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REVIEW

Societal interactions in ovarian cancer metastasis: a quorum-sensing hypothesis

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Abstract The biochemical and biological mechanisms metastatic cancer cells use to function as communities and thwart internal and external growth control mechanisms remain undefined. In this work, we present the hypothesis that cancer cells may use a *Quorum-Sensing* mechanism to regulate multicellular functions and control steps in metastatic colonization. Quorum sensing is a bacterial cell-cell communication process used to track increasing cell-population density and, in response to changes in cell number, coordinate gene expression and behavior on a community-wide scale. Important parallels between the behavior of societies of bacterial cells and societies of malignant cancer cells exist in the bacterial literature. Of relevance to metastasis is the finding that pathogenic bacteria use quorum sensing to determine when their population numbers are high enough to collectively form biofilms in or on host organisms. Biofilms are complex, heterogeneous communities of bacterial cells encased within an extracellular matrix attached to a solid surface. Biofilms exacerbate disease and are refractory to a battery of therapies. We suggest that the quorum-sensing-controlled bacterial biofilm formation process closely parallels the steps in metastatic colonization. Cells migrate toward/on target surfaces (organ-specific homing), show cell-cell and cell-matrix interactions (tumor cell-stromal cell crosstalk), remain subclinical until they can mount an effective attack (dormancy), form complex structures with channels for nutrient flow (vascularized lesions), and contain resistant cells which can cause disease recurrence (persistors). Using ovarian cancer as an example, we present data supporting the connection between metastatic colonization and quorum sensing and discuss the implications for understanding and controlling metastasis formation.

Keywords Metastatic colonization · Ovarian cancer · Quorum sensing

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Abbreviations

d	Density of cells injected
dpi	Days post injection
EPS	Extracellular polysaccharide substance
HA	Hemagglutinin
JNK	c-Jun NH_2 terminal protein kinase
MAPK	Mitogen-activate protein kinase
MKK4/SEK1	Mitogen-activated protein kinase kinase 4/stress-activated protein/Erk kinase 1

MKK4-KR	Mitogen-activated protein kinase kinase 4-kinase inactive
N	Number of cells injected
PCR	Polymerase chain reaction
SAPK	Stress-activated protein kinase
SCID	Severe combined immunodeficient
t	Length of experiment
Y	Yield of experimental metastases

Introduction

In 2008, more than 565,000 deaths from cancer are projected to occur in the United States, most of them from metastatic disease [1]. Metastases are not a direct extension of the primary tumor and are not dependent upon the route of spread (i.e. hematogenous versus lymphatic versus peritoneal dissemination) [2]. Rather, metastasis is defined as dissemination of neoplastic cells from a primary tumor to discontinuous nearby or distant secondary sites where cells proliferate to form overt masses. Of particular interest and clinical relevance is the high recurrence rate in many cancer types after "definitive" therapies such as surgery, radiotherapy, or chemotherapy, demonstrating the urgent need to both identify patients at risk for disease recurrence as well as to develop therapies that specifically target the metastatic process. This discussion will focus on ovarian cancer, although we believe that many of the points presented will apply to other malignancies.

Since the majority of patients with ovarian cancer present with extensive intraperitoneal metastases, it has been difficult to glean information on the natural history of the disease. For the most part, steps in ovarian cancer metastasis have been logically inferred using data from studies of in vitro assays, experimental metastasis assays, and analogies to hematologic metastasis. These observations suggest that cells acquire metastatic competence and are able to survive detachment from the primary tumor, turbulent flow, and exposure to cytokines during transport by the peritoneal fluid. It is also likely that increased motility of cells toward chemical gradients, and physical and biochemical interactions facilitate adherence of cells to secondary sites. Based on patterns of clinical metastases, it is postulated that ovarian cancer cells preferentially adhere to the liver, small bowel, and omentum. Adherent cells that survive and initiate growth can complete the process of metastatic colonization and form detectable overt masses.

The prevailing view is that ovarian cancer metastasis is an inevitable outcome of tumorigenesis and primary tumor growth and is regulated by the same genetic events. However, numerous studies utilizing multiple neoplasms suggest that the process of metastasis is actually regulated by distinct molecular phenomena [3–6] To date, twenty metastasis suppressors have been identified that specifically regulate metastasis formation *without* affecting primary tumor growth *in vivo* and/or are specifically able to inhibit metastatic colonization [5]. Studies of metastasis suppressor proteins are providing insights into this clinically important process [4, 5]. A focus of our laboratory is discerning the mechanism(s) by which metastasis-suppressors impair metastasis formation and how cells may eventually overcome these effects. Over the past decade, we have made several unanticipated findings prompting the metastasis-quorum-sensing hypotheses put forward in this perspective piece. Quorum sensing is a mechanism used by bacterial populations which enables them to adapt to ever-changing environments and carry out complex behaviors. Quorum sensing enables bacteria to communicate, self-organize into cooperative groups, and carry out processes that are successful only because a critical number of cells carry them out in synchrony. The following sections provide specific examples of recent data that prompted the formulation of our quorum sensing hypothesis, a comparison of quorum sensing and metastatic colonization, and examples from the greater literature on metastasis that support a quorum sensing mechanism.

