Laying bare the not-so-sweet tale of a sugar and its role in the spread of cancer

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Cancer has a mighty big bag of tricks that it uses to evade the body's natural defense mechanisms and proliferate. Among those tricks is one that allows tumor cells to turn the intricate and extensive system of lymphatic vessels into something of a highway to metastasis. Yet research unveiled this week may aid in the development of therapeutics that will put the brakes on such cancer spread, and the researchers who completed the study say the findings may extend to other lymphatic disorders.

In the latest issue of the Journal of Biological Chemistry, the team at the VA San Diego Healthcare System and the University of California, San Diego, reports an important advance in the understanding of the molecular machinery needed for lymphatic cell growth.

"In many carcinomas, lymphatic vessels grow and remodel around and sometimes within tumors. This allows tumor cells to go upstream to the lymph nodes," explains assistant professor Mark Fuster, who led the study. Once tumor cells hitch a ride to the lymph nodes, the disease can be more difficult to fight. "We were trying to understand the mechanisms that turn on the growth of lymphatic vessel cells in the laboratory."

To better understand how tumors get lymphatic vessels to construct an entry ramp for cancerous cells, Fuster's team began by looking at a much-studied lymphatic stimulatory protein that is often over-produced by tumors. The protein migrates from the tumor to a layer of cells within

lymphatic vessels known as the endothelium. The tumor-produced protein is officially known as vascular endothelial growth factor C, or VEGF-C for short (pronounced "vej-eff-cee").

"The growth factor VEGF-C lands on a special receiving molecule, or receptor, on the surface of the lymphatic endothelial cells, sending a signal that says it's time for the endothelial cells to replicate and send offshoots," Fuster says. But the team was curious as to whether VEGF-C and its receptor were getting any help from nearby molecules to make that happen. "After all, if there were other players in the mix, that might mean there are other possible drug targets," Fuster explains.

The team focused on a glycan, or sugar, known as heparan sulfate. After some initial clues indicated that destroying the unique sugar on lymphatic endothelial cells would inhibit VEGF-C-dependent growth signaling, Fuster and his team dug in to figure out more about heparan sulfate's role.

"In a cell-based system, we tried to interfere with the components that are involved in making heparan sulfate in lymphatic endothelial cells. We tried inhibiting the production of the sugar and destroying it," Fuster says.

Xin Yin, a postdoctoral research fellow, and Scott Johns, a research associate in the laboratory, both lead authors on the paper, carried out a variety of studies to examine how silencing enzymes in the cell that are responsible for putting the sugar together might alter various cell-growth behaviors and affect VEGF-C's ability to activate its receptor.

"What we found was that giving the glycan-altered cells the VEGF-C resulted in a blunting of the normal growth rate or signaling for growth," Fuster says. "This work shows there may be a key role for heparan sulfate in the initiation of lymphatic vessel-growth responses."

In the setting of cancer, it is thus possible that the presence of heparan sulfate is important for tumor-spurred lymphatic vessel growth: This not only identifies a potential target for anti-cancer drugs, Fuster says, but it may also offer insights about how to stimulate lymphatic vascular growth in diseased parts of the body that, conversely, need lymphatic vessels for normal circulatory and immune functions.

Still, though, Fuster emphasizes that more work remains to be done, because how exactly heparan sulfate interacts with VEGF-C and its receptor remains unclear: "Identifying the importance of heparan sulfate in the growth of living lymphatic systems and identifying its possible importance in mediating the functions of multiple lymphatic growth factors simultaneously remain important considerations for ongoing and future research."

More information: Lymphatic Endothelial Heparan Sulfate Deficiency Results in Altered Growth Responses to Vascular Endothelial Growth Factor-C (VEGF-C), doi: 10.1074/jbc.M110.206664

Abstract
Growth and remodeling of lymphatic vasculature occur during development and during various pathologic states. A major stimulus for this process is the unique lymphatic vascular endothelial growth factor-C (VEGF-C). Other endothelial growth factors, such as fibroblast growth factor-2 (FGF-2) or VEGF-A, may also contribute. Heparan sulfate is a linear sulfated polysaccharide that facilitates binding and action of some vascular growth factors such as FGF-2 and VEGF-A. However, a direct role for heparan sulfate in lymphatic endothelial growth and sprouting responses, including those mediated by VEGF-C, remains to be examined. We demonstrate that VEGF-C binds to heparan sulfate purified from primary lymphatic endothelia, and activation of lymphatic endothelial Erk1/2 in response to VEGF-C is reduced by interference with heparin or pretreatment of cells with heparinase, which destroys

heparan sulfate. Such treatment also inhibited phosphorylation of the major VEGF-C receptor VEGFR-3 upon VEGF-C stimulation. Silencing lymphatic heparan sulfate chain biosynthesis inhibited VEGF-C-mediated Erk1/2 activation and abrogated VEGFR-3 receptor-dependent binding of VEGF-C to the lymphatic endothelial surface. These findings prompted targeting of lymphatic N-deacetylase/N-sulfotransferase-1 (Ndst1), a major sulfate-modifying heparan sulfate biosynthetic enzyme. VEGF-C-mediated Erk1/2 phosphorylation was inhibited in Ndst1-silenced lymphatic endothelia, and scratch-assay responses to VEGF-C and FGF-2 were reduced in Ndst1-deficient cells. In addition, lymphatic Ndst1 deficiency abrogated cell-based growth and proliferation responses to VEGF-C. In other studies, lymphatic endothelia cultured ex vivo from Ndst1 gene-targeted mice demonstrated reduced VEGF-C- and FGF-2-mediated sprouting in collagen matrix. Lymphatic heparan sulfate may represent a novel molecular target for therapeutic intervention.

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