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A need for stratification of metastasis samples according to secondary site in gene expression studies

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Abstract: Comparisons of gene expression profiles between primary tumors and metastasis have revealed genes that are implicated in metastasis formation. However, gene expression studies conducted on metastasis samples from the same primary site usually do not discriminate between different secondary sites. Although the change in the expression of number of genes is expected to be common to metastasis from the same primary but different secondary sites, herein the data that point to substantial differences are presented. Furthermore, the reciprocal communication between metastatic and host cells that is influencing these differences is outlined to emphasize the need for stratification of metastasis samples in gene expression studies.

Introduction

The importance of studying molecular events that lead to metastasis formation is reinforced by the fact that the primary cause of death for >90% of patients with cancer are metastasis at secondary sites (Fares *et al.*, 2020). Still, although the main frameworks of the metastatic cascade have been delineated, the many of the steps involved in metastatic process remain largely unexplored. Gene expression studies (microarrays, RNA-sequencing, RT-qPCR) comparing transcription profiles of primary tumors and corresponding metastasis added to the insights on the molecular events that could be driving invasion, intravasation, survival in the bloodstream, escape from the host immune system, extravasation, and growth at secondary sites–all being the steps of a metastatic cascade. However, majority of those studies do not stratify metastasis samples according to secondary sites. The most common metastasis sites differ according to the primary tumor and include bones, liver, lungs, brain, peritoneum, adrenal glands etc. Since those tissues differ profoundly, it is expected that metastasis from the same primary but different secondary sites are also substantially different by their characteristics including transcription profiles. This is because metastatic cells reciprocally communicate with host cells at a secondary site which is largely influencing their outgrowth, but also the cells, tissue and even physiology of the secondary site.

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In further chapters, this Viewpoint sheds a light on the question whether the gene expression studies that combine metastasis from the same primary but different secondary sites miss a substantial amount of information that is revealed only when they are stratified and, consequently, whether those studies could even provide results that are expected to be biased depending on the compilation of metastatic samples from different secondary sites.

How Extensive is the Communication between Metastatic and Host Cells?

The "seed pre-selection" concept suggests that cancer cells that are primed for metastasis at a certain site (e.g., bone) could be pre-selected among the heterogenous population of cancer cells by tumor stroma (Zhang *et al.*, 2013). This would suggest that metastatic cells from the same primary site could differ from the beginning of their journey to the secondary site. Additionally, after the metastatic cells leave the primary site, on the way to the secondary site they encounter different host tissues. During this route they need to evade the immune system and other obstacles to their survival. To effectively colonize the secondary site, among other specific changes, modifications in the expression of matrix metalloproteinase enzymes and adhesion molecules are expected. Although encounter with different tissues on the route to the secondary metastatic site influences cancer cells extensively, a substantial part of the envisioned differences in transcription profiles between metastasis from

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the same primary but different secondary sites are expectedly cultivated during dormancy and expanded after the metastatic cells escape dormancy. During dormancy, tumor cells need to prepare for growth in the host tissue using reciprocal communication with the host cells. Thus, tumor dormancy, could start the co-evolutionary process at the metastatic site that includes changes in metastatic cells themselves and in the host vasculature, the immune system and other host cells. The results of the continuation of this process are extensive changes in the host cells/tissue and, simultaneously, metastatic cells too. For example, prostate and breast cancer, which are among the most common cancers by incidence and mortality, have high affinity for forming bone metastasis which, in the case of metastatic disease, occur at frequency of 90% and 70%, respectively (Chen *et al.*, 2018; Wong *et al.*, 2019). During metastatic bone disease, the interaction of cancer cells with osteoblasts and osteoclasts leads to osteolytic, osteoblastic, or mixed bone response. Osteolytic response results in destruction of normal bone because of osteoblast inactivation and osteoclast recruitment and activation. Osteoblastic response is the deposition of new bone due to new bone formation with the absence of bone resorption. Osteoclastogenesis and bone resorption that are underlying mechanisms of osteolytic response are frequently started by the release of tumor-derived factors such as parathyroid hormone-related peptide or osteopontin which lead to bone degradation. For what happens afterwards, the term "vicious cycle" is usually used to denote that bone-derived growth factors and calcium that are released by resorbed bone stimulate skeletal tumor proliferation and lead to the recurrence of the whole process. It is obvious from this example that the communication between host and metastatic cells is so influential that it can lead to extensive changes in the host tissue that, in this example, can cause outcomes of bone pain and fractures.

