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The inherent metastasis of leukaemia and its exploitation by sonodynamic therapy

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Contents

Abstract

Nearly all cancers are linked by the inexorable phenotype of metastasis as malignant growths have the capability to spread from their place of origin to distant sites throughout the body. While different cancers may have various propensities to migrate towards specific locations, they are all linked by this unifying principal. Unlike most neoplasms, leukaemia has inherent cell motility as leukocytes are required to move throughout the vascular system, suggesting that no mutations are required for anchorage independent growth. As such, it seems likely that leukaemias are inherently metastatic, endowed with the deadliest phenotype of cancer simply due to cell of origin. This article presents the biology of metastasis development and how leukaemia cells are inherently provided these phenotypic characteristics. It is then proposed how clinicians

Keywords: Metastasis; Leukaemia; Leukocytes; Ultrasound; Sonodynamic therapy; Drug resistance; Chemotherapy

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may be able to exploit the motility of leukaemia and metastatic emboli of other cancer types through an approach known as sonodynamic therapy (SDT), a treatment modality that combines chemotherapeutic agents with ultrasound to preferentially damage malignant cells. As experimental evidence has indicated, SDT is a promising therapeutic approach in need of clinical testing for further validation.

1. Introduction

The heterogeneity and extent of diversity among known cancer types is truly immense. Even within a given cancer type, there are various histologies, aberrant biochemical pathways and mutations that permeate a truly unique disease for an individual patient. However, all cancers are linked by the inexorable phenotype of metastasis. In other words, each cancer has the capability to spread from its place of origin to distant sites throughout the body (with exception to some brain cancers). While such cancers may have various propensities to migrate towards specific locations, they are all linked by this unifying principal. In fact, more than 90% of patient mortality due to cancer is a direct consequence of metastatic progression [1]. Other than aberrant cell proliferation, there is not a single more unifying phenotype in cancer biology.

Cancer occurs after cells in a tissue progressively acquire aberrant mutations to produce progeny with uncontrolled capacities of proliferation [2,3]. This uncontrolled mitosis produces a primary tumour which eventually undergoes metaplasia, followed by dysplasia and then anaplasia, resulting in a malignant phenotype. It is this malignant phenotype that provides a mechanism for intravasation into the circulation, followed by extravasation to a secondary site for tumourigenesis. Such a phenomenon is referred to as metastasis, defined as the spread of a cancer variant from one organ to another non-adjacent organ [4]. As opposed to invasion which involves the migration of cancer cells to adjacent tissue, metastasis necessitates the development of cells capable of surviving highly variable environments as they fragment from the primary tumour and migrate into the vasculature.

Most cancers (~85%) are of epithelial origin, referred to as carcinomas. Epithelial cells are normally held in place by junctions to adjacent cells as well as the basal lamina matrix [3]. Most epithelial cells require such attachments in order to survive, thereby requiring carcinomas to undergo a series of mutations that not only remove this need of cell signalling, but allow the cell to detach from the lamina foundation. However, not all cancers face such an uphill battle. Leukaemia is a highly heterogenic cancer of dedifferentiated leukocytes that causes more disease-related deaths of children (younger than 18 years of age) than any other disease in the United Kingdom or United States [5, Cancer Research UK]. The statistic includes all diseases, and is a sobering reminder of the prevalence of childhood cancer, despite the commendable advancements in cancer therapy. Unlike carcinomas, leukaemia has inherent cell motility as leukocytes are required to move throughout the vascular system, suggesting that no mutations are required for anchorage independent growth. As such, it seems plausible that leukaemias are inherently metastatic, endowed with the deadliest phenotype of cancer simply due to cell of origin. However, its most devastating characteristic may also be exposed as a fatal flaw if chemotherapeutic approaches are able to exploit the ease at which dedifferentiated leukocytes traverse the cardiovascular and lymphatic systems. This article will present the biology of metastatic progression and how leukaemia cells are inherently endowed with this devastating phenotype. Finally, it is indicated how clinicians may be able to exploit the motility of leukaemia and metastatic emboli of other cancer types through an approach known as sonodynamic therapy (SDT), a treatment modality that combines chemotherapeutic agents with ultrasound to preferentially damage malignant cells.

2. The biology of non-haematopoietic metastases

Although it may seem archaic by contemporary standards, the existence of metastasis was accurately predicted by Stephen Paget in his article, "The Distribution of Secondary Growths In Cancer of The Breast," published in a 1889 volume of *The Lancet*. In the article, Paget examined post-mortem data that had been assembled from 735 women with breast cancer and noted that the organ distribution of cancer cells in these patients followed particular migratory patterns. Due to these observations, Paget suggested that the movement of malignancy is not due to chance events; rather some tumour cells (the "seed") grew preferentially in the microenvironment of select organs (the "soil") and that migration to secondary sites resulted only when the appropriate seed was implanted in its suitable soil [6]. Paget's assertion that the microenvironment plays a critical role in regulating the growth of metastases has since been supported by numerous experimental studies; a remarkable insight for the time that has since dramatically improved the understanding of cancer biology.

In order for normal cells to transition into malignant potency, there are several crucial aberrations that need to occur. Such characteristics include mitosis in the absence of external growth stimulatory signals, substantial growth in spite of exogenous inhibitory signals, angiogenesis (excluding various haematological malignancies), the potentiation of cell immortalization, and finally, the capacity of invasion and metastasis [2]. This is typically the last step in malignancy (Fig. 1A), and is the phenotypic characteristic that kills most patients [7]. It should be noted that there is a considerable difference between invasion and metastasis. Invasion refers to the ability to thrust aside and displace

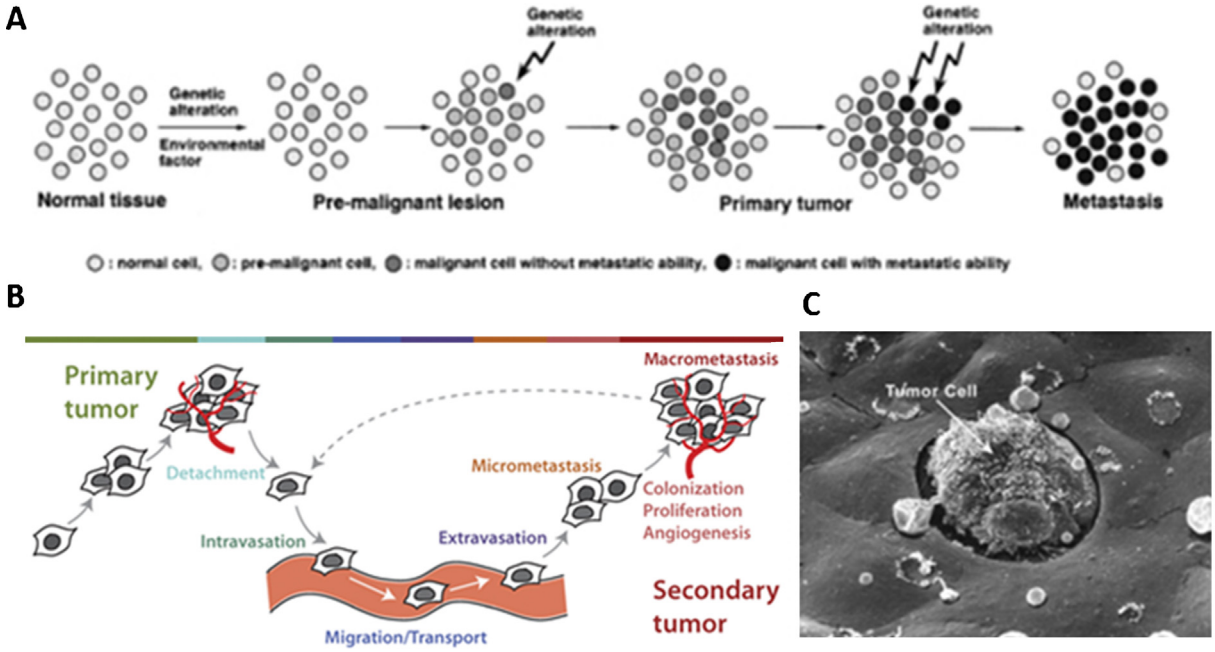

Fig. 1. The pathogenesis of cancer. (A) The steps by which mutations convert normal tissue into cells of metastatic potential. The last step of carcinomas and sarcomas is typically a series of acquired mutations that confer migration to distant sites (metastasis). (B) The progression of metastatic emboli requires that cells from the primary tumour acquire enough mutations to fragment off, and survive the heterogeneity of the circulatory system. Once the cells reach a secondary site, they must find the environment suitable for developing a subsequent tumour. (C) A metastatic cancer cell can cross the endothelial cell layer of a blood vessel during intravasation to gain access to the circulatory system. This vasculature highway enables the cell to find another suitable environment in which to form a secondary tumour site.
Top image courtesy of [3]. Bottom left image courtesy of [8]. Bottom right image courtesy of [2].

