Site-specific metastasis formation

Chemokines as regulators of tumor cell adhesion, motility and invasion

Adit Ben-Baruch

Department of Cell Research and Immunology; George S. Wise Faculty of Life Sciences; Tel Aviv University; Tel Aviv; Israel

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The metastatic spread of tumors is a well-coordinated process in which different types of cancers tend to form metastases in defined organs. The formation of site-specific metastases requires full compatibility between the intrinsic properties of the tumor cells and the tumor microenvironment. It was recently found that chemokines which are expressed in specific loci promote the adhesion, migration and invasion of tumor cells that express the corresponding receptor(s). Of the different members of the family, the CXCL12 chemokine and its cognate CXCR4 receptor are the prototypes of this process, although other members of the family (e.g. CCR7 and CCR10) also play a role in determination of the metastatic spread. This commentary addresses the fundamental roles of chemokines and their receptors in site-specific metastasis, with emphasis on CXCL12-CXCR4. The article also describes some of the efforts that were performed thus far in order to identify the intracellular components involved in this process. The focus is put on the roles played by proteins that regulate adhesion and migration of tumor cells in response to CXCL12, including mainly focal adhesion kinase (FAK), Pyk2/RAFTK and members of the Rho family of GTPases (RhoA, Rac, Cdc42). This is followed by discussion of open questions that need to be addressed in future research, and of the potential therapeutic implications of the findings that are available to date in this field.

The metastatic spread of tumors is not random. While certain types of cancer preferentially metastasize to particular organs, others “favor” different remote sites for metastasis formation. To form metastasis, tumor cells have to successfully complete a defined set of events that requires adequate properties of the tumor cells and of the target organs.1-4 Of the different steps involved in this complex process, major emphasis was recently given to the events occurring at the target site, in which tumor cell arrest is followed by extravasation through the vessel wall, migration and invasiveness in the host organ, and finally by tumor cell proliferation and angiogenesis. Together, these steps enable the “seeding” of the cancer cells in distinct target organs and formation of site-specific metastases.1-4

Therefore, to succeed in the metastatic process, appropriate tumor cell properties need to join forces with a tumor-supporting microenvironment at the target site. In line with the “Seed and Soil” theory postulated by Stephen Paget more than a century ago, an ample number of studies now indicate that full compatibility between the tumor cells and their surrounding milieu at the target organ is essential for site-specific metastasis formation.1-3

The extensive research that was performed on the metastatic process has led to identification of pivotal microenvironmental factors that may dictate the failure or success of the metastatic cascade. In this context, the focus was put on members of the chemokine superfamily. Chemokines are low molecular weight proteins whose activities are exerted primarily in the immunological context, where they induce the migration of leukocytes in response to chemotactic gradients. Chemokines regulate leukocyte homing to lymphoid organs in the course of normal hematopoiesis (homeostatic chemokines) or promote the extravasation of leukocytes to damaged/infected sites in inflammation (inflammatory chemokines). As such, chemokines that are released at specific sites either constitutively (usually homeostatic chemokines) or inducibly (mainly inflammatory chemokines), promote the adhesive properties of leukocytes that express the corresponding receptors, and activate the motility apparatus of such cells. Due to their chemotactic properties, the chemokines are viewed as indispensable regulators of leukocyte homing to specific sites in the body, and of the immune integrity of the host.5-8

Concurrently with the identification of the roles played by chemokines in immunity and inflammation, it became evident that specific chemokines are expressed in organs that are preferential sites of metastasis formation. These observations raised the possibility that chemokines that are present at specific organs promote the adhesion and migration of tumor cells that express the corresponding receptor(s), by that supporting tumor cell invasion and the establishment of metastases at these specific loci. In such a case, the chemokines may promote the “seeding” process of cancer formation at the corresponding site.
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Focused on the chemokine CXCL12 which is expressed in lymph nodes, liver, lungs and bone, organs which constitute preferential metastatic sites in breast cancer. The study has demonstrated that breast tumor cells express CXCR4, the corresponding receptor for CXCL12 and that the chemokine induced migration and invasion properties in the tumor cells. Furthermore, by interfering with the intact activity of the CXCL12-CXCR4 axis, the authors have

cells in specific target organs and thereafter may also increase their ability to propagate at these sites. Such activities of the chemokines may thus contribute to site-specific metastasis formation, and may constitute an important determinant of the metastatic cascade.

