Influence of tissue components on reliable mRNA quantification via RT-qPCR in FFPE breast cancer tissues

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Study outline

Three different set-ups were designed in order to clarify the interference of non-invasive tumor tissue components with the stability of the RT-qPCR-based MammaTyper® marker expression results.

Impact of tumor cell content on relative gene expression

To analyze the impact of the tumor cell content on the quantitative marker result, RNA was isolated from paired whole surface tissue sections containing different tumor cell contents (20-39%, 40-59% and 60-79%) and their corresponding whole tissue sections. Similar expression patterns between whole tissue and tumor-enriched samples for the four marker genes human epidermal growth factor receptor 2 (HER2), estrogen receptor 1 (ESR1), progesterone receptor (PGR) and marker of proliferation (MKI67) were obtained considering FFPE tissue sections with a tumor cell content of at least 20%. Microdissection of breast cancer samples for the MammaTyper® assay is recommended for sections containing less than 20% malignant tumor cells. RNA contribution of normal tumor-adjacent tissue is very low compared to hypercellular invasive cancer resulting in low influence on tumor marker expression quantification.

Effect of adipocytes on relative gene expression

For estimating the effect of larger areas of adipocytes on the MammaTyper® test results, pairs of non-dissected samples and macrodissected samples (tumor-enriched sections and adipocyte-enriched sections) were compared with respect to their relative gene expression. We could clearly demonstrate that due to the low RNA contribution of adipose tissue MammaTyper® test results are not influenced by this tissue component.

Effect of DCIS on relative gene expression

To study the impact of DCIS on the MammaTyper® test results, 24 non-dissected whole tissue sections, corresponding whole tissue sections without DCIS and microdissected-enriched DCIS samples were compared with respect to their relative gene expression of the four marker genes ERBB2, ESR1, PGR and MKI67. Most of the tumor-adjacent DCIS samples display a different gene expression pattern as the residual, tumor-containing tissue of the same breast cancer sample (e.g. HER2). However, due to the low total RNA yield from DCIS tissue, even high amounts of tumor-adjacent DCIS do not affect gene expression quantification of the invasive tumor tissue.

Conclusion and Perspective

We performed a detailed analysis of tissue parameters for reliable mRNA quantification via RT-qPCR in FFPE breast cancer samples. We could show that neither DCIS nor larger areas of adipocytes adjacent to the invasive tumor region are influencing the quantitative single marker result of the MammaTyper® test.

In addition, we could demonstrate a good agreement of the expression levels of the individual MammaTyper® genes in tumor-enriched samples and corresponding whole surface breast cancer sections. A tumor cell content of at least 20% is recommended and validated for the MammaTyper® test.