
Article:
Rainero, E. and Norman, J.C. (2015) Endosomal integrin signals for survival. Nature Cell Biology, 17 (11). 1373 - 1375. ISSN 1465-7392

https://doi.org/10.1038/ncb3261

Survival of circulating cancer cells is an inside job

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An exciting new study from Alanko et al. indicates that following cell detachment, endocytosed integrins can drive assembly of functional signalling complexes on the cytoplasmic face of endocytic membranes. This keeps circulating cancer cells alive to increase their metastatic capacity.

Many cell types rely on integrin-mediated contact with the extracellular matrix (ECM) for growth and survival. Therefore, when anchorage-dependent cells become detached from the ECM they cease to thrive and begin to die via a form of programmed cell death called anoikis. When cells are attached, integrins engage with the ECM and cluster at the plasma membrane to form large macromolecular signalling machines termed 'focal adhesions' or 'focal contacts'. The clustering of ligand-engaged integrins into focal adhesions promotes recruitment of signalling and cytoskeletal adaptor proteins, such as focal adhesion kinase (FAK) and talin respectively to integrin cytoplasmic domain (Fig. 1A). These adaptors, in turn, mediate mechanical links to the actin cytoskeleton to allow force transmission, but also bring-in numerous signalling moieties that lead to activation of pathways which support cell growth and suppress anoikis. Prominent integrin-activated signalling pathways are the Ras-PI3K-PDK1-Akt and Ras-Raf-MEK-ERK axes and, although the mechanistic details of how integrin engagement leads to their activation are a little obscure, there is copious evidence that following cell detachment the activity of these pathways is reduced and this increases the likelihood of cells entering anoikis. To metastasise, cancer cells must leave their organ of origin and travel through the lymphatics and circulatory system to colonise other organs. This entails loss of adhesive contact with the ECM and so, to complete this journey, cancer cells must develop mechanisms to circumnavigate anoikis. One way in which cancer cells suppress anoikis is via the acquisition of activating mutations in anti-apoptotic pathways, such as the Ras-PI3K-PDK1-Akt axis, or by loss of tumour suppressors, such as PTEN. This report from Alanko et al. indicates that there is another route to anoikis suppression that does not necessitate mutation-based activation of pro-survival signalling pathways. Alanko et al. have elegantly shown that following cell detachment integrins move from the cell surface to populate endosomes, and then remain in an active state within these compartments. This allows integrins to recruit

the signalling adaptor FAK and other focal adhesion proteins (such as talin and vinculin) to endosomal membranes which can then command anti-apoptotic signalling at this intracellular locale (Fig. 1B). Importantly, the study goes on to show that this process may be linked to the acquisition of metastatic capacity in cancer. Indeed, Alanko et el. find that a Rab subfamily GTPase (Rab21), which drives actively-signalling integrins to endosomes, is required for breast cancer cells to colonise the lung – an experiment that provides encouraging evidence that integrin endosomal signalling dictates metastatic potential *in vivo*.

It is now well established that growth-factor receptors signal from endosomes in a way that influences anoikis. For instance, sorting nexin 1 (SNX1) has been shown to be downregulated during colon cancer progression, and this correlates with acquisition of anoikis resistance (1). Mechanistically, downregulation of SNX1 drives endocytosis-dependent activation of EGFR and MAPK pathway, leading to survival in absence of adhesion. More recently, Rab7 has been shown to promote EGFR/Akt signalling at late endosomes to sustain anchorage-independent growth in breast cancer cells (2). However, none of these studies definitely link the loss of adhesion with anoikis resistance, nor do they explain how integrin signalling may be reprogrammed in response to cell detachment.

