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Review

Cancer Research

Cell Membrane Fluid–Mosaic Structure and Cancer Metastasis

Garth L. Nicolson

Abstract

Cancer cells are surrounded by a fluid–mosaic membrane that provides a highly dynamic structural barrier with the microenvironment, communication filter and transport, receptor and enzyme platform. This structure forms because of the physical properties of its constituents, which can move laterally and selectively within the membrane plane and associate with similar or different constituents, forming specific, functional domains. Over the years, data have accumulated on the amounts, structures, and mobilities of membrane constituents after transformation and during progression and metastasis. More recent information has shown the importance of specialized membrane domains, such as lipid rafts, protein–lipid complexes, receptor complexes, invadopodia, and other cellular structures in the malignant process. In describing the macrostructure and dynamics of plasma membranes, membrane-associated cytoskeletal structures and extracellular matrix are also important, constraining the motion of membrane components and acting as traction points for cell motility. These associations may be altered in malignant cells, and probably also in surrounding normal cells, promoting invasion and metastatic colonization. In addition, components can be released from cells as secretory molecules, enzymes, receptors, large macromolecular complexes, membrane vesicles, and exosomes that can modify the microenvironment, provide specific cross-talk, and facilitate invasion, survival, and growth of malignant cells. *Cancer Res; 75(7); 1169–76. ©2015 AACR.*

Introduction

Cell membranes represent important cellular barriers and first-contact structures of normal and cancer cells. Extracellular signals from ions, hormones, cytokines, enzymes, growth and motility factors, receptors, extracellular matrix (ECM), other stromal elements, and subcellular membrane vesicles must first interact with the cell membrane to initiate signaling processes. Therefore, cell or plasma membranes are cellular filters that can selectively transmit signals and substances from outside cells and from adjacent cells into a cell's interior. Conversely, they can also release signals and molecules to other cells and the micro- and macro-environment in a complex process that has been termed "social cell biology" (1). In addition, cells are compartmentalized into organelles by various intracellular membrane structures that are responsible for biosynthesis, energy production, replication, transportation, recycling, destruction, secretion, and other cellular activities.

Cell membranes are intimately involved in the biochemical events that define cancers, and in particular, they are intensely involved in cancer metastasis (2). In addition, the establishment of metastases also requires a complex interplay between malignant cells, normal cells, stroma, and ECM in their new microenvironments, and these interactions are primarily mediated through cell membranes (3).

Department of Molecular Pathology, The Institute for Molecular Medicine, Huntington Beach, California.

Corresponding Author: Garth L. Nicolson, The Institute for Molecular Medicine, P.O. Box 9355, S. Laguna Beach, CA 92652. Phone: 949-715-5978; Fax: 714-596-3791; E-mail: gnicolson@immed.org

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Physical Properties of Cell Membranes

An important concept that maintains cell membrane structure is that amphipathic membrane components self-associate to exclude water interactions on their hydrophobic surfaces, whereas the hydrophilic portions of their structures interact with the aqueous environment (4). Thus, membrane glycerolphospholipids self-assemble to form lipid bilayers (5) due to the energy provided by the hydrophobic effect and van der Waals forces (6). Membrane integral globular proteins interact with membrane lipids through their acyl structures due to hydrophobic forces and much less to hydrophilic interactions between lipid head groups and protein hydrophilic amino acids (4, 6, 7).

Membrane proteins are operationally of three types: integral, peripheral, and membrane-associated (7). Integral proteins are globular and tightly bound to membranes by mainly hydrophobic forces and intercalated into the membrane lipid bilayer, whereas peripheral membrane proteins are bound to membranes by electrostatic or other forces. Peripheral membrane proteins can be removed from membranes without destroying basic membrane microstructure and are important in providing membrane attachment sites, scaffolding, tethering or membrane-supporting structures, membrane curvature-promoting components, and attachment points for soluble enzymes and signaling molecules (7, 8).

When membrane are distorted, deformed, compressed, or expanded, different forces and components react to the physical perturbations (9, 10). For example, certain peripheral membrane proteins can bind and cause deformation by forming crescent-shaped α-helical bundles that bind to membranes via electrostatic and some hydrophobic interactions (9), causing curvature by bending membranes to fit peripheral protein structure (9, 10).

