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Ready to migrate? Reading cellular signs of migration in an epithelial to mesenchymal transition model

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Abstract: The epithelial to mesenchymal transition (EMT) is a cellular program that drives de-differentiation of cells in both physiological and pathological processes. One of the characteristics of cells describing an EMT is the (re)acquisition of a motility capacity that allows them to migrate through the original tissue as well as to other sites in the organism. The molecular mechanisms that control the EMT are rapidly emerging and here we add to the idea that the adaptation required for cells to commit to the EMT includes adjustments of the translation machinery and metabolic pathways to cope with a high demand of extracellular components.

Introduction

The epithelial to mesenchymal transition (EMT) is a highly conserved cellular outcome considered as a driving force of migration-dependent processes and characterized by a number of morphological, structural, and molecular changes (Trepat *et al.*, 2012; Yang *et al.*, 2020). In order to prepare and adapt to variable and stressful conditions encountered during migration, cells must put in place concerted molecular and cellular programs, some of which are the focus of this piece.

Several *in vitro* and *in vivo* models have been developed to study different aspects of EMT (Greco *et al.*, 2021). While the latter gives insightful information about the interplay among cells and their environment, *in vitro* approaches using cell-based models are advantageous to answer specific molecular and biochemical questions under well-defined conditions. Over the last years, we have been characterizing global differences in gene expression and metabolism in EMT using a MCF7-derived breast cancer cellular model based on the overexpression of truncated forms of the MKL1 protein (also known as MRTFA) that results in an EMT-like phenotype. While MCF7 control cells remain close to the epithelial edge of the EMT spectrum, cells expressing a constitutively active N-terminal domain-lacking (ΔN200) MKL1 show characteristics of basal cells (Flouriot *et al.*, 2014; Kerdivel *et al.*, 2014) that can be associated with the breakdown of some features of intercellular cooperation (Aktipis *et al.*, 2015).

Main Text

We recently reported that EMT-like MKL1 ΔN200 cells show extensive molecular and biochemical modifications (Fernández-Calero *et al.*, 2020). Indeed, our transcriptomic and translatomic results suggest a general boost in translation that could be sustained by the significant increase in the expression and activity of several components of the protein synthesis machinery and translation-associated factors (Fig. 1) (Fernández-Calero *et al.*, 2020). Higher ribosome biogenesis was previously detected in other EMT cellular models, such as non transformed mouse mammary gland epithelial NMuMG and MMTV-PyMT mouse-derived Py2T mammary cell lines treated with TGFβ, and MCF7 cells cultured under hypoxic conditions, as well as in Wnt-driven EMT delamination and migration of neural crest cells in chick and mouse embryonic development (Prakash *et al.*, 2019). Altogether, this suggests that modification of translation is a common characteristic of EMT. In addition, MKL1 ΔN200 cells display deep metabolic rewiring consistent with an enhanced Warburg-like effect

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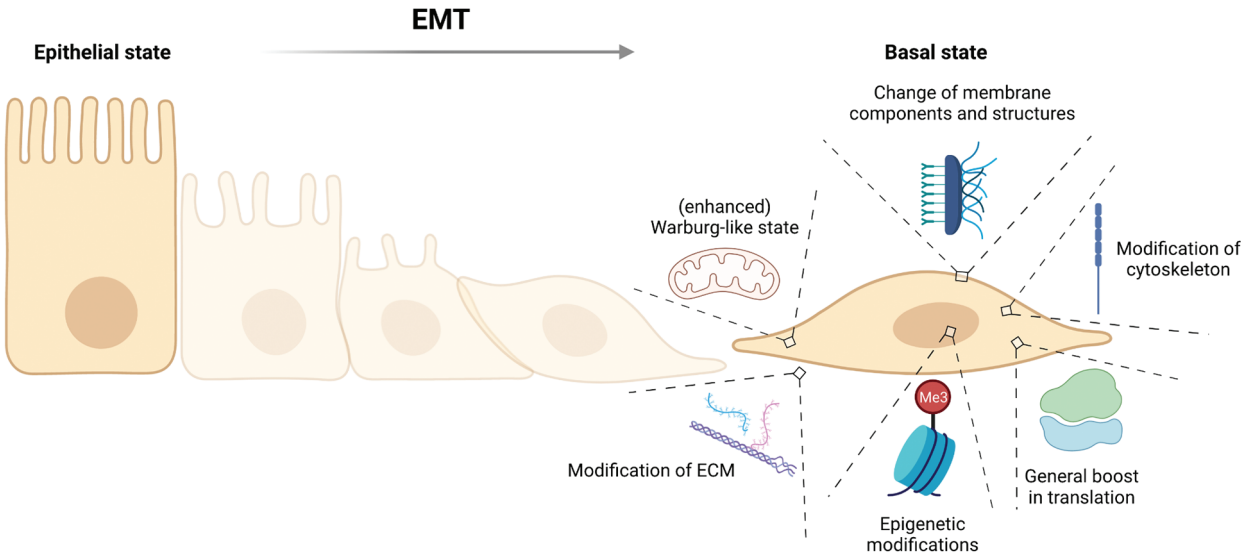