Liver is another common secondary site for cancer metastasis. Primary cancers that form liver metastasis include colorectal, pancreatic, melanoma, lung, and breast cancer. The complexity of the local host tissue and metastatic cell communication arising from the number of different cell types that through this communication sustain metastatic cell growth is exemplified by the case of liver metastasis. In liver metastasis, these interactions play important roles in the engraftment, survival, and growth of the metastases. Various cells participate in this communication including liver sinusoidal endothelial cells, Kupffer cells, hepatic stellate cells, parenchymal hepatocytes, dendritic cells, resident natural killer cells and other immune cells like monocytes, macrophages and neutrophils (Tsilimigras *et al.*, 2021). Firstly, tumor cells enter the sinusoidal vessels and encounter the resident Kupffer cells, natural killer cells, and the liver sinusoidal endothelial cells which results either in tumor cell death or their extravasation in the perisinusoidal space of the liver. After extravasation, in a dynamic process, hepatic stellate cells, activated by Kupffer cells, enable endothelial cell migration which, in combination with VEGF released by tumor cells or activated Kupffer cells, promotes neovascularization. Although the primary role of the immune system is to eradicate tumor cells, its components can be recruited by cancer cells to support their own growth (Janssen *et al.*, 2017; Wu *et al.*, 2020). In a setting of the secondary liver cancer, innate and adaptive immune cells with tumor suppressing, but also tumor-promoting roles are recruited. These interactions can promote rapid tumor growth which is further potentiated by the interaction with hepatocytes and the growth factors that they release (Tsilimigras *et al.*, 2021). From this example, it is evident that the interplay between liver host and metastatic cells is highly entangled and involves many of the cells that are specific for a host organ and are not found in other tissue types.

Another example of metastatic cells recruiting many of the cells that are specific to the target tissue is taking place in the lungs. Common cancers that metastasize to the lungs include breast, colon, prostate, and bladder cancer. In lungs, even smoking exposure has the pro-metastatic effect, emphasizing the role of the local environment in metastasis formation. Metastatic cells growth in lungs is potentiated by many of the cells of the immune system and the local cells of the lungs which start signaling cascades that allow the establishment of immunotolerant niches which promote the growth of metastasis (Stella *et al.*, 2019).

To conclude, it is evident on the examples of bone, liver, and lung metastases, that reciprocal communication between metastatic and local host cells is extensive and largely influences the cells from the secondary site. Simultaneously, it makes foundations for the significant contribution of the local environment to the formation of the metastatic niche and subsequent potentiation of metastatic cells expansion.

Genomic and Transcription Profiles of Metastatic Cells Show Secondary Site-Specific Changes

In the previous chapter, influence of the metastatic cells on the local host tissue biology on the example of bones is briefly outlined. Additionally, their ability to recruit local cells in metastatic niches is exemplified by the liver and lung metastasis. But how do metastatic cells change in response to this crosstalk? One of the examples is the ability of prostate cancer metastatic cells to acquire an osteoblastic phenotype, termed osteomimicry. Osteomimicry is the ability of tumor cells to resemble resident bone cells (osteoblasts) by expressing bone matrix proteins. Through this shift toward an osteoblast-like gene signature they acquire ability to modulate bone cell crosstalk. This is an evasive strategy adopted by prostate cancer cells to disguise themselves in bone and to achieve metastatic cell survival (Furesi *et al.*, 2021).

Another example of changes reflected in metastatic cells genome and transcriptome that are a consequence of a specific, local communication with the host tissue comes from the field of brain metastasis. It is estimated that 20% of all patients with cancer will develop brain metastases (Achrol *et al.*, 2019). Most brain metastases occur in patients with lung, breast and colorectal cancers, melanoma, or renal cell carcinoma. In a similar way to liver and lung metastases, brain tissue also provides many of the local characteristics that are influencing metastatic cells. For example, when human cancer cells were xenografted into different organ sites of immunocompromised mice, transcriptomic data indicated that the brain microenvironment induced complete reprogramming of metastasized cancer cells which resulted in

a gain of neuronal cell characteristics (Park *et al*., 2011). Further to this, although clonally related primary tumor and brain metastasis pairs shared a common ancestor, a distinct evolution pattern occurred at the metastatic site. This was shown through whole-exome sequencing of matched primary tumors and brain metastases from a variety of solid tumors (Brastianos *et al*., 2015). Further studies showed that shared gene alterations common across brain metastases included genes involved in axonal guidance (Saunus *et al*., 2015).

