Supplement

Thu Thuy, N. ., Van Hung, N. ., Van Chu, N. ., Phong Thu, L. ., & Trung Tho, L. . (2025). Relationship of transgelin expression with clinicopathological characteristics and disease-free survival in HER2-positive breast cancer. *Biomedical Research and Therapy*, *12*(8), 7697-7707. https://doi.org/10.15419/55xvxh21

Hematoxylin and eosin staining

The paraffin-embedded tissue specimens were cut into 5-μm serial sections. The sections were dewaxed and rehydrated with xylene and ethanol, respectively, followed by staining with Hematoxylin for 5 min and 1% acid ethanol for 3 seconds. The sections were then rinsed in distilled water and stained with eosin for 3 min. Dehydration and hyalinization were then subsequently performed. Sections were visualized using a light microscope (Olympus, Tokyo, Japan).

Immunohistochemical staining

Formalin-fixed, paraffin-embedded tissue sections were stained with an automatic immunohistochemical staining device (Ventana BenchMark ULTRA autostainer) as follows:

1. Tissue sections were cut at 3–5 μm thickness and mounted on positively charged slides.
2. The computer and the automated immunohistochemical staining machine were started.
3. The pre-installed general staining program was initiated.
4. The specific processing and immunohistochemical staining protocol was configured on the machine.
5. The primary antibody to be used was selected.
6. Paraffin was removed using Ezpred solution (a reagent specifically designed for the machine).
7. Antigen retrieval was performed using CC1 solution at 95°C for 30 minutes.

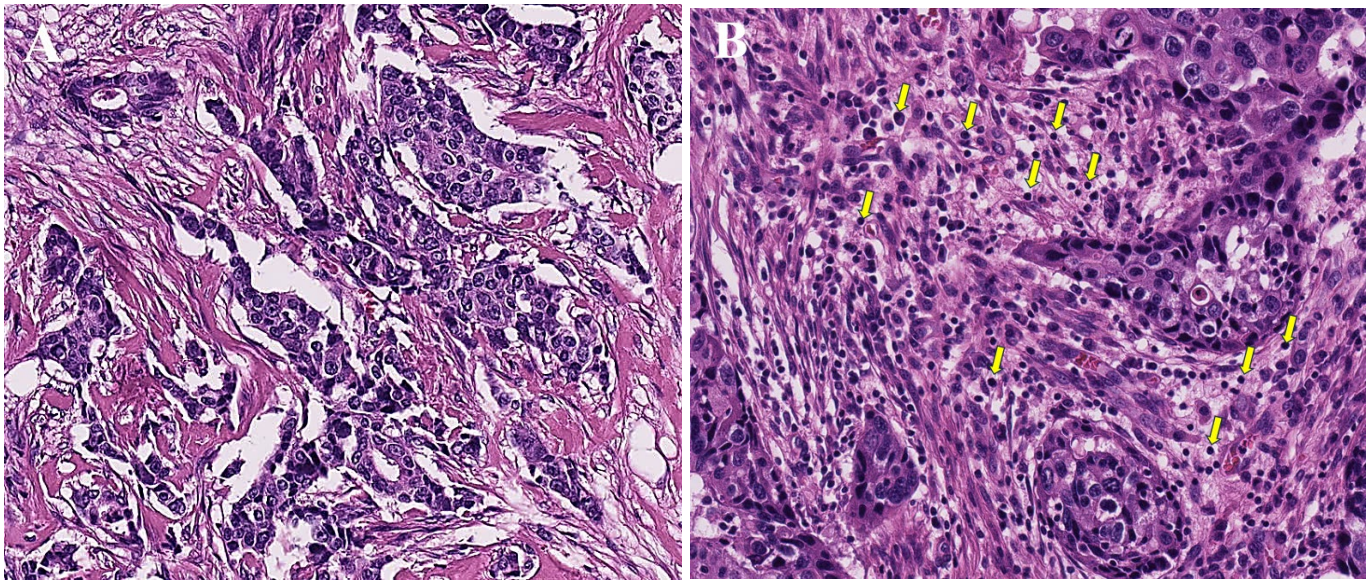
Slides were incubated with primary antibodies against Transgelin (Scytek, RA0451-C.1)) at a dilution of 1:100 and CD8 (Diagnostic Biosystems, Mob117) at a dilution of 1:200 according to the manufacturer's instructions.

8. Immunoreactivity was visualized using a Diamino Benzidine (DAB) detection kit.
9. After the staining process was completed, the slides were removed and washed with

a detergent solution to eliminate the LCS oil layer.

10. Nuclear counterstaining was performed with hematoxylin.
11. Coverslips were applied.

    Slides were examined under a light microscope.



Supplementary Figure: Tumor-infiltrating lymphocytes in HER2-positive breast cancer. Staining Hematoxylin and eosin in original magnification 20x: Low (**A**) and high (**B**).