Commentary:

Quis custodiet ipsos custodies: Who watches the watchmen?

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Should this be said again? No cell is an island and in tissue-specificity and cancer, context is supreme.

Decades ago, seminal recombination experiments illustrated the dominant role of mammary mesenchyme in directing epithelial development [1-3], and strongly suggested that the microenvironment plays a significant role also in the manifestation of carcinoma. More direct evidence for such functions came from a study demonstrating that an unadulterated microenvironment can suppress the malignant phenotype and re-direct tumor cells to give rise to normally functioning tissues and indeed healthy mice [4]. One may wonder why such a stunning finding did not convince the scientific community to pay more attention to the role of context. The answers are complex, not the least of which is that concomitantly with this finding, the roles of oncogenes and mutations were being discovered. That excitement carried the day, specially because no one subsequently determined whether or not these mice generated from malignant cells contained tumorigenic mutations, and no new group reproduced the work. The following decade saw the discovery that even potent oncogenes could be ruled by context [5], and another couple of decades later it was shown that similar reprogramming of metastatic melanoma by an embryonic microenvironment was possible [6]. There are many more examples which are not as clear cut, but are nevertheless compelling. The extensive literature of two-stage carcinogenesis, namely initiation and progression, indeed clearly indicates that "initiation" and DNA damage alone are not sufficient to allow carcinogenesis.

Implicit in these findings is: once a tumor or an oncogene, not always a tumor or an oncogene. A renewed focus on the tumor microenvironment as a therapeutic target [7] has also led to the recognition that markers within the microenvironment could have predictive power. Two recently published reports identifying 'stromal signatures' in breast cancer patients prognostic

for patient survival [8] and predictive of response to chemotherapeutic treatment [9] provide proof of this concept. In the current issue of *The American Journal of Pathology*, two independent studies [10, 11] identify a novel stromal marker, caveolin (Cav)-1, which predicts clinical outcome of breast cancer patients irrespective of its expression in tumor epithelium.

Cav-1 is a scaffolding protein essential to the structure of caveolae, "little caves" or invaginations in cellular plasma membranes [12]. Cav-1 recruits and arranges lipids and proteins to these membrane sites to function in endocytosis and signal transduction [12]. The observation that Cav-1 expression is attenuated in oncogenically transformed cells [13] led to exploration of whether Cav-1 loss in mammary epithelium was causative. Although mechanistic data suggested that Cav-1 null mice exhibited aberrant epithelial growth [14], and that forcing Cav-1 expression in breast cancer cell lines inhibited growth and metastases in xenograft models [15], a clinical link proved elusive. However, MMTV-PyMT tumors transplanted into the fat pads of Cav-1 knockout mice displayed significantly enhanced growth (vs. wild-type mice) [14], motivating investigation of whether stromal Cav-1 expression correlates with human breast cancer patient survival.

This is precisely what Sloan et al. and Witkiewicz, Dasgupta, et al. demonstrate in this issue of AJP. Using tissue microarray data in conjunction with breast tumor sections and extensive patient survival data, Sloan et al. demonstrate that strong stromal Cav-1 expression is associated with smaller breast tumor size and grade, and is highly predictive of increased survival (Fig. 1). Patients with positive expression of stromal Cav-1 had a 91% ten year survival rate, vs. a 43% survival rate for patients lacking stromal Cav-1 expression. Importantly, there was no correlation between Cav-1 expression in the tumor epithelium and clinical outcome in either tissue arrays or tumor sections [10].

Witkiewicz, Dasgupta, and colleagues independently investigated the clinical significance of stromal Cav-1 expression in a breast tumor tissue microarray. The presence of stromal Cav-1 was strongly associated with tumor size, local spread to lymph nodes, and progression-free survival in Tamoxifen-treated and –untreated patients. Again, tumor Cav-1 expression did not correlate with either of the described metrics [11]. Strikingly, both studies found that stromal Cav-1 expression predicted patient survival independent of estrogen receptor (ER), progesterone receptor (PR), or HER2 status [10, 11]. Results from these two clinical studies suggest that stromal Cav-1 expression may be a new independent prognostic factor for long-term survival and disease recurrence for breast cancer patients, and the Tamoxifen data suggest that expression of this stromal marker may also predict resistance to treatment.

