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The regulatory effect of semaphorin 7A on proliferation and migration in human umbilical vein endothelial cells

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Abstract

Semaphorin 7A (SEMA 7A), a factor originally identified as regulating axon growth, has recently been implicated as a pro-angiogenic factor. The molecular mechanisms for this ability to stimulate angiogenesis have not been identified. This study examines if SEMA 7A can have a direct effect on vascular endothelial cells or whether it indirectly induces angiogenesis through stimulation and recruitment of macrophages as has been suggested. Using a human umbilical vein endothelial cells (HUVECs), the ability of SEMA 7A to affect proliferation and migration was examined. HUVECs were exposed to SEMA 7A directly or to conditioned media collected from macrophages exposed to SEMA 7A and a cell proliferation assay was performed. Preliminary results suggest that direct exposure of HUVECs to SEMA 7A resulted in a significant decrease in proliferation. Additionally, our results suggest that macrophages exhibited a slight stimulation of migration in response to SEMA 7A.

Introduction

Angiogenesis is the process by which new blood vessels are produced from existing vessels (reviewed in Bianco and Gerhardt, 2013). Many different factors work together to regulate this very important process including growth factors such as members of the vascular endothelial growth factor family (VEGF). Another family of molecules that has recently been identified as having both pro- and anti-angiogenic characteristics, are the semaphorins (Neufeld et al., 2012) which were originally identified as factors that regulate axon path finding in both a positive and negative manner. Studies suggest that in vivo expression of exogenous semaphorin 7A (SEMA 7A) resulted in both angiogenesis (Ghanem et al., 2011) and macrophage recruitment (Holmes et al., 2002). The molecular mechanisms of this pro-angiogenic effect of SEMA 7A is not fully known and it is not clear whether this activity can be attributed to a direct effect on the vascular endothelial cells or was indirectly due to the ability to recruit macrophages to the area. Interestingly, SEMA 7A is a membrane bound molecule that has an RGD binding domain, which can bind to beta-1 integrin. In axons, SEMA 7A has been shown to bind to beta-1 integrin and stimulate the ERK pathway (Pasterkamp et al., 2003). Activation of the ERK pathway has been shown to stimulate proliferation and migration, which are key factors in angiogenesis. Beta-1 integrins are present on endothelial cells, and have been shown to support VEGF ERK signaling (Senger et al., 2004). This suggests that SEMA 7A may stimulate angiogenesis through direct stimulation of the ERK pathway in endothelial cells rather than through indirectly recruiting macrophages to the area to release pro-angiogenic factors. Using an in vitro tissue culture model, the ability of SEMA 7A to stimulate angiogenesis directly (figure 1A) in human umbilical vein endothelial cells (HUVECs) or indirectly (figure 1B) through macrophage stimulation were examined. Upon exposure to SEMA 7A, early events in angiogenesis were measured through proliferation and migration assays.

Direct Effects of SEMA 7A on Proliferation

Using an in vitro Fssue culture model, the ability of SEMA 7A to affect proliferation and migration was examined. HUVECs were exposed to SEMA 7A directly or to conditioned media collected from macrophages exposed to SEMA 7A and a cell proliferation assay was performed. Preliminary results suggest that direct exposure of HUVECs to SEMA 7A resulted in a significant decrease in proliferation. Additionally, our results suggest that macrophages exhibited a slight stimulation of migration in response to SEMA 7A.

Macrophage Transwell Migration

Figure 5 VEGF and SEMA stimulate migration of macrophages. Using RAW 264.7 macrophage cells, a Transwell migration assay was performed as described in figure 3. The results suggest that consistent with the literature, soluble SEMA 7A, when placed in the lower chamber, stimulates chemotaxis.

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Figure 1 Diagram of the direct and indirect models examined in this study. In this study, an in vitro tissue culture system was used to determine whether SEMA 7A can (A) directly act on HUVECs or whether it indirectly promote angiogenesis by inducing macrophages (RAW 264.7 cells) to release pro-angiogenic factors.