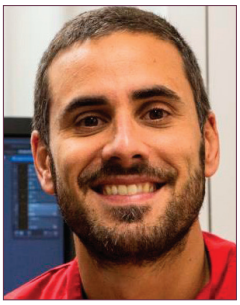
Breast cancer brain metastasis: The complex bench-to-bedside proceedings



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Advances in new strategies for cancer treatments have markedly improved the life expectancy of patients. However, therapy of secondary spread (metastasis) of tumours remains far from satisfactory; currently, metastasis of primary tumours causes 90% of total cancer deaths [1].

However, one particular tumour type – breast cancer – shows a significant increase in patient survival, life expectancy doubling in the last 40 years according to Cancer Research UK, 2015. This type of cancer has the ability to metastasise to several distant organs, such as lung, bone, liver and brain [2]. Clinical data indicates a huge variability in the incidence of brain metastases, usually in the range of 10 to 40% depending on breast cancer subtype [3]. Nevertheless, one feature is common to all of these reports, i.e. patient survival is measured in just months once cancer cells disseminate to the brain.

Part of the meagre improvement conferred by current therapies is the lack of experimental models that recapitulate accurately this multistep disease and, thus enable reliable analysis of different therapeutic approaches. We can find numerous in vitro and in vivo models in the literature for the study of different primary and secondary tumours, which can be categorised based on tumour type, animal background, tumour induction route, etc. With the caveat that each animal model carries its own advantages and limitations [4], we will describe different pre-clinical studies in which mouse models for the study of breast cancer brain metastasis have proved useful.

Mouse models in brain metastasis research

The initial stages in the design of novel therapeutic drugs comprise a thorough pre-clinical work up based on multiple in vitro and in vivo validations. In the case of brain diseases, there are several groups working on the elaboration of 3D in vitro models, in an attempt to epitomise the complex architecture of the central nervous system and the blood-brain barrier (BBB) in particular [5,6]. However, to the present day, none of these models seem to provide a sufficiently representative approach to completely replace in vivo validation. Therefore, animal models remain an inevitable tool for the study of neurological diseases and subsequent therapy development.

Although it is impossible to fully extrapolate results obtained from a rodent-based study to the actual human response, current techniques allow researchers the use of xenograft animal models. These are based on the injection of human cancer cells into immunocompromised animals, and thus represent a semi-humanised approach. This allows one to take a step closer to understanding the interactions between human cancer cells and the brain. We will describe how some of these humanised animal models have shed light on the biology behind brain metastasis progression and how this has potential clinical applications.

Molecular targeted therapy

One of the newest approaches to the treatment of cancer metastasis is molecular targeted therapy (MTT). This concept relies on the idea of targeting proteins specifically involved in tumour growth and dissemination. A particular family of transmembrane proteins that play a critical role in the successful colonisation of circulating tumour cells in distant organs are the cellular adhesion molecules (CAMs). This ubiquitous family of proteins has pivotal roles in almost all phases of human biology (such as proliferation, migration, apoptosis, survival, etc.), as well as in many diseases [7]. Despite the complexity of metastasis, which comprises a number of different stages, there is evidence that CAMs are actively involved in many, if not all, steps of the metastatic cascade [8,9].

The privileged location of CAMs on the cancer cell surface makes them attractive targets for clinical trials [10]. Two primary approaches to evaluating the potential of anti-CAM therapies have been used in our lab. The first approach involves blocking antibodies against particular CAMs that are known to be involved in tumour progression. The aim has been to block the interactions between CAMs expressed on tumour cells and their counter ligands in the tumour microenvironment [11]. The second approach has used interference RNA (iRNA) techniques to knockdown gene expression of specific CAMs so as to determine their impact on tumour progression by modulating the interaction of metastatic cells with brain cell populations [12].

Targeting different stages of brain metastasis

The design of experimental studies to investigate brain metastasis can be focused at different phases of the metastatic cascade. Some studies are aimed at treating the early stages of colonisation in the brain, whilst others have been designed to target tumour colonies once they have reached a compromising size within the central nervous system. In the first case, metastatic tumour cells are introduced into the bloodstream by intracardiac or intracarotid injection, and allowed to disseminate to the brain as would occur in patients. In the second case, tumour cells can be introduced directly into the brain by stereotaxic microinjection to bypass the initial seeding stages, and thus focus on downstream tumour proliferation within the brain environment.

Early stage diagnosis and treatments

The diagnosis of brain metastasis and subsequent treatment may vary depending on the number of metastatic colonies, total tumour volume, and their location within the central nervous system. Therefore, early diagnosis is vital to detect tumours at a size when conventional therapies, such as surgery or radiotherapy, can be most effective on tumour progression. Improvement in early diagnostic methods would give clinicians a greater window of opportunity to apply such therapies and increase patient life expectancy beyond a few months.

