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Appendix E1

Supplemental Materials and Methods

Animal Models

All mice were kept in a pathogen-free animal facility with free access to water and food within a 12-hour light-dark cycle. Male mice from the same litters aged 7–8 weeks and weighed 24–31 g were visually inspected without gross evidence of discreet tumors before used. For all experiments and procedures, systemic anesthesia was induced with intraperitoneal injection of a mixture of ketamine (50 mg/kg; Ketaject, Phoenix Pharmaceutical, St Joseph, Mo) and xylazine (5 mg/kg; Bayer, Shawnee Mission, Kan), and analgesic carprofen (5 mg/kg; Norbrook, Northern Ireland) was administered subcutaneously.

The spleen was exposed by a surgical peritoneal incision, whose longitudinal axis measured 5 mm in length and cut perpendicularly to that of the spleen. For our study, the colorectal adenocarcinoma cell suspension (CT26 cells for Balb/C and MC38 cells for C57BL6 mice) was slowly implanted into each mouse over one minute via direct intraparenchymal splenic injection using a 25-gauge needle within a volume of 200ul at a concentration of $1.0*10^6$/mL (35,36). Splenectomy was performed 5 minutes posttransplantation eventually followed by abdominal closure. Mice were killed using an overdose of carbon dioxide within a chamber system (SMARTBOX CO2 chamber system; EZ Systems, Palmer, PA).

RF Ablation Protocol

RFA was applied following administration of the anesthesia, analgesia, and laparotomy procedure 24 hours after cell transplantation. Ablation lasted for 5 minutes on the exposed left liver to produce coagulation measuring 8 × 6 × 4 mm in diameter corresponding to a volume equivalent to 3.5% ± 0.02 of the whole liver. To avoid piercing trauma, the 21-gauge needle was placed onto liver surface with a 1-cm exposed tip, whose temperature was maintained at 70°C ± 1 (average ± SD) by automated titrating (∼1 Watt energy output/4 milliampere) with a clinically compatible RF generator (CC-1 Cosman coagulation system; Radionics, Burlington, Mass). To complete the RF circuit, the animal was placed on a standardized metallic grounding pad (Radionics). A sham procedure was performed as control, including laparotomy, hepatic exposure, and placement of the nonactivated electrode applicator.

Inhibitor preparation

PHA (PHA-665752, Sigma-Aldrich, St. Louis, MO) was originally obtained in powder form and mixed in 0.9% NaCl to achieve a dose of 0.83 gm/kg in 300ul saline and was IP injected 2 hours before ablation. S3I powder was mixed and dissolved in 0.9% NaCl to reach a dose of 10 mg/kg in 300ul saline.

Division of Tasks

All animal experiments were conducted by researchers (L.H., M.S.) with at least 1 year of experience in performing tumor implantation, RF ablation and surgery in these models. Cell culture and tumor preparation was performed by A.M. (5 years experience). The ratings on tumor load and tumor area ratio was performed by two authors (L.H., M.S.) by consensus without interval of readouts. Data analysis (interpretation of the immunohistochemical stains) was performed by three authors (L.H., E.G., and S.N.G.) with 2, 25, and 15 years of experience, respectively, with a consensus review performed whenever discrepancy was noted. Statistical analysis was performed by three authors (L.H., M.S. and S.N.G.).

References

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