OBY: From the campus of Harvard Medical School, this is Think Research, a podcast devoted to the stories behind clinical research. I'm Oby.

BRENDAN: And I'm Brendan, and we are your hosts. Think Research is brought to you by Harvard Catalyst, Harvard University's clinical and translational science center.

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BRENDAN: Heart valve diseases affect roughly 3% of the US population. For those with heart valve disease, open heart surgery to replace the valve is the most common treatment. Unfortunately, open heart surgery is costly, invasive, and extremely time-sensitive. Through a unique partnership with Japanese pharmaceutical company, Kowa, Dr. Elena Aikawa and her teams are using multidisciplinary cutting-edge technologies to find drug targets that will reduce the need for valve replacement, and the burden of heart valve diseases.

Dr. Aikawa is the director of the vascular biology program at the center for Interdisciplinary Cardiovascular Sciences, the director of the heart valve translational research program at Brigham and Women's Hospital, and a professor of medicine at Harvard Medical School. Dr. Aikawa, thank you very much for joining us. Welcome to the show.

ELENA AIKAWA: Oh, thank you very much. Very happy to be here.

BRENDAN: So you're the director of the vascular biology program at the Center for Interdisciplinary Cardiovascular Science, and you're also the founding director of the Heart Valve Translational Research Program at Brigham and Women's Hospital. Could you describe the goals of each of these programs?

ELENA AIKAWA: We'll be happy to. Let me start with the CICS, which is Center for Interdisciplinary Cardiovascular Sciences. So we launched our center in December 2009 at the Brigham and Women's Hospital in collaboration with the Japanese pharmaceutical company, Kowa. And this is a unique model of academic-industry partnership, which is focusing on target discovery for new therapies.

Despite high unmet needs for new therapies, the entire process, from target discovery to preclinical and clinical development of new compounds, takes a very, very long time-- sometimes decades. To speed the process, we establish a new paradigm of fully-integrated drug discovery research at CICS. And at CICS, academic investigators and pharmaceutical scientists from Kowa work side by side, which is unique. And the goal of the cardiovascular calcification problem at CICS that I'm leading is to develop new classes of drugs to treat vascular and valvular calcification through the speedy translation from the target discovery phase to the drug development.

On the other hand, the Heart Valve Translational Research program was launched in 2015 in recognition of the growing clinical burden caused by heart valve disease, and the uniqueness of the heart valve tissue. Because of that, the Division of Cardiovascular Medicine has developed a one of a kind program solely devoted to heart valve research. The mission of by Heart Valve Translational Research program at the Brigham is to increase awareness of heart valve disease, in order to stimulate research into disease mechanisms and therapeutic target discovery.

The heart valve represents unique and highly dynamic tissues. They are distinct from other cardiovascular tissues, and require a multidisciplinary approach to fully integrate and comprehend the various components of valvular pathology and physiology. Therefore, a fully integrated holistic approach is required to connect all aspects of heart valve disease. The Heart Valve Translational Program facilitates collaborations between basic researchers and clinicians within cardiovascular medicine and Brigham and Women's Hospital, and incorporates the strengths of engineering and network medicine throughout the Boston area, to establish a holistic research program dedicated to finding therapeutic options for patients with heart valve disease.

BRENDAN: So the CICS is in collaboration with Kowa pharmaceuticals, and that was started in-- what year did you say?

ELENA AIKAWA: 2009.

BRENDAN: 2009. OK. So then six years or so later, you started the Heart Valve Translational Research program. What was the inspiration for starting the Heart Valve Research program?

ELENA AIKAWA: That's a very, very good question, actually. When I came to the Brigham in 1991, my mentor was Dr. Frederick Sean in pathology department. I am pathologist by training, so I came here from Moscow, Russia, and joined the Dr. Sean Lab. And Fred Sean is a known researcher in heart valve disease, and he inspired me to look into heart valve disease, like those early times.

And I knew that for heart valve disease, there are no treatments except heart valve surgeries, and this is something that inspired me to develop this program to find therapeutic options. And I can talk about the [INAUDIBLE] later.

BRENDAN: OK, great. And so the work you're doing-- you're focusing on heart valve disease, and also specifically calcific aortic valve disease. Can you tell us about this disease and how it's currently treated?

