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Transfer of malignant traits as opposed to migration of cells: A novel concept to explain metastatic disease

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ABSTRACT

Metastatic disease is believed to develop following dissemination of cells to target organs. Inability of this theory to effectively explain certain phenomena such as patterns of metastatic spread, late metastasis formation, different gene patterns between primary cancer and metastasis have brought forward the need for alternative models. Recent discoveries have strengthened the validity of theories supporting a humoral transfer of malignant traits as opposed to migration of malignant cells to explain metastatic disease in cancer patients.

In light of this new evidence, we would like to highlight a model that offers a new perspective to explain cancer metastasis. In the system that we theorize, genetic material released by cancer cells would travel, either free or packed in exosomes, through the blood. Target cells located in organs deriving from the same embryological layer might uptake this genetic material due to expression of specific receptors. Interplay with the immune system would determine the fate of these oncofactors and would regulate their ability to circulate in the blood, integrate in the genome and be transcribed. We also hypothesize that the expression of cell membrane receptors such as integrins, to which cancer exosomes ligate might be mediated by inherited or acquired oncosuppressor mutations.

Background

Metastatic disease is the leading cause of morbidity and mortality related to cancer and is generally believed to develop following dissemination of neoplastic cells to target organs (seed to soil hypothesis) [1,2]. The validity of this concept as the only method to explain metastasis has however been questioned due to several reasons including the inefficiency of the steps involved (separation from the primary tumor, intravasation, survival in the circulation, extravasation and successful colonization in the secondary organs) [3,4], the little number of circulating tumor cells (CTCs) (less than 0.1% cells remain viable and less than 0.01% of these surviving CTCs can produce metastasis) [5–7], long latency periods prior to overt metastasis formation [8], poor correlation between bone marrow micrometastases and their clinical manifestations [9] and gene expression patterns different between primary cancers and metastases [10].

If on one hand recent studies have highlighted the role of tumor micro-vesicles (TMV), oncosomes and cancer exosomes in facilitating the migration and engraftment of circulating cancer cells [11–13], on the other hand it has also been shown that such cargo entities might be involved in a horizontal transfer of malignant traits through incorporation of humoral factors released by primary tumors. This model of horizontal transfer would be independent of cell migration and would rely on factors carried in circulating microvesicles (such as nucleic acids, micro-RNA, mutated and amplified oncogene sequences and retrotransposon elements) and delivered to normal cells located in target organs, promoting the activation of survival and mitogenic signaling pathways, which eventually would allow these cells to acquire cancer cell characteristics [14,15].

Pioneering works done by Garcia-Olmo in 1999 described this mode of horizontal transfer of oncogenic traits and called it the "genometastasis hypothesis" [16,17]. Proponents of this theory demonstrated that immortalized mouse fibroblast cell lines (i.e. NIH3T3 cells that are p16INK4a/p19ARF deficient) and immortalized human cell lines (HEK293) would acquire malignant traits after exposure to cancer patients' sera [16–20].

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Recently our group reported, for the first time, on the successful transformation of BRCA1 knocked out (BRCA1-KO) fibroblasts into colon cancer cells, pancreatic cancer cells [18,21], ovarian cancer and hepatocellular carcinoma (unpublished data) after exposure to sera of patients with the above mentioned cancers. This discovery strengthens the notion that metastases might not be exclusively due to cancer cells dissemination but may actually be a process reproducible in primed cells, located in target organs, through the incorporation of key factors released by the primary tumors. The effect was reproduced also when the BRCA1-KO fibroblasts were exposed to exosomes isolated from the serum of patients with cancer, confirming the important role of cargo entities such as exosomes in delivering the cancer traits [21].