Integrin endocytosis and recycling has been studied in some detail in the past few years and, in some cases, the way in which these processes influence signalling pathways to alter cell migration is well-described. For instance, the Rab11 effector, RCP coordinates recycling of integrins with receptor tyrosine kinases (RTKs), to promote Akt-mediated phosphorylation of RacGAP1 (3) (4) (5) (6). Phosphorylated RacGAP1 then locally promotes RhoA activation at the tip of cellular protrusion, thus promoting invasiveness (4). Furthermore, tensin and Arf4-dependent internalisation of ligand-engaged α5β1 integrin to late endosomes is necessary for recruitment of mTOR to lysosomal membranes, providing a link between adhesion and nutrient signalling (7). Despite these advances, there is very little known about how integrins traffic following detachment of cells that are normally adherent. Moreover, it has not been clear whether integrins can recruit adaptors to signal from endosomal membranes as has been shown to occur for RTKs. Alanko et al.'s findings convincingly show that this can indeed occur in a way that is physiologically and pathophysiologically relevant, and further demonstrate that focal adhesion adaptors previously thought to be recruited only to adhesion sites at the plasma membrane can physically interact with integrin cytodomains which are exposed on the cytoplasmic face of isolated endosomes.

This description of integrin signalling at endosomal membranes provide a rationale for how cell detachment may help to suppress anoikis in cancer cells, and also poses a number of interesting questions for future research. Prominent open questions are: given the established role of mechanical tension and receptor clustering in integrin signalling, how can this signalling be maintained within an endosome where there is no apparent means for force generation? What dictates

the contribution of ‘classic’ focal adhesion signalling versus integrin endosomal signalling, and which integrin-containing signalling platforms are specifically assembled on endosomes? Lastly, we feel that it will be important to investigate this process in the context of mutations (such as those leading to constitutive activation of RTKs and their downstream signalling) that are already known to suppress anoikis, as this may provide indications as to whether strategies aimed at targeting endosomal integrins may be combined with existing anti-oncogenic signalling therapies to kill circulating cancer cells.

Acknowledgements

Work in JN’s laboratory is supported by Cancer Research UK and Breast Cancer Now. AR is supported by Cancer Research UK and The West of Scotland Women’s Bowling Association

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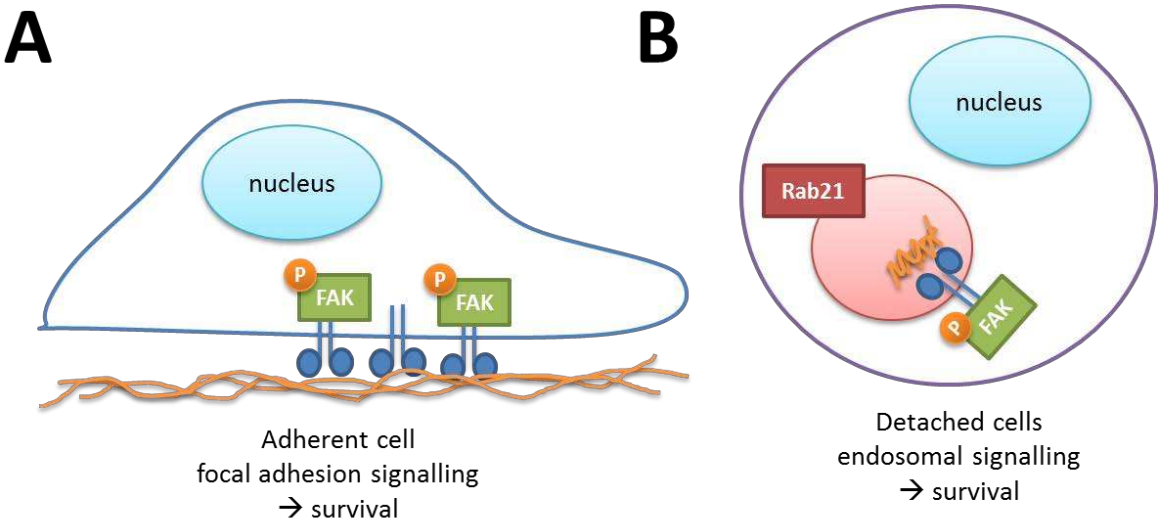

Figure 1. Integrins can drive assembly of pro-survival signalling complexes at the plasma membrane and at endosomes

(A) Integrins can cluster into focal adhesions at the plasma membrane to recruit and activate focal adhesion kinase (FAK) which promotes cell survival.

(B) Following detachment, Rab21 drives endocytosis of activated integrins and this leads to their accumulation within endosomes. Internalised integrins then recruit active phosphorylated FAK to endosomal membranes to promote signalling to suppress anoikis.