will tear the vein. Most difficult cases of severe bleeding occur due to this simple mistake. After the needle is removed, immediately apply firm pressure using a cotton swab. However, do not press too hard, as this will likely open the injection site further and exacerbate bleeding. We recommend using an absorbent, coagulative swab, such as a PVA Surgical Spear (Allegiance, IL), to hasten clot formation. It is extremely important to monitor the mouse for any adverse effects from the injection, its current temperature, and its breathing. If blood loss is a concern, a small (up to 500 µl) injection of isotonic saline or PBS can be given subcutaneously in a dorsal flank. We actually recommend doing this in every animal *prior* to the injection in anticipation of possible excessive blood loss. Once bleeding has been controlled, carefully replace the organs that have been removed and gently close the wound. Be careful not to overly stretch or bend the mouse, since the clot that has formed on the inferior mesenteric vein is already exposed to considerable blood pressure and could rupture if subjected to additional trauma.

Problems: Embolism in the liver and lungs and rupture of the vessel with massive bleeding at the site of the injection.

X. Spleen injection

Many investigators choose this model for the development of liver metastases because bleeding is easier to control, the injection can be performed by a single person, and complications are extremely rare. Nevertheless, there can be major problems after spleen injection, including massive bleeding caused by deep penetration of the spleen, use of a large gauge needle, or inadvertent tearing of the spleen during isolation and injection. Embolisms can often occur, especially when the cells are not well shaken, or a too high cell number (cells are in the liver and/or lungs within seconds after injection). All of these problems can be avoided with adequate caution. We have had minimal problems with injections of between two and four million cells and recommend as slow an injection as possible.

To perform the injection, place the mouse on its right side. In nude mice, the dark- maroon spleen can be seen through the skin. Make a 1 to 2 cm incision on the animal's left lateral side, exposing the spleen and pancreas tail. After the spleen is removed from the abdominal cavity, the injection site should be directly under the capsula peripheral to the splenic hilum. The needle should not be introduced more than 1 cm into the spleen. We recommend a 27-gauge needle for this injection. After the slow injection of one to two million cells in an injection volume of 50 to 100 µl, the needle should be removed carefully and the injection site compressed for at least 2 minutes with a cotton swab. A successful injection is shown by the appearance of a small, discolored bolus at the injection site for approximately 30 seconds. Sometimes splenic bleeding will not stop easily, so careful use of a surgical spear or cotton swab can hasten coagulation. Do not close an animal until the bleeding has stopped. The spleen will typically not coagulate on its own and the animals will slowly suffer from internal blood loss and die. Once the bleeding is stopped, replace the organs, wash the abdominal cavity, and close the wound. Spleen injections normally produce local splenic tumors in 2 to 3 weeks. Usually, it takes more time for metastases to appear in the liver (4-6 weeks), when compared to a direct portal vein or liver injection

Problems: Embolism in the liver and lungs and bleeding at the site of the injection. Although the leakage of tumor cells at the injection site can be avoided, it is possible.

XI. Liver injection

Liver injection is ideal for local tumor development in a single liver lobe. The left upper lobe is the commonly used site of injection because of its size and ease of isolation. The direct injection is easy to perform, but the liver tissue, especially the capsula, is quite fragile and therefore should never be handled harshly, such as with metal forceps. A transverse incision should be placed directly across the xyphoid process and extended for approximately 2 cm. The injection should be placed close to the margin of the lobe, to prevent a direct injection into larger blood vessels, and just under the capsula. The liver is quite easy to compress, and therefore care must be taken to ensure that the needle is actually inside the lobe and neither so close to the surface that the injection leaks or bursts, nor so deep that the injection simply passes out the other side. A successful injection is shown by a quick and spreading brown-yellow discoloration that is evident even after an injected volume of as little as 25 µl. The original tissue color should return after 1 to 2 minutes. After extraction of the needle, the injection site should be compressed for 1 minute with a cotton swab to prevent tumor cell leakage out of the liver and into the abdominal cavity. Bleeding complications are very rare and caused mainly by excessive pressure or lacerations made during the injection.

Problems: Embolisms caused by deep injections, infarction of liver tissue in the injected lobe at high doses ($1x10^6$ cells), and leakage of cancer cells, leading to peritoneal carcinomatosis.