Hypothesis

We hypothesize that metastatic disease might occur via transfer of malignant traits from the primary tumor to primed cells located in organs deriving from the same embryological layer in which the primary cancer occurred. The different stages of carcinogenesis such as initiation, promotion and progression might not represent events limited to the cells forming the primary tumor, but may actually be a process reproducible in primed cells, located in target organs, through the incorporation of key factors released by the primary tumor. In order to be receptive, the target cells must carry a transformation-predisposing hit (i.e. mutation in an oncosuppressor or oncogene), which would favor the incorporation of these onco-factors. Based on our previous work [21], we speculate that the oncosuppressor genes might protect the integrity of the cell genome not only by repairing DNA damages but also by blocking foreign material uptake at the level of the cell membrane with subsequent DNA integration of cancer-derived factors. This onco factors would preferentially target cells located in organs deriving from the same embryological layer. We speculate that the immune system might regulate the trafficking of these factors and might inhibit their transcription when they get integrated in the genome of target cells. Failure of this control mediated by the immune system might be responsible for late metastases.

Evaluation of the hypothesis

Oncosuppressor as gatekeepers

The oncogenic potential of cancer patient sera on either immortalized cells or single oncosuppressor mutated cells has already been demonstrated in several studies performed on both murine and human cells [16–19,21]. These cells incubated with either sera or media from cancer cells displayed oncogenic properties, such as increased proliferation, enhanced anchor-independent growth in soft agar and formation of tumors after subcutaneous injection into NOD/SCID mice. This malignant transformation seems to be secondary to the transfer and delivery of circulating genetic material which either free or packed in cargo entities such as exosomes or TMV bodies may potentially act as an endocrine or paracrine messenger, able to affect the genome of recipient cells [22]. It was also shown that normal cells are refractory to the transforming potential of cancer patient serum, confirming the concept elucidated already by Knudson that target cells must be first primed or initiated to undergo transformation [18,19,21,23]. The "initiation" represented by the mutation of an oncosuppressor gene, would be a prerequisite for target cells to be able to integrate key genes, shed by the primary tumor, and become susceptible to the effect of the factor(s), circulating in the bloodstream of patients with metastatic cancer. The integration of these factors would trigger a cascade of events that eventually would lead to the malignant transformation of the target cells [21].

Clinical data show that epithelial cells with a single mutation form adenomas only and further accumulation of mutations is necessary in order to induce carcinogenesis [24,25]. This evidence supports our hypothesis that a single oncosuppressor mutation might be the predisposing factor that allows uncontrolled access of mutating elements into the cells. In other words, we speculate that cells with normal genome might behave as a closed system.

Tumor suppressor genes function to restrain inappropriate cell growth and division, as well as to stimulate cell death to keep cells in proper balance [26]. In addition, some of these genes are involved in DNA repair processes, which help prevent the accumulation of mutations in cancer-related genes [27–29]. The evidence that cells with normal genome don't undergo malignant transformation when exposed to cancer patient sera as opposed to cells with a single oncosuppressor mutation [16–19,21] in which an increased exosomes uptake has been confirmed, paves the way to fascinating hypothesis on a potential unknown function of the oncosuppressor genes.

We think that oncosuppressor genes might protect the genome of the cells by impeding the uptake of foreign circulating genetic material at the level of the cell membrane. We hypothesize that when oncosuppressor genes are not functioning properly, the cells might express some membrane proteins or receptors that would allow cancer exosomes to enter the cells, deliver its genetic cargo and damage the genome. Unpublished data from our research laboratory seems to confirm this notion. We have verified that knocking down the *BRCA1* gene in human fibroblasts causes expression of new receptors such as alpha4/beta6 integrin complex, galectin, tetraspanin and epiplakin, which are not normally expressed in non mutated fibroblasts. The alpha6 beta4 integrin complex is associated with aggressiveness and invasion and it is thought to be one of the main mediators in the process of carcinoma invasion and metastasis [30–32]. We think that these molecules might constitute some of the receptors to which the exosomes might ligate to deliver their cargo inside the cells. Current experiments are being performed to study this hypothesis and verify its validity.

