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Tumor-stroma biomechanical crosstalk: a perspective on the role of caveolin-1 in tumor progression

Fidel Nicolás Lolo[1,#], Víctor Jiménez-Jiménez[1,#], Miguel Sánchez-Álvarez[1,#] and Miguel Ángel del Pozo[1,*]

[1] Mechanoadaptation and Caveolae Biology lab, Cell and Developmental Biology Area. Centro Nacional de Investigaciones Cardiovasculares (CNIC). Madrid, Spain

[#] These authors contributed equally

*** Corresponding author:** madelpozo@cnic.es

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ABSTRACT

Tumor stiffening is a hallmark of malignancy that actively drives tumor progression and aggressiveness. Recent research has shed light onto several molecular underpinnings of this biomechanical process, which has a reciprocal crosstalk between tumor cells, stromal fibroblasts and extracellular matrix remodeling at its core. This dynamic communication shapes the tumor microenvironment; significantly determines disease features including therapeutic resistance, relapse or metastasis; and potentially holds the key for novel antitumor strategies. Caveolae and their components emerge as integrators of different aspects of cell function, mechanotransduction and ECM-cell interaction. Here, we review our current knowledge on the several pivotal roles of the essential caveolar component caveolin-1 in this multidirectional biomechanical crosstalk, and highlight standing questions in the field.

1. INTRODUCTION

When describing alterations indicative of tissue insult and inflammation, the roman encyclopaedist Celsus (c. 25 BC – c. 50 AD) included "***tumor***" (swelling and stiffening; along with *calor* (warmth), *rubor* (redness), and *dolor* (pain)) as one of its cardinal signs. Ever since, and especially for the case of abnormal mass growth, swelling and stiffness have constituted an important diagnostic and prognostic parameter in classical medicine. The existence of links between stiffening and tumor progression was early demonstrated when studying the relevance of interstitial fluid pressure in an experimental rabbit model of epithelial carcinoma[1]. Subsequent studies corroborated that tumors exhibit differential, measurable mechanical features, and these correlate with aggressiveness and tumor progression (reviewed in [2–4]). Indeed, mechanical forces profoundly influence cell function regarding not only their motility, but also their differentiation state and proliferation[5–7], and mechanical properties of tumors affect their progression, vascularization, drug delivery and therapeutic resistance and propensity to metastasize[8–13]. Enabled by emerging technologies, the systematic study of tumor biomechanics across scales has soared in recent years, and their intervention is intensely explored as a potential opportunity to improve antitumor therapeutics[14, 15].

1.1. Extracellular matrix remodeling in the tumor microenvironment: a reciprocal process defining tumor mechanical properties

Apart from 'passive' sources of mechanical force, such as interstitial pressure and compression from adjacent structures, mechanical properties of solid malignant tumors arise from their dense, desmoplastic extracellular matrix (ECM). The ECM is a three-dimensional network of different proteins and glycoproteins providing structure, mechanical support and biochemical regulation to the cells of a given tissue. As befits the exquisite specialization according to the different tissues ECM belongs to[16], tumoral ECM exhibits a differential composition, and apart from basic structural components such as collagens, the tumor stroma is commonly enriched for interstitial, structural ECM components that can have a profound impact on tumor cell behavior and the sculpting of the tumor microenvironment, including fibronectin (FN), tenascin-C (TnC), or osteopontin[17–21]. The use of unbiased quantitative proteomics approaches has allowed for describing tumor-specific changes in the ECM proteome (termed *matrisome*) and assert their potential to derive signatures of prognostic value[22–27]. Importantly, many of these tumor-enriched ECM proteins have the intrinsic capacity to activate inflammatory signaling and proliferation, and metastatic behavior[17, 18, 27–32]. In addition, ECM components of the basement lamina (an amorphous ´net´ which separates epithelial cells from their surrounding and delimitates tissue compartments) are profoundly altered (or even partially absent) in tumors, contributing to tumor cell migration and metastasis[33].

The ECM has a complex composition and spatial organization as a mesh of fibers, and thus adapts non-linearly to force loads[16, 34, 35]. As aforementioned, tumor ECM does not only display abnormal abundance and composition: it is also commonly arranged in specific fashions (highly anisotropic patterns, with thick, tense bundles of ECM fiber components frequently aligned centripetally) that critically contribute to define the mechanical properties of the tumor stroma—which in turn impact on tumor cell growth—and provide an appropriate scaffold for tumor cell migration[17, 36, 37]. A key step in the assembly of tumoral ECM networks is the biogenesis of FN fibers (hereon termed *fibrillogenesis*). FN fibers act as ´template´ scaffolds for the assembly of collagen fibers, which ultimately confer rigidness and support a major share of mechanical load[38–41]. Other relevant ECM proteins (thrombospondin, osteopontin, TnC) also co-align with these anisotropic patterns, and potentially take part on their generation[17, 39]. Metalloproteases, modifying enzymes and crosslinking mediators such as lysyl-oxidases further contribute to ECM remodeling through specific activities of regulated cleavage and processing, glycosylation and other posttranslational modifications, and the establishment of specific patterns of branching. All of these processes also contribute to define the mechanical properties of the ECM. The dysregulation of these activities often underlies protumorigenic ECM remodeling [6, 39, 42].

Fibrillogenesis and biomechanical ECM remodeling are driven by both mechanical forces (either cell-generated tension on the substrate, or long-range pressures), and spatial arrangement of the cells channeling these forces. Cells exert mechanical forces primarily through actomyosin contraction. The involved structures are connected to and align with ECM fibers through different anchorage molecules such as integrins, which are in turn coupled to signaling pathways governing cytoskeletal dynamics. Integrins are heterodimeric (alpha and beta subunits) transmembrane adhesion molecules serving a dual function: (i) mechanical (linking the ECM to the actin cytoskeleton); and (ii) biochemical (sensing cellular adhesion and then transducing it bidirectionally through effector proteins)[43]. A prominent transduction pathway relies on talin-vinculin complex stabilization and focal adhesion-kinase (FAK) activation at focal adhesions bound to stiffening ECM, which in turn enhances Rho kinase (ROCK) and downstream Rho activity to drive actin bundle stabilization and actomyosin contraction. Importantly, these pathways engage in crosstalk with other major signaling networks (growth factor signaling, ERK-Ras pathway, MAP kinases) which control cell state, survival or proliferation[2, 17, 39, 41, 44–46].

