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Changes in cell and tissue organization in cancer of the breast and colon

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Most cancers arise in epithelia, the tissue type that lines all body cavities. The organization of epithelia enables them to act as a barrier and perform vectorial transport of molecules between body compartments. Crucial for their organization and function is a highly specialized network of cell adhesion and polarity proteins aligned along the apical–basal axis. Comparing breast and intestinal tissue as examples of common cancer sites, reveals an important contribution of polarity proteins to the initiation and progression of cancer. Defects in polarity are induced directly by mutations in polarity proteins, but also indirectly by changes in the expression of specific microRNAs and altered transcriptional programs that drive cellular differentiation from epithelial to more mesenchymal characteristics. The latter is particularly important in the metastatic process.

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Introduction/background

Epithelial tissues act as boundaries between the body and the outside world. They form the external surface of the body and line the cavities of most internal organs. Epithelia also constitute much of the tissue in glands where epithelial cells have evolved a specialized capacity for polarized secretion of fluids. Epithelial tissue is also the most common site for the development cancers. Carcinomas arise from epithelial tissue and account for as many as 90 percent of all human cancers. Two of the most common cancers in humans occur in breast and colonic epithelium. Carcinomas are characterized by changes in normal cell and tissue organization of epithelia and their progression is usually accompanied by increasing disorganization.

In this review we will compare and contrast the general features of the normal organization of the epithelium in the breast (or mammary gland) and intestinal tract and describe recent studies that have furthered our understanding of how this organization changes in cancer.

