

PLOS Science Wednesday: Hi reddit, we're Katherine and Marni and we found that most DNA changes in breast cancer metastases occurs in the original tumor, while the metastatic tumors are comprised of clones from the original – Ask Us Anything!

PLOSScienceWednesday ¹ and r/Science AMAs¹

¹Affiliation not available

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Hi Katherine and Marni, and thank you for doing this AMA.

The observation of metastases that appear to derive from multiclonal seeds composed of multiple cells strikes me as very interesting. A couple of questions related to this observation:

- It seems you think this is happening because you observe the same, presumably clonal (based on sequencing depth), mutations in the metastatic tumor as you do in the parental tumor. Can you correct me if I missed a part of your argument? Also what coverage did you get in your sequencing, and how did you try to overcome issues with sampling error (due to heterogeneity in the tumor, and sequencing only a small biopsy) in the primary tumor?
- How do you envision the cells metastasizing? As one clump (I imagine there are physiological restraints on how big a clump can be) or as single cells which reunite at the metastatic site?
- Do you think the observation of the multiclonal metastatic tumor suggests some sort of interdependence between subclones? If so, would understanding the nature of this interdependence help to generate hypotheses for targeted therapies?
- What about your RNA-seq data? Despite relatively few private mutations, do the metastatic tumors exhibit a strikingly different expression phenotype? To what extent does microenvironment influence expression profiles of these cells, and do you think this has therapeutic implications?

[SirT6](#)

Thanks for your questions. We found sets of mutations, or clones, within different percentage of cells in the primary (as measured by the variant allele frequency) that were also observed in the metastatic tumors. Many of these clones were shared with some but not all of the metastases, indicating distinct populations of cells. All tumors, including the primary and metastases had evidence of multiple clones, with many of the metastatic clones derived from multiple clones within the primary which had then moved/metastasized to other sites of the body. The genome coverage was between 29X to 72X for the



samples sequenced. Bulk tumor RNA/DNA sequencing is always limited by sampling error (i.e. sequencing only a small biopsy or only one section of the tumor). Previous work by others have compared multiregional sequencing in primary breast cancer which demonstrate the vast heterogeneity within each primary. Despite this limitation, our analysis still demonstrates multiple clones (genetic heterogeneity) in the primary which is reflected in the metastases.

Our group has not been investigating the mechanism of how multiple cells may be metastasizing but we would refer you to two excellent, recent publications in PNAS where the authors Au et al (doi: 10.1073/pnas.1524448113) and Cheung et al (10.1073/pnas.1508541113) experimentally assess mechanisms of polyclonal seeding.

Your third bullet raises a very interesting question which we have also discussed; however, this is out of the scope of our current study. We would refer you to the Cheung et al. (10.1073/pnas.1508541113) study which investigated this interdependence further.

When we looked at the RNA expression profiles of our tumors compared to over 1000 breast cancers from TCGA, we found the metastases were more like their primary tumor than they were to other breast cancers - primary or metastasis. When we collected the metastases, we also collected normal tissue when we could and performed RNA sequencing on those as well. We found that the metastases looked much more like the primary breast cancer than to normal tissue from the site where the metastasis occurred. The dominant expression profiles were similar. However, this does not suggest that the microenvironment isn't playing a role, and we definitely agree both the stroma and the immune component are very important. We haven't specifically looked at the microenvironment role in these samples yet.

Hi Katherine and Marni. Thanks for doing this AMA.

Just a simple question for you two. What attracted you to this field in Biology and cancer research? What tips do you have for any young women who may be considering venturing into STEM fields?

[KriosDaNarwal](#)

Marni: As a little girl, I knew I wanted to be a physician - using my scientific knowledge to help people. As many of us, I was personally affected by a close family member with cancer and wanted to know why - why did some cells go awry in my family member? Why was she so sick? And if we knew the underlying cause, why couldn't we cure it like a simple strep throat? This led to a passion of mine for both medical care and research. Through research, we can directly discover something that may help push the needle just a little bit towards "cure". I would encourage any and all women with a passion for science and the curiosity and drive to pursue a career in STEM fields. My advice for women (and men as well!) would be to involve yourself in opportunities that excite you. Identifying a mentor in your field who is truly invested in your success will allow for career guidance at critical steps: applying to graduate school, identifying the right project, and successfully applying for funding.