The third class of membrane proteins is often isolated with cell membranes, but they are actually not membrane proteins (7).

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These are cytoskeletal and associated signaling structures at the inner cell membrane surface, and at the outer surface they include certain ECM components. These membrane-associated components are parts of dynamic structures involved in stabilizing membranes (and thus cells) and immobilizing membrane components. They are especially important in cellular activities, such as cell adhesion and motility, growth, endocytosis, exocytosis, signal transduction, and other important activities (11, 12).

The Fluid–Mosaic Model of Cell Membranes

First proposed in 1972, the fluid–mosaic membrane model (8) has proven its usefulness in describing basic nanoscale cell membrane structure for over 40 years (11). Although this model has been remarkably consistent with data collected on biologic membranes since 1925, it was inevitable that the original model could not explain aspects of membrane dynamics. For example, the concepts that membrane domains and cell membrane–associated structures are important in controlling the lateral mobilities and distributions of cell membrane proteins were not yet discovered (11–14).

Models of cell membrane structure produced a few years after the original model (8) were much less homogeneous (7). They contained additional information on protein and lipid aggregations and their segregation into membrane domains, cytoskeletal, and ECM interactions, among other features (7, 11). Nonetheless, in subsequent revisions of the fluid–mosaic model, all of the basic elements at the nanoscale level were retained (11–14). However, the arrangements of lipids and proteins into more compact structures and domains that maximized their mosaic nature along with the addition of dynamic hierarchical membrane organization produced a much more detailed description of its organization (Fig. 1A; refs. 11–14).

By sorting different membrane lipids and integral membrane proteins into specialized membrane domains based on their physical properties, Mouritsen and Bloom (15) proposed that such sorting was based primarily on hydrophobic interactions and some hydrophilic interactions. This prevents hydrophobic mismatches between lipids and proteins, thus preventing membrane distortions (15).

The fluid–mosaic membrane model also accounted for cell membrane asymmetry (8). Cell membranes are asymmetric in the display of their components (11, 16). The finding of asymmetric distributions of various lipids, proteins, and glycoproteins between the inner and outer leaflets of cell membranes is likely universal (11, 16, 17). Moreover, the disruption of membrane asymmetry in cell membranes is associated with cell activation, adhesion, aggregation, apoptosis, recognition by phagocytic cells, among other events. Of note, it is also associated with pathologic processes (17, 18).

Cytoskeletal- and ECM–Cell Membrane Interactions

What the original fluid–mosaic membrane model lacked was the integration of this structure with other cellular elements (7, 11). Cytoskeletal and ECM interactions are known to alter cell membrane macrostructure by restrictions in the freedom of movement (lateral mobility) of membrane proteins and also causing global movements of these and other components by tethering them to cellular or extracellular structures (7, 11, 12). This process can result in endocytosis of some macromolecular complexes at the cell surface. Receptor clustering, domain formation, submembrane plaque assembly, internalization, acidification of the resulting endosomes, degradation, and membrane recycling are all part of normal membrane recycling (19, 20). The mobility of integral membrane components can also be controlled by cell–cell and cell–ECM interactions (21).

Cell adhesion and receptor complexes that are immobilized by ECM or various interactions are capable of communicating signals that are transmitted through a dynamically assembled cytoskeleton or generating mechanical forces that can move cells or resist exterior mechanical stresses (22). This serial assembly of specialized components (ECM, integral membrane proteins, peripheral membrane proteins, adaptor proteins, cytoskeletal elements, among others) may have evolved to convert biochemical signals into mechanical forces that are important in cellular behavior. Although many of the membrane peripheral proteins have been identified as components involved in cytoskeletal interactions with membranes (23), membrane lipids are also important in these interactions as specialized lipid domains or "lipid rafts" (20, 24).

Membrane domains are dynamic structures that can be generated by ligand or ion binding, hydrophobic interactions (or other events) and can assemble into complex transmembrane superstructures. These complexes recruit additional peripheral proteins at the inner cell membrane surface to form transmembrane plaques that are competent for initiating cellular signaling via enzymatic processes or undergoing further attachment to cytoskeletal elements (3, 22, 25, 26).

