PROSPECT
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Modulators of Cancer Cell Invasiveness

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ABSTRACT

Cell invasiveness is essential for cancer metastasis. Many proteins, and more recently also non-coding RNAs, particularly microRNAs (miRNAs), have been reported to affect the cell invasiveness of various cancers. There is an apparent gap between the high number of these macromolecules and the low number of signaling pathways experimentally verified to control cancer invasiveness. We have brought together these various proteins and RNAs because we could not find any publication that filled this important gap. We have noted 589 proteins, 28 miRNAs, and 1 long non-coding RNA that are reported to modulate invasiveness in cells of various cancers. Interestingly, 44 proteins enhance invasiveness in cells of some cancers, but suppress it in cells of others. Almost all of the proteins that show experimentally verified activation/inhibition effects on, or binding interactions with, each other are linked together in a single network, in a "hub-and-spoke" architecture. The accumulated data show trends that point to anticipated future results and highlight gaps in what is known about invasiveness signaling. Identification of cancer invasiveness signaling networks is important for combination and personalized targeted therapies of cancers. J. Cell. Biochem. 111:791–796, 2010. © 2010 Wiley-Liss, Inc.

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Cell invasion is essential in embryogenesis, wound healing, and angiogenesis. It is also required for cancer metastasis. Identification of cancer invasiveness-modulating signaling networks is needed to understand the mechanism and regulation of cancer invasion. It is also necessary for strategies of targeted therapies, including combination and personalized therapies, of various cancers. Much remains unknown about the factors and signaling networks that control cell invasiveness in various types of cancers [Mareel and Leroy, 2003; Giehl et al., 2004; Sliva, 2004; Condeelis et al., 2005; Wang and Zhang, 2005; Christofori, 2006; Wang et al., 2007; Zöller, 2009]. To plan strategies to identify signaling pathways that control a phenotype, first it is necessary to have an overview of the factors that affect such phenotype.

For brevity, we focused on publications that deal only with: (a) invasiveness in solid cancers, (b) proteins and RNAs, (c) evidence stemming from sequence-specific functional assays based on nucleotide or amino acid sequences, (d) cancer cells, and (e) primarily human cells. This necessary focus meant the exclusion of (a) stromal cells, although they participate in cancer invasion, and (b) peptides, such as neurotransmitters. We have noted 618 cancer invasiveness-modulating factors, consisting of 589 proteins, 28 microRNAs (miRNAs), and 1 long non-coding RNA (Supplementary Tables I and II). We have categorized each invasiveness-modulating protein according to its most distinctive or specific property, in descending order of priority: molecular function, localization, or biological function (e.g., "proteinases," "plasma membrane," and "adhesion," respectively). Factors in the "other protein modifications" category include: crosslinking, ubiquitination, stabilization, isomerization, deacetylation, demethylation, and molecular chaperoning. Adapter and scaffold proteins are primarily part of the "other regulators of proteins" category. The "transport" category refers to transport of glucose, potassium chloride, zinc, iron, and lipophilic molecules. The "other proteins" category covers a carbonic anhydrase, a prion protein, and mostly proteins of unknown function.

Many factors act directly, as integral members of signaling pathways that regulate invasiveness. Other factors, such as mRNA processing proteins, may contribute only indirectly, by their role in the formation of a member of such pathway. As expected, the present tally includes known functional partners, such as extracellular ligands and their corresponding plasma membrane receptors.

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Of 589 invasiveness-modulating proteins, 457 enhance invasiveness (Supplementary Table I), and 177 suppress it (Supplementary Table II), in cells of various cancers. In each protein category there are more enhancers than suppressors of invasiveness, except in proteolysis regulators and adhesion-related proteins. There are no secreted proteinases that only inhibit invasiveness (Supplementary Tables I and II). In over 20 protein families, various members of the same family have opposite effects on invasiveness (Supplementary Tables I and II).