MKK4: a map kinase kinase that functions as a metastasis suppressor

As a key member of the stress-activated protein kinase (SAPK) signaling cascade, MKK4 can phosphorylate both the JNK and p38 MAPKs, resulting in the activation of transcription factors and/or phosphorylation of other regulatory proteins [7]. Using SKOV3ip.1 cells, a metastatic human ovarian cancer cell line that lacks significant endogenous MKK4, we showed that ectopic expression of hemagglutinin (HA)-tagged MKK4 reduces overt experimental metastasis formation by 90% in a kinase-dependent manner, and that MKK4 signals through p38, and not JNK, to suppress in vivo metastatic colonization [8, 9]. As is the case with other metastasis suppressors, SKOV3ip.1 cells expressing HA-MKK4 have no detectable alterations in growth rate or apoptosis under a variety of in vitro growth conditions [8]. Thus, the suppressive effect of MKK4 on metastatic growth is dependent on in vivo activity of the protein. Interestingly, animals injected with MKK4-expressing cells show a 70% improvement in survival as compared to controls, but these animals will eventually succumb to disease burden [8, 9].

The above findings raised some important questions. What are the biological mechanisms responsible for MKK4-mediated suppression of metastatic colonization? Can MKK4-expressing cells become resistant to MKK4's

effects? As described in the following sections, we conducted studies designed to examine how MKK4-expressing cells ultimately bypass MKK4-mediated suppression. What was especially puzzling was that MKK4's ability to block metastasis appears to be dependent on cell number. To our knowledge, no other study has examined the effect of cell number on metastasis suppressor function. Further, in recent years there have been very few studies investigating the potential role of cell number on metastatic efficiency or other hallmark behaviors of metastatic cells. Therefore, our goal was to formulate a testable hypothesis regarding cell number and interactions with the host microenvironment that would be supported by the metastasis literature and our data regarding the mechanism by which MKK4-suppressed cells can overcome suppression.

Societies of cells can bypass MKK4-mediated suppression of SKOV3ip.1 ovarian cancer metastatic colonization

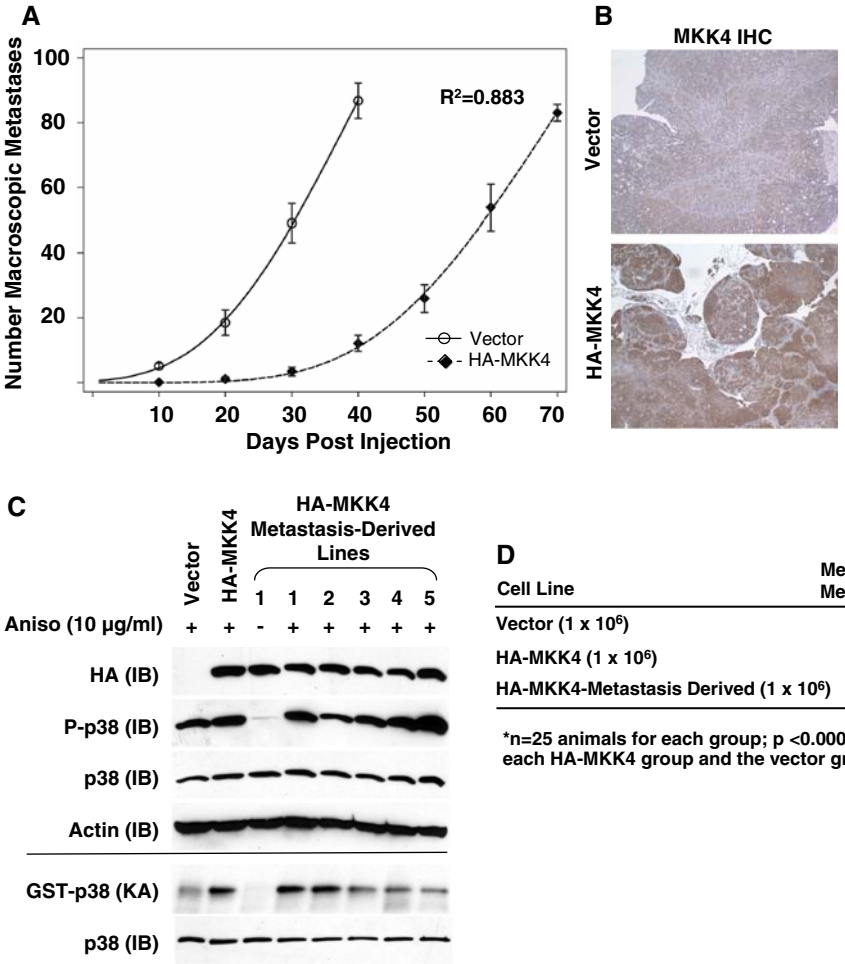
We have previously demonstrated that expression of HA-MKK4 in SKOV3ip.1 cells significantly reduces the number of overt implants following intraperitoneal injection and extends animal survival [8, 9]. Eventually, however, even mice injected with HA-MKK4-expressing cells develop macroscopic metastases and succumb to their disease burden. Recently published studies from our laboratory examined the biological mechanism of MKK4-mediated metastasis suppression and eventual outgrowth of cells. Specifically, using an in vivo time course assay, Lotan et al. showed that both vector-only and HA-MKK4-expressing metastases were able to be mathematically modeled using the same Gompertzian equation (Fig. 1, Panel A). The HA-MKK4 growth curve was simply shifted in time relative to the vector-only curve, with metastasis formation delayed by an average of 30 days [10].

