Lactate driven upregulation of BACH1 promotes metastatic dissemination in triple negative breast cancer

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SAN ANTONIO BREAST CANCER SYMPOSIUM

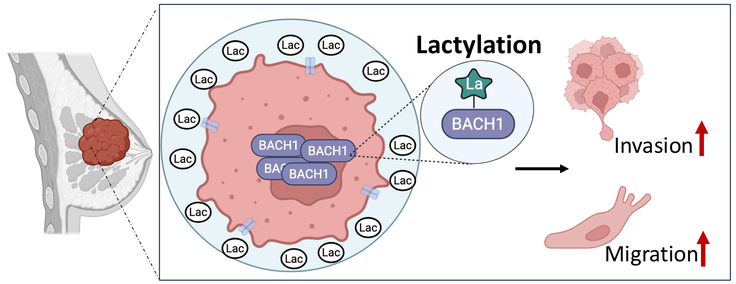
Introduction and Purpose of Study

Triple negative Breast cancer (TNBC) is the most aggressive subtype of breast cancer. Heme-regulating transcription factor, BACH1, is highly expressed in TNBC and promotes invasion and metastasis. BACH1 can be intrinsically regulated at the transcription, translation and post-translational levels. Limited studies focus on extrinsic factors, such as nutrients found in the tumor microenvironment, that could regulate BACH1. Previous studies have identified lactate accumulates in the tumor microenvironment including that of TNBC tumors. Lactate's immune suppressive effect drives metastasis and tumor invasion. Recent studies identified that protein lactylation, a post-translational modification, can be derived from lactate. However, limited studies have identified how lactate can affect metastasis regulators in TNBC. Identification of lactate mediated regulation of BACH1 would enable a novel way of targeting BACH1 stability thus reducing metastasis related progression in TNBC.

Hypothesis

Lactate promotes TNBC metastasis by lactylating and upregulating BACH1

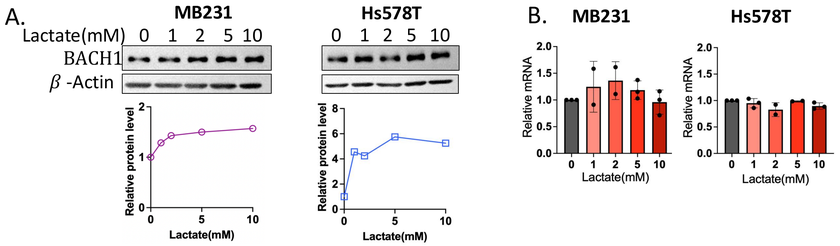
Working Model



Lactate accumulation in the breast tumor microenvironment leads to BACH1 lactylation, resulting in increased cellular invasion and migration.

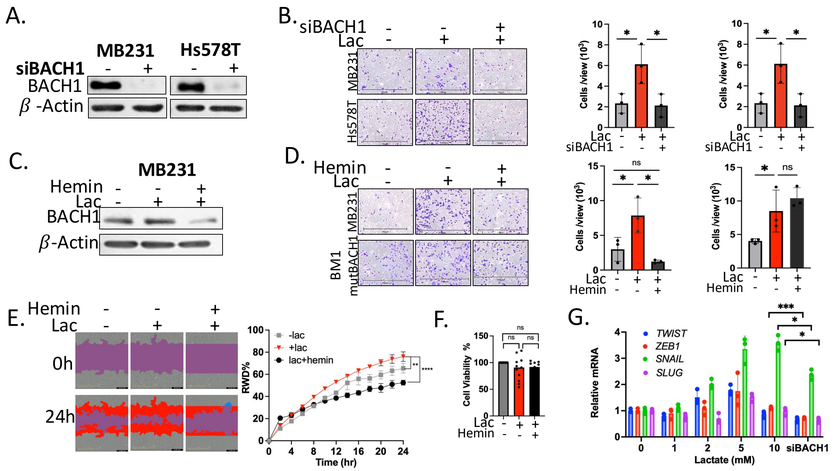
Results

Figure 1. Lactate increases BACH1 protein expression not mRNA expression



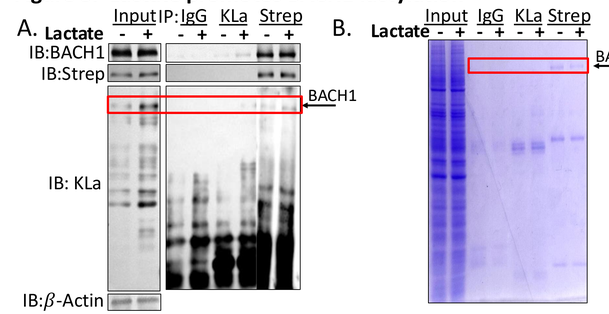
A)BACH1 protein expression **B**)mRNA expression following lactate treatment for 24 hours under 1mM glucose conditions. Error bars ±SEM.

Figure 2. Lactate promotes BACH1 mediated invasion and migration



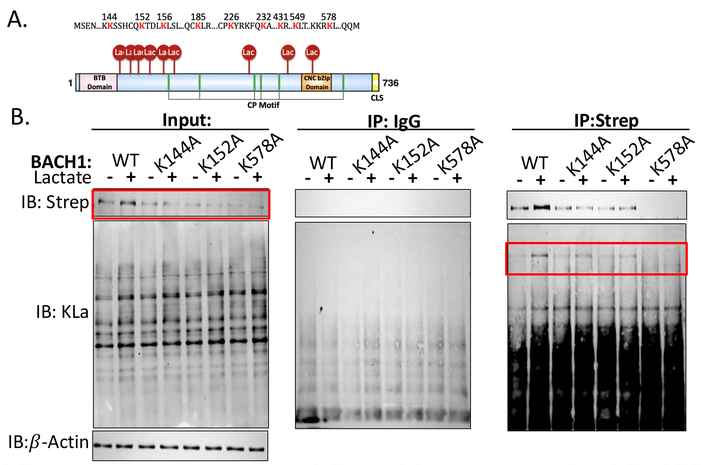
A),C)representative protein blot for cell lines used for invasion assay in (B) and (D). **B), D**) staining and quantification of invaded cells **E)** Scratch wound with quantified relative wound density(RWD). **F)** Calcein AM assay measuring cell viability. **G)** Relative mRNA level for EMT associated genes in control and knockdown(siBACH1 under 10mM lactate) Hs578T cells. Student's t-test p-value * p<0.05,**p<0.005,***p<0.0005. Error bars ±SEM.

Figure 3. Lactate promotes BACH1 lactylation



A) Immunoblotting of IP samples with BACH1, Strep-tag and Pan KLac antibodies. Arrows indicate BACH1 size
B)Coomassie blue staining of samples loaded in(A)

Figure 4. Targeting lactylated lysines on BACH1 reduces its lactylation



A) BACH1 lactylation sites with representative diagram of BACH1 motifs. **B**) Cell lysates(Input-left) of WT-strep BACH1 HEK293T cells and lysine(K)mutant-strep BACH1 were immunoprecipitated with control IgG(middle) or anti-Strep(right), followed by immunoblotting Strep and Pan- Lysine Lactylation(KLa), B-Actin immunoblotted on Input as loading control.

References

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