Immunostaining Protocol: P-Stat3 (Xenograft and Mice)

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[Abstract] We sought to understand the mechanisms behind the potent effect of stromal TGF-beta program on the capacity of colorectal cancer (CRC) cells to initiate metastasis. We discovered that mice subcutaneous tumors and metastases generated in the context of a TGF-beta activated microenvironment displayed prominent accumulation of p-STAT3 in CRC cells compared with those derived from control cells. STAT3 signaling depended on GP130 as shown by strong reduction of epithelial p STAT3 levels upon GP130 shRNA-mediated knockdown in CRC cells.

Materials and Reagents

1. Paraffin sections (subcutaneous tumors samples or liver metastasis from nude mice respectively injected subcutaneously or intrasplenic with CRC cells)
2. XILOL
   Note: Xylol also referred to as xylene or dimethylbenzene is a solvent used in histology as a clearing agent to remove paraffin from dried microscope slides prior to staining.
3. MilliQ H_2O
4. Wash buffer (Dako, catalog number: K800721)
5. Rabbit anti-P-Stat3 (Cell Signaling Technology, catalog number: 9145S)
6. BrightVision poly-HRP anti- Rabbit (Immunologic, catalog number: DPVR110HRP)
7. Envision FLEX antibody diluent (Dako, catalog number: K8006)
8. Peroxidase Blocking Solution (Dako, catalog number: S202386)

9. ImmPACT DAB (Vector Laboratories, catalog number: SK-4105)
10. DPX mounting media (Sigma-Aldrich, catalog number: 06522)
11. Hematoxylin
12. Tris/EDTA (pH 9.0) (see Recipes)

Equipment

1. Oven
2. Immunostaining apparatus

Procedure

1. Stove samples at 65 °C just before starting the immunostaining technique. Remove the samples from the oven when the wax present in sections is completely undone.
2. De-waxing and rehydration: Place slides in a rack to perform following washes (bath).
   a. XILOL: 10 min
   b. XILOL: 10 min
   c. XILOL: 5 min
   d. 100% EtOH: 10 min
   e. 100% EtOH: 5 min
   f. 96% EtOH: 5 min
   g. 90% EtOH: 10-15 times
   h. 80% EtOH: 10-15 times
   i. 70% EtOH: 10-15 times
   j. 50% EtOH: 10-15 times
   k. 25% EtOH: 10-15 times
   l. H_2O MilliQ: 10-15 times
3. Antigen retrieval.
   a. Tris-EDTA Buffer (pH 9.0)
   b. Time: 20 min in boiling water
4. 3 washes 5 min with 1 ml 1x wash buffer.
5. Blocking endogenous peroxidase.
   a. 200 ml Peroxidase Blocking Solution
   b. Time: 10 min
6. 3 washes 5 min with 1 ml 1x wash buffer.
7. Incubation with primary antibody.
   a. Antibody: Rabbit anti-P-Stat3

b. Dilution 1/200 in Envision FLEX antibody diluent
c. 200 µl/sample
d. O/N 4 °C

8. 3 washes 5 min with 1 ml 1x wash buffer
9. Incubation with antibody BrightVision
   a. Antiboby: BrightVision anti-Rabbit
   b. 150 µl/sample
   c. Time: 45 min at room temperature
10. 3 washes 5 min with 1 ml 1x wash buffer
11. Revealed with ImmPACT DAB.
    a. 200 µl/sample
    b. Time: 10 min
12. 3 washes 5 min with 1 ml distilled water.
13. Hematoxylin (1 ml) counterstaining, time: 2 min.
14. Rinse in distilled water bath.
15. Rinse in TAP water bath.
16. Dehydration and mounting with DPX.

Representative data

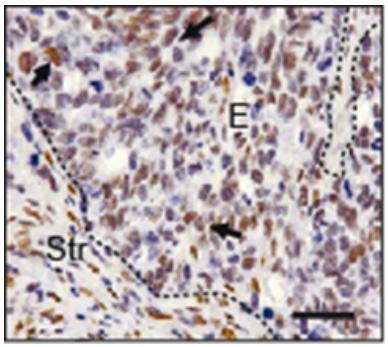

Figure 1. p-STAT3 staining of liver metastases generated after intrasplenic injection of CRC cells. Note strong staining in epithelial cells (arrows). E: epithelial cells, Str: stromal cells. Scale bar = 50 µm.

Recipes

1. Tris/EDTA (pH 9.0)
   12 g Tris
   3.7 g EDTA in 10 L MilliQ

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