**Blood endocan as a biomarker for breast cancer recurrence**

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**Supplementary Information**

**Supplementary Figures**

|  |  |
| --- | --- |
| **a** |  |
|  | |  |  | | --- | --- | | **IHC** | **Blood endocan** | | **a** | Not detectable | | **b** | 5.60 ng/mL | | **c** | 4.41 ng/mL | | **d** | Not detectable | | **e** | Not detectable | |
| **b** | **c** |
|  |  |
| **d** | **e** |
|  |  |

**Figure S1** Pathological assessment of endocan expression in primary breast tumors removed by the surgery. **a**, Representative tissue section from a breast cancer patient who showed blood endocan levels below the cutoff value (1.68 ng/mL). **b** & **c**, Representative tissue section from breast cancer patients whose cancer cells expressed endocan. **d** & **e**, Representative tissue section from breast cancer patients whose endothelial cells expressed endocan. The table summarizes blood endocan levels for individual patients listed in the panel from **a** to **e**.

|  |  |  |
| --- | --- | --- |
| **Athymic nude** |  |  |
| m214 & m236: <0.156 ng/mL @Day 131 | |
| **NSG** |  |  |
|  |  |
| m257: 1.19 ng/mL @Day 58  m256: 2.14 ng/mL @Day 58 | |

**Figure S2** Bioluminescence imaging of athymic nude mice, NSG mice, and their tissues excised after *in vivo* imaging at the endpoint. Exposure times used are listed on the upper left. Blood endocan levels at the endpoint are also listed for the individual mice.

**Supplementary Tables**

**Table S1** Clinical characteristics of breast cancer patients whose blood was collected before and after the surgery.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Clinicopathological features | Total patients, *N* (*N* = 16) | Endocan negative population, *N* (%) (*N* = 8) | | Endocan positive population, *N* (%) (*N* = 8) | |
| Age at blood collection |  |  |  |  |  |
| Median (years) | 53 | 52 |  | 52 |  |
| (Minimum-Maximum) | (42-83) | (46-83) |  | (42-76) |  |
|  |  |  |  |  |  |
| Menopausal status |  |  |  |  |  |
| Pre-menopausal | 9 | 4 | (44.4%) | 5 | (55.6%) |
| Post-menopausal | 7 | 4 | (57.1%) | 3 | (42.9%) |
|  |  |  |  |  |  |
| BMI |  |  |  |  |  |
| BMI<25% | 12 | 6 | (50.0%) | 6 | (50.0%) |
| BMI≧25% | 4 | 2 | (50.0%) | 2 | (50.0%) |
|  |  |  |  |  |  |
| ER expression |  |  |  |  |  |
| <90% | 0 |  |  |  |  |
| ≧90% | 16 | 8 | (50.0%) | 8 | (50.0%) |
|  |  |  |  |  |  |
| PgR expression |  |  |  |  |  |
| <10% | 2 | 1 | (50.0%) | 1 | (50.0%) |
| 10%≦ PgR <90% | 4 | 2 | (50.0%) | 2 | (50.0%) |
| ≧90% | 10 | 5 | (50.0%) | 5 | (50.0%) |
|  |  |  |  |  |  |
| Tumor size |  |  |  |  |  |
| Tis\* | 1 | 1 | (100.0%) | 0 | (0.0%) |
| T1 | 9 | 5 | (55.6%) | 4 | (44.4%) |
| T2 | 6 | 2 | (33.3%) | 4 | (66.7%) |
|  |  |  |  |  |  |
| Lymph node metastasis |  |  |  |  |  |
| N- | 11 | 6 | (54.5%) | 5 | (45.5%) |
| N+ | 5 | 2 | (40.0%) | 3 | (60.0%) |
|  |  |  |  |  |  |
| Pathological Stage |  |  |  |  |  |
| 0 | 1 | 1 | (100.0%) | 0 | (0.0%) |
| I | 7 | 4 | (57.1%) | 3 | (42.9%) |
| II | 8 | 3 | (37.5%) | 5 | (62.5%) |
|  |  |  |  |  |  |
| Ki67 labeling index |  |  |  |  |  |
| <20% | 11 | 6 | (54.5%) | 5 | (45.5%) |
| ≧20% | 4 | 2 | (50.0%) | 2 | (50.0%) |
| Unknown | 1 | 0 | (0.0%) | 1 | (100.0%) |

\* Tis: Ductal carcinoma in situ (DCIS) or Paget’s disease with no associated tumor mass.

