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Orthotopic models of cancer for preclinical drug evaluation: advantages and disadvantages

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Abstract

Considering the enormous effort that has taken place over the years to discover new chemotherapeutic drugs for treating the common cancers, the conventional murine and xenograft test systems used to test efficacy for drug development have identified only a limited number of useful agents that are active clinically at well tolerated doses. In recent years, considerable effort has been made to develop more clinically relevant models by the use of orthotopic transplantation of tumour material in rodents. It has been shown that it is now possible to transplant tumour material from a variety of tumour types into the appropriate anatomical site and often these tumours will metastasise in a similar manner and to similar locations as the same tumour type will in human cancer. As yet, although a body of literature has amassed on the technique itself and its implications for metastasis, there are relatively few laboratories using these test systems in drug development programmes. Nevertheless, given the expertise now being developed and some interesting observations being made on the role of the tumour site on response to therapeutic agents, it is likely that the use of orthotopic systems will strengthen our ability to select the most appropriate molecules for recommended use in clinical studies.
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1. Introduction

The selection of appropriate animal model systems in which to assess novel therapies for cancer remains controversial, but as drug discovery programmes become more geared to rational drug development, where molecules are designed to interact with a specific clinically relevant target, the selection of the appropriate *in vivo* test system is crucial to establishing the worth of such molecules. In the past, murine tumour systems were used for drug screening with mouse leukaemias being utilised as prescreens [1]. These grew very rapidly, had a high growth fraction and proved to be sensitive to a number of agents that were subsequently shown to have more activity against leukaemias and lymphomas than against solid carcinomas and sarcomas and to be toxic to the bone marrow [2]. As a result of these early screens, there is a general misconception that tumours in rodents are sensitive to drug therapy and are easy to cure. In reality this is untrue and back in 1987 Corbett and colleagues [3] pointed out that most of the agents that had entered the clinic at that time had poor or no activity against the majority of transplantable solid tumours in mice. Modest activity is often seen, but this is usually at the expense of host toxicity [4]. In current times, new drugs are not screened for activity in panels of tumours until there are considerable mechanistic and *in vitro* data available. The National Cancer Institute (NCI) in the United States of America (USA) has had a major influence in moulding the modern strategy for drug screening ensuring that information on cytotoxicity levels and potential targets of new molecules is available at an early stage [5,6]. If a molecule has successfully passed the various tests that suggest it may have potential for further in-depth study, an appropriate *in vivo* model must be sought. This has traditionally been a panel of often poorly characterised human tumour xenografts implanted subcutaneously (s.c.) in nude mice. Although this type of model is relatively easy to operate in that it is technically straightforward and generates lots of data it is not necessarily the most

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relevant to clinical cancer. There are publications that suggest that correlations between xenograft data and clinical activity are good [7], but there are publications that give the opposite view [8]. One major drawback of models that utilise s.c. tumour implants is that they clearly do not reproduce the primary site of the common human cancers nor do they represent the common sites of metastasis. Humans develop lung cancer in the lung, colon cancer in the colon, breast cancer in the breast etc and the common malignancies metastasise preferentially to specific sites, e.g. colon tumours metastasise to the liver. In an attempt to address these issues, orthotopic transplantation of tumour material to the appropriate anatomical site has been established. There seems to be a general view appearing amongst researchers involved in the drug discovery and development process that the use of models that more closely reflect the biological features of cancer growth and metastasis in humans will provide better prediction of potential clinical activity. This short review attempts to address some of the advantages and disadvantages of adopting this procedure for drug discovery and development.

