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THE TISSUE TRANSGLUTAMINASE ACTIVITY DOES NOT STRENGTHEN CELL ADHESION DURING MECHANICAL STRETCH

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Abstract. Tissue transglutaminase activity (tTGA) has been shown to be important in cell adhesion to extracellular matrix (EM). However, it is not well know if tTGA improves cell adhesion during mechanical strains imposed on the EM. We have compared the adherence properties of NIH 3T3-fibroblast cell line on fibronectin (FN) and on an EM that lacks the 42KD tTG-binding site. The cells were plated on silicone membranes and were stretched 10% also. Results indicate cell loose their morphology in the absence of tTG binding site and mechanical stretch exacerbates this poor response. The presence of tTGA is important in initial cell adhesion but does not strengthen the cell adhesion to EM.

Keywords: Cell adhesion; extracellular matrix; cyclic stretch

I. INTRODUCTION

The cell adhesion to the extracellular matrix (EM) is extremely important because it involves EM recognition, cell spreading and migration. Its has been shown also that cell adhesion can invoke anti-apoptetic signaling cascades. The EM adhesion process is primarily mediated by integrins that are covalently bound to specific motifs present on the EM. Of interests are the β1 and β3 integrins that are expressed in most tissues and can facilitate adhesion to fibronectin matrices. It has been shown that signalling through integrins can initiate outside-signaling cascade promoting the formation of focal adhesions and ultimately the cell spreading and survival. Various studies have shown that on fibronectin (FN) substrates the cell adhesion is primarily mediated by a 110 KD sequence comprising of a classical RGD motif. Recently, however, it has been suggested that tissue-transglutaminase (tTG) activity (tTGA) also facilitates and/or strengthens the integrin binding to the FN through a separate 42-KD gelatin binding domain present on the FN [1]. This suggestion that tTGA has little to do with cross-linking but facilitates/strengthens the integrin binding to FN and perhaps independently activate the outside-in signaling cascade is compelling. Despite tTGAs are abundantly expressed in most tissues, whether the primary role of the tTGA is to facilitate or to strengthen the integrin binding to FN is not well understood. Ideally these two activities should improve cell survival.

In this study this role of tTGA in cell adhesion to the EM was investigated. For this purpose, cells were cultured on substrates that had 110Kda sequence but either lacked/or preserved a 42-KD binding sequence of the tTGA. This helped to elucidate if tTGA facilitated the cell adhesion. Secondly, cyclic stretch was imposed on cells grown on these matrices. This experiment isolates the strengthening role of tTGA on cell adhesion.

II. METHODOLOGY

NIH 3T3 fibroblasts were cultured on plates coated with 2% gelatin. The cells were maintained in a medium containing 10% Fetal Bovine Serum, Dulbecco's Modified Essential Medium (DMEM, Gibco), Penicillin and Streptomycin (100U/ml each). The cells were incubated at 37C 5% CO_2 in air and 100% humidity. The cells were grown until confluence and then made quiescent for 16-18 hours by changing to medium to a low serum supplement (0.3% FBS). On the day of the experiment the cells were washed twice with PBS, incubated for 10 minutes with dissociation medium (5 mM EDTA in half strength PBS). This allowed preservation of the tTGA because trypsin rapidly degrades tTG. The cells were then detached and re-suspended in starvation media containing DMEM and antibiotics. The cells were then plated on the silicone membranes coated with RGD and with a synthetic RGD polymer (sRGD) which does not contain the 42 KD fragment (Sigma, St. Louis, MO)

The stretch apparatus was a custom-built servo device that provides uniaxial stretch to thin silicone membranes, was also prepared in house. Specific grooves etched on the membranes (250μm x 250μm x 35μm) allowed both to calibrate the measured strain and also provided extra support for uniform stretch of the extracellular matrix. A microcontroller governed the servomotor to yield a desired pattern of stretch to the silicone membrane. The membranes were coated with the 10μg/ml FN and 10μg/ml sRGD in 50 mM Tris-HCL and were let to passively absorb the substrate in 4 C for 36 hours. The coated membranes were then blocked with 3% BSA for one hour in room temperature and subsequently washed with starvation media. The cells were then allowed to attach to the substrates for 4 hours after which they were either subjected to a %10 sinusoidal stretch (2 Hz) or no-stretch for further 4 hours.