ELENA AIKAWA: The various heart valve diseases combined affect almost 3% of total populations in the US, and this is more than 7 million Americans, with an incidence of greater than 10% in elderly patients. The aortic valve stenosis occurs due to development of calcific aortic valve disease, which characterizes by formation of large calcific nodules involving leaflets. These nodules caused leaflet stiffness and immobility, which results in heart failure, and there is no drug therapy to prevent or treat calcific aortic valve disease. And only effective treatments are invasive and costly-- open heart surgery-- or transcatheter valve operation to replace the aortic valve.

And if valve is not replaced within next two years after the onset of symptoms, the patients could develop a heart valve failure and die. And the outcomes of heart disease, or calcific aortic valve stenosis are worse than in many invasive cancers, so it's devastating disease. And imagine if you have a patient who is 30, 40 years old, and she had open heart surgery with a very big scar from throat to abdomen, and it's not very pleasant for a woman, and for a man as well.

So it's very difficult to live with this scar forever, and we still don't have means to create the bioprosthetic valve, which can last the whole entire life of the patients after operation. And most likely that patients after surgery or replacement of heart valve with bioprosthetic valve will need to have second replacement within 10, 12 years. So if you're young and healthy valve placement, most likely, you will need to have another one.

So now, surgeons and companies who develop devices develop transcatheter valve replacements, which also involve practically the same kind of valve, bioprosthetic valve, which are used for open heart surgery. Those valves will most likely not survive for a long time in the patient, and would calcify the same way as the bioprosthetic valve calcified within maybe even a shorter period of time. So that would require another operation or another transcatheter valve.

BRENDAN: OK. So you're saying that even the replacement valves suffer from this calcification that a natural, biologically-- or, like, the original valve would suffer from?

ELENA AIKAWA: Yes, exactly, and that is a big problem in the field. And it still is not-- we don't know how to deal with this problem yet. Many various labs and companies are working on it, but still there is no solution.

BRENDAN: And besides that-- you know, so even without the classification problem, people still have to get their replacement valves replaced again? It's not a-- if you do have a valve replacement it's not a lifetime fix.

ELENA AIKAWA: That's exactly what's happening. It's not lifetime [INAUDIBLE], and the patients most likely need another surgery. Or if a patient has chest surgery, so most likely, the patient cannot have transcatheter valve replacement when she or he older. Let's say the surgery was when she or he was 60, and then the next one is when it's 80, so it should be, again, second open heart surgery, because usually, surgeons don't do-- at least that's what I learned from literature, that surgeons usually don't do first open heart surgery and then transcatheter. So it's very hard. And

Imagine that if you pediatric patient-- same thing. You know, maybe a pediatric patient would have several open heart surgeries, because valves, which are made by companies, bioprosthetic valves, they're not growing with pediatric patients together, right? So the valve-- if patients, let's say 10-year-old, valve will not grow when the patient is 20 years old, so it needs to be played even if it's still functioning well. Because of that, we are working very hard at CICS and within heart valve translational research program to find some kind of drug which can prevent or slow progression of calcification. So that's my goal, and I hope to retire-- before my retirement, I will find some solution for that, and make some patients happier.

BRENDAN: Right. So like you said, you're working on a drug that can treat patients with this calcific aortic valve disease. And you talked about how the heart valve is a very kind of specific tissue. Can you talk a little bit about how-- like, what happens when the valve calcifies, and maybe what makes the heart valves so particular or hard to treat.

ELENA AIKAWA: There are so many aspects which make valve treatment is very difficult, and may involve issues very special. For example, heart valves contain specialized set of cells which are very heterogeneous, or very different, from one another. They have been shown to inhibit both genotypic and phenotypic differences from other cells within cardiovascular system. And these cells, interestingly, sense and respond to changes in the mechanical properties of the highly dynamic valvular tissues to maintain homeostasis.

You understand that valve is moving thousands of times within, you know, minutes. It's constantly moving. No other tissue that same thing, right? And under this pathologic pressure-- or even not pathologic pressure-- cells undergo osteogenic changes or osteogenic transformation. This is one theory. And the other theory that cells-- they release extra cellular vesicles that could serve as an [INAUDIBLE] of hydroxyapatite or calcium.

