

Fibulin-5 Controls Angiogenesis By Inhibiting Integrin-induced ROS Generation

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Fibulin-5 (Fbln5) is an extracellular matrix (ECM) integrin-binding protein that has been implicated as a regulator of angiogenesis; however, the mechanism of action that underlies this activity is unclear. Reactive oxygen species (ROS) are potent stimulators of angiogenesis. We identified a novel pathway by which Fbln5 functions to control angiogenesis by inhibiting the production of reactive oxygen species (ROS) in an integrin dependent manner. We show that mouse embryonic fibroblasts (MEFs) harvested from *Fbln5*-null (*Fbln5*^{-/-}) mice produced higher levels of ROS than *wild-type* (*WT*) MEFs due to increased activation of $\alpha\beta 1$ integrins by fibronectin. Previously, *in vivo* studies using *Fbln5*-null (*Fbln5*^{-/-}) mice showed an increase in vascular invasion into implanted polyvinyl alcohol sponges in *Fbln5*^{-/-} mice compared to *wild-type* (*WT*) animals. We found that treatment with the antioxidant NAC reduced the level of angiogenesis into matrigel plugs from *Fbln5*^{-/-} mice but had no effect on matrigel plugs from *WT* animals supporting the idea that Fbln5 regulates angiogenesis by inhibiting ROS production.

To understand how the loss of Fbln5 and the subsequent increase in ROS production would affect tumor growth, we performed tumor studies in mice. We found that subcutaneous and orthotopic pancreatic (Pan02) tumors in *Fbln5*^{-/-} mice grew significantly slower, had higher levels of ROS and surprisingly had decreased angiogenesis compared to tumors from *WT* littermates. We further showed that tumor growth and angiogenesis could be reverted by antioxidant treatment. We concluded that the loss of Fbln5 expression and the subsequent increase in ROS formation in the tumor microenvironment resulted in chronic oxidative stress and decreased endothelial cell survival. These results suggest that Fbln5 may provide a unique target for anti-cancer therapy.