Interestingly, 44 proteins of various categories enhance invasiveness in cells of some cancers, but suppress it in cells of other cancers, suggesting that this is a widespread occurrence (Supplementary Tables I and II). We will refer to them as "bimodal" proteins, for brevity. These observations result from qualitative experiments, but quantitative measurements would not alter the conclusion. Of these 44 proteins, 32 proteins either stimulate or inhibit invasiveness only in cells of different cancers, spanning over 15 protein categories (Supplementary Tables I and II). The trend of the data suggests that at least additional members of over 15 protein families already known to affect invasiveness (Supplementary Tables I and II), will turn out to be bimodal. The other 12 of these 44 proteins are bimodal in cells of cancers of the same organ but different patients (cell lines) as well as in cells of different cancers: a scaffolding protein (caveolin 1); plasma membrane receptors (integrins β1 and β4); other plasma membrane proteins (cadherins E and P); growth factors (IGF1, VEGFA); transcription factors (AR, ID2); a secreted proteinase (tPA); a hormone (GNRH1); and a transport protein (LCN2) (Supplementary Tables I and II).

It is not known how one protein enhances invasiveness in cells of one cancer and inhibits it in cells of another cancer. It could be postulated that the mechanism is that such protein acts through one signaling pathway in one cancer cell type and another signaling pathway in another cancer cell type. If so, it should be noted that signaling between some pairs of invasiveness modulators is downregulatory in cells of some cancers but is upregulatory (possibly via different pathway intermediates) in cells of other cancers [DeLassus et al., 2008, 2010]. If a factor at one cellular concentration promoted invasiveness but inhibited it at a higher level, then decreased invasion: (a) induced by depletion of the factor would appear to show that the factor only enhances invasiveness; and (b) induced by overexpression of the factor would seem to demonstrate that it only suppresses invasiveness. In principle, another potential concern would be that the observed invasiveness stimulation or inhibition could depend on the method used to manipulate the cellular concentration of accessible wild-type copies of the factor in question. Neither of these two reservations applies to at least 34 of the 44 proteins listed here as bimodal because these 34 proteins promoted invasion in cells of some cancers and inhibited it in cells of others when using similar methods to alter cellular levels of the protein in question (Supplementary Tables I and II).

The frequency of bimodal proteins is an average of about 1 of 10 in most protein categories (Supplementary Tables I and II). However, bimodal factors are rare in some categories: 1 of 46 kinases, none of 13 plasma membrane proteinases (tested in 28 cancer cell lines), none of 16 protein modifiers (tested in 22 cancer cell lines), 1 of 29 cytoskeleton-related proteins (tested in 34 cancer cell lines), and none of 28 miRNAs (tested in 42 cancer cell lines; Supplementary Tables I and II). This trend suggests that not all members of at least these factor categories will turn out to be bimodal. Within each protein category, there is no obvious difference between the bimodal proteins and the proteins in which bimodality has not been observed (Supplementary Tables I and II). There is also no apparent difference between the cancer types, which show bimodality and those in which it has not been detected (Supplementary Tables I and II). For most bimodal proteins, the ratio of cancer types showing invasiveness stimulation versus cancer types showing invasiveness inhibition is $\sim$1, but it is 12 for a secreted proteinase (uPA) (Supplementary Tables I and II).

Another level of complexity is that some segments of a given protein may stimulate cancer cell invasiveness while other fragments of the same protein may have the opposite effect. For example, various sequences of collagen IV (extracellular matrix) promote melanoma cell invasion but another sequence of collagen IV inhibits it [Pasco et al., 2005]. Bimodality may reflect differences between: (a) cancers originating from various organs; (b) various subtypes of cancers originating from one organ; or (c) various patients afflicted by one cancer subtype.

Toward the goal of estimating the number of unidentified invasiveness-modulating signaling pathways, an important question is to what extent is each of the present proteins experimentally linked to others in this group. Of 589 invasiveness-modulating human proteins, the numbers of proteins that have experimentally tested interactions with others in this group are 177 for activation/inhibition effects and 251 for binding interactions, in the STRING database [Jensen et al., 2009] (Supplementary Tables I and II). In this database, 314 proteins do not show any experimentally verified activation/inhibition effects on, or binding interactions with, other proteins in this group, and 95 have only 1 or 2 of such links (Supplementary Tables I and II). Some proteins show substantial numbers of experimentally determined interactions with others in this group but have not been experimentally identified as members of invasiveness signaling pathways. Examples are: an extracellular matrix protein (IBSP), hormones (PRL and CGA), and carbohydrate metabolism proteins (LGALS3 and MGAT5) (Supplementary Tables I and II).