Along this rationale, the study by Zlotnik and his colleagues was the first to establish a firm link between chemokines and site-specific metastasis formation.9 In their study, the researchers focused on the chemokine CXCL12 which is expressed in lymph nodes, liver, lungs and bone, organs which constitute preferential metastatic sites in breast cancer. The study has demonstrated that breast tumor cells express CXCR4, the corresponding receptor for CXCL12 and that the chemokine induced migration and invasion properties in the tumor cells. Furthermore, by interfering with the intact activity of the CXCL12-CXCR4 axis, the authors have

Figure 1. Chemokines and additional microenvironmental factors in site-specific metastasis. Chemokines are important contributors to site-specific metastasis formation and they constitute a part of a very complex microenvironment that interacts with tumor cells that have reached the potential metastatic sites. In this respect, only a full compatibility between the chemokine/additional microenvironmental factor (one or more) and the corresponding properties of the tumor cells would support a successful and full-blown metastatic process. (A) The microenvironment counterpart of the process: successful establishment of metastasis is dictated by factors that are expressed at the microenvironment of the potential metastatic site(s), chemokines and others. (#1) Full compatibility between the factors that are found at the potential metastatic site (chemokines such as CXCL12 and others) and receptors that are expressed by the tumor cells supports the successful establishment of metastases. (#2, #3) The lack of essential chemokine/s, or of other tumor-supporting factor(s) reduces the efficacy of metastasis formation, or leads to failure of this process, despite the fact that receptors for both are expressed by the tumor cells. (B) The tumor counterpart of the process: successful establishment of metastasis is dictated by the array of receptors that are expressed by the tumor cells. Although the potential metastatic site is enriched with the essential tumor-promoting chemokines/additional microenvironmental factors, the metastatic process can not reach its maximal potential or fails, because the tumor cells lack the expression of the required receptor(s) for these factors (or express non-functional receptors). (C) The microenvironment at the potential metastatic site is diverse and complex, including a large array of pro-malignancy factors [chemokine(s) and other(s)] whose activities complement each other, therefore together amplifying the metastatic process. (#1) A microenvironment enriched with all the potential tumor-supporting factors can interact with tumor cells that express functional receptors for all these factors, together leading to the most intensified levels of metastasis. (#2, #3, #4) A microenvironment that lacks one or more of the essential factors would not enable the formation of full-blown metastatic process, although the tumor cells express the required receptors.
shown that formation of metastases in preferred organs was significantly inhibited, indicating that this chemokine and its receptor significantly dictate the specificity of the metastatic spread of breast tumor cells.

In the years that followed, the above study was pursued by an extraordinarily high number of investigations, together establishing a new concept in the field of site-specific metastasis formation. Our current understanding of the metastatic process suggests a very important role to the chemokine-chemokine receptor axis, whereby chemokines that are produced at specific organs increase the adhesive, migratory and invasive properties of tumor cells that have reached these sites and express the corresponding receptor(s), by that promoting site-specific metastasis formation.\textsuperscript{10-16}

The initial observations that were made on the contribution of the CXCL12-CXCR4 pair in breast cancer were soon followed by the demonstration that CXCR4 is expressed by almost all cancer types, suggesting that the CXCL12-CXCR4 pair may be involved in site-specific metastasis formation in a large number of malignant diseases. Alongside with the CXCL12-CXCR4 axis, other chemokines and their receptors were implied in organ-specific metastasis: it was suggested that CCR7 expression by tumor cells facilitates lymph node infiltration by the cancer cells and that CCR10 is involved in skin-directed metastasis by melanoma cells.\textsuperscript{10-15}

