Published in final edited form as:

Dev Dyn. 2013 October ; 242(10): 1202–1209. doi:10.1002/dvdy.24017.

Laser Mediated Cell Ablation During Post-Implantation Mouse Development

Jesse R. Angelo and Kimberly D. Tremblay[†]

Department of Veterinary and Animal Science, University of Massachusetts, Amherst, MA 01003

Abstract

Background—Laser mediated cell ablation is a powerful tool that has been used to understand cell fate in a variety of externally developing organisms but has not been used during mammalian post-implantation development.

Results—We describe a method pairing laser ablation with murine embryo culture and establish parameters that can be used to precisely ablate cells in the selected field with minimal disruption to adjacent cells or the underlying cell matrix. Ablation of a large domain of endoderm, followed by ~1 day of culture results in a phenotypically normal embryo and gut tube, indicating that laser ablation is compatible with normal development. We next focused on one of the three precursor populations that have been shown to produce the liver bud. Ablations of a single progenitor domain results in a unilateral delay in the liver bud while the contralateral side is unaffected.

Conclusions—We demonstrate that laser ablation is a specific and useful technique for studying cell fate in the mouse embryo. This method represents a powerful advance in developmental studies in the mouse and can be used to provide information on the specification of organs, differentiation, cell migration and vital tissue interactions during development.

Keywords

laser ablation; liver development; endoderm; embryo culture; mouse

Introduction

Selective cell ablation is an effective tool for the study of differentiation, cell migration, and tissue interaction. Current ablation techniques used in the vertebrate embryo center on genetic approaches such as tissue specific *Diphtheria toxin A-chain* (DTA) expression (Lee et al., 1998; Hu and Cross, 2011; Kopp et al., 2011; Stuckey et al., 2011) in mouse, as well as toxin–antitoxin strategies such as complimentary *parD Kid-Kis* expression (Nehlsen et al., 2010) and targeted expression of cytotoxic compounds such as *E.coli* nitroreductase (Slanchev et al., 2005; Curado et al., 2007) in fish. While these techniques are specific and reproducible, they lack the flexibility to target a genetically indiscrete portion of tissue. Additionally, genetic ablation techniques carry the inherent investment of generating and maintaining transgenic animals.

[†]To whom correspondence should be addressed: Dr. Kimberly D. Tremblay, University of Massachusetts, Amherst, 661 N Pleasant Street, Amherst, MA 01003, Office: 413.545.5560, Fax: 413.545.6326, kdtrembl@vasci.umass.edu.

Chemical ablation agents offer efficient cell destruction without the need to create animal lines. Tissue specific compounds, such as those available for neural ablation, offer highly specific, temporally restricted cell destruction but are only applicable to a narrow range of tissue types (Ciutat et al., 1996; Rite et al., 2005; Sai et al., 2009). Non-tissue specific agents, such as hydroxyurea, are dependent on developmental events, allowing for selective lineage ablation and temporal but not spatial specificity (Armstrong et al., 1998; Sweeney et al., 2012).

Laser-mediated cell ablation has been used to study cell lineage, regeneration, and tissue specific signaling of externally developing organisms such as *C. elegans* (Yanik et al., 2004; Fang-Yen et al., 2012; Schulze et al., 2012), zebrafish (Kohli and Elezzabi, 2008; Zhang et al., 2012), frogs (Mondia et al., 2011) and *Drosophila* (Kiehart et al., 2000; Supatto et al., 2005; Soustelle et al., 2008; Abreu-Blanco et al., 2011). An advantage of laser ablation is that it can be used with both temporal and spatial specificity and is hindered only by the target cells' accessibility. If a cell population is not visually distinct, genetic or physical cell labeling can aid with identification of the target cell population (Yanik et al., 2004; Thayil et al., 2008; Fang-Yen et al., 2012). During mouse development, the use of lasers for cell ablation has been confined to pre-implantation embryos. Infrared lasers have been used to destroy individual blastomeres from a 4-cell embryo and to remove trophectoderm from expanded blastocysts (Tanaka et al., 2006; Cortes et al., 2008; Sanmee et al., 2011) without damaging the remainder of the embryo.