A recent publication that compared metastasis samples from the same primary but from different secondary sites showed that prostate cancer metastasis from bones, lymph nodes and liver differ substantially in transcription profiles, although a change in a group of genes is shared. Generally, changes in gene expression that were site-specific grouped in gene ontology terms that are reminiscent of processes that take place in the target organ (Samaržija, 2021). This is in line with the report that showed the induced expression of genes that are physiologically associated with liver function in liver metastases. The authors hypothesize that this was likely a consequence of overshooting adaptation to the host site (Hartung *et al*., 2019). To further refine differences in prostate cancer bone metastasis, recent studies showed that even within bones, three different types of metastatic cells were recognized by differences in gene expression pattern, morphology, and clinical behavior. However, these differences were traceable back to the primary tumor (Thysell *et al*., 2019) which could possibly be explained by scenario in which all three types of bone metastasis go through same changes in the bone metastatic niche, but they all keep original differences. In a further study, the same group identified two proteomic phenotypic subgroups within bone metastases from prostate cancer patients. These subgroups were related to disease prognosis (Iglesias-Gato *et al*., 2018). This is another evidence that supports the need for stratification of metastatic samples in gene expression studies, since, like seen here, even metastasis from the same primary and secondary sites differ so profoundly that these differences could be even further explored with the purpose of improving treatment of metastatic prostate cancer (Thysell *et al*., 2019).

Among the groups of genes whose expression expectedly changes the most in cancer cells at the metastatic site are metabolic genes. To become compatible with the metabolic pattern of the surrounding tissue, the cancer cells need to adapt its own network of metabolic genes in the process of the metabolic adaptation of metastatic organotropism (Wang and Luo, 2021). In this way, the cancer cells most efficiently acquire energy, nutrients, metabolites, proper pH and adjust to the levels of oxygen that are all specific to the secondary site.

In the summary of this brief chapter, it needs to be emphasized that not only metastatic cells largely influence the host cells, but in this reciprocal communication they are also a subject to extensive, secondary site-specific changes that lead to their expansion.

Conclusions

Bone metastases underline the ability of metastatic cells to change extensively the host tissue. Additionally, on the example of liver and lung metastases, it is evident that metastatic cells can recruit many of the cells of the host tissue to promote their own expansion. Many of those cell types are secondary site specific. On the other hand, changes in the metastatic cells themselves that are result of this crosstalk, are extensive, which is exemplified by osteomimicry in bones or the adaptation of the transcription programs to mimic the processes of the target organ which is, for example, documented in brain and liver metastases. These brief outlines presented in this Viewpoint support the notion that communication between host and metastatic cells is very extensive and influential for the fates of both, the target tissue, and the metastasis. Because of the entangled relations in the metastatic niche, all the contributors to metastatic growth are influenced and co-evolve simultaneously. Although the studies showed that a substantial number of changes in gene expression are shared among metastasis from the same primary but different secondary sites, the data presented in this Viewpoint underline the importance of the documented differences for the expansion of metastatic cells within a niche. However, the extent to which metastatic cells change in response to secondary site host cells could be secondary-site specific. This means that it could be more pronounced for some metastatic niches (those in brain, for example) than the others. In any case, gene expression studies that would be designed in the way that they interrogate the differences in metastases instead of pooling metastatic samples from the same primary but different secondary sites would refine our knowledge on metastatic cells and processes. They would also potentially offer insights that could help in understanding of the biology of metastasis from different sites and contribute to efforts to find solutions for metastasis targeting. Although metastases are by far the most common cause of cancer-related deaths, cancer therapies are mainly designed in a way that they target primary tumor growth only, and little attention is given to pathways governing metastatic outgrowth (Weber, 2013). However, recent work emphasizes the importance of making therapeutic advances in treating metastases at all stages (Ganesh and Massagué, 2021). To achieve this, more thorough understanding of this multi-step process is needed.

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References

Achrol AS, Rennert RC, Anders C, Soffietti R, Ahluwalia MS et al. (2019). Brain metastases. *Nature Reviews Disease Primers* **5**: 5. DOI 10.1038/s41572-018-0055-y.

Brastianos PK, Carter SL, Santagata S, Cahill DP, Taylor-Weiner A et al. (2015). Genomic characterization of brain metastases reveals branched evolution and potential therapeutic targets. *Cancer Discovery* **5**: 1164–1177. DOI 10.1158/2159-8290.CD-15-0369.