adjacent tissues, while metastasis is the fragmenting of tumour cells from a primary tumour that enables migration to secondary sites *via* the circulatory system [3,8]. In other words, primary tumours invade adjacent tissue systems, while secondary tumours invade distant tissue systems through the settlement of migratory emboli. Metastasis of solid tumours consists of five consecutive steps which will be described in detail: invasion and migration, intravasation, circulation, extravasation, and finally, the combination of colonization, proliferation, and angiogenesis at the secondary tumour site (Fig. 1B and C). These steps will later be compared to the inherent metastasis of leukaemia to denote similarities and differences in migratory patterns.

It should be noted that while metastatic spread through the cardiovascular or lymphatic systems are the most common migratory routes, cells can also move in a transcoelomic manner in which the spread of a malignancy into body cavities can occur *via* seeding the surface of the peritoneal, pleural, pericardial, or subarachnoid spaces. Examples of transcoelomic activity include ovarian tumours that spread transperitoneally to the surface of the liver, as well as mesothelioma and primary lung carcinomas migrating through the pleural cavity, causing malignant pleural effusion [9]. In addition, the accidental transfer of cancer cells during surgical intervention is an artificial form of metastasis that presents an ever posing threat to patients in the clinic [2].

2.1. Invasion and migration

In order for a primary tumour to become metastatic, neoplastic cell dissemination to different organs is an inherent necessity. Dissemination is a complex cell motility phenomenon, requiring the molecular coordination of protrusion, chemotaxis, invasion, and contractility; all in an effort to achieve directed cell migration. The epithelial-mesenchymal transition (EMT) is an important early step in carcinomas for the conversion of primary tumour cells into a migratory population that is capable of systemic metastasis [9,10]. In effect, epithelial cells lose their cell polarity and cell–cell adhesion as they transform into mesenchymal stem cells (MSCs), gaining migratory and invasive properties of such cell types. When the cell reaches its target secondary site, it then reverts using the mesenchymal–epithelial transition (MET), allowing the carcinoma to seed in a distant organ. Although the EMT/MET transition is a natural phenomenon found in wound healing, it is aberrantly overused in tumourigenic epithelial cells [11–14].

In order for metastatic cells to traverse the distance between the epithelial lining and the endothelium leading up to intravasation, the development of substantial motility stands out as a necessity. High resolution multiphoton imaging of tumours *in vivo* has shown that malignant cells use both collective and single cell motility in their migration to the circulatory system. Breast carcinoma cell

motility is characterized by solitary amoeboid movement at speeds significantly higher than other types of cell motility (~4 μm/min) [10]. The explanation to such high rates of travel is truly profound; invasive tumour cells form pseudopodia *in vivo* in response to epidermal growth factor (EGF) secreted by tumour-associated macrophages, as part of the cancer cell/macrophage paracrine loop described in breast carcinomas [20,21]. Filamentous (F)-actin rich pseudopodia are the defining morphological feature of fast moving amoeboid cells and are involved in chemotaxis to direct neoplastic cells towards blood vessels before intravasation [10].

2.2. Intravasation

Once metastatic carcinoma cells successfully detach from their epithelial lining, they must gain access to the circulatory system if they are to invade nonadjacent organ systems. While proteases degrade the extracellular matrix (ECM), establishing a basis for invasion, the tumour cells themselves must have the capacity to migrate down this path to reach the endothelial lining of the circulatory system. The macromolecular explanations for the acquisition of cell motility are rather straightforward as the remodelling of the actin cytoskeleton, along with the formation and dissolving of adhesion complexes are integral to generating this novel phenotype. As a result of the EMT-MET transition, the malignant cells are capable of developing lamellipodia that stretch in the moving direction [2,23]. On the front end of the cell, integrins build new focal contacts between ECM and lamellipodium, while the connections on the end are dissolved. By this approach, the cell moves and continuously secretes proteases on its march to the endothelium. On the edge of a lamellipodium, spiky structures known as filopodia also form by the reorganization of microfilaments [23]. The formation of filopodia is vital for cell motility as they form focal adhesions with the substratum, linking it to the cell surface. Migrating cells almost always display filopodia as they are involved in the sensation of chemotaxis, resulting in changes to directed locomotion [24].

Although cell motility is integral for intravasation, it is not until the malignant cells break through the lumen of lymphatic or blood vessels that this step of metastasis is complete. Malignant cells can either directly or indirectly reach the cardiovascular system, as metastatic spread can initially begin in the lymphatic system. While classical cancer biology typically focuses on intravasation of haematological origin, it has been elucidated that lymphatic spread is just as relevant to metastasis. Integral to the purification of blood, the lymphatic system collects the interstitial fluid, moving the fluid through the lymph nodes and lymphatic vessels until it returns to cardiovascular circulation. It has even been proposed that carcinomas often spread initially *via* the lymphogenous system, whereas sarcomas tend to spread *via* the haematological system [30]. This may be due to the fact that the penetration of lymphatic vessels is easier due to the absence of a continuous basement membrane and a coating layer of pericytes, as well as their weak inter-endothelial connections [30].

2.3. Circulation

Cells that manage to break through the endothelium of the circulatory system gain access to a vast network of vessels, substantially increasing the likelihood of secondary tumours. Under normal physiological conditions, most cells activate a special form of apoptosis known as anoikis in the case they are unable to adhere to solid substrate or other cells (Fig. 2) [31]. In fact, cancer cells that are still anchorage dependent at the time of entering the circulation have anoikis activated, thereby destroying the cells [32]. While most cells would not survive and proliferate in the heterogeneous environment of the vasculature due to the lack of a solid substrate, malignant cells typically acquire enough mutations to become anoikis resistant (Fig. 2). In addition, if malignant cells are not yet independent of exogenous growth factors which are secreted by stroma cells, apoptosis is likely to be induced due to the absence of such growth factors in circulation [3].

Even when malignant cells have acquired these advantageous adaptions, they are still highly vulnerable in the vasculature. In smaller vessels, such as the arterioles or capillaries, high shear forces often occur. This can cause significant damage to neoplastic cells, as they do not have the unique cytoskeletal structure of erythrocytes that permits such mechanical stress [3]. Such forces are likely to be a primary reason for metastatic inefficiency. Further, the heterogenic environment of circulation, characterized by extremely high concentrations of oxygen and lymphocytes, substantially impair the progression of malignant cells [2,3]. Therefore, the presence of these adverse conditions actually provides a breeding ground for particularly aggressive and resistant cancer cells due to selection pressures [2]. Malignant cells antagonize the potentially toxic conditions of the vasculature by forming microemboli, an aggregation of tumour cells with thrombocytes and erythrocytes [7]. The formation of microemboli is mainly due to tissue factor, a protein highly expressed in malignant carcinoma cells, but not on benign tumour or healthy epithelial cells. Tissue factor interacts with special proteins in the plasma to activate thrombin which converts fibrinogen into fibrin, thereby resulting in cell conglomeration [7]. The importance of metastatic emboli has been analyzed in knock-out mice which lacked specific components of the clumping-cascade [33]. Melanoma cells were administered intravenously (i.v.) into the knock-out mice to assess metastatic efficiency. In comparison to the control group, metastasis was decreased by >90%. By shielding neoplastic cells from the unstable environment of circulation, emboli formation becomes an integral mechanism for the survival of cancer metastases as they continue their migration towards a secondary location.