XII. Pancreas injection

The pancreas injection is used to establish a localized tumor model, similar to liver and spleen injections. Fortunately, unlike vascular or direct spleen/liver injections, injection into the pancreas normally does not lead to vascular embolism. The location of the incision for this injection procedure is very similar to that for the spleen injection. For this injection, we recommend a 27-gauge needle and a maximum volume of 50 µl. After the incision is made, remove the pancreas tail, and holding the

tail with a cotton swab, pull it carefully out and away from the spleen. Insert the needle up to 1 cm into the pancreas and be sure that the needle is inside the pancreas. Then slowly inject the tumor cells. A successful injection is shown by the formation of a small bubble after approximately 25 µl of the injectable has been injected. After the needle is pulled out, the injection site should be compressed with a cotton swab for 10 to 20 seconds, with care taken not to allow tumor cells to spill into the abdominal cavity. Usually bleeding is not a problem. Replace the organs and close the mouse.

Problems: If the injected volume is too high, the pancreatic tissue may rupture. Cell leakage, with subsequent peritoneal carcinomatosis, is possible, but not as common as after an intrahepatic injection.

XIII. Surgical orthotopic implantation into the pancreas or liver

The purpose of SOI is to establish consistent tumor size and location in a large group of animals. After practice, the model is easy to establish. Most of the implantations can be performed using a micro-needle holder and 6-0 to 8-0 absorbable suture, even for individuals with no prior skills, though it does depend to some extent on the experience, the suture size, and the need to operate under a microscope.

The first step in this model is to establish the subcutaneous cancer cell line as a stock for the following SOI. To do this, we injected 4 to 8 x 10^6 cells in 100 µl in the dorsal flanks of 4-week-old nude mice (see subcutaneous tumor models). Before the specimen is harvested, the skin should be aseptic, and if the animals will be killed, the incision should be very large to avoid contact of the tumor with the non-sterile skin environment. After the tumor is removed with sterile instruments, wash the tumor several times in a sterile Petri dish (or culture plate) with sterile PBS, until the washing solution remains clean. The necrotic tumor tissue, seen in large tumors, located in the middle must be cut away since it is unsuitable for implantation; if you cannot see the necrotic tissue, cut away tissue from the approximate middle of the tumor. The remaining tumor can minced with scalpels into approximately 1.5 mm^3 pieces.

For the SOI into the pancreas, mice are anesthetized and positioned laterally on their right side. A small left abdominal flank incision should be made similar to that made for a spleen or pancreas injection, and the pancreas tail with the spleen should be carefully removed. The tumor piece (kept in PBS) should be carefully put on the tip of a needle and transplanted into the pancreas tail, using a 6-0 PDS II surgical suture (Ethicon) and micro-needle holder (Medicon, Germany). Pancreatic tissue is very soft and the contact of tumor tissue with the pancreas is poor. Therefore, to stabilize the tumor pieces, several knots (we recommend 3) should be made in the suture, so that the fragment touches the pancreas gently, yet stably. The knots should not be pulled too tightly, to prevent necrosis of the pancreas. After the implantations are done, the pancreas is carefully returned to the peritoneal cavity and the wound is closed in one layer. The procedure is not overly invasive, nor does it involve severe blood loss, so surgical death rates should be below 5%.

The pancreas tail implantation is the most convenient pancreas model. However, the choice of the head or tail depends on the goal of the experiment. Problems with implantation in the head are that it can lead to bile duct, pancreatic duct, and intestine/stomach compression, with corresponding clinical symptoms. In contrast, because of the adhesions that form, implantation in the tail can be difficult for when the animals have to be reopened for multiple treatments.

The implantation of tissue in the liver is more complicated, because of the induced bleeding and the soft and fragile properties of liver tissue. We recommend using an 8-0 suture to attach the tumor pieces directly to the left upper lobe in a procedure similar to a direct liver injection. When using a large diameter suture, the needle required is too large and can damage the liver. It is quite difficult to perform surgery using fine sutures without a microscope or other visual assist device, and therefore special equipment may be required. It is possible to use a 6-0 suture, but the tissue damage is high and can lead to complications such as hematoma, necrosis, bile duct compression, and ascites.

Problems: The adhesions that form can cause ileus, or the tumor can grow into the stomach (ileus), spleen (bleeding), or intestine (ileus). The bile duct or blood vessels, especially at the pancreatic head, can also be compressed.

XIV. Discussion

By following the techniques described here using many commercially available tumor cell lines, we routinely achieved a tumorigensis rate of 90 to 100%. Many of these models, especially intraorgan and vascular injections, develop fast growing and highly lethal tumors. Survival will depend on the initial tumor size, distribution, and cell line, in addition to the health and age of the animal. However, for a tumor model to be successful, correct follow-up is also important. After finishing surgery, nude mice are usually hypothermic and should be placed under a heat lamp until they are actively awake. For longer surgery, a heat pad is recommended. For the first 3 postoperative days, mice should be inspected daily for bleeding, herniation of the intestines, skin infection, or loose staples. Mice heal quickly, and in most cases, staples can be removed on the fifth postoperative day.

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