Whole genome sequencing and quantitative proteomics reveal HPV integration and HER2 overexpression in a patient with cervical cancer: Comprehensive omics analysis driving clinical treatment decisions

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Abstract

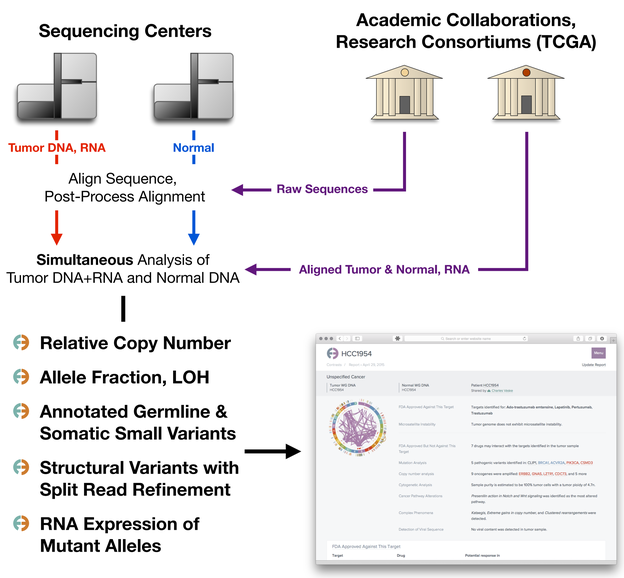
Introduction: Selection of drugs to treat patients with cancer is typically based on the anatomical site in which the tumor is located. Here we report a treatment decision for a patient with relapsed, advanced cervical cancer that was based on a comprehensive omics analysis using whole genome sequencing (WGS) combined with quantitative proteomics.

Methods: The patient was a 44-year-old female whose disease had progressed following surgery and more than 4 lines of chemotherapy. WGS was performed on the patient's formalin-fixed, paraffin-embedded (FFPE) metastatic tumor sample and a matched-normal reference sample. Quantitative proteomics was performed on the FFPE tumor sample by Selected Reaction Monitoring Mass Spectrometry and was quantitated at the atomolar level.

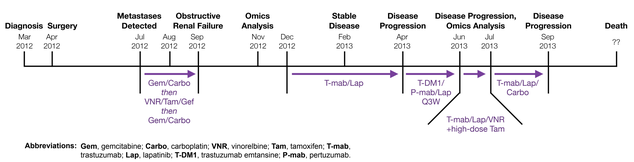
Results. WGS found somatic mutations and rearrangements and reads mapping to human papillomavirus type 18 (HPV 18). Mutations more commonly found in breast cancer (*ERBB2*, *CDH1*, and *CLTCL1*) were noted. The HPV 18 genome was integrated into chromosome 17 in close proximity to a 7-fold amplification of the *ERBB2* gene. Proteomic analysis of the FFPE tumor validated and quantitated overexpression of HER2 protein resulting from *ERBB2* gene amplification, with 11,322 amol/µg of tissue protein. Clinically observed ranges for breast or gastric cancer are 150-500 amol/µg, with levels above 750 amol/µg correlating with FISH-positive amplification and clinical efficacy of trastuzumab (unpublished observation). Based on these comprehensive omic findings, trastuzumab, a therapy approved for breast and gastric cancer, was administered. The patient experienced a reduction in the size of her tumor (by CT/PET) and stabilization of her disease for 5 months.

Conclusion. WGS and proteomic profiling of this patient's disease identified, confirmed, and quantitated an appropriate target for pharmaceutical intervention. The patient presented with cervical cancer; however, the WGS analysis pointed towards a potentially causative integration of the HPV 18 genome resulting in *ERBB2* amplification along with genomic mutations more commonly found in breast cancer. Proteomic analysis further validated and quantitated the HER2 expression resulting from *ERBB2* gene amplification, leading to the patient's treatment with trastuzumab. Our findings argue for the use of comprehensive omics analysis to guide decision support for personalized management of cancer care with therapies determined based on a quantitative proteomic signature, independent of anatomical tumor type.

Sequencing Analysis Pipeline



Case Details



Abbreviations: **Gem**, gemcitabine; **Carbo**, carboplatin; **VNR**, vinorelbine; **Tam**, tamoxifen; **T-mab**, trastuzumab; **Lap**, lapatinib; **T-DM1**, trastuzumab emtansine; **P-mab**, pertuzumab.

- 44-year-old woman with poorly differentiated cervical adenocarcinoma
- Surgery (Sep 2011): radical hysterectomy, including bilateral salpingectomy and lymphadenectomy, with preservation of the ovaries
  - Wall invasion to outer third of cervix; horizontal spread, 2.7 cm
  - No lymphatic, vascular, or parametrial invasion; lymph nodes, negative
- CT/PET, biopsy (Jul 2012): multiple pelvic masses detected (SUV_{max}, 43.9)
  - Received carboplatin/gemcitabine followed by vinorelbine/tamoxifen/gefitinib (4 doses QW) followed by carboplatin/gemcitabine (1 dose)
- Obstructive renal failure (Sep 2012): treated with ureteral stents
- Biopsy of metastatic tumor (Nov 2012): omics analysis
  - Detection of HER2 amplification/overexpression
  - Received trastuzumab/lapatinib
- Disease stabilization for 14 months
- CT/PET (Apr 2013): disease progression with pleural effusions and severe hydronephrosis
  - Placement of nephrostomy tubes
  - Received adotrastuzumab emtansine/pertuzumab/lapatinib Q3W
- CT/PET, biopsy (Jun 2013): disease progression, minimal genetic changes in tumor
  - Received trastuzumab/lapatinib/vinorelbine and high-dose tamoxifen (4 weeks) followed by carboplatin/trastuzumab/ lapatinib
- CT/PET (Sep 2013): disease progression