Inflammation is one of the hallmarks of cancer, CAMs being main contributors to its onset and progression during metastasis. These molecules are very sensitive to the presence of tumour cells and their expression is significantly upregulated in the tumour microenvironment [8,11,13]. This idea formed the basis of a number of studies exploiting the presence of different CAMs, including VCAM-1 (CD106). In several models of brain metastasis, we have been able to localise micrometastases in the brain, prior to BBB disruption, through the use of contrast agents targeting VCAM-1 that can then be detected by magnetic resonance imaging [14]. This pre-clinical work is now being translated to a Phase I/IIa clinical trial.

Following the same idea of using MTT for early diagnosis, our group is now developing new approaches to facilitate delivery of therapeutic agents to micrometastases in the brain. The BBB is a natural hurdle that stops most current anti-cancer drugs from crossing into the brain, and this is particularly a problem in the early micrometastatic stages, when the BBB is completely intact, but tumours may be more amenable to treatment if they can be accessed. We have shown that, in addition to various CAMs, the vessels associated with micrometastases express high levels of another type of protein, tumour necrosis factor receptor 1 (TNFR1), which, when activated by systemically administered TNF-like agents, provokes a disruption in BBB integrity [15]. This is selective and specific to sites of micrometastases, allowing anti-cancer drugs to access the brain parenchyma at these tumour sites. Thus, this strategy holds promise for treating micrometastases that are diagnosed early, even when the BBB is still intact. This work is currently in the late stages of pre-clinical development and will be a firm candidate for future clinical trials.

Another possibility is to target the early interactions of circulating tumour cells with the vascular endothelium, via CAMs, as a potential therapeutic strategy. Such an approach would be designed to prevent adhesion of circulating tumour cells to the cerebral vasculature and subsequent extravasation into the brain parenchyma. For instance, VCAM-1 and ALCAM (CD166) are 2 inmmunoglobulin-like CAMs intimately involved in leukocyte and tumour cell interactions with the lumen of blood vessels [11]. Their counter ligands, VLA-4 and ALCAM, respectively, are expressed in many types of tumour cells, including breast cancer cells. To explore this potential therapeutic strategy in a pre-clinical mouse model of breast cancer brain metastasis, tumour cells were pre-treated with antibodies against either VLA-4 or ALCAM. These pre-treated cells were injected intracardially into mice and allowed to disseminate to the brain. As a result of the CAM neutralisation, a significant decrease in subsequent colonisation of the brain was seen (Figure 1). The results suggest the potential for antibody therapy in patients with breast cancer at risk of suffering from brain metastasis.

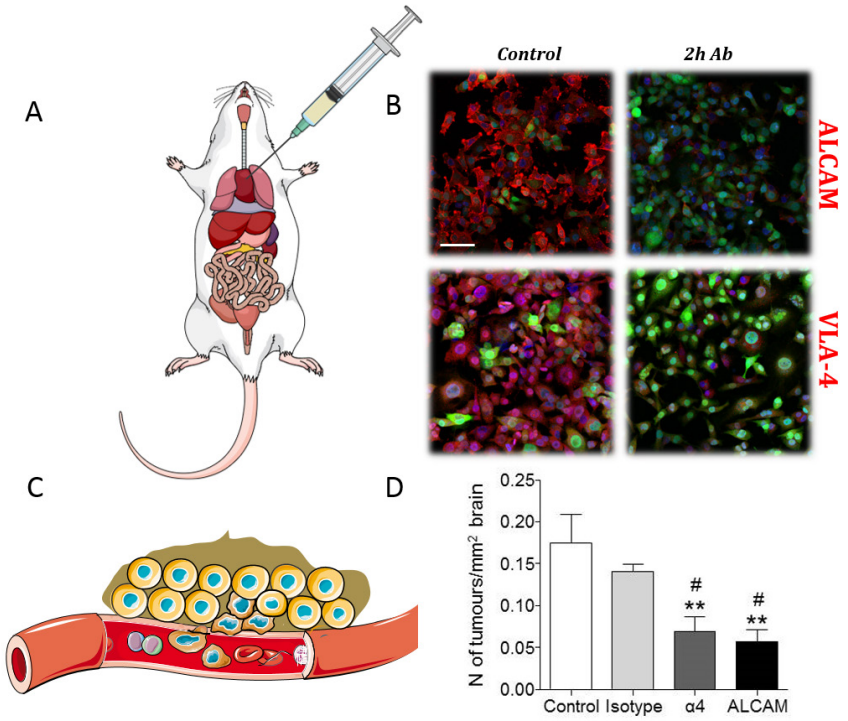

Figure 1. A. Schematic of intracardiac delivery of tumour cells. B. Illustration showing the extravasation process of circulating tumour cells into the brain milieu. C. MDA231Br cells (human breast cancer cells) in green, showing expression of ALCAM and VLA-4 (in red) before (control) and after (2h Ab) antibody treatment. A significant reduction in CAM expression is evident after treatment. Nuclei of tumour cells in blue. Scale bar 50μm. D. Graph showing significant reduction of metastatic colonies 21 days after intracardiac injection of tumour cells pretreated with neutralising antibodies compared to controls; MDA231Br cells were treated with either anti-ALCAM or anti-VLA-4 (Đ4) antibodies.