The possibility that chemokines and their receptors contribute to site-specific metastasis has led to an extensive research in many cancer types. Not only that the expression of CXCR4 was detected in a large number of cancer types, in many of the cases CXCL12 induced adhesion, migration and invasion by the tumor cells. These in vitro findings suggested that CXCL12 promotes tumor cell activities that are required for the completion of steps that are essential for disease progression, leading to formation of metastases at specific organs. Indeed, such roles were confirmed for the CXCL12-CXCR4 pair in several of the tumor cell systems that were analyzed, by studies in animal model systems showing that impairment or induction of the activities of this axis significantly affected metastasis formation. Finally, the roles of CXCL12-CXCR4 in elevating site-specific establishment of metastases were substantiated in specific malignant diseases by clinical studies, correlating the degree of CXCR4 expression and its specific pattern of intracellular localization with formation of metastases at remote and specific organs in cancer patients. Together, these findings identified a key determinant that dictates the ability of specific tumor cells to establish metastases in a well-coordinated and site-directed process.\textsuperscript{10-16}

However, as attractive as the role of the CXCL12-CXCR4 pair in organ-specific metastasis may be, several ambiguities still remain. The major difficulties emerge from the tumor-wide nature of CXCR4 expression, and from the implications of this phenomenon. The many different cancer types that express CXCR4 and adhere/migrate/invasive in response to CXCL12 do not necessarily share the same metastatic pattern in cancer patients. Some form metastases in all or part of the organs that are enriched with CXCL12, while others establish metastases in other loci, in which CXCL12 is not a predominant constituent of the tissue. Obviously, it is possible that the CXCL12-CXCR4 pair acts alongside with other chemokine-chemokine receptor pairs, and that the end result is dictated by the equilibrium that exists in the target organs between different members of the family, acting on tumor cells that express the corresponding receptor/s. Therefore, the final consequence may reflect the activities of several chemokines and their receptors, including for example CCR7, CCR10 and/or CXCR7, the recently identified receptor for CXCL12.\textsuperscript{16,17}

However, based on the multifactorial nature of malignant diseases, it is quite obvious that chemokines and their receptors are not the only determinants of site-specific metastasis formation, and that intrinsic properties of the tumor cells other than chemokine receptors come into play, by responding to additional microenvironmental stimuli at specific body targets (Fig. 1).

The take home message is therefore that the roles played by chemokine receptors and their ligands in dictating the metastatic spread of tumors are important, but that they are one of several determinants that are involved in the site-specific metastatic process. The implications are mainly at the therapeutic arena, where the inhibition of the CXCL12-CXCR4 pair, for example, may prove inefficient in preventing the establishment of metastases. In addition, when the CXCL12-CXCR4 pair is considered as a test case, we need to clearly characterize the levels at which this pair acts, in order to identify potential targets for inhibition. One approach would be to inhibit the expression of CXCL12 and/or of CXCR4, by that taking the risk of impairing immune activities that are absolutely dependent on this pair. An alternative attitude would be to down-regulate the factors that induce CXCR4 expression by the tumor cells and/or the intracellular mechanisms leading to CXCL12-induced adhesive, migratory and invasive properties in tumor cells.

The latter approach is challenging because it demands precise understanding of the mechanisms that regulate the expression of CXCR4 and of the signaling pathways it induces in tumor cells, together providing the correct conditions for successful "seeding" of the tumor cells at the target organ. Although a number of studies have identified factors that induce CXCR4 expression by tumor cells,\textsuperscript{10-16} this avenue of research is just at its beginning. Furthermore, the molecular mechanisms that are induced by CXCL12 via CXCR4 are far from being elucidated, and only in a limited number of cases there is initial understanding of the intracellular mediators involved in tumor cell adhesion, migration and invasion in response to this chemokine.

