Bread Cancer consists of numerous intrinsic molecular subtypes, providing the basis for clinical treatment decisions. Lately, it has become increasingly recognized that factors other than intrinsic cancer characteristics, such as immune composition in the tumor microenvironment, have important effects on treatment choices and efficacy. bioSyntagma has developed a method, the Molecular Fingerprint (mPrint), that enables multiplexed analysis of spatially defined regions in formalin fixed, paraffin embedded (FFPE) tumor samples allowing for analysis of gene signatures unique to the tumor microenvironment. This method was applied to (molecularly) defined sets of breast tumors and used to evaluate four different tumor regions of interest (ROIs): 1) Visible Carcinoma Proper (with cancer cells), 2) Inflammatory Tumor-Related (spare cellularly), 3) Interfaces between viable tumor and inflammatory components (tumor-infiltrative microenvironment) and 4) Tissue away from the normal breast tissue. This approach was compared to whole tissue specimens from each patient block. Each ROI and whole tissue sample was analyzed by high throughput qPCR for a panel of 248 genes using Takara Bio SmartChip qPCR (Takara Bio, Mountain View, CA). Sequence-unique oligos from each patient were also analyzed using IHC for three target markers and correlated with qPCR results for validation of the method.

Overall, molecular expression was observed in general expression trends between selected IHC and RNA expression. qPCR data was further analyzed using hierarchical clustering analysis and showed that morphologically defined ROIs clusters completely different than traditional clustering of entire tissue samples. Notably, patient clustering based on morphological regions is independent of the intrinsic cancer subtype, as determined by molecular profiling of whole tissue samples, as well as independent of trends in tumor Mutational Burden (TMB) and Microsatellite Instability (MSI). These findings suggest that current methods of patient stratification based on whole tumor molecular subtyping (normal breast tissue). This approach was compared to whole tissue sections from each patient block. Each ROI and whole tissue sample was analyzed by high throughput qPCR for a panel of 248 genes using Takara Bio SmartChip qPCR platform (Takara Bio USA). Sequence-unique oligos from each patient were also analyzed using IHC for three target markers and correlated with qPCR results for validation of the method.

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