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Welcome to Yale Cancer Answers with your host doctor Anees Chagpar. Yale Cancer Answers features the latest information on cancer care by welcoming oncologists and specialists who are on the forefront of the battle to fight cancer. This week, it’s a conversation about genetic and environmental influences in colon cancer with Doctor Caroline Johnson.

Doctor Johnson is assistant professor of Epidemiology in the Department of Environmental Health Sciences at the Yale School of Public Health and Doctor Chagpar is a professor of surgical oncology at the Yale School of Medicine.

Caroline you can start off by telling us a little bit about your research? I use a technology called metabolomics to investigate specific differences in metabolism that affect colon cancer, development progression and even response to therapeutics. So particularly in my research I’m interested in examining
metabolism in patients that develop tumors that occur on the right side of the colon, so that is the area of the colon between the appendix and slightly up from there in the rectum and ascending colon, because those patients have the poorest survival and what we've seen in the literature is actually female patients have much higher incidence of tumors that occur in this region of the colon, so we've been using metabolomics to get a better understanding of the metabolism of these tumors. So maybe we can stop there for a second and just kind of dig a little bit deeper into what exactly metabolomics is and how that works. It's the study of all the small molecules that are present within a sample so we can take a biological sample such as a blood sample, or even a tumor tissue, and we can analyze it in an agnostic manner. So we examine basically all the different levels of all the small molecules that might be within that sample and this is similar to genomics or transcriptomics. So small molecules are basically metabolites that are within our
bodies that come from the processing of things like dietary products, and they produce vital components that are needed for our bodies for different biological processes, such as growth and healing, immune responses, energy, and even sleep, so metabolomic analysis can also really show us about the metabolism of an individual and it can also show us metabolism of things like environmental chemicals and drugs as well within an individual and that could be produced by the bacteria or even the microbiome within an individual. And this technology is particularly important for cancer because we know that metabolites can affect how a tumor grows as tumor cells need nutrients and energy and the tumors themselves produce metabolites. So metabolomics can really provide us great insight into how an individual produces metabolites and how this might propagate tumor growth as well. So basically you’re kind of looking at all of these metabolites to gain some insight.
Tell us what sample you used to look at these metabolites. One can imagine that there may be many options that you would have whether it's looking at the stool or whether it's looking at tumor tissue, or whether it's looking at blood. So what exactly do you do to try to gain this insight?