Cell ablation in post-implantation mouse embryos is hindered by the stringent requirements of a viviparous model. Here we present a method for selective cell ablation, coupled with whole embryo culture, that allows for the selective ablation of tissues or portions of tissues on the ventral surface (exterior) of the early post-implantation embryo. These tissues include those with inductive properties such as the node, notochord and anterior visceral endoderm (AVE) as well as portions of the visceral or definitive endoderm. In this study, a microscope guided infrared laser is used to specifically ablate a 100–200 μm^2 area of definitive endoderm in 8.25–8.5 days *post coitum* (dpc) embryos which are then cultured for ~1 day to examine how ablation of a particular precursor population within the endoderm affects gut tube formation and organ specification. While ablation of a dorsal gut tube precursor population does not result in any identifiable morphological changes to the gut tube, we find that ablation of one of the two symmetric lateral liver precursor populations results in a liver bud that is smaller and lacks differentiation markers on the ablated side while maintaining normal growth and gene expression on the contralateral side. Taken together, our results demonstrate that laser ablation is compatible with normal development and that ablation can be used to assess cell fate during mouse development. Because of the precision of laser-mediated cell ablation, we anticipate that in addition to cell fate, this technique will facilitate a closer examination of the requirement of early inductive tissues during development.

Results and Discussion

Optimization of Laser-Mediated Ablation in Post-Implantation Mouse Embryos

Ablation within the definitive endoderm was performed on embryos with 4–14 somite pairs (S). A 300 mW infrared laser mounted on the 20X objective of an inverted microscope

equipped with an x, y-plane motorized stage was used (Fig. 1A–C). Because the laser is designed to ablate cells on the surface of a tissue culture dish, the embryo/target tissue must be securely positioned as close to the bottom of the dish as possible. The embryo is secured in the dish using a silicone holder submerged in 37°C media and positioned with the use of a dissecting microscope (Fig. 1D–E). The dish is transferred to the inverted microscope where correct positioning is confirmed under the 4X objective (Fig. 1F) before switching to the 20X objective for ablation (Fig. 1G).

Laser-mediated cell ablation is accomplished by rapidly heating discrete areas to high temperatures. The extent of damage caused by the laser is controlled by three parameters: pulse duration, percent of power used and distance of the sample from the laser's focal point. If a cell lies outside the focal plane, only a fraction of the laser's 300 mW potential is received by that cell. Cells within the laser's shallow depth of field are subjected to temperatures ranging from 80°C at the edge of the beam waist to 140°C at its center. This is illustrated in Figure 2A, which depicts a transverse section through an ~8.5 dpc embryo with the light path of the laser (gray), the 10 µm beam waist (black ellipse) and the generated heat gradient (orange scale).

To establish optimal parameters for cell ablation in whole embryos, laser power was kept constant at 100% and embryos were subjected to a range of pulse durations (25–200 µs) every 10 µm over a similarly sized total area. Whole mount views of an embryo treated with different pulse durations on equivalent areas of endoderm/visceral endoderm, followed immediately by propidium iodide (PI, a membrane impermeant DNA stain) and Hoechst incubation illustrate the range of affects elicited by pulse duration (Fig. 2B). The amount of PI integration at 25 µs is negligible and at 50 µs is low, indicative of sub-threshold pulses. With 200 µs pulse durations, substantial non-specific cell loss was observed (discussed more below). Pulses in the range of 100–150 µs appeared optimal based on the consistent, high degree of PI staining.

Under optimized conditions, damage to the targeted definitive endoderm is observed with bright-field microscopy as small circles in the tissue (Fig. 2C, C' between arrows) which are easily visible upon addition of trypan blue (Fig. 2D, D' between arrows), a membrane impermeant dye that is excluded from live cells but produces a blue stain in cells with disrupted membranes. Section analysis reveals that trypan blue is found only on surface cells, and is excluded from underlying cells, highlighting the laser's specificity (Fig. 2E, arrowheads). FOXA1, which is expressed in the definitive endoderm, notochord and floor plate, clearly demarcates all of the trypan blue stained cells, further demonstrating the specificity of the ablation. The clusters of FOXA1 and trypan blue-positive endoderm (Fig. 2E–F, arrowheads) represent the round clumps of dead cells that appear after laser treatment (Fig. 2C–D').

To further investigate the specificity of ablation we investigated the effects of optimal and greater than optimal pulse durations on histological architecture. The pre and post-ablation images of a similar region of three different embryos subjected to a range of pulse durations are shown in Figure 3 (A–C, 140 µs; D–F, 200 µs; G–I, 300 µs). With 140 µs pulses, damage is limited to surface cells with no apparent disruption of underlying tissue architecture. As

