

## CHAPTER IX: PECTIN

What I'm going to quickly do is to give you a sense of the anti-tumor affects of carbohydrates, focusing on pectin, but I want to draw a parallel between glycosaminoglycan and pectin, because much of our work began focusing on the important roles of GAGs in cancer biology.

And, this slide essentially summarizes that eventually got us to think about the role of GAGs in this process and how similar kinds of molecules play a role in regulating various biological processes.

So, here is a cell, a representative cell, with the glycosaminoglycan chains. So, one of the things that we found, and I'm summarizing several pieces of data that came together in this slide, is the fact the tumor cells expressed unique distinct sequences, and I've just illustrated them as green and blue here. Sorry, green and red here.

The green sequences of GAGs are those that enable tumor cells to achieve a phenotype where they can rapidly grow. On the other hand, the red being those that can actually dramatically inhibit these tumor cells to proliferate. What we found when we analyzed a whole host of different tumor cells is that these tumor cells had a balance between sequences that enabled these tumor cells to dramatically acquire the phenotype property to grow, with those that would inhibit these tumor cells from growing.

So, in other words, the way these cells express these polysaccharides on the cell surface, it was not only an ensemble, but an ensemble where you had structures that could activate growth factor signaling, leading to dramatic proliferation of these tumor cells, or fragments that inhibited the presentation of these growth factors.

So, it gave us a sense that it's very important to frame the property of the sugars on these cells, the function of sugars in regulating the various signaling pathways. We're looking at proliferative pathway, we're looking at apoptotic pathway, you're looking at migration in a more holistic way, rather than individual pieces, and the fact that these compartments do not, because, obviously, they were being displayed on these cell surfaces in a very interesting way.

So, we have done studies, and we're continuing to do studies with the glycosaminoglycan family of these, of heparin, heparin sulfate, to truly understand how various heparin preparations could be derived to modulate tumor growth, obviously, with the point of view of keeping inhibition in mind. And, different heparin fragments are screened, and one of the interesting properties we observed is that they seem to be tumor specific, if you take lung carcinoma and compare it with melanoma, there are distinct classes of these GAGs having different endpoints. And, part of what we're now trying to do is understand the structure function relationship and be able to figure out what kinds of pathways are affected. Obviously, depending on what the tumor type is, the pathway is going to be pretty distinct.

So, that got us to think about pectin because there were these observations made in literature that, if you take the peel from citrus fruits, whether it's lemon or orange, and these are, again, complex molecules that I pointed out earlier, not only do you have a smooth region, but you also have a heavy region. In other words, you have a linear part and you have a branching part. And, the variety of different molecules that add to this complexity, because they are different kinds of building blocks that are used by plants for cell cell communication.

But, the interesting thing about pectin is they have several galactose binding moieties. And, the way the galactose residues are presented in these pectins, in the context of these two domains of these pectins, the biological functions or the biological consequences would be different. Analogous to the glycosaminoglycan example that I spoke about.

So, this was a challenge. Because, part of what we wanted to do was get to the structure of these molecules and try to correlate how different parts of components of pectins were truly influencing the biological activity.

And, I just want to give you a quick flavor of where we are with this work. This is R21 and then IGMS, they recently funded us. But, part of the idea was to access the structure of these molecules to eventually get to the biology.

Just to very quickly tell you, we have been able to generate a variety of different pectin preparations, starting from the basics. We instead of buying pectin from GNC or sigma, where we actually screened for a variety of different activities, we found that, depending on who was selling the pectin, the activity was all over the place, because it wasn't standardized. Our first goal was to figure out how to standardize. But, we also began doing that by saying let's go to the source, let's go to the peel. Imagine if you had a day here you could think of isolating the peel, doing a twenty minute microwave or twenty second microwave, followed by a hydrolysis, and then you could start structurally characterizing the various fragments, and then begin to tease about the biology.

But, I'm going to give a quick summary here. At a fairly nontoxic concentration we found that these different fractions of pectin were indeed having anti-tumor affects, whether they were delivered IV or IP. And, one of the interesting

observations we also made was the fact that it seemed to affect angiogenesis, as you can see this picture.

