Supplementary Materials & Methods

Immunohistochemistry

Slides were deparaffinized and rehydrated in 100% xylene (3X, 5 min), 100% ethanol (2X, 3 min), 95% ethanol (1X, 3 min), 70% ethanol (1X, 3 min) and distilled water (1X, 3 min). Antigen retrieval was performed by heating slides in citrate buffer at 90°C for 30 min. Slides were washed in PBS (3X, 5min), incubated for 30 min in blocking solution (PBS, 5% donkey serum, 1% BSA, 0.2% Triton X-100, 0.05% Tween 20), and incubated in primary Ab solution overnight at 4°C. Slides were then washed with TBST (3X, 15 min), incubated with secondary Ab (anti-rabbit-BIOT, 2 μg/ml, SouthernBiotech, #6440-06) in blocking solution for 30 min at RT, and washed again with TBST (4X, 15 min). Endogenous peroxidase was blocked with 0.3% hydrogen peroxide in methanol for 30 min at RT prior to rinsing 3X with PBS. Signal was amplified using Vectastain® Elite® ABC HRP Kit (Vector Laboratories, #PK-6100) according to manufacturer's instructions, and then washed with PBS (3X, 5min). Signal was visualized using a DAB peroxidase (HRP) Substrate Kit (Vector Laboratories, #SK-4100) following the manufacturer's recommendations. Each slide was incubated with DAB solution for 1-5 min until strong signal appeared, then the reaction was stopped by dilution with PBS. Slides were washed with PBS (2X, 5 min), distilled water (2X, 2 min), stained in hematoxylin (12 s, VWR, #10143-150) and rinsed with tap water. Staining was fixed in Bluing Solution (0.1% sodium bicarbonate) for 1 min and rinsed in distilled water (2X, 2 min) prior to dehydration in graded alcohols and xylene. Slides were mounted using Permount solution (Fisher Scientific, #SP15100) and subsequently analyzed for Podxl staining and localization.

Immunohistochemical scoring

The OC TMAs (219 cases) were initially scored based on number of stained cells, intensity and staining pattern. Number of stained cells was represented by an overall percentage of stained cells in the tumour, and then translated into a scoring scale of 0 (no staining, <1%), +1 (intermediate, 1-50%) and +2 (high, >50%). Intensity was scored based on a scale of 0 (negative), 1 (weak), 2 (intermediate) and 3 (strong). We then combined +1 and +2 cases with weak or higher staining and grouped them as PODO83 or PODO447-positive, respectively. The dominant staining pattern of each case was scored as A (apical), AL (apicolateral), L (lateral), B (basal), BL (basolateral), and CY (cytoplasmic). We then combined A, AL, B and BL groups as M

(membranous), and compared CY versus M staining for each of the mAbs. For the tissue cross-reactivity (TCR) TMAs, the frequency of stained cells was identified as negative (Neg), very rare (<1%), rare (1-5%), rare to occasional (5-25%), occasional (>25-50%), occasional to frequent (>59-75%), and frequent (>75-100%). Blood vessel (endothelium) staining was included as part of the overall score for each individual tissue.