noted in Figure 2, this treatment level resulted in clusters of PI labeled tissue, representing the clumps of dead cells and cell debris (Fig. 3C, between white arrowheads). E-Cadherin, a component of adherens junctions, is normally present at areas of epithelial cell-cell contact, but is disrupted in the ablated region (Fig. 3C, between white arrowheads). Normal epithelial morphology is maintained in the contralateral side of each section (Fig. 3C, F and I, between green arrowheads). Laminin, a secreted component of the basement membrane, appears similarly localized in the ablated area and contralateral side, suggesting that the extracellular matrix is minimally perturbed with 140 μ s pulses (Fig. 3C). Pulses of 200 μ s lead to less specific ablations that decimate endoderm, leaving observable gaps in the embryo (Fig. 3F–F', between white arrowheads) and damage the underlying somitic tissue (yellow arrowhead). With 300 μ s pulse durations, extensive non-localized tissue damage occurs, resulting in a gross disruption of tissue architecture including loss of the endoderm and underlying tissues (Fig. 3I, I'). Whole mount views of the embryo before (Fig. 3G) and after (Fig. 3H) 300 μ s pulses reveal a distortion of the embryo at the ablated areas, indicating that the damage was immediate. The non-specific damage resulting from 200 or 300 μ s pulses renders these pulse durations unsuitable for experimental use. Notably, even the most severe pulse durations did not result in apoptosis of neighboring tissues outside the field of ablation, as assessed by immunofluorescent staining for active Caspase-3 (data not shown). Taken together, these results suggest that with optimized laser settings, laser ablation can be used to specifically ablate target tissue in the context of the whole embryo without significant damage to adjacent cells.

Tissue Ablation is Compatible with Normal Embryo Development

If laser ablation is to be an effective tool for studying developmental processes, then the ablated embryos must retain the ability to develop normally. To explore the systemic effects of laser-mediated ablation, embryos were subjected to partial gut tube precursor ablation at 6 S (n=5), 8 S (n=2), 9 S (n=2), and 11–14 S (n=5), with similar results observed regardless of starting age. Figure 4A depicts a representative 6 S embryo in which a 100 X 150 μ m area of definitive endoderm over the first somite on the left side was ablated using 120 μ s pulses. Fate mapping studies have revealed that this area will give rise to the dorsal gut tube rostral to the dorsal pancreas bud (Tremblay and Zaret, 2005; Angelo et al., 2012). The proximity of the ablated area to the future dorsal pancreas bud allowed us to locate the ablated domain after 27 hours of culture (Fig. 4B). All endoderm-ablated embryos formed a liver bud and both a dorsal and ventral pancreas bud (Fig. 4C–E). Each bud displayed appropriate expression of the liver and pancreas-specific markers, HNF4 α and PDX1 respectively. Furthermore, FOXA1 labeling of the ablated area demonstrated no apparent disruption in the epithelium of the dorsal endoderm, suggesting that the ablated area underwent repair without disrupting the orderly emergence of adjacent foregut organs.

Endoderm Ablation to Assess the Requirement of Liver Bud Precursors

Given that laser-based ablation of large regions of the endoderm can be performed without altering organ bud emergence we next sought to investigate the requirement of identified endodermal organ progenitors on organ budding. We previously identified 3 discrete domains of unspecified ventral foregut endoderm in the early somite embryo (8.25–8.5 dpc) that contribute to the bilaterally symmetric liver bud (Tremblay and Zaret, 2005; Angelo et

al., 2012). Two of these domains include similarly sized areas within the right and left lateral endoderm that flank the somites prior to gut tube formation. Our fate mapping experiments suggested that these left and right lateral domains contribute to the left and right side of the liver bud, respectively. To determine the requirement of the endogenous liver precursor domains for liver bud development, we ablated an area of approximately $140 \mu\text{m}^2$ encompassing much of the right or left lateral progenitor domain leaving the contralateral domain intact (Fig. 4F, between arrows). To discern the ablated and contralateral sides after culture, we labeled the contralateral endoderm with DiI and cultured the embryos for ~27 hours (Fig. 4G). At the end of culture, embryos were imaged and sectioned to examine liver bud development.

We found that liver precursor ablation resulted in distinct phenotypic characteristics in the normally symmetric liver bud (Fig. 4H–I). The side of the liver bud to which the ablated cells were believed to contribute always appeared smaller and displayed fewer signs of differentiation than the DiI-positive contralateral side ($n=6/6$). For example, in the embryo depicted in Fig. 4F–I, marked growth and expression of the differentiation marker, HNF4 α was mainly confined to the DiI-positive contralateral side of the liver bud (Fig. 4H–I, compare with C). Importantly, expression of the endoderm marker FOXA1 revealed that a continuous layer of endoderm surrounded the ablated side of the liver bud, suggesting that the initial wound successfully healed.

Given the observation that ablation of one of the lateral liver precursors always resulted in a smaller liver bud on that side, we next performed a more detailed analysis of control and manipulated embryos, without DiI labeling (Fig. 4J–Q; $n=7$). In control embryos, FOXA1 is found throughout the entire gut tube and the symmetric HNF4 α /AFP expressing liver bud (Fig. 4J–L). Although FOXA1 is apparent throughout the gut tube and liver bud of ablated embryos (Fig. 4M), the liver differentiation markers HNF4 α and AFP are appropriately expressed on the contralateral side but are not apparent on the ablated (right) side (Fig. 4N–O).