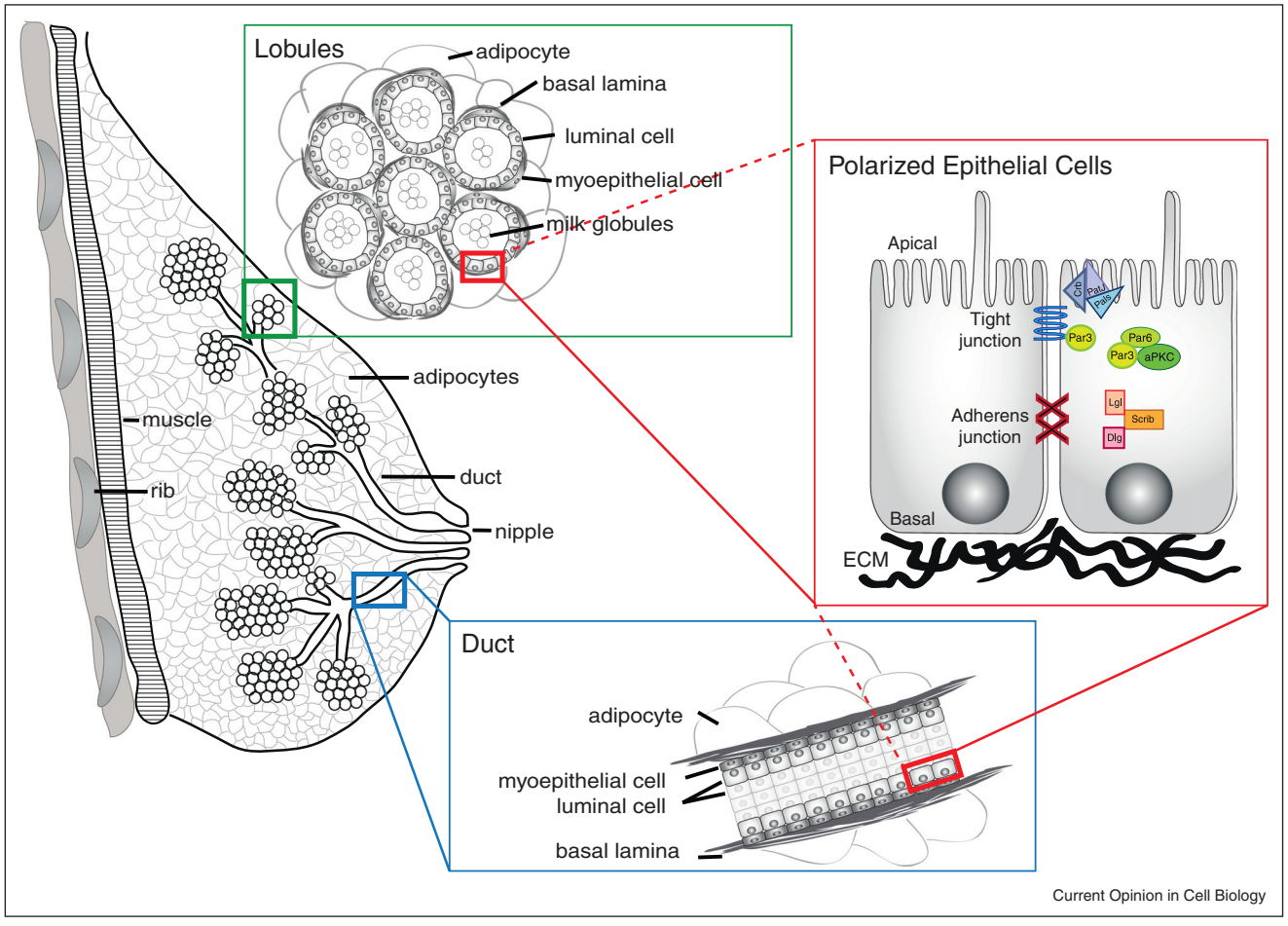
Normal epithelial organization

The organization of the breast and the intestinal tract is exquisitely well suited to perform the functions of these tissues. The main function of the breast is production and secretion of milk during lactation whereas, in contrast, the intestine absorbs nutrients and packages waste products for excretion (Figures 1 and 2). In both tissues, these roles require polarized epithelia and both the inner luminal epithelial layer of the breast and the epithelial layer of the intestinal tract are composed of tightly connected cells, held together by specialized adherens junctions formed by cadherins, which connect cells via homotypic interactions between their extracellular domains (Figures 1 and 2) [1]. Tight junctions form above adherens junctions towards the luminal surface and physically separate the apical membrane of cells, facing the lumen, from the basolateral membrane that interfaces with the interior of the body. This polarity of cellular organization allows vectorial transport across the epithelial layer, secretion to the outside surface of the body in breast and absorption into the body in the intestine. Integrins and other transmembrane proteins on the basal surface physically anchor epithelial cells to the underlying basement membrane, an organizing scaffold comprised of specific extracellular matrix (ECM) molecules that are produced by the epithelium itself and surrounding fibroblasts. The selective distribution of ion channels to either the apical or the basolateral domain is an integral part of this polarity and ensures correct ion transport and the associated flow of water.

Cancer in epithelia

In breast tissue, cancer arises predominantly from the luminal epithelial cells that line both the ducts and milk-producing lobules, and less frequently from the outer layer of basal cells. In the intestine and colon, cancers arise in the epithelium of the crypt [2,3]. The prevalence of epithelial cancers in general may be due to the frequency with which the cells in these tissues divide. For example, over a woman's lifetime the breast undergoes many (~450) cycles of growth and involution in response to hormonal cues during each menstrual cycle in addition to the dramatic changes that occur with pregnancy. In the intestine, epithelial cells are also continually turned over.

