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Breast Cancer: Characterization of Hormone Receptor Status Using Mass Spectrometry Imaging

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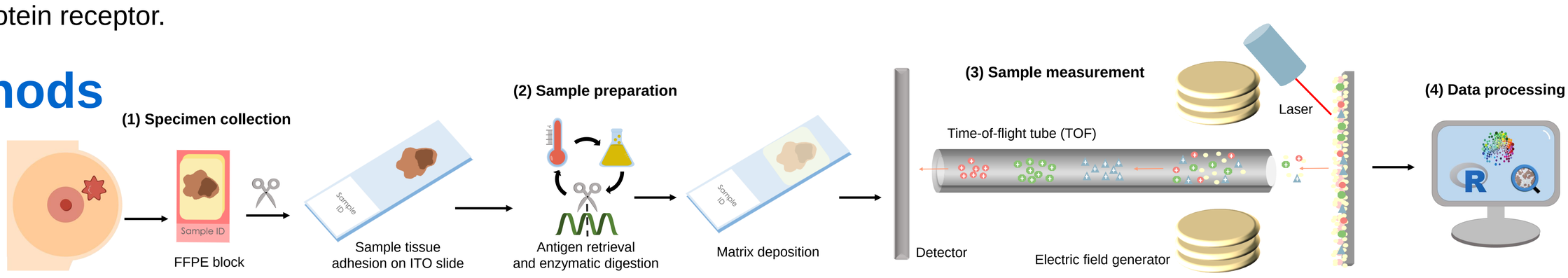
Aims

Breast cancer diagnosis and subsequent treatment choice revolve around the characterization of the status of three different receptor proteins: estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor 2 (HER2).
Triple-negative breast cancer (TNBC) is usually associated with poor outcomes as it presents lower response rates to traditional treatment approaches. In this study, we have evaluated the proteomic content of breast cancer samples to better understand the molecular signatures of each protein receptor.

Methods



Tissue microarrays comprising samples from breast cancer samples (n=1191) were subjected to on-tissue tryptic digestion and incubated in a humid environment (**Figure 1**) followed by matrix application (α-cyano-4-hydroxycinnamic acid) using an automated sprayer (TM sprayer, HTX Technologies). Samples were analyzed utilizing a Bruker RapifleX MALDI-TOF mass spectrometer with a pitch size of 50 μm^2.
Subsequently, the matrix was removed, sections were stained by hematoxylin and eosin, and scanned for meticulous histopathological annotation. The receptor statuses were also all re-evaluated by the same pathologist to avoid inter-personal bias. Data analysis was performed using SCiLS Lab (Bruker) and statistical analysis was performed on R.

Results

Table 1. Supervised classification outcome.

Classification Results (%)				
Model	**ER**	**PR**	**HER2**	**TNBC**
LDA	90.6	81.2	77.7	95.9
RF	95.5	92.2	93.4	97.9
KNN	97.3	95.2	95.2	98.7
SVM	95.6	90.8	88.8	98.8

Table 2. After ROC analysis, the features were identificatied by MS/MS and literature search.

m/z	Tentative ID	Sequence
1198.7	Actin *	AVFPSIVGRPR
805.4	COL4A3	ALEFVAR
771.4	UNC79	GPVESKR
771.4	COL1A2	GASGPAGVR
1428.7	Vimentin	SLYASSPGGVYATR
1495.7	Vimentin	TYSLGSALRPSTSR

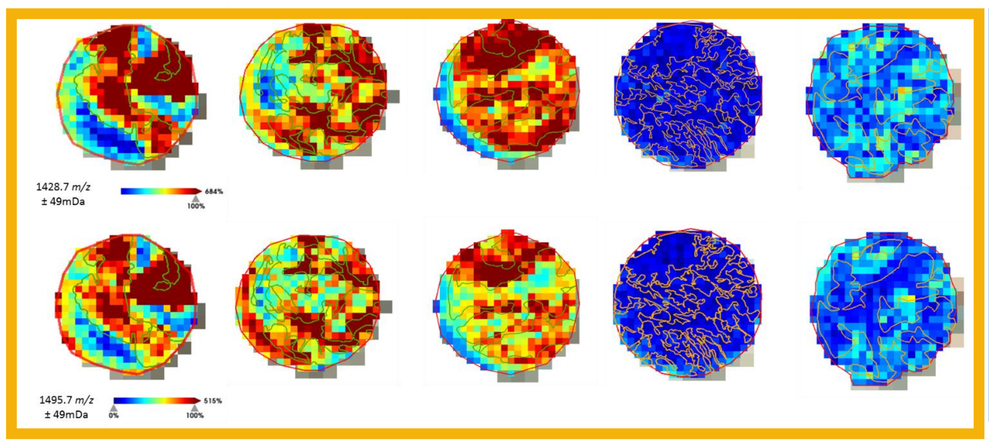


Figure 2. On-tissue distribution of molecular features obtained by ROC-AUC calculation. Histological annotation of the tumor regions (TNBC in green, non-TNBC in orange) could be correlated with the distribution intensity of the peptide fragments m/z 1428.7 and 1495.7.

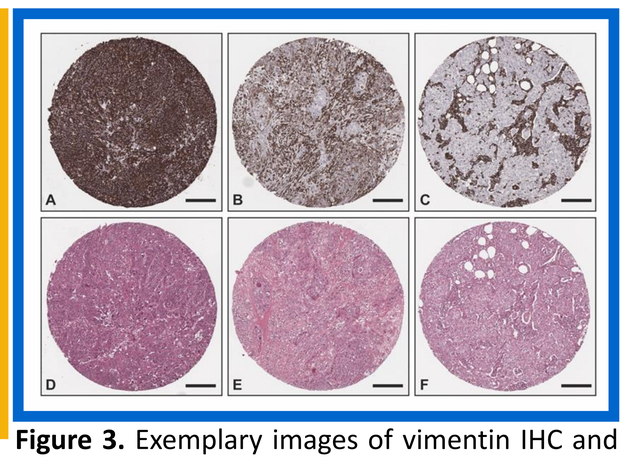


Figure 3. Exemplary images of vimentin IHC and corresponding H&E: (A,B,D,E) TNBC with positivity within the tumor and strong positivity within the tumor stroma; (C,F) non-TNBC with no vimentin staining within the tumor and strong positivity within the tumor stroma.

From the supervised classification outcome (**Table 1**) there is an indication that TNBC presents further diagnostic – and possibly treatment – targets when compared to the individual receptors. Upon analysis of the individual molecular features (**Table 2**) and the tissue histomorphology (**Figure 2**), we could conclude that Vimentin, and COL1A2 were overexpressed in TNBC.

While COL1A2 was also overexpressed in ER and PR, Vimentin was solely overexpressed within the tumor cells of TNBC, as could be validated by immunohistochemistry analysis (**Figure 3**).

Conclusions

In this study, we have demonstrated that MSI is an effective technique for subtyping breast cancer and a valuable tool for discovering new tissue markers. Vimentin was identified as a protein overexpressed in TNBC tumor cells, unlike their receptor positive counterparts. We anticipate that further investigations into the role of vimentin will reveal new pathways in the development of this disease, leading to the identification of novel treatment targets.

References:
1. Gonçalves et al., I*nt. J. Mol. Sci.* **2023**, *24*, 2860

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