As with any exciting study, intriguing data raise a number of questions which sow the soil for future studies. Principle amongst these questions is whether Cav-1 is a surrogate or a functional biomarker (summarized in Figure 2):

An argument for Cav-1 being a functional biomarker is that its absence may reflect the physical absence of a Cav-1-expressing cell type (Fig. 2, Scenario 1). While Cav-1 was not expressed in the normal mammary epithelium, both groups observed Cav-1 expression in myoepithelium, endothelium, and fibroblasts [10, 11]. Whereas endothelial cells and fibroblasts have demonstrated roles in promoting tumor progression [7], myoepithelial cells (MEPs) function as natural tumor suppressors [16, 17]. In a three-dimensional model of normal acini, it is the MEPs that confer polarity to luminal cells [18], and in a xenograft model of breast tumor progression, the presence of normal MEPs prevents conversion of the ductal carcinoma in situ (DCIS) phenotype to invasive ductal carcinoma [19]. This 'guardian' function of normal MEPs begins to be lost in situ and MEPs surrounding DCIS are in fact quite abnormal [20]. As tumors progress, MEPs are mysteriously

reduced or absent (e.g., in invasive breast tumors) [21]. Whether MEPs have apoptosed, transdifferentiated, or migrated away is unknown, but it is quite possible that Cav-1 disappears with them. Indeed, enhanced tumor growth and invasion (assessed by tumor cell-positive lymph nodes) observed by Witkiewicz, Dasgupta, et al. to correlate with loss of Cav-1 expression are also noted consequences of MEP loss [22].

If not a surrogate biomarker, Cav-1 may instead be a functional biomarker directly responsible for the tumor suppressor functions of MEPs (Fig. 2, Scenario 2). Carcinoma-associated MEPs lose the ability to deposit an integral component of laminin-rich basement membrane which surrounds breast epithelium, potentially robbing epithelial cells of signals crucial to maintaining their architecture [18], and secrete chemokines which may foster tumor growth and invasion [20]. Loss of Cav-1 expression from MEPs, perhaps induced by factors secreted by either transformed epithelial cells or disrupted stroma, may skew their secretory profile and ultimately promote an invasive phenotype.

Witkiewicz, Dasgupta, et al. make a case for Cav-1 loss exerting its effects in the fibroblast component of the microenvironment (Fig. 2, Scenario 3) [11]. This group has recently shown that loss of Cav-1 induces a carcinoma-associated fibroblast (CAF) phenotype [23], which actively participates in tumor progression [24, 25]. Loss of Cav-1 expression may directly mediate transition to the CAF phenotype and promote tumor growth by either attenuating the activity of a tumor suppressor (e.g., retinoblastoma tumor suppressor [23]), activating TGF-β expression, and/or modulating TGF-β receptor activity [26, 27].

Regardless of which scenario may be operating, it is of interest that neither study positively correlated stromal Cav-1 expression with distant metastases (i.e., M-stage). Further.

the offspring of Cav-1 null mice and Her-2/neu mice (which develop mammary-specific tumors) established by Sloan et al. [10] developed tumors faster and required more rapid sacrificing than Her-2/neu counterparts, but did not show increased lung metastases. In light of the survival data, however, the simple question remains: why do patients lacking stromal Cav-1 expression die so fast? It is well accepted that metastatic growths are the cause of breast cancer-related deaths, so determining whether lack of stromal Cav-1 expression at the primary site mediates escape from tumor dormancy at the secondary site in already established mouse models [14] may yield intriguing results. Elaborating upon such studies by deleting Cav-1 in specific cell types (e.g., MEPs, adipocytes) could reveal whether Cav-1 expression is crucial only within certain cell populations and also pinpoint which cell type(s) to use for interrogation of the molecular mechanisms by which reduced Cav-1 expression enhances tumor growth and invasion.

