RABbing cancer the wrong way

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Hypoxia, a reduction in the normal level of tissue oxygen tension, is a common characteristic of solid tumors, endowing them with malignant, aggressive, and treatment-refractory properties (1, 2). The family of hypoxia-inducible heterodimeric transcription factors (HIFs) (3) mediates, at large, the biological effects of hypoxia by regulating the transcription of a large number of target genes whose elevated expression in patient tumors correlates with poor prognosis and treatment failures (4). It is well established that hypoxia drives malignant progression and metastasis by promoting angiogenesis, metabolic adaptation, and overall tumor fitness (5); however, the precise molecular mechanisms underlying this process are not fully elucidated. Furthermore, the signaling pathways, which are activated by hypoxia and facilitate the multi-step metastatic cascade that involves local matrix remodeling, cancer cell intravasation, extravasation, and homing to distant sites (6), are not well understood. In PNAS, Wang et al. provide significant insights into this process. The authors identified a new mechanism by which hypoxia induces breast cancer metastasis by stimulating microvesicle (MV) biogenesis and shedding from the primary tumor cells (7). The authors showed

conclusively that this process is dependent on HIF expression, which in turn promotes RAB22A transcription (7). RAB22A is one of the more than 60 human Rab GTPases, which are membrane-bound proteins that function as molecular switches, oscillating between their active and inactive states to spatiotemporally integrate intracellular signaling and membrane trafficking events (8). Although a few members of the Rab family of proteins have been implicated in carcinogenesis, such as RAB25 (9), very little is known about the potential role of RAB22A—best known for its role in endocytic trafficking—in tumor progression or metastasis. Wang et al. identify a pivotal role for RAB22A in MV biogenesis and metastasis promotion. RAB22A knockdown (KD) completely eliminated the hypoxia-induced MV formation, and although its reduced expression had no effect on the primary tumor, it significantly impacted the metastatic tumor burden by lowering the number and size of lung metastases. These data describe a new mechanism by which hypoxia uses RAB22A-coated MVs as the “currency” to affect the metastatic transaction.

Tumor cells are known to use different modes of intercellular communication through

the secretion of diverse types of membranous vesicles termed extracellular vesicles (EVs); EVs are further subcategorized based on their size, biogenesis and release mechanism, and content into exosomes, MVs, and apoptotic bodies (10). Although consensus in the literature is still lacking on the exact criteria used to define each EV subpopulation, it is well established that these secreted vesicles carry information from the cell of origin in the form of protein, RNA, or DNA, which on transfer to neighboring cells can influence the phenotype and function of recipient cells (Fig. 1). In cancer, these secreted EVs have been shown to promote chemoresistance (11) and metastasis through the intercellular transfer of nucleic acids, oncogenic transcription factors (12–14), and the formation and promotion of the premetastatic niche (15). It is conceivable that each of the EV subtypes bears a specific protein and lipid composition and carries a select set of functional information from the cell of origin. However, regardless of the exact nomenclature and size and content criteria used to describe these secreted EVs, the concept provided by Wang et al. “subscribes” to the simple mathematical Law of the Lever, first described by the ancient Greek mathematician Archimedes in the third century BC (16). According to the law, a small input force can generate a much larger downstream output force. Similarly, Wang et al. show that when the locally secreted RAB-coated MVs are taken up by neighboring cells, the process of metastasis is enhanced; this evokes the concept of a small local input (MV shedding) resulting in an amplified and detrimental—in this case—larger output (metastatic phenotype), through the leveraging of this newly identified function of RAB22A.

These data further suggest that, clinically, patients whose tumors express high levels of RAB22A protein would be more prone to metastasis. To that end, the authors mine two large breast cancer expression datasets and clearly demonstrated that the expression of RAB22A mRNA is associated with decreased overall survival in women with breast cancer. Although these data show that RAB22A expression is an independent

Fig. 1. Schematic representation of the HIF-RAB22A-MV-metastasis axis. Hypoxia induces the HIF pathway, which in turn up-regulates RAB22A transcription. RAB22A protein then translocates to the MV outer membrane and promotes MV formation under hypoxia. Hypoxic donor cancer cells secrete these RAB22A-coated MVs, the uptake of which by neighboring recipient cells promotes cancer spread and metastasis.

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adverse prognostic biomarker for breast cancer patients, its significant association with the expression of other HIF target genes such as VEGF, P4HA1, and GLUT1 confirms the mechanistic studies presented in this paper, showing that RAB22A expression is HIF1 dependent and that HIF protein expression is required for the initiation of the hypoxia-RAB-metastasis pathway.

This newly identified axis begs the question: what might be the effects of these RAB22A-coated MVs, not only on intercell communication, but also on the tumor microenvironment? Remodeling of the tumor microenvironment—comprising extracellular matrix (ECM) and nontumor cells such as endothelial, immune, and fibroblast stromal cells—represents a key step in tumor progression and metastasis. One of the first, and most critical, steps in metastasis is the degradation and crossing of ECM by tumor cells, a complex process that involves cross-talk and multidirectional signaling involving many different cell types present in the 3D tumor microenvironment where the primary carcinoma resides (17). In addition, emerging data reveal a critical role of hypoxia on ECM remodeling and metastasis through the effects of HIF on the composition, physical and mechanical properties of ECM, and the recruitment and reprogramming of different cell types, all of which work in concert to enhance metastasis (18). Experiments performed by Wang et al. demonstrate that RAB22A KD in triple negative breast cancer cells resulted in decreased invasion in vitro and decreased lung colonization in vivo. However, the contribution of the tumor microenvironment in this outcome has not been addressed (Fig. 1). Elucidating the potential molecular cross-talk between RAB22A and ECM in breast cancer invasion and metastasis will have a great impact on designing better therapeutic strategies to treat, and potentially prevent, metastasis.

As the overwhelming majority of cancer mortality is attributed to metastatic disease, the data presented by Wang et al. identify the HIF-RAB22A-MV axis as an important mediator of breast cancer metastasis, one

Wang et al. identify a pivotal role for RAB22A in MV biogenesis and metastasis promotion.

that is clinically relevant and potentially “actionable.” We can envision targeting each of the components of this axis, starting with HIF at the helm. The quest for identification of specific HIF small molecule inhibitors, which started soon after its discovery (3) and was based on compelling evidence showing HIF’s critical role in tumor progression and metastasis, has been very challenging. Despite multiple attempts to identify such small molecule inhibitors, most of the drugs shown to affect HIF do so indirectly by modulating

upstream pathways that regulate HIF’s protein synthesis or function (19). Although currently there is no clinically approved HIF-specific inhibitor, the data by Wang et al. provide strong support for the further clinical development of HIF inhibitors in the context of targeted combination therapies.

Moreover, the data by Wang et al. suggest that inhibiting RAB22A function can be a promising therapeutic strategy. However, in the absence of specific RAB22A inhibitors, alternative strategies targeting the cytoskeletal pathways that mediate MV plasma membrane translocation and secretion should be considered. Interestingly, the microtubule cytoskeleton has been recently implicated in the intracellular trafficking of RAB22A cargos (20), suggesting that a therapeutic combination of HIF-targeted therapies with the microtubule inhibitors, already used as the standard of care in the treatment of metastatic breast cancer, should be greatly synergistic.

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