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Purification of Signaling Protein May Boost Tissue Engineering

The purification of a powerful signaling molecule that coaxes cells to mature may also signal the beginning of a new era in tissue engineering.

In newly published studies in the journal *Nature*, researchers show that purified Wnt protein, long known as a potent trigger of development and cell proliferation, can also cause blood-forming stem cells to proliferate.

"With these studies, we can now imagine isolating and expanding a patients stem cells using activated Wnt proteins before they are treated with chemotherapy which destroys their immune system."

— **Roel Nusse**

The discovery suggests novel ways to enhance stem cells to restore the blood-forming systems of cancer patients whose cells have been destroyed by chemotherapy.

The research on Wnt activation and its role promoting the development of blood-forming (hematopoietic) stem cells was reported in two related articles published online on April 28, 2003, in *Nature*.

Howard Hughes Medical Institute investigator Roel Nusse and Karl Willert at Stanford University School of Medicine led one team that reported that the addition of a lipid molecule is necessary for the activation of Wnt proteins. Co-authors on that article include Jeff Brown and Esther Danenberg, as well as researchers in the laboratory of Irving Weissman, also of Stanford Medical School. Weissman led a second group of scientists who reported in *Nature* that activated Wnt is crucial for self-renewal of hematopoietic stem cells. Lead authors on that paper were Tannishtha Reya and Andrew Duncan, now at Duke University Medical Center.

According to Nusse, researchers had not isolated active Wnt proteins before—a long sought-after goal since the proteins play important roles in embryonic development and in controlling the proliferation of stem cells.

Nusse and Harold Varmus, President of Memorial Sloan-Kettering Cancer Center and a member of the HHMI medical advisory board, were members of one of the research teams that discovered Wnt genes. They identified Wnt genes as oncogenes activated in mouse breast cancer.

Pluripotent embryonic stem cells are immature cells that have the capability to mature into a wide range of blood and tissue cells. They may hold the key to restoring compromised immune systems and even regenerating tissues and organs damaged by disease or trauma. Even though such stem cells have been isolated, inducing them to proliferate for use in treatment has been only marginally successful.

“It has always been predicted that the Wnt proteins could act as growth factors that could be added to cells and direct them into a particular growth pathway without genetically changing the cells,” said Nusse. Central to this process, he said, has been purifying and characterizing the active form of Wnt proteins, which has proven especially frustrating.

“We now understand that this protein is modified by the attachment of a lipid after it is produced, which gives it a tendency to stick to cell membranes where it is active,” said Nusse. “However, that means the protein also was not soluble and would stick to containers, so standard purification techniques didn't work.”

Nusse and his colleagues developed techniques that used detergents to render the protein soluble, and also robust cell assays that would measure the biological activity of the protein. “It was a real challenge to get the methods to work,” said Nusse, “requiring all kinds of biochemical tricks.”

In their latest work, Nusse and his colleagues showed that one member of the Wnt protein family, mouse Wnt3a, is activated by the attachment of a lipid called palmitoyl to a particular amino acid on the protein. The lipid is necessary for activation, as is the presence of the attached amino acid, cysteine, which is conserved in all Wnt proteins, said Nusse. The researchers may well have found the key mechanism of activation for the multitude of Wnt proteins.

Next, Nusse, Weissman and their colleagues tested the effects of the activated Wnt protein on hematopoietic stem cells. They found that the protein greatly enhanced stem cell proliferation in the test tube, while maintaining the stem cells in their immature state. The researchers also observed that Wnt-treated stem cells retained their activity and were able to reconstitute the blood-forming systems of mice that had been irradiated to destroy their hematopoietic cells.

The accompanying *Nature* paper by Reya, Duncan, Weissman and their colleagues demonstrated that the Wnt signaling pathway plays a crucial role in hematopoietic stem cell self-renewal. The researchers showed that the protein specifically affected by Wnt—beta catenin—is necessary for stem cell proliferation, as is the Wnt protein itself.

“With these studies, we can now imagine isolating and expanding a patient's stem cells using activated Wnt proteins before they are treated with chemotherapy which destroys their immune system,” said Nusse. “Those proliferated cells could then provide a powerful way to restore the hematopoietic system. And since Wnt is a specific growth factor and doesn't fundamentally alter the nature of the cells, there is no danger that the cells will take on unwanted properties.”

According to Nusse, over-activation of the Wnt signaling pathway due to genetic mutation has been implicated in some cancers. Thus, he said, discovery of the nature of Wnt activation in cells will enable researchers to mimic the cancer process experimentally, to study its mechanism.

Nusse and his colleagues are now studying other Wnt proteins to determine whether they require the same mechanism for activation and whether they, too, can trigger proliferation of other types of stem cells. In some ways, Nusse and his colleagues have a head start - in collaboration with HHMI investigator Jeremy Nathans at The Johns Hopkins University School of Medicine they have already identified the specific cell surface receptors, known as Frizzleds, to which the Wnt proteins bind to activate the cells.

“We're trying also to understand why cells respond to particular Wnt proteins by looking at the expression of the specific receptor for the Wnts,” said Nusse.