And it's interesting-- it has been reported by our pathology colleagues at the Brigham and Women's Hospital that osteogenesis process-- and by osteogenic process, I mean real bone formation, and sometimes, you can see the bone within the tissue, within the valve tissue, and you can see even bone marrow within that bone. So it's like real bone.

But this process is very, very rare, OK? So the other processes are more degenerative formation or nucleation-- of the calcium. And we're seeing that this second process is more common. For example, as I mentioned, our colleagues in the pathology department showed that bone formation is very rare in both bicuspid and tricuspid valves. Approximately 10% of patients will have, you know, bone formation. But the other have more degenerative abundant amount of calcium.

And interestingly, the bone formation never happens in bioprosthetic valves. So suggestion that 90% of calcification would occur through the extracellular vesicle associated mechanism. And in my lab, we study the formation of calcification through these extracellular vesicle-associated mechanisms. Every cell in your body releases extracellular vesicles. So you're sitting here, you're walking around, your cells are constantly release extracellular vesicles to keep them at stasis.

And vesicle cells are very good communicators between cells and extracellular matrix. So they're talking to each other, and with the cells and surroundings. When some pathological conditions occurs, like [INAUDIBLE], diabetes, or chronic kidney disease, when you have high levels of phosphate or calcium, so vesicles also start to have pathological cargo inside them, OK? And they tend to calcify, and they tend to nucleate calcium and phosphate and hydroxyapatite molecule, and become calcification.

So we've found that mitochondria is involved in this process. During mitochondrial calcium signaling, alkaline phosphatase activation kind of increases. And alkaline phosphatase is very important molecule, which kind of early marker of calcification. And this alkaline phosphatase would load to extracellular vesicle via molecule so called sortilin.

And we published this paper in *JCI* in 2016. This loading of alkaline phosphatase within the vesicles would increase extracellular vesicles' calcification potential.

And then another molecule comes, another molecule which we discovered in the lab, so called annexin I. And annexin I promotes aggregation of those vesicles. So imagine you have several vesicles, which are loaded with very highly phosphogenic potential, like alkaline phosphatase, and then they start to aggregate with each other and start to grow when they're trapped in extracellular matrix within the collagen or something like that. So this paper was just accepted to *Science Advances* a couple of weeks ago.

And we published another paper in *Nature Materials* several years ago, when we demonstrated that aggregated extracellular vesicles would kind of nucleate hydroxyapatite and form this microcalcification.

BRENDAN: So you're saying-- just to recap what you were just talking about. So the extracellular vesicles, they're cells, or they're particles that are moving around our bodies all the time. And when somebody has a disease, like kidney disease, diabetes, those extracellular vesicles have extra stuff in them that promotes this bone growth or calcification and--

ELENA AIKAWA: If they [INAUDIBLE] calcification potential, yes.

BRENDAN: Yes. And so when they get together, that's when-- or are they attracted to each other somehow because of their common contents?

ELENA AIKAWA: Good question. So we think what happens, that they express annexin I on the surface of extracellular vesicles. Because what is extracellular vesicles? It's actually mini-me of the cell, right? Because extracellular vesicles burst from the cell, which is already pathological, and the membrane is pathological as well.

So extracellular vesicles will burst from the cell. And this membrane of that vesicles contain annexin I. And annexin I is a [? tesserin ?] protein which promotes aggregation. So once you have expressed annexin I, then other vesicles would tend to aggregate with surrounding vesicles.

That's how we think they aggregate with each other. And with process of aggregation is very important for formation of microcalcification, because otherwise vesicles are very, very tiny. They're just 100 to 300 nanometers. Very, very small.

So the microcalcifications, I'm talking about approximately 15 microns. So they're much larger than extracellular vesicles. And in order for microcalcification to be harmful, they need to be certain size-- as I just mentioned, 15 microns-- certain shape, they need to be elongated. And once they elongate, they develop very high stress concentration from the pull.

And if they're sitting in the fibrous plaque, or other sorts of plaque, which could be very, very thin, it causes very high stress concentrations, high mechanical stress, and could lead to their rupture. So that microcalcifications are bad for other sclerotic lesions, but probably they don't affect so much valve dynamic. Right?

