

## **Therapeutic targeting in breast cancer for the treatment of lymph node metastasis: PHACTR1.**

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Metastasis is the major cause of death from cancer (1); metastasis to the lymph nodes in breast cancer is dangerous, relatively common, and in one study ranged from 10-60% based on molecular subtype (2-4). We predict therapeutic index is intrinsically linked to differential expression which can be comprehensively and blindly determined using whole transcriptome data (5). We pioneered understanding of metastasis to the lymph nodes as an intermediate biological and anatomical counterpart to the CNS metastasis in humans (6-12). Here we utilize whole transcriptome technologies (13, 14) to measure total transcription in the lymph node metastases and primary tumors of humans with breast cancer for discovery and insight into clonal evolution through disease progression. We identify here a therapeutic target based on its differential expression and up-regulation upon metastasis to the lymph nodes in humans with breast cancer, PHACTR1, as a candidate therapeutic target for the medical management of lymph node metastasis.

We utilize genomic and transcriptomic technologies to study the genomic sequences (DNA), the transcriptome (RNA), and epigenetic modification (eg., CpG-DNA) of humans with 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 in breast cancer, and adeno and squamous forms of NSCLC in lung cancer, "regional" metastasis to the lymph nodes, metastasis to distant sites, including the lungs, the liver and the brain, and the circulating tumor stem cell. Here we measure total transcription in metastasis to the lymph nodes to discover and describe a therapeutic target identified through rigorous study of the lymph node metastatic transcriptome in breast cancer: a therapeutic target that is up-regulated and metastasis-specific, providing ideal therapeutic index to minimize toxicity and maximize efficacy: PHACTR1.

## Results

**Figure 1:** PHACTR1 is differentially expressed in lymph node metastasis in humans with breast cancer.

### I. Lymph node metastases and primary tumors; human breast cancer.

$n=18$  primary tumors from humans with breast cancer

$n=16$  lymph node metastases (tumor) from humans with breast cancer

ID	p-value	t	B	logFC	Gene	Rank	%DE
213638_at	1.56E-01	-1.4519835	-5.12807	-0.15859269	PHACTR1	3401/22277	84.7

Through quantitative comparison of total transcription in the primary tumors of the breast and in lymph node metastases of humans with breast cancer (13), we discovered differential expression of phosphatase and actin regulator 1, encoded by *PHACTR1* in metastasis to the lymph nodes in humans with breast cancer (**Chart 1**). The expression of PHACTR1 changed more than nearly 85% of the human lymph node metastatic transcriptome when considering all transcripts whose expression was measured - in this case, 22,277 transcripts ("Rank"). Note the negative fold-change indicating increased quantity of PHACTR1 messenger RNA in lymph node metastases, demonstrating up-regulation of PHACTR1 during disease progression and dissemination in breast cancer.

### II. Lymph node metastases and primary tumors; human breast cancer.

$n=36$  primary tumors from humans with breast cancer

$n=36$  lymph node metastases (tumor) from humans with breast cancer

ID	p-value	t	B	logFC	Gene	Rank	%DE
213638_at	7.20E-03	-2.765582	-2.54958	-0.5390672	PHACTR1	562/22277	97.5

Through measurement of total transcription in the lymph node metastases of humans with breast cancer as compared to primary tumors of the breast, we validated differential expression of PHACTR1 in metastasis to the lymph node in breast cancer (**Chart 2**) (14). The expression of PHACTR1 here changed more than 97% of the lymph node metastatic transcriptome when considering all transcripts whose expression was measured - in this case, 22,277 transcripts ("Rank"). Note the negative fold-change indicating increased quantity of PHACTR1 messenger RNA in lymph node metastases, demonstrating up-regulation of PHACTR1 during disease progression and dissemination in humans with breast cancer.

Thus, differential and increased expression of PHACTR1 defines the lymph node metastatic transcriptome in human breast cancer.

## Discussion

Adjunctive treatments in medical oncology limit the emergence of resistant tumor clones during treatment with a second agent (whether neoadjuvantive chemotherapy or a targeted therapy like trastuzumab). Inhibitors of PHACTR1, immunoglobulin or small molecule based (once evaluated for toxicity and safety) can immediately be tested for efficacy in patients with lymph node metastasis who have failed previous treatment, with the ultimate goal of identifying the most effective inhibitors of lymph node metastasis in humans with breast cancer who have not yet progressed but are at predicted high risk, or those whose metastasis has not yet become unmanageable due to metastasis size, number or location. A multi-kinase approach delivered in conjunction with chemotherapies that target dNTP synthesis, replication of the daughter strand and activity at the spindle at anaphase, targeting *CDKN* inactivation and ATP-binding cassette pump expression in resistant cases, is most likely to be most effective in limiting tumor clone resistance (15).

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## Methods

We utilized GSE44408 (13) for this tumor transcriptome study, measuring whole transcription in metastasis to the lymph nodes and in primary tumors from humans with breast cancer (along with GSE57968 [14] for target validation) using microarray data (published) and R-based computational methods.