Unexpectedly, almost all of the proteins that show experimentally verified activation/inhibition effects on, or binding interactions with, each other are linked together in a single network (Figs. 1 and 2), visualized using Cytoscape [Shannon et al., 2003]. For instance, in the binding category of STRING, 251 of the 589 proteins had corresponding entries in STRING and showed 522 direct binding interactions with each other, and 231 of the 251 proteins interacted with each other forming a single network. To estimate if this can occur by random chance, we extracted 600 random proteins from STRING, asked how many are connected, and repeated this extraction test 100 times. This is a more stringent test because the invasiveness-controlling proteins were not generated from the STRING database; extracting random proteins from the database biases the set toward finding interactions. Nonetheless, in our computational test the largest network observed consisted of 32 proteins and occurred only once. The average network size was 25 proteins, compared to the 251 observed in invasiveness modulators. These results indicate that the

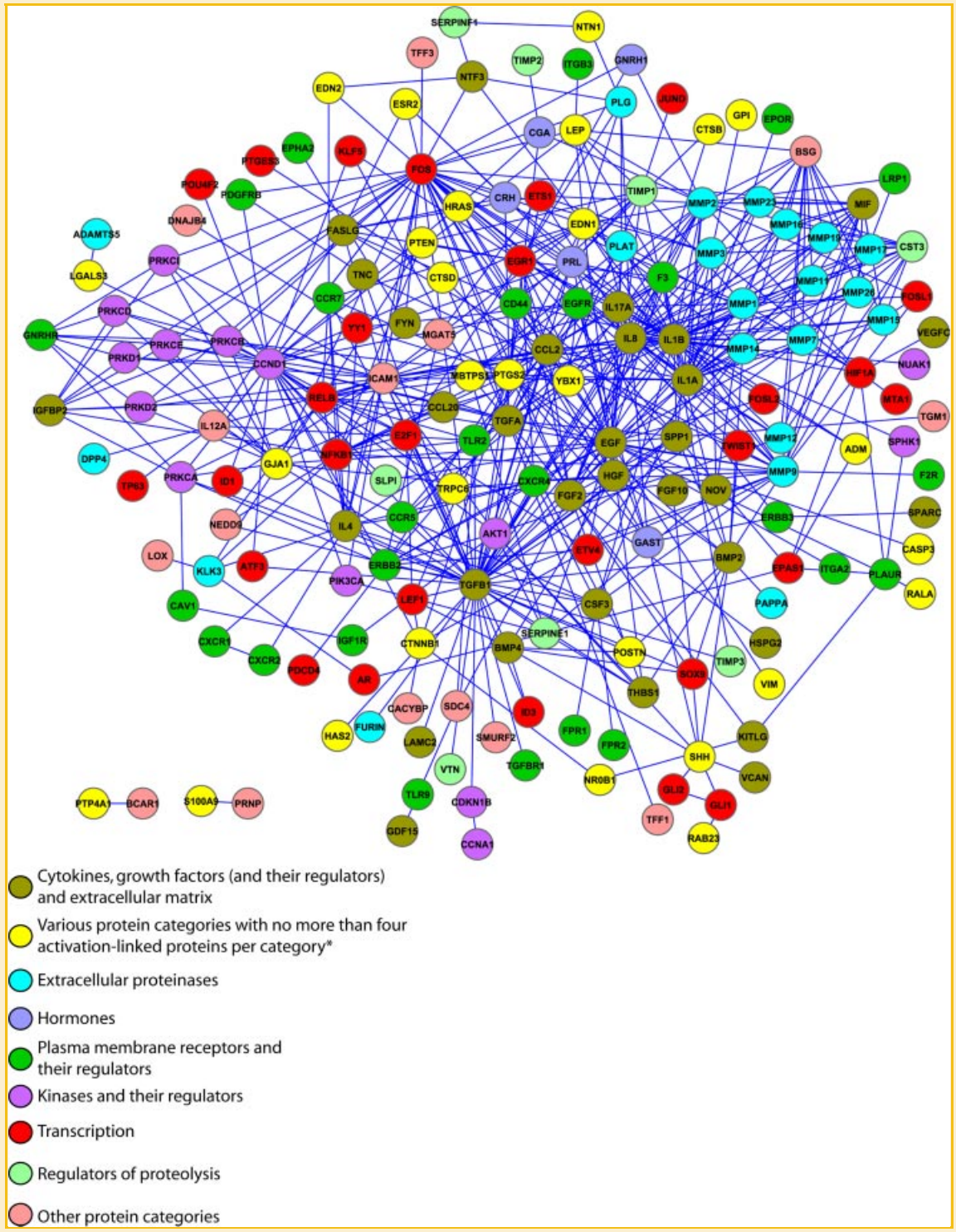

Fig. 1. Experimentally determined activation and inhibition effects between the invasiveness-modulating proteins in the present group (STRING database) [Jensen et al., 2009]. The interaction network model was generated with Cytoscape [Shannon et al., 2003]. *This includes: proteins involved in adhesion, calcium metabolism, cytoskeleton, gap junction, carbohydrate metabolism, ion channel, lipid metabolism, and vasoregulation; other extracellular ligands; GTPases; nuclear membrane receptors; phosphatases; and intracellular proteinases.