Figure 1



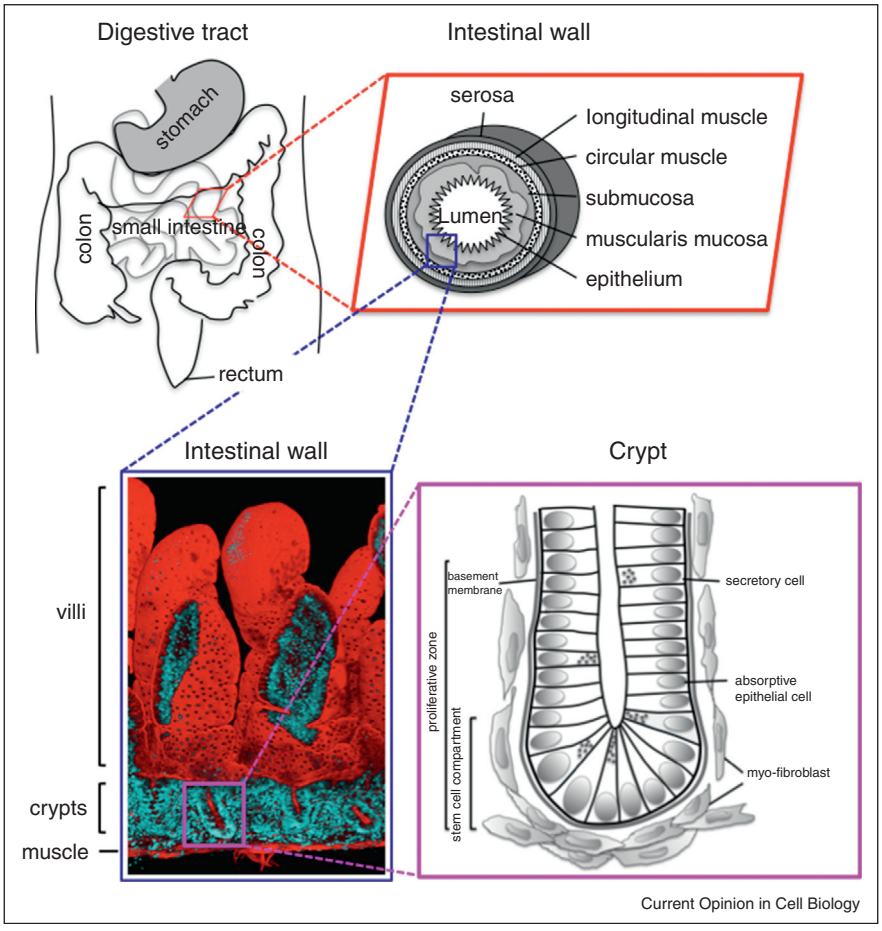
Schematized view of the tissue organization in breast with the features described in the introduction. Breast tissue overlies the ribs and chest muscles. The milk producing glandular epithelia of a woman's breast is contained within adipocyte tissue. A breast consists of 15–20 epithelial lobes, each develops numerous milk-producing lobules upon pregnancy (green inset). Each lobule and lobe is connected to the nipple via ducts (blue inset) that transport milk. The lobules and ducts consist of a bilayered epithelium comprising an inner layer of milk-producing luminal epithelial cells and an outer layer of myoepithelium that contracts to generate milk flow. Luminal epithelial cells are polarized containing apical domains that face the lumen and basolateral domains that interface with the interior of the body (red inset). Junctional proteins mediate adhesion between cells and are regulated by three sets of polarity regulators: Scribble (Scrib)/lethal giant larvae (Lgl)/discs large (Dlg) proteins; Crumbs/PALS/PATJ proteins; and the partitioning defective 3 (Par3)/Par6/atypical protein kinase C (aPKC) proteins. Fibroblasts and immune cells infiltrate the adipocyte tissue and assemble around the epithelium, which is surrounded by basal lamina (not shown).

The estimated 1.1 million crypts in the mouse intestine produce about 10 million cells per hour (about 5 billion in humans) and this is balanced by the loss of cells from the tissue [4]. Given these staggering numbers it is not surprising that, even with exceedingly low endogenous rates of mutations, tumorigenesis in both the breast and the intestine increases with age.

Abnormal, uncontrolled proliferation is one of the first signs of cancer, and is the consequence of genetic changes that confer on cells the ability to divide and survive in the absence of appropriate matrix anchorage. This allows rapid, unconstrained cellular expansion. In the breast epithelium this produces unpolarized cells no longer confined by their intercellular interactions. These cells have the capacity to invade the underlying stroma by either undergoing an epithelial to mesenchymal transition (EMT) and migrating individually, or staying bound in small clusters and migrating collectively through the basement membrane and connective tissue, eventually breaching blood and lymphatic vessels for metastasis to secondary sites (Figure 3).

In the intestine, increased proliferation and decreased differentiation are common features of early tumor stages. However, initially cells remain relatively well polarized and continue to form glandular structures similar to normal tissue. Only at later stages, when tissue organiz-

Figure 2



Schematized view of the tissue organization in intestine with the features described in the introduction. The intestine resides in the abdominal cavity and is essentially a long tube that connects the stomach to the rectum. It is divided into small and large intestine (colon). Both regions contain a number of different tissue layers (red inset). The outermost layer, the serosa, covers the intestine and is followed by two muscle layers that are perpendicular to each other. The outer longitudinal muscle layers run parallel to the intestinal axis and the inner, circumferential muscle layer circumnavigates the intestinal wall. The next layer is the sub-mucosa, which consists mostly ECM, contains blood and lymphatic vessels. The muscularis mucosa consists of myo-fibroblasts that reside directly underneath the basement membrane that underlies the epithelium lining the intestinal lumen (blue inset). The epithelium in the small intestine is folded into villi and adjacent crypts (purple inset) shown in a 200 μm thick vibratome section of human small intestine, stained with phalloidin and DAPI to reveal F-actin and nuclei respectively. In colon only crypts are present. Crypts contain proliferative stem cells at their base that produce the different cell types normally present in the epithelium including secretory and absorptive epithelial cells. The epithelium in each crypt is surrounded by myo-fibroblasts.