2. Principles of orthotopic transplantation

Orthotopic transplantation of colon tumours in mice has been around for many years [9] and there is now a wealth of literature describing tumour material being implanted into most of the common sites in which cancer arises. Of course the technique not only needs to be technically feasible, but it must be ethically acceptable. It is clearly very straightforward to implant breast cancer tissue into the mammary fat pad of mice, but much more demanding on surgical skills to implant prostate cancer tissue into the prostate of a mouse for example. Early experiences were largely restricted to colon cancer and a number of general observations have been made using colon models. Orthotopic transplantation of murine colon adenocarcinoma resulted in metastatic growth in the liver [9]. At that time, the role of this type of model for new drug evaluation was not proven. Other studies since the 1950s had indicated that it was possible to grow tumours in rodents in other sites e.g. by injecting tumour cells into the left ventricle or intravenously (i.v.) [10]. Much of the effort at that time was to investigate the various stages of metastatic spread rather than to develop models for drug discovery purposes. In the case of therapeutic studies, it is important to fully characterise the model system in advance to ensure the therapeutic target is present in the model and this can be easily illustrated by a series of procedures carried out in this laboratory with a syngeneic murine model system. For this work, we utilised one of a mouse colon model panel developed in the mid-1970s [11]. These are adenocarcinomas of the colon originally induced in Naval Medical Research Institute (NMRI) mice by dimethylhydrazine. They have an advantage over some other murine systems in that they consist of a series of tumours that possess different growth characteristics, differentiation state [12] and chemosensitivity patterns to commonly used anti-cancer drugs [11]. For these early studies, we selected the MAC15 tumour. These tumours are locally invasive at the s.c. site (Fig. 1a) and previous studies had demonstrated that when a piece of this tumour was implanted intraperitoneally (i.p.) it was possible to drain ascitic fluid that contained tumour cells from these mice. These tumour cells could be grown in culture or injected i.v. when they grew in the lungs of recipient syngeneic hosts to form poorly differentiated lung colonies [13]. Using this model, it was possible to examine the influence of tumour site on response to anti-cancer drugs [14] without the necessity for intricate surgery—at first sight a very useful addition to an *in vivo* test system, although it did not address a fundamental question in colon cancer therapy viz the response of liver metastases. This needed a different approach and, in the first instance, we inoculated MAC15 cells from ascitic fluid into the wall of the caecum. This resulted in the successful growth of a poorly differentiated adenocarcinoma in the caecal wall (Fig. 1b) with metastatic deposits in the liver (Fig. 1c, d) [15]. If on the other hand, tumour pieces from the s.c. serially transplanted MAC15 were implanted into the caecal wall, a well-differentiated tumour grew at the transplantation site (Fig. 1e) and well-differentiated metastatic deposits were identified in the liver (Fig. 1f, g). Clearly, the cells recovered from the ascitic fluid did not retain the differentiation state and did not reproduce the morphology of the original tumour. Although the poorly differentiated model may well have been useful for some drug molecules e.g. standard anti-proliferative agents, it would have been less useful for evaluation of molecules that target specific stromal components of the tumour such as the tumour blood supply. As a result of the expanding literature on orthotopic transplantation and general consideration of the clinical relevance of tumour site, a number of factors that are important for drug responses have now been identified. One example is from the studies of Fidler and colleagues [16,17] who investigated the response to doxorubicin (dox) and 5-fluorouracil (5-FU) in three tumour types growing in different anatomical sites. Response to 5-FU was similar independent of site whereas only s.c. tumours responded to dox. These authors demonstrated that the difference in sensitivity to dox was probably due to overexpression of *mdr1* mRNA in the resistant sites. These experiments demonstrate the need for thorough biological investigation of the model systems employed in order to interpret the effects of therapy, an area often overlooked in preclinical drug studies.