Cell membranes should be considered completely integrated mechanostructures within tissues. They continuously interact with and link various intracellular structures to components outside the cell while receiving signals and contacts from the microenvironment and passing these signals on to elicit appropriate cellular responses. They also send out messages, maintain cell polarity, and mechanical properties while undergoing constant turnover of their constituent components. Thus, the basic structure of cell membranes has evolved from the original homogeneous concept to one that contains specific "domains" of varying sizes that form specific membrane regulatory and mechanical structures that are linked to other intra- and extracellular structures (11, 12) that are involved in many cellular properties characteristic of normal and cancer cells (3, 11).

Cell Membrane Lipid and Protein Interactions

In addition to their asymmetric distributions across the membrane, membrane lipids are also unevenly distributed in the membrane plane (5, 27, 28). Cholesterol is particularly important in cell membrane organization and is often found in specific membrane domains (27–29). This is thought to be due, in part, to cholesterol's affinity for both the fluid and solid phases of membranes (29). Cholesterol partitions into liquid-ordered/-disordered phases to roughly the same extent and changes the properties of the lipid phases (30). Sphingolipids are also important in the formation of ordered membrane lipid domains (31). Sphingomyelins and phosphatidylcholines constitute more than one half of plasma membrane phospholipids and form the main partners for cholesterol (32). Indeed, sphingomyelins and cholesterol are critically important in formation of small, ordered

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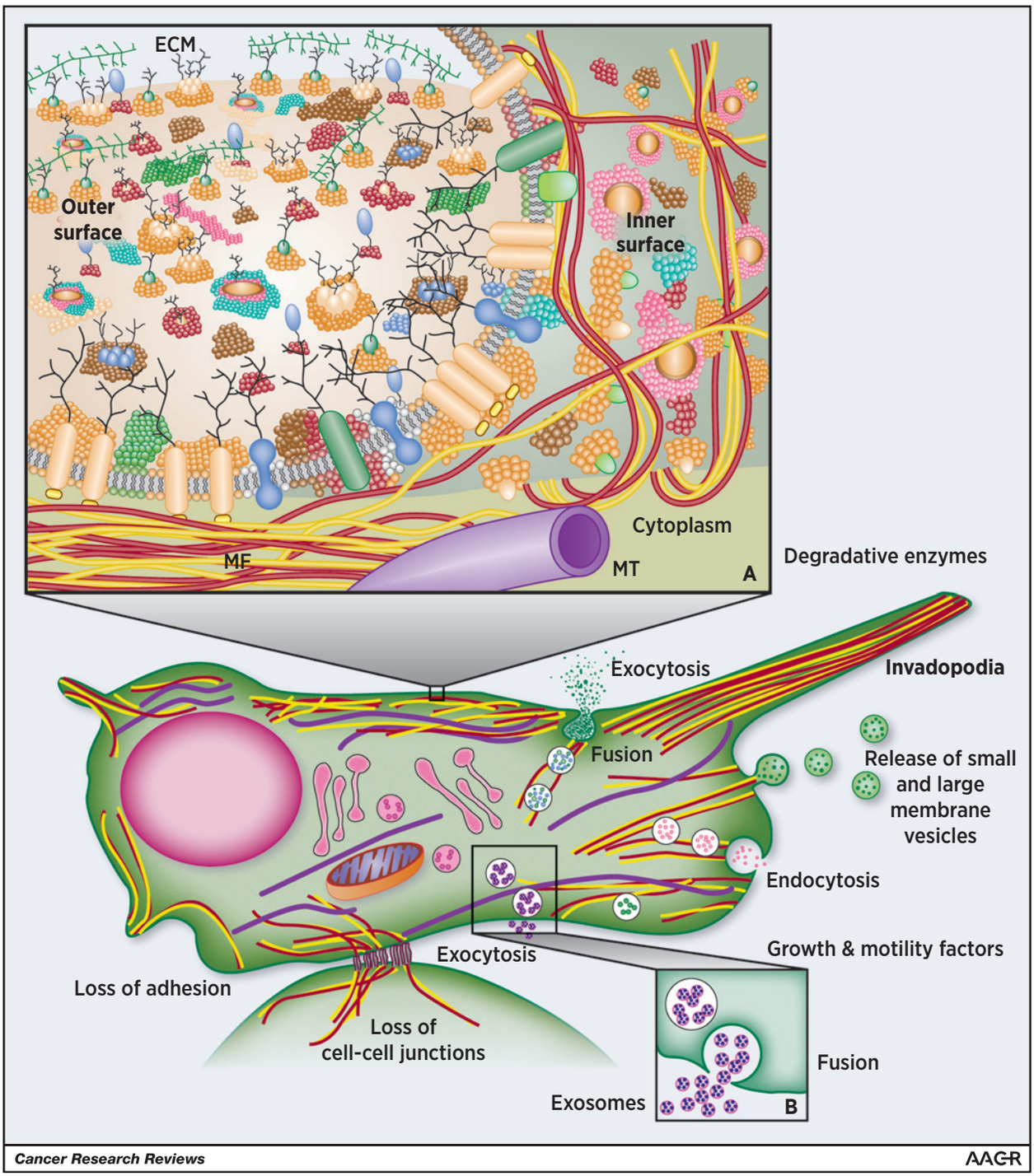

