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REVIEW

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Drug Development Against Metastatic Cancers

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While combinational diagnostic and treatment strategies over the past decades have significantly improved the overall survival of cancer patients, metastatic cancer remains a leading cause of death in developed countries. The lack of successful treatment strategies for the disease is in large part due to the complexity of the metastatic transformation, which embodies extensive cellular and extracellular alterations, enabling metastatic cancer cells to reach and colonize other organs. The mode of action for the majority of anti-cancer drugs used in clinics today is primarily tumor growth inhibition. While they are effective in destroying cancer cells, they fall short in blocking metastasis. Here we discuss the evolution of past and current anti-cancer drug development, the limits of current strategies, and possible alternative approaches for future drug development against metastatic cancers.

INTRODUCTION

Modern drug development has been successful in treating diseases with well-understood mechanisms, such as bacterial infections and hypertension. In these cases, targeting specific factors to generate agonists or antagonists for or against the specific functions can cure diseases or alleviate the symptoms of diseases. The successes of these types of drugs have significantly improved the lifespan of people around the world. In comparison, there remain few options for patients with metastatic cancers. The intense research and drug development effort over the past few decades have yet to successfully produce chemotherapeutics that effectively and specifically inhibit metastasis. Perhaps it is time to consider alternative approaches.

THE BRIEF HISTORY OF ANTI-CANCER DRUGS DEVELOPMENT

Anti-cancer drugs became a subject of intense research after World War II. The early drugs used were based on exposure observations without having a precise understanding of the mechanisms of action. For example, nitrogen mustard [1] was used and found effective in treating non-Hodgkin's lymphoma [2], initially based on the observation of lymphatic suppression of soldiers who died from an accidental release of stockpiled mustard gas during World War II. Subsequent retrospective studies identified the mode of action to be alkylation by irreversible binding to the alkyl group on DNA [3-5]. Since then, a number of alkylating compounds with stronger potency have been developed [6]. Another example is the discovery and improvement of anti-folate agents for cancer treatment. The idea was derived from the observation that folates, such as vitamin B9, enhanced the proliferation of acute lymphoblastic leukemia (ALL†) [6]. Based on this observation, methotrexate, a potent dihydrofolate reductase inhibitor, was developed to treat a range of cancers [7-9] and remains in clinical use today.

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†Abbreviations: ALL, acute lymphoblastic leukemia; ABL, Abelson Murine Leukemia oncogene; BCR-ABL, Breakpoint Cluster Region- Abelson Murine Leukemia; EMT, epithelial-mesenchymal transition; EGFR, Epidermal Growth Factor Receptor; VEGFR, Vascular Endothelial Growth Factor Receptor; CRISPR, Clustered regularly interspaced short palindromic repeats; PNC, Perinucleolar compartment.

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Subsequent waves of genome-toxic and cytotoxic drugs developed from natural products or synthetic chemistry aimed to contain the uncontrollable growth of cancer cells. The modes of action of these drugs include DNA damage, cell cycle blockage, and apoptosis stimulators, etc. [6]. Some of these drugs are still key chemotherapeutics used today as adjunct treatment for a wide range of cancers. While the strategy of killing cycling cells has markedly improved cancer patient survival, the indiscriminate nature of cytotoxicity also generates serious undesirable side effects for patients and increases the risks of secondary cancers [10,11]. Many of these compounds are carcinogens themselves, particularly the genome toxins, which induce DNA damage and genome instability.

Over the past three decades, extensive research in cancer biology has revealed many factors that could be involved in the transformation of normal cells into cancerous ones. These include factors in signaling cascades that regulate the cell cycle, growth, extracellular attachment, and cell mobility and invasion [12]. Gene and gene products that either enhance or suppress tumor cell growth have been identified and characterized. These understandings provide the basis for the development of drugs that target specific factors believed to play key roles in cancer cell growth. These undertakings led to a generation of new target therapeutic drugs, which have advanced into the clinic over the past decade. One of the first drugs developed was imatinib mesylate, an inhibitor of ABL tyrosine kinase [13]. It shows efficacy in treating chronic myeloid leukemia [14,15], caused by a chromosome translocation (Philadelphia chromosome) [16] that generates the BCR-ABL fusion protein [17]. Subsequently several other drugs that inhibit the EGFR receptor [18] or the angiogenic ability of tumors [19,20] became available. Unfortunately, these drugs are only effective against tumors whose growth is dependent upon the targeted signaling process. For example, only 10 to 15 percent of non-small cell lung carcinoma patients show partial remission when treated with gefitinib, an EGFR tyrosine kinase inhibitor [18,21]. In addition, these drugs quickly become tolerated by tumors as remaining cancer cells with pre-existing or acquired mutations of the targeted factors become resistant to the treatment [6].