probability of observing 522 interactions in 600 randomly chosen proteins is 2^{251}–2^{25}, or 1 in 3.6×10^{75}.

We found that the number of interactions versus the observed frequency of proteins with a given number of interactions their observed frequency is approximately linear on a log–log plot, as expected for a power–law distribution: log (Pr) = gamma log (k), with the estimated value of gamma = 0.6 for the activation/inhibition graph and 0.7 for the binding graph. A network where

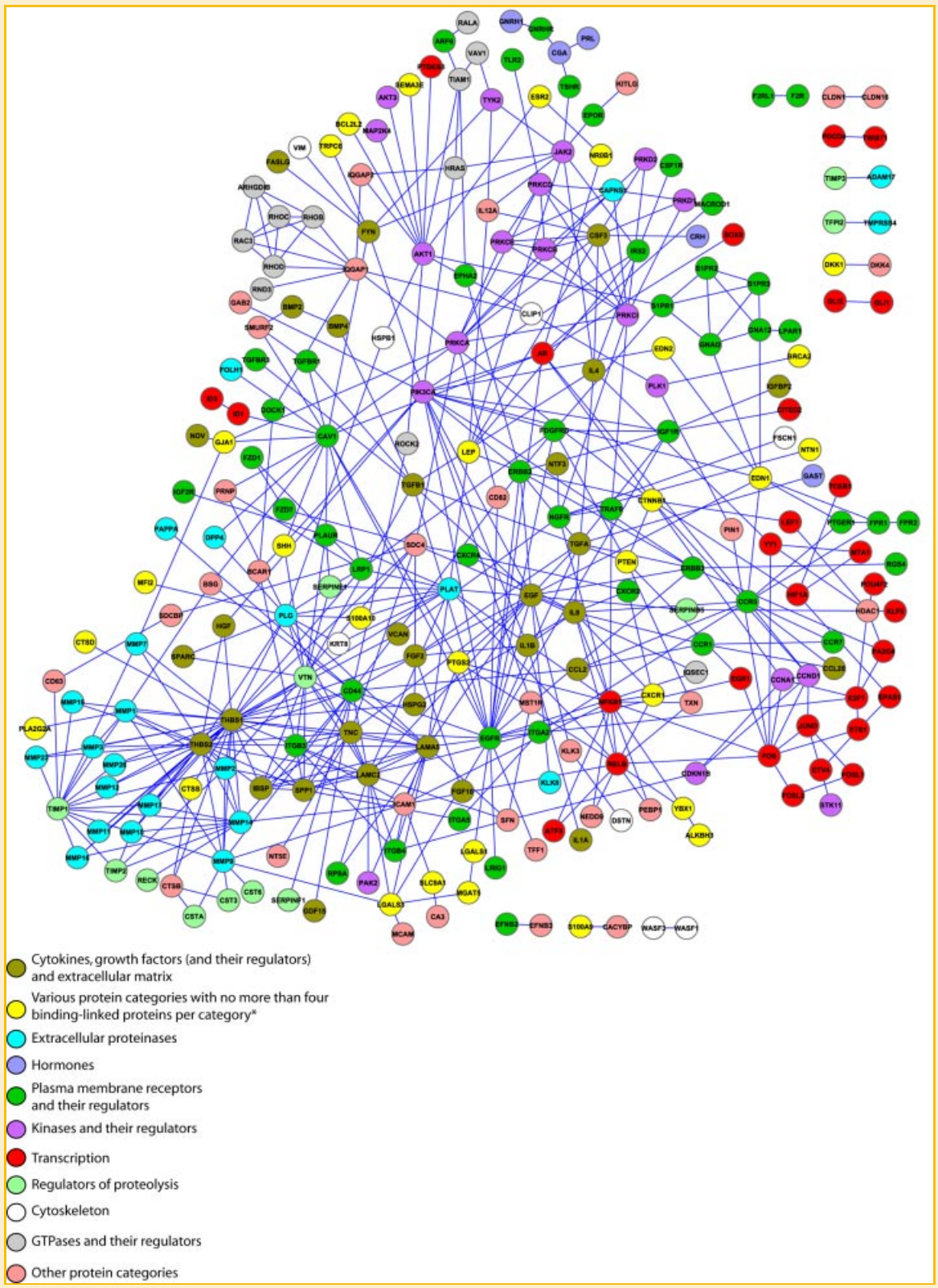

Fig. 2. Experimentally identified binding interactions between the invasiveness-regulating proteins in the present set (STRING database). The interaction network model was generated with Cytoscape. *This includes: proteins involved in adhesion, calcium metabolism, gap junction, carbohydrate metabolism, ion channel, lipid metabolism, and vasoregulation; other extracellular ligands; nuclear membrane receptors; phosphatases; and intracellular proteinases.

gamma has a value >0.5 is considered a "hub-and-spoke" architecture [Albert and Barabási, 2002]. Thus, the invasiveness-modulating proteins interact in a "hub-and-spoke" organization. In such an architecture most members are directly connected to a few members, suggesting that most of these proteins are directly regulated by (or regulate) a few proteins of this group. These two results support the notion that the invasiveness-regulating proteins that are experimentally linked to each other are members of a small number of invasiveness signaling pathways.

The ratio of the number of proteins in this set that activate/inhibit versus bind a given protein ranges from 26:1, pointing to proteins that may influence others in this group about invasiveness through signaling pathways rather than directly, to 0:24, pointing to proteins whose effects about invasiveness on others in this set may have not been detected yet (Supplementary Tables I and II). The profile of activation/inhibition effects (Fig. 1) differs substantially from that of binding interactions (Fig. 2), among experimentally determined links between invasiveness-modulating proteins. These differences include what proteins are involved in activation/inhibition effects versus binding interactions, as well as what proteins show many or few activation/inhibition effects on, or binding links to, others in this set (Figs. 1 and 2). In terms of both activation/inhibition effects and binding links, all of the tested matrix metalloproteinases (MMPs) cluster in a single group, while other extracellular proteinases spread outside this cluster (Figs. 1 and 2). This supports a model in which MMPs influence each other directly and other extracellular proteinases do not affect each other or MMPs, particularly not directly. In contrast, kinases show more clustering in activation/inhibition effects than in binding interactions, and transcription proteins exhibit the opposite (Figs. 1 and 2).