Katherine: I was born when my dad was in graduate school getting his PhD and was often around the lab. Science was something I was always interested in and I enjoyed working with my dad on science fair projects as a kid. When I was in high school, I got a job at a research facility near my house to get hands on experience doing research - both lab and field work. I found as many ways as possible to get involved in school or outside of school in science activities. I had a strong interest in genetics and that was my motivating interest in applying for graduate school. My background was mostly plant research, but once in grad school, I found an advisor and lab I really liked that studied breast cancer and it stuck. My advice is to seek out opportunities and become involved. Similar to what Marni said, finding a mentor to help and support you is a great as well and can help you figure out opportunities.

Thank you for your work focusing on Triple Negative tumors. I am a TN BC survivor of 4 years, very happy to be alive. I found the TP53 connection interesting.

TP53 was the only shared somatic mutated gene between the two patients and was present in every tumor specimen sequenced.

Could TP53 be used to target treatments?

Are you working on any further studies with TN? or aware of others that are in process?

Do you think it would help to save tissue from tumors for further testing as more targeted tests and treatments might be done as research develops?

Those sneaky little clones travelers making their way to distant sites to grow need to be eradicated. Is there anything available yet to do this for people who are survivors? Do you expect there will be? Isn't this the hope of the future? How close are we?

In the meantime, I use my mind to imagine the eradication and prevention of any malformed cells.

[bansheeink](#)

TP53 is the most commonly mutated gene in all of cancer, and especially in breast cancer. TP53 is more commonly mutated in the more aggressive breast cancers. Many researchers have worked tirelessly to try and identify therapeutic interventions to target TP53; however, this protein is incredibly complex with diverse functions across the cell. TP53 function can be completely disrupted in some cancers (as in classic tumor suppressor) but also can acquire missense mutations and thus gain new functions (similar to an oncogene). It is involved in both cell death and cell growth. This all complicates research in identifying both the role of TP53 in breast cancer metastasis as well as successful therapeutic interventions.

As for saving tissues from tumors for future therapy: we as researchers have seen the power of new technologies over the last decade, allowing DNA and RNA sequencing from tissues that was not possible before. We urge the proper collection and storage of tissue, as it is impossible to predict how this tissue could be used in future studies.

First off, cheers for your discovery! Now I'm interested in how this might help us fight (breast) cancer in future. What possible conclusions can we draw?

[Orriz](#)

Our study is limited in that we have only looked at two patients so far, but is intriguing to find that most of the mutations observed in the metastases of these triple negative breast cancers were also in the primary. These tumors were highly aggressive and are not representative of all of breast cancer: Patient A1 was diagnosed with metastatic disease. We think that it suggests that better targeting of the heterogeneous primary tumor might help reduce metastatic disease. Our finding of multiple clones in the primary also begs for development of new targeted approaches to wipe out the potentially metastatic clones. At this point we cannot conclude if this mechanism is similar in less aggressive breast cancers or other tumor types. Indeed in other tumors like renal cancer, they show that the acquisition of mutations are required for metastasis to occur. Future research in larger cohorts of metastatic breast cancer patients are needed to identify therapeutic targets to block this process.

Could you highlight some of the tools and technologies you use to do this kind of research?

Is it really a lot of genetic sequencing? exome sequencing? flow cytometry? Curious.

[FUCKYOUINYOURFACE](#)

We performed both DNA whole genome sequencing and RNA sequencing to perform these experiments with the Illumina HiSeq. Many scientists contributed to robustly analyze these results with a variety of computational methods.

Hi Katherine and Marni, and many thanks for doing this AMA.

Are there any hints in your data about tissue targeting? For example, are there similarities in the multiclonal population that can cross the blood-brain barrier? That goes to bone? Etc.

[3-2-1_liftoff](#)

Our current dataset is limited by only having two patients with shared sites including the lung and liver metastases. On-going work in both the United States and Europe looking at >200 women with matched primary and distant metastatic sites will hopefully address your question. A much larger cohort is needed to statistically determine why specific subtypes of breast cancer tend to metastasize to different organs (i.e. ER+, luminal tumors are more likely to first relapse in the bone while HER2-enriched tumors are more likely to relapse in the liver, and basal-like tumors are more likely to relapse in the lung and brain).

