Ruprecht-Karls-Universität Heidelberg
Medizinische Fakultät Mannheim
Dissertations-Kurzfassung

Functional consequences of Tie1-silencing in endothelial cells

Autor: Elias Karl Loos

Institut / Klinik: Gemeinsamer Forschungsbereich "Vaskuläre Biologie" der Medizinischen Fakultät Mannheim, Universität Heidelberg (CBTM) und dem Deutschen Krebsforschungszentrum Heidelberg (DKFZ-ZMBH Allianz)

Doktorvater: Prof. Dr. H. Augustin

The Ang/Tie system is a key regulator of angiogenesis and vessel maturation during embryogenesis as well as vascular homeostasis during adulthood. Loss of Tie1 *in vivo* results in embryonic lethality due to severe vascular and lymphatic defects, manifested as edema formation and hemorrhage. However, Tie1 remains an orphan receptor with no known ligand binding to it nor leading to autophosphorylation. Therefore, alternative signaling mechanisms have been proposed, offering new directions to understand the role of Tie1. Tie1 heterodimerization with Tie2 leading to inhibition of Ang-1/Tie2 signaling or regulation of Ang-2/Tie2 agonistic properties have been reported. Pro-angiogenic VEGFA has been described to induce Tie2 phosphorylation and proteolytic processing of Tie1, resulting in a membrane-anchored cytoplasmic and a soluble extracellular domain of the receptor. Despite strong evidence of these mechanisms, little is known about the functional consequences of loss of Tie1 in EC being exposed to VEGFA. Therefore, this study aimed at investigating how absence of Tie1 alters VEGF-induced EC function, focusing on sprouting angiogenesis, migration, proliferation and apoptosis *in vitro* as well as an angiogenesis assay and hemangioma formation *in vivo*.

The study demonstrated that VEGFA-induced sprouting angiogenesis *in vitro* is independent of Tie1, but not of VEGFR2, as previously described. EC migration *in vitro* was impaired independent of VEGFA following Tie1 silencing. Furthermore, cell viability and cell cycle transition were enhanced in Tie1-silenced EC *in vitro*. This was consistent with the finding that pERK levels were increased in Tie1-silenced EC. EC apoptosis was induced by several reagents *in vitro*, including serum starvation, Desferrioxamine, a reagent mimicking hypoxia, and Staurosporine, a potent inhibitor of multiple protein kinases. Silencing of Tie1 inhibited apoptosis in EC exposed to these reagents and partial rescue of apoptosis in EC was mediated in a VEGFA/VEGFR2-dependent mechanism. VEGFA was shown to induce Tie2 phosphorylation in EC, which was in part dependent on Tie1, as silencing of Tie1 decreased pTie2 levels *in vitro* following VEGFA stimulation. Phosphorylation of Akt, a key mediator of Tie2 downstream signaling, was not affected in unstimulated EC, but was slightly decreased upon VEGFA stimulation in Tie1-silenced EC. Interestingly, Tie1-silencing downregulated EC-intrinsic VEGFA expression on mRNA level.

To establish if *in vitro* findings are manifested *in vivo*, a spheroid-based angiogenesis model was performed. In this model, Tie1-silenced EC displayed impaired vessel formation as demonstrated by reduced CD34-positive microvessel density and αSMA pericyte coverage. Additionally, polyoma virus transformed and immortalized EC, termed Endothelioma cells from $Tie1^{-/-}$ and $Tie1^{+/+}$ mouse embryos, were used in the spheroid-based *in vivo* angiogenesis model. Interestingly, the Endothelioma cells formed tumor-like lesions resembling hemangiomas. Endothelioma cells from $Tie1^{-/-}$ and $Tie1^{+/+}$ produced different hemangioma-like lesions according to their distribution of intratumoral hemorrhagic and solid areas with $Tie1^{-/-}$ hemangiomas displaying increased hemorrhagic areas.

In summary, it was demonstrated that Tie1 acts on EC to influence apoptosis, migration and proliferation signaling *in vitro* through an unrecognized mechanism. However, evidence was provided to suggest that this might be, at least in part, due to Tie2 activation. Additionally, impaired vessel formation in a spheroid-based *in vivo* angiogenesis assay using Tie1-silenced EC was reported. Lastly, reduced hemagioma integrity using transformed EC from $Tie1^{-/-}$ embryos was observed.