[PHYSIOLOGY]

UNRAVELING THE WEB

Attacking undesirable veins and arteries could fight cancer and blindness

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Imagine veins and arteries that branch out, grow, split and spread. Normal during embryo development, in adulthood this formation and proliferation of blood vessels might be at the source of serious problems, such as blindness and cancer. The biochemist Ricardo Giordano, from the Chemistry Institute at the University of São Paulo (USP), has been finding ways of localizing and exterminating these blood vessels that sprout out of place and in an untimely fashion.

He has developed a peptide (protein fragment) that brings together highly desirable qualities in a potential pharmaceutical substance to fight these problems: the molecule is able to find blood vessels that should be created – and it does this circumventing the body's defenses, which are unable to recognize the peptide as an intrusive substance to be fought. The molecule, known as $_{D}$(LPR) because it is made out of leucine, proline and arginine, was the result of the work of a couple of Brazilian researchers that jointly coordinate a laboratory at the M. D. Anderson Cancer Institute in Texas, USA: the molecular biologist Renata Pasqualini and the oncologist physician and researcher Wadih Arap. During the course of 10 years of post-doctoral work in this lively environment full of equipment, minds and motivation to discover proteins that can have an impact on human diseases, Giordano used the ZIP cold concept developed by Renata and Arap: each type of cell, in each of the body's tissues, has a unique molecular signature that can be recognized by

specific peptides, just like the number 05415-012 designates a two-block area in which postmen will find the offices of this journal.

And this worked, as shown in the article in the March issue of the journal *PNAS*. To build trackers capable of overriding the radar of the immune system, Giordano used a conceptually simple trick based on the two categories of peptides, characterized for having certain chemical groups that go toward the right (D) or the left (L). "Nature chose to make proteins in the L form," explains the biochemist. Therefore, he chose their mirror image, D. As this is not found in nature, the body's immune system does not recognize it. It is as if the peptides that go around the blood and the cells were all left-handed. The enzymes in charge of destroying impurities, which are akin to left-hand gloves, do not fit on right hands and therefore allow them to escape. Thus, $_{D}$(LPR) goes undetected, yet it does not fail to fulfill the role of its mirror twin, RPL.

The task, in this case, is to inhibit the production of the vascular endothelial growth factor (VEGF), which is the substance mainly responsible for the proliferation of blood vessels. "However, one can't totally inhibit the activity of this growth factor: the VEGF's basal function is important in order to maintain the blood vessels," Giordano states. He therefore looked for a right hand that might affect only the generation of new vessels, which was successfully achieved by $_{D}$(LPR) on premature retinopathy, as the *PNAS* article shows,

This is the cause of the musician Stevie Wonder's visual handicap.Premature retinopathy mainly affects those babies that had to spend some time in an incubator when they were born. As the oxygen pressure is very high (about 70%) inside this type of equipment, when the child is taken into the natural atmosphere, with about 20% of oxygen, the retina cells interpret this as a shortage of oxygen and produce more VEGF. The outcome is a vascular web in the retina density so that it obstructs vision. Giordano showed that the peptide $_{D}$(LPR) can find such a formation of undesirable blood vessels, recognizing

specific molecules in the membrane of the vascular cells. "Because it's small, the peptide is cheaper to produce and the likelihood of causing side-effects is small, because the cell's exterior is the most selective part; therefore, the action is localized."

When it fits into the cell's surface, $_{D}$(LPR) interferes with the VEGF activation chain, thus inhibiting exaggerated vascular proliferation. In Giordano's trials, the system worked on culture cells and on live mice. Because it is small, stable and water-soluble, the peptide developed by the biochemist has everything to be successful in the eyes of those who suffer from this type of eyesight problems, if it eventually does, indeed, result in a medical drug.

THE PROJECT

Identification of new molecular markers in angiogenic retinas and rational design of new therapeutic agents for eye diseases with a vascular component - no. 2008/54806-8

TYPE
Young Researcher

COORDINATOR
Ricardo José Giordano - IQ/USP

INVESTMENT
R$ 774,669.76

Besides retinopathy among premature babies, vascular proliferation in the retina also causes wet macular degeneration, the main cause of partial loss of vision connected with aging. A $_{D}$(LPR) drug might perhaps be applied in the form of eye drops, which would be a relief as compared to the current macular degeneration treatment, which consists of injections applied directly on the eye. Wadih Arap has already had to have an eye injection due to a detached retina and has warned that it is awful.

Tracking a bomb - In the laboratory that he created last year upon returning from Texas and being hired by USP, Giordano is looking for new VEGF regions in mice that could function as therapeutic targets. The benefits of this could extend way beyond eyesight illnesses. Vascular proliferation or angiogenesis, stimulated by VEGG, is also what characterizes malignant tumors, which secrete angiogenesis factors to encourage the production of blood vessels that, in turn, feed the masses of cancerous cells. "If we manage to fight this process, which normally doesn't occur in adults, we will have yet one more weapon to fight cancer," predicts the researcher.