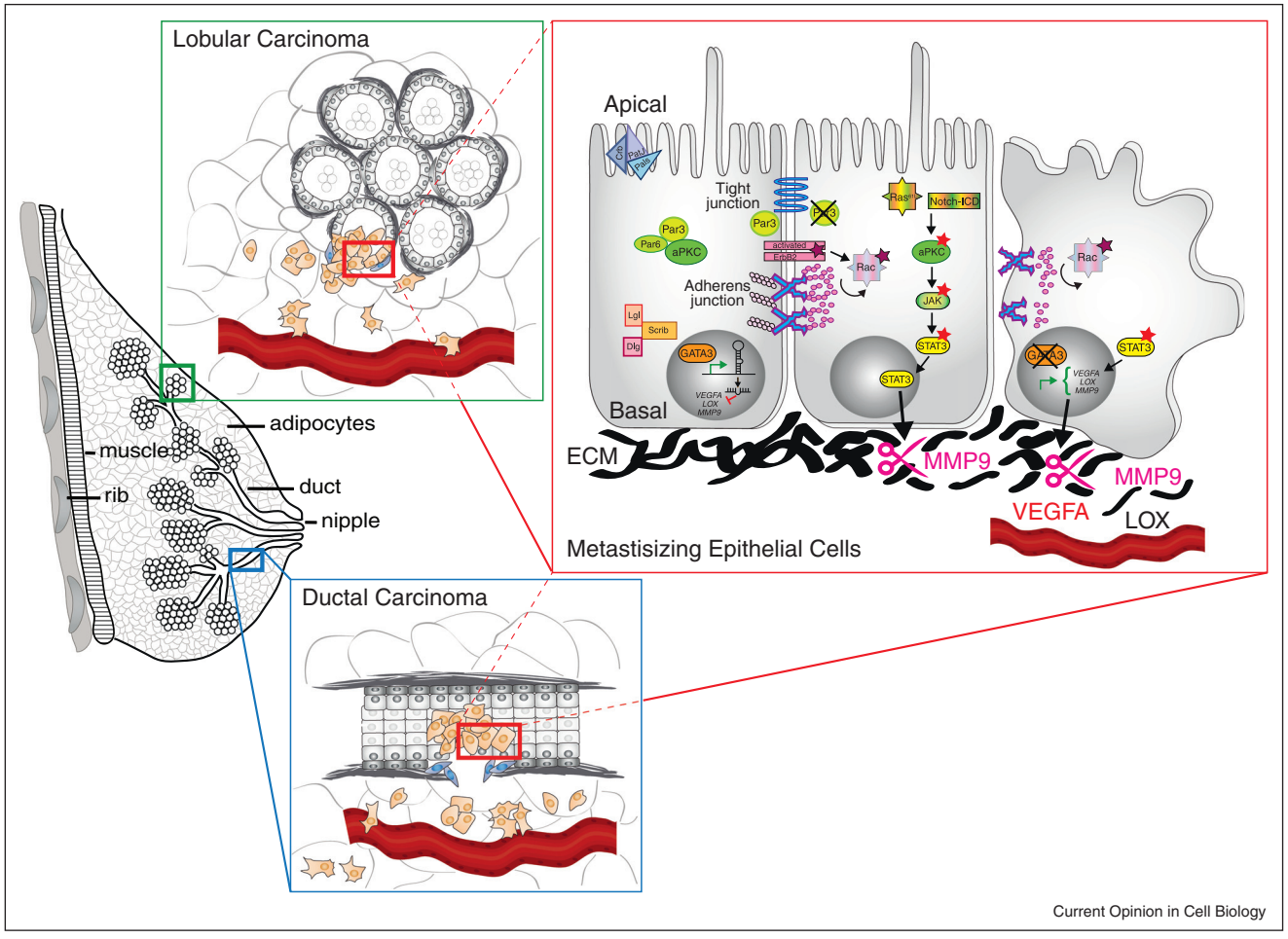
ation is more aberrant is cellular polarity lost and cells invade the surrounding stroma (Figures 2 and 4). Important for the spread of all cancers is the microenvironment, which evolves with the growing tumor and plays a crucial role in promoting this invasion by supplying growth factors, chemokines and even migratory cues to newly forming blood vessels.

