

DOWNREGULATION OF FLI1 IN HUMAN MICROVASCULAR ENDOTHELIAL CELLS PROMOTES CELL SURVIVAL AND LEADS TO CORD FORMATION IN 3D COLLAGEN GELS

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INTRODUCTION. Fli1 (Friend leukemia integration-site 1) is a member of the Ets family of transcription factor, and is frequently found in Ewing sarcomas and peripheral neuroectodermal tumors. In our preliminary study we observed downregulation of Fli1 expression in endothelial cells and fibroblasts in invasive breast carcinomas. Fli1(-/-) mice die at embryonic day 11.5, with a loss of vascular integrity leading to cerebral hemorrhage, suggesting that Fli1 may play a role in regulation of vascular system(1,2).

The aim of this study was to investigate the role of Fli1 in human dermal microvascular endothelial cells (HDMEC).

METHODS. HDMECs were isolated in our laboratory from newborn foreskins and propagated in EGM-2 MV with 5% fetal bovine serum (CAMBREX, USA). Adenoviral vector expressing small interfering RNA (siRNA) corresponding to Fli1 gene was generated for this study. Human Endothelial Cell Gene Array (HS-036, SuperArray Bioscience, USA) and previously described 3D angiogenesis model (2) were used

RESULTS. Fli1 specific siRNA suppressed Fli1 mRNA expression level by 72% as compared to non-silencing siRNA. To identify gene programs regulated by Fli1 in HDMECs, we used Human Endothelial Cell Biology Gene Array. Out of 102 genes, 40 genes produced a detectable signal. Upregulated genes included ADAM17, Ang-2, Flt-1, COX-2, Tie-2, ICAM-2, Integrin alpha 5, EPI/LACI and GDCF-2, while downregulated genes included Tsp-1, annexin A5, IL-8, Caspase 1 and RIP. This data suggested that Fli1 may regulate cell migration and cell survival pathways.

To investigate the functional significance of Fli1 downregulation, we utilized 3D angiogenesis model. Confluent cells were overlaid by acellular collagen

layer and then covered by a collagen layer containing adult dermal fibroblasts. In this model control HDMECs (GFPAd) produce cords in the presence of 5% serum, while no cord formation is observed in the absence of serum. In contrast, HDMECs with reduced Fli1 expression (Fli1Ad) produce cords in the presence or absence of 5% serum. Formation of immature cords by HDMECs (FliAd) was also observed in serum-free medium in the absence of fibroblasts (Fig. 1).

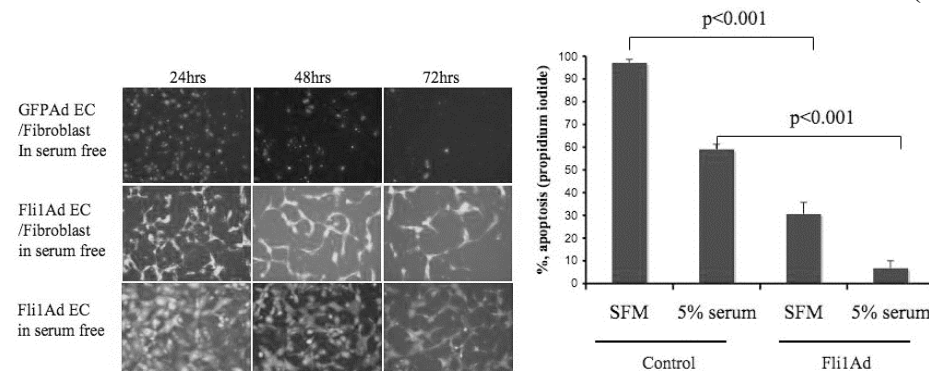


Fig.1 Fli1Ad HDMECs form cords in the presence or absence of 5% serum (left panel). Percentage of apoptotic HDMECs at 48 hrs. (right panel).

Similar experimental conditions were used to analyze cell survival. To detect apoptotic cells HDMECs were stained with propidium iodide, while live cells were detected by staining with Hoechst33342. Control and FliAd HDMECs were overlaid by acellular collagen layer and incubated for 48 hours in the presence or absence of 5% serum. The percentage of apoptotic cells in control HDMECs was 59.1% and 97.1% and in Fli1Ad 6.7% and 30.6% in the presence or absence of serum, respectively.

DISCUSSION. This study demonstrates that downregulation of Fli1 may play an important role in tumor angiogenesis by modulating cellular programs involved in cell migration and cell survival.

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