PLOS Science Wednesday: Hi Reddit my name is Hunter Underhill, and I discovered a new way to use circulating tumor DNA to improve cancer diagnostics and disease monitoring – Ask Me Anything!

PLOSScienceWednesday [1] and r/Science AMAs[1]

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Thanks for taking time to do this AMA.

I have a couple of questions.

1. Your article says that malignant tumors shed DNA into circulation. Is this specific to malignant tumors or do benign tumors also shed DNA into circulation? If so, is there a way to use your methodology to differentiate between malignant and benign tumors?

2. How do you envision these techniques being combined with personalized medicine? Do you think this could be a non-invasive way to establish what specific mutations are in an individual cancer to develop individualized treatments?

RedQueenConflicts

Thank you for the questions! Regarding the first question, there is a recent paper by Lehmann-Werman et al. (PNAS, 2016, 113:E1826-34) describing the detection of cell-free DNA from a variety of tissue types based on methylation patterns. YM Lo's group has done a lot of work looking at cell-free fetal DNA in maternal plasma (e.g., Sci Transl Med, 2010, 2:61ra91). These studies along with other works strongly suggest that most any tissue has the potential to deposit DNA into the circulation, including benign tumors. In our study, the cell-free DNA deposited by malignant tumors tended to have a shorter fragment length than the background cell-free DNA. Notably, the shorter fragment length size was not specific to malignant tumors and overlapped in part with the normal background cell-free DNA. A reasonable analogy for identifying circulating tumor DNA amongst cell-free DNA is the proverbial needle in a haystack. Isolation of a specific cell-free DNA fragment size simply removes a large portion of the haystack so that the search for the needle is simplified. However, the needle is still mixed in with the remaining portion of the haystack and is indistinguishable. Variant analysis via sequencing or ddPCR (or other modality of choice) is necessary to identify mutations associated with malignant tumors to truly find the needle. Selection of a specific fragment length that corresponds to an expected fragment size associated with malignant tumors increases the mutant allele frequency within a sample to improve the sensitivity of sequencing/ddPCR. So I do not think that fragment size selection alone will be able to differentiate between malignant and benign tumors, but I do think that fragment size selection will improve the sensitivity of detecting mutations associated with malignant tumors, thusly

improving our ability to discern malignant from benign.

Regarding the latter question, I think one of the strengths of fragment size selection is that it is not directed at a specific variant. Once a fraction containing a desired fragment length is isolated from a sample, the fraction may be subsequently analyzed for either a single variant (e.g., ddPCR) or sequenced to identify any number of variants. As such, fragment size selection lends itself very strongly to individualized medicine by affording the opportunity to follow a known mutation in a patient to monitor response to therapy, while also surveying for new mutations that may represent evolution of the tumor that inform resistance/susceptibility to current or alternate therapies.

Thanks for doing this AMA, very interesting paper!

I don't really have a feel for how much circulating DNA is typically found in a person, and how cancer affects this.

Could you give a rough idea (maybe pg measurements) just how much DNA a healthy person should have floating around in their body? Is this primarily limited to the blood, or do we find it distributed throughout the tissues? And how much does this amount change during cancer - or does it stay roughly the same, just with alterations in fragment length such as you describe?

jamimmunology

Thank you for the questions! In a normal healthy person, we usually get on average about 20-30 ng per 8 mL of plasma, although the range of values can be much wider. In healthy individuals (i.e., tumor-free), factors such as exercise, inflammatory response to a viral illness, etc., may increase the amount of cell-free DNA that is present. In our cancer patients, we usually get about double the amount of cell-free DNA seen in healthy individuals, but a reasonable estimate of the ranges that we see in cancer patients is between 20 ng to > 1ug per 8 mL of plasma – a very wide range! The amount of cell-free DNA present in cancer patients may be altered by tumor burden, chemotherapy, radiation, etc. along with the factors that alter cell-free DNA in healthy individuals. As such, increases in cell-free DNA in cancer patients is not all due to circulating tumor DNA and it is difficult to determine causality as there is a lot of inter-subject variability. Even using mutant allele frequency may not be a strong measure of tumor burden as tumor cells tend to be genetically heterogenous. This is an active area of investigation.