We next examined the size of the ablated and contralateral side of liver buds from individual embryos. The size of the ablated side is always smaller than that of the contralateral side (Fig. 4P). The narrow size range of the ablated side may be indicative of the amount of regrowth the liver precursors are able to accomplish over the culture period of 27 hours or could be indicative of the inability or delay of the ablated side to undergo the rapid proliferation that is typical of the liver bud at the end of culture [9.5 dpc; (Duncan, 2003)].

To determine if the growth of the contralateral side was comparable to that of control embryos we measured the area of the left and right sides of control cultured embryos ($n=9$). Although the average area of the ablated side of liver buds was significantly different from that of the contralateral side, the average size of the contralateral side of the liver bud is not statistically different from that of either the left or the right side of liver buds from control embryos (Fig. 4Q). These results suggest that while the left and right side of the liver bud normally grow at the same rate, the growth and differentiation of one side is independent of the other.

Conclusions

In summary, targeted cell ablation is a simple yet powerful tool for use with mammalian embryos. By pairing laser ablation with whole embryo culture, we have demonstrated that it is possible to study the fate of specific tissue domains that are not genetically distinguishable, and therefore inaccessible by genetic methods. Temporal and spatial-specific ablations, such as those demonstrated herein by laser ablation, can shed light upon the fate and requirement of embryonic tissues, competence and re-patterning of “naive” tissues and can be used to examine embryonic tissue repair.

Laser ablation followed by whole embryo culture can be used to examine the developmental requirements of a variety of inductive tissues that are on the surface of the early developing mouse embryo including the AVE, node, and notochord as well as the fate or requirement of portions of tissues that line the surface of the conceptus such as the visceral and definitive endoderm. While classic tissue extirpations designed to understand the requirement of the AVE and node have been performed in the mouse (Thomas and Beddington, 1996; Davidson et al., 1999), the precision provided by inverted microscope guided laser ablation can now be used to determine the requirement of these inductive tissue, or discrete portions of these tissues, without altering adjacent tissues or even the underlying extracellular matrix. Furthermore, we suggest that this technique can be used to better understand the implicit requirements of endoderm organogenesis, narrowing the temporal window of inductive interactions and gaining more specific insight into the signals necessary for organ growth *in vivo*.

Experimental Procedures

Embryo dissection and culture

The Institutional Animal Care and Use Committee (IACUC), at the University of Massachusetts, Amherst approved all animal studies. CD-1 (Charles River) females were mated with CD-1 studs and the morning of the copulation plug defined as 0.5 days post coitum (dpc). After removal from the uterus, embryos were immediately dissected in warmed and equilibrated (at least one hour in a 5% O₂, 5% CO₂, 37°C incubator) dissection media [DM: 10% Fetal bovine serum, 90% DMEM (Lonza, 12-709)] under a Nikon SMZ1500 dissection microscope equipped with a 37°C stage. After dissection, embryos were held in a 4-well dish (Nunc) in a 5% O₂, 5% CO₂, 37°C incubator in culture media [CM: 75% rat serum (Valley Biomedical, AS3061), 25% DMEM (Lonza, 12-614) supplemented with Pen/Strep (Gibco), non-essential amino acids (Lonza) and GlutaMAX (Gibco)]. After laser manipulation, each embryo was temporarily incubated in one well of a 4-well dish in CM (as above) until all embryo manipulations were complete. For culture, embryos were placed into individual glass bottles with 1–1.5 ml of fresh 37°C equilibrated CM and placed into roller culture as described (Angelo et al., 2012). At the end of culture the extraembryonic tissues were removed, the quality of each embryo noted, the embryo photographed, the somites counted and the embryos fixed in 4% paraformaldehyde overnight at 4°C.