That’s a really good question, basically we can take anything, we can take a blood sample or stool sample, or a tumor tissue, and we can obtain these from patients, and we can extract all the different metabolites out of these biological samples. And what we end up with is sort of a mixture of anywhere from maybe 3000 up to you know 10 to 20,000 different molecules that could be present within this sample within my research so far, we have primarily examined tumor tissues from patients, so with collaborations with both Sloan Kettering Cancer Center and also Yale Cancer Center, we obtained over 200 tumor tissues from patients where these tumors had been obtained during surgery and we were able to...
analyze these tissues to examine which metabolites were present and how they were different between different patients. So how they were different between both women and men and from patients with right sided colorectal cancer and also from tumors that occurred in other regions of the colon as well? And if all of these patients had cancer, one would imagine that you’re really looking at the metabolomic profile of tumors in these patients is that different than what you would expect in normal colon? So are there some metabolites that you would expect only in tumors versus in healthy tissue? Yeah, that’s a great question, so we know that tumors have very sort of increased rapid growth, so we tend to see metabolites linked to energy metabolism and sort of making those or encouraging those building blocks to be built to build new cells so we know there’s a lot of what they call metabolic rewiring that happens within a tumor compared to a normal tissue. And within my research, we were really interested in looking at the tumors themselves and how they differed between male and female patients because
what is quite interesting about colorectal cancer and all cancers, they tend to have a higher incidence in male patients, but what we see is that in the right side of the colon, women tend to have this higher incidence, so we wanted to see what was different metabolically about these tumors that occur specifically in women with right sided colorectal cancer and what we saw was that they had this very different metabolic profile where they tended to generate energy differently and they use one metabolite school, disparaging that seemed to be much higher within this set of patients than compared to male patients that had right sided colon cancer, and also patients that had tumors in the other side of the colon. So we’ve really gone after this metabolic pathway to understand more about this side of metabolism and potentially how it could in the future be potentially targeted for perhaps a precision medicine approach for these groups of patients. That’s interesting that women have a metabolite that
0:07:35.659 –> 0:07:38.014 processes energy differently than men.
0:07:39.804 –> 0:07:43.388 I just wonder when I think about
0:07:43.388 –> 0:07:46.509 Asparagine I started thinking about
0:07:48.493 –> 0:07:53.12 nucleic acids and amino acids
0:07:53.242 –> 0:07:57.136 that form the building blocks of
0:07:57.136 –> 0:08:01.409 cells and whether these could be
0:08:01.409 –> 0:08:05.289 manipulated based on dietary factors,
0:08:05.29 –> 0:08:06.61 for example.
0:08:06.61 –> 0:08:12.97 So when we think about how cells use energy,
0:08:12.97 –> 0:08:14.785 sometimes that may be
0:08:14.785 –> 0:08:19.02 mediated in part by people’s dietary intake,
0:08:19.02 –> 0:08:21.785 did you look at that as a
0:08:21.785 –> 0:08:23.623 potential difference in male
0:08:23.623 –> 0:08:25.318 versus female patients?
0:08:25.77 –> 0:08:27.12 Within our cohort
0:08:27.12 –> 0:08:29.82 we didn’t have information on diet,
0:08:29.82 –> 0:08:32.058 but that’s very much something that
0:08:32.058 –> 0:08:34.758 would be useful to have something
0:08:34.758 –> 0:08:37.018 like a food frequency questionnaire,
0:08:37.02 –> 0:08:38.82 which is sometimes collected
0:08:38.82 –> 0:08:41.07 for different biobanks
0:08:41.07 –> 0:08:43.317 and in different cohorts.
0:08:43.32 –> 0:08:46.881 Yes, exactly I think it’s
0:08:46.881 –> 0:08:48.629 really important here,
0:08:48.63 –> 0:08:52.014 but I think Asparagine does come from
0:08:52.014 –> 0:08:54.427 many many different dietary sources
0:08:54.427 –> 0:08:58.38 and it actually has been seen
0:08:58.38 –> 0:09:00.936 to be produced potentially,
0:09:00.94 –> 0:09:02.806 or metabolize by the microbiome as
0:09:02.806 –> 0:09:05.47 well and it can be produced internally
0:09:05.47 –> 0:09:07.725 through your own biochemical processing
of other metabolites through an enzyme called Asparagine synthetase. So, biologically, it can come from your internal processing, but it can also come from dietary sources, and it can come from microbial processing as well. So, as with many metabolites that are present within tumors and also present within the colon, we always have to take into account all these different biological sources of where they can come from. So we can either manipulate them and try and sort of, potentially reduce the effects of the disease, or improve therapeutic response. And it seems to be so multifactorial when you think about where all of these metabolites can come from, and all of the different processes both within normal cells as well as within cancer cells, which raises the question, do women normally have more of this metabolite even outside their colon cancers? I think in this context what we’ve begun to see...
is that Asparagine might be increased in these patients because these tumors may be nutrient deplete and this is something that we still have to look into, so we can’t really confirm this, but just from our metabolomic studies, it seems to be indicating this. And this is maybe due to differences in blood supply to the tumor, or something else. Less oxygen that might be getting to the tumor. And when we look at the other processes that are going on within these samples we see that the generation of other energy metabolites is different as well, which could be indicating that there could be something particular about how these tumors might be growing in this area of the colon. So at the moment we don’t have normal colon tissues from from individuals, but that’s something that we do want to look at to see if the patients that do not have colon cancer, if the colon tissues have these different metabolites that could be different between men and women, and could affect the development of these tumors. You kind of wonder as
whether this is cause or effect. So in other words, is it that you had a tumor which was growing, which then caused this altered metabolomic profile, or was it that you had some other processes that were going on that altered your metabolomic profile, which then spurred on the cancer? Did you gain any insight into that question? I think it’s probably more of the latter. We see that Asparagine is produced internally. As I mentioned through this enzyme asparagine synthetase, and this enzyme is controlled somewhat by another gene mutation of aging mutant Kras, so it could be that these tumors have this oncogene and it could be affecting these metabolites, so it could be an effect that we’re seeing, but it is probably a combination of many things, that includes this potential mutation to this gene. But also it could be the way that the tumor is growing as I mentioned within the colon as well and all together, all these different processes are causing this effect of this increase in Asparagine that seem to help
propagate the tumor when it might be under these stress conditions where it’s not able to obtain nutrients in a normal fashion, so I think this is what could be happening. And also as I mentioned as well, this combination of the microbiome present as well within the colon that could be affecting how this metabolite is being processed. And it’s an interesting puzzle to think about how metabolomics works along with genetic mutations and so on when we think about colon cancer. We’re going to take a short break for a medical minute. Please stay tuned to learn more about genetic and environmental influences in colon cancer with my guest Doctor Caroline Johnson. Support for Yale Cancer Answers comes from AstraZeneca, working to eliminate cancer as a cause of death. Learn more at astrazeneca-us.com. This is a medical minute about head and neck cancers, although the percentage of oral in head and neck cancer patients in the United States is only about 5% of all diagnosed cancers,
there are challenging side effects associated with these types of cancer and their treatment. Clinical trials are currently underway to test innovative new treatments for head and neck cancers, and in many cases less radical surgeries are able to preserve nerves, arteries and muscles in the neck, enabling patients to move, speak, breathe and eat normally after surgery. More information is available at yalecancercenter.org.