Chen W, Hoffmann AD, Liu H, Liu X (2018). Organotropism: New insights into molecular mechanisms of breast cancer metastasis. *npj Precision Oncology* **2**: 4. DOI 10.1038/s41698-018-0047-0.

Fares J, Fares MY, Khachfe HH, Salhab HA, Fares Y (2020). Molecular principles of metastasis: A hallmark of cancer revisited. *Signal Transduction and Targeted Therapy* **5**: 28. DOI 10.1038/s41392-020-0134-x.

Furesi G, Rauner M, Hofbauer LC (2021). Emerging players in prostate cancer–bone niche communication. *Trends in Cancer* **7**: 112–121. DOI 10.1016/j.trecan.2020.09.006.

Ganesh K, Massagué J (2021). Targeting metastatic cancer. *Nature Medicine* **27**: 34–44. DOI 10.1038/s41591-020-01195-4.

Hartung F, Patil A, Meshram RJ, Weber GF (2019). Gene expression signatures of site-specificity in cancer metastases. *Clinical and Experimental Metastasis* **37**: 159–171. DOI 10.1007/s10585-019-09995-w.

Iglesias-Gato D, Thysell E, Tyanova S, Crnalic S, Santos A et al. (2018). The proteome of prostate cancer bone metastasis reveals heterogeneity with prognostic implications. *Clinical Cancer Research* **24**: 5433–5444. DOI 10.1158/1078-0432.CCR-18-1229.

Janssen LME, Ramsay EE, Logsdon CD, Overwijk WW (2017). The immune system in cancer metastasis: Friend or foe? *Journal for Immunotherapy of Cancer* **5**: 79. DOI 10.1186/s40425-017-0283-9.

Park ES, Kim SJ, Kim SW, Yoon SL, Leem SH et al. (2011). Cross-species hybridization of microarrays for studying tumor transcriptome of brain metastasis. *Proceedings of the National Academy of Sciences* **108**: 17456–17461. DOI 10.1073/pnas.1114210108.

Samaržija I (2021). Site-specific and common prostate cancer metastasis genes as suggested by meta-analysis of gene expression data. *Life* **11**: 636. DOI 10.3390/life11070636.

Saunus JM, Quinn MCJ, Patch AM, Pearson JV, Bailey PJ et al. (2015). Integrated genomic and transcriptomic analysis of human brain metastases identifies alterations of potential clinical significance. *Journal of Pathology* **237**: 363–378. DOI 10.1002/path.4583.

Stella GM, Kolling S, Benvenuti S, Bortolotto C (2019). Lung-seeking metastases. *Cancers* **11**: 1010. DOI 10.3390/cancers11071010.

Thysell E, Vidman L, Ylitalo EB, Jernberg E, Crnalic S et al. (2019). Gene expression profiles define molecular subtypes of prostate cancer bone metastases with different outcomes and morphology traceable back to the primary tumor. *Molecular Oncology* **13**: 1763–1777. DOI 10.1002/1878-0261.12526.

Tsilimigras DI, Brodt P, Clavien PA, Muschel RJ, D'Angelica MI et al. (2021). Liver metastases. *Nature Reviews Disease Primers* **7**: 27. DOI 10.1038/s41572-021-00261-6.

Wang C, Luo D (2021). The metabolic adaptation mechanism of metastatic organotropism. *Experimental Hematology and Oncology* **10**: 30. DOI 10.1186/s40164-021-00223-4.

Weber GF (2013). Why does cancer therapy lack effective anti-metastasis drugs? *Cancer Letters* **328**: 207–211. DOI 10.1016/j.canlet.2012.09.025.

Wong SK, Mohamad NV, Giaze TR, Chin KY, Mohamed N et al. (2019). Prostate cancer and bone metastases: The underlying mechanisms. *International Journal of Molecular Sciences* **20**: 2587. DOI 10.3390/ijms20102587.

Wu M, Ma M, Tan Z, Zheng H, Liu X (2020). Neutrophil: A new player in metastatic cancers. *Frontiers in Immunology* **11**: 565165. DOI 10.3389/fimmu.2020.565165.

Zhang XHF, Jin X, Malladi S, Zou Y, Wen YH et al. (2013). Selection of bone metastasis seeds by mesenchymal signals in the primary tumor stroma. *Cell* **154**: 1060–1073. DOI 10.1016/j.cell.2013.07.036.