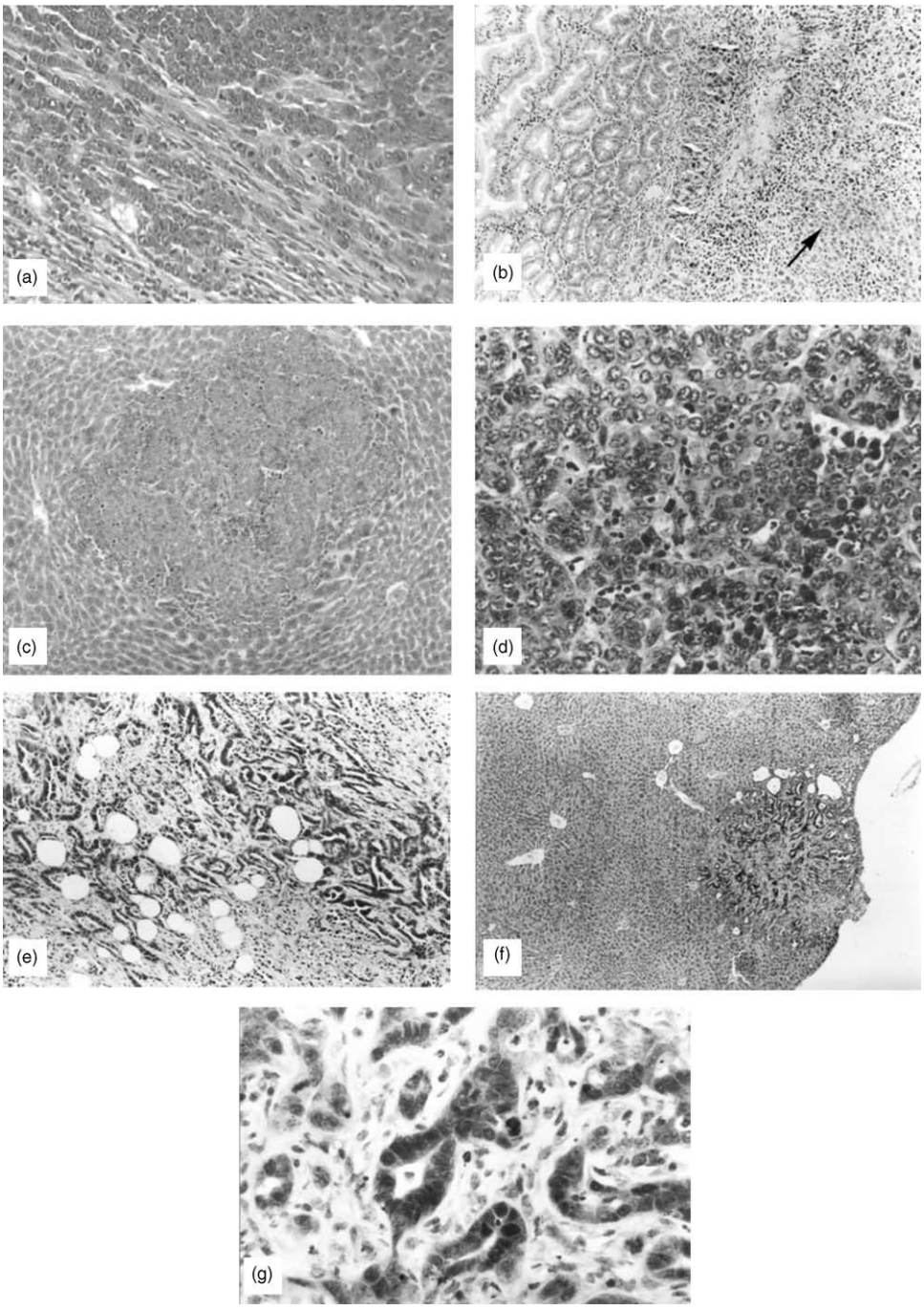

Fig. 1. Histological sections of murine colon tumours (H&E). (a) Local invasion in subcutaneously transplanted MAC15A tumour. (b) Poorly differentiated appearance of orthotopically implanted MAC15A cells in the mouse caecum (↑). (c) Poorly differentiated colon metastasis of orthotopically implanted MAC15A cells. (d) High power of (c). (e) Well-differentiated MAC15 tumours implanted in mouse caecum. (f) Single well-differentiated metastasis of orthotopically implanted MAC15 tumours. (g) High power of (f) showing glandular appearance of metastasis. (original images b–g courtesy of Ramakrishnan, 1983 [15]). MAC15A cells were derived from ascitic fluid [13].