The emergence of anisotropic ECM arrangements also derives from the regulated alignment of cells into organized patterns. Abercrombie and Heaysman early described the influence exerted by fibroblasts on neighboring cells, through a phenomenon they termed ‘contact inhibition of locomotion’ (CIL), by which cells undergo repolarization and direction change upon contacting each other[47]. These mechanisms, required for physiological tissue patterning, also

rely on actomyosin contractility and partially explain how fibroblasts tend to spontaneously co-align[48–50]. A recent study from Sahai and coworkers shed light on gene networks driving these mechanisms, through a collective behavior they termed 'cell collision guidance'[51]. Specifically, they identified transcription factor TFAP2C-dependent expression of Rho GTPase 3 (RND3), which localizes to and downregulates actomyosin contraction at cell-cell collision points, as required for fibroblast alignment and generation of anisotropic ECM patterns. Of note, computational modeling from long-term cell imaging revealed that alignment *precedes* confluence, and that polarization and persistent migration alone, are not sufficient to explain fibroblast co-alignment.

Stromal ECM remodeling feeds both from and into the biochemical and mechanical adaptation of surrounding cells, engaging on self-sustained positive loops as part of what Bissell and colleagues labeled as ´stromal dynamic reciprocity´, SDR[52]. Tissues require continuous monitoring and reinstating balance among all its components in response to any change or challenge, for maintaining homeostasis. Thus, different cell populations and the ECM receive information from each another, and produce a response that feeds back onto the rest[53–55]. In tumors, primary changes on ECM mechanical properties (as a result for example of damage or inflammation, or compressive forces from surrounding tissues upon growth) can be sensed by mechanotransduction systems in tumor cells or stromal cells, which promote adaptive responses (for example, further deposition of ECM, or contraction), which in turn remodel the ECM onto anisotropic patterns, conferring to it more stiffness, that can further activate cell mechanoadaptation. This principle is pivotal not only to understand the impact of tumor stiffening on disease progression, but also to rationalize how certain challenges to homeostasis on tumor-free tissues (such as damage or inflammation) promote tumor initiation and progression even if their direct action is transient and eventually resolved[54]. Among cell components within the tumor microenvironment, cancer-associated fibroblasts emerge as predominant players in this reciprocal biomechanical crosstalk with ECM remodeling.

1.2. Cancer-associated fibroblasts: master regulators of stromal remodeling

Fibroblasts are a remarkably heterogeneous cell type, generally considered of mesenchymal origin, servicing interested tissues and maintaining ECM structure[56]. While fibroblasts in healthy tissues are mostly quiescent and display very low metabolic rates, physical damage or acute/chronic inflammation of the functional organ parenchyma, induce an "activated" state in quiescent fibroblasts as part of a coordinated wound healing response, aimed at regenerating and repairing the tissue[57]. Central features of this response are the robust induction of protein synthesis activity or "secretory phenotype" to produce both paracrine signals and new ECM components, and the acquisition of contractile activity[58, 59].

Although certain tumor cell types have a certain capability to depose and remodel ECM[24, 60], stromal fibroblasts displaying sustained aberrant activation are the predominant effectors of ECM biogenesis and biomechanical remodeling within the tumor[46]. The origin of these Cancer-Associated Fibroblasts (CAFs) is incompletely understood, and recruitment of mesenchymal precursors from stem cell niches, as well as activation of local cell populations (such as fibroblasts, as well as endothelial cells through endothelial-to-mesenchymal transition) have been proposed as relevant mechanisms[46, 61]. CAFs often comprise a major share of the mass of a solid tumor, and emerge as key drivers of tumor progression, not only through stromal remodeling but also as central regulators of angiogenesis, tumor immunity or tumor cell metabolism[46]. While a unique signature exclusive to these heterogeneous mesenchymal populations is lacking, CAFs most frequently display 'markers´ of an activated phenotype: α-smooth muscle actin (α-SMA), fibroblast activation protein (FAP), vimentin and desmin, ECM components such as tenascin-C and periostin, platelet-derived growth factor receptors (PDGFRs), secreted protein acidic and rich in cysteine (SPARC), integrin α11 or fibroblast-specific protein 1 (FSP1)[46, 62, 63]. Their sustained activation is not well understood, but epigenetic reprogramming is likely a driving component[52, 64, 65]. Tumor cells can act as primary activators of CAFs through paracrine signaling pathways priming positive feedback between both cell types, most notably transforming growth factor-β (TGF-β), hepatocyte growth factor (HGF), platelet-derived growth factors (PDGF), fibroblast growth factor (FGF-2), and chemokines (CXCL12, CXCL10, CCL21, CCL25, CXCR4 or CCR9)[46, 59]. An additional emerging mechanism through which tumor cells instruct CAF activation (and viceversa) is exosome secretion[46, 66].

Importantly, CAF-dependent ECM remodeling onto anisotropic patterns constitutes a potent fibroblast-activating event, and substrate mechanical properties are a key component of this activation[67]. In a seminal study, Calvo and coworkers demonstrated that the activation of the transcriptional mechanotransducer Yes-associated protein (YAP) is a prominent feature of CAFs, required for their sustained activation through SDR[68]. YAP is a conserved transcriptional factor first described as a key effector of the Hippo/Wnt developmental pathway[69–71], for which responsiveness to metabolic stimuli and mechanical cues (most notably, substrate stiffness) have been subsequently demonstrated[72–78]. Specifically, substrate stiffness (and surrounding context, including confluency and cell spreading area) promote YAP/TAZ activation and translocation to the nucleus, where they transactivate, together with a defined transcription regulation machinery, functionally coherent gene subsets to drive cell adaptation and differentiation[71, 75, 76, 78]. Importantly, these transcriptome signatures are markedly enriched for ECM components and remodeling enzymes, as well as cytoskeletal components and other

regulators of interest such as components of the TGF-β pathway[71, 77]. In CAFs, YAP is regulated by substrate stiffness-driven actomyosin contraction, and required for the expression of key components of the actomyosin contractile machinery (including anillin actin-binding protein (ANLN), Diaphanous-3 (DIAPH3) and myosin light chain 2 (MLC2)). Thus, YAP drives a feed-forward loop between stiffness-driven CAF activation and further ECM remodeling that is essential for the thriving of this tumor stroma population and its biomechanical remodeling activity. Importantly, because YAP also feeds into TGF-β signaling and cooperates with other pathways such as metabolism and stress responses [71–74, 76–79], it also provides a central hub to integrate that mechanical crosstalk, with paracrine tumor cell-CAF communication and other features (nutrient availability, ROS levels, immunity) of the tumor microenvironment.

