Periostin in lymph node pre-metastatic niches governs lymphatic endothelial cell functions and metastatic colonization

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**Supplementary figures:**
Supplementary Figure 1: 3D analyzes of the POSTN network and lymphatic vessels. Immunofluorescence of the complete LN at pre-metastatic (PM) (2 weeks) and metastatic (M+) (4 weeks post implantation) stages compared to ctrl (POSTN in red and LYVE1 in green). Bars = 500 µm.
Supplementary Figure 2: POSTN is increased in pre-metastatic LNs of mice implanted with CaSki cells. Morphometric analysis of POSTN and LYVE1 lymphatic vessels in pre-metastatic LN as compared to the control. A) POSTN, LYVE1 and cell nuclei were stained in red, green and blue respectively in the control and pre-metastatic (PM) stage. Bars = 250 µm. B) Scatter graphs use scatter plots to represent POSTN, LYVE1 and colocalization density (in percentage) assessed by a computer assisted method (n ≥ 9). Results are expressed as mean ± SD, and statistical analyses were performed using a Wilcoxon-Mann-Whitney test (*p<0,05; **p<0,01). C) Spatial distribution analysis of LYVE1, POSTN, and the colocalization of both stainings from tissue edge to tissue center. Statistical analyses were performed using a Kolmogorov Smirnov test (*p<0.05). D) qRT-PCR performed on E6-E7 HPV proteins from LNs samples. The primary tumor was used as a positive control.
**A** HEV-POSTN-DAPI

Ctrl (1 week)

PM (1 week)

**B**

HEV density (%)

|        | Ctrl | PM |
|--------|------|----|
| 0      | 0    | 0  |
| 1      |      |    |
| 2      |      |    |
| 3      |      |    |
| 4      |      |    |

**C**

HEV staining area distribution

|        | Ctrl | PM |
|--------|------|----|
| 0      |      |    |
| 0.2    |      |    |
| 0.4    |      |    |
| 0.6    |      |    |
| 0.8    |      |    |
| 1      |      |    |
| 1.2    |      |    |
| 1.4    |      |    |
| 1.6    |      |    |

**D** HEV-POSTN-DAPI

Ctrl (2 weeks)

PM (2 weeks)

**E**

HEV density (%)

|        | Ctrl | PM |
|--------|------|----|
| 0      |      |    |
| 1      |      |    |
| 2      |      |    |
| 3      |      |    |
| 4      |      |    |

**F**

HEV staining area distribution

|        | Ctrl | PM |
|--------|------|----|
| 0      |      |    |
| 0.2    |      |    |
| 0.4    |      |    |
| 0.6    |      |    |
| 0.8    |      |    |
| 1      |      |    |
| 1.2    |      |    |
| 1.4    |      |    |
| 1.6    |      |    |

**G** HEV-POSTN-DAPI

Ctrl (4 weeks)

M+ (4 weeks)

**H**

HEV density (%)

|        | Ctrl | M+ |
|--------|------|----|
| 0      |      |    |
| 1      |      |    |
| 2      |      |    |
| 3      |      |    |
| 4      |      |    |

**I**

HEV staining area distribution

|        | Ctrl | M+ |
|--------|------|----|
| 0      |      |    |
| 0.2    |      |    |
| 0.4    |      |    |
| 0.6    |      |    |
| 0.8    |      |    |
| 1      |      |    |
| 1.2    |      |    |
| 1.4    |      |    |
| 1.6    |      |    |

Lmax:

- Ctrl (1 week): 0.95 ± 0.20 mm
- PM (1 week): 0.86 ± 0.16 mm

Lmax:

