Obesity & Cancer Webinar Series

Obesity, Microbiome, and Cancer: Mechanistic Considerations Webinar
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Webinar Questions and Answers

Moderator:

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Panelists:

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Liza Makowski, Ph.D.
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Answered Questions

1. **I'm curious about when you talk about the obese microbiome, my understanding is you're talking about the fecal microbiome. And I'm wondering how much the adipose tissue for a biome contributes to that?**

   Christian Jobin, Ph.D.: Right. The obesity, the tissue we had—adipose tissue—was coming from a colitis-Crohn's disease patient, not an obese patient. But there have been studies done with obese patients with type 2 diabetes and hypertension where they found in the adipose tissue the presence of microbiome. We're not quite sure what is the function of these bacteria in terms of disease outcome. Do they participate in the systemic inflammation? Do they promote the obesity secondary disorder? So, we're not 100 percent sure what's happening. For IBD, these microbes seem to have an impact, so if one resects the colonic tissue but doesn't take care of the mesenteric fat, patients often relapse into inflammation within five years. So, they are hiding in plain sight. And even if you remove the inflamed tissue, these bugs are there and come back and promote inflammation. You could almost view them as a reserve force somehow. I'm not sure if it will be the same for obesity

or type 2 diabetes, and so on. But you would think that these microbes could participate in the systemic or pathology associated with obesity.

2. **I was interested in the inulin-fed mutant mice and whether or not they were hyperphagic or what their food intake was compared to the non-inulin-fed.**

   Matam Vijay-Kumar, Ph.D.: Yes. TLR5KO mice are hyperphagic, whether we provide lab chow or a purified diet enriched with inulin. We did not do calorie restriction (pair feeding) in the liver cancer study because it is a six-month study. It is technically challenging to do pair feeding for six months. Interestingly, calorie restriction prevented metabolic syndrome in TLR5KO mice, suggesting it may be beneficial to alleviate liver cancer.

3. **I'm curious about passive microbiota transfer in humans.**

   Christian Jobin, Ph.D.: We know there is oral and rectal transfer for different diseases. But for obesity/CRC, I don't think so; obesity is not a communicable disease per se. It's kind of fascinating that one could transfer the phenotype from an obese patient to a mouse, at least increase their calorie intake for the same amount of food. So there is a microbiome component to the obesity that is transferrable to experimental model. But is it for relative, for example? Is it communicable through fecal-oral route? The answer will be no.

4. **Can the gut microbiome of an obese individual affect the microbiota of others in their community—family, roommates—who are not obese and eat healthy diets? This may relate to some studies looking at people who interact together who may also have similar phenotypes.**

   Christian Jobin, Ph.D.: Obesity is not an infectious disease, but as mentioned before, the phenotype could be transferred using pre-clinical models. There is a case report of FMT for C. diff treatment where the person received microbes from a relative who had a higher BMI. It was reported that the patient who received that microbiome also developed a higher BMI and associated obesity pathology. But this is a case report. I think no one has ever observed such a phenotype in large study. Actually, FMT has been a very, very safe modality for recurrent C. diff.

5. **By eliminating fat intake, will that decrease (in your model system, I presume) bile salts in the intestine, thereby decreasing cancer risk?**

   Matam Vijay-Kumar, Ph.D.: It is an excellent question. Actually, the diet we used is not a high-fat diet. It is a regular 10 percent fat diet. We didn't go below 10 percent fat. Already the TLR5KO mice, which developed liver cancer, were on the verge of essential fatty acid and vitamin D deficiency because of cholestasis. I think, giving a completely fat-free diet or

low-fat diet may reduce liver cancer risk, but it may increase the risk of essential fatty acid and fat-soluble vitamin deficiency.