FIGURE 1. Biochemical, molecular and cellular adaptations detected in truncated MKL1-dependent MCF7-derived breast cancer cellular model during the epithelial to mesenchymal transition (EMT)-like process. The well-differentiated and polarized epithelial cells change their morphology and cell contacts, and through a series of intermediate states, develop a de-differentiated and basal state able to migrate. For instance, basal cells have increased expression of several components of the protein synthesis machinery and translation-associated factors such as eIF1, eIF2, eIF3, eIF4, eIF5, eIF5A, eEF1A, eEF1B, eEF1G, and eEF2. This is accompanied by a metabolic rewiring defined by changes in the expression of enzymes involved in glucose metabolism and Tricarboxylic Acid Cycle (TCA) (glucose transporters, phosphoglucokinase, pyruvate dehydrogenase kinase, and mitochondrial isocitrate dehydrogenase isoforms) that are in line with the measured higher concentration of oxaloacetate, fumarate and α-ketoglutarate (AKG). Basal cells also express significantly higher levels of KDM2A, KDM3A, KDM6A, JARID2, and JMJD6 dioxygenases, which could lead to epigenetic modifications (histone demethylation). Membrane structures such as lamellipodia and focal adhesions are also modified, as well as the cytoskeleton through disassembly of E-cadherin and increased expression of, for example, actins ACTA and ACTB and keratins KRT10-17. Finally, basal cells produce and export more extracellular matrix (ECM) proteins, mainly collagens and enzymes involved in their processing (COL3-11-12-13-16-24, AKG-dependent collagen prolyl-hydroxylases P3H2 and P4H2, and precollagen lysyl-oxoglutarate dioxygenases PLOD1-3), laminin (LAMA, LAMB) and fibronectin (FN1).

characterized by impairment of Tricarboxylic Acid Cycle (TCA), rise of glucose consumption and glycolysis, and flux redirection towards oxidative steps of the Pentose Phosphate Pathway (Fig. 1) (Fernández-Calero *et al.*, 2020). This behavior was also detected in different cellular models of EMT (Kondaveeti *et al.*, 2015; Liu *et al.*, 2016; Morandi *et al.*, 2017; Martínez-Reyes and Chandel, 2020). This phenotype is potentially promoted by HIF-1α (and other regulatory factors) (Semenza, 2017), as suggested by changes in the expression of glucose transporters, phosphoglucokinase, pyruvate dehydrogenase kinase, and mitochondrial isocitrate dehydrogenase isoforms. In addition, using metabolomics approaches, we detected higher concentration of oxaloacetate, fumarate, and mainly, α-ketoglutarate (AKG) in MKL1 ΔN200 cells, which is consistent with modified usage of TCA enzymes (Fernández-Calero *et al.*, 2020 and references therein), as exemplified by the switch from IDH3 to IDH2 isoform that enables cycle reversal.

The above-described rewiring is associated with a non-proliferative state of MKL1 ΔN200 cells as pointed out by maintenance of cell number and repression of proliferation-associated genes (Prakash *et al.*, 2019; Fernández-Calero *et al.*, 2020). Rather, cells augment their size, which is accompanied by development of cellular structures and triggering of molecular programs that are both related to migrating phenotypes.

Does this context support cell migration?