1.3. The impact of mechanical forces on tumor cell behavior

Cancer has traditionally been considered a genetic disease, whereby cell proliferation is increased due to perturbations of their hereditary (i.e. genetic) material. While this paradigm was soon challenged and phenomena such as metabolism and tissue development and organization were proposed as candidate drivers of cancer disease[80–82], the advent of molecular biology and early genomics eras consolidated a “gene-centered” perspective of tumor cell biology until very recent times, attracting the lion share of our attention to a growing list of oncogenes and tumor suppressors, and mechanisms leading to genomic instability, as the central drivers of these diseases[83–93].

However, evidence from multiple sources supports that genetic mutations (including those affecting most prominent oncogenes) do routinely occur in disease-free individuals. Thus, tumorigenesis and tumor progression imply the failure of mechanisms curbing uncontrolled proliferation and ensuring tissue homeostasis, one of which is stromal architecture and mechanical properties[94–96]. Cell context is essential in determining the influence of genotype on phenotype[92]. Modulation of ECM stiffness and composition can induce malignant phenotypic traits[97], and revert already established malignant phenotypes of genetic origin[98]. Programs engaged by the mechanical properties of the tumor microenvironment largely overlap with major signaling pathways driven by oncogenes, and can critically determine transformation, cytoskeletal rearrangement, stress survival, motility and metastasis[99], amplifying or even mimicking the effects of a ´driver´ genetic perturbation[3, 100]. For example, substrate stiffness can have a dramatic impact on a cell´s response to TGF-β[101]. Stromal stiffness is also critical in determining tumor cell contractility and for cells to apply pulling, enabling cell motility and metastasis[35, 99, 102]. As aforementioned, these signaling nodes intersect with major growth factor signaling pathways contributing to tumor progression[102]. Tumor cell extravasation and metastatic invasion is also critically regulated by blood flow shear[103, 104].

ECM stiffness and composition drive epigenetic regulation and cell differentiation, which drive tumorigenesis[102, 105]. Mechanical cues are a pivotal component of cell-cell and cell-ECM interactions driving developmental decisions. YAP plays a fundamental role at initial steps of development (differentiation of trophoectoderm from inner cell mass during preimplantation[106]), as well as during the differentiation of other cell types and tissues (insulin-producing β–cell[107], cardiomyocytes[108], neuron-astrocyte decision from neuroblasts[109]). Importantly, mechanical input can be essential to this role, as is the case for mesenchymal differentiation onto either adipocyte or osteoblast[110]. These findings link tumor mechanoadaptation with C.H. Waddington´s view of cancer as a disruption of the epigenetic landscape driving developmental decisions[82].

An additional, perhaps often overlooked mechanism through which ECM remodeling can influence tumorigenesis, is metabolic reprogramming. Metabolic switches commonly observed for different types of cancer, both regarding energy usage and anabolic rewiring (´Warburg effect´ (i.e. high glycolytic rates in normoxia and nutrient availability)[111], enhanced anaplerosis[112], coordinated overdrive in lipid, nucleotide and protein anabolism[113, 114], etc) are not mere ´side-effects´, but mechanisms actively contributing to tumor cell proliferation and aggressiveness. The bidirectional ties between mechanotransduction and mechanoadaptation, and metabolic regulation, are just starting to be understood, and recent research suggests that conditions of enhanced cell tension can promote protumoral metabolic rewiring, including enhanced glycolysis[115, 116]. Conversely, cell metabolic regulation feeds onto cell mechanoadaptation programs. Again, a paradigm of these bidirectional relationships is embodied by the mechanotransducer YAP, because YAP activity modulates cell metabolic state but can be itself regulated by metabolic pathways[72–74, 76, 116–118].

2. CAVEOLAE: CELL MECHANOSENSING STRUCTURES

The eukaryotic plasma membrane (PM) is a complex organelle composed of many different biochemical species, including lipids, sugars and proteins, constituting a natural barrier and gate for communication between the cell inside and the extracellular environment. Rather than constituting a homogeneous structure, these components are compartmentalized into different domains that perform specialized functions, with a precise temporal and spatial regulation, initially coined collectively as *lipid rafts*[119]. Among them, caveolae emerge as key membrane domains onto which cell signaling, membrane organization and cell mechanoadaptation converge. Caveolae are invaginated nanodomains (60-80nm in diameter) with a specific biochemical composition of lipids and proteins[120–124].

2.1. Structure of caveolae

The characteristic flask-like, 'omega'-contoured shape of typical caveolae structures requires core scaffolding protein components (caveolin-1 (CAV1) in most tissues, and Cavin-1 (CAVIN1, also known as PTRF)) interacting with specific lipid species (most notably, cholesterol) to stabilize required PM curvatures[125–130]. CAV1 is a transmembrane, hairpin/shaped protein, embedded in the cytoplasmic leaflet of membranes, with its amino- (N-) and carboxyl- (C-) terminal domains facing the cytosol [121, 123, 131]. Tyrosine residue 14 (Y14) in the N-terminal domain is reversibly phosphorylated in response to different cues including mechanical stimuli[132, 133], and modulates caveolae internalization[134], tumor cell growth suppression[135] or, importantly, fibroblast polarity and migration and ECM remodeling[136–138] (see below). The C-terminal domain, on the other hand, contributes to PM anchoring[130, 139–141]. A key structural feature of CAV1 is its Caveolin Scaffolding Domain (CSD, residues 82-101), which has been shown to be important for CAV1 protein-protein interactions and signaling regulation[124, 142–144]. Importantly, CAV1 directly binds cholesterol, presumably through a specific motif within its CSD, termed CRAC (cholesterol recognition amino-acid consensus) motif[139, 145–147]. This ability favors cholesterol condensation[148], which is critical for caveolae stability; indeed, cholesterol sequestering molecules (such as methyl-β-cyclodextrin) disrupt caveolar structures[126, 149]. Interestingly, changes in PM cholesterol organization can intrinsically contribute to cancer progression and invasion[150].