Of these 589 proteins, only some have been experimentally identified as members of a specific invasiveness-controlling signaling pathway [Mareel and Leroy, 2003; Giehl et al., 2004; Sliva, 2004; Condeelis et al., 2005; Wang and Zhang, 2005; Christofori, 2006; Wang et al., 2007; Zöller, 2009]. For many other proteins in over 18 protein categories, their connection to a specific invasiveness signaling pathway has not been experimentally established (Supplementary Tables I and II).

MicroRNAs are non-coding, single-stranded, ~21–23 nucleotide long RNAs that control gene expression, in mammals typically by inhibition of protein synthesis. Of 28 invasiveness-regulating human miRNAs, 19 inhibit invasiveness (Supplementary Table II) and nine stimulate it (Supplementary Table I) in cells of various cancers. Since each miRNA tends to control ~100 protein genes, the identity of such genes will add another challenge to the study of cancer cell invasiveness regulation. Of the miRNAs known to modulate invasiveness, the connection to invasiveness signaling pathways is only known for some of them, such as miR-200, miR-10b, and miR-21 (Supplementary Tables I and II). Only one long non-coding RNA has been reported to affect invasiveness thus far (Supplementary Table I).

The universality of the functions of the present invasiveness-modulating proteins and RNAs in various cancers is unknown. Some evidence supports the idea that the role of some factors is not universal in all cancers. First, different signaling pathways regulate a given cancer trait in various cancers [Wood et al., 2007; Jones et al., 2008]. Second, signaling pathways between invasiveness modulators differ in cells of different cancers [DeLassus et al., 2008, 2010]. Third is the bimodal proteins discussed above. Other evidence suggests that some factors may play universal roles in at least some cancers. First is the hub-and-spoke architecture of the present activation/inhibition and binding networks discussed above. Second, 1 miRNA (miR-21) and 37 proteins in 15 protein categories modulate invasiveness in cells of cancers of at least four different organs (Supplementary Tables I and II). This trend to regulate invasiveness in cells of cancers of various organs suggests that at least these factors are universal regulators of invasiveness in some cancers.