We have not sought to study cell-free DNA outside of the circulation, so I am unable to comment on its presence in specific tissues. Of note, however, cell-free DNA is present in CSF, but the fragment size is more genomic (>1 kb) and very different than what we see in plasma.

Hi Dr. Underhill, and thank you for taking the time to do this AMA.

I was hoping that you could elaborate a bit on what you think the ultimate impact of circulating tumor DNA will be in the clinic. The technology is often touted as offering an actionable, non-invasive biomarker with implications in diagnosis, prognosis and monitoring treatment response.

Personally, I've always been a bit pessimistic about the ability of CT DNA to live up to this potential. My reasoning is essentially, with a disease like cancer, there is no point in cutting corners. So while, in theory, non-invasive sounds nice -- it is much more important to have accurate and interpretable biomarkers. And in that case, I just don't see CT DNA beating out other technologies at this point. For instance, I would place a lot more faith in any sequencing data I get from a biopsy/resected sample than I would from a CT DNA sample. Similarly, disease progression, seems much better monitored by imaging modalities. Maybe, I could see an argument for CT DNA helping to monitor minimal residual disease, but I would want to know a lot more about the sensitivity of this assay.

Thanks!

SirT6

Thank you for the questions! You raise a lot of very interesting points. Although circulating tumor DNA is a very active area of research, the field has enormous room to grow. So I currently agree with you that the technology is not quite where it needs to be for diagnosing and monitoring cancer, but it is moving there very rapidly. In my mind, there are two key elements that need to be addressed. First is improving the sensitivity of detecting circulating tumor DNA. Continued advances in technology will enable us to detect cancers earlier when only a very small amount of circulating tumor DNA is present relative to the large background of normal cell-free DNA. There are many steps in this process, but hopefully, fragment size selection will come to represent one of these steps. And second, identifying variants most commonly associated with individual types of cancer so that cancers can be diagnosed via cell-free DNA. A necessary part of variant identification in cancer is determining the concordance between variants associated with the primary tumor and variants present in circulating tumor DNA. This is an active area of research and various groups are working on the development of panels that cover common variants to survey cell-free DNA for the presence/absence of different types of cancer.

As an MR physicist, I have a strong interest in imaging. However, imaging has its limitations – expense, variability in image interpretation, variability in imaging protocols, travel time to scanners, length of study for an MRI scan, contrast agent injection, etc. Personally, as both an MR physicist and a geneticist, I think the coupling of cell-free DNA (i.e., genotyping) with imaging (i.e., phenotyping) will provide the strongest advance in personalized medicine. Being able to diagnose and monitor patients non-invasively through a blood draw affords the opportunity for patients to be evaluated/followed remotely without having to travel to surgical centers for a biopsy or imaging centers for routine follow-up – this is less of an issue in big cities, but does present a major problem for patients in rural areas. However, imaging will always have a central role in cancer care as it is necessary to fully characterize (e.g., location, size, number of lesions, etc.) new and recurrent disease.

Do you have any projects you're working on on the side? I work in IT for example, and I'm often setting up stuff on my homelab or buidling PC's or hacking stuff etc.. do scientists run their own experiments on their own personal projects?

ROGUETWOSTANDINGBY

All of our studies were approved by the IACUC at the University of Washington and the IRB at the University of Utah.

Do you have any advice for young people interested in pursuing a career in medicine/medical research?

Mg515

From my perspective, I think two of the most important things are to study something that truly interests you and try to work in a lab/clinic that not only does good science/medicine, but has good people. Try to surround yourself with brilliant, but kind people (both in medicine and in the lab) that question you, challenge you, and listen to you. I think once these elements are in place, you will find yourself willfully and happily doing the very hard work necessary to achieve your medical/research goals.

Thanks for doing this AMA.

I have a couple of questions.

1. As you mentioned, you have found a distinct size difference in DNA fragment length between circulating tumor DNA and cell-free DNA. Have you investigate the reason for the difference between them, does that mean some function gain or loss?