So but another bad thing about microcalcifications is that they can grow. Because if vesicles can aggregate, microcalcification can also coalesce with each other and grow, and eventually become larger macrocalcification, and this is what is harmful for the valve.

BRENDAN: So you've been working with Kowa for about 11 years, and you also work closely with scientists from Kowa who come to the lab and work alongside your postdocs and researchers. So I was wondering if you could talk about that relationship, and that model, and how common is that? And maybe what the advantages of that are and how it helps you in your work.

ELENA AIKAWA: Yeah, this is also very interesting and important question, because in our knowledge, in my knowledge specifically, there is no other incident of this kind of model, in US, at least, yet where academic investigators and investigators from pharmaceutical company working together in the shared space. So I think it's very unique.

And the reason why we actually came to this idea, why we're doing that, because to kind of make this gap between academia and industry smaller, or even don't have this gap, right? So because the scientists from Kowa are learning from academic people how to look broadly and how to create the mechanistic data, and we are learning from them how to do some sophisticated experiments which are fixated with targeted discovery.

In general, we maintain a very active and pleasant environment. Without this environment, without understanding each other within this small space, it's impossible to create something innovative. We just know boundaries between groups, and we, most of all, welcome out-of-the-box ideas. You know, anyone who come up with crazy idea, we are really, really excited, and we start discussing it and see how fruitful it would be.

For example, in my field, in calcification field, we lack animal models. So there are no very good animal models for development of aortic valve calcification, so we needed it to overcome it. And another problem in calcification field, that calcifications, or microcalcifications cannot be seen by imaging modalities, because they are so small, as I mentioned.

Extracellular vesicles approximately 100 to 300 nanometers, microcalcification, 15 microns. But all of these are still below the resolution of current imaging modalities, right? So that is very big problem. So we needed to create something so we can follow our formation of microcalcification.

And one of my postdocs, his name is Josh Hutcheson, he is now an independent investigator, assistant professor in Florida International University. But when he worked with me, he said, you know, why don't we try to use some hydrogels? And don't use the cells.

So he took the hydrogels, or collagen fibers, and then seeded those collagen fibers with vesicles which he isolated from calcifying some muscle cells. And then vesicles started to aggregate with each other and form microcalcification.

So because we could look at this close up, and because of availability of high resolution technology microscope in Harvard Imaging Center, we were able to kind of follow our formation of microcalcification, and for the first time, see how microcalcification form at the level of individual extracellular vesicles. So no one done them before, but we did it, and now it's a widely used technology.

Another technology which I really like to use, and now I think it's used not only in our group, but also around the world, is so-called molecular imaging. So molecular imaging applies molecular imaging agent, which has fluorescence. And once it binds to, for example, to hydroxyapatite in your body-- it could be bone, or it could be calcification, it could be microcalcification.

It binds to it and provides fluorescent signal. And then you can just look at this signal in vivo, while mouse is still alive. You know? You can also have a section from the certain tissue, and if you have any signs of calcification, you would see fluorescence in this calcified particle. And you can also use the same agent for in vivo cells. And we could also use it even for extracellular vesicles.

So these kind of ideas, which can help you to look deeper in some calcification aspects, are always very welcome.

I always look for postdocs in my group who would have very diverse backgrounds. For example, several people, including Josh Hutcheson, and I have two more, Samantha Hopkins and Mark Blaser, who has biomechanical background. And it's different from, for example, background of Marcus Rogers, who is also in my group now, who has background in inflammation lipids, and so on. So combining these backgrounds sometimes gives you very unique ideas and provides some more creativity.

We were thinking about this from the beginning. And Masanori Aikawa, who is founding director of the center, he has this kind of idea from the beginning that we need to work together. We need to work within the same space. We need to learn from each other.

There are many enterprises when academia works with company, right? The company would send the call and say, OK, we're interested in some new target for, let's say, calcification or inflammation. And the academic investigators would send their proposals to the company. Company will choose which proposal is the best, and fund this investigation from certain groups, right?

But this is different. We work so close, we are the same group, right? We are the same family. We have people who represent Kowa at the center, who are administrators, and we have our own administrators, so they kind of communicate with each other very closely.