3. Advantages of an orthotopic system

Given the fact that many tumour types can now be grown successfully in orthotopic sites [18–21], what are the real advantages of such systems for drug discovery and development? It is now clear that the process of metastasis is more efficient in orthotopically implanted tumours and closely mimics human metastasis [22]. There are impressive studies describing metastasis of orthotopic tumours to clinically relevant sites e.g. prostate and breast to bone [23,24]. One of the most obvious advantages then of orthotopic systems is that attempts to target processes in local invasion e.g. inhibition of proteases or interfering with the process of angiogenesis

can be carried out in a more clinically relevant site. The identification of different receptor expression in endothelium from different organs [25] clearly demonstrates the usefulness of using appropriate sites for design and investigation of novel targeted anti-angiogenesis therapies. Studies with anti-vascular drugs have been carried out in models of colon cancer. 5,6-dimethyl xanthenone acetic acid (DMXAA) [26] and Combretastatin A-4 phosphate (CA-4) [27] have been shown to cause vascular shutdown and haemorrhagic necrosis in vascularised colon tumours in orthotopic and metastatic sites in mice [28,29]. Blood flow effects with CA-4 occurred well below the maximum tolerated dose (MTD) whereas DMXAA had a narrow therapeutic window. In subsequent clinical trials, CA-4 appeared to be more effective at causing changes in blood flow in human tumours. More recently, another combretastatin analogue, combretastatin A-1 phosphate, has been shown to be more effective than CA-4 at causing haemorrhagic necrosis in two models of human colon tumour liver metastasis [30]. It remains to be seen whether data from orthotopic tumour systems give a better indication of potential clinical activity of these antivascular drugs.

Orthotopic transplantation has occasionally been useful in identifying potential pitfalls of s.c. tumour models. There have been a number of publications indicating that it is possible to potentiate the activity of standard and investigational agents in experimental tumours by combination with a variety of vaso-active agents. This approach relies on the described lack of smooth muscle and innervation of neovasculature in solid tumours [31]. Many agents alter blood flow in tumours [32], but most studies utilised the anti-hypertensive hydralazine that is effective at reducing blood flow in transplantable tumours in rodents [33,34]. Hydralazine was shown to enhance the effectiveness of bioreductive drugs such as RSU1069 [35], Tirapazamine [36], EO9 [37] and mitomycin C [38] and a couple of direct-acting cytotoxic agents, melphalan [39] and tauromustine [40]. These studies were all carried out in s.c. transplanted models and although such studies indicated a potential therapeutic strategy this has not been shown to work clinically. Studies in rodents with primary malignancies [41] indicated less of an effect with hydralazine and studies from this laboratory indicated that hydralazine was more effective at shutting down blood supply to a s.c. transplanted murine colon tumour than to the same tumours transplanted orthotopically [42]. Although the approach of altering tumour blood flow for therapeutic gain in humans seems to be little studied at present, Rowell and colleagues [43] showed by the use of SPECT and ^{99m}Tc-HMPO that single dose oral hydralazine caused the blood flow through human lung tumours to increase rather than decrease. Experimental evidence suggests that orthotopically transplanted tumours may be more appropriate models in which to investigate these physiological strategies than the usual s.c. transplanted tumour models.

To date, considering the large body of published information on orthotopic systems, few studies have been concerned with investigating the effects of chemotherapy in general and even less have been used in preclinical studies of novel drugs. However, some of these studies suggest that the results are likely to better reflect the activity in patients. The Hoffman group which is prolific in the area of orthotopic tumour research, using an *in vivo* model of small-cell lung cancer (SCLC), showed that cisplatin had significant effects against lung tumours, but was ineffective against the same tumours growing s.c. [44]. Mitomycin C was ineffective against the lung tumours thus reflecting the clinical situation. The authors concluded that their data suggested that tumours grown orthotopically reflect the clinical effects of drugs on human SCLC more closely than the tumours growing s.c. The Fidler group made the equally valid point that human colon xenografts growing s.c. in nude mice often respond to dox, whereas human colon cancer does not [17]. Despite these very interesting studies, there is a need for more in-depth assessment of the potential advantages of orthotopic over s.c. tumours by examining currently useful chemotherapeutic agents against common cancer types.