These data raise the question of whether the eventual outgrowth of HA-MKK4-expressing cells is the result of selection for variant clones that have lost or inactivated MKK4 or another mechanism such as a population-wide adaptation to the consequences of SAPK signaling [10]. Published data reproduced in Fig. 1, Panel B illustrate that overt metastases arising from HA-MKK4-expressing SKOV3ip.1 cells at ~65 dpi do, in fact, continue to express HA-MKK4, indicating that in vivo selection for deletion or decreased expression of MKK4 has not occurred [10]. To further expand upon these findings, 25 independent metastasis-derived cell lines were established and 100% of them retained expression of HA-MKK4 protein which could be artificially activated in vitro (representative data shown in Fig. 1, Panel C [10]). Compared to vector-only cells, MKK4 metastasis-derived lines remained suppressed in the ability to form overt experimental metastases when re-injected into naïve mice (Fig. 1, Panel D [10]). Taken together, these recently published in vitro and in vivo data strongly suggest that eventual outgrowth of HA-MKK4-expressing cells is not due to selection for clones of cells that have permanently altered their MKK4 signaling status, but rather, is due to adaptation of the population to the biological consequences of SAPK signaling [10].

Bypass of MKK4-mediated suppression is related to size of the metastatic foci and/or population number

We have recently published data showing that HA-MKK4-expressing cells display decreased proliferation as measured by BrdU incorporation and phospho-histone-H3 staining (Fig. 2, [10]). We were intrigued by our observation that a shift from low proliferation to increased proliferation coincides with lesions reaching an area of approximately 10 mm^2 (i.e. Log 4 μm^2; Fig. 2 [10]). We speculated that the number of cells within an HA-MKK4-expressing lesion might be vital to whether MKK4 could exert a suppressive effect. To test this notion we revisited findings from our in vivo time course assay which suggested that once a critical threshold of cells was reached, SKOV3ip.1-HA-MKK4 cells overcome suppression (Fig. 1 Panel A, [10]). We speculated that if suppression was related to the number of cells colonizing the target organ, then increasing the number of cells injected should bypass MKK4-mediated suppression.

Using our standard assay, the metastatic ability of 1×10^6 SKOV3ip.1-vector and SKOV3ip.1-HA-MKK4 cells as well as SKOV3ip.1-HA-MKK4-KR cells, which express a kinase inactive mutant of MKK4 (HA-MKK4-KR), was assessed. The HA-MKK4-KR-expressing cells behave in analogous fashion to vector-only controls since the protein cannot phosphorylate downstream targets. The number of overt metastases in each group was assessed and the average number of metastases per group versus time displayed in Fig. 3. Interestingly, when 1×10^7 SKOV3ip.1-HA-MKK4 cells were injected, we observed metastasis formation similar to that produced by 1×10^6 SKOV3ip.1-vector or SKOV3ip.1-HA-MKK4-KR cells. These previously unpublished in vivo data support the notion that a critical threshold number of SKOV3ip.1 cells can bypass suppression and initiate growth, which prompted several questions. Are there examples of autonomous single-celled organisms that show variable behaviors depending on their cell number? If so, what is the mechanism by which these organisms coordinate and execute functions as a population? Finally, do such multicellular communities



D

Cell Line	Mean Number of Metastases ± SD
Vector (1 x 10^6)	29.6 ± 8.1
HA-MKK4 (1 x 10^6)	1.3 ± 1.7*
HA-MKK4-Metastasis Derived (1 x 10^6)	4.8 ± 5.5*

*n=25 animals for each group; p <0.0001 between each HA-MKK4 group and the vector group.

Fig. 1 HA-MKK4-expressing SKOV3ip.1 cell lines derived from macroscopic metastases retain expression of functional MKK4 in vitro and remain suppressed for metastasis when reinjected into naïve mice. (**a**) HA-MKK4-expressing cells are delayed in forming macroscopic metastases. The number of ≥1 mm metastases present as a function of time was determined by injecting SKOV3ip.1-vector (solid line, circles) clones or SKOV3ip.1-HA-MKK4 (dashed line, diamonds) clones using our standard intraperitoneal metastasis assay [10]. Means and standard errors at each time point are presented and represent the data from 12 to 15 mice per timepoint. Nonlinear regression revealed that a 3-parameter Gompertz model fit the 165 data points well ($R^2 = 0.883$), indicating that the shape of the growth curve was similar between the two groups. (**b**) Immunohistochemical staining for MKK4 in macroscopic metastases derived from vector-only and HA-MKK4-expressing at 30 and 65 dpi respectively. SKOV3ip.1 cells have low endogenous MKK4 levels, as seen here in vector-only cells. MKK4 was consistently detected in macroscopic HA-MKK4 metastases. (**c**) Clonal cell lines were derived from 10 independent SKOV3ip.1-HA-MKK4 macroscopic metastases and screened for the presence of the HA-tag, and endogenous phospho-p38, p38, and actin following stimulation with anisomycin (representative data are shown). In vitro kinase assays show that HA-MKK4 is functional in vitro and it phosphorylates the GST-p38 substrate in a manner similar to parental cell line controls (positive control, second lane). As a loading control, the blot was also probed for the GST-p38 substrate (bottom panel). (**d**) All five metastasis derived cell lines in panel *B* were re-injected into 5 mice each for the standard end-point metastasis assay. These cell lines were equally suppressed for macroscopic metastasis formation at 30 dpi compared to parental lines ($P < 0.0001$ for both groups compared to vector control). (This figure was adapted from data originally published by Lotan et al. [10])

