MedicalXpress

Secret to healing chronic wounds might lie in tiny pieces of silent RNA

March 22 2010

Scientists have determined that chronic wounds might have trouble healing because of the actions of a tiny piece of a molecular structure in cells known as RNA.

The Ohio State University researchers discovered in a new animal study that this RNA segment in wounds with limited blood flow lowers the production of a protein that is needed to encourage skin cells to grow and close over the sore.

In a parallel experiment using human skin cells, the researchers silenced the RNA segment with an experimental drug and saw those protein levels rise. The skin cells multiplied as a result.

The combination of findings suggests that targeting this RNA segment with a drug that could be used topically on skin might offer new strategies for treating chronic wounds, which are sometimes fatal and cost the U.S. health-care system an estimated $25 billion annually.

The research appears this week in the online early edition of the Proceedings of the National Academy of Sciences.

RNA in cells is responsible for using instructions from DNA to make proteins, but the piece of RNA identified in this study has a completely different role. It is a microRNA, a small segment of RNA that blocks an important protein-building process. The RNA segment in question is known as miR-210.

The research involves wounds that are ischemic, that is, they heal very slowly or are in danger of never healing because they lack blood flow and oxygen at the wound site. These types of wounds affect about 6.5 million patients each year, and are common complications of diabetes, high blood pressure, obesity and other conditions characterized by poor vascular health.

"When blood supply is inadequate, many things are deficient at the wound site, including oxygen. That leads to a condition called hypoxia," said Chandan Sen, professor and vice chair for research in Ohio State's Department of Surgery and senior author of the study. "We have shown that hypoxia induces miR-210, which actually blocks the ability of the cells to proliferate, a step necessary for the wound-closure process."

Sen's lab has studied the effects of low oxygen on wound healing for years, but just now has been able to identify the sequence of events connecting low oxygen and the inability of skin cells to grow. Sen, who is also executive director of the Comprehensive Wound Center at Ohio State, said this is the first publication to suggest microRNAs regulate the healing process in chronic wounds.

Sen and colleagues created ischemic and non-ischemic wounds on mouse skin for comparison. To create the ischemic wounds, they established a flap of skin with limited blood flow and placed the wound in the middle of the flap.

The scientists used a number of technologies - including laser Doppler imaging and hyperspectral scanning - to demonstrate that the wounds received differing levels of blood flow. Additionally, they used a specialized probe to measure actual oxygen levels in the wounds and showed that oxygen in the ischemic wounds on the mice closely matched oxygen levels measured in chronic human wounds in clinical settings.

In these ischemic mouse wounds, the researchers observed that the hypoxic, or low-oxygen, conditions led to the presence of a specific type of protein called hypoxia inducible factor-1a, or HIF-1a. This protein can turn genes on and off and, in this case, appears to influence the behavior of at least one microRNA as well.

By placing markers in the wounds for these substances, the scientists could observe their relationships in the wound. The presence of HIF-1a in low-oxygen conditions led to the activation of the miR-210, and that microRNA in turn lowered levels of the protein needed to kick skin cells into action. This protein is called E2F3.

In contrast, the non-ischemic wounds on the mice showed abundant levels of the E2F3 protein and healed normally within about seven days.

To test these relationships further, the researchers set up experiments using a line of human skin cells most responsible for closing over a wound.

Under normal oxygen levels, the scientists manipulated the cells to activate the HIF-1a protein that normally is induced by hypoxia. When the HIF-1a was present, the cells contained high levels of the miR-210, which in turn lowered levels of the E2F3 protein.

The researchers further manipulated these conditions by inserting an experimental drug into the cells called an antagomir, a synthetic molecule that renders microRNAs inactive and which was designed to act specifically on miR-210. When the miR-210 levels were lowered with the antagomir, the E2F3 protein levels rose and the skin-cell growth increased significantly. When the miR-210 levels were artificially raised using a molecule that mimics its behavior, the skin cells' growth was compromised.

"MicroRNAs are induced only by certain conditions. They have specific profiles in the daily biology of a given organ, but under conditions of an injury, certain microRNAs wake up," Sen said. "Once it sees hypoxia, miR-210 wakes up, and then it governs what happens with the E2F3 protein after that."

Antagomirs are a burgeoning area of drug development research. They are considered potent agents that can interfere with microRNA behavior throughout the body. Their use in wound healing, on the other hand, might benefit from the need to use them only topically on the skin, Sen said. He plans to investigate their effects on wounds in further animal studies.

Provided by The Ohio State University