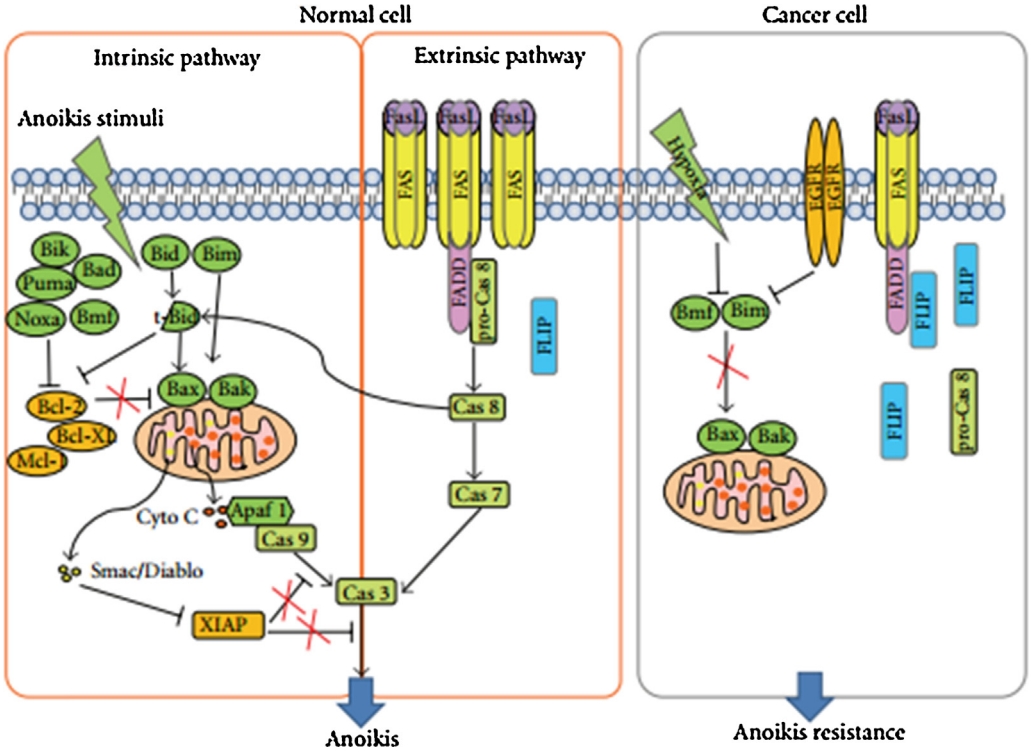

Fig. 2. The anoikis cascade and development of resistance. When detached from ECM, normal cells induce anoikis through both intrinsic and extrinsic pathways. Upon cell detachment, FAS and FasL are upregulated and FLICE-like inhibitory protein (FLIP) is downregulated, resulting in the activation of caspase-8. This is followed by activation of caspase-7 and caspase-3. Loss of cell adhesion also activates proapoptotic Bcl-2 proteins (Bik, Puma, Bad, Noxa, Bmf, Bid, Bim, Bax, and Bak) that inactivate antiapoptotic Bcl-2 proteins (Bcl-2, Bcl-xL, Mcl-1), thereby inducing mitochondrial membrane permeabilization through apparent Bax/Bak oligomerization. Such activity causes mitochondria to release cytochrome c, activating caspase-9 and subsequently caspase-3. Smac/DIABLO is released and inhibits XIAP (inhibitor of apoptosis) perpetuating caspase-3 activation. Once this occurs, anoikis is inevitable. However, increased FLIP expression in malignant cells inhibits the extrinsic pathway. Further, oncogenic expression induced by epidermal growth factor receptor (EGFR) and hypoxia often seen in neoplastic cells downregulate Bmf and Bim, inhibiting the mitochondrial pathway in suspended cells. In this regard, cells acquire anoikis resistance, substantially increasing the likelihood of anchorage independent growth.
Image courtesy of [31].

2.4. Extravasation

Eventually, partially due to chemokine signalling, partially due to trial and error, the metastatic cells approach an organ that is suitable for secondary growth. Before reaching that organ, the cells must find a way to exit the circulatory highway. If cancer cells survive the adverse conditions in the vessels and reach larger venous blood vessels, they are carried further by the bloodstream until they enter the capillary network of the lungs. There is a high probability that the cancer cells will become entangled in the capillary system of the lungs due to the sheer difference in size between malignant cells and the small vessels that must be successfully traversed. For comparison, cancer cells are often ~20 μm in size, in relation to the capillaries that are ~3–8 μm [2]. Unlike erythrocytes that are designed to meander through the narrow passages, malignant cells are not very elastic and often form microthrombi. Therefore, the probability that the metastatic emboli will become lodged in the arterioles is very high. It is nothing short of miraculous that any malignant cells are able to successfully traverse the circulatory system. Unfortunately for the patient, even a highly unlikely event becomes probable when enough cells reach this metastatic state.

In addition, the ever resilient cancer cells have mechanisms to greatly increase the odds in their favour. It is likely that neoplastic cells nearing the lung capillaries discard a tremendous amount of cytoplasm in order to form smaller, but still viable cells that are capable of passing through the small vascular network. It has also been elucidated that some cancer cells can avoid the capillary network by using arterial–venous shunts, bypassing the capillaries altogether [7]. Either way, when malignant cells leave the lung capillaries and reach the general arterial vessels, they have access to a substantial variety of body tissue.

Once the cells have reached a favourable secondary site, extravasation will occur in two different ways. One possibility is that neoplastic cells begin proliferating in the lumen of a vessel. The rapidly growing tumour will eventually compromise the vessel wall, thereby providing an accessible route into the tissue [7]. The second possibility for malignant cells to penetrate an organ is similar to intravasation