exhibit any behaviors that resemble those associated with metastatic cancer cells?

Quorum sensing regulates population-dependent behaviors of bacterial societies

In order to adapt to ever-changing environments and carry out complex behaviors, bacteria have developed the ability to communicate and self-organize into cooperative groups. In a process called *quorum sensing*, populations of bacterial cells function together to carry out processes that are successful only because a critical number of cells carry them out in synchrony [11–13]. Two seminal studies, one in the 1960s [14] and one in the 1970s [15] suggested the concept that bacteria could communicate and act in groups. Prior to this work, bacteria had been viewed as asocial organisms that exhibited only individual behaviors. A

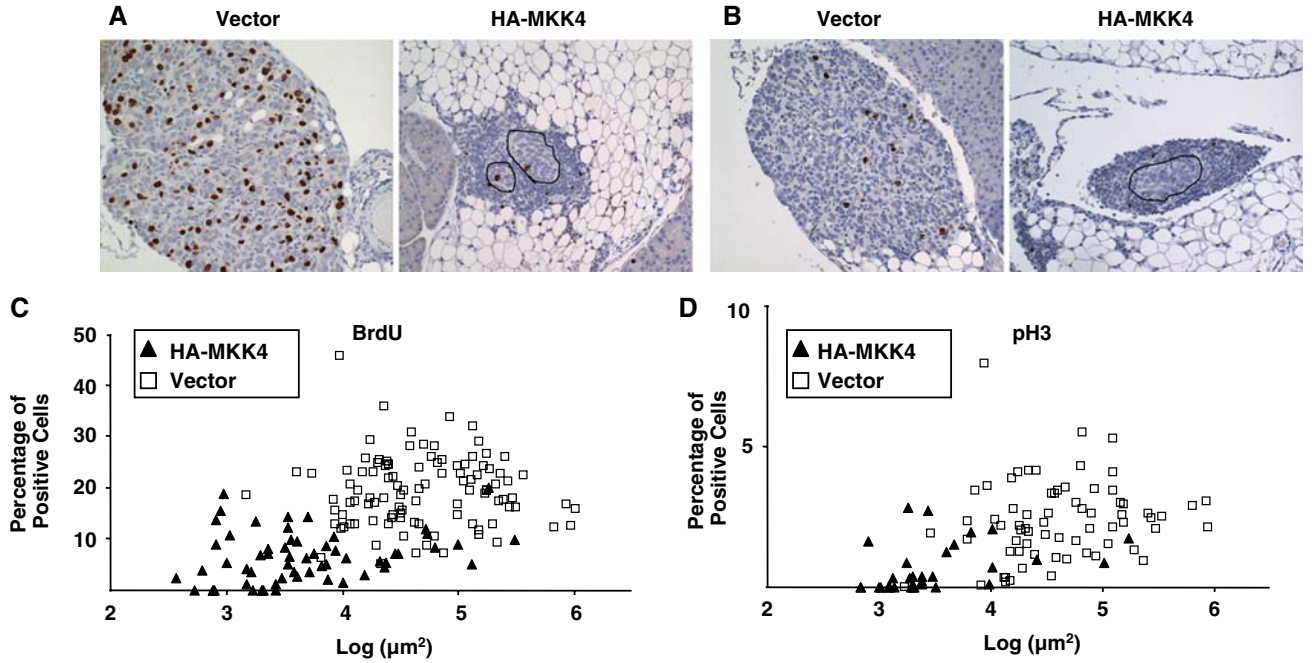


Fig. 2 HA-MKK4-expressing microscopic metastases show decreased proliferation as assessed by BrdU incorporation and pH3 staining at 14 dpi. A. BrdU was injected intraperitoneally 4 h prior to the experimental endpoint. Immunolabeling for BrdU in vector-only and HA-MKK4-expressing SKOV3ip.1 microscopic metastases at 14 dpi (100× magnification). (**b**) More than 160 microscopic metastases were scored for size (in μm^2) and percent BrdU-positive cells using a computer aided image analysis system. Both size and BrdU incorporation were significantly decreased in HA-MKK4-expressing metastases compared to vector-only metastases ($P = 0.0003$ and $P < 0.0001$ respectively) (**c**) Immunolabeling for pH3 in vector-only and HA-MKK4-expressing SKOV3ip.1 microscopic metastases at 14 dpi (100× magnification). (**d**) More than 100 microscopic metastases were scored for size (in μm^2) and percent pH3-positive cells a both size and pH3 immunostaining for mitotic cells were significantly decreased in MKK4-expressing metastases compared to vector-only metastases ($P = 0.0008$ and $P = 0.004$ respectively). (Figure reproduced from data originally published by Lotan et al. [10])