Given the striking prognostic finding of Cav-1 loss in the tumor microenvironment, a final point of discussion is whether stromal Cav-1 also provides a meaningful therapeutic target. Forced expression of Cav-1 in transformed mammary epithelial cells significantly inhibits their growth [14]; thus, exploring the biological functions and molecular regulation of Cav-1 in developing mammary stroma as well as in normal adult mammary stroma may further motivate the development of strategies to enhance tissue specific Cav-1 expression in breast cancer patients. For now, the two studies presented in this issue of AJP provide additional validation that the microenvironment is an important and potentially powerful source of clinical information to predict patient outcome, and demonstrate specifically that stromal Cav-1 may be a valuable clinical marker. Determining whether stromal Cav-1 functions to directly suppress tumor growth and the factors which regulate its expression may also reveal novel therapeutic avenues and help unveil who is watching the vigilant watchman.

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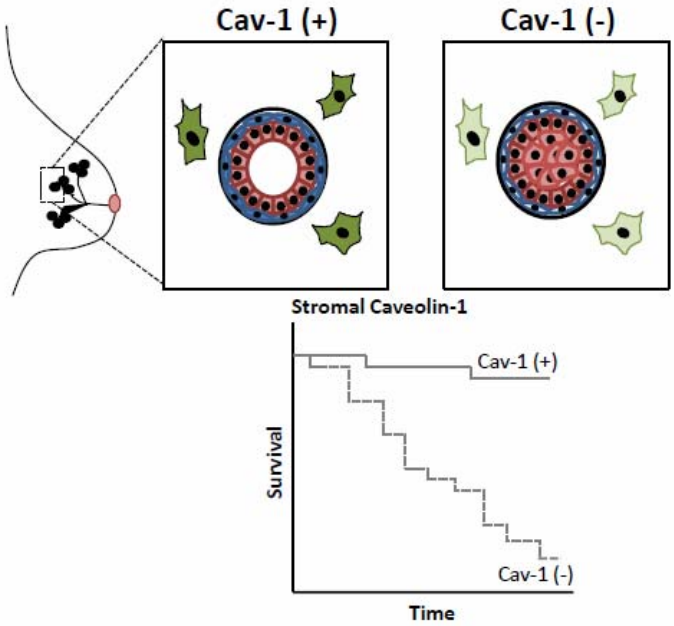
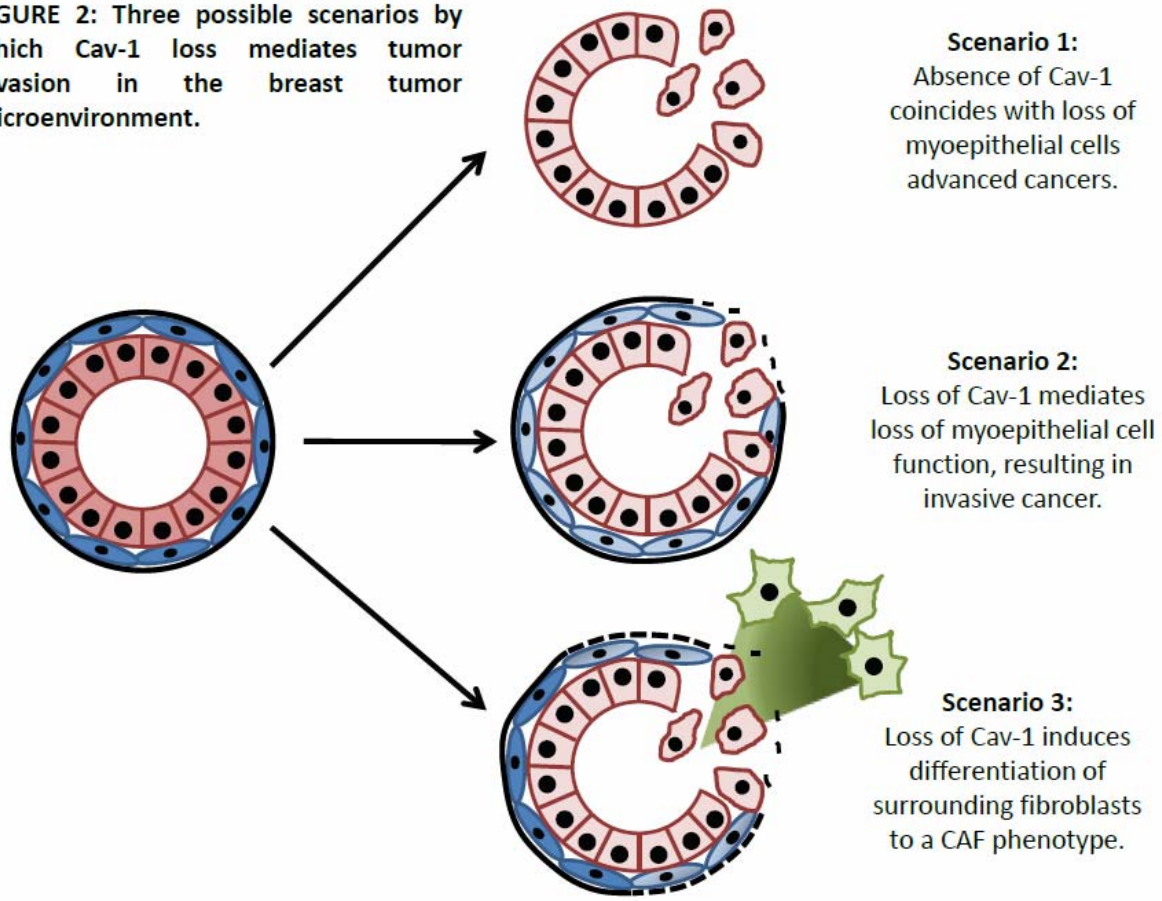
Figure Legend.

