Egfl7 promotes tumor escape from immunity

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Keywords: endothelium, escape from immunity, angiogenesis, adhesion molecules, extravasation

Egfl7 is an endothelial-specific gene which expression is deregulated in human cancers. We showed that Egfl7 promotes tumor escape from immunity by downregulating the expression of leukocyte adhesion molecules in endothelial cells, thus repressing immune cell extravasation into tumors.

The tumor blood vessel endothelium forms an imperfectly tight barrier which actively protects the tumor mass from the immune system. Tumor escape from immunity is achieved, in part, by the downregulation of leukocyte adhesion molecules normally expressed by activated endothelial cells. This endothelium anergy limits the adhesion and infiltration of effector immune cells within the tumor.1 We found that Egfl7 induces this mechanism in endothelial cells, and thus promotes tumor escape from the immune system.2

Egfl7 (also named VE-statin) is a secreted protein specifically expressed by endothelial cells during embryogenesis.3 Egfl7 represses smooth muscle cell migration, suggesting a role for this protein in vascular maturation. Egfl7 is also an endogenous repressor of endothelial elastogenesis via its interaction with lysyl oxidases and repression of their catalytic activity.4 A few reports showed that egfl7 is deregulated in human cancer; its expression is not anymore restricted to the endothelium in human tumors, but is also detected in cancer cells themselves. Further, Egfl7 expression levels in human hepatocarcinoma, glioma, and colon cancer correlate with a higher tumor grade, with a poorer prognosis and higher metastatic scores,5,6 suggesting that Egfl7 is associated with tumor progression.

In order to understand its role during tumor growth and metastasis, we overexpressed Egfl7 in breast cancer 4T1 and lung adenocarcinoma LLC1 cells, and analyzed the resulting effects in vitro and in vivo. Overexpression of Egfl7 had no effects on tumor cell proliferation, migration, or clone formation in vitro, suggesting that the protein has no major effects on tumor cells themselves. On the other hand, when implanted in Balb/c or C57Bl/6 mice, tumors expressing Egfl7 grew faster and induced a higher incidence of lung metastasis than controls. Egfl7 increased the tumor vascular density, endothelium permeability, necrosis, and hypoxia. On the other hand, Egfl7 had no effects on the Ki67 proliferation index of tumor cells or on apoptosis.

More interestingly, when tumor cells were implanted in immune-deficient SCID-Bg mice, Egfl7 had no effects on tumor growth and metastasis anymore, suggesting that the effects of Egfl7 were strictly dependent on the existence of an intact immune system in the receiving mice. Furthermore, control tumors grew in SCID-Bg mice as fast as tumors overexpressing Egfl7, while tumors expressing Egfl7 grew faster and induced a higher incidence of lung metastasis than controls. Egfl7 increased the tumor vascular density, endothelium permeability, necrosis, and hypoxia. On the other hand, Egfl7 had no effects on the Ki67 proliferation index of tumor cells or on apoptosis.

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A detailed analysis of the morphology of tumors grown in immunocompetent mice revealed the presence of numerous individualized cells in the blood vessels lumens of tumors expressing Egfl7, while a small number of such cells were infiltrated within the tumor tissues. On the contrary, most of these cells infiltrated the tissues of control tumors and few were observed in the blood vessels. In parallel, RNA and protein arrays analyses showed that IFNγ expression was severely reduced in tumors overexpressing Egfl7 when compared with controls, suggesting that these tumors were depleted of immune cells. Immunostainings confirmed that T-lymphocytes had massively infiltrated control tumors whereas tumors overexpressing Egfl7 were much less infiltrated and most T-lymphocytes were sequestered in the blood vessels lumens (Fig. 1). This was repeatedly observed with all immune cells tested, namely CD4 and CD8 T-lymphocytes, B-lymphocytes, dendritic cells, macrophages and NK cells, which were down-represented in tumors overexpressing Egfl7 when compared with control tumors. This implied that Egfl7 affected the infiltration of all types of immune cells, with no particular specificity. However, Egfl7 had no direct effects on immune cells, as it was not able to decrease the basal or induced proliferation of T lymphocytes, nor the basal or induced activation of T-lymphocytes, dendritic cells, and NK cells. Second, the effects of Egfl7 on immune cells were locally confined to the tumors and not systemic since the spleens of mice bearing control or tumors expressing Egfl7 were equally well populated.

From all these observations, it became highly probable that Egfl7 affected the extravasation of immune cells from the...
blood circulation into the tumors. An analysis of expression of leukocyte adhesion molecules confirmed this hypothesis and showed that endothelial cells from tumors expressing Egfl7 expressed much less ICAM-1, VCAM-1 and E-selectin than controls (Fig. 1). We found that expression of these adhesion molecules was directly controlled by Egfl7 in endothelial cells, as repressing endogenous egfl7 using RNA interference promoted the expression of ICAM-1, VCAM-1 and E-selectin and lymphocyte adhesion to these cells, suggesting that Egfl7 is a constitutive repressor of endothelial cell activation.

When switching to the human disease, we noticed that in human breast cancer tumors expressing high levels of Egfl7, blood vessel endothelial cells expressed less ICAM-1 and VCAM-1 than in tumors expressing low levels of Egfl7. In addition, there was a direct and inverse correlation between the levels of expression of egfl7 and those of ifnɛ, these two points suggesting that Egfl7 promotes tumor escape from immunity in human cancer as it does in mice.

Egfl7 is therefore an endogenous regulator of endothelial cell activation which, when expressed in a tumor context, favors tumor growth by downregulating immune cell extravasation. Tumor escape from immunity mediated by the endothelium is undoubtedly an interesting process to consider for the design of therapeutic tools aimed at preventing cancer progression and, following our observations, Egfl7 represents a new target for interfering with these processes. It is worth noting that since Egfl7 maintains the normal endothelium in a non-activated state, interfering with this property when targeting Egfl7 might produce severe adverse side effects in normal organs.

Figure 1. Control tumors (top) show a large infiltration of CD3ε+ T-lymphocytes (brown) and express high levels of ICAM-1 (green) and VCAM-1 (red). Tumors expressing Egfl7 (bottom) have a reduced infiltration of T-lymphocytes which mainly remain in the blood circulation (arrows), blood vessels express much less ICAM-1 and VCAM-1 (inserts).

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