What was the previous understanding/consensus about metastasis?

[MarsNirgal](#)

There is not much consensus in the field of understanding about metastasis. The seminal paper first describing DNA sequencing of a matched tumor and metastasis was in renal cell carcinoma, demonstrating branched evolution with a single cell of origin seeding distant metastasis. A study of basal-like breast cancer, the matched metastasis, and a xenograft show high percentage of shared genetics across all 3 tumors. Single cell sequencing of one breast cancer with one matched liver metastasis suggest a single cell seeded the distant site. This is corroborated by a large panel of matched primary and brain metastases sequenced, showing continued evolution and acquisition of resistance mechanisms in the brain metastasis specifically. A larger study in prostate cancer suggests metastasis can seed other metastases. Other studies in non-small cell lung cancer, colorectal cancer, and ovarian cancer all shed light on the cancer evolution through metastasis. Few studies, however, have more than 1-3 matched metastases with the original tumors. Furthermore, advances in DNA and RNA sequencing provide even greater depth of coverage and only further illuminate the genetic complexity of cancer. Further research with >3 matched metastases and primaries across multiple subtypes of breast cancer is needed. Parallel studies in immunocompetent in vivo models are needed to better understand mechanisms of metastasis. There is no consensus, but there might not be a consensus, as there are likely diverse mechanisms of metastasis and therapeutic resistance across different types of cancer.

Hey, great paper! Very interesting to see how you could reconstruct the different clones based on the VAF of variants in common.

How did you differentiate between true and false negatives when doing your pairwise comparisons? I've considered restricting such comparisons in regions that are sufficiently covered in both samples, or "rescuing" variants with limited evidence in one sample when there's a good call in another samples and I'm looking forward to hearing more opinions on the matter!

[Crankt](#)

False negatives are extremely difficult to address; however, we did reinterrogate all mutations from the metastases in the primary tumors. We had bioinformaticians who visually inspected the alignment of reads to address this concern. Upon review, we identified low percentage clones in the primary that would typically be filtered when using a VAF cutoff of 5% or greater. Another method that we researched was compiling all alignments from one person (i.e. all 6 tumors sequenced) to achieve >400X coverage at positions and subsequently call mutations. Others have published on similar methods and demonstrate the power of using sequencing from related tumors to lower the false negative rate.

Thank you for doing this AMA, I enjoyed reading the results of your study and it looks very well done. My question has to do more with the primary tumor than the metastasis. How uniform is the genome of the primary tumor, given that cancer is considered a collection of mutations and how would you go about assessing the genome of a tumor?

[Diltron24](#)

A very elegant study published in 2015 by Lucy Yates and colleagues (DOI: 10.1038/nm.3886) investigates the genetic diversity in primary breast cancer. They show that heterogeneity of primary breast cancers varies, but that it is correlated to tumor size.

How much money do you get from the Susan B Komen foundation for your research? How much support do they give to breast cancer research, in your opinion?

[SnapdragonGunmetal](#)

The UNC Tumor Donation Program was founded in 2001 by Dr. Lisa Carey. Between 2001 and 2010, a few patients on an ad hoc basis graciously donated their tumors at the end of their lives for this study. In 2010, Susan G. Komen awarded Dr. Carey a Scholar Award to formalize the program and provide the resources necessary to perform this research. We are extremely indebted to the Komen foundation for their generosity and the impact that they have in our community through both research funding, outreach, and patient support.

Thanks to both of you for working to help understand/cure this dreadful disease. I have a question; please forgive me if it's been asked previously or has been answered in your research.

I take it that your research is based on findings from two patients.

Is there a reason you only used two patients?

Will you be repeating this research with a greater number of research subjects?

Will you research this subject using patients with er+, non basal cell cancers?

Thank you again for all that you are doing.

[sjack827](#)

At the time we started the study we had samples for two patients. In 15 years, we now have collected 55 patients covering a larger spectrum of breast cancer types. Getting these types of samples is incredibly difficult and we could not have done it without the generosity of these patients who participated. We, along with others, are in a multi-center collaboration to study all of these tissues

along with women from outside UNC.