INFLUENCE OF GRP RECEPTOR-INDUCED PHOSPHORYLATION OF FAK TYROSINE 397 ON CELL DEFORMABILITY AND BLEBBING

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INTRODUCTION

In the context of cancer, differentiation describes the degree to which tumor cells histologically resemble the non-malignant tissues whence they originated. We have proposed that gastrin-releasing peptides (GRP) and its receptor (GRPR) are morphogens, serving to promote tumor cell differentiation when aberrantly expressed in colon cancer [1]. Since better-differentiated cells metastasize less frequently, this suggests that GRP/GRPR expression should affect cells properties contributing to this process. Metastasis fundamentally requires tumor cells to traverse (i.e., transmigrate) through tight spaces such as endothelial gaps in order to access the blood stream.

Since preliminary and circumstantial data suggest that GRP's morphogenic properties are mediated via focal adhesion kinase (FAK) [2], the goals of this study are to assess the role of GRPR-induced FAK activation on epithelial cell deformability, and to see if this biophysical parameter could be an indicator for greater malignancy or tumor cell transmigration.

MATERIALS AND METHODS

Cell Culture

293 cells are a non-malignant epithelial cell line that natively expresses GRP and GRPR. The cells were cultured in RPMI 1640 media supplemented with 10% fetal bovine serum. The cultures were maintained at 37 C in a humidified atmosphere consisting of 95% air and 5% CO_2. To dissect out the role of FAK, 293 cells were modified to inducibly express the dominant negative enzyme FRNK under control of a Tet-On (or dox-sensitive) promoter. For this purpose, 5 days after being seeded, the cells were washed using RPMI 1640 media and dox medium was added. The cultures were put back in the incubator overnight. To remove the cells from their substrate, shear stress was applied and the cells were then suspended in RPMI 1640 media.

Experimental Setup

To characterize cell deformability, the micropipette technique is used [3]. Micropipettes of ~8 μm in diameter were formed from 1mm-outer diameter, 0.5 mm inner diameter capillary tubes [4]. Pipettes were pre-treated with Fetal Bovine Serum and connected to a manometer. Pipette pressure was applied using the displacement of two water-filled reservoirs. Experiments were viewed with an interference contrast video microscope and recorded on videotape along with the pressure and time.

Deformability Characterization

Epithelial cells are aspired at a constant pressure of 600 Pa (6 cm-H_2O) into a micropipette (see Fig. 1).

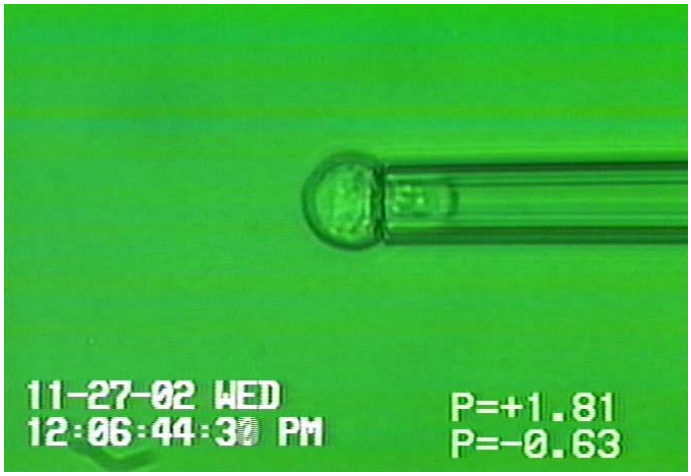

Fig.1: Cell entering the micropipette (Pipette diameter = 8 microns, cell diameter = 18 microns)

Cell deformability is assessed on whether the cells flow inside the micropipette completely or not. The ratio, α, of cell diameter over micropipette inner diameter is recorded to ensure that cells are subjected to a similar amount of deformation.

RESULTS AND DISCUSSION

Figure 2 summarizes the data. It is found that 18.6% of the control cells entered completely inside the micropipette (N=39, N is the total number of cells analyzed) as opposed to 35.9% for cells exposed to dox (N=43). Further investigation of the data reveals that when the diameter ratio α is smaller than 2.5 (relatively smaller cells), the percentages of cells capable to flow inside the micropipette are 33.3% and 42.3% for control and dox cells, respectively (Fig. 3). On the other hand, when α is larger than 2.5 (relatively larger cells), no control cells are found to be able to flow inside the micropipette, whereas 23.1% of the dox cells can. Statistical analysis shows that cells in presence of dox are more deformable (p=0.04), in particularly for the relatively larger cells (p=0.01).

