EFFECT OF FOCAL ADHESION KINASE ON FEK 293 CELL DETACHMENT

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INTRODUCTION

Adhesion to the extracellular matrix (ECM) is directed by the integrin family of receptors. Attachment of the cell's integrin receptors to an ECM ligand leads to the formation of well defined structures linking the ECM to the cell's actin cytoskeleton. These points of attachments are called focal adhesions and they anchor a complex actin stress fiber network. These networks are dynamic as cells move and respond to their environment. This dynamic process is regulated largely in part by protein tyrosine kinases. Focal Adhesion Kinase (FAK) is an important protein tyrosine kinase that serves in the regulation of the flow of signals from the ECM to the actin cytoskeleton. FAK also has been shown to mediate cell growth and survival as a result of its part in cell adhesion [1]. Scientists have demonstrated that FAK is over expressed in a variety of cancers [2]. Because of its role in cell growth, survival, motility, and adhesion, it has been hypothesized that the over-expression of FAK contributes to metastatic properties of tumor cells [2]. Further information can be found on our site at http://www.uic.edu/com/dom/gastro/labvideos. Tumor metastasis is a complex process, therefore it is important to determine the effect of FAK in all aspects including cell detachment, adhesion, and migration. The objective of this study is to analyze the effects of FAK on cell detachment of a well defined Fetal Epithelial Kidney 293 cell line (FEK 293).

MATERIALS AND METHODS

Three distinct populations of cells were used: FEK 293 cells transfected with HYG1 vector (HYG1), FEK 293 cells transfected with FRNK vector and treated with doxycycline (FRNK), FEK 293 cells transfected with HYG1 vector and treated with Methyl Ester (ME). The FEK 293 cell line normally expresses FAK. The HYG1 vector is a placebo vector that does not change the normal cell function or the normal expression of FAK. Therefore the HYG1 cells are used as a control in these experiments. The FRNK vector provides a sequence for the production of a protein FRNK in a tetracycline on/off system. When activated FRNK is produced and interrupts the production of FAK, leaving almost no FAK expressed in the cell. Methyl ester treatment also interrupts the use of FAK by the cell. However, it does so by preventing the phosphorylation of FAK which is required for activation.

The instrument used for the adhesion assay is the Rheoscope [3, 4]. This device has a cone plate configuration. There are many advantages for using this type of instrument to shear and detach adherent cells. The rheoscope produces a well defined homogenous laminar flow, and only a small volume of media and number of cells are required for each assay. Due to the design, photographs and video may be taken while the cells are exposed to shear and cell detachment can be observed.

Previous experiments using this type of device have studied the effect of a physiological shear stress on endothelial cell detachment [5, 6]. This value can be as high as 100 dynes/cm^2 in arterioles [7, 8]. For our experiments a much smaller shear stress was needed. Because tumor cells that detach and metastasize usually enter the circulatory system through small blood vessels, only a shear stress corresponding to that in capillary beds, venules, and even the lymphatic system should be required [9]. In venules, the shear stress is shown to be approximately 5dynes/cm^2 or less [7,8]. The lymphatic system is shown to produce shear stresses less than 3.5dynes/cm^2 [8].

For the present experiments, the respective cells were seeded on glass cover slips at an average density of 8 cells/mm^2. The HYG1 cells were not treated. The FRNK cells were treated with doxycycline at the concentration of 2μg/ml. The ME cells were treated with 1μM methyl ester. The cells were then incubated for approximately 24. Before the assay was performed the cover slips were washed by 2 drops of media from a transfer pipette while holding the cover slip at a 45 degree angle. The cells were counted manually using an upright microscope. The cover slip was placed into the chamber of the rheoscope and sheared for 15 seconds. The shear rates applied were 2.4 s^{-1}, 10.5 s^{-1}, 18.3 s^{-1}, 28.1 s^{-1}, and 39.5 s^{-1}, corresponding to shear stresses 0.055 dynes/cm^2, 0.24 dynes/cm^2, 0.42 dynes/cm^2, 0.64

dynes/cm^2, and 0.90 dynes/cm^2 respectively. The cover slip was washed again after shearing to remove any non-adherent cells and counted. For all cell types used 10 assays were performed at each shear rate.

