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Cancer cell invasion: Caveolae and invadosomes are partners in crime

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During cancer progression, tumor cells need to disseminate by remodeling the extracellular tumor matrix. A recent study sheds light on the intricate cooperation between caveolae and invadosomes that facilitates the spread of cancer cells.

For the invasion and metastasis associated with cancer progression, primary tumor cells must breach the basement membrane and infiltrate through the extracellular matrix (ECM) and tumoral stroma. The ECM is a complex 3D network composed of different proteins, glycoproteins, and polysaccharides[1]. The spatial organization and the stiffness of the ECM determine the mechanical properties of the tumoral stroma and influence cell dissemination and metastasis[2]. ECM remodeling is therefore a crucial process in tumor progression. This remodeling requires the action of invadosomes, specialized cellular protrusions with collagenolytic activity mediated by membrane type I matrix metalloproteinases (MT1-MMPs)[3] that facilitate cell dissemination by weakening the collagen matrix[4]. However, key questions regarding the precise role and activation mechanism of invadosomes remain to be addressed: specifically, how does the plasma membrane sense ECM rigidity to activate invadosome function?

Caveolae are specialized plasma membrane invaginations with a specific protein and lipid composition; their formation requires CAV1 (caveolin-1) and CAVIN1[1]. These structures are highly sensitive to membrane tension and therefore play an important role in cellular mechanosensing and mechanotransduction[1,5]. CAV1 has been widely studied in the context of cancer development and progression, and its role varies based on cancer type and tumor stage[2,6]. The relationship between invadosomes and caveolae was previously not clear: although evidence indicated a role for CAV1 in regulating invadosome function and formation[7], insights into the mechanism of caveolae-modulated ECM degradation and the crosstalk between caveolae and invadosomes remained elusive. A recent study by Monteiro *et al.*[8] published in *Nature Cell Biology* now elucidates an intricate relationship between invadosomes and caveolae in tumor cell dissemination through remodeling of the collagen matrix.

These authors used an invasive human breast cancer cell model (MDA-MB-231 cells), which expresses high levels of CAV1, and grew these cells on collagen

fibrils to study the organization of caveolae and invadosomes at the contact sites between the plasma membrane and the underlying constricting collagen fibrils. The authors detected invadosomes by staining for cortactin, TKS5, F-actin, and MT1-MMP, which are enriched at invadosomes[9], and identified caveolae by staining for the caveolar markers CAV1 and CAVIN1. Their first observation was the localization of CAV1 clusters (in addition to invadosomes) along the collagen fibrils in elongated structures (Figure 1).

Monteiro *et al.*[8] observed a surprising non-overlapping distribution of caveolae and invadosomes along the fibrils, with an alternating localization of both structures. Since invadosomes possess collagenolytic activity, which modifies the geometry and organization of the collagen matrix[9], they determined whether the alternating distribution of caveolae and invadosomes is sensitive to collagen fibril organization and curvature. First, the authors confirmed in their cell model that collagen fibrils were more curved beneath the cells as a result of invadosomal collagenolytic activity. At the fibril level, they observed that caveolae were predominantly associated with aligned collagen regions, whereas invadosomes were found in highly curved and softened collagen regions due to the remodeling activity of invadosomes (Figure 1).

So how do caveolae and invadosomes at contacting regions with collagen fibrils act together to facilitate tumor cell dissemination? Monteiro *et al.*[8] revealed that caveolae were required for invadosome formation and collagen cleavage. Disruption of caveolae led to a reduced number of invadosomes, a decrease in their function, and an increase in collagen alignment, thereby affecting ECM remodeling. On the other hand, disruption of invadosomes affected caveolae association with matrix fibrils, increasing the recruitment of caveolae to collagen. These findings suggest a dynamic interplay between these two structures.

Next, the authors attempted to address the mechanism involved. How do cells sense collagen matrix rigidity in order to promote its degradation and tumor cell dissemination? They examined the recruitment dynamics of caveolae and invadosomes to the fibrils and found that the association of caveolae with collagen fibrils preceded invadosome formation. Given that caveolae are mechanosensitive structures, the authors postulated a role for caveolae in sensing the mechanical cues from the ECM. They demonstrated that collagen-fibril-induced bending of the plasma membrane drives caveolae recruitment in an ECM-independent manner and that this recruitment promotes invadosome formation and activity.

