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Communication between tumor cells and fibroblasts as a prognostic factor of NACT in TNBC

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Introduction

Neoadjuvant chemotherapy (NACT) with cytotoxic drugs is the standard of care in aggressive breast cancer, including the triple negative subtype (TNBC), however, patient outcome is variable. Response to therapy is orchestrated by an intricate interplay between cancer, stromal and immune cells collectively forming the tumor microenvironment.

We have recently uncovered a feedback mechanism of tumor cells and fibroblasts, involving IFNB1 signaling, that supports tumor cells in the recovery from chemotherapy-induced stress (references 1, 2).

Aim of the Study

Here, we wanted to assess whether targets of IFNB1 signaling in fibroblasts and tumor cells would qualify as predictive markers of pathological complete response (pCR) after NACT, and as prognostic markers for the course of the disease.

Patients

A prospective consecutively enrolled cohort (2000 - 2021) was available with an overall pCR rate of 46%. pCR was defined by no invasive cancer cell in breast or axilla (ypT0 N0). The median follow-up for EFS was 36.2 months (6-154) and for OS 39.3 months (6-214).

Table 1: Selected patients and tumors characteristics

	pCR n=16 (100%)	non-pCR n=14 (100%)
Age		
< 35 yrs	4 (25.0%)	2 (14.3%)
35 - 50 yrs	4 (25.0%)	6 (42.9%)
50 - 75 yrs	6 (37.5%)	6 (42.9%)
> 75 yrs	2 (12.5%)	0 (0.0%)
Tumor size at time of diagnosis (cT)		
< 2 cm	6 (37.5%)	5 (35.7%)
2 - 5 cm	9 (56.3%)	7 (50.0%)
≥ 2	1 (6.3%)	2 (14.3%)
Nodal status at time of diagnosis (cN, in part pN)		
Negative	10 (62.5%)	9 (64.3%)
Positive	6 (37.5%)	5 (35.7%)
Grading		
G2	1 (6.3%)	4 (28.6%)
G3	15 (93.8%)	10 (71.4%)

Methods

RNA-sequencing data from *in vitro* experiments found the GO-term GO:0051607 'defense response to virus' significantly enriched. Twentyfour genes intersected between differentially expressed genes and the genes of this GO-term. We selected three of the encoded proteins

a) interferon induced with helicase C domain, IFIH1
b) interferon alpha-inducible protein, ISG15
c) 2'-5'-oligoadenylate synthetase, OAS1

to test their expression in human specimens of TNBCs after NACT by immunohistochemistry (IHC) using the histo-score (H-score).

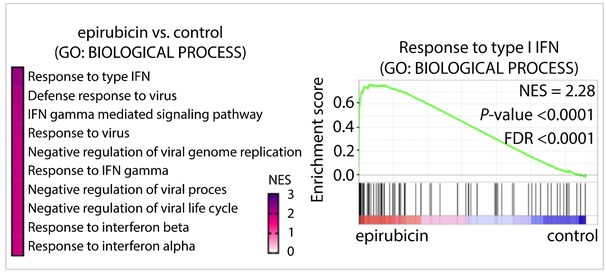

Figure 1: GSEA analysis (MSigDB v.4.0.3) of differentially expressed genes from RNA-sequencing of cancer associated fibroblasts, which had been in co-culture with breast cancer cells that had previously been treated with epirubicin or without (control). The top 10 significantly enriched GO terms are shown (left). Enrichment curve for the top biological process (Response to type I interferon) on the right. *P*-value was calculated by random permutation testing.