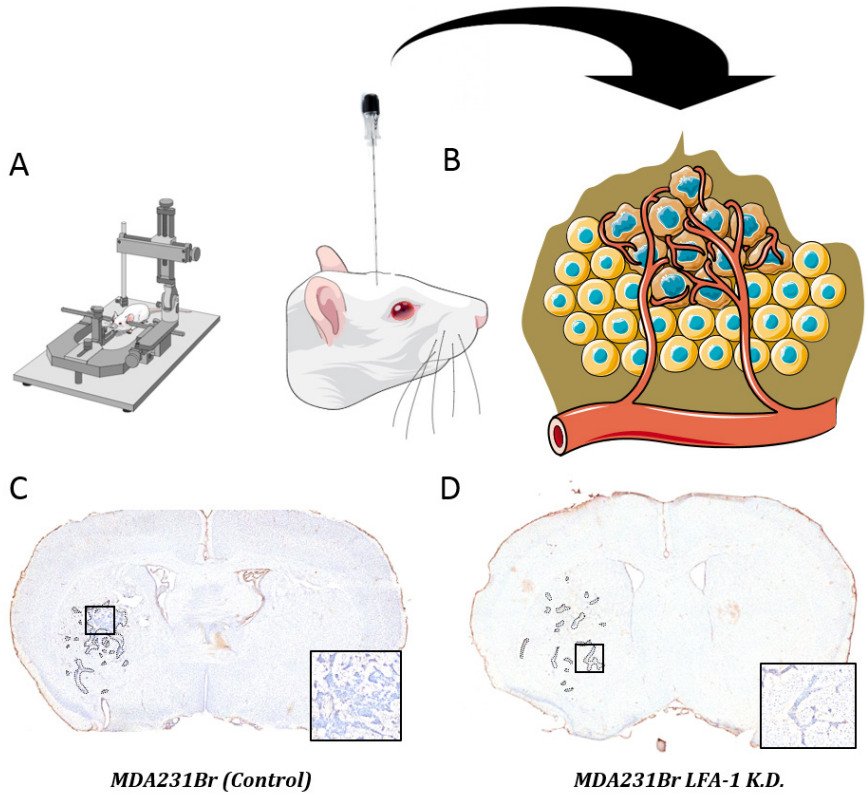

Figure 2. A. Schematic of the stereotactic frame and the intracerebral implantation of tumour cells. B. Illustration of the secondary tumour growth within the central nervous system. C-D Coronal images of mouse brain sections showing the extent of tumour colonies (dotted lines) following intracerebral injection of either parental MDA231Br cells (left) or the same cells with LFA-1 knockdown (right). A clear reduction in tumour growth was seen in animals injected with LFA-1 knockdown tumour cells compared to the control group.

Late stage treatments

A late diagnosis means that tumours are bigger and more aggressive, surgery and radiotherapy become largely ineffective, and prognosis is dismal. Usually, when patients experience signs and symptoms of primary tumours, metastatic spread is already present in different organs. Epidemiologic studies show that most cancer patients with a late diagnosis have lower chances of survival compared to patients where the treatments started in the early stages of cancer initiation (Cancer Research UK, 2016). Nevertheless, one feature of the later stages of brain metastasis growth that may facilitate treatment, despite the advanced stage, is disruption of the BBB. Thus, if more effective therapeutics can be developed/identified, then BBB disruption might well deliver such agents to the tumour microenvironment.

An important detail that must be taken into account in brain metastasis therapy is the role of the tumour/brain microenvironment. Tumour cells have the ability to manipulate the host tissue and drive it into a more tumour-supportive phenotype. As a result of this co-option of brain cell function, combination therapy targeting both the tumour cells themselves and elements of the local host response is becoming popular for the treatment of tumours in such an aggressive state. Thus, neo-adjuvant therapies, such as MTT against CAMs expressed on metastatic cells, may supplement frontline treatments (surgery or radiotherapy) and enhance patient survival.

To explore the potential of this approach, direct intracranial implantation of metastatic tumour cells into the brain has proven useful [12,16]. Some of the advantages that this model offers are the precise implantation of tumour cells into a known location and the potential to study tumour growth at later time points than is possible with the intracardiac models described above; in those models systemic dissemination of tumour cells can lead to serious deterioration in animal welfare over longer time-courses.

