QUANTITATIVE INTRACELLULAR SIGNALING THROUGH THE EPIDERMAL GROWTH FACTOR RECEPTOR AT DIFFERENT INTEGRIN BINDING CONDITIONS

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INTRODUCTION:

Cell migration is a crucial event in many pathological and physiological states such as cancer and wound healing respectively. One of the key parameters that regulates cell migration is cell-substratum adhesion linkages. This aspect influences the main cell migratory events of rear-detachment, stabilization of membrane extensions and attainment of cell-contractile force generation. It has been reported that these aspects are altered upon stimulation with epidermal growth factor (EGF) as a function of different substratum adhesiveness as determined in fibroblasts using surfaces coated with different fibronectin concentrations. Cell locomotion speed and membrane protrusion activity are highest at intermediate adhesiveness and lowest at the two extremes. However, the precise biochemical intracellular signals governing these biophysical events that are altered have not been elucidated. We aim to understand and quantify in a "systems" manner these key intracellular biochemical switches, which will have widespread applications including designing a mathematical model that would predict cell behavior depending on extracellular signals.

Methods:

NR6 wild type cells, expressing human epidermal growth factor receptor were grown on surfaces coated with different (increasing) fibronectin concentrations representing increasing surface adhesiveness. The concentrations used were 0.1, 0.3, 1 and 3 ug/ml of fibronectin. These coatings cover the biphasic aspects of EGF-induced motility. Activation status of a number of intracellular proteins was assessed using immunoblotting, upon stimulation with EGF for different time periods. These signals involved in cell motility included- Epidermal growth factor receptor (EGFR), Phospholipase Cγ, Focal adhesion kinase, ERK (p44/42) MAPKinase, Myosin light chain, Shc (p52/46) and m-calpain. These molecules are activated by phosphorylation and this can be used as a marker of flux through the specific pathway.

Results:

At 5 minutes of EGF treatment, phosphorylated ERK (p44/42) MAPKinase, phosphorylated-m-calpain and phosphorylated Shc showed the strongest signal at intermediate fibronectin concentration (1ug/ml) whereas phosphorylated –EGFR showed the highest signal at the lowest fibronectin concentration of 0.1 ug/ml and progressively decreased with increasing fibronectin concentration of the surfaces.

At 1 hour of EGF treatment, phosphorylated–EGFR continued to show the highest signal at the lowest Fibronectin concentration of 0.1 ug/ml which progressively decreased with increasing fibronectin concentration. However, phosphorylated- Phospholipase Cγ signal increased step-wise as the surface fibronectin concentration increased with a highest signal at 3 ug/ml. Phosphorylated-ERK (p44/42)MAPKinase signal was extremely low at 1 hour of treatment with EGF. All these immunoblots were compared with fraction of total effector present (e.g. phospho-ERK with total ERK) etc., the levels of expression of which (of total) were found to be relatively unchanged throughout the treatment conditions.

Conclusions:

Phosphorylated-ERK(p44/42) MAPKinase, phosphorylated -Shc signal and m-calpain activity show a biphasic curve with highest signal/activity being at an intermediate adhesiveness that corresponds with the biphasic curve of cell locomotion speed and membrane protrusion activity. Future experiments would aim to quantify these signals using specific enzyme activity assays and ELISA. We also aim to quantify the signals that are altered in prostate cancer cells upon stimulation with different ligands for the epidermal growth factor receptor.

2003 Summer Bioengineering Conference, June 25-29, Sonesta Beach Resort in Key Biscayne, Florida