Code name: VEGF111

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VEGF111 is a new mutant of VEGF-A (*vascular endothelial growth Factor A*), which is essential to the development of the vascular system. In December 2007, The *Journal of Cell Biology* published an article written by the team of ULg researchers who discovered it. VEGF111, the source of both deleterious effects and therapeutic hopes, now begins its path as another of medicine's mysteries.

The most developed animal species are all provided with a vascular system. The principal actor involved in its development is a molecule called vascular endothelial growth factor A (VEGF-A), the founding member of the VEGF family, which also includes VEGF-B, VEGF-C, VEGF-D, VEGF-E and placental growth factor. VEGF-A is, however, frequently called by its "family name" (VEGF) rather than by its "given name".

Human beings possess approximately 30,000 *genes*, a number of which encode several *proteins* (more than 100,000 have been enumerated). Such is the case for the VEGF-A gene. A gene's ability to encode several proteins which may have opposing activities is, among other things, the result of alternative splicing. In this mechanism, pre-messenger RNA may produce different messengers RNA through the elimination of certain non-encoding fragments, the *introns*, to allow for the joining together end-to-end of the exons, which are generally encoding fragments. Now constituted solely of *exons*, the messenger RNA is then exported to the cytoplasm for protein translation.
Through the interplay of combinations in the selection of potentially encoding fragments of the gene and, correlativelively, in the removal of unretained fragments (i.e. introns), a dozen mutants of protein VEGF-A have now been identified: VEGF206, 189, 165, 121, etc. VEGF111 can now be added to the list of these isoforms. VEGF11 was recently discovered and investigated by researchers at the Laboratory of Connective Tissue Biology of the University of Liège (ULg)(1). This led to the publication of an article in the December 1, 2007 issue of The Journal of Cell Biology (JCB)(2).

(1) The Laboratory of Connective Tissue Biology is a member of the GIGA-Cancer Research Center.

Genotoxic Stress

This new protein presents very particular characteristics within the VEGF family which could account for its possible role in the acquisition of resistance to anti cancer treatments. However, it could also act as a therapeutic agent in ischemic pathologies that are myocardial (infarction) and peripheral (cerebral vascular injury, arteriopathy of the lower limbs, etc.) in nature, as well as in the healing of chronic wounds and the treatment of neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS).
It has been experimentally proven that reducing the production of VEGF-A in mice by half causes the animals' death. "This underlines the key role this protein plays in the vascularization process" notes Betty Nusgens, director of the Laboratory of Connective Tissue Biology of the University of Liège. In addition, it has been determined that VEGF-A is involved in several human pathologies: in some cases, it is in excess while in others, it is deficient. In the case of a surplus of VEGF-A, an excess number of blood vessels are formed. This undesired angiogenesis is a special ally of cancer. Malignant tumors induce the neoformation of a capillary network in their vicinity; they take the oxygen and nutrients necessary for their development from this environment, then continue to use them to invade neighbouring tissues and disseminate as metastases. Excessive vascularization is also involved in macular degeneration and in certain retinopathies potentially leading to blindness.

Other diseases are associated with VEGF-A deficiency: in ischemic diseases of the lower limbs for example, such a deficiency may cause ulcers to develop. It is also implicated in angina and myocardial infarction, as well as in erectile difficulties resulting from insufficient blood supply in the corpus cavernosum penis. Charles Lambert, researcher at the Laboratory of Connective Tissue Biology, comments that "From a therapeutic point of view, certain pathologies therefore demand that VEGF action be inhibited and others that it be increased". VEGF111 (containing 111 amino acids) was discovered incidentally by Pierre Mineur, another member of Betty Nusgens' research team, during a study on the impact of ultraviolet radiation on cells. The fact that the protein is not present under normal conditions and that UV-B damage cellular DNA seems to indicate that it is produced in response to genotoxic stress (i.e. that which is toxic for the genome). This led the researchers to examine whether other genotoxic agents such as drugs used in chemotherapy induce VEGF111 expression.
It was found that such was indeed the case in human cells in in vitro culture as well as in mice transplanted with human mammary tumor cells. Whether this also applies to man remains to be proven.