Using intracranial models of brain metastasis, we have shown that CAMs also play an important role in metastasis growth in the late stages of metastatic disease. Tumour cells use these proteins to interact with surrounding brain cell populations (e.g. astrocytes, microglia, neurons, endothelial cells), and blockade of certain CAM-based tumour-brain interactions has shown promising results in our pre-clinical studies [12]. We have now demonstrated that using iRNA against LFA-1 (CD11a/CD18, $\alpha L\beta 2$), another CAM expressed in human breast cancer cells, disruption in signalling with its cognate ligand ICAM-1 on brain cells significantly reduces tumour growth (Figure 2).

Translational approaches

Drug development in oncology has gained considerable momentum in the clinic. MTT, with its ability to harness the body's immune response, is also making significant progress. However, despite the large investment by industry in designing and implementing new strategies to target cancer cells, Phase I and Phase II clinical trials have a significant failure rate. One explanation for this apparent lack of success is the need for improved experimental design at the pre-clinical stage. Animal models are one of the most powerful tools to fill that gap from bench to clinic, but these do not always fully recapitulate the clinical situation. Thus, care must be taken in developing and applying these in the most appropriate and representative manner, which may involve the use of several different models. As previously described, treatments based on antibody and iRNA techniques against a particular set of CAMs show promise as new approaches to reduce brain metastasis onset and progression. The current existence of drugs against some of these proteins, such as Natalizumab (anti-VLA-4) or Efalizumab (anti-LFA1), for the treatment of other diseases, such as multiple sclerosis and psoriasis [17,18], offers the possibility of repurposing these agents for the treatment of brain metastasis. A major advantage of this approach would be access to their previous clinical history and a substantial reduction in the time taken to reach the clinic; development of new drugs varies from 10 to 17 years from inception to clinic, whilst repurposing of drugs can reduce this time to ›10 years.

Conclusions

Current treatments for patients suffering metastatic spread to the brain are based on different combinations of surgery, radiotherapy and chemotherapy. There is clear evidence for the benefit of combined,

rather than single, therapies in this situation. Our pre-clinical work suggests that CAM-based therapies may be an effective immunotherapeutic approach to target metastatic growth in the brain. The next steps will investigate the role of such agents in combination with other therapies, such as radiotherapy or chemotherapy, as a potential neoadjuvant route to the treatment of brain metastasis.

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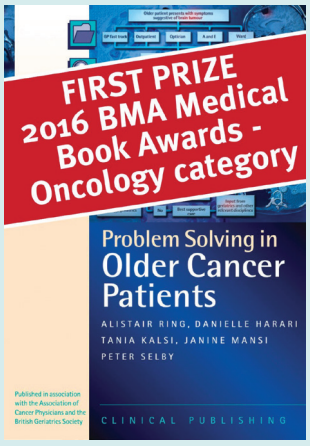
BOOK REVIEW

Problem Solving in Older Cancer Patients

Alistair Ring, Danielle Harrari, Tania Kalsi, Janine Mansi, Peter Selby. Published by: Clinical Publishing. ISBN No: 978-1-84692-110-0. Price: £39.95.



This 310 page book is published in association with the Association of Cancer Physicians and the British Geriatrics Society. The book is aimed at the Multi-disciplinary team managing the older cancer patients. I feel this book is eminently suitable for the Oncology Specialist Registrar though Consultant Oncologists, Nurses and Allied Health Professionals will appreciate it.

The numerous contributors are from the UK. The book is divided into two sections: Section One: Perspectives and Section Two Case Studies.

Section One: Perspectives, contains 18 chapters devoted to the challenges of treating the elderly cancer patient; using surgery, radiotherapy and systemic chemotherapy. Other factors such as patient selection for treatment, ethics and capacity for consent and palliative care are considered.

Section two: Case- studies comprises 32 chapters which discuss the management of a wide range of patient scenarios. For each case the case history is presented followed by several thought provoking questions. Each question is answered in detail, citing trial evidence where necessary. This is followed by a conclusion and learning points in clear bullet point format. References and examples of further reading are listed at the end of each chapter. I found that the selection of cases were typical of those seen in the out-patient clinic, for instance prostate, renal, breast, colorectal carcinomas. I felt that the decisions about treatment were balanced considering that it very easy to over treat an elderly patient and send them into an irretrievable downward spiral of complications. Experience often dictates that, "less is more."

Overall I found this text to be readable. A lot of information is presented in tables and highlighted boxes. The book revealed the importance, of involving many health care professionals including the General Practitioner in the overall management of the patient, and of good communication between the professional teams. Given the increasing incidence of cancer and that of the elderly population surviving with an increasing number of co-morbidities, I consider this to be a relevant and useful book to the Oncology Trainee in particular. It is also a useful read for other members of the oncology team.

Dr Karin Baria, Retired Consultant Oncologist.