You’re listening to Connecticut Public Radio. Welcome back to Yale cancer answers. This is doctor Anees Chagpar and I’m joined tonight by my guest doctor Caroline Johnson and we’re talking about genetic and environmental influences in colon cancer and right before the break, Caroline was telling us about her studies looking at metabolomics. That is to say the study of different metabolites. Looking at gender differences in right sided colon cancer. So Caroline, I wanted to dig into that a little bit more because we started to talk about whether these metabolomic changes
0:15:18.51 –> 0:15:21.853 are what drives the colon cancer or
0:15:21.853 –> 0:15:24.403 whether the colon cancer is what
0:15:24.403 –> 0:15:26.949 drives the metabolomic changes,
0:15:26.95 –> 0:15:30.106 and you had mentioned that the
0:15:30.106 –> 0:15:33.185 metabolomic changes may be in part
0:15:33.185 –> 0:15:35.813 related to mutations in KRas,
0:15:35.82 –> 0:15:39.708 but we know that Kras and oncogenes
0:15:39.708 –> 0:15:43.217 may spur on cancers as well.
0:15:43.22 –> 0:15:46.268 I wonder whether these two processes
0:15:46.27 –> 0:15:48.69 are independent of each other.
0:15:48.69 –> 0:15:50.326 That is to say,
0:15:50.326 –> 0:15:52.78 Kras causes metabolomic changes and
0:15:52.864 –> 0:15:55.499 also causes separately tumor development
0:15:55.499 –> 0:15:58.85 or whether these are Interrelated.
0:15:58.85 –> 0:16:02.238 Do you have any sense on that?
0:16:03.35 –> 0:16:06.83 I think they are interrelated and the
0:16:06.83 –> 0:16:09.674 findings that we have seen
0:16:09.674 –> 0:16:12.266 linking Mutant Kras and Asparagine
0:16:12.266 –> 0:16:15.529 have been seen in other cancers as well.
0:16:15.53 –> 0:16:18.666 So you know the mutant carriers is very
0:16:18.666 –> 0:16:22.248 common in pancreatic cancers and there is a
0:16:22.25 –> 0:16:24.77 clinical trial right now
0:16:24.77 –> 0:16:26.86 actually that I saw yesterday
0:16:26.86 –> 0:16:28.532 for targeting Asparagine by
0:16:28.532 –> 0:16:30.649 using a drug
0:16:30.65 –> 0:16:33.674 along with other first line chemo.
0:16:36.36 –> 0:16:39.37 So we do know that the mutant
0:16:39.37 –> 0:16:41.26 Kras does regulate other
0:16:41.26 –> 0:16:43.36 genes and signaling pathways that
0:16:43.36 –> 0:16:45.04 does affect Asparagine production.
0:16:45.04 –> 0:16:48.064 So I think it’s probably a case of mutant
Kras affecting Asparagine levels.

