QUANTITATIVE IMAGING AND DEEP PROFILING OF COLLAGEN STRUCTURE IN ASIAN TRIPLE NEGATIVE BREAST CANCER

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Background and Objectives

Stromal biology has been poorly studied compared to the cancer cell compartment and the immune cell population, until recent findings highlighted the indispensable influence of stromal reaction to the efficacy of treatment including immunotherapy. Collagen fibers are the major structural extracellular matrix component in breast and other tumours, and increased stromal collagen fibers have been found to facilitate breast tumour formation, invasion, and metastasis.

Materials & Methods

Stromal remodeling is featured by collagen realignment in the stromal compartment, collagen fibers and basal membrane, which can be identified, quantified and visualized by state-of-the-art stain-free pathology imaging technologies Second Harmonic Generation and Two Photon Emission (SHG-TPE). We profiled 388 triple-negative breast cancer (TNBC) patients with 68 SHG-TPE parameters and correlate with clinicopathological parameters.

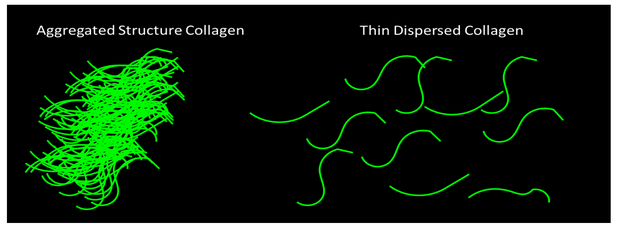

Fig. 1 The cartoon illustrated the two kinds of collagen fibres that can be visualized, segmented, differentiated and quantitated in the unstained tissue section – AGGREGATED collagen and Thin collagen.

Results

Among all the parameters, high Collagen Fiber Density (CFD) showed marginal association with better disease free survival and overall survival (DFS p=0.047; OS p= 0.044). Furthermore, we developed algorithms to further differentiate the collagen fibers into “aggregated collagen fibres” and “thin collagen fibres” based on the complexity and texture of the fibers. Interestingly only aggregated CFD is associated with better prognosis (DFS and OS, p=0.014; p= 0.033) but not thin CFD. On the other hand, we found that every incremental 1 percent of aggregated/thin collagen ratio was associated with better DFS (HR 0.97, 95% CI 0.96-1.00, p= 0.015). The addition of aggregated/thin collagen ratio to clinicopthological features significantly increased the prognostic value for DFS ($\Delta LR\chi^2$= 8.4, p=0.004), compared to clinicopathological features alone (histological grade, tumour size and lymph node status).

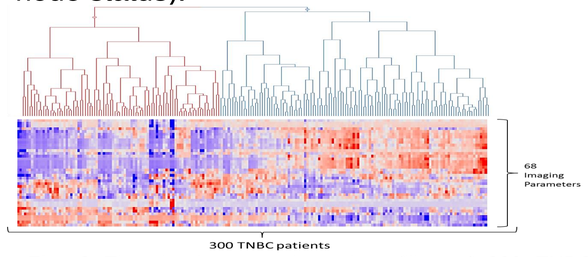

Fig. 2 The dendrogram showed profiling of 300 TNBC patients by using 68 SHG fundamental imaging parameters of AGGREGATED collagen demonstrated two unsupervised clusters which are highlighted in blue and red respectively. The heat map is colored by the quantitative imaging parameters with the highest numbers in red and the lowest numbers in blue.

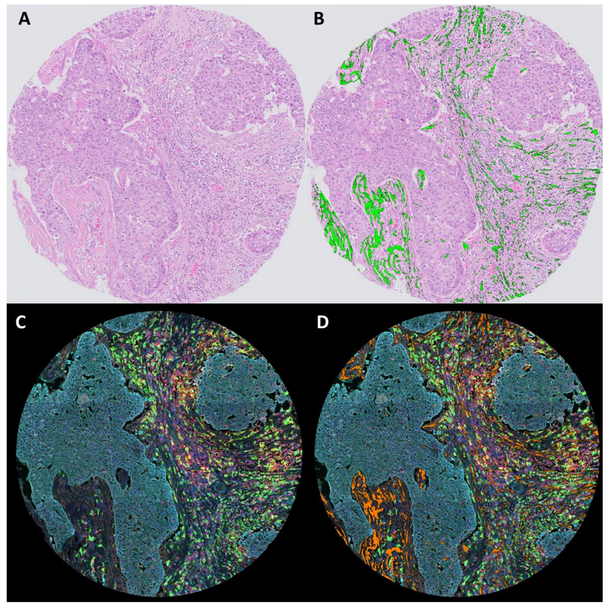

Fig. 3. A) Representative H&E image of a triple negative breast cancer. B) The merged image of the Second harmonic Generation pathology imaging system and the corresponding H&E image A, showed green colored collagen fibres surrounding the tumour nests. This might suggest this is a “immune excluded tumour” phenotype.
C) Representative Multiplex IHC image of a triple negative breast cancer. (PD-L1: Dark Green, PD-1: Light Green, CD68: Red, CD8: Pink, CK: Cyan, CD20: Brown) D) The merged image of the Second harmonic Generation pathology imaging system and the corresponding multiplex IHC image C, showed Orange colored collagen fibres surrounding the tumour nests. This might suggest this is a “immune excluded tumour” phenotype.

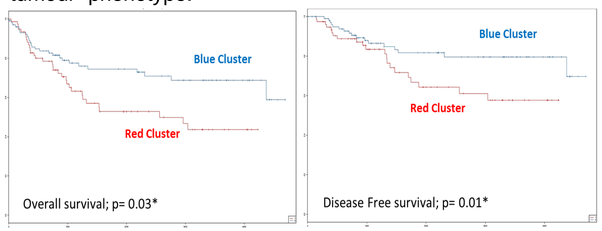

Fig. 4 Kaplan-Meir curve showing TNBC patients in Blue cluster (defined by AGGREGATED collagen as shown in Figure 3) had significantly favourable A) OS (*p*=0.03) and B) DFS (*p*=0.01).

Variable	DFS		OS	
	$\Delta LR\chi^2$	*P* value	$\Delta LR\chi^2$	*P* value
CP + AGGREGATED/Thin Collagen ratio vs. CP	8.40	0.004*	3.50	0.061

Table. 1. The likelihood ratio ($\Delta LR\chi 2$) of one of the representative SHG parameter (AGGREGATED/Thin Collagen ratio) to predict clinical outcome is significantly higher than clinicopathological parameters (CP).

Summary and Discussion

Our automated image analysis pipeline with SHG offers a novel platform to quantitate stromal collagens of the tumor microenvironment to better predict the clinical outcome of patients with triple negative breast cancer. With breast cancer as proof-of-concept for our findings, we plan to apply and validate in other cancers, potentially translating to clinical applications in the imminent future.

SHG is widely used to assist diagnosis of fibrosis disease. Our data showed potential application on cancer setting however validation with bigger cohort and other cancers are warranted. Further study might need to focus on prediction of treatment response such as immunotherapy.

Acknowledgement

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