Figure 1.
A hypothetical cancer cell undergoing change to an invasive phenotype and beginning the process of invasion. A, a representation of the cell membrane that contains membrane domain structures and membrane-associated cytoskeletal and extracellular structures. The cell membrane has been peeled back at the right to reveal the bottom membrane surface and membrane-associated cytoskeletal elements that form barriers (corrals) that limit the lateral motions of some of the integral membrane proteins. In addition, membrane-associated cytoskeletal structures are indirectly interacting with integral membrane proteins at the inner membrane surface along with matrix or ECM components at the outer surface. Although this diagram presents possible mechanisms of integral membrane protein mobility restraint, it does not accurately represent the sizes and structures of integral membrane proteins, lipid domains, or membrane-associated cytoskeletal structures. B, exosomes or intracellular membrane vesicles are released from larger vesicles that fuse with the cell membrane. MF, microfilaments; MT, microtubule. Reprinted from BBA Biomembranes, 1838 (6), Nicolson GL, The Fluid–Mosaic Model of Membrane Structure: Still relevant to understanding the structure, function and dynamics of biological membranes after more than 40 years, 1451–1456, Copyright 2013, with permission from Elsevier.

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lipid domains ("lipid rafts") that are generally surrounded by liquid-phase lipids (32, 33).

The formation of lipid rafts is a dynamic and reversible process that confers functional signaling properties to cell membranes. As mentioned, lipid rafts are characterized by enrichments of cholesterol and sphingolipids, which are held together by hydrogen bonds, charge pairing, and hydrophobic and van der Waals forces (32–34). Their constituents can quickly exchange with bulk membrane lipids as well as with lipids in other rafts (35). Lipid rafts are generally nanosized (<300 nm diameter, most ~10–200 nm) domains that can contain integral and peripheral membrane proteins. The proteins sequestered into membrane rafts can turn these domains into functional signal transduction structures important in endocytosis, cell death regulation, and other events relevant to cancer therapy (34, 36).

Integral membrane proteins can interact within different membrane domains, but they must also interact with membrane lipids to produce an intact plasma membrane (8, 11, 15, 37). Specifically, portions of their structures must directly pair with the acyl chains of membrane phospholipids or the hydrophobic portions of other membrane lipids. This is accomplished by hydrophobic matching (15, 29, 37). The concept of hydrophobic matching between the hydrophobic core of the lipid bilayer and hydrophobic stretches of amino acids in integral membrane proteins is essential for understanding how cell membranes form a stable structure (11, 29, 38). If the hydrophobic portions of this structure are mismatched, an elastic distortion of the lipid matrix around the integral membrane protein occurs (15, 29, 37). This can produce protein conformational changes, potentially effecting protein function and protein–protein interactions, such as protein aggregation into membrane super-structures (37, 39). In addition, there are other physical forces, such as lateral pressure forces, lateral phase changes, membrane curvature, ionic interactions, among other forces, that must be considered to produce an overall tensionless membrane structure (11, 39).