6. **Have you yet been able to examine the ICB microbial signature in humans?**

   Liza Makowski, Ph.D.: No, we have not been able to do that. I think that would be fantastic in the same way that you could look at a gene signature in different subtypes with survival. We have not been able to do that. One thing I found in the field—as I come from kind of a different angle since I'm new to the microbial field—is that there are so many different papers, and there's lots of contradictory or inconsistent information. Even in our own study, when we looked at jejunum compared to cecum, there were different responses in terms of obesity or response to immunotherapy. I think we have to be a little bit more careful in our comparisons and be really clear when we're publishing with regard to details. How we're treating our mice, the diets, and where we're isolating microbes from must be spelled out. There need to be additional studies for consistent findings across models, so we can summarize things in a clear, consistent way across different models and then compare that to patients.

   Christian Jobin, Ph.D.: If I may add [a comment] because it's a very important question, and Liza is right. I mean, there is not that much clarity as to what is driving a responder signal. If you want to benchmark that, what is driving responsiveness or non-responsiveness, [with a] benchmark that could be used in the clinic as a tool for screening patients, the answer is a flat no. But there is data out there. We did a couple of machine learning analyses of published data for PD1 treatment in humans, downloaded all the data, looked at signals, and there was no clear predictability in the phylogeny—if you use phylogeny as a signal—entry signal to the machine learning, no predictability. In the gene function, a little bit but not enough to say, I could screen now. So I think we need bigger cohorts. The geography of this cohort is not homogenous, so you have cohorts in Europe, Asia, different parts of the U.S., and they are relatively small, 100, 150. So, it's been difficult to get clarity on that. But there's always a signal coming all the time, you know, with fecal bacteria, Ruminococcus, Akkermansia. Those are the suspects, and they are used for treatment but not for predictability. So, there's more work to do for sure.

7. **What is the impact of a high-fat diet on large bowel transit time and hence the ability of colonic bacteria to form particular metabolites? And how does this change when moving the obese patient to a more healthful diet?**

   Christian Jobin, Ph.D.: Wow. I have no idea. That's interesting; I mean, I will not even know for a mouse, so even less for a patient. If the transit is increased, we know that the microbiome, if you start having a very fast transit and you look at microbiome, it would be very different because these communities don't have time to set up some sort of a shop. They're always in constant motion. If you do a microbiome analysis on a specimen that has

very high-transit, loose stools, you will have a very different population than if the person has a slower transit. Now, how does it link to a high-fat diet and different movement? I don't know. I have no idea. Maybe the other panelists will answer that.

Matam Vijay-Kumar, Ph.D.: I have a comment on that. If a person takes a high-fat diet, some of the fat may not be absorbed. So, they may display steatorrhea and this can ease bowel movement that prevents constipation. There is a high chance that such a change in bowel movement can affect the gut microbiota and their metabolites.

8. **How to delineate whether gut microbiome changes cause changes in a host's inflammatory status or the host's inflammatory status causes gut microbiome changes? This is the cause-and-effect question, I suspect.**

Matam Vijay-Kumar, Ph.D.: I think inflammation can cause changes in microbiota composition, and microbiota dysbiosis can cause inflammation. Microbiota dysbiosis is kind of a catch-all term, whereby even small alterations in microbiota composition at the taxonomical level can be regarded as microbiota dysbiosis. So, I think both are possible. In our mouse study, we demonstrated that the transfer of gut microbiota alone is sufficient to cause metabolic syndrome by inducing low-grade inflammation in recipients.

Christian Jobin, Ph.D.: That's correct. I go back to IBD inflammatory bowel disease, as a flagship of studies in microbiome and host response. Experiments have been done by different groups where they take feces from a patient in remission or patient with active inflammation. You put that in a model. Then you could see and read some inflammatory response to the host. The host was fine up until it met these microbes, and then the host reacts in a way that—a kind of phenocopy where the microbe was coming from, which means inflammation or no inflammation. What I was showing here is that if you target inflammation with molecules, then the microbes change. Microbes could induce inflammation. Inflammation could change the microbial composition. If you see that as an amplification loop, and somehow you disrupt that loop, you may gain homeostasis eventually. IL1, NSAIDs have been studied for inflammation/microbiome interaction. So, I don't think microbiome is an epiphenomenon of inflammation but rather an amplification component.

