

## Featured Publication Note

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# Endothelial myosin in tumor invasion: a three-dimensional FRET study

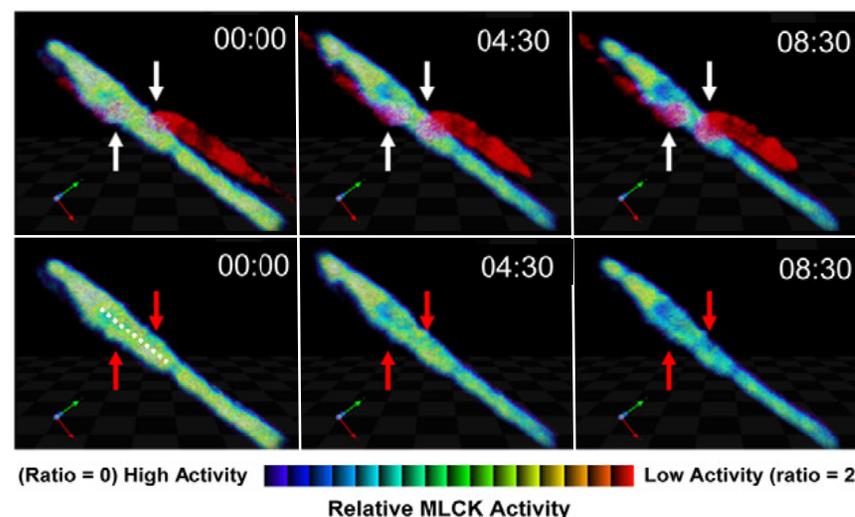
The endothelium is conventionally considered to be an important line of defense against tumor dissemination; however, recent research has suggested that endothelial cells may play a significant role in the invasiveness of cancer cells.

In this study, researchers have investigated the transient and localized tumor-mediated signaling events between invasive breast cancer cells and the underlying endothelial cells. A novel approach was used, which integrates vascular engineering with 3D time-lapse FRET imaging.

A 3D vascular network was constructed in collagen gels using endothelial cells that express a FRET-based  $\text{Ca}^{2+}$ -calmodulin-sensing biosensor. This reports the activity of endothelial  $\text{Ca}^{2+}$ -calmodulin dependent MLCK in the cell during tumor intravasation. MLCK (myosin light chain kinase) is a key regulator of endothelial permeability. After introducing metastatic breast cancer cells into the gel, they showed that tumor transendothelial migration occurs via both paracellular and transcellular routes.

The researchers also found that the invading tumor cell triggers MLCK activation in the endothelial cell. The figure shows the real-time endothelial MLCK activity during invasion by two breast cancer cells, displayed using ratio imaging. 3D ratio images were constructed using *Volocity 5.0 software*. Persistent invasion by breast cancer cells triggered a regional and marked decrease of FRET, indicating the activation of MLCK (see *Volocity movie*). The most pronounced MLCK activity (FRET loss) occurred at the site of endothelial-tumor contact, suggesting that the invading tumor cells could locally affect the endothelial MLCK activity.

Activation of endothelial MLCK at the invasion sites was shown to lead to regional diphosphorylation of myosin-II regulatory light chain (RLC) and myosin contraction. This study highlights the active role of endothelial cells, and the important role of endothelial myosin-II function, in tumor intravasation.



**Figure: Breast cancer cells invade the vasculature and induce endothelial MLCK activation.**

A portion of blood vessel is simultaneously invaded by two MDA-MB231 breast cancer cells (red cells, white arrows). Relative MLCK activity was displayed by means of ratio imaging using *Volocity*. The ratio comprises the numerator  $F_{\text{FRET}}$  (YFP emission, with CFP excited) and the denominator  $F_{\text{YFP}}$  (YFP emission, with YFP excited). Using the intensity of the denominator (which serves as the internal control of MLCK relative concentration, irrespective of FRET), ratio colors were displayed in 16 different intensity levels by the intensity-modulated display. This allows the simultaneous display of MLCK activation and protein concentration profiles. Loss of FRET during MLCK activation turns the ratio color 'bluer'. The bottom three panels show the identical 3D FRET ratiometric images, but with the red channel (representing the cancer cells) digitally removed to highlight the FRET changes in the endothelial cell underneath.

### Movie:

<http://jcs.biologists.org/content/vol123/issue3/images/data/431/DC1/Movie3.mov>

Figure adapted with permission from the *Journal of Cell Science*: Khuon S, Liang L, Dettman R W, Sporn P H S, Wysolmerski R B and Chew T-L (2010). Myosin light chain kinase mediates transcellular intravasation of breast cancer cells through the underlying endothelial cells: a three-dimensional FRET study. *Journal of Cell Science*; 123: 431 <http://jcs.biologists.org/cgi/content/short/jcs.053793v1>