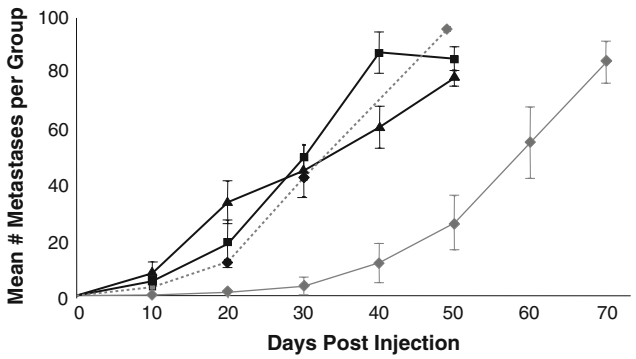


Fig. 3 Increased MKK4-expressing SKOV3ip.1 cell number can bypass metastasis suppression. 1×10^6 cells of three SKOV3ip.1-vector (black line, triangles), three SKOV3ip.1HA-MKK4-KR (black line, square), and four SKOV3ip.1-HA-MKK4 (gray line, diamonds) clonal cell lines were injected intraperitoneally into female SCID mice. Data from each group of clones were pooled, with the average number of metastases per group versus time displayed. Notably, when 1×10^7 SKOV3ip.1-HA-MKK4 (dashed gray line, diamonds) cells were injected, numbers of metastases formed were similar to the number produced by injection of 1×10^6 SKOV3ip.1-vector or SKOV3ip.1-HA-MKK4-KR cells

surge in studies of bacterial social interactions, primarily in the past decade, has shown that hundreds of diverse bacterial species are capable of cell-cell communication, via a chemical lexicon, and furthermore, that bacteria coordinate group behaviors and act in many respects like higher-multicellular organisms [16]. Quorum sensing involves the production, release, and detection of chemical signaling molecules called autoinducers. As a population of quorum-sensing bacteria grows, the concentration of released autoinducer increases proportionally with cell number. When the extracellular autoinducer concentration reaches a critical threshold level, the group detects the molecule and responds to it with a population-wide alteration in gene expression. Thus, linking alterations in gene expression to autoinducer levels enables bacteria to act like multicellular organisms. Quorum sensing controls processes including the production and secretion of virulence factors, sporulation, bioluminescence, and DNA exchange [17–19]. Quorum sensing also regulates the development of complex structures called biofilms.

A biofilm is a community of bacterial cells adhered to a surface or to each other which becomes encased in a self-produced polymeric matrix. Bacteria living in biofilms have increased resistance to antimicrobial agents and are better able to withstand environmental stress [20]. Studies have identified some of the environmental cues that signal the cells to initiate biofilm formation, the proteins that

mediate this response, and the physical processes involved in forming these complex structures. The biofilm formation process appears similar to metastatic colonization. A comprehensive review of the salient literature enabled us to compare these two seemingly disparate processes. A summary of our findings is presented in Fig. 4. Specifically, we present a comparison between steps in metastatic colonization (upper panel), bacterial biofilm formation (middle panel), and a summary of characteristics common to both processes (lower panel). It should be noted that in order to specifically address the biological data presented in Figs. 1–3, we have focused our discussion on metastatic colonization. Thus, the model summarized in Fig. 4 does not consider the potential contributions of a quorum sensing-like mechanism on tumor initiation and growth which are biologically and molecularly distinct processes [5].

In response to environmental signals such as cellular stress, bacteria can initiate biofilm formation. As in metastatic colonization, to complete this process cells must: (1) escape from the primary site and move to secondary sites; (2) adhere to and survive on target surfaces; (3) form microcolonies; and (4) develop into complex multicellular structures [20–23]. Both free-swimming bacteria and those dispersed from a mature biofilm can initiate this process. In the latter case, bacteria produce enzymes to degrade the polymeric matrix to facilitate escape. Surface attachment is determined by both turbulent flow and by physiochemical properties of the system. Motile bacteria can also use flagella to reach a target surface. Attachment is mediated by adhesins, secreted polysaccharides, and structures such as pili. Once cells have attached, they no longer require flagella, and instead utilize pili-mediated twitching motility which allows the bacteria to move along the surface and organize into monolayers [21, 22]. Multicellular behaviors are initiated by microcolonies**,** communities of bacterial cells that are three to five layers deep and are embedded in an extracellular polysaccharide substance (EPS) [23]. After surface attachment and microcolony formation, mature biofilm structures can form [20]. The end result of this quorum-sensing-process is the development of structures which are strikingly similar to metastases. This leads us to the notion that as with bacteria, overt metastasis formation could be mediated by a quorum-sensing-signaling circuit analogous to those previously identified [13, 16, 17, 24, 25].