This review focuses on recent developments in our understanding of four key changes in cell and tissue architecture during epithelial tumorigenesis: firstly, loss of cell polarity; secondly, collective cell migration; thirdly, EMT; fourthly, cell/stromal interactions and extracellular matrix (ECD) remodeling. Because most tumors originate in epithelial cells, we focus on these cells and do not include discussions about immune cells, which infiltrate most tumors and play an important part by contributing significantly to the microenvironment [5,6]. Our increasingly sophisticated knowledge of the complex changes that accompany tumor initiation and growth are ushering in exciting times and we are currently witnessing the tangible benefits basic research has brought to society as this knowledge is translated into molecule-based diagnostics and therapies.

Changes in polarity in cancer

Loss of apical–basal polarity is one of the hallmarks of epithelial cancers and it occurs in the early stages of tumor

Figure 3

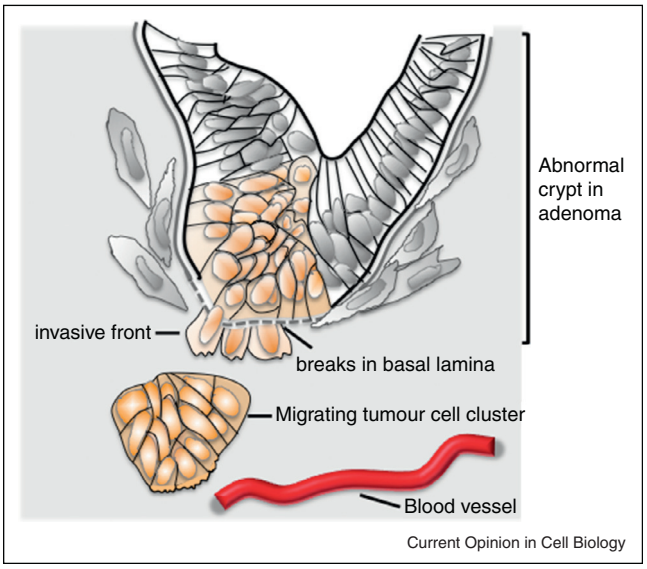


Relationship between cellular and tissue changes in breast cancer. Invasive lobular carcinoma (ILC) (green inset) originates in the luminal cells of milk-producing lobules. These cells will proliferate uncontrollably (tan cells) and eventually penetrate through the myoepithelial cells (blue cells) and basal lamina to gain access to blood vessels, allowing tumor cells to metastasize to other parts of the body singly and in clumps. Infiltrating ductal carcinoma (IDC) (blue inset) originates in the luminal epithelial cells of the milk ducts and has the same capacity as ILC to spread to other parts of the body. Cancer cells undergo numerous changes, eventually losing their polarized orientation and their contact with other cells to metastasize (red box). Recent studies show that two key changes are the loss of PAR3 and GATA3, leading to changes in the microenvironment surrounding the tumor, including increased angiogenesis.

progression in breast tissue, but later in colorectal cancer where it correlates with the appearance of invasive migratory cells at the tumor margin and the appearance of tumor cell islands [7]. Polarity is established in mammalian epithelia by the coordinated actions of three sets of proteins referred to as polarity regulators: the Scribble (Scrib)/lethal giant larvae (Lgl)/discs large (Dlg) proteins that establish and maintain the basolateral membranes; the Crumbs/PALS/PATJ proteins that regulate apical membrane biogenesis and maintenance; and the partitioning defective 3 (Par3)/Par6/atypical protein kinase C (aPKC) protein complexes that mediate tight junction formation and regulate their function at the apical-basal border (Figure 1) [8]. The key role these protein complexes play in creating and maintaining proper cell polarity suggest that the reverse may also be true, and that their disruption could play similarly pivotal roles in the loss of cell polarity observed during tumor progression. Yet, how these protein complexes participate in cell transformation and whether loss of cell polarity is causal to human cancer are questions that are still under active investigation.