MKL1 ΔN200 cells acquire migration-related features exemplified by modification of cytoskeleton components (actins ACTA and ACTB, keratins KRT10-17), disassembly of E-cadherin (Kerdivel *et al.*, 2014), and adjustment of cell-cell junction types, lamellipodia-like structures and focal adhesions (Fig. 1) (Fernández-Calero *et al.*, 2020). Noteworthy, the latter could be fueled by the preferential expression of many integrin genes (particularly ITGA1 and ITGA5), several of which have been associated with EMT and metastasis (Gharibi *et al.*, 2017; Deng *et al.*, 2019; Janiszewska *et al.*, 2020). Moreover, these cells display increased expression of genes implicated in synthesis and export of extracellular matrix (ECM) proteins, mainly collagens and enzymes involved in their processing (COL3-11-12-13-16-24, AKG-dependent collagen prolyl-hydroxylases P3H2 and P4H2, and precollagen lysyl-oxoglutarate dioxygenases PLOD 1-3), laminin (LAMA, LAMB) and fibronectin (FN1) (Fig. 1) (Fernández-Calero *et al.*, 2020). Secretion of ECM components is a well-known characteristic of cancer cells that contribute to (re)define the composition of the matrix to facilitate cell migration and tumor progression (Naba *et al.*, 2012, 2014). Importantly, the high demand of protein synthesis imposed by the production of ECM components could be sustained by the above-mentioned high expression of translation-associated factors, which is in turn promoted, attractively, via favoring their own translation (Fernández-Calero *et al.*, 2020). Of note, these observations are consistent with the description of circulating tumor cells derived from breast tumors that over-express ribosomal proteins (Ebright *et al.*, 2020). Furthermore, the high concentration of AKG, which is a key hub metabolite for several processes, could also add to the phenotypic

adaptations induced by MKL1. For instance, AKG favors biosynthetic processes since it is used to hydroxylate collagen's prolines and lysines at the endoplasmic reticulum (Krane, 2008; Rappu *et al*., 2019) and supports biosynthesis of lipids exported towards the extracellular matrix (ECM) and required to increase cell size (Reitman and Yan, 2010; Martínez-Reyes and Chandel, 2020). Moreover, AKG promotes epigenetic modifications through activation of histone demethylation by specific dioxygenases (Tsukada *et al*., 2006; Salminen *et al*., 2015; Liu *et al*., 2017; Rhoads and Anderson, 2020) and regulates the redox state through NADP to NADPH conversion at the expense of NADH oxidation (Mullen *et al*., 2014; Ju *et al*., 2020; Sharma and Ramanathan, 2020).

The above-mentioned attributes are coherent with the phenotype of detaching cells. However, are cells actually migrating in our model? Our experiments showed that although MKL1 ΔN200 cells continuously expand their size when maintained in regular liquid medium, the population viability decreases over time. However, if cells are cultured in a moving-favorable context such as Matrigel, they remain alive and migrate (Fernández-Calero *et al*., 2020). This result points towards the importance of migration as an alternative and viable output for such cells that occurs in particular scenarios. Indeed, the experiments performed suggest that the composition and nature of the pre-existing environment are essential for cells to develop their fully motile capacity. Therefore, further studies are required to define to what extent MKL1 ΔN200 cells depend on the matrix and if its nature induces extra changes on gene expression towards more migration-associated signatures.

Indeed, ECM composition drives, for example, stem cell migration and differentiation resulting in fundamental consequences on cell and tissue functions and is also decisive in fibroblast proliferation and migration during wound healing and tissue regeneration (Jiang *et al*., 2016). ECM composition underlies ECM stiffness, geometry interaction with cell receptors, as well as several physicochemical parameters that together work as major determinants of cell fate and behavior in the tissue (Druso and Fischbach, 2018; Winkler *et al*., 2020). This is particularly relevant for breast cancer cells, since their ability to metastasize and invade new tissues largely depends on the physicochemical characteristics of the ECM where the original tumor had developed (Spill *et al*., 2016; He *et al*., 2017; Watson *et al*., 2021). Therefore, cells and ECM constitute a single unit sustained by a bidirectional interplay that relies on mutual modifications and that ultimately leads to their own constant variation and adaptation (van Helvert *et al*., 2018; Winkler *et al*., 2020).

Conclusions and perspectives

As any other cellular program, migration requires a concerted adaptation plan. In reaching such a state, cells modify central features of cooperation that characterize multicellularity to sustain detachment and migration, and the above-described mechanisms provide, at least in part, the context and fuel to dialogue with the ECM and face this stressful quest. Moreover, the regulation of gene expression at the translation step appears strategic for cells to cope with continuous cues rising throughout migration and colonization of new environments, and to be ready to proliferate immediately once they have colonized a secondary site. Addressing these questions requires a combination of *in vivo* and *in vitro* approaches to further analyze the steps followed by soon-to-migrate cells and to characterize the cell-environment system as a whole, which would contribute to unravel the relationship between cancer-related EMT and ancestral networks, and to identify putative targets to block dissemination of migrating tumor cells.

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