Thus far, many of the different studies that addressed the mechanisms involved in CXCL12-induced adhesion/migration/invasion show that actin filaments are polymerized following stimulation by the chemokine. Beyond this point, most of the investigations were sporadic and have analyzed several potential regulators of adhesion and migration. These included integrins, focal adhesion kinase (FAK), Pyk2/RAFTK, paxillin, Yap and members of the Rho family of GTPases, including RhoA, Rac1 and Cdc42. The number of studies that looked in depth into these issues, and directly associated the activation of these elements with functional regulation of chemokine-induced tumor cell invasion, through increased adhesion and migration, is surprisingly small. This is mainly so when one considers the importance of this field of research and its potential therapeutic implications.
However, a more intensive and relatively informative research was performed on the mechanisms involved in CXCL12-CXCR4-induced adhesion/migration/invasion in two of the malignant diseases, breast cancer \(^9,18-23\) and melanoma \(^24-27\). The studies in these two systems have provided initial insights into the complex net of interactions that exists between the different proteins that coordinate processes of cell adhesion and motility in response to CXCL12. In breast cancer, the different studies provided evidence to the direct involvement of FAK, Pyk2 and phosphatidylinositol 3 kinase (PI3K) in CXCL12-induced migration of the tumor cells. \(^18-20\) In parallel, the studies by the group of Ganju have shown that CXCL12 upregulated the phosphorylation of a large number of proteins that are involved in formation of focal adhesions and in tumor cell motility: FAK, Pyk2, paxillin, Crk and Crk-L.\(^18,19\)

This group has also shown that PI3K was directly associated with the tyrosine phosphatase SHP2 and that SHP2 had an important role in the regulation of tumor cell chemotaxis. Combined with their observations regarding the roles of Cbl in control of motility, the authors suggested that stimulation of breast tumor cells by CXCL12 leads to migration processes that require FAK, Pyk2, PI3K, Cbl and SHP2. Furthermore, the authors suggested that Cbl, SHP2 and PI3K form a multimeric complex in response to CXCL12 stimulation, and that this complex is important for tumor cell motility.\(^19\) Since CXCL12 was also found to up-regulate matrix metalloproteinases (MMP) 2 and 9 in breast tumor cells,\(^19\) it is possible that the chemokine leads to increased tumor cell migration which is accompanied by matrix degradation, together supporting site-specific invasion and metastasis formation.

In parallel to the studies that were done in breast cancer, major efforts were put by Texido and his colleagues to decipher the mechanisms involved in CXCL12-induced metastasis formation by melanoma cells. This group has provided a sequential and well-designed series of studies on the mechanisms contributing to CXCL12-induced adhesion and migration of the tumor cells. These investigations focused mainly on the activation of RhoA, Rac1 and Cdc42, GTPases that control the dynamics of the actin cytoskeleton and serve as major regulators of cell motility. The authors have shown that CXCL12 triggered in melanoma cells the activation of RhoA, Rac1 and Cdc42, however only RhoA and Rac1 were directly involved in melanoma cell invasion in response to CXCL12.\(^24\) CXCL12-induced activation of RhoA and Rac1 has led to up-regulation of MT1-MMP expression, then giving rise to processing of pro-MMP-2 to mature MMP-2.\(^24,25\) Therefore, similar to the findings in breast cancer, the activation of melanoma cells by CXCL12 can increase cell motility and in parallel promote degradation of the extracellular matrix. Together, these two processes can give rise to increased invasion by the tumor cells, which is potently induced by the chemokine.

Further analyses by the same group have shed light on the mechanisms that activate RhoA and Rac in melanoma cells. Their findings demonstrated that stimulation of melanoma cells by CXCL12 triggered the activation of Jak, being an upstream event leading to Vav stimulation.\(^25\) The activation by CXCL12 induced the phosphorylation of Vav1 and Vav2, and Vav1 phosphorylation correlated with increased quantities of Rac, and to a lesser extent of RhoA. Moreover, interference with Vav1 and Vav2 expression in the cells impaired substantially the activation of Rac and RhoA in response to CXCL12 in the melanoma cells and inhibited tumor cell invasion.\(^25\)

Together, these findings indicate that activation of Jak leads directly or indirectly to Vav activation. Thereafter, Vav-induced activation of RhoA and Rac follows, promoting not only tumor cell motility but also degradation of basement membranes through the activation of MT1-MMP and than of MMP-2.\(^24,25\) This complex chain of events is tightly controlled and careful investigation of the RhoA-mediated pathway indicated that it is oppositely regulated by specific G\(\alpha\) proteins: The stimulation of melanoma cells by CXCL12 has led to coupling of G\(\alpha_13\) to CXCR4, followed by Vav-RhoA activation and stimulation of tumor cell invasion; On the other hand, activation of G\(\alpha_{12}\) by different measures gave rise to p190RhoGAP-mediated inactivation of RhoA, and to impairment of invasion,\(^26\) in a process not involving Vav regulation. Similar indications were also found for G\(\alpha_{15}\), where the expression of a constitutively active variant of this G protein induced defective RhoA activation.\(^26\) Therefore, these findings indicate that activated G\(\alpha_{13}\) and G\(\alpha_{15}\) trigger similar RhoA-related functional responses in melanoma cells, and play important roles in regulating the metastatic spread in melanoma.