The current model for the biogenesis of caveolae has a first step on CAV1 synthesis by endoplasmic reticulum (ER)-bound polysomes. CAV1 dimers are then trafficked through COPII vesicles to the Golgi system, where further oligomerization takes place, and finally vehicled through the endosomal compartment towards the PM[121, 151]. Importantly, this CAV1 trafficking route channels cholesterol efflux from the ER, and determines cholesterol relative content across most cell membrane systems in the cell, which in turn can have a profound impact on their functioning. Recent super-resolution microscopy studies suggest that once at the PM, CAV1 oligomers combine onto more complex intermediate-state scaffolds, which eventually coalesce to form caveolae[152]. CAVIN1, on the other hand, acts as an adaptor protein complexed with other cavins[153], wrapping around the oligomerized scaffolds that form caveolae, and defining the striated caveolar coat typically observed by electron microscopy[126, 154, 155]. Caveolae are found in tumor cells, but are usually more abundant in stromal cells, including vascular endothelial cells, smooth muscle cells and fibroblasts[121, 156]. Intriguingly, caveolae can assemble into higher-order clustered structures (*rosettes*) in conditions of relative low PM tension, through specific regulatory mechanisms[130, 157, 158].

2.2. Caveolae and mechanical force sensing and transduction by cells and tissues

Caveolae were first-described more than 60 years ago[120], but their specific functions are still a matter of debate[121], as these structures have been associated with a substantial number of biological processes. However, their role in cell mechanobiology is receiving increasing attention after being described as mechanical stress sensors[121, 122, 159]. Evidence shows that caveolae can flatten in response to a wide variety of stimuli leading to increased PM tension, behaving as elastic structures that re-assemble once tension is released (see Figure 1) [121, 122, 157, 160, 161]. This is crucial for cell and organismal homeostasis, and caveolae-deficient cells and tissues exhibit increased sensitivity to membrane tension changes (i.e. deficient mechanoprotection)[162–164]. Upon caveolae flattening, CAVIN1 is released from the PM and CAV1 scaffolds dissasemble[160, 165], buffering membrane tension and triggering downstream signaling events. Mechanosensititive channels, for instance, can be affected by disruption of caveolae-dependent buffering[166, 167]. Furthermore, caveolae flattening leads to alterations in local lipid composition, which in turn modulates Ras signaling[168].

Several of these effects are likely derived from ECM receptor clustering within caveolae. This is particularly important for β1-integrin-mediated mechanical activation, whereby caveolae regulate activation of the Src-like kinase Csk[137, 169, 170]. Indeed, CAV1 has been implicated in many aspects of β1-integrin biology, particularly endocytosis control[134, 171–173], and this is very relevant for epithelial cell-ECM interaction and associated signaling events, such as EGF sensitivity and migration[174, 175]. Of note, phosphoregulation of CAV1 at its Tyr14 residue has a relevant impact on many of these events[174]. Integrin activity can be directly regulated by PM reorganization[176, 177]; however, clarifying whether this is related to caveolae buffering ability will require further investigation.

The actin cytoskeleton is very sensitive to mechanical forces[178, 179], and a mutual regulation between CAV1 and actin cytoskeleton has been suggested[134, 137, 158, 180]. Caveolae dynamics are tightly coupled to actin stress fibers, and organization of CAV1-dependent invaginations is directly associated to stress fibers-regulators, such as the formin mDia1 and the kinase c-Abl[158, 181]. Furthermore, CAV1 interacts with filamin-A (FLN-A), which links integrins to the cytoskeleton[182]. Thus, increased actin polymerization at the cell periphery leads to caveolae flattening, whereas inhibiting stress fiber formation leads to increased number of caveolar *rosettes* and CAV1 clustering[158, 181–183]. Of note, the levels of CAV1 and caveolar density are regulated by cell stretching through actin-dependent, RhoA-driven mechanisms[135]. RhoA is among the most studied actin cytoskeleton regulator linked to CAV1[134, 137, 178, 184–186], and physical interaction between them has been reported[187]; this has profound implications for tumour biology as discussed below. CAV1 also regulates the activity of other cytoskeleton modulators, such as Cdc42 and Rac1 GTPases[137]. In the case of

Rac1, for instance, CAV1 controls both its PM-targeting by limiting the number of available Rac1-binding sites[134], and promotes Rac1 degradation[187], which also limits downstream signaling. However, whether functions attributed to CAV1 are also dependent on caveolae dynamics requires further investigation. In fact, many of these regulatory processes has been associated with non-caveolar CAV1, including prostate cancer metastasis[187] and primary cilium length control[185]. Finally, the regulation of caveolar rosette disassembly upon tension, dependent on a mechanism involving c-Abl-driven phosphorylation of the Formin-binding protein 17 (FBP17, essential for rosette stabilization) is also tightly coupled to stress fiber dynamics[157].

