Partner up to invade

A secretory complex and a cytoskeletal polarity protein cooperate to make tumor cells more invasive, according to new work from Sakurai-Yageta et al.

To burrow into nearby tissue, tumor cells create membrane protrusions called invadopodia. Within invadopodia, vesicles accumulate that contain metalloprotease enzymes needed to degrade the extracellular matrix. Because a secretory complex called the exocyst is known to link exocytic vesicles to growing membranes, the team tested the effect of depleting particular exocyst subunits in breast cancer cells. They showed that the cells were less able to degrade the matrix on which they were cultured and became less invasive.

The same subunits bound to a protein called IQGAP1, which links actin and microtubules at the leading edge of migrating cells, accumulate in invadopodia and have been implicated in tumor invasion. Linkage of IQGAP1 and the exocyst was needed for metalloprotease accumulation at the invadopodia, though it remains to be determined whether the exocyst directly targets the vesicles. Two important promoters of actin cytoskeletal assembly, Cdc42 and RhoA, promoted the association of IQGAP1 with the exocyst, through direct contact with exocyst subunits. Knockdown of either Cdc42 or RhoA also reduced matrix degradation and tumor invasiveness.

The authors suggest that by linking IQGAP1 to the exocyst, the cell ensures coordination of two central features of tumor invasion: actin reorganization at the invadopodia and exocytosis of protein-degrading enzymes. JCB

A better way to see splicing partners

Splicing factor interactions have been well-characterized in vitro, but less so in vivo. Ellis et al. have now used live cell imaging techniques to study these interactions in vivo and to discover where in the nucleus they occur.

Coimmunoprecipitation can’t capture the dynamic nature of protein interactions, and the resolution of conventional fluorescence microscopy is too poor to distinguish truly interacting proteins from ones that are merely close to each other. In contrast, a positive FRET (fluorescence resonance energy transfer) signal implies two proteins are close enough to interact, and photobleaching or FLIM (fluorescence lifetime imaging microscopy) can detect changes in an interacting pair over time.

Using these techniques, the authors confirmed that the splice site protein, SF2/ASF, and the 5′ splice site factor, U1 70K, interact in a type of nuclear body called a “speckle” and, to a lesser extent, in the nucleoplasm. When transcription was blocked, nucleoplasmic, but not speckle-localized, interactions were diminished. This finding bolsters the case for speckles as storage or assembly sites for splicing factors rather than sites of transcription-associated splicing.

The same localization and response to transcription inhibition was seen for interactions between SF2/ASF and the 3′ splice site binder, U2AF35. But apparently not all splicing factors accumulate in speckles: interactions between HCC1 and either U2AF35 or U2AF65 were most prominent in the nucleoplasm, even when transcription was blocked.

There is clearly still much to uncover about splicing factor interactions in vivo, but by using FLIM-FRET, the authors have a powerful tool to dig deeper. JCB

The mitochondrial connection

Add two, and perhaps three, more skills to the portfolio of multi-talented plectin, say Winter et al.: one isoform of the protein anchors mitochondria to the cytoskeleton and keeps these organelles in shape, and may also create a platform for signaling.

Plectin is a “cytolinker” protein responsible for connecting intermediate filaments with, among other things, actin microfilaments, microtubules, and myosin motors. Its versatility arises from alternative splicing—almost a dozen isoforms are known, which differ at their N termini, affecting the substrates to which they can attach. One isoform, P1b, has been found targeted to mitochondria, but the significance of this linkage has been unclear.

The authors have now found that P1b links mitochondria to the intermediate filament protein, vimentin. P1b colocalized with vimentin, and loss of P1b reduced the amount of vimentin in mitochondrial extracts. Loss of P1b also dramatically altered mitochondrial shape, increasing the number of mitochondria that were highly elongated. Elongation was not caused by alterations in mitochondrial membrane potential, mass, or fusion dynamics. But it may have resulted from a reduction in protein kinase C-δ activation, as this was apparent in cells that lacked P1b, through unknown mechanisms. PKC-δ promotes mitochondrial fission, and the authors suggest that intermediate filament–linked P1b may serve as a scaffold for recruiting PKC-δ, and perhaps other signaling molecules, to regulate mitochondrial fission. JCB

In the absence of P1b, mitochondrial networks become more tubular (right).

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