Figure 1: Stromal Caveolin-1 (Cav-1) expression predicts breast cancer patient outcome. In this issue of *The American Journal of Pathology*, Sloan et al. and Witkiewicz, Dasgupta et al. show that an absence of staining for the structural protein Cav-1 in the breast tumor microenvironment (reflected by reduced shading of myoepithelial cells, blue, and fibroblasts, green) is predictive of poor clinical outcome for breast cancer patients. Importantly, Cav-1 expression in the tumor epithelium does not correlate with patient outcome.

Figure 2: Three possible scenarios by which Cav-1 loss mediates tumor invasion in the breast tumor microenvironment. *Left*: Schematic view of a cross-sectioned normal mammary duct. An inner layer of luminal epithelial cells (red) is surrounded basally by myoepithelial cells (blue) and basement membrane (black). *Right*: Loss of Cav-1 could coincide with or result in 3 distinct scenarios. **Scenario 1**: Absence of Cav-1 coincides with loss of myoepithelial cells (MEPs). MEPs are more often found in benign breast lesions than in advanced carcinomas [21]. Since Cav-1 is expressed by MEPs, MEP loss would be reflected by an absence of Cav-1 staining. **Scenario 2**: Loss of Cav-1 mediates loss of MEP function, resulting in invasive ductal carcinoma. Cancer-associated MEPs behave distinctly from normal MEPs, which function as tumor suppressors [16]. Loss of Cav-1 may directly alter the secretion profile of MEPs such that they are unable to regulate architecture, ultimately resulting in tumor invasion. **Scenario 3**: Loss of Cav-1 induces differentiation of surrounding fibroblasts to a carcinoma-associated fibroblast (CAF) phenotype. Normal breast fibroblasts express Cav-1 [10, 11]. Loss of Cav-1 in fibroblasts could directly initiate their transition to CAFs (green), which secrete a variety of factors to promote invasion and possibly inhibit the production of Cav-1 in other cell types (e.g., MEPs), thereby further promoting invasion by the means described in Scenario 2.

FIGURE 1: Stromal Caveolin-1 (Cav-1) expression predicts breast cancer patient outcome.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