Results

IHC staining has been established for IFIH1, ISG15 as well as OAS1 and stainings of representativeFFPE tissue samples with pCR (n=16) and non-pCR (n=14) have been performed

- all three proteins were detected within cancer cells, stromal cells and tumor infiltrating lymphocytes
- all three markers show lower protein expression in pCR samples compared to non-pCR samples

Survival

- pCR predicted a better clinical outcome
- positive correlation with downregulated IFIH1 and OAS1
- no effect considering ISG15

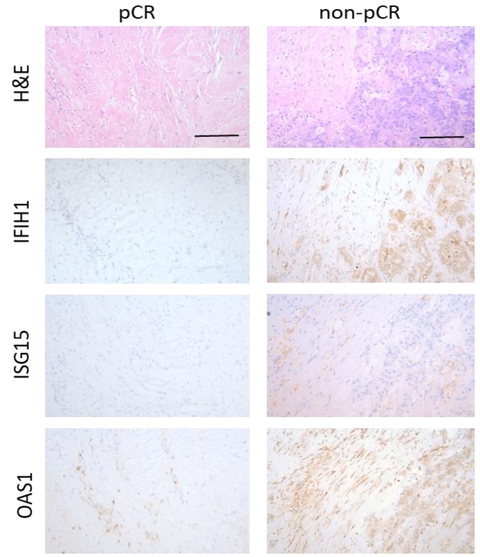

Figure 2: Exemplary histology and IHC stainings of BC samples with pCR and non-pCR respectively. All figures show a 200x magnification and the scale bars depict 100 µm. In non-pCR sample higher expression of IFIH1, ISG15 as well as OAS1 can be demonstrated. The stained cell populations encompass tumor cells, TILs and stroma cells. In pCR sample only low expression of OAS1 can be detected.

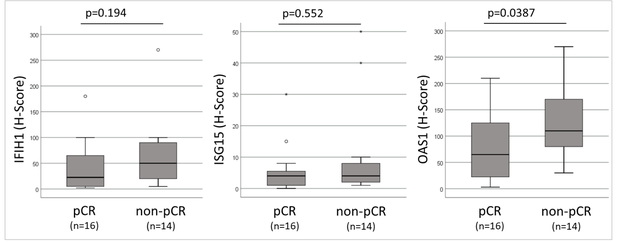

Figure 3: IFIH1, ISG15 and OAS1 expression analyzed by IHC. Boxplots represent the median and interquartile range of protein levels analyzed using the H-score. *p*-values were calculated using Mann-Whitney-U-test.

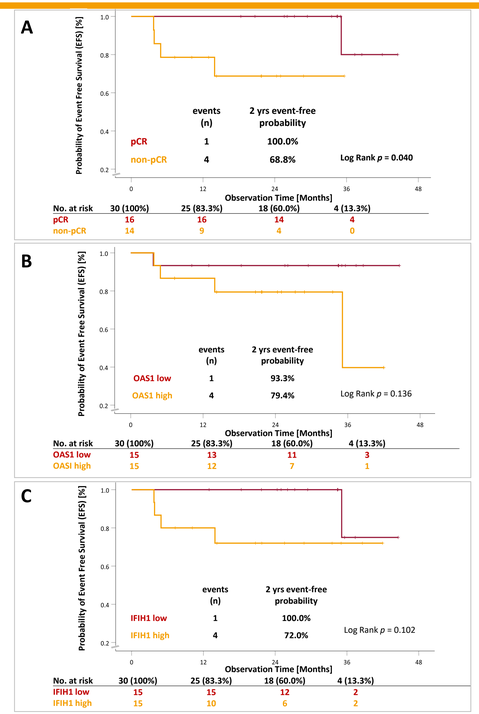

Figure 4: Survival estimates for event free survival (EFS) considering pCR and non-pCR (A), the differential expression of OAS1 (B) and IFIH1 (C)

Conclusion

The expression of markers of an IFNB1-triggered antiviral response have to be validated in the entire TNBC-cohort as an potential interferon-response predictor of survival.

Reference

(1) Maia et al. IFNbeta1 secreted by breast cancer cells undergoing chemotherapy reprograms stromal fibroblasts to support tumour growth after treatment. *Mol Oncol* **15**, 1308-1329 (2021)

(2) Berdiel-Acer et al. Stromal NRG1 in luminal breast cancer defines pro-fibrotic and migratory cancer-associated fibroblasts. *Oncogene* **40**, 2651–2666 (2021)

Contact

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