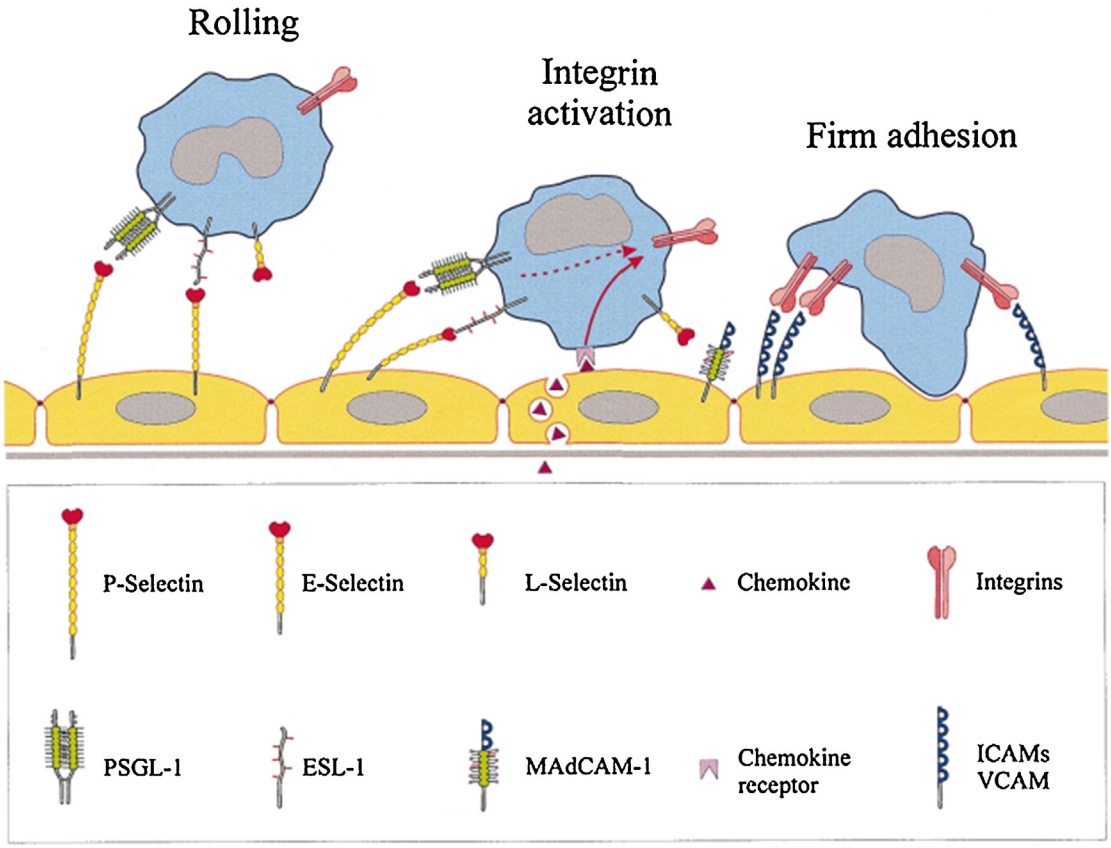

Fig. 3. Leukocyte extravasation due to an immune response. The entry of leukocytes into tissue due to an immune response is controlled by a cascade of molecular interactions. Upon recognition of disturbance or pathogens, resident macrophages in the affected tissue release cytokines such as IL-1, TNFα and chemokines. IL-1 and TNFα enable the endothelium of blood vessels near the site of infection to express cellular adhesion molecules, including selectins. Circulating leukocytes are localized towards the site of injury or infection due to the presence of chemokines. The selectins initiate the tethering of leukocytes to the endothelial cell surface, initiating rolling adhesion. Leukocytes roll along the blood vessel wall and sense activating chemokines, which are deposited on the endothelial cell surface. This interaction results in the activation of leukocyte integrins, which bind members of the immunoglobulin superfamily (Ig-SF) that are responsible for mediating firm adhesion. Such activity enables directed migration on the endothelial cell surface.
Image courtesy of [34].

and includes the degradation of endothelium and basement membrane through proteolytic enzymes. Leukocytes also have the ability to extravasate in great quantity over a short period in order to combat inflammation and infection [35]. In the course of this process known as diapedesis, leukocytes adhere *via* selectins to the endothelium and perform a form of rolling adhesion to interact with endothelial cells, which in turn enables the passage of leukocytes. This is due to the fact that carbohydrate ligands on the circulating leukocytes bind selectin molecules on the inner wall of the vessel with marginal affinity. Such activity enables leukocytes to slow down and begin rolling along the inner surface of the vessel wall (Fig. 3) [34]. During this rolling motion, transitory bonds are formed and broken between selectins and their associated ligands, causing leukocyte motion to halt. Diapedesis involves a variety of molecules including LFA-1 + ICAM-2, CD3 + ICAM-1, as well as PECAM (CD31), and the complete process takes place in less than a minute [35].

2.5. *Colonization, proliferation and angiogenesis at the secondary tumour site*

Now that the metastatic cells have successfully reached an ideal secondary location, they must quickly begin altering the extracellular environment within the organ to establish favourable conditions for a proliferating tumour. The new microenvironment typically does not provide the same survival and growth factors as the original tissue, which enabled the primary tumour to proliferate [7]. Without the special physiological factors of the original site, many metastasizing cells die or reside without proliferation as micrometastases. In fact, the amount of senescent micrometastases in cancer patients usually exceeds the amount of micrometastases that grow to a detectable size [2]. The detection of keratin profiles typically seen with epithelial cells in mesenchymal tissue provides evidence for micrometastases. This technique enables physicians to track down single cell micrometastases amongst 10^5–10^6 surrounding mesenchymal cells [7].

It has been confirmed that the EMT which takes place at the beginning of metastatic invasion is reversed during colonization (referred to as MET) [36]. This transition enables metastasized tumour cells to adopt their original epithelial character. As suspected by Paget years ago, considerable evidence suggests that that molecular factors present in specific organs can influence whether or not metastatic cells will find the environment favourable for colonization [37]. Breast and prostate carcinoma cells often metastasize to bone due to favourable interactions between the malignant and bone cells (osteoblasts and osteoclasts in particular) [38]. This prototypical seed and soil situation is also found in colon carcinomas and other cancers that metastasize to the liver, as specific growth factor receptors needed for sustained proliferation are readily available in the hepatic microenvironment.

After initial colonization and proliferation of secondary tumours, there needs to be development of an adequate blood supply that can sustain the ever increasing metabolic activity of the tumourigenic growth. Since there are usually not enough adjacent blood vessels to recruit, tumours simply make their own. In regard to cancer biology, angiogenesis is the formation of new blood vessels in the direction of a tumour to provide it with nutrients and to remove metabolic waste products [39]. Commonly seen in primary tumours, angiogenesis becomes just as integral to secondary malignancies that exceed the natural blood supply of their microenvironment.

3. The inherent metastasis of leukaemia

Although cancers found more commonly in adults have particularly high mortality rates (pancreatic, liver, and lung), they take much longer to develop malignancy than childhood cancers. In fact, many adult carcinomas require years of incubation before enough mutations have been acquired for detectable symptoms to become apparent [5]. By contrast, leukaemia, the most prolific childhood cancer in the United Kingdom and the United States often only takes a few months to spread throughout the patient. While it is true that leukaemias are often found in adults, many of these cases are in the form of secondary leukaemia, an unfortunate side effect of prior chemotherapeutic intervention [40,41]. In fact, leukaemia has the highest proportion of child *vs.* adult malignancy rates than any other cancer [5]. It also should be noted that many of those adults fall within the 18–30 year old range, still substantially younger than most cancer incidence.

With such a high proportion of young individuals being diagnosed with leukaemia, there must be some phenotypic characteristics that set the haematological malignancy apart from other cancers. This becomes apparent when the nature of leukocytes, the blood cells that dedifferentiate into leukaemic malignancy, is taken into consideration. By default, leukocytes have substantial mobility, circulating the vasculature in patrol or in response of pathogenic disturbance. In a manner similar to transformed malignant cells, leukocytes are also capable of amoeboid migration, meandering their way through the ECM to reach a site of infection or inflammation [34,35]. Therefore, the first two steps of metastasis (invasion and migration, intravasation) are unnecessary as leukocytes are naturally found in systemic circulation, and have the capability to migrate towards endothelial tissue. The classes of leukocytes are incredibly diverse by nature, as a result of being either of myeloid (monocytes and macrophages, neutrophils, basophils, and eosinophils) or lymphoid (T-cells, B-cells, and natural killer, NK cells). Consequently, leukocytes give rise to a profound heterogeneity of cancers [42,43]. As expected, the demands of such a diverse cell type are satisfied by an ever-present population of haematopoietic stem cells (HSCs). Cancers can be thought of as cells that have been reprogrammed to a dedifferentiated state, marked by gene expression typically reserved for embryonic or adult stem cells. As will be explained, this abundant population of stem cells makes blood cells particularly prone to cancer pathogenesis.

In addition, this review has already established that it is necessary for leukocytes to maintain an innate form of extravasation, as they need such characteristics to elicit a rapid immune response [34,35]. Being of the haematopoietic lineage, leukaemias do not require mutations to avoid anoikis, providing further evidence that leukocytes require fewer mutations to become malignant. Taking all of these phenotypic characteristics together, it becomes more apparent why leukaemias are the most prevalent childhood cancers. Based on the cancer biology of other cell types, it can be inferred that leukaemia is endowed with inherent metastatic potential. In a sense, leukaemias often require fewer mutations than cancers of other cell types to spread throughout the patient. It is this inherent metastasis that separates leukaemia from any other malignancy.

3.1. Migratory patterns of leukaemia

If leukaemia was inherently metastatic, it should have characteristic migratory patterns as found in other cancers. Such an answer requires only a scalpel and patience (as well as patients). In fact, autopsy reports describing leukaemia mortality have long been established in the clinical setting [44,45]. In most cases, it appears that leukaemia has a similar seeding frequency of the skin, breast, trachea, diaphragm and all other muscles. Due to their cell type of origin, the highest incidence of metastasis is found in the lymphatic system (lymph nodes and spleen). The excess of metastases at specific sites do not cluster in topographical areas or in anatomical systems, with the exception of metastases in the central nervous and endocrine systems (acute lymphoid leukaemia, ALL). From the unique migratory patterns of ALL, it can be inferred that soil specificity may account for the higher than expected occurrence of metastases in the noted organs for a specific leukaemia. Chronic lymphoid leukaemia (CLL) shows an excess of metastases in

all lymph nodes, kidneys, adrenals and the heart. The unique seeding of CLL suggests a lymphatic route of dissemination, as opposed to a cardiovascular spread of malignant cells [44].