- Ctrl (2 weeks): 0.70 ± 0.15 mm
- PM (2 weeks): 1.00 ± 0.20 mm

*p=0.02*
Supplementary Figure 3: POSTN is not associated with HEVs in (pre)-metastatic LNs. Morphometric analysis of experimental (pre)-metastatic LNs as described in Figure 2. A-D-G) POSTN (red) and HEV (MECA79+ in green) stainings in pre-metastatic (PM) (at 1 week in A and 2 weeks in D) and in metastatic (M+) (at 4 weeks, G). Bars = 250 µm and 50 μm in the right (higher magnification of the insert images). B-E-H) Scatter graphs use scatter plots to represent POSTN and HEV densities (in percentage) (n ≥ 9). Results are expressed as mean ± SD (Wilcoxon-Mann-Whitney test: *p<0.05). C-F-I) Spatial distribution analysis from tissue edge to tissue center. The blue rectangle delineates the area between 0-0.30 mm from the LN border where the cumulative normalized areas of HEV and POSTN were measured and represented in the top right. Maximum distance of migration from the tissue border (Lmax) is indicated. Results are expressed as mean ± SD (Wilcoxon-Mann-Whitney test: *p<0.05; **p<0.01). D-I) All the results are representative of two independent experiments.
Supplementary Figure 4: HEVs are observed inside the tumor area. HEV and cell nuclei were stained in green and blue, respectively. Fontana staining was used to stain tumor cells in metastatic (M+). Bars = 50µm.
A

αSMA  POSTN mRNA  Merge/DAPI

B

FRC  LEC

POSTN (pg/ml)

C

CTRL  VEGFC

POSTN-DAPI

B16F10 CM  Caski CM
**Supplementary Figure 5: POSTN is produced by fibroblasts.** A) Representative POSTN (red) mRNA detection by RNAscope and immunostaining of α-SMA (green) on human LN sections (scale bar=20 µm). Cell nuclei are stained with DAPI in blue. **B)** ELISA of human POSTN performed on FRCs and LECs stimulated by VEGF-C (400ng/ml) or medium conditioned (CM) by B16F10 or CaSki cells (with at least 3 wells per condition in each experiment). Results (pg/ml) are expressed as mean ± SD (Mann-Whitney test: **p<0.01; ***p<0.001 ****p<0.0001). Results represent a set of three independent experiments. **C)** POSTN immunostaining of FRC (in red) stimulated by VEGF-C (400ng/ml), B16F10 CM or CaSki CM. Nuclei were counterstained with DAPI (blue). Bars=50 µm. Results are expressed as mean ± SD and represent a set of two independent experiments (Mann-Whitney test: **p<0.01).
**Supplementary Figure 6: POSTN exerts different effects on LECs and tumor cells**

A) Adhesion assay of LECs (left panel) and B16F10 cells (right panel) on POSTN coated-wells. Results represent a set of three independent experiments (with 12 wells per condition in each experiment) and are expressed as mean ± SD (Mann–Whitney test: ****p<0.0001). B) LEC and B16F10 proliferation analysis using the IncuCyte S3 system. LECs and B16F10 cells were seeded on a POSTN-coated well for 24h and 48h, respectively. The quantification is represented by the percentage of confluence. Results are expressed as mean ± SEM and the Anova two-way significance test was used (* p < 0.05, ** p < 0.01). C) Characterization of LEC and B16F10 cell morphology via cell circularity and area quantification in a POSTN-coated well (10ng/well) compared to the control. Cells were stained with phalloidin (red) and DAPI (blue) used as a marker for the nuclei. One representative experiment out of the 3 analyzed more than 20 cells per condition. Bars=20 µm. Statistical analyses were performed using a non-parametric Mann–Whitney test (***p<0.001). D) Chemotactic response of LECs and B16F10 cells to a gradient of complete medium or POSTN. The rose diagram illustrates the migration direction migration of more than 20 cells. Black arrows indicate the mean migration direction. Histograms represent the migratory speed (µm/min) and the accumulated distance (µm) where results are expressed as mean (n = 3 biological replicates) ± SEM. Statistical analyses were performed using a non-parametric Mann–Whitney test.