Analysis of cancer exosomes uptake between normal human cells and cells with either *p53* mutation or *BRCA1* suppression has shown a higher uptake (three to ten fold) in oncosuppressor mutated cells. Furthermore, we have observed that these exosomes transfer genetic material that transit efficiently to the nuclei corroborating the hypothesis that malignant genetic material can penetrate the nuclei of target cells through the exosomes and integrate in the cell DNA when oncosuppressor genes are not functioning properly [18,21,33].

Exosomes, which were reported to predominantly contain RNA and proteins have been found to also contain >10-kb fragments of double-stranded genomic DNA, mutated cancer genes such as *p*53 and KRAS, and genomic DNA spanning all chromosomes [34,35]. They have been shown to possess the ability to integrate into target cells and transfer also viral infections [36]. Furthermore, they have been shown to possess replicative capabilities that allow a self-replication of the genetic material inside the exosomes itself [37]. These distinctive features of the exosomes make them reasonable candidates for the transfer of malignant traits from cancer cells to target organs.

Role of the immune system

This concept of foreign genes integration is not novel in the biological field. DNA and RNA viruses are well known for their capability to integrate in the genome of cells (i.e. Herpes virus, Human papilloma virus, Epstein Barr virus, Hepatitis B and C virus) and

induce carcinogenesis [36,38]. In our opinion, genetic material released by cancer cells either free or packed would act essentially like viral genetic material and interact with the immune system. This interplay between cancer genes and immune system would eventually determine the outcome of the neoplastic process.

We speculate that cancer genes once released by the primary cancer, travel, packed in exosomes, to the lymphatics before entering the systemic circulation. At the level of the lymph nodes, exosomes interact with the lymphoid cells. The interplay between exosomes and lymphoid cells determines either destruction of the cancer genetic material or induces tolerance of the immune system to these oncofactors. If tolerance ensues, exosomes might be able to penetrate the cells, integrate in the genome of the lymphoid cells and determine malignant transformation of the lymph node. Following tolerance, the exosomes would be able to enter the systemic circulation and travel trough the body. Owing to the tolerance acquired, the immune cells might not be able to detect and clear the exosomes, which eventually would penetrate into the target cells and integrate in their genome. Once integrated, the cancer genes might be either expressed, determining the malignant transformation of the cell, or remain silent and get activated later in time, determining the phenomenon of late metastases (Fig. 1).

This hypothesis provides a more elegant and more convincing explanation for late metastases than current theories based on the conceptual model of dormant malignant cells generated through experimental studies. The notion of a malignant cell able to suppress its unregulated proliferation for an indefinite time has been difficult to validate in vivo, leaving essentially unsolved the pathways and the mechanisms involved in the occurrence of metachronous metastases [39].

The mechanism of integration of cancer genes that we propose, mirrors what it has already been observed, both in vitro and in vivo, in infections caused by viruses such as the Varicella Zoster Virus (VZV) or Herpes Simplex Virus 1 (HSV-1). These viruses are able to establish a life-long latent infection punctuated by periods of virus recrudescence following a decline of the T cell-mediated immunity. Lack of control from the immune system would cause reactivation of the integrated viral genes with active replication of the virus and cell infection even decades after the integration in the nervous ganglia [40–43]. A similar mechanism might be involved in late cancer metastasis and cancer genes once integrated in the genome of target cells might remain silent and be expressed later in time due to a failure of not yet defined homeostatic mechanisms, probably involving the immune system.

The concept that lymph nodes might be hallmarks of generalized neoplastic disorder rather that reservoirs of travelling cancer cells has gained popularity lately due to new evidence that lymph node dissection might not improve overall survival as previously thought and their complete surgical evacuation has little if no impact on the life expectancy of patients [44–47]. These findings, in our view, corroborate our hypothesis that presence of cancer cells in the lymph nodes suggests a mechanism of tolerance that the body has acquired rather than a reflection of the burden of disease.

