Karolinska Institutet

Department of Oncology and Pathology

Making Endothelial Cells Move
- A Study of Angiomotin and Binding Partners

AKADEMISK AVHANDLING
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Abstract

Angiogenesis is a process crucial for tumor growth and metastasis formation and encompasses the control of endothelial directional migration, anastomosis and lumen formation. We have previously shown that Angiomotin (Amot), a membrane-associated scaffold protein, plays an essential role in controlling endothelial cell migration and cell shape. Amot is critical for normal development as more than 75% of *amot* deficient mouse embryos die *in utero* due to vascular defects.

In this thesis, the vascular system of the mice that survive gestation are studied. These mice are apparently normal in regards of body weight, kidney function and reproduction. Further, the vascular system did not exhibit any changes in perfusion or vascular density. However, the growth of Lewis Lung Carcinoma tumor xenograft was significantly impaired in *amot* deficient mice as compared to sibling wt mice. Analysis of the tumor vasculature revealed a marked change in vessel morphology and decreased vascular density and perfusion. These data argue that Amot, besides its role in development, plays a critical role in pathological angiogenesis. Gene expression analysis shows that the loss of *amot* expression leads to indirect effects, which activates NF-κB regulated genes and results in an inflammatory phenotype *in vitro* and *in vivo*.

As the thesis title implies, the function of Amot in endothelial cell migration has been investigated by identifying and studying its binding partners and their functions. The polarity proteins Patj and Mupp1 (plus Pals1) are presented as binding partners to the Amot PDZ-binding motif. This is of interest since establishing cell polarity is a key step in directional endothelial migration. Migration also depends on correct actin polymerization, driven by Rho GTPases. We show that Amot is essential for the localization of RhoA activity at the leading edge of a migrating cell and that the Rho GEF (activator of RhoA) Syx1 also associates to Patj/Mupp1. Finally, we show that the interaction of the PDZ-binding motif to Patj/Mupp1/Syx1 is essential for vessel migration in developing zebrafish embryos.

Furthermore, tight junction-associated tumor suppressor Merlin (nf2) is identified as a binder to the Amot coiled-coil domain. Rich1, a negative regulator of Rac1 (also a RhoGTPase), has been shown to bind to Amot through the same domain. We show that Amot regulates Rac1 activity and subsequent MAPK pathway activation through Merlin and Rich1 and that Merlin and Rich1 compete for the same binding site on Amot, resulting in Rac1 regulation. We hypothesize that Rich1 is inhibited by its binding to Amot, leaving Rac1 in its active state, able to polymerize actin. Upon binding to Merlin, Amot releases Rich1 and Rac1 is inhibited. Finally, we show that Schwann cell tumors, caused by loss of *nf2* expression, exhibit a decreased growth rate upon loss of *amot* expression, leading to prolonged mouse survival. This further emphasizes the potential for Amot as a drug target and indicates that anti-Amot therapy could be used to treat Schwanoma patients.

Amot localizes to the lamellipodia of migrating cells, a region of the cell membrane with specific lipid composition. The Amot coiled-coil domain has been shown to be a conserved lipid-binding domain. I propose a model where migratory cues trigger the change in lipid composition of the cell membrane, concentrating lipids to which Amot binds to the lamellipodia. Amot brings with it the complex of Patj/Mupp1:Syx1, localizing RhoA activity and thus actin polymerization at the leading edge.