Cell Membrane Restrictions on Mobility and Hierarchical Organization

Restrictions on the lateral movements of integral membrane proteins have been attributed to extracellular restrictions, such as ECM, the formation of specialized membrane domains (lipid rafts and larger lipid domains), large protein complexes, and peripheral membrane barriers at the inner membrane surface (Fig. 1A; refs. 11, 12). Jacobson and colleagues (13) have summarized the lateral movements of membrane proteins into distinct categories: (i) transient confinement by obstacle protein clusters (also called protein fenceposts or pickets); (ii) transient confinement into defined domains or corrals by a cytoskeletal meshwork; (iii) directed motion due to attachment to the cytoskeleton; and (iv) random diffusion in the fluid membrane. Therefore, the original description of integral membrane proteins freely diffusing in the membrane plane pertains to only one of these categories (8).

It is now believed that a substantial portion of integral membrane proteins is not capable of free lateral diffusion in the cell membrane; they are confined, at least transiently, to small membrane domains by barriers at the inner membrane surface (11–14). However, integral proteins can escape from one of these domains to an adjacent domain. They can even escape the domains altogether, unless they undergo aggregation and their size prevents extradomain movements. Consequently, the abilities of membrane proteins to move between adjacent domains may be related to their sizes, the sizes of the cytoplasmic barriers, and the complex interactions of these barriers with the cytoskeleton (14) and ECM (11).

The approximate areas of cell membrane receptor domains have been estimated to vary from 0.04 to 0.24 μm^2, and the approximate transit times of membrane receptors in these membrane domains can vary from 3 to 30 seconds (14, 40). Thus, cell membrane domains can range in diameter from 2 to 300 nm. For example, actin-containing cytoskeletal-fenced domains have been found in approximate diameters of 40 to 300 nm, lipid raft domains in the range of 2 to 20 nm, and dynamic integral membrane protein complexes in domains of 3 to 10 nm in diameter (14, 40). The presence of different types of cell membrane domains and the selective presence of membrane proteins in these domains suggest another level of membrane compositional complexity beyond the original fluid–mosaic membrane structure (11–14).

Cell membranes must quickly respond to intracellular and extracellular signals and other microenvironmental events. To do this rapidly, it may be more efficient to have receptors prepositioned in the cell membrane within signaling domains so that they can undergo rapid aggregation into supramolecular signaling structures (14). The partitioning of plasma membranes to limit the dynamics of their integral membrane protein components (at least part of the time) to cytoskeletal-fenced corrals, or tethering them directly or indirectly to membrane-associated cytoskeletal elements, can create relatively stable membrane domains of increased receptor densities (40).

Signal transduction, cell activation, identification, differentiation, and other complex membrane-initiated events may require the presence of enhanced receptor densities within specific membrane domains to selectively promote cellular signaling. Kusumi and colleagues (14, 40) have proposed that cell membranes possess hierarchical architectures that consist of a basic fluid–mosaic membrane plus various membrane micro- and nanosized domains or compartments defined by cytoskeletal fencing and protein fenceposts, lipid rafts and other lipid domains, oligomeric integral membrane lipoprotein domains, and other structures. This complex structure is depicted simplistically in Fig. 1A. Within the hierarchical structure (including membrane domains) protein components are limited in their diffusion rates to those that are five to 50 times slower than when the same components are free to diffuse laterally in the membrane without restraint (41).

Cell Membrane Fusion and Vesicle Transport

Cells package newly synthesized materials for transport to various organelles and to the cells' exterior by incorporating them into small membrane vesicles that are delivered to specific target membrane domains (42). This process is also used to rapidly repair damage to the plasma membrane and other cellular membranes (43). Invasive tumor cells use directed exocytosis and other pathways to release degradative enzymes, bioregulators, and other molecules into the microenvironment to facilitate invasion of ECM and tissue stroma and evade host defenses (Fig. 1;

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refs. 44–46). It is also a mechanism that is used to display newly synthesized receptors, enzymes, and other molecules on the exterior cell membrane surface and facilitate their turnover.