Liza Makowski, Ph.D.: If they're asking how to do some of these studies to answer this question, one might think that you could conduct antibiotic treatments or fecal microbial transplants. And then if you want to look at host changes, you could either do some sort of inducible knockdown or knockout before or after these antibiotic treatments or do a pulldown of an immune cell, like if you're going to pull down a CD8 T cell to determine its role in a signaling network. And then like Dr. Jobin showed, anti-TNF or there's other drugs out there for IL1.

9. **Can you talk more about practical applications or potential future applications of the information and research discussed today?**

Matam Vijay-Kumar, Ph.D.: Based on our mouse studies, we propose that people with microbiota dysbiosis should not take too much of fermentable fiber foods. Of course, it is premature to conclude that fermentable fibers are bad. Nevertheless, it is a well-known fact that IBD patients should abstain from fermentable fiber foods, like FODMAPS, that can aggravate IBD flares, abdominal cramps or flatulence. In our mouse model of liver cancer with gut microbiota dysbiosis, we found that purified fibers can cause liver cancer, thus underscoring that all fibers are not made equal and neither they are universally beneficial to all. In contrast, whole foods containing inulin such as whole chicory or Jerusalem artichoke, which are an excellent source for inulin, do come with a natural inhibitor for bacterial fermentation, i.e., difructose anhydride, which can limit the fermentation and protect from side effects.

Liza Makowski, Ph.D.: It is hard to understand exact roles of the microbiome. But for example, it appears we know obesity can induce immunosuppression, which is actually beneficial for some immunotherapy responses. But we don't want to recommend people being obese when they have cancer therapy. The therapeutic concept may be along the lines if we can mimic or induce some transient signaling pathway to invoke the "obese-like" state, that we're getting clues from the microbiome and clues for these metabolites or inducing crosstalk leading to this immunosuppression. So, we then just use that intervention as a transient therapy when patients are going in for immunotherapy to improve efficacy. Those are examples that could be practical applications that we can learn from looking at our mouse models, comparing that to patient models.

Stephen D. Hursting, Ph.D.: Along the same lines in terms of cancer risk, it's clear obesity is driving the risk of many cancers. Estimates are on the order of 20, 25 percent of cancers you can attribute to obesity. It's a major factor. We've got roughly 43 percent of our adult population now in the obese category. Not all of those are going to be metabolically dysregulated, but a good chunk. And so, this is a major, major problem. Obesity has become probably, arguably the most important preventable risk factor for many of these cancers. It's a major contributor, and it's very difficult to lose weight. I mean, that's a challenging thing. I think, as Liza said, we're not there yet. But if microbiota can be a target that's easily manipulated, and we can figure out how best to do that, then maybe we don't have to lose weight to reverse this obesity problem that we're in, in terms of cancer. And so, I think that's the promise here. It looks like of all of the pathways leading to cancer, it seems like the microbiotal-related ones may be as responsive to diet as anything we study. I think the promise is that we can come up with a way to healthfully change the microbiome in ways that will prevent—kind of disconnect—this obesity–cancer connection. And hopefully a combination of not only the number of calories we consume but maybe the types of food

that we consume, the better, to more healthfully shift that microbiota is going to get us out of some of these issues that we're facing with obesity and cancer.

10. **How might exercise-induced differences between obese and nonobese microbiomes play a role in addition to diet?**

    Stephen D. Hursting, Ph.D.: We usually don't just eat in isolation in any other thing we do. I've been kind of complaining that the nutrition people don't factor in exercise sufficiently, and the exercise people don't factor in diet sufficiently. We really need to get a little more together on that because that is clear—that combination of healthful changes in physical activity and diet are the way to go. We tend to isolate them, and we need to stop that and put them together.

    Christian Jobin, Ph.D.: A safe take-home message is we can't ignore the microbiome when we think about different diseases. Any intervention on a pathology, be it exercise, diet, etc., is bound to alter the microbiome. The real question is the consequences of these changes, and I think more is needed to address that.