A. Measurements and Statistical Analysis

At the end of the experiment the cells were washed twice with PBS, fixed and stained with coomassie blue (Pega DJM, 1980). Cells from random sections of the membranes were photographed at x20 magnification and were classified as healthy or dead based on proper appearance of the cytoplasm around a well-defined nucleus. To eliminate investigator bias, a blinded observer performed these

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Abstract

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counts. A multiple linear regression analysis were performed using the percent of healthy cells present on the substrate as the dependent parameter; the substrate (FN or sRGD) and stretch (yes or no) as the independent parameter. Statistical significance were taken at $p<0.05$ level.

III. RESULTS

Nearly fifty percent (52.9±7.7%) of the NH 3T3 cells were healthy looking when they were plated on a fibronectin-coated membrane (Figure 1). They were however were less likely to be so (15.6±7.0%, $p<0.05$) on membranes coated with the synthetic polymer lacking the tTGA. Stretch on itself increased the propensity cell death by 14.9% but this effect was not significant in the multivariate analysis ($p<0.067$).

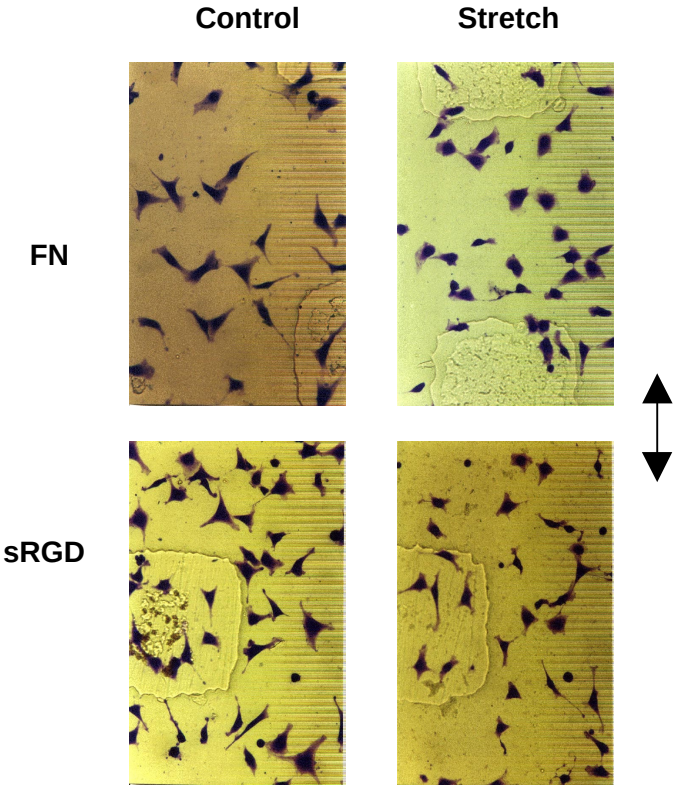

Figure 1. Photomicrographs of the NIH3T3 cells

IV. CONCLUSION

Primary findings of this study are as follows: (1) presence of tTG binding site on the substrate increases chances of survival and (2) the cells were susceptible to the substrate stretch.

This study confirms that the presence of tTG and its 42KD substrate-binding site is important for cell adhesion. However, the results of mechanical cyclical stretch experiment indicate that the bond between tTG and the 42KD-binding site does not have a strengthening affect on the cell adhesion.

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REFERENCES

[1]. Akimov SS, Krylov D, Fleischman LF and Belkin AM. Tissue transglutaminase is an integrin-binding adhesion coreceptor for fibronectin. J Cell Biol vol 148 pp825-38 Feb 2000