**Table S2** Clinical characteristics of breast cancer patients with postoperative recurrence.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Clinicopathological features | Total patients, *N* (*N* = 20) | Endocan negative population, *N* (%) (*N* = 7) | | Endocan positive population, *N* (%) (*N* = 13) | |
| Age at blood collection |  |  |  |  |  |
| Median (years) | 55 | 63 |  | 55 |  |
| (Minimum-Maximum) | (35-78) | (35-78) |  | (41-70) |  |
|  |  |  |  |  |  |
| ER expression |  |  |  |  |  |
| <90% | 9 | 3 | (33.3%) | 6 | (66.7%) |
| ≧90% | 11 | 4 | (36.4%) | 7 | (63.6%) |
|  |  |  |  |  |  |
| PgR expression |  |  |  |  |  |
| <10% | 11 | 4 | (36.4%) | 7 | (63.6%) |
| 10%≦ PgR <90% | 5 | 1 | (20.0%) | 4 | (80.0%) |
| ≧90% | 4 | 2 | (50.0%) | 2 | (50.0%) |
|  |  |  |  |  |  |
| HER2 expression |  |  |  |  |  |
| ≦1+ | 16 | 7 | (43.8%) | 9 | (56.3%) |
| 2+ | 3 | 0 | (0.0%) | 3 | (100.0%) |
| ≧3+ | 1 | 0 | (0.0%) | 1 | (100.0%) |
|  |  |  |  |  |  |
| Subtype |  |  |  |  |  |
| Luminal | 12 | 5 | (41.7%) | 7 | (58.3%) |
| HER2 | 1 | 0 | (0.0%) | 1 | (100.0%) |
| Triple-negative | 7 | 2 | (28.6%) | 5 | (71.4%) |
|  |  |  |  |  |  |
| Ki67 labeling index |  |  |  |  |  |
| <20% | 6 | 2 | (33.3%) | 4 | (66.7%) |
| ≧20% | 14 | 5 | (35.7%) | 9 | (64.3%) |

**Table S3** Effect of the interval between the surgery and the blood collection on changes in blood endocan levels.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Patient # | Pre-surgical endocan level (ng/mL) | Post-surgical endocan level (ng/mL) | UP or DOWN | Interval of time between the surgery and the blood collection (Days) |
| 1 | 5.97 | 10.90 | UP | 29 |
| 2 | 5.66 | 1.03 | DOWN | 32 |
| 3 | 12.45 | 1.47 | DOWN | 32 |
| 4 | 17.31 | 5.60 | DOWN | 46 |
| 5 | 5.60 | 4.38 | DOWN | 59 |
| 6 | 4.41 | 2.20 | DOWN | 64 |
| 7 | 2.24 | 14.51 | UP | 67 |
| 8 | 19.64 | 10.86 | DOWN | 83 |

**Table S4** Endocan, CEA, and CA15-3 concentrations in the blood samples of patients with postoperative breast cancer recurrence.

|  |  |  |  |
| --- | --- | --- | --- |
| Patient # | Endocan level (ng/mL) | CEA level (ng/mL) | CA15-3 level (U/mL) |
| 1 | <0.156 | 5.6 | 23.9 |
| 2 | 0.16 | 2.8 | 12.4 |
| 3 | 0.30 | 4.8 | 8.1 |
| 4 | 0.43 | 3.1 | 85.9 |
| 5 | 0.61 | 4.6 | 17.4 |
| 6 | 0.89 | 2.7 | 14.6 |
| 7 | 1.22 | 1.5 | 14.2 |
| 8 | 2.35 | 9.4 | 189.9 |
| 9 | 3.88 | 1.7 | 5.8 |
| 10 | 4.48 | 0.9 | 5.5 |
| 11 | 5.20 | 1.4 | 25.6 |
| 12 | 5.78 | 0.6 | 18.1 |
| 13 | 5.80 | 1.3 | 4.6 |
| 14 | 6.21 | 23.9 | 8.6 |
| 15 | 6.63 | 1.7 | 27.2 |
| 16 | 9.13 | 10.2 | 95.7 |
| 17 | 9.45 | 6.4 | 30.2 |
| 18 | 10.34 | 2.1 | 6.5 |
| 19 | 10.75 | 23.4 | 34.7 |
| 20 | 10.82 | 4.1 | 9.0 |

Red numbers indicate positive for the respective biomarker.