Furthermore, recent evidence has induced a paradigm shift about the role of immune cells during malignant progression. Whereas the historical viewpoint was that host immunity is protective with regards to cancer, it is now clear that certain subsets of chronically activated innate immune cells promote growth and/or facilitate survival of neoplastic cells [48]. Several studies indicate that limiting or altering the presence of harmful innate immune cells in pre-malignant tissue minimizes oncogene-induced primary cancer development and metastasis [49–51].

The role of B cells as critical adaptive immune cells necessary for innate immune cell infiltration, activation, and responses downstream of oncogene expression has been shown in neoplastic skin. Humoral immunity might play an immunomodulatory role and exert its effect distally via production of soluble mediators that may regulate cancer development via altering circulating cytokine

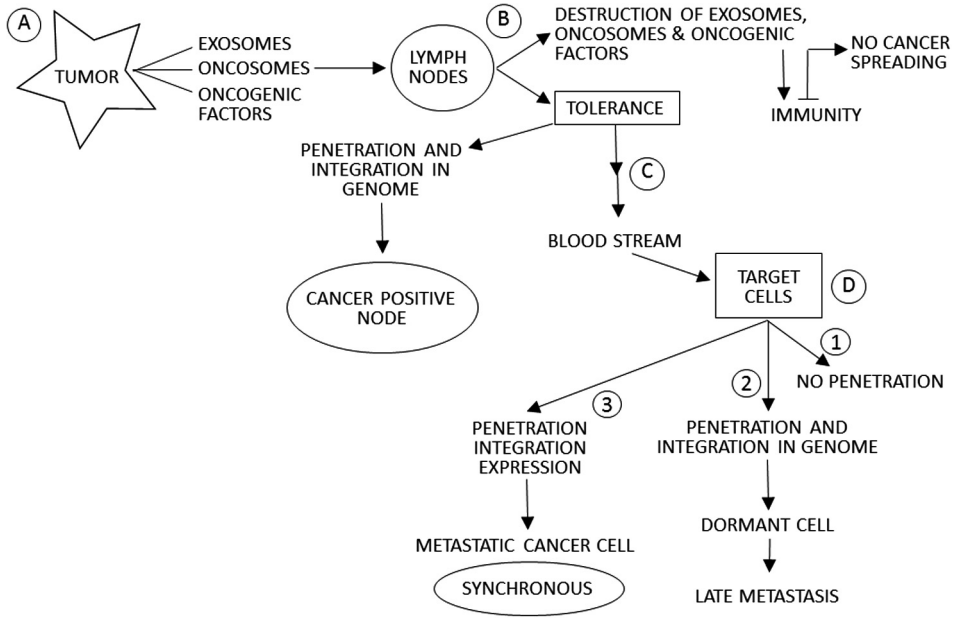

Fig. 1. The Humoral Transfer of Malignant Traits Model. A) The primary tumor releases factors, which travel through the lymphatic system into the regional lymph nodes. B) In the lymph node the cancer factors might be either destroyed by the lymphoid cells with development of immunity and inhibition of the metastatic process or tolerance might ensue with subsequent uptake, integration of cancer genes in the genome of the lymphoid cells and transformation of lymphoid cells into cancer cells. C) Once tolerance is established oncofactors can freely travel through the bloodstream undetected by the immune system. D) Cells located in organs deriving from the same embryological layer may 1) not express specific receptors with subsequent failure of the uptake, 2) may express specific receptors with consequent penetration of the oncofactors in the cell and integration in the genome with no expression. A decline of the immune function later in time would cause reactivation of the integrated genes with malignant transformation of the cell and late metastasis, 3) oncofactors would be uptaken by the target cells. Integration and immediate transcription of the cancer genes would determine synchronous metastasis.

and/or chemokine profiles/levels [48,52]. The immune system can destroy tumors, and yet paradoxically also promotes and sustains cancer and the rules for these choices are unclear [52]. In light of this data, we speculate that the interaction between oncofactors such as exosomes or TMV and lymphoid cells located in the lymph nodes might determine the choice of action that the immune system will adopt when exposed to neoplastic cells. Tolerance to cancer factors might induce activation of specific pathways and integration of cancer genetic material in the lymphoid cells with subsequent malignant transformation of the lymph node. This event will indicate a specific anergy towards cancer genetic material, which will flow freely through the bloodstream and cause transfer of malignant features to receptive cells. A different scenario might be observed if the interaction between cancer factors and lymphoid cells triggers an immune reaction, which would eventually lead to destruction of the oncofactors, development of humoral/cellular immunity with subsequent inhibition of oncofactors uptake at distance (Fig. 1).