In the past year, for example, two papers using breast as a model system have demonstrated a tumor suppressive function for the polarity regulator, Par3. Loss of Par3 in the context of oncogene activation resulted in decreased cell adhesion and increased tumor cell invasion and metastasis. Overexpression of three different oncogenic stimuli was used to drive transformation: the Notch

Figure 4



Relationship between cellular and tissue changes in colon cancer. Intestinal tumors maintain many aspects of the normal glandular organization with polarized epithelia. At the invasive tumor margin, cells lose their normal polarized organization and become migratory (orange). Although individual cells can be observed migrating from tumors, islands of dissociated tumor cells are frequently observed near tumor margins, as is increased infiltration by lymphocytes (not shown) [7,44].

intracellular domain (ICD); an activated form of Ras, H-Ras^{61L}; and a mutant form of Neu/ErbB2 (NDL) that promotes constitutive receptor dimerization [9••,10••]. Overexpression of the Notch ICD and mutant NDL, alone, only increased cell proliferation and caused hyperplasia, but was insufficient to generate tumor cell dissemination. Similarly, on its own, the loss of Par-3 only disrupted cell polarity and caused dysplastic cell growth. It was only when Par3 was silenced in the context of oncogene activation that increased cell invasion and tumor metastasis was observed. However, two different downstream mechanisms were identified. In the context of Notch ICD and Ras^{61L}, knock-down of Par3 activated aPKC, which in turn signaled through the JAK/STAT pathway to induce the expression of matrix metalloproteinase 9 (MMP9) and destruction of the ECM (Figure 3) [9••]. In the context of excessive Neu/ErbB2 activation, loss of Par-3 resulted in the inappropriate activation of Rac, which in turn promoted aberrant actin remodeling that led to a breakdown of adherens junctions, allowing cells to break free [10••]. Yet, even with these genetic changes, epithelial morphology was maintained by both the primary tumor and its metastases, and there was no evidence that the transformed cells underwent an epithelial to mesenchymal transition (EMT) commonly associated with metastasizing cells [9••]. Together, these studies show that the combined deregulation of both polarity and proliferation pathways significantly accelerates tumor growth and enables metastasis, demonstrating the importance of polarity in maintaining tissue homeostasis and governing its integrity. Loss of Par-3 frequently occurs in human breast cancer and is associated with a modest but statistically significant reduction in survival probability [9••].

In the intestine, mutations in the adenomatous polyposis coli (APC) gene are common to most tumors and they occur extremely early in tumorigenesis. Loss of both APC alleles is sufficient for tumorigenesis in this tissue [11]. Despite the extensive direct and indirect links between the APC protein and cell polarity proteins [12], cells in intestinal tissue or tissue explants that lack APC remain polarized [13]. The functional consequence of the interaction of APC with polarity proteins including Dlg and Scrib in tissue is not clear. In cultured cells, APC can modify adhesion and polarity by scaffolding polarity protein complexes and contributing to their assembly at correct locations in the cells [14,15]. However, the situation in tissue seems more robust and the relatively normal cellular polarization in APC mutant tissue and organoid cultures suggests that APC is not required to maintain cellular polarity and tissue barrier function. It is nonetheless possible that subtle changes in polarity complexes are already present in APC mutant tissue at early stages of tumorigenesis, but that they are not sufficient to cause physiologically measurable defects in cell polarity. The necessity to maintain cell and tissue polarity is particularly important in intestinal epithelium. The intestinal lumen with its high bacterial content together with the chemical byproducts of nutrient breakdown is a challenging environment. Exposing these contents to the body would create serious systemic infection and be detrimental to the organism. Thus, the threshold that has to be reached to lose cell polarity may be particularly high in intestinal tissue and it is likely to require a number of different mutations to produce loss of cellular polarity and loss of barrier function in this tissue. Consistent with this idea, tumors in mice harboring only an APC mutation are rarely invasive; however, they become invasive when other oncogenic mutations, for example in Ras or p53, are also present [16••].

