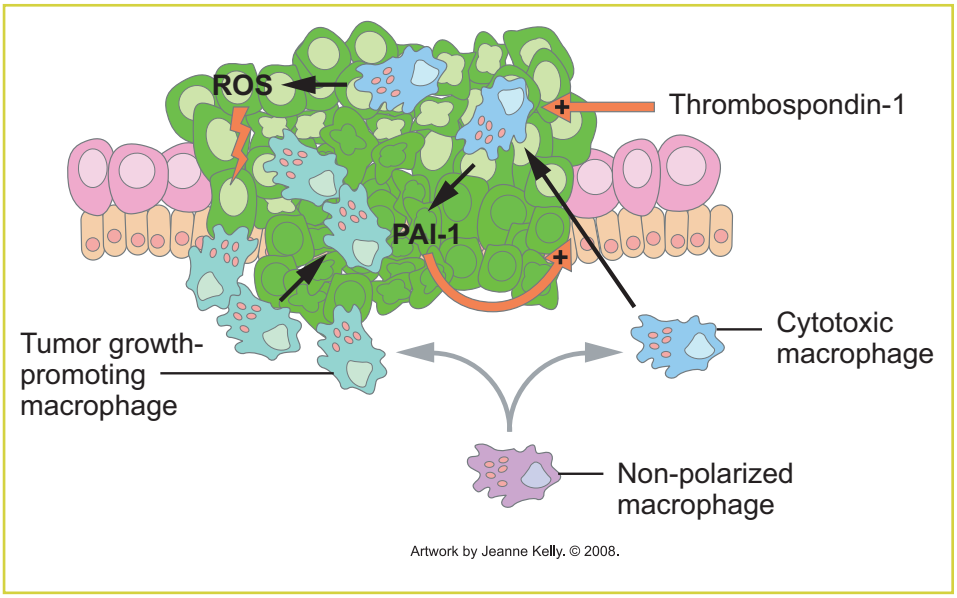
Thrombospondin 1 Wages a Double Hit Against Cancer

Cancer is the result of a complex series of molecular steps that promote uncontrolled growth and erode the body's ability to fight the resulting tumor. Generating a more complete picture of these molecular events should help identify strategies to prevent and treat the disease.

Researchers have found one protein that plays a role in tumorigenesis: thrombospondin 1, or TSP1. Many tumors downregulate expression of TSP1, suggesting that the protein may interfere with tumor progression. This notion is supported by the fact that forced overexpression of TSP1 inhibits tumor growth. TSP1's antitumor activity has long been attributed to its ability to prevent the formation of new blood vessels, which are essential for tumor growth. However, a recent study led by David Roberts, Ph.D., Head of the Biochemical Pathology Section of the CCR Laboratory of Pathology, reveals that TSP1 also may help the immune system find and destroy cancer cells. Post-Doctoral Fellow Gema Martin-Manso served as lead author of the resulting paper, which was published in a recent issue of *Cancer Research*.

To pursue this area of study, Roberts and Martin-Manso collaborated with fellow Laboratory of Pathology researcher Maria Tsokos, M.D., and David Wink, Ph.D., from the CCR Radiation Biology Branch. Using a mouse model, the team found that TSP1-overexpressing tumors exhibit delayed growth and confirmed previous reports that these tumors are heavily infiltrated with immune cells called macrophages. There are two types of tumor-associated macrophages: M1 macrophages are toxic to tumor cells, whereas M2 macrophages can actually promote tumor growth. Analysis of cell markers revealed that the macrophages infiltrating TSP1-expressing tumors were predominantly of the antitumor M1 type.



As shown in the above figure, expression of TSP1 by cancerous tumors leads to recruitment of immune cells called macrophages that, in turn, express PAI-1 and activate cytotoxic macrophages to fatally damage cancer cells with ROS.

The researchers then carried out a series of experiments in cultured cells to determine how TSP1 attracts macrophages to the tumor site. They found that TSP1 stimulates expression of plasminogen activator inhibitor 1 (PAI-1), a protein known to be important for immune cell migration. Interestingly, a closer look at the tumors in the mouse model revealed that the infiltrating macrophages strongly expressed PAI-1, suggesting that this protein may be important for recruitment of macrophages to tumors *in vivo*.

To delve more deeply into the functional effects of TSP1, Roberts and his colleagues cultured melanoma or breast carcinoma cells together with

macrophages. They found that addition of TSP1 dramatically increased the ability of the macrophages to kill the cancer cells, confirming their hypothesis that TSP1 stimulates the antitumor effects of the immune system.

One way immune cells attack is through the production and release of molecules called reactive oxygen species (ROS), which can fatally damage cells. The CCR research team showed that TSP1 causes macrophages to release ROS by binding to the cell surface protein $\alpha_6\beta_1$ integrin, which subsequently increases intracellular calcium levels. Importantly, they also found that ROS could inflict tumoricidal effects on breast carcinoma cells similar to those caused by TSP1.

Together, these studies suggest that tumor expression of TSP1 leads to recruitment of immune cells and subsequent activation of these cells to fatally damage cancer cells with ROS. This explains why so many tumors downregulate expression of TSP1—not only does it prevent the formation of new blood vessels, but it also makes it more likely that the tumor will be detected and attacked by the body's immune system. This insight into the dual role of TSP1 may pave the way for new approaches to cancer treatment.

Reference

Martin-Manso G, Galli S, Ridnour LA, Tsokos M, Wink DA, Roberts DD. Thrombospondin 1 promotes tumor macrophage recruitment and enhances tumor cell cytotoxicity of differentiated U937 cells. *Cancer Res* 68: 7090–7099, 2008.