3. CAV1 AND TUMOR BIOLOGY

3.1. CAV1 in tumor cell biology

CAV1 displays multiple links with the control of cell cycle progression, growth and survival signaling, anabolism, cytoskeletal dynamics and invasiveness of tumor cells, but these effects are highly contextual (often, even contradictory) and depend on tumor cell type and disease stage[188–194]. This is portrayed by the varying expression levels of CAV1 during carcinoma progression: low in early stages (where its role curbing proliferation predominates); but high in advanced stages, where it correlates with invasive phenotypes and therapeutic resistance[194]. Of note, in advanced prostate carcinoma, characterized by robust expression of CAV1 as compared to early subtypes, CAV1 reprograms TGF-β signaling from tumor-suppressive to oncogenic[195]. Several of these links are likely determined by CAV1-dependent mediation of cell-ECM interaction: for example, CAV1 exerts a negative impact on anchorage-independent growth by eliciting integrin internalization and shutdown of Rac1 proliferative signaling upon detachment[172, 196]. These mechanisms may underlie the downregulation of CAV1 expression frequently observed in early stages of epithelial tumorigenesis, where the inhibitory effect of CAV1 on cell cycle progression is prominent and not bypassed yet by concomitant mechanisms. Of note, a number of oncogenic mutations and tumor-associated phenotypes can blunt CAV1 expression[197]. These events may be also linked with the impact CAV1 downregulation has on cell metabolism: CAV1 depletion leads to increased glycolytic rates even in normoxic conditions and CAV1 downregulation correlates with a ‘Warburg effect’ phenotype[198, 199]. The role of CAV1 and caveolae as mechanically regulated organizers of the PM may also contribute to the context-dependent modulation of oncogenic signaling[197]. An intriguing concept is the availability of non-caveolar CAV1 versus PM-localized CAV1 (which increases with tension-driven disassembly of caveolae) as a key determinant of its interaction (for example, through its CSD) with different signaling nodes[197]. Finally, CAV1 and caveolae may also directly confer mechanoprotection to tumor cells when subject to substantial mechanical stress during metastasis[2].

Mechanisms of tissue organization and developmental regulation may hold a key to understand the context-dependent role of CAV1 in tumor cell biology, and its relationship with cell mechanoadaptation and stromal mechanical properties. CAV1 expression levels generally correlate with the physiological exposure of tissues to mechanical stress (high in muscle, endothelium, lung parenchyma, expanding adipose tissue; low in neural tissue and blood cells), and are tightly controlled during development and cell differentiation at different levels, including epigenetically[200]. CAV1 plays an important role controlling cell state transitions driving differentiation in development or tissue repair[200–202]. CAV1 downregulation is required during transition between differentiation states, and is followed by CAV1 upregulation, which stabilizes back the new cellular state[203]. Thus, in tissue patterning during development or repair, CAV1 can exert different effects depending on cell type transitions, and the stimulation context involved[203]. This 'Janus behavior' is a hallmark of the role of CAV1 in carcinoma, for which matrix stiffness is a relevant cue regulating transitions in carcinoma cells[204, 205]. Squamous and small-cell lung carcinoma are discriminated by different epithelial (TP63, CSTA, COL17A2) and neuroendocrine (VGF, SYP, CHGB, INSM1, HMP19, TUBB2B, CACNA2D4, GNB3) markers, respectively, and multiple transdifferentiation processes have been described among these two types of carcinoma upon treatment as a mechanism for drug resistance[206]. These observations support the existence of an axis of neuroendocrine-epithelial differentiation, analogous to the one driving neural crest during development—an EMT process modulated by CAV1—, controlling transitions between these two carcinoma types[207]. Accordingly, CAV1 expression inhibits neuronal differentiation[208, 209], and is one of the transcriptome features that best discriminates small-cell carcinomas from squamous carcinomas, inversely correlating with neuroendocrine expression signatures[206]. CAV1 expression levels can also have a profound impact on tumor cell metabolism[210, 211] and a subsequent study demonstrated a correlation between metabolic status and mechanical properties across patient-derived carcinoma subtypes. Again, CAV1 expression was highly informative for discriminating these different carcinoma subtypes[212]. Thus, CAV1 emerges as a potential central node determining how a cell differentiation state is linked to specific metabolic and/or mechanoadaptive reprogramming, providing in turn a framework to understand its tumor type- and stage-dependent role.

3.2. Stromal CAV1 as a regulator of tumor microenvironment architecture and biomechanical ECM remodeling

In addition to its various roles in tumor cell biology, CAV1 emerges as an important functional regulator of CAFs. Expression levels of CAV1 in the stromal compartment often show correlation with aggressiveness and prognosis in breast, lung, liver or pancreatic tumors[213–221]. However, because of the broad range of processes affecting tumor progression that are or

can potentially be affected by stromal CAV1, including angiogenesis, metabolism and paracrine signaling, the specific direction of this correlation (i.e. whether certain stromal CAV1 levels have a positive or negative impact on the disease) is again highly dependent on context, and challenging to interpret mechanistically. Here, we summarize current knowledge on the roles of CAV1 expression in CAFs with regards specifically to ECM remodeling and its biomechanical reciprocity with CAF activation, which primarily promotes tumor stiffening and metastasis[120]. It must be however observed that pathological ECM remodeling and desmoplasia can in turn affect other aforementioned parameters, such as tumor vascularization and metabolism.

CAV1 expression and functional caveolar structures are relevant for paracrine signaling driving CAF activation. Caveolae provide a platform for the appropriate regulation and integration of different signaling cues, and are enriched in membrane receptors such as epidermal growth factor receptor (EGFR), transforming growth factor β receptor (TGF-βR) and insulin receptor (IR)[222–224]. A key signaling network modulated in a CAV1-dependent manner is the TGF-β pathway, pivotal for CAF activation and ECM remodeling (see above). Both TGF-βRI (ALK1, ALK5) and TGF-βR II localize to caveolae[225, 226], where they interact with CAV1. CAV1 interaction through its scaffolding domain with TGF-βRI was early reported to suppress TGF-β signaling and Smad3 activation[222], in accordance with subsequent studies supporting a TGF-β pathway-curbing role for CAV1[226, 227]. Indeed, CAV1 depletion in different cell models exacerbates TGF-β signaling, promoting epitelial-to-mesenchymal transition phenotypes and notably, the deposition of collagen-rich ECM and fibrosis with a distinct composition[227, 228]. Of note, certain ECM components commonly associated to EMT (such as FN or TnC, of significant relevance for tumor stroma remodeling) are relatively depleted in matrices derived from CAV1-depleted cells, highlighting the concomitant effect CAV1 intervention can have on ECM biogenesis (see below)[227, 229]. Multiple non-exclusive mechanisms include allosteric regulation, modulation of receptor-transducer interaction, receptor internalization and turnover and modulation of downstream signaling nodes such as ERK and MAPKs[187, 227, 230–233]. However, CAV1 can also promote the activity of specific TGF-βRs such as ALK1[226], and exacerbate the activation of mesenchymal cell populations in specific fibrosis models[234]. These studies reflect the high context dependency of the functional impact of modulating caveolae density and CAV1 levels on TGF-β signaling (and, for that matter, on most of its downstream functions). Interestingly, TGF-β signaling regulates back CAV1 expression[227, 235], revealing potential signaling feedback loops between the TGF-β pathway and other networks (including mechanosensing/mechanotransduction) that converge on caveolae.

