Molecular Analysis of Angiogenesis in Space

Ryuichi Morishita, Hiromi Koike
Division of Gene Therapy Science, Osaka University Graduate School of Medicine
2-2 Yamada-oka, Suita, Osaka 565-0871, Japan
TEL: 06-6879-3901  FAX: 06-6879-3909
e-mail: morishita@gts.med.osaka-u.ac.jp

1. Purpose of the present study

It is obvious that angiogenesis is one of key important phenomenon in the maintenance of life, since the knock-out mice of VEGF is lethal. In addition, angiogenesis is related to numerous disease including cancer, ischemic heart disease and diabetic retinopathy, and so on. However, its molecular mechanisms are still largely unknown despite of many efforts of the researchers. Angiogenesis-related disease is also critical for human being living in the aerospace. Therefore, it is quite important to study the molecular mechanisms, considering the development of novel therapy for those diseases. In the process of angiogenesis, the proliferation and/or migration of endothelial cells involved in the formation of 3D structure including matrix degradation is pivotal. Probably, the formation of 3D structure may be related to the influence of gravity. Thus, it is not surprising that the process of angiogenesis might be different between the high and zero gravity. Using aerospace, it might be possible to elucidate the role of gravity on the process of angiogenesis. If the high gravity influences the formation of collateral and angiogenesis, the concept of dimension from the preexisting arteries might be considered. Furthermore, in near future, it would be increased that the people stay in the space for more long time. In some cases, the elderly populations like Mr. Glen will stay in the space. In these circumstances, the stimulation or inhibition of angiogenesis in aerospace might be related the incidence of cancer or cardiovascular disease. From this viewpoint, the present study will provide the important information to the future space plan. Alternatively, the molecular mechanisms of angiogenesis related to gravity will also provide new therapeutic strategy to treat angiogenesis-related disease such as cancer and cardiovascular disease.

2. Materials & Methods

2.1 Effect of gene transfer of angiogenic growth factor on the blood flow

Initially, we performed co-transfection of angiogenic growth factors such as VEGF or HGF with prostacyclin synthase gene, since prostacyclin is well known to dilate the
blood vessels. After gene transfer, the blood flow was measured by laser Doppler system in mice and rabbit hindlimb ischemia model. In addition, VEGF or HGF protein was also measured using ELISA. The capillary formation was also analyzed by the counting using alkaliphosphatase staining.

2.2 Expression pattern of genes in response to angiogenesis under gravity
Mouse (C57/BL6) was exposed to zero-gravity or micro-gravity using free fall instrument or airplane. Then, the aortas were removed after sacrifice, and frozen by liquid nitrogen immediately. Total RNA and PolyA+RNA were extracted from control or experimental mouse. The differential pattern of mRNA expression was examined using microarray.

2.3 Effects of high gravity on angiogenesis
To study the high gravity on angiogenesis, we employed rolling arm experimental instrument in NASDA. After creating hindlimb ischemia model by the surgically removed the femoral artery in mouse (C57BL/6), human HGF (hepatocyte growth factor, an angiogenic growth factor, plasmid DNA (500ug/150ul 0.9% saline) was intramuscularly injected into the ischemic hindlimb at 10 days after operation. Then, mouse was moved into the rolling arm instrument, and rolled for 2 weeks at 3G. During the rolling, to maintain the mouse condition, the experiments were interrupted for 1 hour every 2 days.

3. Results
Co-transfection of genes of angiogenic growth factors (VEGF, HGF) with prostacyclin synthase gene resulted in a significant increase in the blood flow and capillary density as compared to the transfection of single gene (Fig.1). These results clearly demonstrated the vasodilative response regulated angiogenic process. Unexpectedly, using rolling arm, the blood flow was significantly improved in mouse conditioned under high gravity as compared to normal gravity after HGF gene transfer (Fig.2). The microarray demonstrated that the cyclin G and IL-12 were markedly decreased under zero-gravity. Moreover, other unknown genes were also different from the mouse conditioned under zero-gravity and normal gravity. We have still continued to analyze the data in more details. Overall, the high gravity rather than micro gravity seems to stimulate the angiogenesis. Probably, the vasodilatation of the blood vessels by high gravity might be related to the stimulation of angiogenesis under high gravity.
4. Future direction to the study of space environment

The final goal of the present study is 1) to identify the molecular mechanisms of angiogenesis, and 2) to develop the novel therapeutic strategy to treat ischemic disease. Then, it is possible to precise the risk of angiogenesis-related disease such as cardiovascular disease and cancer and treat those disease when the human being live in the space. To achieve the final goal, it is necessary to continue the study using space shuttle in order to elucidate the effects of long time exposure to no gravity.

【List of Publications】
Conference reports
5. Hiromi Koike, Ryuichi Morishita, Naruya Tomita, Yoshiaki Taniyama, Tadashi


Reviews


URL
http://www.med.osaka-u.ac.jp/pub/gts/