But of course, as I mentioned before, asparagine can be modulated by other sources, and also from the microbiome. We have analyzed the microbiome from some tumors from the right sided patients. So from both men and women, there is some microbiota that are correlated with asparagine. Levels only in the female patients. So we do believe there is a multifactorial effect on asparagine production that could be itself propagating the tumors as well by giving them more nutrients, we know that Asparagine can increase the uptake of other amino acids and can affect other processes such as even polymetabolite production or autophagy. Another process is like that. So I believe this is a very wide combined effect. And really the technology metabolomics has allowed us to get an insight into this because we can not only analyze Asparagine, but we can analyze all the other metabolites that could be
affected by asparagine levels as well, it could be affected by mutant Kras so it really is a wider scope or a magnifying glass really into looking more into how these pathways are regulated by both genes and metabolites. Have you found a difference in Asparagine between men and women who are Kras negative? That is to say, they don’t have a Kras mutation. I wonder whether these two are directly linked, so for example, women may have more Kras mutations, and therefore you may be seeing these metabolomic differences or whether these are really separate processes altogether? We haven’t looked at that specifically but what we have done is we’ve looked at survival, and actually there’s many different publicly available data sources that we can look at to look at gene expression and also patient survival. So we looked at mutant Kras we looked at asparagine synthetase and we saw that patients with these genes had much poorer survival if
they were female and they had a right sided tumor so we compared, the Kras mutant to the Kras wild type, and it was again in these different resources we saw that it was always the female patients of right sided colon cancer that had the poorer survival, and we looked at asparagine levels within our own cohorts. And we looked at the survival data because our tumors were collected in the 1990s, so we were able to follow up with survival of the patients. And we saw that it was again, the women with right sided tumors that had poor survival, and increased risk of recurrence if they had high asparagine levels. Interesting and did you look at whether these asparagine levels were higher in tumors that were larger versus smaller, or was it if you looked at two tumors that were identical in terms of their size and their grade, and the level of invasion and their lymph node status, and all of the other markers that we look at for prognosis.
was asparagine independently associated with prognosis? We didn’t have the size of the tumors to sort of understand that, but that’s a very good question. What we did was we had a very small amount of tumor from each patient, but it was the same size for each patient that the biopsy that we had. So we compared between those biopsy sizes. But we did take into account things like the stage of the patient and we saw across the board that it was stage one, stage two and three that had high levels of asparagine in the women with right sided colon cancer, but for men they didn’t have these high levels of asparagine at these different stages, so it tended to be mostly in the women again. And so when you looked at prognosis, did you look at it and found that asparagine was correlated with prognosis? Was that independent of their stage at presentation? Yes, it seems to be independent of stages. So what we really want to do next is we want to obtain blood
samples from patients to see if we can measure asparagine levels. And if this could be potentially a biomarker as well for these patients. So that’s something that we want to validate in a larger cohort.

