Commentary

Migrating with Myosin VI

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One reason to embark in the analysis of global gene expression differences between normal and cancerous prostate epithelial cells is to determine mechanisms that are responsible for prostate cancer development. In this issue of *The American Journal of Pathology*, Dunn et al¹ identify myosin VI as a gene that is consistently overexpressed in prostate cancer compared with normal epithelium. Previous studies of myosin VI have revealed overexpression in ovarian cancer and a role of myosin VI in cell migration, endocytosis, and cell polarity.^{2–4} In addition to these cellular processes, which can all be linked to oncogenesis, the study by Dunn et al¹ identifies a novel function of myosin VI. They demonstrate that a sharp decrease in myosin VI expression causes global gene expression changes and reduces the ability of prostate cancer cells to grow as colonies in soft agar. Based on this new insight about the role of myosin VI in prostate cancer, we examine here the broader function of myosin VI during prostate cancer development and cancer cell invasion.

Myosin VI Is an Early Marker of Prostate Cancer Development

The first clue that myosin VI may indeed be an early target of oncogenic transformation in prostate cancer development is the observation that myosin VI is overexpressed in proliferative inflammatory atrophy (PIA)⁵ and in prostatic intraepithelial neoplasia (PIN).¹ PIN possesses the histopathological features of cancer and is generally recognized as a precursor for invasive carcinoma, but the molecular requirements for progression of PIN to invasive cancer have been difficult to determine. Because myosin VI is uniformly expressed in individual PIN lesions, it is not sufficient for invasion. However, myosin VI expression in PIN strongly suggests that it takes an active role or is at least linked to the selection process of PIN cells that become invasive. Anecdotal evidence supports the concept that invasive cells proliferate slowly,⁶ and a recent molecular study identifies pathways that are responsible for the reciprocal switches from cell migration and invasion to cell proliferation that may be operational in the progression of PIN to

invasive cancer.⁷ Because myosin VI overexpression increases cell migration, myosin VI most likely facilitates the invasion of PIN cells into the stroma.

As demonstrated by Dunn et al,¹ myosin VI is already expressed in the precursor lesion of PIN or PIA. This histological entity is not considered malignant but may be a precursor of malignancy because it is commonly associated with PIN and invasive cancer.⁸ Several cancerous molecular changes are detectable in PIA, including hypermethylation of glutathione S-transferase-pi, gain of chromosome 8, and expression of the cell cycle-dependent kinase inhibitor p16.⁹ In addition, there is high expression of proteins associated with a stress response, such as cyclooxygenase-2¹⁰ and glutathione-S-transferase A1.¹¹ The expression of myosin VI protein in PIA may be associated with a stress response as well, in particular cell stress in response to DNA damage. In this context, the transcriptional activation of the myosin VI gene may occur through a p53-dependent mechanism on activation of p53 in response to DNA damage.¹² Because the functional integrity of p53 is probably preserved in PIN cells and only lost later in cancer progression, p53 can induce myosin VI gene transcription in PIN. Thus, myosin VI overexpression occurs before cells become overtly malignant. It appears that overexpression of myosin VI is tolerated during cancer development as cells acquire additional genetic changes. It is likely that some genetic alterations in cancer cells will interact with myosin VI and that the function of myosin VI changes during the development of PIN and the progression to invasive cancer.

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Myosin VI and Prostate Cancer Invasion

Locally invasive prostate cancers possess a unique architecture by forming a highly branched ductal network. This histological growth pattern strongly suggests the deregulation of branching morphogenesis and the classic microscopic description of prostate cancer as "small glands, back-to-back" results from the two-dimensional view of an excessively branched three-dimensional ductal tree. The growth of prostate cancer relies on cell motility and proteolysis at the tips of ducts, in locations of stromal invasion.¹³ The three-dimensional process of branching morphogenesis by prostate cancer cells is difficult to recapitulate *in vitro*. A major driving force is the prostate stroma, with malignant epithelial cells inappropriately responding to stromal stimuli. Thus, the excessive branching morphogenesis relies largely on aberrant reciprocal interactions between stroma and epithelium. There is a possibility that the overexpression of myosin VI alters the response of epithelial cells to stromal stimuli. There are two subcellular compartments, the endocytic compartment and the leading edge of migratory cells that contain myosin VI. They are both involved in the regulation of cell migration in response to growth factor stimulation.^{4,14–16} Thus, an increased expression of myosin VI could enhance the response of cells to stromal factors and facilitate tumor invasion.