11. **Christian, you mentioned you're looking at microbiome transcriptome, metabolome. Liza, you mentioned that there's multicompartment at play here. And so, there's crosstalk between adipose tissues and immune cells in the microbiome. So, how do we get better at integrating these multicompartment, multilevel types of regulatory factors? Because, it seems to me, we've got to get better at that to start to accelerate the pace, particularly in translational research.**

    Christian Jobin, Ph.D.: For sure, the 16s have been so cheap now that it's just a baseline. You do it because you can do it, but you don't gain that much clarity. Whole genomes getting cheaper, so you put that in the integration, but yet it's static pathways. You don't know if they are functional. Then you say, well, I'm going to have RNA seq, so I will know which genes are induced in the community. So now you're stuck with a big dataset that you try to make sense of through integration of multidimensional data. So, it's not always giving you a clearer understanding. And the metabolites to add to the function. So, the more we add on, sometimes I think the less it becomes clear. Most companies right now are working on microbes that they could identify as a supplement, and they are not targeting microbial genes. They're not targeting a microbial metabolite. They want to replace an ecosystem or do cocktail designer. So, at one point, we'll have to decide, if getting larger data sets contribute to better understanding. Maybe AI could come to the rescue.

    Liza Makowski, Ph.D.: It falls to us when we're publishing that we have to be clear with the mouse models, ages, sex, and where we're getting the microbes from. And then when we're isolating maybe from tissue, we need to be clear about the controls we're using.

Increasingly, I think, as we delve more into the details, then we can start comparing and collating our data. That's the burden of the journal as well as the investigators.

Matam Vijay-Kumar, Ph.D.: I agree with Dr. Makowski. It is already in practice in basic science research. We need to stringently control animal housing conditions, including diet source (regular or sterile), type of bedding material, nestlets, and water. For instance, providing acidified water or regular water to animals can substantially impact microbiota composition. So, all the information should be clearly mentioned in the methods that will help to compare between the studies and [allow for] reproducibility.

Stephen D. Hursting, Ph.D.: There's actually international effort on that. It's the ASPIRE guidelines, and most journals are now picking that up. But it's worth all of us taking a look at that from time to time and instilling that in our students to have that front and center. It's a great point.

12. **Are individuals who have gone through cycles of weight loss and obesity accounted for in these type of studies (also as compared to those who have had surgically induced weight loss)?**

Christian Jobin, Ph.D.: Related to microbial community? Yes, losing weight has an impact on microbiome but it is not clear if intervention modalities (diet, exercise, surgery, etc.) [produce] different impact microbiota. For example, microbial diversity is typically low in obese patients, so would intervention such as adjustable gastric banding or Roux-en-Y-gastric bypass restore this diversity? While most Roux-en-Y-gastric bypass patients remained with low microbial gene richness post-surgery, these patients displayed more pronounced clinical improvements of metabolic function than banding surgery. Not clear why, but this may be related to the capacity of intestinal microbiota to produce a drastically different set of metabolites regardless of diversity.

Liza Makowski, Ph.D.: I do not think that there has been enough history to capture weight cycling in patient data yet. Capturing food intake and patient-reported data are often fraught with measurement or memory bias. Perhaps with more digital scales communicating with smart phone apps, more accurate data can be captured for patients. Some work out of Vanderbilt by Alyssa Hasty addresses weight cycling or metabolic cycling and supports that cycling is a less healthy metabolic state (Cottam et al., 2018).

13. **Please recommend anti-inflammatory foods and supplements for assisting with gut health and anti-inflammation.**

Christian Jobin, Ph.D.: Precision nutrition related to a microbiota and specific beneficial outcome is still a work in progress. I can't really recommend any specific modality but a general concept about complex fiber intake, low sugar and salt intake all have roots in the

microbiome. Dr. Kumar showed that some SCFA may be detrimental for liver cancer, so there may be a context-dependent relation with microbial outputs and diet.