Attacking VEGF is not a new idea. There are some drugs against these factors, based on antibodies, that have been already approved and are in use, but, according to Giordano, they have proven to be less effective than scientists hoped and cause unpleasant side effects, a problem that he hopes to avoid with the targeted peptide that he has developed. "There are hundreds of labs worldwide trying to develop this type of medication; it's a race." For him, more important than getting there first is to develop a pharmaceutical product in Brazil. Not only to have more accessible drugs, but also to own the intellectual property for them, as this might aid further research.

One of Renata's and Arap's priorities now is to continue the tests to develop a drug based on the peptide developed by the collaborator from USP. "We want to establish in São Paulo a branch of the company to which the M.D. Anderson intellectual property is being licensed, in order to get partnerships and invest-

ment to develop drugs," the researcher tells us. One advantage of conducting the clinical trials here is testing the drug's effectiveness on the Brazilian population, a validation that is independent from the effects of the drug on a larger number of patients. In the future, $_D$(LPR) may also prove effective against tumor irrigating blood vessels, but the group has prioritized the study of eye diseases to avoid the huge competition around the war on cancer, whereas, in Renata's words, "there's a vacuum surrounding retina treatments."

The two researchers are in the right place. M. D. Anderson is a huge research center and hospital that specializes in cancer. Here, one of the world's benchmark reference centers where cancer treatment is concerned, and therefore a place that gets the toughest cases, the researchers have access to a large number of patients and huge scientific challenges. Besides doing research, Arap sees hospital patients. In the Texan laboratory, the two scientists that graduated from USP have been using the ZIP code idea to fight cancer and obesity. They have developed a prostate cancer drug that is in the initial stage of clinical trials on humans. "We have already treated six patients," Renata told us. This initial stage, with only a few patients, after the drug has been tested on other species – generally mice, dogs or monkeys – is mandatory in order to assess the treatment's possible toxic effects. By finding the drug in tumor biopsies, the study validates the notion of using ZIP codes against cancer and other diseases – a method that appears to be effective against fat cells, according to an article published by the group in 2004, in *Nature Medicine*.

"If we could fight angiogenesis, we'd have one more weapon against cancer," Giordano says

The peptide finds the specific molecular signature of the tumor or the fat and carries along with it a bomb – the klaklac molecule (*see* Pesquisa FAPESP *nº 115*). "It's a corkscrew-shaped structure rich in negative charges, which attach to the membrane of mitochondria," Giordano describes. Upon destroying the mitochondria, the cells' power plant, klaklak specifically eliminates the undesirable cells, such as the blood vessels that irrigate tumors. In an earlier research stage, Marina Cardó-Vila, a Catalan researcher, worked together with Giordano at the M. D. Anderson center, using similar techniques on different molecules. She showed, in an article also published in the March issue of *PNAS*, that an inverted peptide (D shaped) system, such as that produced by her colleague, effectively inhibits the growth of mammary tumors in female mice.

Breathing space – Besides pharmaceutical potential, Giordano's tracking peptide has also been shown to be an effective research tool. In collaboration with Rubin Tuder, a Lithuanian pathologist who graduated from USP and now teaches at the University of Colorado, he showed, in 2008, in the *Journal of Biological Chemistry*, that the technique allows one to find and destroy blood vessels that maintain the structure of the lungs' alveoli and cause lesions similar to those found in the lungs of smokers with emphysema. In this case, the peptides work as an anti-drug. The idea of this is to produce, in labs, mice with emphysema pulmonary characteristic, in order to study the disease in greater depth.

Tuder is now looking into using the method to help to diagnose pulmonary hypertension, characterized by the proliferation of cells in the vessels of the lungs. This, in Brazil, is linked with schistosomiasis (*see* Pesquisa FAPESP *nº 158*). Today, in order to look inside blood vessels, one must insert a catheter through the groin. The researcher's plan is to bind gold particles, for instance, with tracking peptides. Gold is recognized by CAT scanners, a far less invasive test than catheterization. "I'm trying to identify peptides that locate these lung lesions to aid diagnostic imaging," explains the pathologist. He has already found, in patients' cell cultures, promising molecules for this role and in another two months, he hopes to have further details to relate.

Although the method seems promising to treat major diseases, the researchers do not expect it to be a panacea and they certainly are not planning to inject klaklac loaded peptides to attack undiagnosed tumors preventively. "Cancer is a very difficult disease," comments Renata. "Steps taken are small, the benefits, incremental; but if one doesn't try, nothing will be achieved." ■

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Scientific articles

1. GIORDANO, R. J. *et al.* "From combinatorial peptide selection to drug prototype (I): Targeting the vascular endothelial growth factor receptor pathway." **PNAS.** v. 107, n. 11, p. 5.112-17. 16 Mar. 2010.
2. CARDÓ-VILA, M. *et al.* "From combinatorial peptide selection to drug prototype (II): Targeting the epidermal growth factor receptor pathway." **PNAS.** v. 107, n. 11, p. 5.118-23. 16 Mar. 2010.
3. GIORDANO, R. J. *et al.* "Targeted induction of lung endothelial cell apoptosis causes emphysema-like changes in the mouse." **Journal of Biological Chemistry.** v. 283, n. 43, p. 29.447-60. 24 Oct. 2008.