Dissemination of cancer cells

Invasion of tumor cells into surrounding tissue and their dissemination to distant sites produces metastases and this represents the most challenging problem clinically. A great deal of recent attention has focused on the role of EMT in epithelial cell metastasis [17,18]. However, the studies on Par-3 discussed above highlight the fact that breast tumor cells may not undergo a complete EMT in order to disperse. Instead, clusters of epithelial cells are commonly observed by pathologists, moving through tissue and the blood stream as physically and functionally connected group of cells in a process termed collective

cell migration [19]. These cells display some of the characteristics of EMT including alterations in apicobasal polarity, the ability to modify the extracellular matrix and the capacity to invade, but nevertheless they remain in aggregates. The significance of such clusters was demonstrated over 40 years ago in studies showing more efficient formation of lung metastases after injecting aggregates of mammary tumor cells into animals compared to a similar number of dissociated cells [20]. This early work in animals has been reproduced over the years, including recent studies using high definition, automated microscopy on patient tissue [21,22] or microfluidic capture and analysis of circulating tumor cells from blood samples [23,24••]. The latter study revealed that the clustered cells are not necessarily epithelial, but can be groups of mesenchymal cells that had either proliferated from a single cell, which had undergone an EMT, or had been transformed en masse from epithelial clusters during the process of metastasis [24••]. Thus, to successfully achieve the inherently difficult and inefficient process of dissemination, current data indicate that tumor cells use multiple strategies and consequently invade both as single cells and in clusters, with and without having undergone EMT. The similarities between collective migration and EMT suggest that epithelial and mesenchymal cell states may not always be uniquely represented. Instead, cells may express a range of epithelial and mesenchymal characteristics, driven by regulatory programs such as alternative splicing and epigenetic changes [25,26••], resulting in flexible functionality and form that enables cells to adapt to a wide variety of environments in a dynamic manner as they propagate and spread. The idea that partial EMT within a tumor tissue provides cells with the flexibility required to respond to different environments and permits their dissociation from the tumor is also supported by findings in the intestine, where a subset of EMT markers can be detected even in early adenoma in the intestine despite their epithelial appearance [27]. The partial transition to a more mesenchymal state in early APC mutant tumors may be a consequence of transcriptional changes induced by APC mutations to alter differentiation [27], and may also be responsible for the changes in the number and distribution of different cell types within the epithelium when APC is mutated [13]. The invading margins of intestinal tumors carry many of the hallmarks of EMT suggesting that more complete EMT is associated particularly with invading margins of tumors.

Co-opting the microenvironment

Invading tumor cells migrate into an abnormal environment that has been profoundly remodeled by the developing carcinoma. In the breast, these extracellular changes can be observed as early as the ductal carcinoma *in situ* (DCIS) stage when hyperactive mitogenic signaling in epithelial cells results in secretion of chemokines that attract leukocytes, fibroblasts, endothelial and other cells. These marauding cells accumulate in the stroma surrounding the ducts and lobules where they remodel the ECM and promote tumor growth by participating in reciprocal signaling loops [5]. Many paracrine signaling loops that regulate the microenvironment are well described, and recent research has focused on identifying master regulators that control multiple aspects of the invasive phenotype by regulating microRNAs (miRNAs). While numerous miRNAs have been shown to control EMT during breast tumor progression [28], recently miRNAs have been identified that govern multiple aspects of metastasis, including microenvironmental remodeling, in addition to epithelial plasticity. For example, transcription factor, GATA3, specifies and maintains luminal epithelial cell differentiation in the breast and its loss during tumor progression is associated with poor prognosis [29]. GATA3 promotes luminal differentiation through miR-29b that suppresses the expression of a suite of genes encoding proteins such as vascular endothelial growth factor A (VEGFA), lysyl oxidase (LOX) and MMP9, all of which promote the metastasis of breast tumors to the lung by stimulating angiogenesis, ECM signaling and proteolysis, respectively [30]. The loss of GATA3, which occurs as tumor progress, releases the inhibition provided by miR-29b, leading to the expression of this suite of pro-metastatic proteins (Figure 3). Similarly, miR-148b is downregulated in aggressive breast tumors and has been found to be a major coordinator of malignancy by regulating over 130 genes involved in epithelial cell motility and stromal cell proliferation [31]. Taken together, these studies show that the process of tumor metastasis, previously considered a series of individually regulated events, may actually be under the master regulation of miRNAs that govern many aspects of tumor progression.