A critical event in the delivery of materials via small vesicles is membrane fusion (47). Membrane fusion events are also apparent at the cell level, for example, when adjacent cancer cells undergo fusion with other cancer cells or normal cells to produce aneuploid cells, complementation, and other characteristics found in progressive neoplastic cells (38, 48). Membrane fusion is obviously not a cancer-associated event; it occurs during many normal cellular processes, such as fertilization, myoblast formation, and bone homeostasis (38).

Directed vesicle transport and fusion inside cells depends to some degree on lipid composition, distribution, and acylation. For example, certain lipids, such as the sphingolipids and sterols found in lipid rafts, are concentrated in vesicles destined to fuse with the plasma membrane (42, 49, 50). Specific polyphosphoinositides with their tethered proteins may also help direct vesicles to particular membrane sites (51, 52). Membrane fusion is dependent on specialized membrane-binding fusion machinery composed of specific proteins (SNARE, SNAP, and SM proteins, among others) that pull adjacent membranes together to promote lipid bilayer fusion (53).

The assembly of fusogenic proteins at the cell membrane constitutes a specialized dynamic membrane microdomain called a porosome (54). In some normal cells, porosomes appear ultrastructurally as "pits" measuring 0.5 to 2 μm in diameter containing depressions of 100 to 180 nm (55). Porosomes are responsible for directing exocytosis to particular sites at the cell surface. This is important for directed cell migration and invasion of ECM and stroma as well as for the normal function of cells secreting necessary proteins, glycoproteins, enzymes, bioregulators, and other important molecules.

Cell Membranes and Invadopodia

To facilitate cell invasion, invading tumor cells can extend specialized actin-rich membrane protrusions called "invadopodia" that penetrate into surrounding ECM, stroma, and basement membranes (Fig. 1; refs. 56, 57). These specialized cell structures display and are associated with extracellular degradative enzymes and contain intracellular actin polymers and their regulators, such as cortactin, cofilin, N-WASP, Arp2/3, and facin (57–59). The comparable normal cell counterparts of invadopodia are called podosomes, and these structures are apparent in many normal cells under conditions of ECM and basement membrane invasion during embryogenesis, wound healing, inflammatory responses, and organ regeneration (58, 59). Differences found between invadopodia and podosomes are that they often differ in size, shape, density, and stability—notably, invadopodia are typically stable for much longer periods of time compared with podosomes (59).

Invadopodia are stimulated to form by various factors. These include growth factors, oncogenic transformation, epithelial–mesenchymal transition (EMT), hypoxia, adhesion receptors, chemokines, and degradative enzyme activity. They typically require initial attachment to ECM and linkage to cytoskeletal components to initiate formation (57–59). Beaty and Condeelis (60) have proposed that there are four stages (presumably after ECM attachment) involved in invadopodia formation: (i) assembly of a core actin (and accessory protein) structure, (ii) activation of associated kinases, assembly of actin polymers, recruitment of adhesion components, and their transmembrane linkage, (iii) actin polymerization and invadopodial elongation and stabilization, and (iv) microtuble and intermediate filament recruitment, elongation of the mature structure along with ECM degradation. Although there is some uncertainty as to the exact stage where adhesion receptors are important in invadopodia formation (initially or in the maturation, elongation stages; ref. 60), there is no doubt that adhesion is an important early element of the invadopodia process (56, 59, 61).

Invadopodia may require specialized cell membrane lipid domains to initiate their organization. Indeed, specialized lipid rafts have been found at the sites of invadopodia formation, and similar rafts are known to be involved in membrane vesicle trafficking, exocytosis, and actin polymerization at the cell membrane inner surface (62). Thus, membrane microdomain formation is an important aspect of the formation of invadopodia and podosomes and probably other processes that require directed membrane distortions, adhesion, vesicle fusion, matrix degradation, and other processes.