Quorum sensing provides a unifying and testable model for many long-observed behaviors of metastatic cells

A variety of population-dependent behaviors, such as plasticity of gene expression and phenotype, formation of complex multicellular structures, the switch from a persistent to an active (pathogenic) phenotype, drug resistance, and response to cytokines have been described in metastatic models. In general, these behaviors have been viewed as resulting from the tendency of malignant cells towards heterogeneity. To our knowledge, the concept that these diverse behaviors are the product of a regulated cell signaling mechanism, such as quorum sensing, has not been rigorously tested. Of particular relevance is the observation that cell density at the time of preparation can have a dramatic, nonlinear effect on the number of metastases produced in an experimental metastasis assay (e.g. tail vein or intraperitoneal metastasis assays) [26, 27]. Historically assays are designed to yield a reproducible quantity of overt metastases in a defined period of time. As we considered the literature and standards of practice we formulated the following relationship between yield, number, density, and time. The yield (Y) of experimental metastases for a given model is a function of the number of cells injected (N), the density (d) of cells at the time of preparation for injection, and the time (number of days) of the assay (t). For clarity we will represent this nonlinear relationship as:

$$\boxed{Y \approx f(N, d, t).}$$

Depending on N, metastases can develop shortly after injection/implantation or following prolonged periods. A particular t is selected that gives a reproducible Y, maximizes animal welfare, and fits other experimental constraints. Thus, the Y produced at a given t is highly dependent upon both N and d. To our knowledge we are the first to formalize this relationship, however the biological phenomena have been long-recognized by metastasis researchers.

There are multiple lines of evidence suggesting a relationship between increased population number or cell density and increased metastatic ability in experimental metastases systems. For example, work by Hill et al. demonstrated that cells grown to a larger population size had increased metastasis formation compared to the same number of cells grown to a small population size [28]. The authors of this work found that metastatic variants were generated during the expansion of cells and the mechanism by which this occurred was likely epigenetic and transient. Along the same lines, the effect of cell density on metastatic behavior is illustrated by data from Welch et al. in which the relative number of metastases formed by rat mammary adenocarcinoma clones increased as cells d increased. These critically important but often overlooked data are reproduced in Fig. 5 [27]. Confluence was defined as the maximum number of cells per unit area without causing changes in cell cycle distribution.