Caveolae also allocate different nodes involved in the regulation of inflammatory signaling, such as TNF receptor-associated factor 2 (TRAF2) and tumor necrosis factor receptor-α (TNFR α)[236, 237], which likely contribute to modulate the activated state of CAFs[238, 239].

Finally, it is worth mentioning the role of CAV1 in the control of fibroblast metabolism, which in turn can critically contribute to define the functional state of CAFs and their interaction with the tumor microenvironment (see below).

As aforementioned, among those phenotypes first described as associated with CAV1 genetic deficiency are altered ECM deposition, interstitial space thickening and fibrosis in diverse tissues, most notably vascular wall and lung parenchyma[227, 228, 240–242]. Evidence suggests that CAV1 is a key *direct* regulator of ECM deposition, apart from its role on the modulation of cell states that promote secretory activity, and intervention of CAV1 expression can have a profound, distinct impact on ECM composition beyond the facilitation of transitions towards a secretory phenotype[227]. A feature to be first considered is the fact that CAV1 preassembled oligomers are trafficked through COPII vesicles from the ER to the Golgi before entering the endosomal compartment, instructing the efflux of cholesterol towards the plasma membrane[151, 198, 243]. The impact of CAV1 levels on COPII transport of ECM components has been barely explored, although different studies suggest that collagen deposition is increased in different experimental models of CAV1 deficiency, and cholesterol levels can affect ER exit site dynamics[242, 244–246]. FN is another major ECM component whose metabolism is significantly affected by CAV1 expression: specifically, CAV1 (and presumably caveolae, as suggested by experiments where the cholesterol-squelching molecule methyl-β-cyclodextrin is used) is required for the internalization and turnover of fibronectin monomers and fibrillogenesis, an active process during ECM remodeling[173, 174]. CAV1 Tyr14 phosphoregulation is potentially relevant to these processes[174].

A novel layer of regulation for CAV1-dependent ECM biogenesis has been revealed by the characterization of the role of this protein on exosome secretion[229]. Exosomes are well-established modulators of tumor progression and mediators of the influence of CAFs on the tumor microenvironment, but their direct role on ECM remodeling has been scarcely explored[247]. Intriguingly, a subpopulation of CAV1 molecules is trafficked to the endosomal compartment to enter multivesicular bodies (MVBs), where it can be sorted onto exosomal vesicles for its secretion and direct transfer to other cells. CAV1 itself is required for the specification of exosomal cargo, and CAV1-deficient cells exhibit impaired sorting of non-collagen ECM components (including FN and TnC) onto exosomes—a secretion route allowing for efficient ECM deposition *in vitro* and *in vivo* (in fact, strictly required for certain components such as tenascin-C), which can in turn promote tumor cell invasiveness[229]. The relevance of these findings is highlighted by the fact that, while the ‘classical’ pathway for collagen secretion has long been studied, the secretion routes for non-collagen ECM components have remained puzzlingly elusive[248, 249]. Mechanistically, CAV1 is a pivotal regulator of cholesterol accumulation in the endosomal compartment, which ultimately modulates exosome biogenesis,

morphology and cargo sorting[229]. Indeed, cholesterol trafficking and organization is likely a key mechanism channeling several biological roles of CAV1 and underpinning many of the phenotypes associated with its dysregulation, and its coupling with structures gating ECM secretion is likely[198, 232].

Finally, CAV1 also regulates activities that can determine extracellular ECM crosslinking and organization. Indeed, the levels of several matrix metaloproteinases, as well as collagen maturation enzymes, can be altered upon depletion of CAV1 in different systems[227, 250–253]. High-throughput molecular profiling techniques and their coupling with matrisome isolation procedures will shed light on how these specific ties contribute to CAV1-dependent ECM remodeling and how they relate to each other.

CAV1 and caveolae also emerge as prime regulators of the biomechanical feed-forward loop between CAFs and ECM stiffening. While early studies focused on the metabolic and signaling rewiring CAV1 loss can induce on stromal fibroblasts—thus promoting tumor cell growth through a ´reverse Warburg effect´ and alteration of central pathways such as TGF-β signaling[254]—, Goetz and coworkers focused on the specific impact of this feature on tumor stroma architecture, mechanical properties and functional relevance[136]. CAV1 depletion from different fibroblast models provokes a remarkable alteration on ECM biomechanical remodeling, and CAV1KO/KD fibroblasts generate substrates rich in softer, chaotic collagen matrix, largely devoid of anisotropic, tumorigenic and prometastatic patterns of FN-rich ECM both *in vivo* and *in vitro*. Importantly, these effects could be bypassed by promoting sustained RhoA activity upon p190RhoGAP depletion, indicating that CAV1 levels have an important role in the regulation of actomyosin contraction. These phenotypes correlate with a significant reduction in tumor cell local invasiveness and spontaneous metastasis and with increased end-point survival in breast and melanoma tumor cell xenografting assays, revealing the functional relevance of stromal CAV1-driven biomechanical ECM remodeling in tumors. Interestingly, rescue experiments with different constructs revealed that Y14 phosphorylation is relevant for this ECM remodeling activity. As discussed below, the net effect of stromal CAV1 depletion can comprise other biological effects, such as altered intratumoral angiogenesis, and therapeutic intervention based on stromal CAV1 level modulation should take on account these effects apart from local ECM remodeling. Nonetheless, these studies support a potential value of stromal CAV1 expression as a biomarker for tumor evaluation.