It is evident that the addition of dox to inhibit GRP activation of FAK dramatically enhances cell deformability. This is clearly seen from the results with the relatively larger cells, α > 2.5. These results are not shown because no controls cells were able to completely flow inside the micropipette.

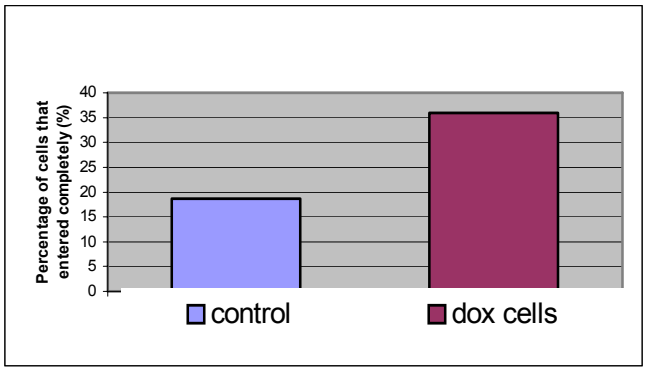

Fig. 2. Cell Deformability

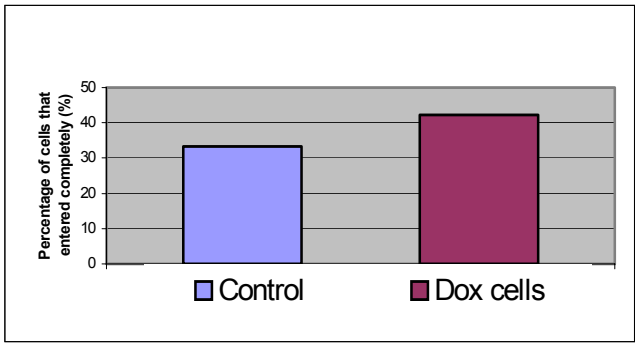

Fig 3. Cell Deformability for cells with diameter ratio ≤ 2.5

Figure 4 show an interesting phenomenon; the formation of blebs. Blebbing is defined as the formation of protusions on cells. Blebbing has been shown to be associated with uncoupling of the plasma membrane from the cortical actin [5]. During the aspiration phase, 25.6% of the control cells and 26.4% of the cells exposed to dox form blebs. The difference is not statistically significant. Movies of cell aspiration and blebbing can be found at:

http://www.uic.edu/com/dom/gastro/labvideos.

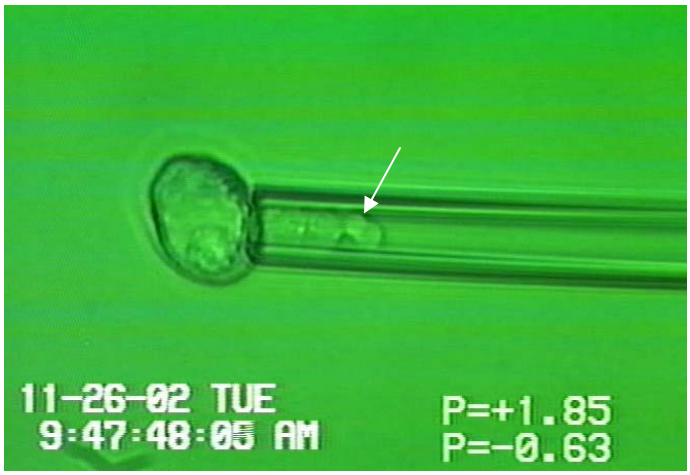

Fig. 3. Cell blebbing
(Micropipette size= 8 microns, cell size = 26 microns)

CONCLUSION

GRP-induced activation of FAK by phosphorylation of Y397 dramatically increases cell deformability. The results indicate that expression of GRP/GRPR interferes with the biophysical properties involved in cell transmigration and metastasis. This finding is consistent with GRP acting as a morphogen, enhancing differentiation, promoting cell motility, and decreasing the likelihood of metastasis.

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