The above findings suggest that it may be possible to inhibit the establishment of site-specific metastasis by targeting well-defined components that regulate tumor cell adhesion/migration/invasion in response to chemokines. Such selected intracellular molecules actually act as balancing factors, and their levels of expression and/or activities may dictate, at least to some extent the success or failure of metastasis formation. Accordingly, using again the CXCL12-CXCR4 axis as a test case, it was shown that breast tumor cell treatment by the tumor suppressor Slit has led to inhibition of breast cancer adhesion, chemotaxis and chemoinvasion.\(^18\) The activity of Slit was mediated by repression of FAK and Pyk2 phosphorylation, inhibition of PI3K and MAPK activation and reduced activities of MMP-2 and MMP-9.\(^18\) Also in breast cancer, it was found that the bisphosphonate Zoledronic acid (ZOL) inhibited the chemotaxis of tumor cells to CXCL12, in a process mediated by inhibition of RhoA and decreased expression of CXCR4.\(^21\) Based on their results, that authors suggested that the ZOL-induced reduction in RhoA activation has led to disorganization of the actin cytoskeleton, that was accompanied by a loss of stress fibers. These experiments in breast cancer manifest the potential strength of maneuvers that inhibit determinants that are required for completion of tumor cell invasion in processes triggered by chemokines via their receptors.

Another example for the potential use of approaches that are based on key molecules in chemokine-induced processes and of their potential use for limitation of metastasis formation was provided in melanoma cells. In this case, the researchers addressed the possibility that inhibition of stimuli that activate G\(\alpha_{13}\) in melanoma cells may reduce CXCL12-induced RhoA activation, and thus may limit the invasive properties of the tumor cells. Indeed, in this system the expression of a constitutively active form of G\(\alpha_{13}\) (G\(\alpha_{15}\)QL) in melanoma cells has led to inhibition...
of RhoA activation in the tumor cells, as well as to inefficient formation of stress fibers and reduced generation of focal contacts. Importantly, although the over-expression of G\(\alpha_{13}\)QL in the tumor cells did not affect the formation of primary tumors, it did lead to a substantial inhibition in lung metastasis formation and to prolonged survival of the mice.\(^{26}\) Together, the findings on Slit and ZOL in breast cancer, and on G\(\alpha_{13}\)QL in melanoma illustrate the potential therapeutic applicability of approaches that target specific components that act as determinants of adhesion, migration and invasion by tumor cells.

The therapeutic potential of such approaches requires a very precise identification of the mechanisms involved in the ability of chemokines to increase adhesive, migratory and invasive properties in tumor cells. Moreover, the current information available in this field suggests that the processes are complex and probably are tumor cell- and/or tumor type-specific. To give one example, RhoA was found to be important in activation of melanoma cell migration and invasion, and possibly also in breast cancer.\(^\text{21,24,25}\) However, such roles for RhoA are not trivial, since the regulatory mechanisms mediated by RhoA and signaling by members of the G12 family of heterotrimeric G proteins may differ in a variety of tumor cell types. Specifically, it was found that G12 proteins play in breast cancer opposite roles to those described in melanoma cells: G\(\alpha_{12}\) and G\(\alpha_{13}\) promoted breast tumor cell invasion, and G\(\alpha_{12}\) signaling was required for metastasis\(^{23}\) (but not for formation of primary tumors). Taken together with recent studies in prostate cancer, glioblastoma and Jurkat T cells, and with studies suggesting a role for G12-mediated signaling in RhoA activation,\(^\text{23,28-30}\) it is possible that the activities of this family of Rho GTPases and of heterotrimeric G proteins depend on the cellular milieu of the tumor cells, and therefore are tumor cell- and/or tumor type-specific. However, one should also consider the fact that the mechanisms may depend on the experimental system used (some of the above did not include stimulation by CXCL12 or any other chemokine) and whether stimulation by a chemokine was included and not, further emphasizing the need for more intensive research in this respect.