Methods

Informed Consent
- The patient provided written informed consent to perform genomic and proteomic analysis

Whole Genome Sequencing
- Formalin-fixed, paraffin-embedded tumor samples and a matched-normal reference sample
- WGS was performed by Illumina (San Diego, CA)
- ~2.5 billion paired sequencing reads resulted in sequencing depths of 45.85x and 30.69x for the tumor and normal samples, respectively
- Burrows-Wheeler Alignment Tool was used to align reads to the modified human reference genome, HG19 (University of California, Santa Cruz, CA)
- Copy-number estimates, somatic variants, and rearrangements were determined as described previously[1]

Quantitative Proteomics
- Proteomic analysis was done using Selected Reaction Monitoring Mass Spectrometry (SRM/MS) as described previously[2]

Gene Panel Analysis
- Cancer gene panel sequencing was performed by Foundation Medicine Inc. (Cambridge, MA)

Fluorescence In situ Hybridization
- Performed by Caris Life Sciences (Irving, TX)

Xenograft experiments
- In immunodeficient mice were performed by Anti-Cancer Inc. (San Diego, CA)

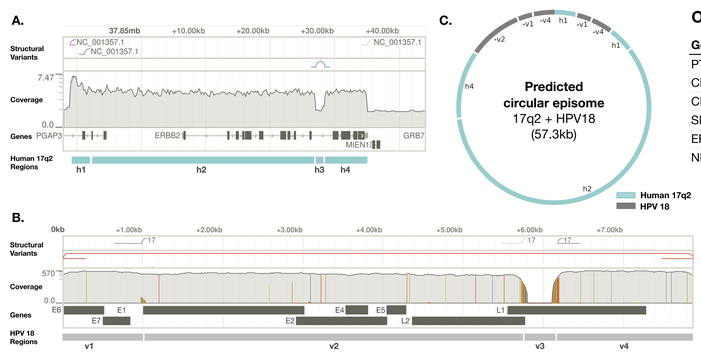
References

Sanborn JZ, Salama SR, Grifford M, et al. Double minute chromosomes in glioblastoma multiforme are revealed by precise reconstruction of oncogenic amplicons. *Can Res*. 2013;73:6036-6045.

Hembrough T, Thyparambil S, Liao WL, et al. Selected Reaction monitoring (SRM) analysis of epidermal growth factor receptor (EGFR) in formalin fixed tumor tissue. *Clin Proteomics*. 2012;9:5.

Castiglioni F, Tagliabue E, Campiglio M, Pupa SM, Balsari A, Menard S. Role of exon-16-deleted HER2 in breast carcinomas. *Endocr Relat Cancer*. 2006;13:221-232.

Results

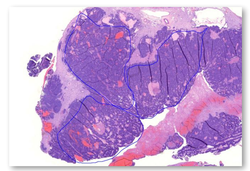


Other relevant alterations identified:

Gene	Alteration	Information
PTPRK	p.R1389*	Tumor Suppressor
CDH1	p.N531S	Tumor Suppressor
CLTCL1	Loss	
SMARCB1	Loss	Tumor Suppressor
EP300	Loss	Tumor Suppressor
NF2	Loss	Tumor Suppressor

- *HER2* was amplified and flanked by structural variants that map directly to the HPV 18 sequence
  - Amplification appears to have resulted in ~18 copies with a minority allele count of 1, implying that the amplification event involved only one *HER2* allele
  - Equal number of copies of *HER2* and *HPV 18*
  - Major capsid gene, L1, is missing
- Configuration of structural variants suggest that a circular episome was formed from regions on 17q2 (including the full coding sequence of HER2) and the HPV 18 viral genome
- Exon 16 (believed to encode a transforming form of the HER2 oncogene[3]) was deleted in 7% of reads (16 of 238)
  - Depth of coverage indicates a late event, occurring after the region was amplified

Outlined areas indicate regions of microdissections

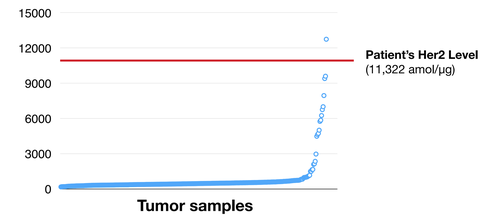


Top 10 protein levels measured in patient.

Gene	Assay Range	Test Result (amol/µg)
KRT7	250 — 100,000	47,333
HSP90A	16,000 — 42,000	23,918
HSP90B	16,000 — 67,000	23,783
Vimentin	7,000 — 100,000	16,422
Her2	150 — 26,000	11,322
E-cadherin	400 — 7,800	1,447
Topo1	938 — 6,385	1,001
KRAS_4B	424 — 35,323	919
Topo2A	392 — 7,193	913
Paxillin	500 — 1,200	734

- Proteomic analysis confirmed tumor overexpression of HER2
  - ~11,322 amol/µg of tissue protein (clinically observed range for non-amplified breast or gastric cancer patients:150-500 amol/µg)
- Treatment with trastuzumab resulted in stabilization of patient's disease

Her2 Protein Levels (amol/µg)



Acknowledgments

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