

Therapeutic targeting in breast cancer for the treatment of lymph node metastasis: GUK1.

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Metastasis is the major cause of death from cancer. Metastasis to the lymph node ("regional" metastasis) generally precedes distant metastasis, and we recently demonstrated that metastasis to the lymph nodes is a likely prerequisite for metastasis to the central nervous system (CNS) in humans with breast cancer (1-8). Here we utilize whole transcriptome technologies (9, 10) to measure total transcription in the primary tumor and in the lymph node metastases of humans with breast cancer to define the lymph node metastatic transcriptome. 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, GUK1, 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: GUK1.

Results

Figure 1: GUK1 is differentially expressed in lymph node metastasis in humans with breast cancer.

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

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

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

ID	p-value	t	B	logFC	Gene	Rank	%DE
213621_s_at	3.34E-02	-2.168081	-3.83813	-0.1230692	GUK1	1488/22277	93.3

Through quantitative comparison of total transcription in the primary tumors of the breast and in lymph node metastases of humans with breast cancer (9), we discovered differential expression of guanylate kinase 1, encoded by *GUK1* in metastasis to the lymph nodes in humans with breast cancer (**Chart 1**). The expression of GUK1 changed more than 90% 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 GUK1 messenger RNA in lymph node metastases, demonstrating up-regulation of GUK1 during disease progression and dissemination in breast cancer.

Thus, we concluded that differential and increased expression of GUK1 likely defined 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 neoadjuvant chemotherapy or a targeted therapy like trastuzumab). Inhibitors of GUK1, 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 (11).

A description of basic chemotherapy approaches supported by our discovery research follows.

1. **Intensification of standard chemotherapy with MDR pump inhibition** at baseline and at resistance
 - a. The pump is the only entity considered at resistance for compensatory clinical inhibition.
2. **Multi-drug** approach targeting DNA replication and cell division: **EZH2, TK1, TYMS, AURKA, and TOP2A**
 - a. Adjunctive option 1: Targeted kinase adjunctive
 - b. Adjunctive option 2: Targeted phosphatase adjunctive
3. **A general solid tumor dual inhibitor** strategy: PARP1/2 inhibition together with CDK4/6 inhibition
4. Genomic medicine-based (tailored) **combinatorial phosphatase inhibition**.
 - a. The number of phosphatases targeted in this approach will depend on tolerability and deliverability of successful targeting combinations.
5. Genomic medicine-based (tailored) **combinatorial kinase inhibition**.
 - a. The number of kinases targeted in this approach will depend on tolerability and deliverability of successful targeting combinations
6. **A complete growth factor inhibition** strategy.
 - a. Targeting angiogenesis through VEGF-A lies at the basis of this fifth chemotherapeutic strategy.
 - b. The approach relies on **accurate and complete description of growth factors induced at the 80-99% range** of the transcriptome in order to completely restrict growth factor signaling
 - c. **A reduced cytotoxic regimen** is ideally administered with the growth factor inhibitor cocktail to induce cell death whilst activating starvation responses
 - d. **Kinase targeting for compensatory inhibition**.
7. **Immunoglobulin-based therapeutic targeting: drug discovery approach**.
 - a. This approach is agnostic to considerations or concepts that prevail in modern medical oncology like cytotoxicity associated with standard chemotherapies like nitrogen mustard based agents, as well as to considerations common when targeting general properties of the cancer cell, like those considered by the multi-drug approach which aims to block DNA replication and cell division
 - b. Instead, it utilizes intensive study of tumor transcriptomes for identification of disease-specific therapeutic vulnerabilities for clinical intervention using targeted, designed monoclonal antibody reagents.
 - c. Targets identified here, rather than being assembled into an immunoglobulin-based therapeutic strategy, can be used as an individual agent as adjunctive treatments in other chemotherapeutic approaches.
8. **Treatments that target the immune system** for control of disease (cancer).
 - a. Class I and II antigen presentation by the tumor through **UBE2L6** modulation
 - b. Natural killer cell tumor surveillance through **NKG7 and HLA-V together with KIR2DL3**
 - i. Gamma delta T cells may function with NK cells in this pathway.
 - c. **Targeting exhaustion** ("reinvigoration") at the tumor microenvironment using pharmacologic inhibition of **CTLA4 AND PD-1**, like involving cytotoxic immune responses by CD8 T lymphocytes
 - i. Novel immunoreceptor targeting TIGIT, LAG-3, VISTA and other surface receptors

1 9. **Rational responses to resistance.**

- 2 a. **CDKN re-activation** through CDK4/6 inhibitor escalation
3 b. CDKN re-activation through **UBE2C and UBE2S** modulation

4 10. Targeting **centers of activity** in the cancer cell.

- 5 a. **Kinetochores**
6 i. SPC24/SPC25, Nuf2, NDC80
7 ii. Other components of the kinetochore plate
8 b. **Cell membrane** during cytokinesis
9 i. PKC isoforms; ECT2.
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Methods

We utilized GSE57968 (9) 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 GSE44408 [10] for target validation) using RNA-sequencing and microarray data (published) and R-based computational methods. When data for GSE44408 is not present, it failed to satisfy validation, and was consulted but omitted.