Overall, the above information illustrates the importance of chemokines and their receptors in site-specific metastatic dissemination and calls for improved characterization of the mechanisms by which they induce adhesion, motility and invasion by tumor cells, leading to site-specific metastasis. Therapeutic approaches that aim at the inhibition of specific intracellular molecular elements require very definite identification of the events occurring downstream of receptor triggering by the chemokine. It is essential to identify the full cascade of events that takes place and to keep in mind that the pathways may be different, or even opposite, in various tumor cells and in different malignancies. It is also very important to take the research one step further, and to elucidate by direct experimental means the roles of specific targets, and of their inhibitors in in vivo model tumor systems. It is also essential to determine whether in cancer patients there are associations between such elements and disease course and progression.

To conclude, the design of therapeutic approaches aimed at inhibition of chemokine-induced metastatic spread and localization depends ultimately on integrated and multidisciplinary research that is based on extensive and thorough experiments. Only such combined efforts may lead to improved understanding of basic mechanisms taking place in chemokine-mediated processes of site-specific metastasis, and to the development of better therapeutic means in the future.

References

1. Friedler JJ. The organ microenvironment and cancer metastasis. Differentiation 2002; 70:498-505.
2. Friedler JJ. The pathogenesis of cancer metastasis: the ‘seed and soil’ hypothesis revisited. Nat Rev Cancer 2003; 3:453-8.
3. Park CC, Bisell MJ, Barcellos-Hoff MH. The influence of the microenvironment on the malignant phenotype. Mol Med Today 2000; 6:324-9.
4. Gassmann P, Enn A, Haier J. Role of tumor cell adhesion and migration in organ-specific metastasis formation. Oncology 2004; 27:577-82.
5. Rot A, von Andrian UH. Chemokines in innate and adaptive host defense: basic chemokine grammar for immune cells. Annu Rev Immunol 2004; 22:891-928.
6. Bacon K, Baggioolini M, Bromeyer H, Horuk R, Lindley I, Mantovani A, et al. Chemokine/chemokine receptor nomenclature. J Interferon Cytokine Res 2002; 22:1067-8.
7. Sallusto F, Mackay CR, Lanzavecchia A. The role of chemokine receptors in primary, effector, and memory immune responses. Annu Rev Immunol 2000; 18:593-620.
8. Zlotnik A, Yoshie O. Chemokines: a new classification system and their role in immunity. Immunology 2000; 121: 127-1.
9. Muller A, Homey B, Soto H, Ge N, Carron D, Buchanan ME, et al. Involvement of chemokine receptors in breast cancer metastasis. Nature 2001; 410:50-6.
10. Zlotnik A. New insights on the role of CXCR4 in cancer metastasis. J Pathol 2008; 215:211-3.
11. Zlotnik A. Chemokines and cancer. Int J Cancer 2006; 119:2026-2029.
12. Ben-Baruch A. The multifaceted roles of chemokines in malignancy. Cancer Metastasis Rev 2006; 25:357-371.
13. Ben-Baruch A. Organ selectivity in metastasis: regulation by chemokines and their receptors. Clin Exp Metastasis 2008; 25:345-56.
14. Balkwill F. The significance of cancer cell expression of the chemokine receptor CXCR4. Semin Cancer Biol 2006; 16:71-7.
15. Wang J, Lobregt R, Taichman RS. The pivotal role of CXCL12 (SDF-1)/CXCR4 axis in bone metastasis. Cancer Metastasis Rev 2006; 25:573-7.
16. Fulton AM. The chemokine receptors CXCR4 and CXCR3 in cancer. Curr Oncol Rep 2009; 11:125-131.
17. Thelen M, Thelen S. CXCR7, CXCR4, and CXCL12: an eccentric trio? J Neuroimmunol 2008; 198:9-13.
18. Prasad A, Fernandis AZ, Rao Y, Ganju RK. Slit protein-mediated inhibition of CXCR4-induced chemotactic and chemoinvasive signaling pathways in breast cancer cells. J Biol Chem 2004; 279:9115-24.
19. Fernandis AZ, Prasad A, Band H, Klosel R, Ganju RK. Regulation of CXCR4-mediated chemotaxis and chemoinvasion in breast cancer cells. Oncogene 2004; 23:157-67.
20. Lee BC, Lee YH, Avraham S, Avraham HK. Involvement of the chemokine receptor CXCR4 and its ligand stromal cell-derived factor 1 alpha in breast cancer cell migration through human brain microvascular endothelial cells. Mol Cancer Res 2004; 2:327-38.
21. Denoyelle C, Hong L, Vannier JP, Soria J, Soria C. New insights into the actions of bisphosphonate zoledronic acid in breast cancer cells by dual RhoA-dependent and -independent effects. Br J Cancer 2003; 88:1631-40.
22. Holland JD, Kochetkova M, Akekawatchai C, Dottore M, Lopez A, McColl SR. Differential functional activation of chemokine receptor CXCR4 is mediated by G proteins in breast cancer cells. Cancer Res 2006; 66:4117-24.
23. Kelly F, Moeller BJ, Juneja J, Booden MA, Der CJ, Daaka Y, et al. The G12 family of heterotrimeric G proteins promotes breast cancer invasion and metastasis. Proc Natl Acad Sci USA 2006; 103:173-8.
24. Bartolome RA, Galvez BG, Longo N, Balleux F, Van Muijen GN, Sanchez-Mateos P, et al. Stromal cell-derived factor-1alpha promotes melanoma cell invasion across basement membranes involving stimulation of membrane-type 1 matrix metalloproteinase and Rho GTPase activities. Cancer Res 2004; 64:2534-43.
25. Bartolome RA, Molina-Ortiz I, Samaniego R, Sanchez-Mateos P, Butelov XR, Teixido J. Activation of Vav/Rho GTPase signaling by CXCL12 controls membrane-type matrix metalloproteinase-dependent melanoma cell invasion. Cancer Res 2006; 66:248-58.
26. Bartolome RA, Wright N, Molina-Ortiz I, Sanchez-Luque FJ, Teixido J. Activated G(alpha)13 impairs cell invasiveness through p190RhoGAP-mediated inhibition of RhoA activity. Cancer Res 2008; 68:8221-30.
27. Bartolome RA, Ferreiro S, Miquelina-Colina ME, Martinez-Prats L, Soto-Montenegro M, Garcia-Bernal D, et al. The chemokine receptor CXCR4 and the metalloproteinase MT1-MMP are mutually required during melanoma metastasis to lungs. Am J Pathol 2009; 174:602-12.

