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Title: Clathrin-mediated endocytosis regulates occludin, and not focal adhesion, distribution during epithelial wound healing **(follow for video) (http://www.youtube.com/watch?v=04fkHeaoi6k)**

Duration: 5.49 mins

Speaker Names (if given): **S1** Dr Josh Rappoport, **S2** Natalie Poulter, **S3** Sarah Fletcher

S1: My name is Josh Rappoport and I am a Lecturer in Molecular Cell Biology at The University of Birmingham in the UK.

S2: My name is Natalie Poulter and I am a postdoctoral fellow in Josh's lab.

S3: And my name is Sarah Fletcher and I am a PhD student in Josh's lab.

S1: In this video we are going to describe our work recently published in Biology of the Cell, "Clathrin-mediated endocytosis regulates occludin, and not focal adhesion, distribution during epithelial wound healing". First I am going to give you some background information.

S2: And then I am going to describe our work analysing the potential for regulation of focal adhesion distribution through endocytosis.

S3: And I am going to discuss our results linking clathrin-mediated endocytosis of the tight junction protein occludin to epithelial wound healing.

S1: My lab is interested in vesicle trafficking, and in particular how endocytosis regulates physiologically relevant processes. One major focus of the lab is the role of vesicle trafficking in cell migration. Many different vesicle trafficking processes have been implicated in different types of cell migration and our primary focus is the pathway of clathrin-mediated endocytosis.

This is the route of entry for a great deal of physiologically relevant cargo including activated growth factor receptors, and cell adhesion molecules. Clathrin-mediated endocytosis is the most well studied vesicle trafficking pathway and we have been able to apply a specialised imaging technique known as total internal reflection fluorescence microscopy, or TIRF microscopy, to study individual structures associated with the cell surface. In this work we have used TIRF microscopy to visualise the endocytosis machinery in combination with proteins known to be important for driving cell migration.

Epithelial cells can form tight monolayers and function as a barrier separating different compartments in the body. However, we can also form these epithelial monolayers with cultured cells.

When wounded, for instance with a scalpel,the epithelial cells will migrate as a sheet to fill in the wound.

However, when endocytosis is inhibited, this wound healing is prevented. So we set out to determine what cargo for endocytosis might be involved in regulating epithelial wound healing.

S2: Focal adhesions are the feet that hold a cell down connecting it with the extracellular matrix. And in a migrating cell new focal adhesions are formed at the front, while towards the rear of the cell focal adhesions disassemble.

Trans-membrane proteins known as integrins cluster together with associated accessory proteins to form focal adhesions which link the extracellular matrix to the actin cytoskeleton inside the cells. As integrins are known cargo for endocytosis we tested whether sites of clathrin-mediated endocytosis were co-incident with focal adhesion structures, potentially driving focal adhesion disassembly.

Using TIRF microscopy we could clearly resolve clathrin and focal adhesions at the bottom of the cell, but found these structures to be distinct. We did not observe colocalisation between the markers for the focal adhesions, and the sites of endocytosis.

This movie clearly shows that the sites where the green focal adhesions are disassembling are devoid of red clathrin.

Similarly when we inhibited endocytosis, we did not observe any change in the distribution of focal adhesions.

Another mechanism previously suggested for regulating focal adhesions is the function of the Src family of protein kinases, and we did detect colocalisation of focal adhesions and a Src family kinase.

Similarly, when we inhibited the function of the Src family kinases, the distribution of focal adhesions was significantly altered. So these results show that endocytosis of integrins during focal adhesion disassembly isn't regulating epithelial wound healing.

S3: So we thought we would look at cell-cell junctions which are required to hold the cells together in a sheet as they migrate. Similar to the way focal adhesions link cells to the extracellular matrix, tight junctions attach adjacent epithelial cells to each other.

Tight junctions are based upon homo-typic interactions that form in the inter-cellular space between several transmembrane proteins.

One of these, which has already been implicated in the process of epithelial wound healing, is the protein occludin.

So we used confocal microscopy to visualise occludin in tight junctions before and after monolayer wounding. As you can see occludin forms a clear band around the cell corresponding to the tight junction region.

So we asked, what would happen to occludinafter wound formation.

As you can see following wound formation the occludin at the wound edge was rapidly lost and appeared to colocalise with Rab5 a marker for the early endocytic system (shown in yellow).

This transient colocalisation was statistically significant and indicative of monolayer wounding serving as a stimulus for occludin endocytosis from the wound edge.

Furthermore, when we inhibited endocytosis this redistribution from the wound edge was prevented.

S1: Therefore, we conclude that endocytosis is important in regulating epithelial wound healing, and while this does not seem to be through internalisation of integrins during focal adhesion disassembly, monolayer wounding is a stimulus for the internalisation of the tight junction protein occludin from the wound edge.

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