The present invasiveness-regulating factors include, for example, 89 plasma membrane receptors (and their regulators), 73 transcription proteins, 47 proteinases, and 28 miRNAs (Supplementary Tables I and II). The final number of proteins and miRNAs that modulate cancer invasiveness may be substantially higher for four reasons. First, more than 230 protein families have members that affect invasiveness (Supplementary Tables I and II) but have additional family members yet to be tested. Second, human cells have, for example, ~1,675 different receptors, ~947 transcription factors, ~434 proteases, and ~721 miRNAs. Third, some proteins that modulate cancer invasiveness are difficult to detect because they are expressed at very low levels [Hegedűs et al., 2008], and might not have been tested yet. Fourth, 89 new cancer invasiveness-modulating proteins were reported in 2009. Thus, it is anticipated that new cancer invasiveness regulators will continue to be detected at similar rates for some time. This, plus the many known invasiveness factors compared to the few experimentally verified invasiveness signaling pathways, suggest that the final number of cancer invasiveness-modulating signaling pathways will be substantially higher. Adding to this complexity, there is substantial signaling pathway crosstalk in some areas of the cancer progression signaling system [López-Otín and Hunter, 2010], but apparently not in others [DeLassus et al., 2010].

Cell invasion requires cell migration. Cell migration is essential in embryogenesis, tissue repair, fertility, immune response, and inflammation. It is also needed for cancer metastasis. Some proteins, which are involved with the cytoskeleton, adhesion or gap junctions, are not known to have extracellular proteinase activity. These proteins modulate migration and are currently understood to regulate also invasiveness (Supplementary Tables I and II). Some of these proteins regulate the formation and function of the plasma membrane protrusions known as invadopodia, lamellipodia, and filopodia [Condeelis et al., 2005; Wang et al., 2007]. We propose that others of these proteins might modulate only migration and/or motility for the following three reasons. First, most studies of invasiveness or of invasiveness and migration use Boyden chamber assays. Boyden chamber cell invasion assays measure the sum of cell migration plus extracellular protein digestion. It is anticipated that, depending on the theoretically possible combinations of effects of a factor on invasiveness and on migration, in some instances Boyden chamber assays may not fully dissect effects on invasiveness from those on migration. Second, testing only invasion might miss a role in migration. Third, the role of a factor in either invasiveness or migration might be missed in wild-type protein overexpression

experiments if in untreated cells the factor is already present at levels that are saturating for the tested phenotype. Thus, to maximally dissect the role of a factor in cell invasiveness versus cell migration, it is necessary to do both invasion and migration assays in cells depleted of that factor. Some of the studies cited here might not have maximally distinguished roles in invasiveness versus migration because it is not clear whether their tests included all three of these parts. Maximal dissection of roles in cell invasiveness versus roles in cell migration or motility would facilitate understanding of their respective mechanisms. It would also help strategically because, if many signaling networks need to be characterized for potential medical application, migration assays are less expensive and easier to scale up than invasion assays.

It seems likely that secreted proteinases, plasma membrane proteinases, and possibly plasma membrane proteinases, which are also intracellular (Supplementary Tables I and II), will remain primarily as signaling endpoint effectors of invasiveness. This might also include some extracellular enzymes that hydrolyze carbohydrates (Supplementary Table I) or other macromolecules. The extracellular proteinases differ in their substrate profiles. Individual manipulation of each of these proteinases affects invasiveness, suggesting that they play non-redundant roles in invasiveness. One possibility is that invasiveness is the sum of migration and motility plus the capacity to digest extracellular proteins. Alternatively, a protein that is not an extracellular proteinase might be a signaling endpoint effector of invasiveness but not affect migration or motility. In that case, invasiveness would be more complex than the sum of locomotion, motility, and digestion of extracellular proteins.

The present overview points to anticipated future results and shows gaps in our knowledge of the complexity of invasiveness signaling in cancers. Identification of the invasiveness signaling networks in various cancers is important to understand the mechanism and control of cancer invasiveness and to plan strategies for combination and personalized targeted therapies of cancers.

