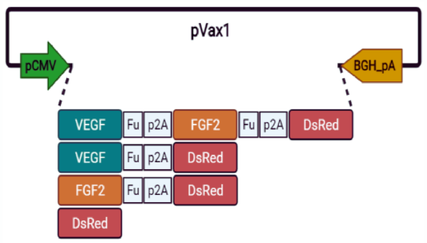
Co-expression of VEGF and FGF2 mediated by multigenic plasmid constructs promotes blood flow restoration in a rat model of hind limb ischemia

Dilara Gatina, Ekaterina Garanina, Margarita Zhuravleva,Angelina Titova, Ilnur Salafutdinov
Institute of Fundamental Medicine and Biology, Kazan Federal University, Russia
sal.ilnur@gmail.com, gatina_dilara@mail.ru

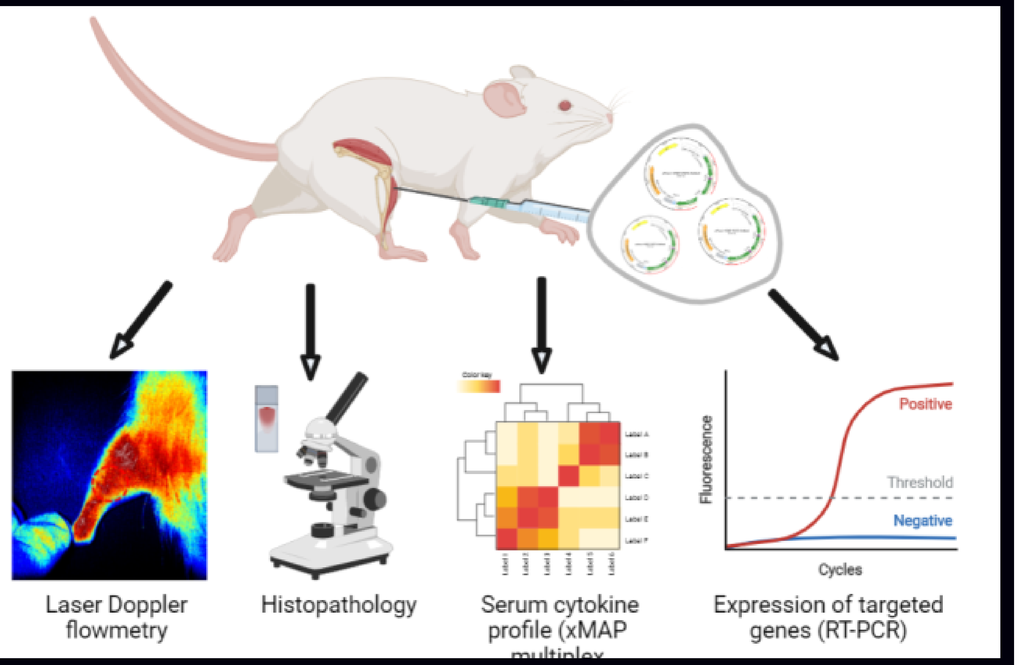
Peripheral arterial disease (PAD) is characterized by stenosis and occlusion of the arteries leading to the poor blood supply to the limb. Patients with PAD suffer pain at rest, intermittent claudication, skin ulcers, and gangrene. The delivery of angiogenic factors to restore tissue perfusion is an attractive strategy, both as a primary and adjunctive treatment for PAD.



Schematic representation of genetic cassettes containing therapeutic proteins (VEGF165, FGF2) and a fluorescent reporter protein (DsRed)

We synthesized multigenic plasmid constructs expressing combinations of VEGF, FGF2, and DsRed genes. In the constructed vectors, gene sequences are linked through the furin-containing 2A-peptide sequence of picornaviruses. Plasmid vector pVax1, approved by the FDA for use in clinical trials.