RESULTS

As shown in Figure 1, the three cell types have distinct differences in detachment properties. The HYG1 control cells detached less readily than those cell types that did not contain phosphorylated FAK. The P values for the results of the adhesion assays performed are listed in Figure 2. The FRNK activated cells and the HYG1 cells showed statistically different adhesive properties at all shear stresses above 0.42dynes/cm^2. This result suggests that the expression of FAK increases the adhesive properties of the cells. A typical effect of shear stress on cell attachment is shown in Fig. 3. The ME treated cells also expressed statistically different adhesive properties above the shear stress of 0.90dynes/cm^2. This result shows that FAK must be phosphorylated to play an active role in cell adhesion. The statistically different results between the ME treated cells and the FRNK activated cells, at shear stress above 0.42dynes/cm^2, was unexpected. This could have occurred if methyl ester did not completely prevent the phosphorylation of FAK.

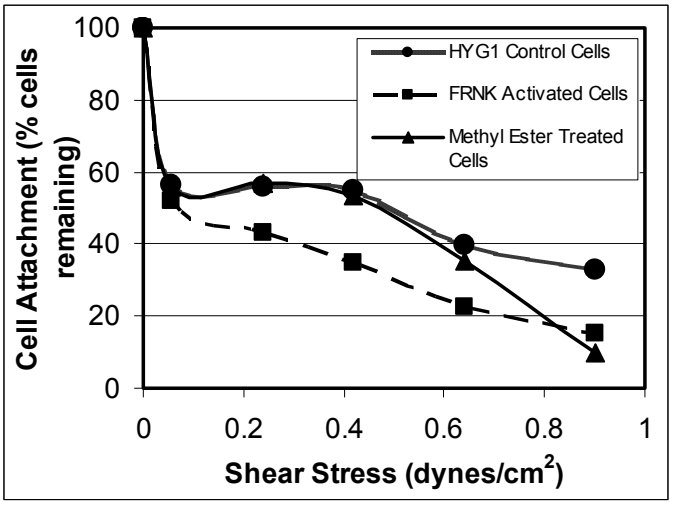

Figure 2. Average percent of cells remaining attached after application of shear stress for 15 sec.

Shear Stress (dynes/cm^2)	Cell Treatment Type		
	Between HYG1 and FRNK	Between HYG1 and ME	Between FRNK and ME
0.055	0.25	0.48	0.31
0.24	0.025	0.46	0.02
0.42	<0.01	0.36	<0.01
0.64	<0.01	0.16	0.01
0.90	<0.01	<0.01	0.01

Figure 3. P Values between the three cell treatment types for different values of shear stress.

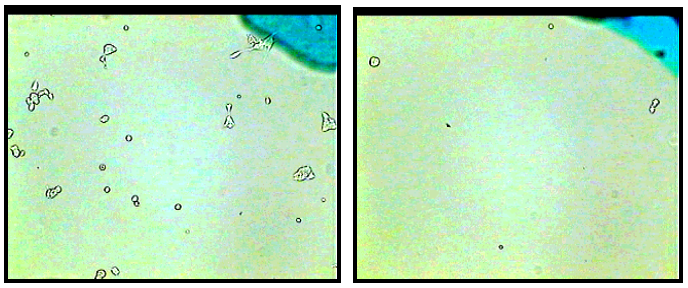

Figure 3. Photograph of FRNK activated cells before (right) and after (left) application of a shear stress of 0.9 dynes/cm^2 for 15 sec.

In conclusion, it has been determined that the expression of the phosphorylated FAK in an epithelial cell produces stronger adhesive properties when exposed to shear stress. However, cells must detach from a tumor readily to metastasize. Therefore, this data opposes the hypothesis that over-expression of FAK is necessary a cause for metastasis. It suggests that FAK plays a part only as a morphogen, not as a mitogen.

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