CAV1 also intersects with a key link enabling self-sustained feed-forward loops between stiffness mechanosensing and ECM remodeling, the transcription factor YAP[255–257]. The influence of CAV1 on YAP mechanoregulation is not derived from indirect inputs such as cell shape, but is operated through actin cytoskeleton dynamics. Of note, these studies also revealed that bypassing YAP phosphorregulation through exogenous expression of a constitutively active

mutant can rescue ECM remodeling and cell contractility in CAV1KO cells[257]. It must be bore in mind that, as with most other aspects of CAV1 biology, CAV1-dependent regulation of YAP is dependent on cell type and context, and CAV1 depletion in other systems such as epithelial cells (where the contribution of inputs other than dynamic substrate stiffening to YAP regulation may be highly relevant) can positively regulate YAP activation[256]. How CAV1-YAP interplay correlates with other CAV1 phenotypes, such as TGF-β rewiring or metabolic reprogramming will constitute interesting future research.

Additional, non-exclusive parameters that can be affected in CAFs by CAV1 expression levels, impacting on ECM remodeling are cell motility and orientation. As aforementioned, these aspects of tissue organization are pivotal for the generation of anisotropic ECM patterns[51]. CAV1 is required for polarized cell migration through mechanisms modulated by p190RhoGAP and Src, and CAV1KO fibroblasts show impaired response to external directional stimuli[137]. Together with its general impact on actomyosin contractility, it is conceivable that CAV1 downregulation may affect the collective behavior required for the emergence of anisotropic matrix arrangement. Future studies will establish whether CAV1 is specifically required for cell collision-driven alignment and tissue patterning

4. CONCLUDING REMARKS

Research summarized above has spurred the interest on tumor cell and stromal mechanobiology, and the role of CAV1 therein, as potential opportunities for both therapeutic intervention and diagnostic/prognostic strategies. Because of the remarkable range of different biological processes CAV1 and caveolae participate of in distinct cell populations of different tumor types, the measurement and targeted modulation of these cell components attracts considerable interest from different lines of study. Approaches for targeted CAV1 expression modulation *in vivo* with translational value developed so far include transduction of a CAV1 CSD-mimicking synthetic peptide[258] and small-interfering RNAs[136], but these tools require additional technologies to achieve cell type specificity, such as targeted nanoparticles or viral vector tools. However, it must be stressed that we still have an incomplete mechanistic understanding as to how all these different activities of CAV1 are coordinated and interplay among each other to determine fibroblast activity, ECM remodeling and tumor progression (summarized in Figure 2). This adds up to the complex relationship ECM remodeling intervention may have with subsequent disease progression: while increasing therapeutic sensitivity, reduction of tumor desmoplasia can promote tumor aggressiveness[259, 260]. A deep, systematic mapping of these functions and integrating multiple, complementary approaches will be required to cover these gaps in our knowledge and achieve personalized medicine.