In the current study, we simulated a surgical model of ischemia. On the 7th day after the simulation of two-stage ischemia, a plasmid solution was injected into the heads of the calf muscle. For the control group, thesame volume of physiological saline was injected. Next, we measured blood flow using laser Doppler imaging in both legs preoperatively and postoperatively on 3,14, and 21 days. We performed pathohistolic analysis of muscle tissue slices and analyzed the expression of target genes by PCR-RV. To analyze the immunological responses of the host rat, we performed cytokine profiling of rat serum using Luminex® xMAP® technology.



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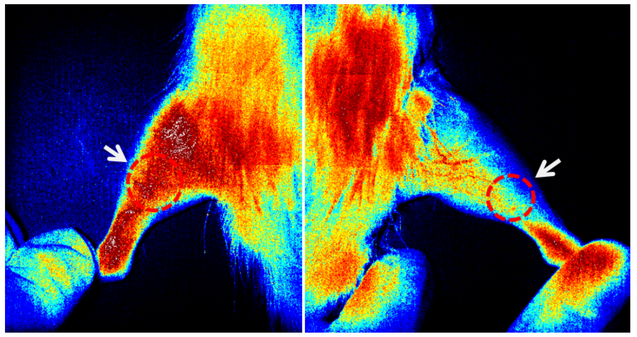

Figure1. Representative figures of blood flow measurements by laser Doppler iImaging in hindlimb ischemia model.On the left, a healthy limb; on the right, the operated limb.

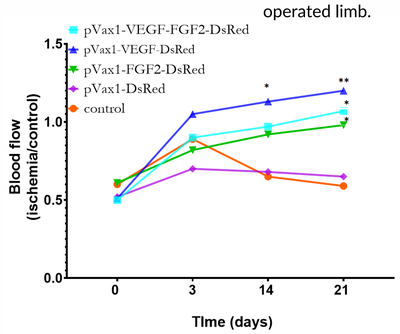

Figure 2. Time course of blood flow recovery by laser Doppler imaging in ischemic muscles of rats during 21 days.

We observed a gradual increase in volumetric blood flow in the experimental groups. On day 21, the maximum increase in volumetric blood flow was in the pVax1-VEGF-FGF2-DsRed group.

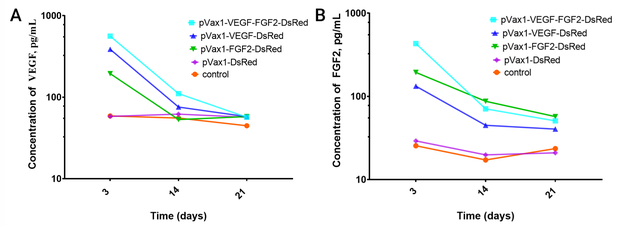

Figure 3. Levels of VEGF (A) and FGF2 (B) secretion in the muscles of experimental and control rats at 3,14, 21 days after ischemia.

The secretion of the target proteins VEGF and FGF2 decreased at 14 and 21 days.

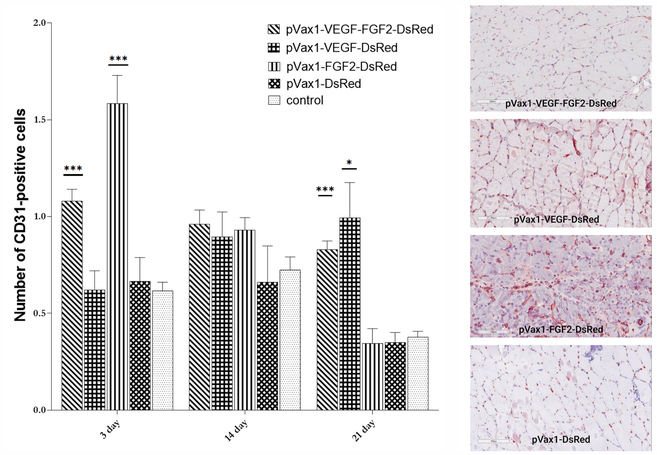

Figure 4. Capillary density at 3,14, 21 days in ischemic rat tissue sections, after injection of plasmid constructs (A). Microscopic images of histological sections stained with antibodies to CD31(B).