Laser ablation

At the time of laser manipulation, each embryo was transferred to a 60 mm tissue culture dish containing 3–4 ml of 37°C DM and imaged on a Nikon SMZ1000 dissection microscope equipped with a 37°C stage. To prepare the buoyant embryo for ablation, the region of the embryo to be manipulated was securely positioned to face the bottom of the tissue culture dish. This was performed by positioning the embryo in the confines of a U-shaped holder cut from RTV615 silicone rubber (Momentive) and the embryo immobilized with a small cube of silicone that fit snugly into the channel of the larger holder. We found that a holder measuring 1.5 X 5 X 8 mm and channel 2 X 4 mm, with a 2 X 2 mm block was best to hold our ~8.5 dpc embryos securely in place. The positioned embryo was then transferred to an Olympus IX50 inverted microscope equipped with an automated stage and laser (Stilleto, Hamilton Thorne). The embryo was imaged at 4X to ensure proper positioning and then viewed through the 20X objective fit with the laser. Unless otherwise noted, pulse durations of 80–150 μ s were used depending on the distance of the target cell population from the focal point of the objective. Individual pulses were performed at 10 μ m intervals, the approximate width of an endoderm cell [(Kaufman and Navaratnam, 1981); and personal communication (JA)], over the entire area of interest. The laser guide, which is easily visible under the microscope, defined the position of the laser on the tissue and the automated stage (Hamilton Thorne) was used to move the embryo in 10 μ m increments. It should be noted that the endoderm of younger embryos (1–4 S) is more sensitive to laser ablation than the endoderm of older embryos (5–14S). A difference in sensitivity to laser ablation by a cell population at different development stages has been noted previously (Thayil et al., 2008). We find that pulse durations of 80–100 μ s is sufficient to induce ablation in endoderm of 1–4 S embryos while the endoderm of 5–14 S embryos requires 120–150 μ s pulses to achieve comparable results. Because of poor developmental outcomes associated with time out of culture, all embryo manipulations are completed within twenty minutes or the embryo discarded. After ablation, each embryo was immediately transferred back into CM and held in a 4-well dish (37°C, 5% CO₂, 5% O₂). When ablations were complete, embryos were subjected to roller culture as described above.

Embryo Staining

DiI labeling was performed on individual embryos after laser treatment (CM-DiI, Molecular Probes) as described previously (Angelo et al., 2012). The position of the labeled cells was recorded manually and documented using epifluorescence and bright field images produced using a MicroPublisher 5.0 RTV camera and QImaging software. After manipulation, each labeled embryo was temporarily incubated in a single-well of a 4-well dish, as noted above, until all embryo manipulations were complete and then placed into roller bottle culture as described above. Embryos that were not placed into overnight culture were labeled with trypan blue (Gibco) by direct application or propidium iodide (50 μ g/L, Sigma) and Hoechst (1:10,000, Molecular Probes) in CM immediately after manipulation. Those to be stained immunofluorescently to assess Caspase activity were placed in CM for two hours after laser manipulation. All embryos were fixed in 4% paraformaldehyde overnight at 4°C for histological processing.

Immunofluorescence

Each embryo used in this analysis was sectioned at 7 μm and all sections from a single embryo collected on one slide. Slides were dewaxed in xylene and rehydrated through an ethanol series. Antigen retrieval was performed by microwave treating in Tris buffer (0.01M Tris base, pH 10), cooled for 1 hr at room temperature (RT), washed with PBS with 0.1% Tween-20 (PBT), blocked with 0.5% milk in PBT for 2 hours and hybridized at RT with primary antibody (in 0.05% milk/PBT) overnight (O/N) at 4°C. After 3 PBT washes the sections were treated with secondary antibody (Molecular Probes; 1:500) at RT for 1 hour. After washing in PBS for 30 minutes the sections were treated with 4', 6-diamidino-2-phenylindole dihydrochloride (DAPI, Molecular Probes, 1:10,000) for 4 minutes and coverslipped with ProLong Gold Antifade Reagent (Invitrogen). Sections were imaged on either a Nikon Eclipse TE2000-S inverted microscope with a Retiga EXi Fast camera or a Nikon Eclipse Ti inverted microscope with an Andor DR-228C camera. Both microscopes use NIS Elements imaging software. Primary antibodies included: mouse anti-FoxA1 (1:1000, [Seven Hills, WMAB-2F83]), goat anti-HNF4 α (1:200, [Santa Cruz sc6556]), rabbit anti-AFP (1:200, [Gentaur, BMDA02]), rabbit anti-Caspase-3 (1:500, [Abcam, ab13847]), rabbit anti-Laminin (1:500, [Sigma Aldrich, L9393]), mouse anti-E-Cadherin (1:500, [BD Biosciences, 610181]), rabbit anti-PDX1 (1:1000, [AbCam, Ab47267]) and guinea pig anti-PDX1 (1:1000, [AbCam, Ab473308]).

Quantification of Liver Area

From each embryo, the number of sections containing liver bud was counted. Sections at the level of $\frac{1}{2}$, $\frac{2}{3}$ and $\frac{3}{4}$ of each bud were chosen for measurement. We previously showed that much of the rostral region of the bud is derived from a midline progenitor population not targeted in our ablations (Tremblay and Zaret, 2005; Angelo et al., 2012). To be confident that we were not measuring contribution from the midline progenitors, area measurements were confined to the posterior half of the liver bud. Measurements were performed using the "Annotations and Measurements" tool in NIS Elements software. For each section, the midline of the liver bud was estimated as arising from the midpoint of the associated gut tube. Using this midline estimate, the size of the ablated and contralateral (control) sides was measured. Measurements for the three levels of the liver bud were averaged for control and ablated sides, respectively, and plotted as area of control vs. ablated per embryo in Figure 4P. The right and left sides of the liver bud in control embryos, which were cultured but not laser treated, were measured in the same manner as laser treated embryos. These data were averaged and plotted in Figure 4Q along with the averages of contralateral (control) and ablated sides of the laser treated embryos presented in Figure 4P. Error bars represent standard error $p = 9.73 \text{ E-}05$ calculated by student's two-tailed, unpaired T test.