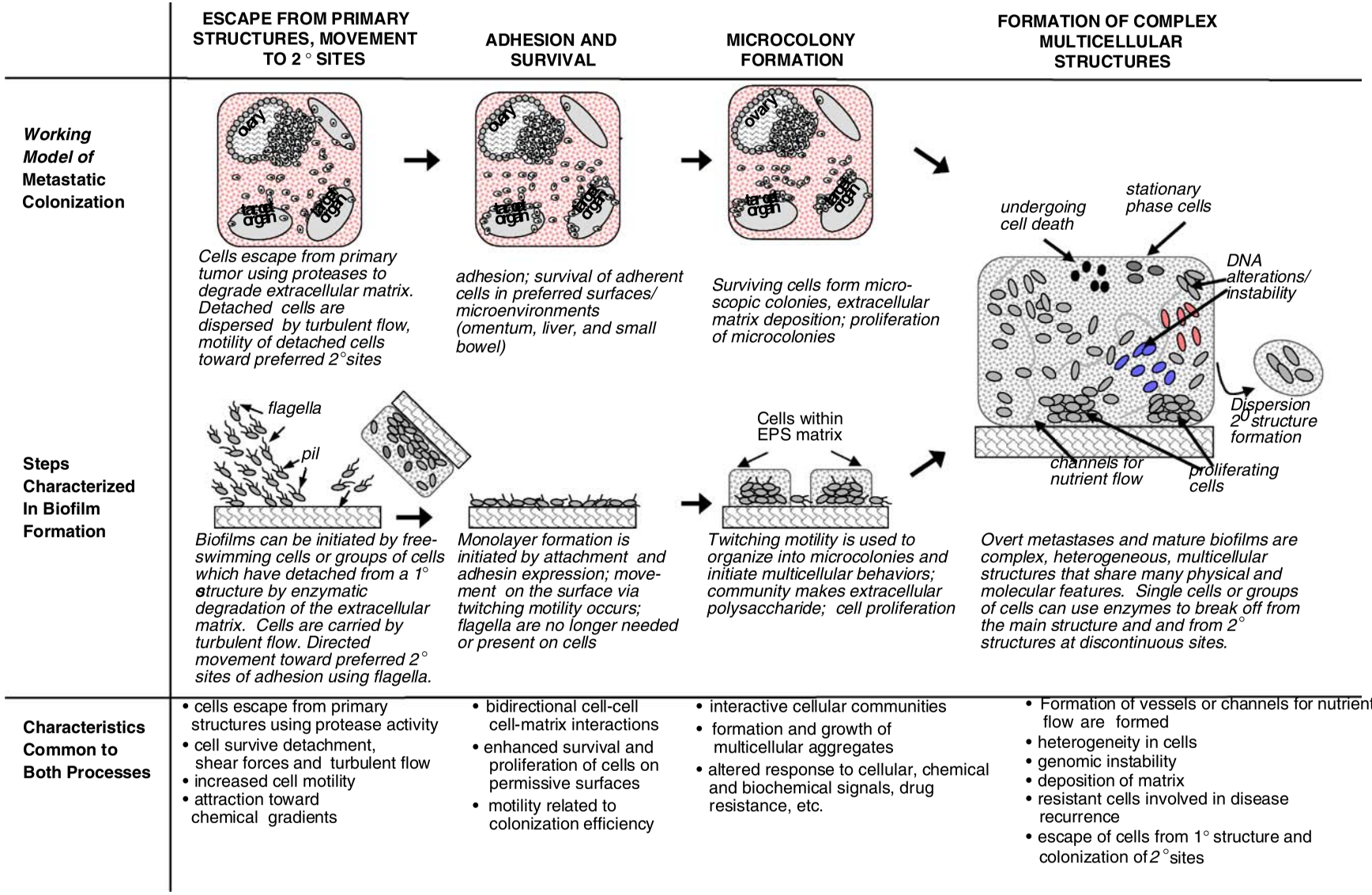


Fig. 4 Comparison of the processes of metastatic colonization and biofilm formation. The process of biofilm formation has many of the hallmarks of metastatic colonization including motility of cells toward appropriate surfaces (organ specific homing), attachment and interaction of cells with each other, surface adhesion and colonization (tumor cell-stromal cell interactions), remaining subclinical until sufficient cell population densities are reached to mount an effective attack on the host (dormancy), formation of complex, heterogeneous, structures containing channels for nutrient flow (formation of vascularized metastatic lesions), and development of subpopulations of therapy-refractory cells which can remain and can cause disease recurrence (persistors)

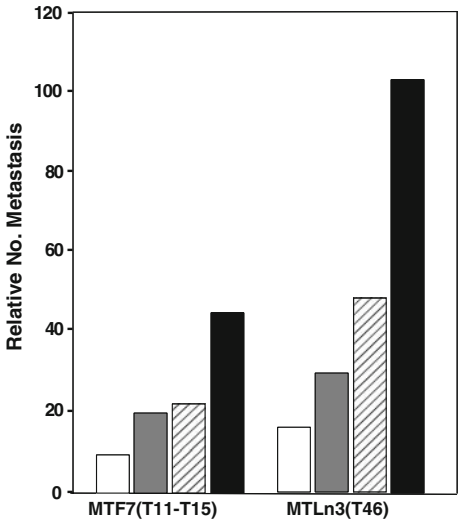


Fig. 5 Relative metastatic efficiency of rat mammary adenocarcinoma cell clones grown to different levels of confluence. Cells were grown to either 20% (white bars), 50% (grey bars), 70% (slashed bars), or 100% (black bars) prior to preparation for experimental (tail vein) metastasis assays. (This figure was adapted from data originally published by Welch et al. [27])

Simply changing *d* by 30% changed *Y* by 50%. These data strongly suggest that some properties required for metastasis are transient and depend on the interactions of groups of cells. Because subtle changes in *N* or *d* can have a large, nonlinear effect on *Y*, most assays/models likely use an excess of cells rather than a minimum *N* needed for a specific *Y* since most studies focus on the ability of an external factor, such as a drug treatment or expression of a gene, to modulate *Y*. In essence, most models of experimental metastasis use a large *N* grown at high-*d*, potentially skewing the cells toward maximal efficiency in putative quorum-sensing-dependent behaviors. In view of quorum-sensing principles, however, these conditions may not represent the environmental complexity in either spontaneous metastasis animal models or clinical disease.

There is further evidence that metastatic cells display quorum-sensing-dependent behavior. As previously stated, quorum sensing provides a cell-counting mechanism that allows bacteria to determine their population size and coordinate group activities. There are multiple lines of evidence supporting the notion that eukaryotic cells may use similar counting mechanisms. We postulate that although malignant cells have derangement of normal growth controls, they nonetheless retain innate counting mechanisms. In bacterial quorum-sensing systems, the concentration of an autoinducer increases proportionally with cell number and when the autoinducer reaches a critical threshold, the group responds with a synchronous change in behavior. Thus, autoinducer activity can be assayed by preparing conditioned medium from cells at high-density and transferring it to cells that are at low-density. Following addition of the high-density conditioned medium, the low-density cells are assayed for the premature activation of behaviors that are ordinarily only expressed by high-density cells.

The ability of the high-density conditioned medium to "trick" low-density cells into exhibiting high-density behaviors is the hallmark of a quorum-sensing mechanism. We were delighted to find that data published by Welch et al. in 1994 show that metastatic cells can exhibit quorum-sensing behavior [26]. These provocative data are reproduced in Fig. 6. In an effort to better understand the effect of *d* on *Y*, Welch et al. tested the possibility that high-density cells secreted a paracrine factor that increased the metastatic efficiency of cells. Representative data using MTLn3 cells are summarized in Fig. 6. Conditioned medium from 70% and 100% confluent plates increased the *Y* by ~50%. Interestingly, conditioned medium from cells at 20% confluence suppressed *Y*. Our quorum-sensing hypothesis suggests that high-density MTLn3 cells secreted an "autoinducer" that caused low-density cells to produce

