Single drug, soft environment can increase platelet production

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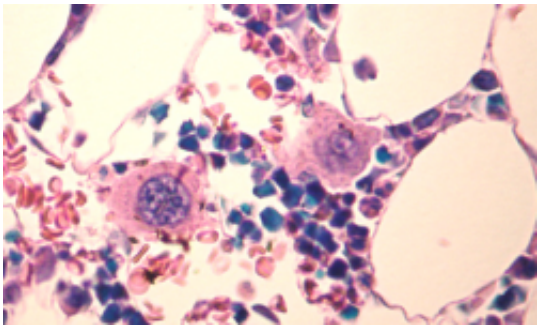

Megakaryocytes in bone marrow

(Medical Xpress) -- Humans produce billions of clot-forming platelets every day, but there are times when there aren't enough of them, such as with certain diseases or during invasive surgery. Now, University of Pennsylvania researchers have demonstrated that a single drug can induce bone marrow cells called megakaryocytes to quadruple the number of platelets they produce.

Jae-Won Shin, a graduate student of pharmacology in Penn's Perelman School of Medicine, and Dennis E. Discher, professor in the Department of Chemical and Biomolecular Engineering in the School of Engineering and Applied Science, led the research. They collaborated with Joe Swift and Ph.D. student Kyle R. Spinler, also of Chemical and Biomolecular Engineering.

Their research was published in the journal Proceedings of the National Academy of Science.

Megakaryocytes are the large bone marrow cells that produce platelets, the smaller cell fragments that form clots to seal blood vessels when the vessels are damaged. The amount of platelets they produce relates to their size. Unlike most other cells, when megakaryocytes copy their DNA, they don't split into two cells but continue to grow larger.

"These cells take the relatively unusual step of becoming bigger and bigger, adding multiple nuclei, which you don't see with other cell types," Discher said. "Mature, multinucleated megakaryocytes are better than uni- or bi-nucleated ones; they have more mass and are ready to make more platelets."

When mature, the megakaryocyte will extend a tendril into a neighboring blood vessel; the flow of blood pulls off pieces of the cell, forming platelets. The motor protein myosin-II plays a number of roles in this process; by inhibiting it with a drug known as belebbistatin, the researchers caused megakaryocytes to make up to four times as many platelets as when it is active.

Myosin-II is responsible for many body systems that require contractile tension, such as flexing one's muscles. In many cells, it is responsible for the integrity of the outer membranes, for cell division and for key aspects of adhesion. Because megakaryocytes are best when they are large, multi-nucleated and fragment easily, inhibiting myosin-II helps produce more platelets in three distinct ways.

"The first factor is when cells normally divide, there is a contracting force between the dividing cells that cleave them apart," Shin said. "But if you inhibit myosin, there is no contracting force and cells grow without dividing. That's how they become multi-nucleated and how the

cell mass becomes bigger.

“The second factor is cytoskeletal stiffness and tension in the cell. When myosin is active, the cell is stiff and tense like well-toned muscle, but if you inhibit the myosin, the cell becomes flaccid and more easy to push around and fragment,” he said.

“The third factor is that cells are able to sense the stiffness of their microscopic environment and react to it, which is also regulated by cellular contractivity,” Shin said. “Adhering to bone inhibits megakaryocyte growth; without myosin-II, they grow as if they were adhering to something soft.”

By testing the megakaryocytes’ growth in different cell culture dishes and gels, the researchers were also able to show that a soft matrix, similar to squishy bone marrow, induced more platelet production than a rigid matrix.

After growing the platelets in Petri dishes saturated with the myosin-II inhibitor blebbistatin, the megakaryocytes were soft enough to spontaneously fragment into platelets. Because platelets need functioning myosin-II to form rigid clots, the researchers subsequently washed away the drug to show that the platelets could still activate.

While the researchers also transplanted blebbistatin-treated human megakaryocytes into genetically modified mice to show that they maintained their increased platelet production within living systems, most of their work was done in vitro to demonstrate that platelets could be successfully synthesized in a lab.

“Platelet transfusions are harder and harder to come by. Not only are there contamination issues, but platelets are also very short-lived,” Shin said. “They only last about a week in a transfusion bag. The ability to

make them in large quantities could save lives.”

Provided by University of Pennsylvania