While more common leukaemias such as ALL and CLL have been categorized for years, the unique dissemination patterns of NK-derived leukaemias were categorized much later [46,47]. Pathologically, NK leukaemias show variable cytological appearances, with frequent angiocentricity (angioinvasion and angiodestruction) associated with zonal necrosis of affected areas. Most cases occur in the nasopharynx and upper aerodigestive tract. However, occurrence in non-nasal sites such as the skin, gastrointestinal tract and testes is also observed [51]. While NK leukaemias have apparent differences in their migratory patterns, they share a common trait with other haematological malignancies of leucocyte origin in the formation of aberrant skin lesions (cutis) [46,47]. Leukaemia cutis refers to the infiltration of transformed leukocytes or their precursors into the skin resulting in readily identifiable cutaneous lesions. Patient examination typically reveals multiple hyperpigmented nodules and plaques involving the face, trunk, and extremities. A diagnosis of leukaemia cutis generally portends a poor prognosis, as it strongly correlates with additional sites of extramedullary involvement [47]. Unlike most cancers, leukaemia has the capability to blanket the dermal layers with cutaneous lesions, providing a profound visual example of its metastatic extent.

3.2. *Uncontrolled haematopoietic stem cells perpetuate leukaemia*

It has been well established that endogenous stem cell populations are often a foreground for tumorigenesis [7]. As is seen in leukaemia, haematopoietic stem cells (HSCs) play a monumental role in malignancy development. During haematopoiesis, the progenitor HSCs receive a complex array of transcription factors and growth signals to differentiate into all known existing blood cells [42]. Within this system, HSCs give rise to either the lymphoid or myeloid lineage as subsequent cell progeny are directed by further differentiation steps to give rise to the desired cell type [48]. However, when improper signalling or mutations occur within HSCs, the likelihood of leukaemia pathogenesis is dramatically increased [49]. Normal haematopoiesis requires complex interactions between the bone marrow microenvironment (the stem cell niche) and HSCs. These interactions are critical for the maintenance of normal HSC activity, and deregulation of this intricate system aberrantly influences HSC self-renewal [49,50]. If this self-renewal is left unchecked, progression into leukaemic stem cells (LSCs) that also possess limitless self-renewal may be generated in the stem cell population, hijacking the homeostatic mechanisms necessary for proper differentiation (Fig. 4A) [49,50]. LSCs are extremely resilient as they take refuge within the sanctuary of the niche during chemotherapy, and consequently contribute to eventual disease relapse [43].

Such deregulation of HSCs is apparent in acute myeloid leukaemia (AML), a leukaemia that has significant clinical relevance due to its prevalence in patients. AML is the second most common leukaemia diagnosed in both adults and children, with a five year survival rate of only 24% [5]. As such, HSC deregulation leading to AML is a common phenomenon in the clinic, and has been meticulously studied in order to further elucidate the pathogenesis of the disease. As for many haematological malignancies, AML has been extensively characterized as a cell autonomous disorder, as the genetic events leading to transformation of the normal haematopoietic cell are found within that cell, and are both necessary as well as sufficient for leukaemic development [49,50]. Aberrant fusion proteins, such as MLL-AF9 or MLL-ENL1 that are expressed as a consequence of chromosomal translocations are typically present in leukaemic blasts derived from patients with AML [43]. Introduction of the subsequent alleles into normal bone marrow cells perpetuates AML in murine models of disease, validating their oncogenic properties [50]. This constructed AML accurately recapitulates the human disease phenotype, including hallmark stem cell properties such as the ability to consecutively replate on methylcellulose in the absence of stroma, and the ability to confer an AML phenotype that can be transplanted *in vivo* [50].

Although all AML cells are thought to harbour the cell-autonomous mutations that are found in its pathogenesis, the subpopulation of AML cells referred to as LSCs are responsible for the long-term repopulating potential as well as the ability to propagate and maintain the AML phenotype [51,52]. It has been estimated that as few as one in a million AML cells possess leukaemia initiating activity [51]. Therefore, AML has a hierarchical organization similar to that of normal haematopoiesis in which there is a rare subpopulation of cells with limitless self-renewal potential that give rise to progeny that lack such potential [51,52]. LSCs in certain types of murine AML (induced by MLL-AF9, 2 MOZ-TIF2, 2 MOZ-TIF3 or MLL-ENL1) have characteristics of progenitor cells with phenotypic characteristics similar to normal granulocyte/macrophage precursors [53]. The leukaemia-initiating cells are more mature than HSCs, but have acquired limitless self-renewal through oncogenic transformation, leading to the activation of essential stem cell genes [53,54]. These observations are intriguing, as it suggests that only a relatively few amount of AML cells are responsible for perpetuating metastatic disease in the patient. This concept opens up the idea of finding therapeutic approaches that specifically target leukaemic progenitors, an approach that has been under considerable investigation (Fig. 4B) [53,55]. If treatments can be successfully devised, the malignant cells actually responsible for maintaining leukaemia cell populations will be eradicated, theoretically neutralizing disease progression.

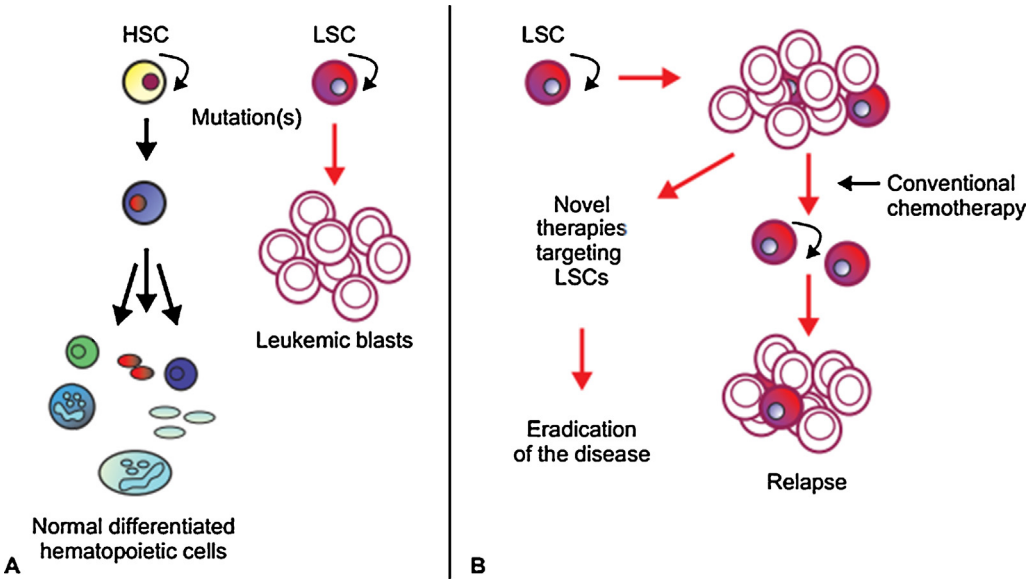

Fig. 4. The leukaemic stem cell model and the need for novel therapeutic approaches. (A) The LSC model proposes that leukaemic blasts originate from a common primitive progenitor that has the capacity to self-renew, akin to normal haematopoiesis. Therefore, such cells are required for maintaining leukaemia cell populations, providing a compelling therapeutic target. (B) Conventional therapies for leukaemia have been designed to eliminate leukaemic blasts. However, these regimens are not as responsive to the LSC population, which eventually recapitulates the disease with drug resistant progeny. The ability to design therapies that can target LSCs should theoretically increase the efficacy of treatments for leukaemias that have been confirmed to rely on LSCs, such as AML. Image courtesy of [55].