THE DIFFICULTIES OF DRUG DEVELOPMENT AGAINST METASTASIS

There are at least two types of cellular populations in a cancer, non-metastatic and metastatic cells. Tumors that are entirely composed of non-metastatic cells are commonly considered benign. Tumors that contain both metastatic and non-metastatic cells are malignant. The growth of the primary tumors with both types of cells often do not pose major health threats except for those growing in sensitive and restrictive organs, such as the brain. The current management of cancer patients combines multiple approaches, including surgery, stem cell transplantation, precision medicine, radiation, hormone-mediated, targeted, immunotherapy, and chemotherapy. Nearly all approaches entail removing or killing the cancer cells to control the tumor growth and to minimize the number of cells capable of metastasis. Stimulation of the immune system serves the similar purpose of reducing the net number of these cells. Together with early and molecular detection, these approaches have helped to improve overall cancer patient survival. However, the incomplete elimination of cancer cells allows opportunities for the cells capable of metastasis to metastasize to other organs, leading to lethality. Therefore, therapeutics that could selectively block metastasis will be very helpful, particularly when used with existing treatment options to limit the lethal cancer metastasis. Why then is the treatment of metastatic cancer such a difficult challenge? The main reasons are the complexity of the disease mechanism and a lack of full understanding of the key responsible pathways and factors.

Transformation into a cell capable of metastasis is a multi-step and complex process. By this process, cancer cells acquire the capabilities necessary to escape the primary tumor, to enter vascular systems, to invade, and to colonize secondary organs [22,23]. What is the mechanism of this process? Enormous effort has been invested to answer this question over the past few decades and the answer remains unclear. Surrogate *in vitro* cell based assays were designed to reflect some, but not all, of metastasis characteristics, including soft agar growth, invasion through proteinaceous gels, and unlimited growth [23]. These assays were used to identify elements that play roles in the transformation process. Thus far, the changes in genome, epigenome, and signaling pathways have been shown to be functionally associated with the process [22,24-29]. These findings, combined with *in vivo* evaluations using cancer tissues and tumor models in animals, have identified genes that either promote or suppress carcinogenesis [22,24-28]. Well-known genes include cancer promotors such as *RAS, MYC, SRC, ABL, ErbB*, and suppressors such as, *Rb* and *P53*, etc. [22,24-28]. Growth factor receptors such as EGFR, VEGFR etc. were found to be important in maintaining the growth of tumors through constitutively activated signaling cascades [30-33]. For the past twenty years, the epithelial-mesenchymal transition (EMT) process has been considered a key mechanism for metastatic transformation because many invasive cancer cells possess markers and characteristics of mesenchymal cells, including the ability to migrate and invade [34].

Adding to the complexity is that a single factor

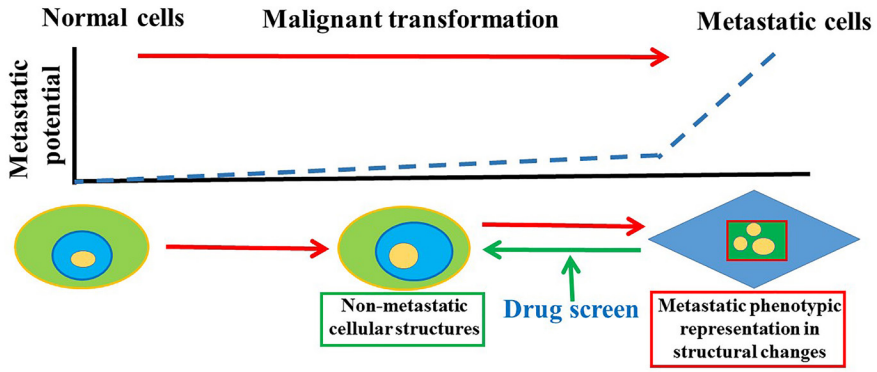

Figure 1. A diagram illustrates the strategy that uses structural features unique to metastatic cancer cells as phenotypic markers for screens to identify small molecules that modify or eliminate the metastatic features back to those of non-metastatic cells.

