

Therapeutic targeting in HER2+ breast cancer to prevent and treat CNS disease: HFE.

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Treatments targeted toward HER2+ breast cancers are limited (1-4). Unbiased discovery approaches have the potential to illuminate subtype-specific vulnerabilities. We performed whole transcriptome differential gene expression analysis of primary tumors of the breast from patients with HER2+ breast cancer, as compared to primary tumors of the breast from patients with breast cancer of other PAM50 molecular subtypes: luminal A, luminal B, basal and normal-like, using published microarray data (5-7). We discovered differential expression of HFE in human HER2+ breast cancer. HFE mRNA was present in higher quantities in tumors of the breast from patients with HER2+ breast cancer as compared to primary tumors of the breast from patients with basal and luminal subtype breast cancers. HFE expression and function is likely informative in providing some level of molecular description of the HER2+ subtype; HFE is a candidate molecule in a targeted therapeutics approach in HER2+ breast cancer.

CRISPR-based reverse genetic screening of human HER2+ organoids or primary tumor cells freshly isolated from patients with HER2+ breast cancer with limited exposure to artificial culture conditions have not yet been described (8, 9). These genetic technologies, though not yet implemented, when done so will likely facilitate discovery of therapeutic vulnerabilities in HER2+ disease with rapidity and ease. Management of major public health problems requires research (bench)-based management in the short run and long run (10). One example of such a major problem is CNS metastasis in HER2+ breast cancer (11-14): this is a type of cancer that affects a human sex with relatively high frequency, a subtype of that cancer that is diagnosed in approximately one quarter of patients diagnosed with that cancer, and a specific complication of the subtype that develops in half of affected patients.

We utilize genomic and transcriptomic technologies to study the genomic sequences (DNA), the transcriptome (RNA), and epigenetic modification (eg., CpG-DNA) of humans with breast cancer. This includes the primary tumor, the source of the transformation - like mutant variants of p53 - subtypes of the primary tumor, including luminal, basal and HER2+ forms, "regional" metastasis to the lymph nodes, metastasis to distant sites, including the lungs, the liver and the brain, and the circulating tumor stem cell. Our hypothesis and working strategy dictates that in the short-run, disease complications and therapeutic limitations are best managed using identification of therapeutic targets by whole transcriptome differential expression analyses (subtraction analysis), and in the long-run, will be enhanced and fully complemented by reverse genetic screening strategies to blindly identify disease-specific, subtype-specific and metastasis-specific therapeutic vulnerabilities augmented by novel immunotherapy approaches. Here we describe one such target identified through rigorous study of the HER2+ tumor transcriptome: a gene product up-regulated, differentially expressed and subtype-specific: HFE.

Results

Figure 1: HFE is differentially expressed in HER2+ breast cancer.

I. Primary tumors of the breast from humans with breast cancer: HER2+ subtype

n=39 primary tumors (breast; human; HER2+)

n=422 primary tumors (breast; human; non-HER2+ subtype, including basal and luminal A/B)

ID	<i>p</i> -value	t	B	logFC	Gene	Rank	%DE
206086_x_at	0.00028876	3.6531492	0.1299	0.21998104	HFE	34/22283	99.8

Through quantitative comparison of total transcription in the primary tumors of humans with HER2+ breast cancer, we discovered differential expression of hemochromatosis, encoded by *HFE* in HER2+ breast cancer in humans (**Chart 1**). The expression of HFE changed more than 99% of the human breast tumor transcriptome when considering all transcripts whose expression was measured - in this case, 22,283 transcripts ("Rank"). Note the positive fold-change indicating increased quantity of HFE messenger RNA in HER2+ subtype tumors, demonstrating up-regulation of HFE during transformation-specific lineage specification of the breast to the HER2+ breast cancer subtype.

II. Primary tumors of the breast from humans with breast cancer: HER2+ subtype

n=10 primary tumors (breast; human; non-HER2+ subtype: basal, luminal A/B and normal)

n=85 primary tumors (breast; human; HER2+)

ID	<i>p</i> -value	t	B	logFC	Gene	Rank	%DE
A_23_P342009	4.92E-01	0.6894142	-5.689887	0.13770349	HFE	17964/31157	42.3

1 In a second microarray dataset (6) from independent investigators and a separate patient cohort, we
2 failed to validate differential expression of HFE in HER2+ breast cancer in humans (**Chart 2**).

3 Nevertheless, we concluded that differential and increased expression of HFE likely defined the
4 transcriptional landscape of the HER2+ breast cancer subtype in humans.

5 **Discussion**

6 Adjunctive treatments in medical oncology limit the emergence of resistant tumor clones during
7 treatment with a second treatment (whether neoadjuvant chemotherapy or a targeted therapy like
8 trastuzumab). Immunoglobulin-based inhibitors of HFE, once evaluated for toxicity and safety, can
9 immediately be tested for efficacy in patients with HER2+ metastasis, with the goal of identifying the most
10 effective reagents for management of HER2+ disease in humans and utilizing these adjunctive agents
11 early in disease to prevent rather than treat CNS disease. We recently used primary tumor transcriptome
12 data to identify multiple phosphatases that we deemed candidate therapeutic targets in management of
13 breast cancer, and fortuitously found their decreased expression was specifically linked to superior overall
14 survival in patients with HER2+ breast cancer (15, 16). A multi-kinase approach delivered in conjunction
15 with chemotherapies that target dNTP synthesis, replication of the daughter strand and activity at the
16 spindle at anaphase, in conjunction with activators of the cyclin dependent kinase inhibitors *CDKN* and
17 inhibitors of multi-drug resistance ATP-binding cassette pump genes in resistant cases, is most likely to be
18 most effective in limiting tumor clone resistance (17).
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Methods

We utilized GSE87049, GSE158309 and GSE74667 for this tumor transcriptome study, measuring whole transcription in HER2+ primary tumors from humans with breast cancer, as compared to primary tumors of non-HER2+ subtype using microarray data (published) and R-based computational methods (GEO2R).