

Cell Clusters in Cancer Metastasis: A Mini Review

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Abstract: Evidence is accumulating that suggests that cancer cell clusters are more likely to lead to metastatic lesions than are single cells. This mini-review explores this issue by examining evidence that indicates that cell clusters possess altered genes and metabolism that promote metastasis. When clusters are disaggregated, the resulting single cells often lose the altered properties characteristic of when they were in clusters. When cluster disrupting agents were injected into mice, metastasis was reduced. Cluster disaggregating agents are described and mechanisms of action of these compounds are explored. Using these agents to target cell clusters might offer a useful therapeutic approach in the treatment of cancer. Cell clusters are also involved in thrombocytosis and biofilm infectivity as well as cancer spread and cluster disrupting agents might be very useful in treating some human conditions in addition to cancer spread. Assays that examine the effectiveness of cluster disrupting agents are also described.

Keywords: Clustered Cancer Cells, Cancer Metastasis, Cluster Disrupting Agents

1. Introduction

The cellular basis of cancer metastasis is not well understood but many studies, some involving cell clusters in the metastatic process, have been reported for decades [1]. Exciting work has been more recently done exploring the issue of what is more likely to give rise to metastatic lesions, single circulating cancer cells or cell clusters [2-13]. Detailed investigations that quantitatively assess the likelihood of single cancer cells versus clusters of cancer cells in causing metastatic lesions are described [2]. The molecular characteristics of cell clusters versus single cells are compared and how these characteristics change when clusters are disaggregated is discussed [2-13]. This mini-review will explore the issue of cell clusters as causes of metastatic lesions and new therapeutic approaches for treating cancer based on this concept are discussed. Cell cluster disrupting agents and their mechanisms of action are described [14-16].

2. Experimental Procedure

Experiments will be presented that utilize cell culture, assays that identify compounds that disaggregate cell clusters, kinetic assays that measure the effects of cell cluster

disrupting agents, *in vivo* injections in mice, metabolic and DNA methylation assays, and statistical evaluations of results.

3. Results and Discussion

In experiments with mice Aceto, et al. [2] calculated that a circulating cancer cell cluster was about 50 times more likely to give rise to a metastasis than a single cell. They found in 323 metastases 171 were derived from clusters while 152 were derived from single cells. They demonstrated this using antibodies that could distinguish between metastases derived from clusters versus single cells. The 50 times figure is based on the finding that clusters were much rarer than single cells [2]. Numerically 2.6% cell clusters (65) versus 97.4% (2486) single cells indicated that clusters were much rarer than single cells (2). These figures documented the calculation of the rarity of cell clusters in causing metastatic lesions. This calculation is the basis for the widely reported contention that cancer cell clusters yield more metastatic lesions than single cancer cells, opening up possibilities for targeting cancer cell clusters as a therapeutic approach for treating cancer.

It is unclear how cancer cell clusters lead to more metastases than single cells. There are a number of possibilities. One study suggests that clusters might produce

less of cell damaging reactive oxygen species, such as peroxides, superoxides, hydroxyl radicals, singlet oxygen and alpha oxygen [3]. Plakoglobin and keratin 14 are more highly expressed in clusters compared to single cells [4] and claudin-11, a protein in tight junctions, was expressed in cell clusters, while E-cadherin, also expressed in clusters, might support cluster survival during metastasis [5].

Cell clustering results in DNA hypomethylation and hypermethylation of specific sites involved in cell proliferation [6]. When clusters were dissociated into single cells the methylation profiles reversed [6]. In some cancers cells in clusters are connected by plakoglobin and CD44 [7, 8], while in others claudin 11 helps cells in clusters adhere [9]. Increased cell-cell attachments are proposed to help cell clusters maintain metastatic properties. Keratin 14 is highly expressed in clusters. Suppression of keratin 14 expression resulted in reduced metastasis. It is believed that suppression of keratin 14 disrupts the activity of genes such as tenacin C, Jagged 1, and epiregulin [4]. Cancer cell clusters also overexpressed transcription factor XBP1, protein disulfide isomerase AGR2, epidermal growth factor receptor HER 3, inhibitor of matrix metalloproteinases TIMP-1, plasminogen activator SERPINE 1/PAI-1, antiapoptotic factor BCL 2 and other genes [10-13]. Clustered breast cancer cells hypomethylate OCT4, SOX2, NANOG and SIN3A genes involved in cell proliferation, while cell clusters hypermethylate polycomb genes implicated in chromatin changes and blocking of cell differentiation [6]. Cells in clusters may be more protected than single cells from the hostile environment in the blood [1], based on their location.

Many compounds were tested on cell clusters. Of 2486 compounds most had no effect on cluster size [6]. Two families of compounds decreased mean cluster size of human breast cancer cells in culture. These included the sodium/potassium-ATPase inhibitors digitoxin and ouabain and tubulin binding agents vincristine sulfate, rigosertib, colchicine, and podofilox [6]. Treating cell clusters with digitoxin or ouabain caused their dissociation while maintaining cell viability and allowing cell proliferation and changes in DNA methylation. Increased intracellular calcium concentration, caused by these compounds occurred, that appeared to lead to an inability of the cancer cells to form functional cell-cell junctions [6, 14, 15]. This appeared to be a mechanism of action of digitoxin and ouabain [6, 14, 15].

The compounds that dissociated cell clusters *in vitro* were tested *in vivo*. Oubain treatment reduced cell clusters, increased single cell numbers and reduced metastatic lesions in mice [6]. Oubain and digitoxin are normally used in the treatment of hypotension and cardiac arrhythmias by inhibiting sodium/potassium ATPase [14-16]. These compounds may be useful in reducing metastasis in humans.

Two recent papers reported in this journal [17, 18] provide a different approach to disaggregate cell clumps. Using a kinetic assay that examines cells and cell clumps from 0 time to 60 min, two reagents were found that disaggregate fixed yeast cell clumps, sodium citrate [17], a known human anticoagulant [19] and sodium sulfate [18]. While fixed yeast

are not cancer cells, their surface properties are similar to live yeast and human cells [20]. This fixed yeast model could identify unclumping reagents that may be useful in treating human cancer as well as thrombocytosis and biofilm infectivity [17, 19, 21]. Thrombocytosis can lead to heart attacks and strokes and biofilms can lead to major infections. Steve Oppenheimer, a Fellow of the American Association for the Advancement of Science, author of this mini-review, received a U.S. Presidential Award from President Obama at the White House, for mentoring the research of hundreds of students whose career outcomes were well documented [22]. This fixed yeast kinetic assay helped facilitate the research of hundreds of students.

4. Conclusions

This mini-review provides evidence that cancer cell clusters give rise to metastases at a higher frequency than do single cells and that cancer cluster disrupting agents may offer a useful approach for treating spread cancer.

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