2. Is your research applicable to specific cancers or broad range of cancers ?

JimballoonX

Thank you for the questions! The reason for the size difference between circulating tumor DNA and cell-free DNA remains unclear. As most of the normal cell-free DNA is derived from circulating cells, we can speculate that the size difference may be due to differences in how cell-free DNA from circulating cells is treated differently from non-circulating cells. We've also considered the possibility that perhaps it's the body's recognition of self vs. non-self. It's interesting to note that a similar difference in fragment size was reported by Lo et al. (Sci Transl Med, 2010, 2:61ra91) when comparing fetal cell-free DNA from the maternal circulation to the maternal cell-free DNA. More investigation is needed in this area as it may have therapeutic implications in cancer.

We studied glioblastoma multiforme and hepatocellular carcinoma in animal models. We then studied melanoma and lung cancer in human patients. The findings were very similar across all four tumor types, which would suggest that our research is applicable to a broad range of cancers. However, a variety of additional cancer types need to be studied before that assumption seems justified.

Why is it necessary to enrich the tumor DNA? Do you sequence the cell free DNA to look for the tumor DNA? Would it be possible to detect known DNA sequences using strand displacement in a FISH-like experiment?

ralph_hunter

Thank you for the questions! In some instances, it is not necessary to enrich for the circulating tumor DNA. For example, in metastatic solid tumors there is generally sufficient circulating tumor DNA present that it can be detected against the background of normal cell-free DNA. Enrichment for circulating tumor DNA may improve sensitivity of detecting non-metastatic solid tumors where the amount of circulating tumor DNA present is relatively low. Another benefit of enrichment is that sequencing is focused on fragment sizes most likely to contain circulating tumor DNA enabling greater depth of sequencing.

We use both sequencing and ddPCR to identify tumor DNA.

It seems reasonable that you may be able to detect known DNA sequences using strand displacement in cell-free DNA. However, you may be limited to only studying a single known mutation per sample – a similar limitation is associated with ddPCR. Sequencing would allow you to survey for multiple variants in a single sample.

What process/instruments did you use to prepare the samples and fragment the DNA?

paraplegic_T_Rex

The samples are inherently fragmented as a function of apoptosis and nuclease-cleaved nucleasome activity and do not require additional fragmentation.

Greetings Dr H. Underhill

Thank you for taking your time and doing this AMA.

The questions I have are not specific to the research presented here....but more on the environment that made it possible for you to do such research.

1. How do you balance doing your research and at the same time deliver health services to the community?

2. What would be your advice to younger persons who want to do medical research but also have service component to their work schedule (like actually seeing patients)?

black0eye0ninja

Thank you for the questions! I am currently 25% clinical and 75% research. That distribution of time allows me sufficient time to see patients, while remaining productive with my research. For physicians in training (i.e., residents/fellows), it is very challenging to find adequate time for research during clinical training. Although some specialties offer 2-3 months as part of the residency/fellowship, this is really insufficient. It may be possible to talk with your program director and ask for additional research time within the program or ask for a 1-2 year leave of absence to work in the lab. Alternatively, once your clinical training is complete, it may be a reasonable option to do a post-doc in a particular field. The downside to the post-doc is that you would be paid a post-doc's salary, which is relatively low compared to what you would make as a physician and your student debt would continue to accumulate. However, you may be eligible to apply to the NIH Loan Repayment Program to help with your student debt while you're in the lab. I think there are some viable options out there, but it will definitely take a lot of work and perseverance on your end to make things work.

How this is related with those who look for fetus DNA on pregnant mothers? (Cell-free fetal DNA (cffDNA)) I mean, in the sense of sharing techniques.

sbassi

Thank you for the question! Lo's group has previously shown that a similar difference in fragment length distribution is also present in cell-free fetal DNA (Sci Transl Med, 2010, 2:61ra91). It seems a reasonable hypothesis that selection of specific cell-free DNA fragment lengths from the maternal circulation may improve the sensitivity of detecting cell-free fetal DNA. The techniques for isolation of a specific cell-free DNA fraction would be similar to what we have done in our tumor patient samples.

Does the available DNA sequence manipulation software servers your purpose on the ctDNA field or do you think there is room to improve with more specific solutions?

sbassi

Thank you for the question! It always seems like there is room for improvement. The largest issues with sequencing cell-free DNA is that you cannot use the traditional variant callers as samples contain a mix of normal cell-free DNA and circulating tumor DNA (and variants may be hetero- or homogenous). We are currently working on developing our own software for variant calling in cell-free DNA samples.