Kinase and phosphatase inhibition as a signal transduction-centric approach to adjunctive chemotherapies for the treatment of gynecologic cancers: CDK1.

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Men and women differ in large part resulting from the presence or absence of gynecologic organs including the ovaries, the uterus, the lining of which is known as the endometrium, and the entry to the uterus (womb), the cervix (1), all of which are targets for development of malignancies (2-7); vulvar cancer is less common (8). Cancer, or unrestricted cell division which develops into a tumor after remaining unchecked, evades active cell death and cell cycle control mechanisms, sustaining its survival through growth factor signals (9) which are transduced to the nucleus to activate and repress transcription of genes (10, 11). These signals are transduced between the plasma membrane and the nucleus by the action of kinases and phosphatases, enzymes that utilize conformational changes and a catalytic site to translate a signal from the extracellular environment to a change in gene expression. Catalytic sites are pharmacologically targetable, and small molecule targeting of the appropriate kinase or phosphatase target will disrupt or block this transduction (12, 13). We utilized whole transcriptome technologies (14, 15) to identify and validate kinase and phosphatase targets in gynecologic oncology, including cancer of the lining of the uterus, or endometrial cancer. Here we describe a differentially expressed, up-regulated, and catalytically available kinase target in endometrial cancer: CDK1.

Keywords: kinase, phosphatase, signal transduction, gynecologic oncology, therapeutic targets in cancer, chemical biology, systems biology of human cancer.

We utilize genomic and transcriptomic technologies to study the genomic sequences (DNA), the transcriptome (RNA), the proteome (total collection of proteins in the cell) and epigenetic modification (eg., CpG-DNA) of human cancer (16-20). This includes the primary tumor, the source of the transformation - like mutant variants of p53 - cancer subtypes, like adeno and squamous forms of non-small cell lung cancer, metastasis to distant sites, including the lymph nodes, the lungs, the liver, the brain, and bones, and the circulating tumor stem cell, which emerges from the primary tumor, disseminates through the body and establishes a novel entity in a separate organ (21-26).

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), administered in combination with FDA approved broad-spectrum chemotherapeutics like CDK4/6 inhibitors, and in the long-run, will be enhanced and fully complemented by reverse genetic screening strategies (27, 28) to blindly identify disease-specific, subtype-specific and metastasis-specific therapeutic vulnerabilities, fully augmented by novel immunotherapy approaches. Here we describe a kinase therapeutic target identified through rigorous study of the endometrial tumor transcriptome: a kinase target that is up-regulated and catalytically available: CDK1.

Results

Figure 1: CDK1 is differentially expressed in endometrial cancer.

I. Primary tumors of the endometrium from humans with endometrial cancer.

n=5 normal endometrial tissue
n=7 primary tumors (endometrium; human)

ID	*p*-value	t	B	logFC	Gene	Rank	%DE
203214_x_at	8.71E-05	-5.7487845	1.77226	-2.17478003	CDK1	96/22277	99.6

Through quantitative comparison of total transcription in the normal endometrial tissue and in primary tumors of humans with endometrial cancer (14), we discovered differential expression of cyclin dependent kinase 1, encoded by *CDK1* in endometrial cancer in humans (**Chart 1**). The expression of CDK1 changed more than 99% of the human endometrial tumor 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 CDK1 messenger RNA in endometrial tumors, demonstrating up-regulation of CDK1 during transformation of the endometrium.

II. Primary tumors of the endometrium from humans with endometrial cancer.

n=2 normal endometrial tissue
n=2 primary tumors (endometrium; human)

ID	*p*-value	t	B	logFC	Gene	Rank	%DE
203214_x_at	0.094248	-2.1106203	-4	-1.301521	CDK1	6380/54674	88.3

Through quantitative comparison of total transcription in the primary tumors of humans with endometrial cancer relative to normal endometrial tissue, in a second microarray dataset (15) from independent investigators, we validated differential expression of CDK1 in endometrial cancer in humans (**Chart 2**). The expression of CDK1 here changed more than nearly 90% of the human endometrial tumor transcriptome when considering all transcripts whose expression was measured - in this case, 54,674 transcripts ("Rank"). Note the negative fold-change indicating increased quantity of CDK1 messenger RNA in endometrial tumors, demonstrating up-regulation of CDK1 during transformation of the endometrium.

Thus, differential and increased expression of CDK1 defines the transcriptional landscape of endometrial cancer in humans.

Discussion

Adjunctive treatments in medical oncology limit the emergence of resistant tumor clones during treatment with a second agent (whether neoadjunctive chemotherapy or a targeted therapy). Small molecule inhibitors of CDK1 kinase, once evaluated for toxicity and safety, can immediately be tested for efficacy in patients with endometrial cancer, with the goal of identifying the most effective kinase inhibitors for management of gynecologic malignancies. An approach that combines kinase and phosphatase targeting with a recently described multi-catalytic strategy that targets dNTP synthesis, replication of the daughter strand and activity at the spindle at anaphase, likely delivered in conjunction with standard chemotherapies and drug resistance pump inhibitors in resistant cases, is most likely to be most effective in limiting tumor clone resistance and disease (29).

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Methods

We utilized GSE63678 for this tumor transcriptome study, measuring whole transcription in primary tumors from humans with endometrial cancer, as compared to normal endometrium (along with GSE39099 for target validation) using microarray data (published or public) and R-based computational methods (GEO2R).