A point that I made earlier is that there's a whole family of galactose binding proteins that are present on cell surface, intercellular parts of the cell and also the extracellular part of the cell, it's called galectins. And, we found that, when depending on that pectin fragments we chose and how we chose them, shown here with galectin-3, their distribution was dramatically affected. And, not only do they affect the tumor cells, but they also affected the antigenic properties. You're looking at a normal vessel, and a vessel with the pectin treated, you can see how the endothelial vessel compartment was affected.

So, the point I'm trying to make was not only was the tumor compartment affected, but the endothelial compartment was affected, and there was a broad role for these molecules, depending on what fractions, what structures, and hence what the target is.

Where we are, I want to very quickly summarize, is the fact that there's a whole host of galectins, galectin one to fifteen, and they have different galactose binding domains, which I said is a key part of the pectin molecule. Galectins play a role in mitogenic property. They play a role in cytostatic properties. They play a role in apoptosis, this cross talk between these two. And, the way these molecules act on the cell surface is form a lattice, so that when you have these pectinic molecules that you present to these cell surfaces, depending on the composition and the epitopes that are presented, you're potentially activating a set of galectins, and the question is which galectin has the

appropriate effect and how do you begin to understand these fairly broad effects not only on the tumor cells, but the endothelial compartment.

So, where we are right now is we really need to understand the specific fractions that affect galectin-1, those that potentially affect galectin-8 and galectin-9. And, we're trying to put a comprehensive picture of how do these pectinic molecules and the various compositions affect distinct pathways, the cross talk between pathways, so that you have synergistic outcome of not only affecting the tumor bed, but the endothelial compartment.

So, we're at the fairly early stages of this work. But, I think what's really exciting is the fact that we've been able to access different structures of these molecules and we're now doing SAR in a methodical way to be able to get the heart of structure function.

But, where I want to go from here is to tell you that, looking at these kinds of molecules, the complexity associated with them, the fact that they modulate a variety of pathways simultaneously, it got us to think about looking at biological processes, not in a reductionist fashion, but how can we take a more integrated way, looking at not only the tumor compartment, but the endothelial compartment, so that we can think of a more effective way for cancer therapy.

What I'm going to show you in the next couple of slides is a process that we put together of taking off the shelf drugs, chemo therapeutic drugs, and anti antigenesis drug – came up with a nanotechnology strategy

of bringing the pieces together so that we could affect biological processes and how glycans in general are modulating.

So, just a very quick primer with regard to cancer biology is that you need the process of transformation to occur with tumor cells. Then, once you have transformation for progression, you need the process of new blood vessels that not only bring nutrients to the primary tumor cells, but enable these tumor cells to escape, leading to the process of invasion and metastasis, and these three pieces really need to be thought of in a more holistic way, when you're looking at cancer biology and cancer progression.

So, with that in mind, what we began focusing, at a time when there were questions asked about chemotherapy and anti angiogenesis is the fact that chemotherapy is toxic, and what does anti angiogenesis therapy do. And, this was something that Judith Folkman proposed in terms of not only doing the chemotherapy in regard to being a strategy for targeting the tumor cells, but as against going to the tumor cells, you attack the supply lines of the tumor cells, like what Napoleon did. Thereby, you starve these tumor cells. And, get to the tumors. But, there have been some challenges and problems with the strategy.

Once you starve the tumor cells, you create a hypoxic environment in the tumor that makes these tumor cells more metastatic, and, therefore, there was a new paradigm that was proposed, and this was right at the sweet spot, when we were looking at these various combinations of glycans and how to think about this problem in a more integrated fashion, that the best way to think about anti-angiogenic therapy is a way to combine anti-angiogenesis with chemotherapy, but leads to a fundamental problem, which is when you cut the supply lines off, less chemotherapy reaches tumor cells. More

needs to be administered. You actually have side effects and high systemic concentration of chemotherapy is a thing you want to do. So, essentially, you get into a catch 22 situation.