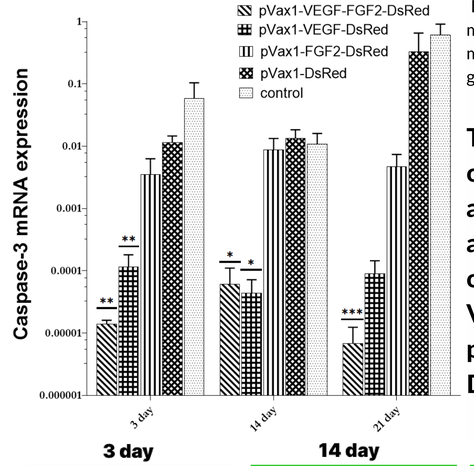

Figure 5. Analysis of Caspase-3 mRNA gene expression in ischemic muscles of control and experimental groups of rats

The lowest mRNA levels of the Caspase 3 gene at all time points studied after ischemia were observed in the pVax1-VEGF-DsRed and pVax1-VEGF-FGF2-DsRed ischemia groups.

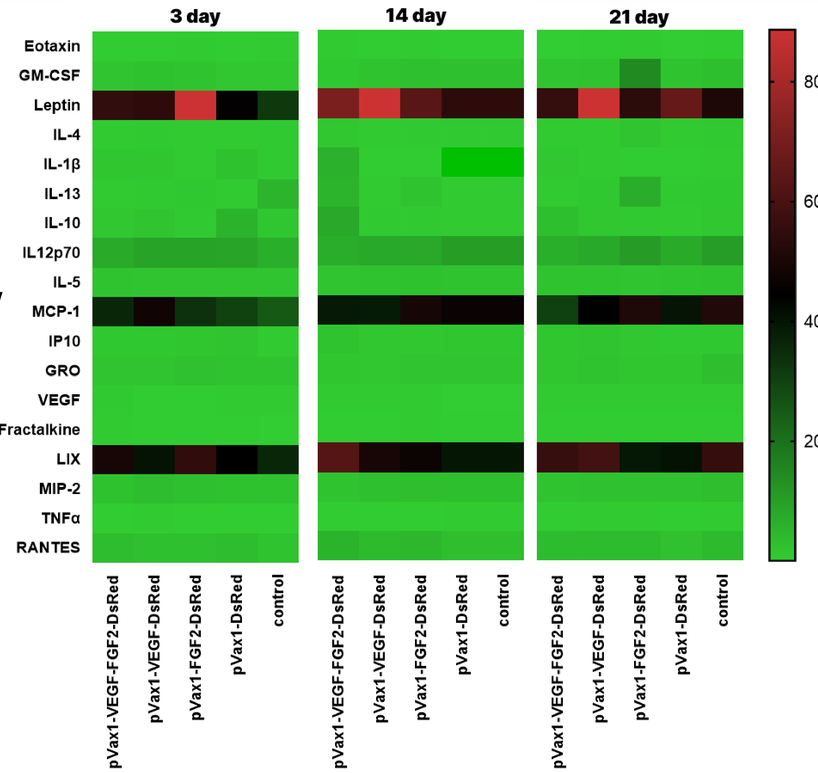

Figure 6. Heat maps of identified cytokines, chemokines, and growth factors in the serum of rats with chronic lower limb ischemia

Quantitative determination of soluble factors in the serum of rats with chronic ischemia of the lower extremities did not show significant differences in the secretion of the studied cytokines.

Conclusion

Co-expression of VEGF and FGF2 stimulates angiogenic and regenerative processes in a rat model of hind limb ischemia. The study of the serum cytokine profile showed the absence of adverse immune effects, indicating the safety of the non-viral constructs used. These results suggest the possibility of using these non-viral constructs to enhance therapeutic angiogenesis in the treatment of ischemic diseases

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