

## **Antibodies to Anionic Phospholipids as Vascular Targeting Agents for Cancer Treatment**

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Anionic phospholipids are largely absent from the surface of resting mammalian cells under normal conditions. Phosphatidylserine (PS), which is the most abundant anionic phospholipid of the plasma membrane, is tightly segregated to the internal leaflet of the plasma membrane in most cell types (1).

PS asymmetry, along with that of phosphatidylethanolamine (PE), is maintained by an ATP-dependent aminophospholipid translocase (probably ATPase II) that catalyzes the transport of aminophospholipids from the external leaflet to the internal leaflet of the plasma membrane (2). Loss of PS and PE asymmetry results from the outward movement of these phospholipids in the plasma membrane and is caused either by inhibition of the translocase or activation of a scramblase that transports all of the lipids bidirectionally. Loss of asymmetry is observed under different pathological and physiological conditions, including apoptosis, cell activation, injury, and malignant transformation.

We hypothesized that anionic phospholipids would become exposed on tumor vasculature because of stress conditions in the tumor microenvironment. Injury and activation of tumor endothelium have been shown to be caused by: (a) tumor-derived interleukin-1 and tumor necrosis factor, which activate the endothelium and induce expression of cell adhesion molecules (3); (b) ROS generated by leukocytes that adhere

to the endothelium (4); and (c) ROS generated by tumor cells themselves as a byproduct of metabolism (3) or as a result of exposure to hypoxia followed by reoxygenation (5). These observations suggested that  $\text{Ca}^{2+}$  fluxes might be generated by these stresses within the tumor endothelium that, in turn, cause exposure of PS, and probably also of PE, through activation a scramblase or inhibition of an aminophospholipid translocase.

To detect cell surface anionic phospholipids, we generated the monoclonal antibodies, 9D2 and 3G4, that react with PS and other anionic phospholipids in the absence of protein cofactors. The antibodies do not bind detectably to neutral phospholipids. The antibodies are more specific for anionic phospholipids than is the natural ligand, annexin V, which strongly binds to PE, in addition to anionic phospholipids. We found that the anti-PS antibodies and annexin V localize specifically to tumor endothelium after i.v. injection into mice bearing various types of solid tumors, including human MDA-MB-231 tumors growing in the mammary fat pads of mice (6). Between 15% and 40% of tumor blood vessels had exposed anionic phospholipids. In contrast, none of the blood vessels in normal tissues had detectable externalized anionic phospholipids. Necrotic regions of tumors were also stained. These findings have since been confirmed in the RIP-Tag spontaneous pancreatic tumor model in a collaborative study with Dr. Donald McDonald and colleagues, UCLA, CA.

The effect of anti-PS antibodies on tumor growth was examined in various murine models, including syngeneic (mouse Meth A fibrosarcoma), subcutaneous xenografts (L540 human Hodgkin's lymphoma) and orthotopic tumors (human MDA-MB-231

breast cancer and human MDA-MB-435 breast cancer). The most potent antibody was 3G4, a mouse IgG3. Treatment of mice with 3G4 antibody resulted in 90%, 65%, 50% and 70% growth retardation of these tumors, respectively. Both small (0.1 cm diameter) and well-established (0.3 cm diameter) tumors were inhibited. Anti-PS treatment induced long-term complete remissions in 50% of mice with Meth A fibrosarcomas and 30% of mice with MBA-MD-231 breast tumors.

Histological examination of orthotopic MDA-MB-231 tumors from mice treated with 100  $\mu$ g 3G4 three days previously revealed vascular damage to tumor blood vessels. The following were evident: 1) Disintegration of vascular endothelium in about 50% of vessels in the tumor; 2) Attachment of leukocytes to tumor endothelium and infiltration of mononuclear cells into tumor interstitium; 3) Occlusion of tumor vessels by platelet aggregates and red cells; 4) A 70% reduction in microvascular density in tumors from 3G4 treated versus untreated mice; 5) Central necrosis of the tumors, with survival of a peripheral rim of tumor cells, typical of a VTA. These findings, together with the finding that the antibodies do not affect the growth rate of various tumor cells *in vitro*, suggest that the primary anti-tumor action of anti-PS antibodies is exerted through effects on tumor vasculature. Other mechanisms, such as antibody-dependent cellular cytotoxicity directed against the tumor cells themselves could contribute to the anti-tumor effect and may be responsible for the killing of tumor cells in the peripheral rim.

In conclusion, antibodies to PS and other anionic phospholipids have potential as therapeutic agents for targeting the vasculature of solid tumors.

## References

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