3.3. Richter's syndrome: evidence of lymphoid leukaemia acting as lymphoma metastases

Although leukaemias and lymphomas are both derived from leukocytes, the haematological malignancies are profoundly different in that leukaemias are inherently "liquid cancers" that do not form solid tumours, while lymphomas form primary tumours in a variety of organ systems. Lymphomas refer to a group of haematological malignancies that develop from lymphocytes, and form solid tumours primarily in the lymphatic system, but can also form in other areas of the body [56,57]. These haematological malignancies are typically broken down into Hodgkin's lymphoma (HL) and non-Hodgkin's lymphoma (NHL), the main difference being Hodgkin's lymphoma is characterized by Reed-Sternberg cells (enlarged B-lymphocytes that are either multinucleated or have a bilobed nucleus) [56]. Further characterization of HL can also include "mummified" erythrocytes (compact nuclei, basophilic cytoplasm, and no nucleoli), as well as popcorn cells (small cells with hyper-lobulated nuclei and small nucleoli) in nodular lymphocyte predominant Hodgkin's lymphoma (NLPHL) [56]. As with most solid tumours, lymphomas have the potential to metastasize once enough mutations have been acquired at the primary tumour site, and secondary sites can range anywhere from the brain to the heart [56–58].

Due to the similarities between leukaemias and metastases from solid tumours, it may be possible that in some cases, leukaemias act very similar to lymphoma metastases, in which further mutations enable the malignancy to seed in an appropriate environment, akin to carcinomas acquiring additional mutations to form tumours at secondary sites. Since lymphomas are strictly of the lymphoid lineage, it should be the case that only lymphoid leukaemias (ALL, CLL, hairy cell leukaemia, T-cell prolymphocytic leukaemia, and adult T-cell leukaemia) are capable of being transformed into lymphomas. Further, transformed leukaemias should have characteristics similar to lymphomas, and have mutual seeding preferences.

Richter's syndrome is a transformation that converts B-CLL and hairy cell leukaemia (can be considered a subset of CLL) into fast-growing diffuse large B cell lymphoma (DLBCL), and in some cases HL, with a frequency of about 5–10% in patients [59–61]. In the majority of these patients, lymphoma clones are perpetuated by transformation of original CLL clones, but some form a separate and independent neoplasm [59]. Patients often present with a rapid clinical deterioration, and average survival is typically between 5 and 8 months post-diagnosis [62]. While the conversion from CLL to lymphoma has not been well-characterized, typical karyotype aberrations have been noted, such as trisomy 12, deletion at 13q14, deletions at 11q22-q23 involving the ATM gene, and deletion at 17p13 involving p53 [59–61]. All of these aberrations are associated with B-cell lymphoma progression [63–66], suggesting that the genetic defects are likely involved in the transformation. Further, Richter's syndrome cells have seeding sites very similar to B-cell [65,66] and Hodgkin's lymphoma [67,68], providing additional support that CLL can be transformed to a tumour-bearing lymphoma under appropriate circumstances.

Although it is a relatively rare phenomenon, Richter's syndrome demonstrates that leukaemic cells have the potential to form solid tumours after appropriate mutations. Although there is a clear distinction between leukaemias and lymphomas, the fact that CLL can be transformed into a lymphoma demonstrates that leukaemias can seed at locations, thereby eliminating the most notable difference between the haematological malignancies. Therefore, lymphoid leukaemias can be viewed as having very similar characteristics to metastatic lymphoma cells, having the propensity to form tumours at distant sites once enough aberrant mutations have been acquired.

4. Exploiting the inherent metastasis of leukaemia

Throughout this article, similarities between solid tumour metastases and leukaemias have been proposed in order to suggest that leukaemia cells are inherently metastatic. This comparison has been made for a very practical reason; if metastatic emboli are comparable to leukaemias, perhaps the unique phenotypic characteristics that link the malignant growths can be exploited to improve current chemotherapeutic approaches. That is, use the most devastating phenotypic characteristics of the malignancy to develop novel therapeutic methods that specifically target and damage circulating cancer cells. Such an approach could not only significantly improve leukaemia chemotherapy, but also treatments for carcinomas that commonly metastasize and form secondary tumours. After all, it is the metastatic phenotype of cancer that subdues most patients.

This form of targeted chemotherapy may be achieved by sonodynamic therapy (SDT), a novel treatment modality that uses ultrasound in combination with specialized chemotherapeutic agents known as sonosensitizers to preferentially damage malignant cells. It has been shown in numerous experiments that ultrasound preferentially damages malignant cells based on the size differential between such cells and those of normal histology [69–71]. This is particularly important for leukaemia and other metastatic diseases, as the malignant cells will be in close proximity to normal blood cells. By targeting the inherent size differential of the malignant cells in comparison to the normal cells in circulation, SDT asserts itself as a therapeutic approach that is both effective and specific. The size differential between malignant and normal cells can be dramatically increased through the use of sonosensitizers that specifically target malignant cells, thereby amplifying the preferential damage of ultrasound [69].

SDT has also been shown to be particularly effective against drug resistant cell lines. One of the most cited shortcomings of chemotherapy in clinical practices is drug resistance acquired by tumours. SDT has been shown to reverse this potent defense mechanism in human leukaemia multidrug resistant cell line K562/A02 [72]. Reversal of drug resistance has also been observed in HepG2/ADM human multidrug resistant hepatocellular carcinoma cells, demonstrating the versatility of SDT [71]. Sonosensitizers often attack cells through multiple mechanisms, creating a potential synergistic effect when agents of different classes are used in combination [69]. Being able to develop treatment regimens in which the synergistic effects of different sonosensitizers are applied can have profound clinical utility. Such treatments could substantially amplify the capability of ultrasound to preferentially damage malignant cells in order to decrease the rate at which drug resistance is observed.

In order to use SDT in the clinical setting, effective measures need to be devised by which to administer ultrasound and chemotherapeutic agents to patients. Seeing that SDT has yet to be tested in the clinical setting, there has been no analysis as to how this treatment modality could be practically applied to patients. Although SDT fundamentally relies on an ultrasound system, there are a variety of ways in which sonication can be delivered. A potentially important approach that takes full advantage of the inherent metastasis of leukaemia is a form of extracorporeal blood sonication (EBS) in which blood is drawn out of the body, as in hemodialysis, and then sonicated before it reenters the patient. This approach will be described in detail, along with using established sonication techniques currently employed in the clinic for the treatment of prostate carcinoma and calcified deposits. In addition, cytochalasin B, a sonosensitizer that could have immediate clinical importance, is also characterized. It is hoped that such efforts will convince academics and clinicians of how the unique characteristics of metastatic disease can be exploited to improve chemotherapeutic approaches in many clinical contexts.

4.1. Extracorporeal blood sonication

Ultrasound inevitably loses some of its intensity as it travels through the human body. If there was a way to remove malignant cells from the body so they could be treated in an extracorporeal environment, there would be no sound inhibitors protecting such cells from the preferential damage of SDT. Although such an approach is unfeasible for most malignancies, leukaemia is unique in that it does not form a primary tumour site. Rather, it flows through the blood, alongside normal cells as it slowly overcomes the natural defenses of the immune system. However, its most beneficial asset can be exploited to become a profound fatal flaw. Since most leukaemias are localized in the blood, it would be rather straightforward to draw the malignant cells out of the body through dialysis. While dialysis is typically used on patients to act as an artificial replacement for lost kidney function due to renal failure, it could just as easily be used to treat leukaemia in an extracorporeal setting. Sonosensitizers would be injected i.v. with some time passing before injection and sonication. The patient would then undergo a typical hemodialysis procedure in which blood is pumped outside of the body, thereby removing the natural sound barriers of human anatomy. There would be nothing standing

in the way between the malignant cells and the ultrasonic waves that are able to inflict considerable preferential damage. In effect, this SDT procedure allows an *in vivo* setting to become almost i*n vitro*. Since the *in vitro* studies of SDT with leukaemia have yielded impressive results [72–75], this may be the most effective way to administer ultrasound to such patients.