is neither necessary nor sufficient to induce the transformation process under experimental conditions. A successful transformation *in vitro* or *in vivo* requires manipulation of multiple genes or gene products [24]. Most recently, the EMT process was found unnecessary for metastasis *in vivo* [35,36], further reinforcing that metastasis involves complex functional networks. This complexity helps in part explain why targeting single genes or gene products has not been successful in treating metastasis. In addition to the complexity of the mechanisms, cancers are also highly heterogeneous diseases, in which cancers or cancer cells from the same organ can be molecularly different among different individuals and even within the same tumors [37]. While the evolution of an individual cancer towards morbid metastasis requires the selection of cancer cells with the right characteristics, cancers from different patients do not necessarily share a common evolution pathway [37]. All these findings reiterate the complexity of metastatic cancers.

FUTURE ANTI-METASTASIS DRUG DEVELOPMENT

What are the future options for drug development against metastatic cancers, given the complexities and the incomplete understanding of the diseases? Unraveling the disease mechanisms clearly remains critically important for the identification of the key factors or networks responsible for metastasis. With the availability of CRISPR technology [38-40] and more sophisticated *in vitro* 3D invasion assays [41], it is possible that the roles of individual genes at normal or mutant forms, singularly or in combination, will be evaluated in human metastatic cancer models in animals. The discovery of key factors responsible for the instigation and/or maintenance of metastasis could then potentially provide productive targets for future development of drugs with selectivity and specificity against metastasis.

Before all that can be achieved, we should look into what stands out in metastatic cancers. Although cancer cells are highly heterogeneous during their evolution and in their individual characteristics, metastatic cells do share a common and lethal feature: the metastatic capability. This unique property distinguishes them from non-metastatic tumor cells or normal cells at any stage of development. Recently, genomic signatures unique to metastatic cancers *in vivo* have been analyzed through comparing genomes from metastatic and non-metastatic cancers [25,26,29,42]. The initial results implicated hundreds of genes in which mutations or changes in their expression levels have been associated with poor patient outcomes [25,26,29,42]. However, the sheer large number of genes, together with the incomplete understanding of their function, make them impractical as direct drug developmental targets, particularly when targeting one or a few genes or gene products at the time may not be sufficiently effective against metastasis.

Given the currently limited understanding and complexity of metastatic mechanisms, an alternative strategy for targeting metastatic cancers is to identify surrogate markers that represent the complex but unique characteristics of metastatic cancer cells. Perhaps cytological markers or specific changes of cellular morphometry in metastatic cancer cells could serve as such markers because complex structural changes could reflect metastatic potential of cancer cells more comprehensively than any single gene or gene product. For example, the changes of nucleolar morphometry have been used as one of the parameters in cancer histological grading for more than a hundred years [43] and grading remains a prognostic marker for cancer. If

a morphological marker could be incorporated into an assay for screening small molecules, it could provide opportunities to identify compounds with selective anti-metastatic properties, potentially with multiple targets (Figure 1).

To identify such a surrogate marker for cancer cells, we have screened a battery of monoclonal antibodies raised against HeLa nuclei and identified perinucleolar compartment (PNC) to be present in cancer cells and absent in normal cells, including embryonic stem cells [44]. PNC prevalence closely correlates with the metastatic potential in a series of human prostate cancer derived cells with defined metastatic potentials [44]. PNC prevalence positively associates with disease progression of examined cancers and negatively associates with patient outcomes [45,46]. We hypothesized that PNC prevalence can be a surrogate marker for metastatic potential of a given cellular population. Using PNC reduction as a phenotypic marker for a high-content screen [47], a lead compound has been identified and optimized, and is being developed as a chemotherapeutic candidate based on its *in vivo* efficacy as an inhibitor of metastasis (unpublished). This approach represents a novel alternative to the current practices to meet the challenge of developing anti-metastasis drugs. Other reliable markers of metastatic transformation could and should be searched for in order to be used in a comprehensive phenotypic marker-based strategy to combat metastatic cancer.

CONCLUSIONS AND OUTLOOK

The enormous effort invested in the battle against cancer has significantly increased the survival rate of cancer patients. However, effectively treating metastatic cancers remains an unmet challenge. With the enormous complexity of the metastatic mechanisms and the lack of full understanding of the key players, it may be time to look to alternative strategies that work with comprehensive phenotypic markers of metastasis to develop effective drugs to treat these complex diseases.

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