The strongest element supporting the conventional model of metastasis is the immunohistochemical similarity between primary cancer cells and the metastatic deposits. However microarray analyses have revealed that the gene expression patterns of primary breast tumors differ from those of their respective lymph node metastases, and that a set of genes exists that is characteristically changed in all of these metastases when compared with their primary tumors [53]. Moreover, some of the CSCs that have been detected in the bone marrow of patients with metastatic breast cancers have shown phenotypical features more in keeping with a possible origin from the bone marrow than an origin from the breast tissue [54,55]. These discrepancies strengthen the notion that similarity does not necessarily imply sameness and metastatic cells although alike to primary cancer cells might not be necessarily deriving from the replicative process of a malignant cell clone.

The novel evidence that BRCA1-KO fibroblasts when exposed to different cancer patients' sera turn their fate and acquire a malignant phenotype, perfectly compatible with the cancer phenotype of the patients [21], is the definite proof that metastases can be reproduced also with transfer of humoral factors. In light of this evidence, the exploration for alternative pathways to explain the metastatic process not only seems rational but also necessary.

Metastatic organ tropism

The pattern of cancer spreading although seems logical and appears to follow routes dictated by anatomical paths, in some types of cancers, the tropism of metastases is hard to explain with the seed to soil model. Anatomy and physiology of the hematologic and lymphatic systems alone hardly explain why melanomas are particularly prone to metastasize to the spleen, brain and meninges or prostate cancer as well as breast cancers are bone-seeking malignancies whereas colorectal cancers are not [56]. Proponents of the seed to soil theory attribute this selective homing to host microenvironment factors, such as local cytokines, adhesive interaction mediated by selectins and integrins and chemokines, which might be responsible for this erratic spread pattern [57].

We speculate that humoral factors produced by cancer cells might have an organ tropism, which follows an embryologically determined route rather than a hematologic or lymphatic path of transmission. As a consequence of that, cancer factors would preferentially be uptaken by cells located in organs deriving from the same embryological layer. Cells deriving from the same embryological layer might express receptors not normally expressed in cells deriving from different embryological layers, which underwent specific gene silencing and different membrane protein expression in the earliest stages of embryogenesis [58]. Cell adhesion molecules expressed by exosomes may guide organotropic metastasis. In this context, combinations of exosome-bound integrins could determine the site of exosomes homing and subsequent delivery of their cell-induced transformation cargo [59,60].

This concept would explain why skin cancers like melanomas have the tendency to metastasize to brain, meninges or adrenal glands, which all derive from the ectoderm. In the same line of thinking, this hypothesis would explain why prostate cancer and breast cancer, which are partially mesodermal in origin, would metastasize preferentially to the bone, which derives from the mesoderm.

Conclusion

Transfer of malignant traits through the blood is a fascinating model that merits further study. Recent experiments have strengthened the validity of this alternative theory, which has the potential to unveil different mechanisms and pathways involved in the metastatic disease. Identification of the oncofactors involved, clarification of the mechanisms behind the uptake of these substances as well as the mechanisms implemented for their integration in the genome is the next challenge and it should be the focus of future research in this field. Elucidation of the molecules and receptors involved in these steps will lead to new therapeutic strategies that might have the potential to reverse the metastatic process, which is ultimately the main cause for cancer related mortalities.

Conflict of interest statement

The authors declare no conflict of interest.

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