Defects in endocytosis trigger cell proliferation, and a regulator of endocytosis, Rab25, is up-regulated in breast and ovarian cancer.² Overexpression of myosin VI might constitute an alternative pro-oncogenic mechanism for altering endocytosis. Myosin VI associates with endocytic vesicles through the tumor suppressor protein disabled 2 (Dab2).^{3,16–18} Dab2 expression is frequently lost in human cancer, and Dab2 heterozygous mice develop hyperplasia and dysplasia of the ovarian surface epithelium.¹⁹ Dab2 binds to the Dab2-interacting protein (Dab2IP), which has potent growth inhibitory activity and which is lost by methylation in different cancer types, including prostate cancer.²⁰ Therefore, prostate cancer cells potentially overexpress the pro-oncogenic protein myosin VI under conditions in which expression of tumor suppressor proteins Dab2 and Dab2IP are decreased. Because Dab2 mediates the association of myosin VI with endosomes,⁴ loss of Dab2 prevents myosin VI association with endosomes, and this might increase the concentration of myosin VI in other subcellular compartments. For example, an increase of myosin VI in membrane ruffles at the leading edge of cells would facilitate cell migration. Theoretically, it should be possible to examine changes in subcellular localization of myosin VI *in vivo* in immunohistochemical staining images. However, immunohistochemical results show diffuse localization of myosin VI throughout the cytoplasm, because myosin VI also associates with the Golgi complex²¹ and secretory vesicles.^{21,22} It is therefore not possible to measure the amount of myosin VI in the apical compartment of cancer cells, where endocytosis occurs, or at the surface of cancer cells at the invasive front. More quantitative methods are necessary to

demonstrate changes in subcellular localization of myosin VI *in vivo* and to determine whether myosin VI concentrations differ in subcellular compartments of benign and malignant prostate epithelial cells.

The overexpression of myosin VI protein potentially augments cell motility at discrete stages of cancer progression. In the initial stages of cancer development, cell motility increases with the acquisition of an invasive phenotype at the transition from PIN to invasive carcinoma. During disease progression, cell motility plays a critical role in the systemic dissemination of cancer cells. It is uncertain, whether the same molecular mechanisms trigger cell movement at different times during prostate oncogenesis and metastasis. The function of myosin VI in epithelial cell migration has been studied in *D. melanogaster*.²³ In fly ovaries, the egg chamber is surrounded by a simple epithelium. During oogenesis, a cluster of distinct follicle cells forms at the anterior pole.²⁴ Myosin VI is highly expressed in follicle cells, and expression is maintained when a small subgroup of follicle cells, called border cells, delaminates from the epithelium and migrates between nurse cells toward the oocyte.²⁵ This process is reminiscent of tumor cell invasion. Initially, the border cells are polarized epithelial cells with cell-cell junctions that consist of distinct membrane subdomains. For migration, the cell polarity changes from apical to planar, proteins from cell-cell junctions reorganize at the invasive edge of the cell to facilitate cell motility and invasion,²⁶ and myosin VI translocates from cell-cell junctions to membrane ruffles at the invasive front of the cells. The correct localization of proteins at tight junctions that maintain cell polarity depends on expression of myosin VI.²⁶ In migratory border cells, migration is significantly impaired in the absence of myosin VI. Similar to observations in border cells, myosin VI knockdown in LNCaP cells slows cell migration.¹ These results demonstrate that myosin VI promotes cell migration *in vitro* and may cause tumor invasion *in vivo*. It will be interesting to examine whether the increase in cell migration follows a disorganization of cell-cell junctions and a loss of cell polarity.

Unanswered Questions

Myosin VI joins a group of genes that are overexpressed in cancer and that can stimulate the motility and invasion of prostate cancer cells. Other genes that belong in this functional category are the cell surface protease hepsin,^{27–30} Trefoil factor 3,^{31,32} genes involved in polyamine biosynthesis,³³ and neuropilin-1.³⁴ Because myosin VI is expressed in precancerous lesions of proliferative inflammatory atrophy,¹ it most likely affects cell motility early during cancer development. Although we have learned from array studies that the expression of several genes that stimulate cell migration increases when cells become cancerous, it is not clear how these genes interact to trigger invasion of prostate cancer cells, whether they are activated in a stepwise process during cancer cell invasion and metastasis, whether they are co-expressed in the same

cancer cells, or whether they function in separate clonal cell populations. With the exception of hepsin,⁶ the migration-stimulating activities of genes that are identified in array experiments have only been tested in cell line experiments. These *in vitro* experiments are unable to capture a gene's migration-inducing function, which requires an organismal context. If the induction of cell migration depends on cell-cell interactions, an organotypic *in vitro* system is needed. Specific to prostate epithelium, three-dimensional organotypic cultures have been difficult to establish in a reproducible fashion, and thus, molecular mechanisms of cell migration and invasion that rely on epithelial and stromal cross-talk are poorly characterized. Therefore, to further investigate the function of myosin VI in prostate cancer development requires an animal model. This is particularly critical in analyzing the role of myosin VI in branching morphogenesis of normal and cancerous prostatic ducts.

It is apparent that myosin VI overexpression can promote oncogenesis by a variety of mechanisms, and future studies are necessary to determine which of these mechanisms operate during the development of prostate cancer. The connection between myosin VI overexpression in the endocytic compartment and the stimulation of oncogenesis also requires additional investigation. It is conceivable that the link between the endocytic compartment and cellular proliferation and migration is through regulation of epithelial polarity. More research is needed to determine whether these compartments are connected through myosin VI or whether myosin VI assumes separate roles in regulating endocytosis, cell polarity, and cell migration. Finally, the connection between myosin VI and the transcriptional machinery deserves further analysis, especially in light of suppression of a tumor suppressor gene by myosin VI. In summary, the observation by Dunn et al¹ that myosin VI is overexpressed in prostate cancer will stimulate more molecular studies to understand the function of myosin VI in prostate carcinogenesis.

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