We have videoconferences bi-weekly or monthly with Kowa scientists when we need to describe them what we found. And in parallel, they doing [INAUDIBLE] screening in Tokyo and they tell us what they found. So it's very dynamic exchange between Kowa and us.

The idea was from the beginning, and I should give all kudos to Masanori, who came up with this. And I think through his passion, persistence, and deep understanding of Japanese culture, he is successful in leading this enterprise. You know, it's very important, understanding of Japanese culture, you know? If you have someone who is not really understanding deeply in the Japanese culture, it would be very, very hard, I believe, to have this center and make it successful. I think within 10 years we really became one of the successful labs at Harvard.

BRENDAN: You mentioned the importance of understanding Japanese culture, and I was wondering if there is anything that you could share about that, like any specific things about Japanese culture that you find very important, or that's necessary for your organization to understand.

ELENA AIKAWA: Yeah. I think two different things. First of all, it's industry, right? It's pharmaceutical industry, which is totally different from academia. Academic people like freedom. They like to do what they like to do. And usually, pharmaceutical companies are telling you what should be done and how it should be done.

And we see these differences between the academic postdocs and Japanese investigators. How Masanori was able to communicate with Japanese side, with industrial side, needed, I believe, very specific understanding, as I mentioned, of Japanese culture, of Japanese people. And understanding Japanese language, and speaking that language fluently and with understanding all nuances.

For example, for me, sometimes it was very hard to understand what the other party wanted, because Japanese people are usually very polite. But sometimes you don't know if you're talking about same thing and they really understand what you're saying, because they would never tell you "no." OK? So it was very hard.

And now, for example, I learned a lot, and I have lots of Japanese scientists who I'm working with. They are kind of my post-doctoral fellows. And first thing when I interviewing them, I would tell them, please, if you don't understand what I'm saying, please tell me, Elena, I didn't get it. Please repeat again. Please say it again. Don't hesitate. This is very, very important.

And until I'm sure that they understand what I'm saying, and I will use paper and pen and draw things. It's kind of like memory notes. You know, like brain map, if you call it. And I would draw everything, and they will leave with this note. And then every time we can come back to it and say, OK, we were discussing this. Did you get it? What did you do? You know, just to make sure.

So it's been hard. But, you know, positive thing about this, that when Japanese fellows are coming in the first year, this is a process, how it goes, like, it's hard, right? We need to make sure to understand each other. But within a year, within two years, three years, they become amazing.

They become fluent in English, they can write papers, and I have several who wrote papers as the first author, with just a revising one, for *ATVB,* which was written by two Japanese fellows. And they can present well, and they can communicate nicely. They become global scientists.

So when they go back to Tokyo, when they go back to Japan, when we have videoconferences, they're sitting now, my fellows are sitting on the other side, and Masanori's fellows are sitting on other side, because they became leaders of the group in Tokyo.

And we are talking about how to make progress, you know, about our progress, about our research. But now they are not fellows anymore. They speak perfect English. They are leaders in the Kowa and they have global vision. And I think this is a very, very important training process which those scientists undergo at our center.

And I think this is our contribution. I believe we trained probably 40, 50 scientists already, and I think that's enormous contribution from Masanori and my effort as well.

BRENDAN: What do you see going forward the next 10 years? What are your hopes and goals, for either CICS or the heart valve translational research program? What are your goals looking forward the next five, 10 years?

ELENA AIKAWA: I'm glad to say that we just signed our agreement and continue for another 10 years, and I believe this is an enormous accomplishment. And it's very important for Kowa and for us as well.

My major goal is to develop a first-in-class drug to treat cardiovascular calcification, particularly valvular calcification. And this is something which is, I don't know if it's possible at all, but I would work for next 10 years just focusing on that at CICS. And if we create even one single drug before my retirement, I would think that I accomplished my dream and I did something very important for patients, and for myself as well.

BRENDAN: Great. Well, Dr. Aikawa, thank you very much for talking with us. It was a pleasure to have this conversation with you.

ELENA AIKAWA: Thank you.

BRENDAN: Thank you for listening. If you've enjoyed this podcast, please rate us on iTunes and help us spread the word about the amazing research taking place across the Harvard community.

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