Influence of culture substrate elasticity on talin recruitment to focal adhesion

Cell migration underlie many physiological processes such as embryological development, immune response and wound healing, and pathophysiological like tumor metastasis. Therefore, understanding the molecular mechanisms behind these processes is a key goal in cell biology.

Currently, most cellular research are conducted on stiff glass or plastic substrates. These kind of substrates do not represent well mechanic properties of a human body, as we are created neither from glass nor from plastic. In case of elasticity only bones have similar stiffness.

It was proven over fifteen years ago that cells not only can feel substrates elasticity, but the environment mechanical properties also have a great impact on cell adhesion, morphology and even differentiation. In our preliminary studies cells showed considerable difference in movement dynamics of their edges between ones plated on elastic substrate, and ones plated on glass. We assume that the difference is a result of different activity of talin1 – a protein involved in creation attachment sites, called focal adhesions, between a cell and a substrate. It is also one of the proteins responsible for sensing mechanical environment around the cell. Furthermore, many studies find talin as an important component of tumorigenesis and metastasis.

The goal of this study is to characterize cells edge dynamics, and its connection to the recruitment of talin1 protein to adhesion sites as a function of mechanical properties of the cellular environment, specifically, culture substrate stiffness.

We hypothesize that molecular mechanisms responsible for differences in cell behavior on substrates of different stiffness are related to talin1. We suspect that binding of talin1 to adhesion sites in cells is either altered or only too brief to create a stable connection between the cell membrane and the substrate**. We will investigate spatial distribution and recruitment dynamics of talin1 in cells plated on elastic substrates.** Moreover, if changes on elastic substrates are connected with talin1 binding, talin1 mediated activation of β-integrins, another protein connected with cell adhesion, may also be impeded. Therefore**, the second aim of this study will be to evaluate the co-localization of talin1 and β-integrin and their interaction in cells plated on elastic substrates.**

To achieve this goal, mouse embryological fibroblast cells, a model cells in cell migration research, will be plated on polyacrylamide elastic substrates of various elasticities. Changes in cells edge movement dynamics, talin1 distribution and recruitment dynamics, and talin1 – β-integrin interaction will be analyzed using various methods of protein labeling and advanced optical microscopy imaging, including confocal microscopy, live cell fluorescence imaging, FRET microscopy and Proximity Ligation Assay.

Current elastic substrates cell migration studies use focal adhesion proteins to monitor other properties of cells. The proposed project aims to underline the biochemical differences in these proteins function, specifically in talin1. The first objective of this study is to perform a wide-range analysis of cell edge dynamics.

Since talin1 is one of the first proteins recruited to adhesion sites during cell migration, careful analysis will allow to concentrate on molecular mechanism of initial stages of migration and creation of connections between cells and the substrate. Analysis of the distribution and spatiotemporal dynamics differences of talin1 within cells plated on different substrates will provide a better description of its role as a mechanotransduction protein, a β-integrin activator, and a focal adhesion protein. Furthermore, analysis of β-integrin – talin1 interactions as a function of substrate elasticity will give additional insight into adhesion initiation.

Importantly, talin plays a role in progression of various types of tumors. Further insight into the molecular mechanisms regulating talin distribution and activity may contribute to discovery of targets and development of new anti-cancer treatments in the future.