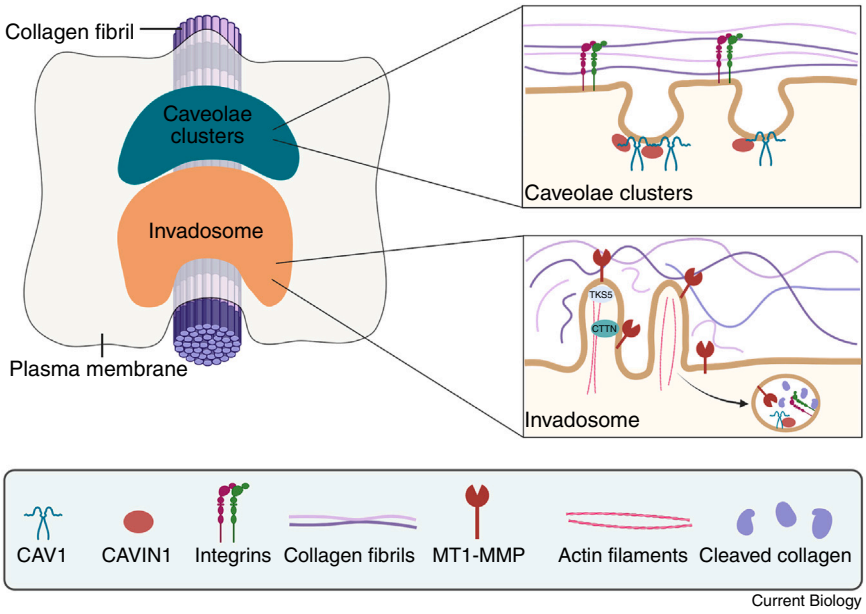

Figure 1. Caveolae and invadosomes intercalate along collagen fibrils to remodel the ECM.
In invasive cancer cells, caveolae and invadosomes organize in an alternating distribution along subjacent collagen fibrils. Clusters of caveolae (bearing CAV1 and CAVIN1) sense matrix rigidity through integrins and regulate invadosome function and formation. At invadosomes (bearing cortactin, CTTN, and TKS5), metalloproteinases degrade extracellular collagen and regulate the internalization of integrins and caveolae. (Figure created with BioRender.com.)

The molecular mechanism by which caveolae sense ECM rigidity was further explored. Caveolar components sense ECM mechanical properties primarily via focal adhesions and integrins[1]: in co-localization experiments, Monteiro *et al.*[8] confirmed the presence of β1 integrin and caveolae at the contact sites between collagen fibrils and the plasma membrane (Figure 1), and found that depletion of β1 and β3 integrins reduced CAV1 association with collagen fibrils. Therefore, integrins, in combination with plasma membrane deformation caused by matrix fibrils, are responsible for caveolae recruitment. Moreover, and in line with previous research[10,11], depletion of caveolar components increased β1 integrin levels at the plasma membrane, indicating a role for caveolae in regulating β1 integrin internalization and recycling.

The authors also demonstrated that invadosomes promote caveolae and integrin internalization, since elimination of invadosomes or inhibition of their collagenolytic activity led to impaired internalization of β1 integrin and CAV1 in intracellular vesicles that also contained proteolytic enzymes, such as MT1-MMP, and collagen. Therefore, caveolae regulate integrin internalization and recycling via a mechanism dependent on invadosomes and the proteolytic cleavage of collagen. However, as previously mentioned, caveolae have been widely shown to regulate β1 integrin endocytosis and recycling[10,11], so the effect of caveolae on β1 integrin recycling could be a general mechanism that is not restricted to invadosomes (Figure 1). Furthermore, given that integrin trafficking is mediated via multiple pathways — including clathrin-dependent endocytosis, clathrin-independent endocytosis (CLIC-GEEC) and caveolae-mediated endocytosis[12] — and that caveolae regulate the CLIC-GEEC pathway[13], it would be interesting to address whether β1 integrins are internalized directly by caveolae in the newly described process.

Finally, the authors explored the consequences of this link between

caveolae and invadosomes in the context of 3D invasion, confirming the alternating distribution of both structures along the collagen matrix previously observed in their 2D model. Moreover, they found that depletion of CAV1 decreased the invasion of breast cancer cell spheroids in an *in vitro* 3D collagen matrix model. This is consistent with previous studies demonstrating that caveolae and CAV1 are required for ECM remodeling and tumor invasion[1,6]: Goetz *et al.*[6] showed that CAV1 stimulates *in vivo* tumor cell invasion through ECM remodeling. Co-localization between caveolae and cytoskeletal-related structures involved in cellular migration and invasion has also been observed during leukocyte transcellular migration through endothelial cells[14]. In both contexts, the caveolae clusters play a role in cellular migration and depend on CAV1 expression.

This promising study from Monteiro *et al.*[8] raises some open, unresolved questions, such as the effects of CAV1 on invadosomes and tumor cell dissemination *in vivo*. Since CAV1 affects breast cancer cell invasion through several mechanisms, including the endothelial-to-mesenchymal transition, cytoskeletal remodeling and integrin trafficking[15], it would be interesting to explore the *in vivo* role of the collaboration between invadosomes and caveolae in tumor cell migration. Another key driver of cancer progression is tumor stiffness[2], which also regulates invadosome activity[16]. Therefore, how do different degrees of collagen stiffness modulate this invadosome–caveolae interplay? Moreover, what is the role of other ECM proteins apart from collagens? This question is pertinent given that invadosomal MT1-MMP also degrades laminin, a process critical for basement membrane breaching[17].

Another intriguing avenue of future research would be to explore the differences with other triple-negative breast cancer cell lines that express very little or no CAV1, such as MDA-MB-468. Are differences in invasion dependent on caveolae or are caveolae-independent mechanisms also relevant for ECM remodeling and tumor progression? Is the cooperation between invadosomes and caveolae also relevant in cancer-associated fibroblasts, which are known to lead tumor cell invasion[18]? CAV1 expression in these fibroblasts can also drastically modify tumor progression[6], so the cooperation could also affect invadosome-related structures in macrophages and stromal fibroblasts. Also, little is known about the molecular mechanisms involved, but the transcriptional co-activator YAP is known to regulate ECM remodeling[19] and to be regulated by caveolae[20], so could YAP act downstream of invadosome activity?

In conclusion, Monteiro *et al.*[8] reveal that caveolae are able to sense the rigidity of the ECM via integrins in a tumor-invasive context and determine the subjacent localization of invadosomes that will weaken and remodel the matrix, allowing tumor cells to escape from the primary tumor (Figure 1).

DECLARATION OF INTERESTS

The authors declare no competing interests.

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