

The up-regulation of CTGF is involved in high-glucose-induced fibronectin production, but not in the increased accumulation of hyaluronan in ECM of dermal fibroblasts

Natalia Yevdokimova¹, Sergij Podpryatov²

¹ Molecular Immunology Department, Institute of Biochemistry, 9 Leontovich str, 01601, Kyiv, Ukraine

Phone: +380 44 234 59 74, Fax: +380 44 279 63 65, E-mail: berezan@mathber.carrier.kiev.ua

² Department of Surgery, First City Hospital, Kyiv, Ukraine

BACKGROUND: The imbalance in the homeostasis of ECM is considered to be a hallmark of diabetic skin connective tissue. The elevated blood glucose level is thought to be the main cause of this phenomenon, but the mechanisms mediating high glucose effect are poorly understood. Although the up-regulation and activation of TGFbeta1 are known to play an important role in the hyperglycaemia effects in various tissues, other growth factors may also be implicated, especially in case of skin ECM derangement in diabetes, when TGFbeta1 is hardly involved. We were interested to study whether the activation of angiotensin II / receptor type 1 pathway with the consequent involvement of CTGF may be the possible cause of high-glucose-induced matrix abnormalities in cultured dermal fibroblasts.

METHODS: Cell culturing, ELISA, semi-quantitative RT-PCR, Western blotting, metabolic labeling and analysis of hyaluronan molecular size distribution.

RESULTS: High glucose treatment of cultured human dermal fibroblasts led to the following: (1) the angiotensin II receptor type 1 (AT1) was up-regulated at the level of mRNA and protein, unlike the receptor type 2; (2) the generation of angiotensin II and the mRNA expression of all components of the local renin-angiotensin system were not altered; (3) the mRNA and protein expression of CTGF were up-regulated, and this effect was cancelled by the blockage of AT1 with losartan; (4) the fibronectin production was increased, also cancelled by losartan, while an anti-CTGF-neutralizing antibody only partly reduced it; (5) the incorporation of ³H-glucosamine in high-molecular-weight (>2000 kDa) pericellular hyaluronan was increased, but was insensitive to both losartan and anti-CTGF-neutralizing antibody treatment; (6) the mRNA expression of hyaluronan synthases (HAS)- 1, -2, -3 was not altered; (7) TGFbeta1 expression, the secretion of total and active TGFbeta1 were not changed.

CONCLUSIONS: The up-regulation of AT1 and the consequent increase of CTGF expression, independently of TGFbeta1, participate in high-glucose-induced fibronectin production in cultured human dermal fibroblasts. In contrast, the increased accumulation of pericellular high-molecular-weight hyaluronan was not determined by the up-regulation of AT1 and CTGF. Since the expression of HAS1, HAS2 and HAS3 was not changed under the action of high glucose, we suppose the involvement of decreased hyaluronan degradation in the observed enrichment of fibroblast ECM with high-molecular-weight hyaluronan. Future studies are necessary to determine the reasons of this phenomenon, which may be relevant to impaired wound healing in diabetes.