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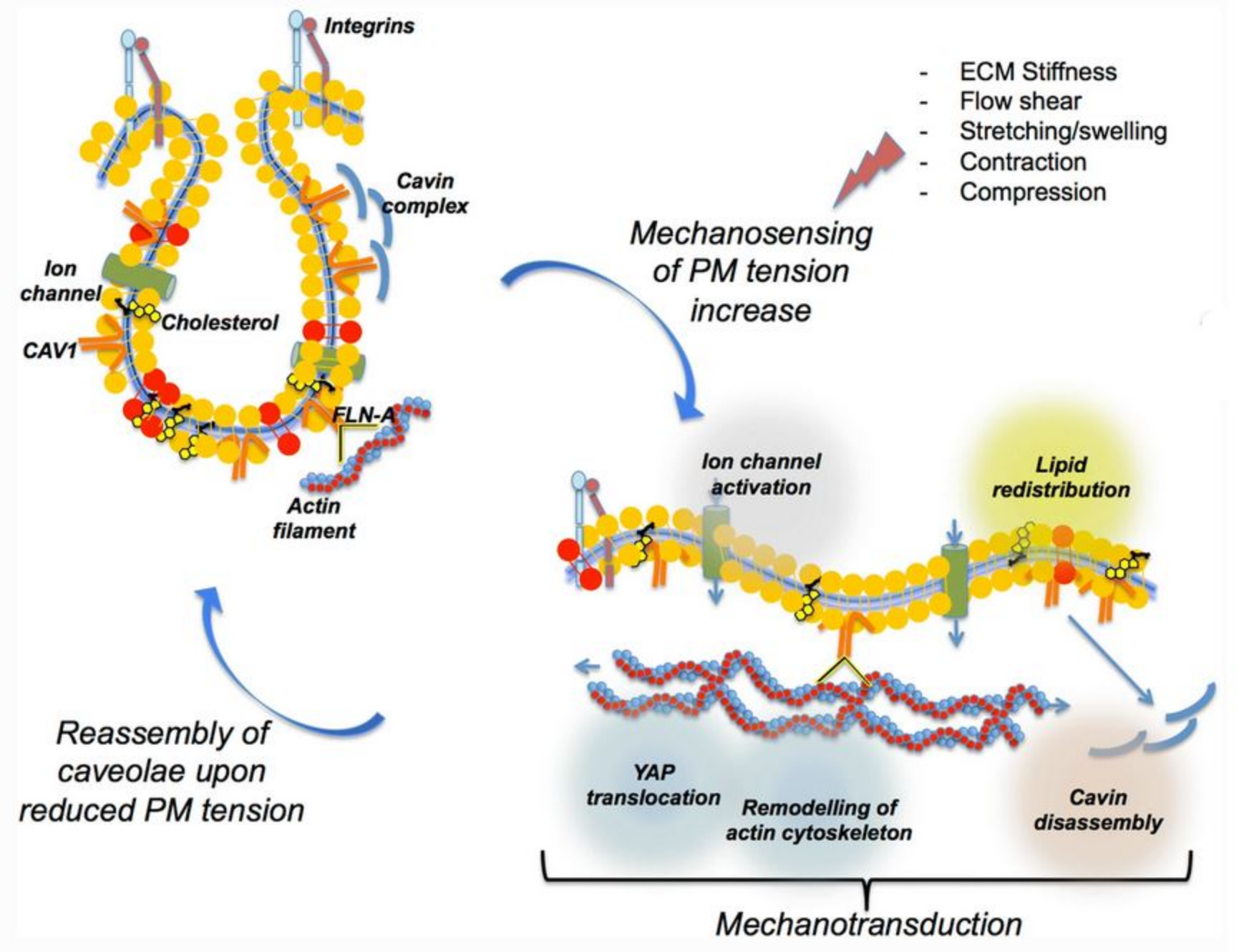
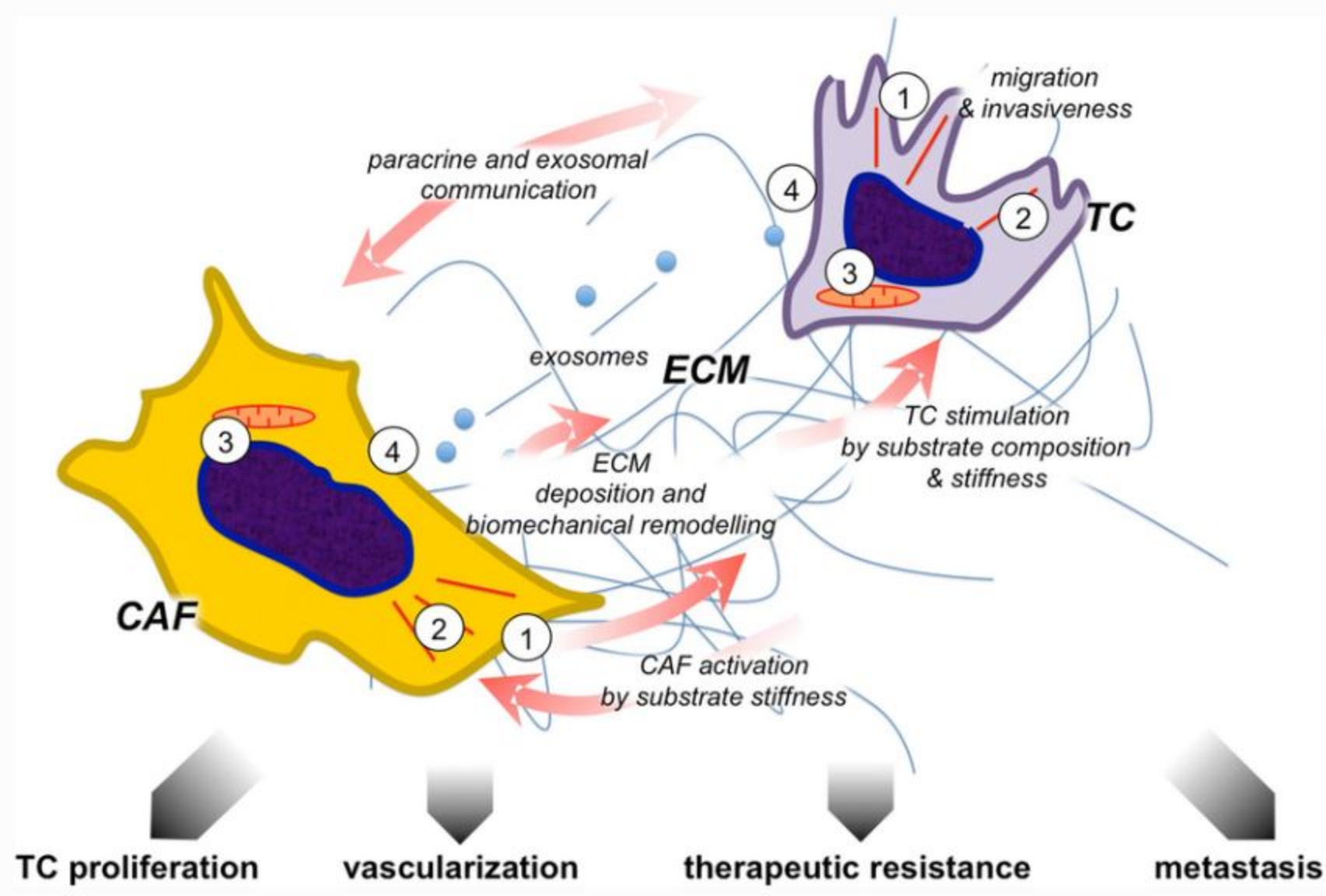
FIGURE LEGENDS:

Figure 1. The main caveolar components (CAV1, CAVIN1, ion channels, cholesterol, phosphatidylserine, PS; Filamin-A, FLN-A, linking with actin filaments) are depicted. Upon different mechanical inputs (i.e. increased ECM stiffness, contractility, swelling, actomyosin contraction), caveolae at the plasma membrane flatten, triggering downstream events (mechanotransduction): CAVIN1 disassembly and translocation, ion channel activation, lipid redistribution, YAP nuclear translocation, actin cytoskeleton remodeling.

Figure 2. Overview of reciprocal interactions between cancer-associated fibroblasts (CAFs), tumor cells (TCs) and extracellular matrix (ECM) within the tumor microenvironment, and potential impact on tumor progression. General roles of CAV1 on CAF and TC behavior in ECM stiffness mechanotransduction are highlighted: (1) cell adhesion, substrate recognition and mechanosensing; (2) mechanotransduction and actomyosin contraction; (3) gene expression, metabolic and differentiation state reprogramming; (4) organization of signaling nodes and efferent paracrine and exosome-mediated signaling.


Integrins
Cavin complex
Ion channel
Cholesterol
CAV1
FLN-A
Actin filament
- ECM Stiffness
- Flow shear
- Stretching/swelling
- Contraction
- Compression
Mechanosensing of PM tension increase
Ion channel activation
Lipid redistribution
YAP translocation
Remodelling of actin cytoskeleton
Cavin disassembly
Mechanotransduction
Reassembly of caveolae upon reduced PM tension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ECM
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CAF
CAF activation
by substrate stiffness
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vascularization
therapeutic resistance
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