For cell invasion to occur, invading cells must have the ability to degrade matrix barriers and migrate along invasion pathways generated by ECM restructuring and destruction. Thus, ECM degradation is an important step in the metastatic process (44, 61–64). Various ECM-degrading enzymes are associated with isolated invadopodia (56, 59), and degradative enzymes appear to be released by exocytosis near sites of invadopodia (61, 65). In addition, cell membranes at invadopodia sites appear to bind to and mechanically orient loosened matrix components parallel to cell surfaces to assist in mechanical force generation into ECM tunnels (61, 65). Therefore, cell membranes, degradative enzymes, and invadopodia can mechanically and enzymatically restructure ECM to facilitate cell movement and invasion by reshaping ECM into tube-like structures in which cancer cells can invade. Eventually collective cell movements can form into a massive multicell invasive structure that penetrates along the tubes generated by invadopodia and then single cells (65).

Cell Membranes, Extracellular Vesicles, and Exosomes

Tumor cells naturally release small (0.1–2 μm diameter) extracellular membrane vesicles (EV) derived from budding plasma membranes and separately even smaller (<100-nm diameter) microvesicles called exosomes from exocytosed intracellular vesicles (Fig. 1; refs. 66, 67). The released membrane vesicles can contain various molecules, such as small fragments of DNAs, microRNAs, proteins (enzymes, biomodulators, and receptors), and carbohydrates (67–69). Along with their enriched plasma membrane components and various cell receptors, they can mediate a form of communication by transfer between tumor cells, resulting in exchange of cellular materials. They can also mediate communication between tumor and normal cells in the microenvironment (67–69). This is not a unique property of tumor cells—vesicles released from normal cells are found in virtually every extracellular fluid where they appear to play a role in normal cell communication and regulation of inflammation, coagulation, development, and other normal physiologic processes (69, 70). In various cancers, they can also affect tumor interactions with the microenvironment and promote progression, angiogenesis, invasion, and metastasis (69, 70–72).

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Various factors can influence the release of EV and exosomes. Among these are cellular energy, intracellular levels of calcium, changes in membrane phospholipids, and other regulators of cytoskeleton-membrane interactions, membrane-acting enzymes, and other effectors of exocytosis, hypoxia, and oxidative and shear stresses (67, 71). Release of EV and exosomes are also affected by invadopodia, which can enhance their release and drive tumor invasive behavior (72). Released EV and exosomes are quite heterogeneous in their composition and reflect cell-to-cell variations in the cargos, membranes, and cells from which they are derived (70–73). Of significance is that most normal and benign cells do not release large quantities of EV and exosomes until they progress to invasive, metastatic phenotypes (73).

Tumor cell–released EV and exosomes can have profound effects on tumor microenvironment (70–72). In addition to their delivery of nucleic acids, bioactive phospholipids, degradative enzymes, receptors, growth and motility factors, and other cargo that can affect invasion and metastatic colonization, these vesicles can also stimulate angiogenesis, stromal reactions, and release of paracrine and other bioactive molecules from normal surrounding cells that condition the tumor microenvironment (67, 70, 71, 73). For example, the observation that signaling microRNAs in microvesicles can enhance endothelial cell migration and promote angiogenesis indicates that these vesicles have important molecular communication properties (74). Shed tumor membrane vesicles can break down, releasing their contents to the extracellular environment, and this has important consequences for invasion and cell motility. An example is the EV-release of a tumor-derived membrane glycoprotein (EMMPRIN) that stimulates fibroblast release of matrix metalloproteinases that facilitate tumor invasion and metastasis (75). EV and exosomes can also be involved in recruiting lymphoid cells that stimulate tumor growth. For example, blocking exosome secretion by inhibiting small GTPases results in decreased tumor growth and lung metastasis in a breast cancer model by decreasing neutrophil-stimulated tumor cell proliferation (76).

Tumor cell–released EV and exosomes may also protect neoplastic cells from harmful chemicals such as chemotherapeutic drugs, oxidized phospholipids, irradiation, immune responses, and cell death signals (67, 77). Treating human and animal lung cancer cell lines with irradiation and hypoxia that do not stimulate apoptosis results in enhancing the release of EV that activate and chemoattract stromal and endothelial cells. Once activated with tumor-derived EV, the stromal cells then release several pro-angiogenic factors. When this stromal cell–conditioned media were used to stimulate tumor cells, the result was enhanced metastatic potential *in vivo* (78).

