THE EFFECTS OF CLASS-3 SEMAPHORIN PROTEINS ON ANGIOGENESIS *IN VITRO* SYSTEM

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Semaphorins are a large family of secreted, transmembrane, or GPI-anchored proteins that are found in variety of tissues and organ systems: nervous, cardiovascular, endocrine, gastrointestinal, immune, reproductive, respiratory, and other systems [1,2]. Semaphorins are best known for their roles in nervous system development, but they also are involved in tumorigenesis by regulating angiogenesis process which in abnormal conditions is a key mediator in cancer development [3,4]. Recent research works suggest that class-3 semaphorins (Sema3) fulfill important regulatory roles in multiple forms of cancer [5]. Depending on the specificity of the tissue, malignancy of the tumor, receptors on the surface of the cytoplasmic membrane, growth factors and ability of proteases to cleave semaphorins, Sema3 can promote or inhibit tumor angiogenesis processes [6]. For example, Sema3C can inhibit angiogenesis in pathologic retinopathy but also can function as angiogenesis promoting protein in gastric cancer [7,8].

The aim of this study was to investigate the effects of Sema3 (B, D, E, and G) on angiogenesis process *in vitro* system. First, expression vectors encoding Sema3 (B, D, E, and G) and a green fluorescent protein Venus or EGFP were constructed. Their construction was verified by performing restriction analysis. Then, human embryonal kidney cells 293FT were transfected with these vectors. The transfection efficiency was checked with fluorescent microscope and the expression of Sema3 (B, D, E and G) proteins in cells and cell media was confirmed by reverse transcription polymerase chain reaction (RT-PCR) and western-blot analysis. The medium of transfected cells, as a source of Sema3 proteins, was collected and used in angiogenesis *in vitro* assay with human umbilical vein endothelial cells (HUVEC). After 16 hours of incubation photos of microcapillary structures formed by endothelial HUVEC cells were taken (Fig. 1). Then, the parameters of microcapillary structures such as number of meshes, mean mesh size, number of junctions, number of master junctions, number of master segments and total length of master segments were analysed with „ImageJ Angiogenesis analyser“ program. Due to the inhibitory function of Sema3A on angiogenesis process [9], in our study it was used as negative control. Finally, statistical analysis (Student's t test) of the resulting quantitative data was performed using GraphPad Software Inc.'s Prism 8 software. The results revealed that among Sema3 (B, D, E, and G) proteins, Sema3B significantly promoted the formation of microcapillary structures. Other proteins (Sema3D, Sema3E and Sema3G) did not show any significant effects on the angiogenesis process *in vitro* compared to control.

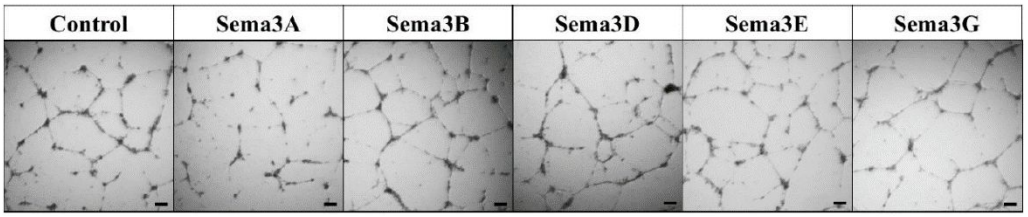

Fig. 1 Analysis of Sema3 effects on formation of the microcapillary HUVEC structures
Photos were taken after 16 hours of incubation. The black line in the lower right corner of the photo represents the 100 μm scale

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