However, the sound intensities used in EBS will likely have to be carefully monitored. There is very little standing in the way between the blood and the high intensity ultrasound being administered. While normal erythrocytes and leukocytes are more resistant to SDT, they are not invulnerable. Sufficient sound intensities will cause just as much damage to these cells as the malignant cells that are within close proximity [76]. Therefore, the sound intensity used in EBS might have to be considerably reduced. Nevertheless, it still provides the most direct route for sonicating leukaemic blasts that are the hallmark of leukaemia pathology. Further, there have been ultrasonic probes already devised that could be used to sonicate bone marrow containing aleukaemic cells as well the LSCs shown to be the driving force behind many haematological malignancies, as will be described in the following section. The combinatorial approach of EBS with such probes could provide a method to comprehensively sonicate almost all areas where metastatic disease (including carcinomas) may reside.

4.2. Using focused ultrasound to target leukaemic stem cells in the bone marrow and solid tumours

Although EBS is a potential avenue for treating leukaemias and solid tumour metastases, sonication of the blood would yield little benefit for other cancers that are often concentrated at a primary tumour site. Further, patients typically have leukaemia cells that remain trapped in the bone morrow, particularly LSCs that perpetuate blast formation. In addition, aleukaemic blasts can remain in the bone marrow, severely perturbing normal production of blood cells. Without the luxury of directly sonicating the blood that can transport blasts and circulating LSCs to an extracorporeal environment, another sonication approach needs to be devised. One that is capable of scanning patients for concentrated pockets of neoplastic cells, and then sonicating these areas with high-intensity ultrasound.

In fact, such technology already exists and could be readily applied in the clinic with a few minor adjustments. Ultrasonic probes are devices capable of delivering high frequency or high intensity ultrasound to localized areas, and are commonly used in the medical field for diagnostic imaging (high frequency) or even breaking up calcified kidney stones (high intensity). Medical ultrasonography uses a substantial variety of ultrasonic probes, and many operational systems are available for testing with SDT. As an example, high intensity focused ultrasound (HIFU) probes are currently being used in the clinic for extracorporeal shock wave lithotripsy (ESWL), and in the treatment of prostate carcinomas. The lithotripter used in such procedures breaks up cell aggregates or calcified stones using multiple focused beams of externally applied, focused, high intensity acoustic pulses [77–80], which could be readily converted for SDT-mediated treatment protocols. ESWL and HIFU-mediated ablation of prostate carcinoma can actually be seen as potential evidence of SDT efficacy, as the methods break up concentrated deposits through inertial cavitation (the process of creating microbubbles in liquids, such as cellular cytoplasm), just as in SDT. The benefit of SDT over HIFU-mediated ablation resides in the fact that SDT activates chemotherapeutic agents in addition to producing inertial cavitation, providing additional mechanisms by which to damage malignant growths.

Due to the advances in medical imaging, it is now possible to readily locate primary tumour sites, providing the basis for sonochemotherapeutic protocols. By injecting sonosensitizers i.v. or subcutaneously (s.c.) at tumour aggregates prior to treatment, ultrasonic probes can be locally applied to the affected site, thereby allowing a potentially site-specified chemotherapeutic approach. Although it may not apply directly to leukaemia, combining the drug activation of SDT with ultrasound-guided drug injection, as is performed in endoscopic ultrasound-fine needle injection (EUS-FNI) [81,82], may be particularly beneficial for solid tumours, including mediastinal lymphomas. This is further supported by research that indicates EUS can be used to improve the delivery of photodynamic therapy (PDT) in porcine models [83,84], which acts by similar mechanisms to SDT. As such, this therapeutic method has apparent clinical implications outside haematological malignancies, as a great diversity of cancers could be treated using this method. Further, using focused ultrasound to target neoplastic cells caught in the bone marrow may be a method by which to specifically target LSCs, although the preferential damage SDT elicits towards normal leukaemic cells still needs to be confirmed with LSCs. It should be noted that leukaemia cells are preferentially damaged in the presence of human haematopoietic stem cells (hHSCs) and normal leukocytes [73], suggesting that preferential damage within the confines of the bone marrow (or the blood for circulating LSCs) is feasible. The extent of neoplasms that can be treated through this form of SDT will only be determined through further preclinical and eventual clinical evaluation.

4.3. Cytochalasin B as the prototypical sonosensitizer

By default, malignant cells have a perturbed cytoskeleton due to the effects of dysplasia and subsequent anaplasia [7]. With so many alterations present in malignant cells, the cytoskeleton provides an ideal opportunity for preferential damage. Specifically, one of the most intriguing possibilities is to preferentially lyse malignant cells based on their considerable size differential to cells of normal histology. This fact suggests that enlarging malignant cells to increase their already noticeable size difference with normal cells may be a method by which to attain preferential damage.

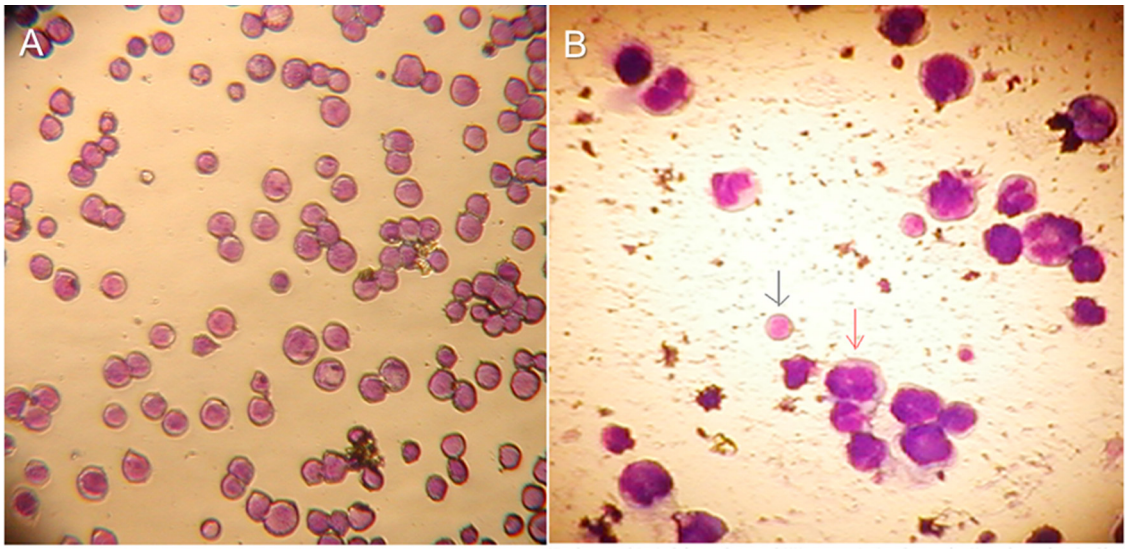

Fig. 5. Effects of 1.5 μM cytochalasin B on U937 cells 48 h post-administration. (A) Untreated U937 cells typically have a size distribution of 13–18 μm. (B) U937 cells 48 h after treatment with 1.5 μM cytochalasin B are enlarged (19–40 μm) and multinucleated. Grey arrow indicates a mononucleated cell. Red arrow indicates an enlarged and multinucleated cell. Nuclei were visualized with Wright–Giesma stain. Photomicrographs were taken under identical conditions of magnification (100×). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
Images are from the laboratory of the author.

Cytochalasin B is a microfilament-disrupting agent that disrupts the actin cytoskeleton and inhibits cytokinesis by interfering with formation of the contractile ring, as well as the development of the cleavage furrow [69]. Consequently, the cell does not divide and an immature actin cytoskeleton remains. However, the cell continues to form nuclei and eventually becomes enlarged and multinucleated (Fig. 5). Such cells have more DNA targets, increasing the likelihood of apoptosis when combined with nucleic acid-directed agents, as demonstrated by my laboratory with doxorubicin (DOX) and 5-flourouracil (5-FU) (unpublished data), and by O'Neill (1975) with cytarabine [85]. Further, the multinucleated cells have a large cell volume, making them more susceptible to physical agitation [73].