That’s something we’re looking into right now to collect these samples. When we were talking about cause versus effect, it really gets to your next steps, right? So if we think that asparagine is really an effect, in other words, you have a tumor that then causes asparagine levels to go up, such that those asparagine levels are predictive of prognosis, certainly thinking about can we use this as a biomarker, especially if it can be found in something simple like a blood sample or a stool sample, might be helpful. On the other hand, if we think about it being more of a cause, that is to say, if you have high levels of asparagine that then sets off a cascade that leads to worse tumors and worse prognosis, then the concept might shift not only to be a biomarker, but to really think about
this as a therapeutic target.
So where do you kind of come down on your next steps with regards to that?
That’s a really good question.
We are currently designing studies to look at the effect of asparagine on tumor growth.
Providing a different cell line, and animal models asparagine to see if it does propagate tumor growth.
There was a study out in Nature a couple of years ago where they in a different cancer model, in a breast cancer model, they fed mice asparagine in their diet and they saw that it actually caused the primary tumor to metastasize.
So there’s been a number of studies that have looked into asparagine and have seen that it can propagate tumor growth.
So we had we have a study that has been funded by the American Cancer Society where we will be looking at the effect of both the gene that produces asparagine so asparagine synthetase, and we’ve developed some cell lines where we have the knockout of this gene, and we will be injecting this into mice and also to feed them
asparagine to see if it will actually affect tumor growth so hopefully in the future down the line we can sort of test to see if any of the asparagine reducing drugs could be used as a therapeutic to reduce asparagine levels in colon cancer patients, potentially.

It's so interesting when you talk about that study in breast cancer where feeding asparagine led to increased metastasis. One of the obvious questions I'm sure all of our listeners want to know is what foods out there are high in asparagine? That's something we're looking into as well. As with any sort of food source, there are many different components within a vegetable or within anything that you eat. I think if it was going to be given as a therapeutic, I don't know if diet is really the best way to approach it. It could be better to potentially try and reduce asparagine levels, and that's what I mean as using it as a preventative measure.
so encouraging people to eat less foods that are high in asparagine.

Which brings us to the question which foods are those?

At the moment we don’t really know which foods have high asparagine levels. That’s something that we would need to look into ’cause you know each food product does contain many different amino acids and other products, and it tends to be some food products that may have higher asparagine levels have other beneficial properties.

Yeah, that’s a really interesting point, but I think that perhaps targeting maybe a therapeutic standpoint from using something like asparagine. AIDS might perhaps be more effective, but definitely the diet would be something that would be useful to look into for these patients.

Yeah, because they kind of wonder whether women just naturally gravitate towards eating foods that are higher in asparagine or whether they process those differently such that they end up with higher levels of asparagine versus men, and so understanding how
they metabolize those foods, might play a role, but can you comment that in looking at the enzymes that breakdown asparagine and also those that increase asparagine, did you find a difference between men and women in terms of their natural enzymes? Even outside of the cancer patient? We haven’t looked at the expression levels of those, but that’s a really interesting point. We do know that the asparagine synthetase is associated with poor survival if it’s a higher expression only in women with right sided colorectal cancer. But I think also having a look more deeply at the microbiome because we know that there are many species within the microbiome that can also metabolize asparagine. This could be, you know, another therapeutic that could be explored as well, and I think having a more in depth look at the microbiome that could be present within the stool sample or within the tissue samples within patients is also really important. The other question that comes to mind is while your...
research is really focused on the differences between men and women, one wonders, especially when you think about the potential role for asparagine in mediating prognosis. I'm going back to that study that you said was published in Nature in the breast cancer model, whether if you look at men with colon cancers, whether men with higher levels of asparagine do worse than men with lower levels of asparagine have you looked at that? We have and it doesn't seem to be the case, so it seems to be sort of what we've seen is the opposite way round. The with you for male patient has higher levels of disparaging. They tend to do better. So it's really perplexing interesting, you know, and it's really fascinating, so it's something that you know where we're looking into within my lab in different models, so hopefully we'll get better insight into this in the next couple of years or so. Doctor Caroline Johnson is assistant
professor of Epidemiology in the Department of Environmental Health Sciences at the Yale School of Public Health. If you have questions, the address is canceranswers@yale.edu and past editions of the program are available in audio and written form at yalecancercenter.org. We hope you’ll join us next week to learn more about the fight against cancer here on Connecticut Public Radio.