Chemokines in tumor cell adhesion, migration and invasion

The authors discuss the role of chemokines in tumor cell adhesion, migration, and invasion, and the potential therapeutic applicability of approaches that target specific components that act as determinants of adhesion, migration and invasion by tumor cells. They highlight the importance of chemokines and their receptors in site-specific metastatic dissemination and calls for improved characterization of the mechanisms by which they induce adhesion, motility and invasion by tumor cells, leading to site-specific metastasis. Therapeutic approaches that aim at the inhibition of specific intracellular molecular elements require very definite identification of the events occurring downstream of receptor triggering by the chemokine. It is essential to identify the full cascade of events that takes place and to keep in mind that the pathways may be different, or even opposite, in various tumor cells and in different malignancies. It is also very important to take the research one step further, and to elucidate by direct experimental means the roles of specific targets, and of their inhibitors in in vivo model tumor systems. It is also essential to determine whether in cancer patients there are associations between such elements and disease course and progression.

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28. Kelly P, Stemmler LN, Madden JF, Fields TA, Daaka Y, Casey PJ. A role for the G12 family of heterotrimeric G proteins in prostate cancer invasion. J Biol Chem 2006; 281:26483-90.

29. Lepley D, Paik JH, Hla T, Ferrer F. The G protein-coupled receptor S1P2 regulates Rho/Rho kinase pathway to inhibit tumor cell migration. Cancer Res 2005; 65:3788-95.

30. Tan W, Martin D, Gutkind JS. The Galpha13-Rho signaling axis is required for SDF-1-induced migration through CXCR4. J Biol Chem 2006; 281:39542-9.