Background

Molecular subtyping of breast cancer has become an integral part of standard evaluation of breast cancer patients. According to the 12th St Gallen International Breast Cancer Conference (2011) Expert Panel systemic therapy recommendations follow the subtype classification. The assessment of the molecular subtypes requires combining data from analyses on ER, PR, HER2 and cell proliferation markers, but their immunohistochemical (IHC) testing carries an up to 20% risk of erroneous results. Similarly, assessment of cell proliferation by Ki-67 staining is hampered by lack of standardization of laboratory methods and agreement on cutoffs.

Here we tested the prognostic value of objective quantification of ESR1, PgR, HER2 and the proliferation marker RACGAP1 using RT-qPCR and compared the results with local and central IHC assessments.

Conclusions

Molecular subtyping of breast cancer by RT-qPCR using RNA isolated from FFPE tissue was successful in this large cohort. RACGAP1 mRNA expression distinguished high and low risk luminal breast cancers. In a multivariate analysis RACGAP1 mRNA expression, outperformed the subset of luminal tumors, high expression of ESR1 and RACGAP1 mRNA by RT-qPCR and compared the results with local and central IHC assessments.

Material & Methods

RNA was extracted from FFPE tumor tissue of 917 patients who participated in the FinHer II trial. ESR1, PgR, HER2 as well as RACGAP1, TOP2A and Ki67 mRNA expression were measured using RT-qPCR.

The molecular subtypes (luminal, HER2-enriched and triple-negative) were determined by predefined cutoffs. Prognostic significance of factors was assessed using univariate and multivariate analyses. The RT-qPCR results were compared with local and central IHC results.

Summary

1) HER2 mRNA showed a bimodal distribution with 197 (21.4%) out of the 917 tumors being above the predefined cut-off. HER2 mRNA expression increased in parallel with HER2 protein expression. Overall concordance of HER2 mRNA testing with central IHC and CISH was good, while local IHC testing suffered from high false positive rates. RACGAP1 mRNA expression was the greater the higher the histological grade.

2) ESR1 and PgR mRNA levels correlated negatively with the histological grade (r=−0.38 and r=−0.33; p<0.0001), whereas HER2 and RACGAP1 mRNA were correlated positively (r=0.10 and r=0.49; p=0.002 and p=0.0011 for each, respectively). RACGAP1 mRNA was negatively associated with ESR1 and PgR mRNA (r=0.17 and r=−0.26, respectively; p<0.0001 for each).

3) Molecular subtypes determined by RT-qPCR using predefined cut-off values were highly prognostic for overall survival (OS) (p<0.001). The 5-year OS rate for patients with luminal cancer was 94% and 86% for HER2-enriched cancer and 84% for triple-negative cancer.

4) In the subset of luminal tumors, high expression of RACGAP1 identified a population of patients who were at a high risk of death (5-year OS 82% versus 95%; p=0.0011).

5) In a multivariate analysis RACGAP1 mRNA expression, nodal status and chemotherapy type were independent prognostic factors, whereas IHC of ER, PgR, Ki-67 and histological grade were not significant.

References