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REFERENCES

Albert R, Barabási AL. 2002. Statistical mechanics of complex networks. Rev Mod Phys 74:47–97.

Christofori G. 2006. New signals from the invasive front. Nature 441:444–450.

Condeelis J, Singer R, Segall J. 2005. The great escape: When cancer cells hijack the genes for chemotaxis and motility. Annu Rev Cell Dev Biol 21:695–718.

DeLassus GS, Cho H, Park J, Eliceiri GL. 2008. New pathway links from cancer-progression determinants to gene expression of matrix metalloproteinases in breast cancer cells. J Cell Physiol 217:739–744.

DeLassus GS, Cho H, Hoang S, Eliceiri GL. 2010. Many new down- and up-regulatory signaling pathways, from known cancer progression suppressors to matrix metalloproteinases, differ widely in cells of various cancers. J Cell Physiol 224:549–558.

Giehl KMA, Wedlich D, Beil M, Seufferlein T. 2004. From tumorigenesis to tumor progression: Signaling pathways driving tumor invasion and metastasis. In: Wedlich D, editor. Cell migration in development and disease. Weinheim: Wiley. 299–340.

Hegedűs L, Cho H, Xie X, Eliceiri GL. 2008. Additional MDA-MB-231 breast cancer cell matrix metalloproteinases promote invasiveness. J Cell Physiol 216:480–485.

Jensen LJ, Kuhn M, Stark M, Chaffron S, Creevey C, Muller J, Doerks T, Julien P, Roth A, Simonovic M, Bork P, von Mering C. 2009. STRING 8—A global view on proteins and their functional interactions in 630 organisms. Nucleic Acids Res 37:D412–D416.

Jones S, Zhang X, Parsons DW, Lin JC-H, Leary RJ, Angenendt P, Mankoo P, Carter H, Kamiyama H, Jimeno A, Hong S-M, Fu B, Lin M-T, Calhoun ES, Kamiyama M, Walter K, Nikolskaya T, Nikolsky Y, Hartigan J, Smith DR, Hidalgo M, Leach SD, Klein AP, Jaffee EM, Goggins M, Maitra A, Iacobuzio-Donahue C, Eshleman JR, Kern SE, Hruban RH, Karchin R, Papadopoulos N, Parmigiani G, Vogelstein B, Velculescu VE, Kinzler KW. 2008. Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. Science 321:1801–1806.

López-Otín C, Hunter T. 2010. The regulatory crosstalk between kinases and proteases in cancer. Nat Rev Cancer 10:278–292.

Mareel M, Leroy A. 2003. Clinical, cellular, and molecular aspects of cancer invasion. Physiol Rev 83:337–376.

Pasco S, Brassart B, Ramont L, Maquart F, Monboisse J. 2005. Control of melanoma cell invasion by type IV collagen. Cancer Detect Prev 29:260–266.

Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T. 2003. Cytoscape: A software environment for integrated models of biomolecular interaction networks. Genome Res 13:2498–2504.

Sliva D. 2004. Signaling pathways responsible for cancer cell invasion as targets for cancer therapy. Curr Cancer Drug Targets 4:327–336.

Wang G, Zhang W. 2005. The signaling network of tumor invasion. Histol Histopathol 20:593–602.

Wang W, Eddy R, Condeelis J. 2007. The cofilin pathway in breast cancer invasion and metastasis. Nat Rev Cancer 7:429–440.

Wood LD, Parsons DW, Jones S, Lin J, Sjoblom T, Leary RJ, Shen D, Boca SM, Barber T, Ptak J, Silliman N, Szabo S, Dezso Z, Ustyanksky V, Nikolskaya T, Nikolsky Y, Karchin R, Wilson PA, Kaminker JS, Zhang Z, Croshaw R, Willis J, Dawson D, Shipitsin M, Willson JKV, Sukumar S, Polyak K, Park BH, Pethiyagoda CL, Pant PVK, Ballinger DG, Sparks AB, Hartigan J, Smith DR, Suh E, Papadopoulos N, Buckhaults P, Markowitz SD, Parmigiani G, Kinzler KW, Velculescu VE, Vogelstein B. 2007. The genomic landscapes of human breast and colorectal cancers. Science 318:1108–1113.

Zöller M. 2009. Tetraspanins: Push and pull in suppressing and promoting metastasis. Nat Rev Cancer 9:40–55.