Coopting the micro-environment for their oncogenic purposes is also part of the invasive process in intestinal tumors. Changes in the expression of metalloproteases are at least partially mediated by alterations in transcription that result from loss of APC and consequent increase in β-catenin, and so contribute to the ability to tumor cells at the tumor margin to modulate their environment and facilitate their dissemination [32•,33]. In addition, changes in a host of miRNAs have been detected in human tumors and many of them promote EMT [34]. For example, downregulation of the miRNA-200 family, particularly at the invasive front of colorectal cancers, correlates with the breakdown of basement membranes [35]. The epigenetic silencing that causes reduction in miRNA-200 is not a static process and depends on the cellular micro-environment [36]. Similarly, loss of miRNA-212 via hypermethylation or loss of heterozygosity may be able to stimulate migration and invasion by inducing EMT markers [37••]. The likely target for miRNA-212 is manganese superoxide dismutase (MnSOD), and in its absence, MnSOD levels rise to

increase the influx of H_2O_2. This, in turn, causes increases in hypoxia inducible factor 1-α (HIF-1α) and stimulates EMT leading to metastasis [37••]. A rise in HIF-1α is also predicted to suppress the expression of APC reinforcing transformation further [38•].

The contribution of mechanics to cancer cell spread

Once tumor cells dissociate from the tumor they have to employ a host of different tools to navigate through the extracellular matrix, enter and exit blood or lymphatic vessels and reach distant sites. In addition to the biochemical modifications that facilitate processes involved in these steps as described above, the mechanical properties of both the tumor cell and its environment also play a role. For instance, migration of cells through their surrounding matrix is limited by the pore size of the matrix [39••]. Below a certain pore size, cells have to remodel the matrix before they can migrate efficiently. The mechanical properties of the cell and importantly its nucleus dictate the threshold for the pore size that prevents cell movement without ECM remodeling such that cells with more pliable nuclei can migrate through smaller pores. The finding that cells become softer (more easily deformable) as they become more metastatic is consistent with the idea that metastatic cells migrate more easily through dense ECM [40•]. Conversely, the mechanical properties of the ECM have a profound effect on the ability of cells to migrate through it. Cells with mesenchymal characteristics are more responsive to this effect and they migrate more efficiently through stiffer ECM material than cells with epithelial characteristics, particularly in three-dimensional matrices [41•,42••].

Understanding the relationship between biochemical and mechanical changes that accompany tumor initiation and progression holds great promise as diagnostic tools. This will be greatly aided by the development of new technical and computational tools to measure and understand the mechanical properties of cells and tissues and their dynamics. Diagnostic tools that measure mechanical properties of tissue are already in use to detect and classify breast tumors [43]. The ability to link such measurements with more detailed information about cell and tissue architecture and the underlying molecular changes holds great promise not only for diagnostic purposes but will also aid greatly in revealing how results obtained in cultured cells relate to situations *in vivo*.

Concluding remarks

Our understanding of cancer, particularly the cellular changes that accompany cancer progression, has increased phenomenally over the last 50 years. Together with immense technological advances this has enabled much improved detection and treatment of some cancers for instance breast cancer. In this case, early detection and molecular markers that allow predictions about therapeutic response have improved patient outcome enormously. Nonetheless, for other cancers, like colorectal cancers, despite a vast amount of molecular and genetic insights gained, our ability to stratify patients to provide treatments that are most likely to be successful or identify patients that are most likely to have recurring tumors remains limited. Having available organ-specific tissue models that are amenable to genetic and biochemical manipulation provides means to integrate results from existing cell biological research with more physiologically complex systems. This may reveal new approaches for how best to capitalize on the detailed insights available about the mechanisms that govern cell polarity.

Acknowledgements

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- •• of outstanding interest

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This paper sheds light on how loss of p53 enhances tumourigenesis caused by mutations that activated Wnt signaling and also how inactivating p53 can contribute to EMT. The data show that the p53 transcriptionally activated miRNA-34 directly represses β-catenin and a subset of Wnt target genes. Loss of the transcriptional activity of p53 increases the invasiveness of cells and the relationship between p53, miRNA-34 and metastatic potential is corroborated by finding in human tumours.

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Using microfluidic herringbone–chip to capture circulating tumor cells (CTCs) from blood with an antibody cocktail, the authors of this paper examined samples from patients with metastatic breast cancer and observe EMT features that vary according to histological subtype. They also noted that clusters of CTCs were more likely to be characterized by the expression signature of active TGF-beta signaling and attached to platelets that may be the source of TGF-beta. These features of clustered CTCs may explain why they were also more likely to express mesenchymal markers as TGF-beta has been shown to induce EMT.

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