4. Disadvantages of orthotopic models

It is relatively easy to identify disadvantages of orthotopic transplantation, the most obvious limitation being technical skill. The procedures are generally far more difficult and time-consuming, hence more expensive than for conventional s.c. models. Endpoints for determining the effects of therapy are more complex than the normal tumour measurement in s.c. models and ensuring that animal suffering is kept to a minimum, although essential, can be difficult. As imaging studies are developed, improved and become more widely available, the endpoint becomes less of a problem and animal usage can be reduced as the ability to follow the effects of therapy sequentially in individual animals becomes possible.

Of course the major goal of drug discoverers is to develop effective therapies for metastatic deposits of the major common malignancies. Very often the surgeon has successfully removed the primary tumour, but the patient will die later of metastatic disease. In our experience, metastasis from an orthotopically transplanted colon tumour in a mouse can be a late event, at least the mouse may succumb to the primary tumour prior to significant metastasis to the liver being obvious. The identification of a suitable endpoint other than the scientifically and morally unacceptable one of survival

can be difficult. However, if the "primary" deposit is surgically excised and the mouse allowed to recover, metastatic deposits occur in the liver after a few weeks. This makes the procedures more complex, but certainly produces a good model of large bowel cancer.

The development and establishment of stable green fluorescent protein (GFP)-expressing cell lines that permit detection and allow visualisation of growth of the tumour and metastases in live tissue [45] has had a major impact on research with orthotopic models. At the time this work represented a significant improvement over the use of the *Escherichia coli* β galactosidase (*lacZ*) gene to identify metastases where it was necessary to use histological preparations of tissues [46]. A number of publications have now appeared on the use of GFP to monitor orthotopic and metastatic growth of tumours non-invasively and this technique would lend itself to preclinical drug evaluation. A non-invasive imaging technique for monitoring luciferase-expressing human prostate tumours and metastasis in nude mice after i.p. inoculation of luciferase has also been described [47] and a particularly interesting approach is the use of *in vivo* bioluminescent imaging to study bone metastasis in a model of prostate cancer [24]. Animal positron emission tomography (PET) studies have been successfully used to monitor the effects of the anti-vascular agent CA-4 in murine liver metastases [48]. Although these imaging studies have indicated the potential for monitoring tumour growth non-invasively, the techniques are not yet widely available and it is still unclear whether the use of orthotopic versus s.c. tumours results in a better prediction of clinical response. The matrix metalloprotease inhibitor, Batimastat, was shown to reduce tumour progression in an orthotopic model of colon cancer [49], but this compound has subsequently been shown to be disappointing in clinical trials, so it appears that orthotopic tumours can still overestimate potential clinical efficacy.

5. Conclusions

In assessing the question as to whether orthotopic models are better for drug studies than the more conventional s.c. tumours, it is necessary to first think about the questions we are attempting to answer [50]. What do we need from a preclinical tumour model used in drug discovery? The most important question can be answered in a simple mouse system i.e. can effective drug concentrations based on previous *in vitro* data be achieved *in vivo* and are these concentrations tolerated without major toxicity? Assuming this to be in the affirmative, the next question to address is: Is the molecule reaching and, more importantly, interacting with its designated target? A prerequisite of any model then, whether it be a s.c. or orthotopic tumour, must be that not only is the target present, but that it is possible in that model to demonstrate evidence of the mechanism of action i.e. a pharmacodynamic endpoint. The best approach to selecting an animal model for drug efficacy studies is to design the model to address each question. It is likely that a number of clinically relevant targets will be better represented by orthotopic tumour systems that mimic the morphology, microenvironment and growth and metastatic patterns of human cancer. Angiogenesis is one of the areas that appears appropriate for more in-depth study in this regard.

In conclusion, there have been very significant developments in orthotopic transplantation techniques for studying the process of cancer metastasis. It is clear that the appropriate microenvironment leads to the development of the metastatic phenotype and there are further opportunities to investigate the molecular events associated with cancer progression. However, there is still some way to go in determining the real advantages these techniques may have in improving our ability to design and evaluate the most effective molecules for treating human metastatic disease. The time is right to fully characterise these models to establish their real worth as predictors of therapeutic outcome of clinical disease.

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