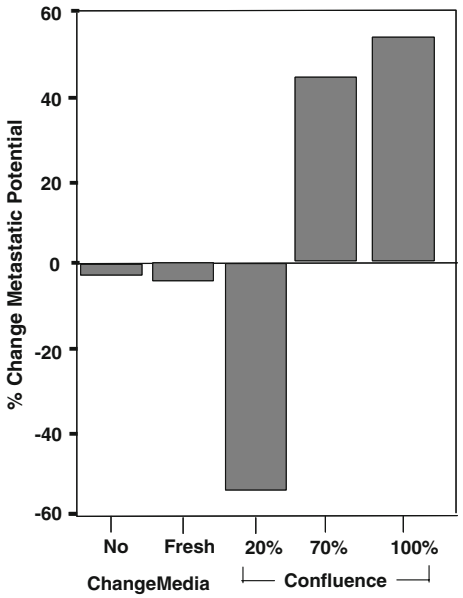


Fig. 6 Percentage change of experimental metastatic efficiency of low-density MTLn3 cells following exposure to conditioned medium collected from MTLn3 cells at various levels of confluence. Conditioned medium was collected following 48 hours of continuous culture from MTLn3 cells seeded to yield levels of 20%, 70%, or 100% confluence at the time of conditioned medium collection. All recipient cultures were seeded 48 h previously to yield 50% confluent cultures. Conditioned medium was added for 4 h prior to preparation for experimental metastasis assays. As controls, either the medium was not replaced, or it was replaced with fresh medium. The results were confirmed using serum-free medium. (This figure was adapted from data originally published by Welch et al. [26])

the *Y* of high-density cells. Further, one can use the conceptual and experimental framework of quorum sensing to design studies to identify both known and novel signals that modulate metastatic efficiency. We believe that re-examining salient findings in the metastasis literature from the viewpoint of quorum sensing enables a fresh and different perspective.

Translational potential

While specific proteins and cellular phenotypes have been associated with metastasis, there is no well-characterized in vivo empirical model that integrates these attributes into a coherent understanding of the unique aspects of ovarian cancer metastasis. Such a model would serve as a framework for the integration of clinical and experimental data and enable quantitative assessment of the temporal and spatial events in ovarian cancer metastasis. From a practical standpoint, a comprehensive model of ovarian cancer behavior which considers a quorum-sensing component may allow us to understand some of the tumor biology that is unique to this cancer type and that baffles clinicians. It is known that surgical cytoreduction of metastatic implants to less than 1 cm or to microscopic disease results in prolonged patient survival. This finding has heightened the debate concerning whether it is the biological properties of the individual patient's tumor that allows maximal cytoreduction or surgical effort. If the model presented here is correct, it could result in improved patient survival by explaining a shift in the tumor environment after surgery such that there are smaller populations of cells able to take advantage of quorum sensing. Such findings would highlight the need to decrease cell density in ovarian cancer populations, possibly by direct treatment with intraperitoneal chemotherapy to reduce cell-dense populations or by repetitive attempts at surgical cytoreduction.

In acknowledging the possibility that a quorum-sensing-like phenomenon exists in cancer cells, we may go forward by targeting new mechanisms used by cancer cell populations to improve patient survival. For instance, development of drug-resistant metastases is a common clinical problem. Data from the bacterial studies have shown that blocking quorum-sensing signaling molecules in drug resistant bacteria may restore susceptibility to antimicrobial therapy [20, 23, 29, 30]. If this phenomenon likewise exists in ovarian cancer cells, it provides an unexplored target for antimetastatic therapy. Further, if ovarian cancer populations utilize quorum-sensing signaling molecules, it may be feasible to target disruption of production or detection of these signals to enhance response to established forms of treatment such as chemotherapy.

Final thoughts

The prevailing dogma remains that metastasis is the result of the "drive" of malignant cells towards growth [31]. Mechanisms based on this view established a model where acquisition of metastatic ability is the product of mutation-selection cycles and derangement of cell growth, selecting for the most aggressive (malignant) clones. This parallels the view that long dominated the field of microbiology. Indeed, even today the concept of quorum sensing remains relatively or completely unknown to many outside the field of microbiology. With respect to cancer, the prevailing view is that cancer cells are "lone agents" and disease is caused by the most malignant cells. As pointed out by Heppner [32] "this view does not consider population biology and plasticity of cancers. Many lines of evidence suggest that the behavior of metastases, like other mixed populations, may not be governed simply by the behavior of its most deviant members. Instead tumors (*and metastases*) [1] may be cell societies, ecosystems in which the various members (clones) interact to produce a group dynamic that define overall behavior" [33]. Further, the mutation-selection paradigm does not account for the dynamic character of metastatic cells readily observed in a variety of cancer types [34–36]. We propose that it would be beneficial to revisit seminal data in the metastasis literature from the viewpoint of quorum sensing. In this view, selection may be for the population that is best able to coordinate its efforts in order to sense and respond to changing conditions and overtake its host, rather than a deterministic process that selects for the most aggressive malignant cells. It is anticipated that other models can be formulated that can provide a theoretical framework for testing the complex and dynamic behaviors of cell interacting in populations. However, it is our view that a model which addresses and incorporates these issues, and we suggest that a quorum-sensing model does, could provide insight into novel molecular mechanisms underlying the metastatic process and may provide novel therapeutic targets.

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