Preferential damage of malignant cells is facilitated by the fact that normal cells exposed to cytochalasin B exit the cell cycle and enter the G_0 state until sufficient actin levels are restored [69]. Therefore, only malignant cells that have lost the ability to enter this resting state become enlarged and multinucleated, providing an ideal target for sonication. To put the size differentials into perspective, normal erythrocytes are typically between 6 and 8 μm and leukocytes range between 10 and 15 μm (the occasional macrophage grows up to 20 μm) [73]. By contrast, cytochalasin B-treated U937 monocytic leukaemia cells routinely grow in excess of 20 μm with some reaching nearly 40 μm in diameter after enough exposure [69]. These cells have reduced cytoskeletal integrity and are ideal targets for sonication. As demonstrated by my laboratory, U937 cells enlarged and multinucleated through administration of 1.5 μM cytochalasin B for 48 h are much more sensitive to low frequency (23.5 kHz) ultrasound than untreated U937 cells, and substantially more sensitive than normal hHSCs and human leukocytes [69].

Cytochalasin B also appears to have a considerable influence on mitochondrial activity. When U937 cells were exposed to the same concentration of cytochalasin B (1.5 μM), the cells exhibited a 4-fold increase in comparison to U937 cells of typical histology, as assessed by MTT assay [73]. Such a dramatic increase in metabolic rate inherently suggests using mitochondrial-directed agents in tandem with cytochalasin B during ultrasound treatments. Indeed, reactive oxygen species (ROS) agents often target the mitochondrial-induced apoptotic pathway of leukaemia cells [70,71,74], providing a viable method to develop synergistic treatments. This approach could be further supported with nucleic acid-directed agents, as cytochalasin B-treated U937 cells are considerably multinucleated. It is very likely that only a single nucleus will have to undergo apoptosis in order to destroy the malignant cell; having so many nuclei present greatly increases the chances of this event.

4.4. The utility of sound energy: sonodynamic therapy vs. photodynamic therapy

Activating chemotherapeutic agents through the use of an outside energy source is not a novel concept. In fact, SDT is extremely similar to PDT, a proven method that is currently being used in the clinical setting [86,87]. The major difference between SDT and PDT is the energy source used to activate the chemotherapeutic agent (sound *vs.* light). In PDT, light is used to excite porphyrins and other endogenous

molecules that emit photoluminescent light after returning to the ground state [88]. Such activity produces excess ROS levels that inflict substantial damage on the cellular integrity of malignant cells, eventually inducing apoptotic mechanisms.

While PDT has indeed been shown to be effective against particular squamous carcinomas, the effective range of the treatment does not extend far past the skin barrier [88]. Consequently, PDT has limited utility in cancer therapy. SDT uses ultrasound that can easily penetrate deep tissue layers where some malignancies reside. Although it retains the ability to produce ROS in the form of sonoluminescent light, SDT does so through inertial cavitation. When microbubbles implode, they give off substantial amounts of energy (in upwards of 5000 K and 800 atm), a phenomenon that produces sonoluminescent light, subsequently leading to the production of ROS [69]. The energy released from microbubble implosion also severely damages malignant cells through subsequent hydrodynamic shear forces, destroying cytoskeletal structures of cells and tumour vasculature [69].

Although PDT can effectively activate ROS agents and other species dependent on a light-activating source, cytoskeletal alterations and perturbed tumour vasculature networks have not been observed. Therefore, PDT is also limited in the variety of sensitizing agents that are available. By contrast, ultrasound provides a source of physical perturbation that can be further enhanced when combined with cytoskeletal-directed agents. My laboratory has shown that microfilament- or microtubule-disrupting agents (cytochalasin B and vincristine, respectively) produce higher rates of neoplastic cell lysis than either the corresponding stabilizing agents (jasplakinolide and paclitaxel, respectively) or untreated cells when combined with low frequency ultrasound (20 and 23.5 kHz, unpublished data). Further, there exists the potential of combining microfilament- and microtubule-disrupting agents in combination with ultrasound to further damage malignant cell populations, as cytochalasin B and vincristine demonstrate considerable drug synergy [89].

5. Conclusion

The inherent metastasis of leukaemia is apparent, as it is derived from leukocytes that are designed to cover vast distances in the human body in a very short amount of time. While carcinomas and other cancers must acquire specific mutations to gain this profound mobility, leukaemias need only become tumourigenic before being defined as a metastatic disease. Without having the need to develop mutations that permeate invasion and migration, followed by intravasation, these haematological malignancies are free to circulate throughout the body as malignant growths. Further, leukocytes have an innate form of extravasation, suggesting it is less complicated for dedifferentiated blasts to escape the circulatory system. While leukaemias do not form primary tumours, they are capable of rapidly crowding out healthy blood cells as their unchecked growth sequesters valuable resources. Eventually, the patient's immune system will become entirely compromised as healthy leukocytes have been replaced by a sludge of white, dedifferentiated cells, the hallmark that gave leukaemia its name ("leukos haima" is white blood in Greek). Nevertheless, lymphoid leukaemias have the propensity to form secondary tumours once enough mutations have been acquired (Richter's syndrome), which have very similar characteristics to lymphomas. It should also be noted that myeloid leukaemias have been found to form solid tumors, referred to as myeloid sarcomas, but these are unrelated to lymphomas.

However, the most beneficial phenotypic characteristics of leukaemia can be exploited by novel chemotherapeutic approaches. Specifically, SDT is capable of sonicating the circulatory highway that leukaemic blasts use to migrate towards distant tissue sites. Ultrasound can be used in combination with chemotherapeutic agents that substantially amplify the preferential damage inflicted on malignant cells. As indicated by experimental evidence, ultrasonic waves produce remarkable antitumour effects under appropriate conditions. However, such effects are not always widespread and varying levels of resistance are observed [69]. That is why SDT is such a sensible prospect, as it significantly enhances the efficacy of sonication, while still demonstrating preferential damage towards malignant cells. Every mechanism by which ultrasound destroys malignant tissue can in fact be amplified when an appropriate sonosensitizer is administered [69]. Such drugs often attack cells through multiple mechanisms as well, creating a potential synergistic effect when sonosensitizers of different classes are used in combination. It has even been shown to reverse acquired drug resistance in multiple cell lines, mitigating one of the most prolific defense mechanisms of cancer.

That is not to say all of the efforts put into developing SDT should be directed towards leukaemia, as there are other malignancies that take the lives of many more individuals each year. In fact, SDT could serve a tremendous utility in the clinic by eradicating metastatic emboli and micrometastases of other cancer types. Doing so might dramatically delay metastatic growths from forming in the patient, allowing clinicians to focus on the primary tumour, which can be treated with known adjuvant or neoadjuvant therapeutic approaches (pending on the performance status of the patient). It just should be noted that SDT has found particular promise with leukaemia and that many lives could be saved if effective treatments are developed. At any rate, the idea of combining ultrasound with drugs that amplify the ways in which it preferentially damages malignant cells is gaining more legitimacy as successful studies have vindicated the potential of SDT. By using the synergistic effects of ultrasound and sonosensitizers, SDT is proving to be a viable treatment modality that has the capability to revolutionize the way in which chemotherapy is administered in the clinical setting. Such an approach exploits the inherent metastasis of leukaemia,

turning implicitly advantageous phenotypic characteristics into a fatal flaw. However, further preclinical testing is still required before clinical evaluation is initiated. Only through these necessary studies will enough evidence be compiled to conclude whether SDT has substantial clinical potential.

Conflict of interest

The author declares no conflict of interest.

Reviewers

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Biography

Matthew Trendowski graduated from Syracuse University (Syracuse, NY, USA) Summa Cum Laude a year early in 2014, while still successfully completing all of the requirements for the Renée Crown University Honors Program. In addition, he was awarded the Donald G. Lundgren Memorial Award for Outstanding Scholarship & Research, given to the most outstanding Biology major graduating at Syracuse University. He has numerous publications in the fields of cancer biology and chemotherapy, and has given talks at prominent research conferences. Matthew intends to earn a MD/PhD, with an emphasis on pharmacology and drug discovery in relation to cancer therapy.