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## Effects of Electrical Stimulation on Proteins Related to Signal Transduction, c-Src and Focal Adhesion Kinase in Fibroblasts <sup>+</sup>

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Abstract

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Abstract: In the field of acupuncture and moxibustion, electrical stimulation of the skin and muscles 9 is known to increase blood flow and metabolism locally and maintain the body in a sustained 10 healthy state. However, little is known about the changes in cellular morphology in response to the 11 electrical stimuli or how the localization of specific proteins is regulated by such stimulation. To 12 gain greater understanding in this respect, the present study examined the effects of electrical 13 stimulation on the cytoskeletal system of cultured fibroblasts. Cultured fibroblastic cells were 14 subjected to periodic electrical stimulation for 0 (unstimulated control), 2, 5, and 20 hours. After 15 approximately 2 hours, the stress fibers and focal adhesions in the cells had become enlarged, the 16 stress fibers exhibiting an increase in thickness, while the cells had a contracted appearance. During 17 20 hours of periodic stimulation, both the stress fibers and focal adhesions gradually became larger 18 and thicker. After the electrical stimulation, the cells exhibited increased staining of focal adhesions 19 with anti-phosphotyrosine antibody (PY-20). They also exhibited increased staining of tyrosine-20 phosphorylated focal adhesion kinase (FAK) (pY397) and tyrosine-phosphorylated c-Src (pY418), 21 indicating that the electrical stimulation had affected proteins related to signal transduction. ELISA 22 analysis showed that 20 hours of electrical stimulation gradually increased the activity of tyrosine-23 phosphorylated c-Src until it was approximately tripled, whereas 5 hours of electrical stimulation 24 approximately doubled the activity of tyrosine-phosphorylated FAK, this being the maximum 25 reached. These results strongly suggest that electrical stimulation induces changes in the activation 26 of c-Src and FAK signaling-related proteins and affects the formation of the cytoskeletal system. 27

Keywords: Electrical stimulation, Src, FAK, focal adhesion kinase, cell adhesion, cytoskeleton, stress28fiber, focal adhesion29

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