Acknowledgments

Grant Support Information: NIH R01 DK087753 and the American Diabetes Award 1-10-BS-178

The authors would like to thank Dr. Jenny Ross for her technical expertise. We would also like to thank Dr. Jesse Mager and members of the Tremblay and Mager labs for their discussion and support. This work was supported by R01 DK087753 and the American Diabetes Award 1-10-BS-178.

References

- Abreu-Blanco MT, Verboon JM, Parkhurst SM. Cell wound repair in *Drosophila* occurs through three distinct phases of membrane and cytoskeletal remodeling. *J Cell Biol.* 2011; 193:455–464. [PubMed: 21518790]
- Angelo JR, Guerrero-Zayas MI, Tremblay KD. A fate map of the murine pancreas buds reveals a multipotent ventral foregut organ progenitor. *PLoS ONE.* 2012; 7:e40707. [PubMed: 22815796]
- Armstrong JD, de Belle JS, Wang Z, Kaiser K. Metamorphosis of the mushroom bodies; large-scale rearrangements of the neural substrates for associative learning and memory in *Drosophila*. *Learn Mem.* 1998; 5:102–114. [PubMed: 10454375]
- Ciutat D, Caldero J, Oppenheim RW, Esquerda JE. Schwann cell apoptosis during normal development and after axonal degeneration induced by neurotoxins in the chick embryo. *J Neurosci.* 1996; 16:3979–3990. [PubMed: 8656292]
- Cortes JL, Sanchez L, Catalina P, Cobo F, Bueno C, Martinez-Ramirez A, Barroso A, Cabrera C, Liger G, Montes R, Rubio R, Nieto A, Menendez P. Whole-blastocyst culture followed by laser drilling technology enhances the efficiency of inner cell mass isolation and embryonic stem cell derivation from good- and poor-quality mouse embryos: new insights for derivation of human embryonic stem cell lines. *Stem Cells Dev.* 2008; 17:255–267. [PubMed: 18447641]
- Curado S, Anderson RM, Jungblut B, Mumm J, Schroeter E, Stainier DY. Conditional targeted cell ablation in zebrafish: a new tool for regeneration studies. *Dev Dyn.* 2007; 236:1025–1035. [PubMed: 17326133]
- Davidson BP, Kinder SJ, Steiner K, Schoenwolf GC, Tam PP. Impact of node ablation on the morphogenesis of the body axis and the lateral asymmetry of the mouse embryo during early organogenesis. *Dev Biol.* 1999; 211:11–26. [PubMed: 10373301]
- Duncan SA. Mechanisms controlling early development of the liver. *Mech Dev.* 2003; 120:19–33. [PubMed: 12490293]
- Fang-Yen C, Gabel CV, Samuel AD, Bargmann CI, Avery L. Laser microsurgery in *Caenorhabditis elegans*. *Methods Cell Biol.* 2012; 107:177–206. [PubMed: 22226524]
- Hu D, Cross JC. Ablation of Tpbpa-positive trophoblast precursors leads to defects in maternal spiral artery remodeling in the mouse placenta. *Dev Biol.* 2011; 358:231–239. [PubMed: 21839735]
- Kaufman MH, Navaratnam V. Early differentiation of the heart in mouse embryos. *J Anat.* 1981; 133:235–246. [PubMed: 7333952]
- Kiehart DP, Galbraith CG, Edwards KA, Rickoll WL, Montague RA. Multiple forces contribute to cell sheet morphogenesis for dorsal closure in *Drosophila*. *J Cell Biol.* 2000; 149:471–490. [PubMed: 10769037]
- Kohli V, Elezzabi AY. Laser surgery of zebrafish (*Danio rerio*) embryos using femtosecond laser pulses: optimal parameters for exogenous material delivery, and the laser's effect on short- and long-term development. *BMC Biotechnol.* 2008; 8:7. [PubMed: 18230185]
- Kopp JL, Dubois CL, Hao E, Thorel F, Herrera PL, Sander M. Progenitor cell domains in the developing and adult pancreas. *Cell Cycle.* 2011; 10:1921–1927. [PubMed: 21558806]
- Lee P, Morley G, Huang Q, Fischer A, Seiler S, Horner JW, Factor S, Vaidya D, Jalife J, Fishman GI. Conditional lineage ablation to model human diseases. *Proc Natl Acad Sci U S A.* 1998; 95:11371–11376. [PubMed: 9736743]
- Mondia JP, Adams DS, Orendorff RD, Levin M, Omenetto FG. Patterned femtosecond-laser ablation of *Xenopus laevis* melanocytes for studies of cell migration, wound repair, and developmental processes. *Biomed Opt Express.* 2011; 2:2383–2391. [PubMed: 21833375]
- Nehlsen K, Herrmann S, Zauers J, Hauser H, Wirth D. Toxin-antitoxin based transgene expression in mammalian cells. *Nucleic Acids Res.* 2010; 38:e32. [PubMed: 20007149]
- Rite I, Machado A, Cano J, Venero JL. Divergent regulatory mechanisms governing BDNF mRNA expression in cerebral cortex and substantia nigra in response to striatal target ablation. *Exp Neurol.* 2005; 192:142–155. [PubMed: 15698628]
- Sai Y, Chen J, Wu Q, Liu H, Zhao J, Dong Z. Phosphorylated-ERK 1/2 and neuronal degeneration induced by rotenone in the hippocampus neurons. *Environ Toxicol Pharmacol.* 2009; 27:366–372. [PubMed: 21783966]