Liza Makowski, Ph.D.: I always preface any statement by stating that my degree may be in nutrition but I am not a dietitian. Following guidelines from physicians and public health groups such as American Heart Association or American Diabetes Association is a good place to start. Asking your physician for a referral to a registered dietitian is the best place to start for nutrition advice by a trained professional. Avoid online or social media fads. A simple fact that whole grains, fruits, and vegetables are better than taking a supplement is important to remember. Supplements are not FDA-regulated, so there is zero control of what is being sold, whether that bottle costs $3 or $300 from a friend or from a fancy grocery store.

14. **For the studies showing that higher obesity is associated with better survival, what is the comparison group? If compared to lean patients who may be lean because they have more aggressive disease and are losing weight, then disease aggression is a confounder. How is this accounted for in these studies?**

    Liza Makowski, Ph.D.: I did not conduct these studies. Your concern is an important one about sicker patients with perhaps weight loss from cachexia may be confounding the comparison to obese patients. Most studies use BMI to determine weight and without reviewing each study, I am not sure of exclusion criteria or determination of, for example, sarcopenic obese patients, etc. More controlled and standardized work needs to be done to determine adiposity and lean mass accurately (cost effectively and rapidly), quantify inflammatory mediators, changes in body weight, and recent or habitual dietary intake to properly address this question about confounding.

15. **Other than differences caused by diet, are there exercise-induced differences between obese and non-obese microbiomes?**

    Liza Makowski, Ph.D.: Some recent human and mouse work on exercise can be found here: Yang et al., 2021 and Dupuit et al., 2021.

16. **Has anyone studied the effect of any widely used/available human dietary probiotics on reducing cancer in mice or other models?**

    Liza Makowski, Ph.D.: There is an RFA called Bugs and Drugs. We should see many papers coming out soon on pro- and pre-biotics. Some work has shown that individual microbes provided to mice or content correlated to patient outcomes improve immunotherapy. Akkermansia muciniphila improved sensitivity to immune checkpoint inhibitor immunotherapy in murine models. Higher Akkermansia muciniphila content correlates with

immune checkpoint inhibitor therapeutic response in breast cancer (Routy B, Le Chatelier E, Derosa L, et al., 2018) and (Goedert JJ, Jones G, Hua X, et al., 2015).

17. **Is there research inquiring into differences in cancer-related pathways in obese patients versus overweight patients? How relevant is BMI in patients?**

    Liza Makowski, Ph.D.: BMI is convenient for population-level or high throughput-type analyses for many human subjects. BMI is also imperfect due to individual differences in lean mass (muscle mass) or adiposity (fat mass). It also doesn't capture adipose distribution (visceral or apple shape vs subcutaneous or pear shape). Waist-to-hip ratio, bioelectrical impedance analysis, DEXA, or MRI are used to capture adiposity and weight with varying degrees of accuracy. Therefore, when reading any literature, paying close attention to measurement, discussion of measurement error, and definition of obese vs. overweight vs. lean, and how subjects are stratified is critical.

18. **Very interesting discussion on the cause-and-effect issue and how to incorporate that into study design. If designing a human study, how best to ensure that the patients don't already have microbial-related inflammation?**

    Christian Jobin, Ph.D.: Microbiome analysis are one component of human studies/clinical trials, which typically include blood, serum, and tissue analysis. So inflammation is not called solely on microbiota feature.

    Liza Makowski, Ph.D.: I would say that it is hard to rule out baseline inflammation unless you have a standard cut-off or biomarker. A longitudinal study of the same patient or subject over time will help determine if interventions are effective to increase or decrease microbial-related inflammation.

19. **To study gut microbiome in cancer patients, there is a logistic question about collecting fecal samples before the patients receive any treatment to minimize treatment impact on gut microbiome analyses. What logistic methods do you recommend for collecting fecal samples from cancer patients in a timely and patient-compliant manner?**

    Christian Jobin, Ph.D.: Longitudinal studies are powerful since each subject served as internal control, but compliance is an issue. Need to make stool collection as "friendly" as possible (prepackage, easy collection kit). We used in the past FOBT card that could be smeared and sent in an envelope if DNA is needed.

    Liza Makowski, Ph.D.: Agree with Dr. Jobin, if collections can occur before therapy, after therapy, and at different times for follow-up, this longitudinal approach is ideal.