What we thought was, think about approaching this problem analogous to how glycans function. They attack different compartments; they are different in different ratios. There's obviously some sort of crosstalk. Could we integrate thinking of the tumor bed and the antigenic bed in some interesting way so that we can bring things together?

So, the idea was a balloon in a balloon. It was essentially an inspiration when the post doc in my group looked balloon inside a balloon, with the idea that several drugs, such as liposomal drugs to nano particle therapies, were beginning to emerge in terms of chemotherapy. Our idea was to create a nano cell, essentially leverages the vascular permeability property of the tumor micro environment, tumor sucks things in. And, then once things are trapped in. So, how does it really work?

So, the inner particle has the chemotherapeutic agent. The outer particle has the anti-angiogenesis agent. The idea being that, as against targeting two different compartments in a separate fashion, you're doing it in a more integrated way, analogous to having multiple things targeted at the same time.

So, what we did, just to very quickly tell you, is conjugated doxorubicin in the inner core and combrestatin on the outer core. Doxorubicin is a chemotherapeutic agent. Combrastatin is a vascular shutdown anti-angiogenesis agent. The whole idea being that we can look at rates, kinetics, of the various compartments, bring the pieces together, in

many ways, it was inspired by how glycans function, different structure, different sequences, modulate the rate and kinetics of various signaling molecules.

The long and short of it is we came up with a screening technique to look at both the tumor compartment and the antigenesis compartment so that we can develop a rapid screening approach to incorporate various different molecules, with the different release kinetics, so that we can affect the tumor bed.

So, here's a very quick movie that visually captures the whole thing. So, what you're looking at is a tumor growing. And, as you zoom in, here are the tumor cells that are going through the process of transformation into progression. So, they secrete angiogenesis factors that essentially bring in new blood vessels to the tumor micro environment. And, the tumors not only get the proper nutrients, but that's the way, it also provides the opportunity for the tumor to escape from that micro environment to metastasize.

In many ways, this is an extracellular event of how a variety of these processes, in many sense, are regulated by glycans.

Now, anti-angiogenesis therapy was exciting, because the thought was, if you shut the supply lines off, you could starve the tumors to death. But, when it's done in combination with anti-angiogenesis drug, with chemotherapy, the fact that you shut the supply lines off enables, limits the ability of these chemotherapeutic drugs to access the tumor micro environment.

So, that leads to these tumor cells to become more hypoxic, and then you have much more antigenesis.

So, here is nano cells, size match, to the micro environment. As I said, one of the permeable properties of tumors was exploited. This is the outer core and this is the inner core. The outer core has the anti-angiogenesis drug, the inner core has the chemotherapeutic drug.

Since rates, kinetics, concentration become important, the loading of each of these drugs become important, you have a range of possibilities in terms of how you can leverage the anti-angiogenesis part. Similarly, you can do that with the chemotherapeutic part. Now, given the vascular permeability property of the tumors, these nano cells get trapped in the tumor micro environment. The outer core releases the anti-angiogenesis drug, eventually leading to a vascular shutdown. And, then, the inner particles are trapped in this micro environment.

And, one of the interesting things you can do with the inner particles if you could have a pro-drug effect, that is, keep it inactive chemotherapy, natural product, activate it, depending on specific enzymes that the tumor cells make. In this case, we made an elastase of the tumor micro environment cleaved the linkage between the polymer and the chemotherapeutic drug, as shown here, with the idea being that once you have these enzymes that are secreted by tumor cells, they release the active chemotherapeutic drug, which eventually would, you know, affect not only the anti-angiogenesis bed, but the tumor bed.

So, the key thing really was the fact that, not only could we show through various combinations of what the efficacy of this drug, but the most important thing with the survival is the fact that, unfortunately, we have to stop these experiments in sixty days, given the way these experiments were done. But, the key point being that this kind

of thinking of integrating the various pieces truly had an affect on how to look at strategies for therapeutics, in large part, trying to frame it from the understanding of what are the roles of glycans and how glycans do.