- Sanmee U, Piromlertamorn W, Vutyavanich T. Effect of co-culturing of half-destroyed and intact 4-cell mouse embryos in varying ratios on subsequent in vitro development. *Theriogenology*. 2011; 75:1682–1687. [PubMed: 21356553]
- Schulze J, Houthoofd W, Uenk J, Vangestel S, Schierenberg E. Plectus - a stepping stone in embryonic cell lineage evolution of nematodes. *EvoDevo*. 2012; 3:13. [PubMed: 22748136]
- Slanchev K, Stebler J, de la Cueva-Mendez G, Raz E. Development without germ cells: the role of the germ line in zebrafish sex differentiation. *Proc Natl Acad Sci U S A*. 2005; 102:4074–4079. [PubMed: 15728735]
- Soustelle L, Aigouy B, Asensio ML, Giangrande A. UV laser mediated cell selective destruction by confocal microscopy. *Neural Dev*. 2008; 3:11. [PubMed: 18442390]
- Stuckey DW, Di Gregorio A, Clements M, Rodriguez TA. Correct patterning of the primitive streak requires the anterior visceral endoderm. *PLoS ONE*. 2011; 6:e17620. [PubMed: 21445260]
- Supatto W, Debarre D, Moulia B, Brouzes E, Martin JL, Farge E, Beaurepaire E. In vivo modulation of morphogenetic movements in *Drosophila* embryos with femtosecond laser pulses. *Proc Natl Acad Sci U S A*. 2005; 102:1047–1052. [PubMed: 15657140]
- Sweeney ST, Hidalgo A, de Belle JS, Keshishian H. Hydroxyurea ablation of mushroom bodies in *Drosophila*. *Cold Spring Harb Protoc*. 2012; 2012:231–234. [PubMed: 22301647]
- Tanaka N, Takeuchi T, Neri QV, Sills ES, Palermo GD. Laser-assisted blastocyst dissection and subsequent cultivation of embryonic stem cells in a serum/cell free culture system: applications and preliminary results in a murine model. *J Transl Med*. 2006; 4:20. [PubMed: 16681851]
- Thayil AK, Pereira A, Mathew M, Artigas D, Blanco EM, Loza-Alvarez P. Decrease in laser ablation threshold for epithelial tissue microsurgery in a living *Drosophila* embryo during dorsal closure. *J Microsc*. 2008; 232:362–368. [PubMed: 19017235]
- Thomas P, Beddington R. Anterior primitive endoderm may be responsible for patterning the anterior neural plate in the mouse embryo. *Curr Biol*. 1996; 6:1487–1496. [PubMed: 8939602]
- Tremblay KD, Zaret KS. Distinct populations of endoderm cells converge to generate the embryonic liver bud and ventral foregut tissues. *Dev Biol*. 2005; 280:87–99. [PubMed: 15766750]
- Yanik M, Cinar H, Cinar H, Chisholm A, Jin Y, Ben-Yakar A. Neurosurgery: functional regeneration after laser axotomy. *Nature*. 2004; 432:822. [PubMed: 15602545]
- Zhang J, Jeradi S, Strahle U, Akimenko MA. Laser ablation of the sonic hedgehog-a-expressing cells during fin regeneration affects ray branching morphogenesis. *Dev Biol*. 2012; 365:424–433. [PubMed: 22445510]

Bullet Points

- We establish parameters for laser ablation that, in conjunction with post-implantation mouse embryo culture, allows for normal development.
- Laser ablation produces a controlled and immediate disruption of tissues on the outside of the developing embryo without altering adjacent tissue layers or the underlying extracellular matrix.
- Ablation of one of the three previously identified liver bud progenitor domains results in a unilateral delay in liver budding, demonstrating that laser ablation can be used to study cell fate in the mouse.