Recently, the use of EV and exosomes for modulating the microenvironment or producing new therapeutics for targeting of specific bioactive molecules to specific sites, such as brain, has been proposed (79). Whether such approaches prove useful clinically remain to be seen, but this novel approach could be an interesting way to target drugs to secondary sites.

Cell Membranes and the Invasive Phenotype

The transition of an epithelial to mesenchymal cell phenotype or EMT in carcinoma cells has been proposed as one of the striking changes that accompany invasion and metastasis (80, 81). Unlike the multiple genetic changes that are typical of *in vivo* transformation and tumorigenesis, EMT appears to be primarily epigenetic and driven for the most part by microenvironmental signals (82). Changes that occur, such as loss of cell adhesion and cell junctions, modifications in cell shape, acquisition of cell motility, release of growth and motility factors and degradative enzymes, among other changes, are characteristic of invasive, malignant cells at the primary site (Fig. 1; refs. 80, 81, 83). At least three different regulatory pathways control cell polarity, adhesion, cell junctions, and other related properties (82). These pathways are driven by tumor microenvironment cross-talk and signaling events starting at the cell membrane surface and then along the relevant signaling circuits inside cells (83). Moreover, once malignant carcinoma cells have metastasized to secondary sites, they can apparently revert back to epithelial-like morphologies along with reexpression of epithelial markers, indicating that EMT has transient and potentially reversible characteristics (80, 81). Although EMT changes have been associated with invasion and metastasis in animal tumors, there remains an ongoing debate on whether this phenomenon is truly representative of malignant pathways in human cancers (84). Indeed, Tarin (85) has argued that invasive cell phenotypes occur after normal tissues are damaged and during embryogenesis and that there is a lack of convincing pathologic evidence in humans that EMT occurs when carcinomas metastasize.

A new approach to developing therapies for highly malignant carcinoma cells has been to target metastasis-related changes, such as cell surface components involved in cell junctions, adhesion, motility, growth, signaling, and other signatures of an invasive phenotype. One place that this could be important is in the identification and typing of circulating tumor cells (CTC; ref. 86). Enumeration of CTC and typing CTC biomarkers are being developed to assess risk of metastatic disease and hopefully predict the effects of therapy to prevent metastases (87). CTC are thought to be directly related to stem cells, the presumptive source of metastases, so Zhang and colleagues (88) isolated CTC from patients with breast cancer without brain metastases, grew them in culture, and subjected them to metastasis assays in immunosuppressed mice. They found that CTC from patients with breast cancer could be selected for a unique "brain-metastasis signature" ($EpCAM^-$/$HER2^+$/$EGFR^+$/$HPSE^+$/$Notch1^+$) that may explain, in part, the ability of CTC to form brain metastases. Using the unselected parental $EpCAM^-$ CTC and the brain-metastasis marker-selected CTC, they found that only the latter were highly invasive and capable of forming brain metastases when xenographed into nude mice (88). Although this report involved only a few patients, future studies may be able to unlock the CTC-targeting signatures for metastasis to specific secondary sites. Such information could prove to be more useful than biopsies in predicting metastatic disease, especially site-specific metastases, and eventually preventing metastases from forming and treating the metastases that have formed (86, 87).

Final Comments

Although tremendous progress has been attained over the last decade in ascertaining the structural and functional roles of cell membranes in malignant processes, much remains to be discovered at the cellular and molecular level. In particular, we know

very little about how microenvironmental signals and cross-talk can rapidly change the phenotypes of cancer and normal cells within the tumor microenvironment and how various signaling pathways, from the cell membrane to various cellular organelles, and *vice versa*, control these complex interactions. Uncoding the plasticity of this process may be essential in explaining metastatic behavior (89).

We are just beginning to understand the cell surface and surrounding properties of malignant cells (and normal cells) that are important in explaining metastasis to secondary sites as well as the properties of target organs for metastatic colonization. This information will not be easily forthcoming, but it will be essential in the eventual development of new therapeutic approaches to limit or destroy metastases. It will also be important in reducing the symptoms of cancer and eliminating the adverse effects of cancer therapy (90).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Garth L. Nicolson

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