Background: Breast cancer is the leading cause of cancer-related mortality in women. Triple negative breast cancer (TNBC) is one of the most aggressive subtypes, which is often accompanied by metastasis, where requires development of new blood vessels (neovascularization) to disseminate tumor cells throughout the body. We recently reported that down-regulation of exchange factor directly activated by Cyclic AMP (cAMP) as known as EPAC1, leads to a reduction in proliferation and inhibition of cell migration. Methods: Here, using gene knockdown by siRNA and a 3-dimensional in-vitro TNBC co-culture model with Human Vascular Endothelial Cells (HUVECs), we studied microvascular density and the role of EPAC1. Angiogenesis probe profiling was employed to examine protein networks that were regulated by EPAC1 in the TNBC cell line, MDA-MB-231. Immunofluorescence along with Electric cell-substrate impedance sensing (ECIS) was used to study vascular permeability. To confirm these findings in a translational context, we employed a human tumor xenograft assay that predicts clinical response to therapy (CancerScan™) and studied CD34+ nodes to determine how neovascularization associates to drug response. Results: We determined that downregulation of EPAC1 in the TNBC cell line, MDA-MB-231, leads to reduction of proteins involved in migration, metastasis, angiogenesis and adhesion. Interestingly, we determined that loss of EPAC1 leads to increased expression of negative regulators in these same pathways. Immunofluorescence imaging showed that EPAC1 downregulation in MDA-MB-231 cells leads to diminished expression of proteins such as Pafkin, NFNA, MMP-9 and tubulin, indicated that EPAC1 role in vascular permeability. Finally, CancerScan™ demonstrated a link between response to therapy and microvascular density, which was reduced under drug pressure in patients that were predicted to respond to treatment. Conclusion: Our results suggest that EPAC1 is a driver of microvascular density in the tumor microenvironment, a feature that may play a key role in distant metastasis and therapy failure. Ex-vivo modeling of neovascularization may be a novel strategy to predict clinical response and distant metastasis.

CAMP Regulated EPAC1 Supports Microvascular Density, Angiogenic and Metastatic Properties in a Model of Triple Negative Breast Cancer

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Brain Metastasis and NeuroVascular Disease Modeling Lab

Fig. 1 A CancerScan™ is a multi-dimensional, live tissue imaging platform to study personalized response to anticancer therapy at the individual patient level. 1) The explorative tissue assay relay mediates the entire tumor ecosystem, including microenvironment components, stroma, and immune contexture. 2) Drugs are interrogated by quantitatively assessing phenotypic response to therapy via a multi-dimensional set of assays. 3) Phenotypic response to treatment siRNA was performed on ~2000 patients, who received the same treatment in clinic that was tested in the exploratory assay. The results from the exploratory assay were correlated to patient clinical response using a machine learning algorithm. M-Score was defined, which predicts positive vs. negative responses. 4) To date, more than 4000 patient samples have been evaluated by CancerScan™. A Subset of the clinical assay performance was published, showing 99% clinical correlation.

Fig. 3 Down regulation of EPAC1 reduces Angiogenesis. A) Tube formation assay was performed to check the effect of silencing EPAC1 in MDA-MB-231 cells on endothelial cells (HUVECs). B) Western blot analysis of EPAC1 shows that it is significantly upregulated in Western blot. C) Tube length and number of nodes were measured to confirm that effect, which indicates a significant inhibition in in vascular development [Fig 1C].

Fig. 2 Decreased MVD after treatment is associated with better predicted treatment outcome, ex vivo B) Patient meta data. C) Representative IHC are shown in the left panel, quantitated CD34+ nodes/field are shown in the right panel. N4, p<0.05 C) Schematic illustrates the steps taken to study neovascularization in the explant model. Briefly, IHC is performed to study CD34+ microvascular nodes, which are quantified per field in the vaccine control cohort and the drug treated cohort (dotscatter). D) Predicted responders (M-score ≥2) and predicted non-responders (M-score ≤2) were quantified for CD34+ per field, and represented as percentage between the vaccine control and drug-treatment. Lower panel compares the change of CD34+/field between responders and non-responders. p<5.

In-vitro Cell Culture Model – MDA-MB-231 Breast cancer cells

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A) Representative image of prostate array. Pre-conditioned cell culture media was obtained from MDA-MB-231 TNBC cells that were treated with siRNA scrambled control or siRNA targeting EPAC1 B) volcano plot shows the probes that are statistically significantly upregulated by a Log2 fold change of 0.1 or higher (blue bars). Red bars indicate the cut-off for statistical significance, p<0.05.

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1. Recruiting the tumor microenvironment
2. Multi-Dimensional Phenotypic Interpretation of Tumor
3. Trained and Validated with 2000 Matched Patients
4. Published Assay Performance
5. Clinical Outcome

Table 1 & 2

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