Figure 1. Inverted microscope guided laser-ablation

A–C) The laser, conjugated to a 20X objective on an inverted microscope (A) is viewed from above (B) and below the tissue culture dish (C). D–E) Because precise and efficient ablation occurs at the inner surface of the tissue culture plastic, the buoyant embryo is positioned with a silicone rubber holder (D) and further stabilized with a silicone plug (small block in E). F) Correct positioning (foregut facing the bottom of the dish) is verified at higher magnification. G) The field of view during manipulation allows for clear identification of the notochord (No), somites (S) and ventral foregut lip (VF). The white asterisk is placed on the ectoplacental cone in D–F.

Figure 2. Laser-mediated ablation of surface cells

A) An illustrated transverse section of an 8.5 dpc embryo. Neural tube (blue), notochord (green), mesenchyme (orange) and endoderm (red) are drawn to scale. The predicted area of laser penetration (black ellipse) lies within the focal plane of the 20X objective. The light path from the objective is represented by grey triangles. The maximum temperature is generated at the center of the focal plane and decreases away from the focal plane as indicated (red-orange scale to the right). B) Similar sized areas were pulsed on the endoderm/visceral endoderm border of an 8.5 dpc embryo with durations ranging from 25 to 200 μ s as indicated. After treatment the embryo was stained with both propidium iodide (PI), which produces a red fluorescence in the nucleus of cells with ruptured membranes, and the nuclear stain Hoechst. C–D') Whole mount views of an embryo treated with 150 μ s pulses targeted to the exposed endoderm before (C–C') and after (D–D') addition of trypan blue (TB). E–F) Transverse sections through an embryo treated as above (C–D) then sectioned and subjected to immunofluorescence, demonstrate that TB staining (E–F arrowheads) is limited to FOXA1 expressing cells (green, F) on the embryonic surface.

Figure 3. Optimized ablation parameters maintain embryo integrity

A–B, D–E and G–H) Whole mount views of individual pre- (A, D, G) and post-ablation (B, E, H) embryos. The indicated pulse durations were used on a similar sized area, on the left lateral endoderm of each embryo (between white arrows). With 300 μ s pulses the ablated area bulges outward (compare G to H), indicating an immediate loss of tissue integrity. The red dot seen in some images is the laser-guide. C, F, I) Each ablated embryo was transversely sectioned and subjected to immunofluorescence analysis of CDH1 (red), a cell junction marker, and the basement membrane marker Laminin (green). The ablated area is located between the white arrowheads. The area outlined by each box is viewed at higher magnification (C', F', I'). The area between the green arrowheads demarcates the undisturbed contralateral side of the embryo. C–C') Pulse durations of 140 μ s result in loss of endoderm cell junctions and clustering of cell debris without significant disruption of the basement membrane. F–F') 200 μ s pulses produce gaps in the basement membrane and fragmentation of the underlying somites (yellow arrowhead). I–I') 300 μ s pulses extirpate the endoderm and underlying basement membrane and fragment the underlying somites (yellow arrowheads).

Figure 4. Ablation of putative liver precursors, but not adjacent endoderm, impairs liver bud development

A–E) The endoderm fated to contribute to the dorsal gut tube proximal to the liver and pancreas buds was ablated in this 6S embryo (between arrows in A). After culture through 25 S (B), section analysis reveals normal expression of the endoderm marker FOXA1, the liver bud (lb) differentiation marker HNF4α and the ventral (vp) and dorsal pancreas (dp) marker PDX1 (C–E, arrow in D indicates the region typically colonized by the ablated dorsal endoderm). F–G) Much of the left lateral liver progenitor domain of this 6 S embryo was ablated (between arrows in F) and the contralateral (right) side labeled with DiI. Whole mount views of the resultant 24 S embryo after culture reveals localization of the DiI labeled cells (G). Section analysis reveals that the HNF4α (green) expressing liver bud cells are confined to the DiI-positive (red) contralateral side of the embryo (H–I). J–O) Section analysis of a control (J–L) and a right liver progenitor ablated embryo (M–O) after culture. The control embryo expresses the liver bud markers HNF4α (grey, K) and AFP (red, L) throughout the liver bud. Although FOXA1 is found throughout the liver bud of ablated embryos (M) both HNF4α and AFP expression are confined to the prominent contralateral (left) side of the tissue (N–O). P) A size comparison of the ablated and contralateral side of

liver buds from individual embryos manipulated at the somite stage indicated and cultured through the tissue budding stage. Q) A comparison of the average sizes of the right and left side of control (n=9) and that of the ablated and contralateral side of the liver precursor ablated embryos displayed in P.