From integrin-binding RGD peptides to vascular homing peptides

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This Japan Prize recognizes the discovery of the RGD cell attachment sequence and of the cell surface receptors that recognize this sequence in extracellular matrix proteins. The origins of the RGD story go back to when I was a postdoctoral fellow at Caltech 1968-1970. Some of the Caltech researchers postulated the existence of a zipcode-like recognition system that would guide cell positioning during development and in the maintenance of tissue architecture. I was already at that time interested in cancer, and it seemed to me that if a recognition system guiding cell movements and positioning really existed, there would have to be something wrong with it in cancer because cancer cells do not follow positional rules. I decided that this is what I would work on having established my own laboratory.

Together with Dr. Antti Vaheri, who later on became Professor and Chairman of the Department of Virology at the University of Helsinki, we designed experiments to isolate cell surface proteins that might mediate cell recognition. These experiments resulted in the discovery of a protein that was present on the surface of normal fibroblasts, but not on retrovirally-transformed fibroblasts. We also found early on that the protein was present in normal plasma. Together with Dr. Deane Mosher, who went to work with Vaheri, we later named the protein fibronectin. It turned out that ours is not the only claim to the discovery of fibronectin, several other laboratories could make a that claim as the identity of fibronectin became clearer.

We spent the next few years characterizing the properties of fibronectin in normal and malignant cells, and, in 1977, Dr. Eva Engvall and I found that fibronectin binds to denatured collagen (gelatin), a discovery that made it possible to isolate essentially unlimited quantities of fibronectin from plasma. Armed with this ability, we set out to study the functionally active domains of fibronectin, soon focusing on its cell attachment domain. This work really picked up in speed when a talented postdoctoral fellow, Michael Pierschbacher, joined the laboratory. Using a monoclonal antibody he made, we were able to isolate a small fragment of fibronectin that promoted cell attachment and that upon sequencing turned out to contain 108 amino acids. We next tested synthetic peptides that covered this sequence, and by following the activity and making the peptides shorter and shorter, ended up with an active tetrapeptide. We also showed that fourth (C-terminal) amino acid could be varied, making the key sequence a tripeptide-arginine-glycine-aspartic acid, or RGD. This peptide has since been shown to be a key recognition sequence for cell attachment in a broad range of species ranging from Drosophila to human.

We proposed that fibrinogen and collagens could function as RGD-dependent cell attachment proteins and that viruses might use fibronectin mimicry to bind to mammalian cells. We also suggested that RGD peptides might be useful in blocking adhesion-dependent biological and disease processes such as platelet aggregation, and invasion and metastasis of malignant cells. These predictions turned out to be accurate.

At the time, we discovered the RGD sequence, the cellular receptors that mediate attachment to fibronectin and other adhesion proteins had not been identified. Having the RGD peptides from the cell attachment site of fibronectin at hand, Robert Pytela, an Austrian

postdoctoral fellow in my laboratory, succeeded in isolating two RGD-binding receptors, a fibronectin receptor and a vitronectin receptor. These receptors became founding members among a family of receptors now known as integrins.

The fibronectin and vitronectin receptors recognized different protein ligands, but in each case, the recognition was based on the RGD sequence. I found this extremely exiting, it was exactly what I had set out to look for in 1970: a cell surface recognition system that would resemble immune recognition. It took 15 years, but the mission was accomplished and we were able to put together the story in a well-cited review published in *Science* (Ruoslahti and Pierschbacher, 1987).

RGD and the RGD paradigm have generated drugs that are used to treat diseases. The availability of the various RGD-directed integrins allowed us to show that RGD peptides could be designed to be selective for individual RGD-directed integrins (Pierschbacher and Ruoslahti, 1987). Other integrins are similarly inhibited by peptides with sequences related to RGD (an aspartic acid residue, in particular, is shared by the various integrin ligands). Indeed, pharmaceutical companies have developed RGD-type compounds that are far more potent than our original RGD peptides and are highly specific for a single integrin. A modified RGD peptide and an RGD peptidomimetic that inhibit platelet aggregation are on the market for prevention of restenosis after angioplasty. Compounds that inhibit the $\alpha 4\beta 1$ integrin are in use for suppression of inflammatory reactions, and inhibitors of the $\alpha v\beta 3$ integrin show promise as anti-angiogenic agents. Other applications are likely to emerge.

I have continued working on the same paradigm that led me to fibronectin and RGD: how do cells find their appropriate place in the body and what goes wrong with malignant cells that metastasize? We were using peptide libraries displayed on phage to identify RGD-type peptides for individual integrins, and it occurred to me that we could use phage libraries in live mice to detect vascular specificities that might be involved in tumor metastasis. Indeed, we have shown that every tissue we have analyzed puts a specific signature on its vasculature and have identified a tumor molecule, metadherin, that binds to lung vasculature and is involved in metastasis. We have also used the *in vivo* phage screening method to isolate peptides that specifically home to tumors, and have shown that coupling of drugs and drug-like molecules to homing peptides can increase the potency of the drug and decrease its side effects. The RGD sequence and integrins have already had an impact on clinical medicine. I hope that these new peptides and their vascular receptors will also prove useful in the treatment of disease.