Resistant and diffusible

Whether VEGF111 is simply an "ordinary" mutant of VEGF-A is still unknown. The circumstances under which it is produced are themselves peculiar. Moreover, VEGF111 is the only VEGF-A human isoform not to have the amino acid sequence encoded by exon 5. This sequence, however, contains the principal sequences of molecule cleavage by plasmin and matrix metalloproteases, proteolytic enzymes capable of fractioning and thereby degrading it. Charles Lambert explains that "...we hypothesized that VEGF111, unlike other known mutants of VEGF-A, was resistant to proteolysis. We treated it experimentally, as well as VEGF165 and VEGF121, with both pure plasmin and an ulcer exudate rich in proteolytic enzymes. We observed that isoforms 165 and 121 underwent a complete and rapid degradation while their homologue 111 was in no way altered."

Except for VEGF165b, which is the only isoform not to be provided with exon 8a and thus acts as an angiogenesis inhibitor, all other mutants of VEGF-A are pro-angiogenic in that they favor the neoformation of blood vessels. VEGF111 notwithstanding, they are permanently found in the blood, albeit at very low concentrations. As underlined by both Charles Lambert and Betty Nusgens, their presence is not fortuitous. Furthermore, vascularization processes, although far from commonplace, do indeed appear in adults, in particular during the menstrual cycle and all forms of tissue repair.
If it can be demonstrated that what takes place in mice also occurs in man, VEGF111 expression would be induced, among other things, by chemotherapeutic agents administered in human clinical practice. The resistance of the protein to proteolysis would then make it a major pro-angiogenic factor, refractory as it is to degradation processes affecting its peers. Betty Nusgens adds that "if this were so, it would constitute a significant obstacle in so far as it could participate in the acquisition of resistance characteristics to anti cancer treatments".

Anti-VEGF therapies are already being administered as adjuvants in some chemotherapy treatments. However, the proteolysis-resistant nature of isoform 111 raises the question as to whether it would be useful to develop a targeting strategy centered on the annihilation of this specific molecule. This question is especially pertinent as current broad-spectrum treatments must sometimes be discontinued because of the appearance of side effects such as edemas or haemorrhages.

Another characteristic of VEGF111 is the absence of encoded sequences for exons 6 and 7, one it shares with mutant 121. These sequences, present in all other VEGF-A isoforms, are involved in their sequestration by the extracellular matrix. In other words, VEGF111 is a diffusible protein: it acts on targets both close to and far from the site where it has been produced. In particular, it acts on distant endothelial cells, thus favoring, as the ULg researchers have demonstrated in mice, the development of blood vessels close to the tumor and the subsequent development of metastases.

The Laboratory of Connective Tissue Biology plans in the very near future to join forces with clinical services to study VEGF111 expression in patients receiving chemotherapy or radiotherapy treatment. The Laboratory
also intends, in collaboration with researchers from the University of Ghent and the Nuclear Energy Research Center in Mol, to perform a study on the impact of cosmic radiation on astronauts and air pilots and navigators. This radiation, very energized at altitudes above the earth's athmosphere, is less filtered and consequently becomes a genotoxic agent capable of giving rise to the expression of VEGG111 and other potentially deleterious molecules.

As stated by Charles Lambert, "astronauts going to Mars might not only suffer from significant bone and muscle loss and a weakening of the immune system, but potentially run the risk of developing cancer". NASA and ESA (the European Space Agency) are particularly attentive to this issue; they have each just launched a radiobiology program aimed at evaluating the effect of different types of radiation and particles on human beings in their earthly environment."

Hope for therapeutic applications

VEGF111 could in principle be both an "enemy" (if it induces resistance to cancer treatments) and a "friend" (if its pro-angiogenic properties prove useful in treating ischemic pathologies, certain neurodegenerative diseases or chronic wounds). It has indeed been demonstrated that patients suffering from amyotrophic lateral sclerosis (the most famous of whom is the astrophysicist Stephen Hawkins) have lower levels of VEGF-A than healthy subjects. VEGF111, the variant which should prove itself to be the most active as it is resistant to proteolytic degradation, might constitute a therapeutic tool in the treatment of this particular disease, but research is still in the preliminary stages.
Betty Nusgens notes that "a clear parallel undoubtedly exists between the development of the vascular and the neurological systems. Research carried out by Peter Carmeliet at the Catholic University of Leuven has also demonstrated that VEGF plays an essential role in the development of the nervous system, in particular through its association with neuropilin, a membrane receptor present therein."

Moreover, mice genetically modified to produce less VEGF are known to experience locomotor difficulties and problems in maintaining equilibrium. It is not yet known whether VEGF111 will prove useful in the treatment of myocardial infarction, arteriopathies, ALS or chronic wounds, but the researchers at the University of Liège believe it is definitely worthwhile continuing the investigation.