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Rajagopal N. Aravalli
Jafar Golzarian
Erik N. K. Cressman

Animal models of cancer in interventional radiology

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R. N. Aravalli · J. Golzarian ·
E. N. K. Cressman (✉)
Department of Radiology,
University of Minnesota Medical School,
MMC 292 Mayo,
420 Delaware Street SE,
Minneapolis, MN, 55455, USA
e-mail: cress013@umn.edu
Tel.: +1-612-6265540
Fax: +1-612-6265580

Abstract Animal models will play an increasingly important role in oncology research, especially for solid tumours such as hepatocellular carcinoma that are resistant to chemotherapy. Many models have been used, but there is a need for increased awareness of the limitations of these models and also a need for guidance for future model development.

Keywords Interventional oncology · Tumour ablation · Hepatocellular carcinoma · Animal model

With the rapid growth in interventional cancer treatment and imaging around the world, the question naturally arises: which treatments are 'the best'? Patients, physicians, researchers, grant agencies and companies are all interested in the answer to this question. Many studies are undertaken and published. Most of these studies are of the retrospective sort. Very often they are sponsored by manufacturers. The inevitable result is that they come with significant attendant limitations.

Prospective, randomized human clinical trials are expensive, time-consuming, and very difficult to perform. Further compounding the problem, there may be no ethical way to conduct a trial that would answer the question at hand. We must keep in mind our true goals: survival benefit or palliation. We must be certain the benefits are real and that they exceed the risks of procedures. It is not sufficient after an intervention to show a change in some parameter. Examples often used include a smaller tumour, a decrease in enhancement with contrast, a change in some imaging parameter such as diffusion-weighted imaging, or even 'no evidence of disease'.

This is not to say that ablations, embolizations, etc. make no difference. Indeed quite the contrary is true. Nevertheless, this is the setting in which we use animal models in an effort to improve our methods and outcomes. We must exercise caution in what models we use, how we design our investigations and in how we interpret the data [1]. We are in essence trying to mimic an extremely complex, poorly understood process with a much simpler one. We succumb readily to unjustified extrapolations from the results because the temptation is strong and yet quite subtle. So subtle in fact, that it often goes unrecognised.

It has been noted that we have cured cancer in a mouse a thousand times over, yet still millions of people die of malignancies each year. Even more patients put up with all manner of what are essentially systemic poisons and terrible side effects in an effort to forestall the inevitable. There has been a sharp increase over the last several years in the use of minimally invasive, image-guided ablation therapies and the research that is published. It is therefore timely to ask some pertinent questions such as (1) What constitutes a good animal model? (2) What are some of the most commonly used animal models? and (3) What would make an improved animal model? In short, are the conclusions we are drawing from our models resulting in changes that are improving survival and symptom relief for our patients?

The following discussion summarises a few articles in the literature and a few observations made along the way. We hope this will provide a basis for further discussion, improve understanding and perspective of the existing literature, encourage more critical examination of future work in this arena, and finally, offer some direction with regard to the design and conduct of future studies. The

focus here is on models used to imitate hepatocellular carcinoma (HCC), but many of the same considerations could be applied to other tumours such as renal cell, prostate, or breast cancer, etc. We will begin with a more general discussion, list and examine a few specific models, and summarise the situation with an eye toward future model development.

To address the first question concerning what constitutes a good animal model, we must first obtain a larger picture with more background and context. Animal models, or more broadly, biomedical models, have been defined as 'surrogates for a human being, or a human biologic system, that can be used to understand normal and abnormal function from gene to phenotype and to provide a basis for preventive or therapeutic intervention in human diseases' [2].

In 1985 the National Research Council in the United States articulated the rationale as follows: appropriateness as an analog, transferability of information, genetic uniformity, background knowledge of biological properties, generalizability of the results, ease of experimental manipulation, ecological consequences and ethical implications. Paraphrased, the traditional view would entail considerations such as relevance (though this often gives way to other factors as we will see), practical considerations such as cost, time to develop the condition under query, reproducibility, familiarity and technical ease.

More recently, additional aspects have entered the picture. These include the ability to study the condition at the cellular level in addition to the tissue and whole-organism level, attractive imaging parameters, and the ability to treat using human-scale devices. In addition to the time required to develop a treatable mass (tumour kinetics), the survival time in untreated animals may help to refine the choice further. The newest areas to reach prominence have reflected the explosion in technology regarding molecular and cell biology, and the various 'omics': genomics, proteomics, metabolomics and so forth [3].

From the standpoint of practical considerations, certain of these constraints are clearly at odds with each other. Consider the inverse relationship of cost and statistical power of a study. Zebra fish are cheap and plentiful [4], but of course do not lend themselves readily to catheter or ablative techniques. Rodents and rabbits are more expensive but somewhat challenging to catheterize. Pigs, dogs and primates, while costly, are much more in line with the human scale for devices. The physiology is also much closer to human physiology. The trade-off with larger animals of course is that statistical power will in most cases be weak with the usual numbers involved.

Prioritizing the following criteria could easily be the subject of a separate discussion. On a conceptual level an optimal animal model for studying the outcome of interventions on a solid tumour such as HCC should entail the following: It should closely mimic the human disease on a molecular basis. It should derive from a relevant cell line that lends itself to easy propagation, characterization, and storage and study in vitro. It should be reliable and predictable in tumour generation and growth kinetics. It should allow survival differences to be manifest (no short-term mortality from spontaneous or iatrogenic metastatic disease). It should allow accurate assessment of treatment effects (i.e. minimal spontaneous necrosis). It should be readily imaged. It should occur in a similar background setting as the human disease. It should be inexpensive and avoid any requirement for immune suppression or inbred clonal genetic identity in order to facilitate ease of use on a widespread basis.

This is an extremely demanding list and is reminiscent of the old phrase in computer science, 'Fast, cheap, and reliable: pick any two of the three.' Unfortunately there are more than three important factors. As far as cost is concerned, however, the point can be argued that a bad model is worse than no model at all. The danger lies in valuable resources being expended deriving invalid conclusions and diverting those resources from other areas that might move the field ahead. Again, adapting a phrase from computer science, it is a case of 'garbage in, garbage out'. It is thus incumbent on us in research to consider very carefully what we do, how we do it, and the conclusions we draw from our efforts.

Historically, many models have been used for HCC: mice [5, 6] and rat models [7] are indeed plentiful, but there are also the ground squirrel, many avian variants of hepatitis, the tree shrew [8, 9], rabbit, guinea pig [10], woodchuck, dog and pig models and even simian models [11], including the macaque [12] and marmoset [13]. From the previous discussion and qualifications, it becomes clear that the term 'model' is very loosely applied.

Two general strategies have been used and can be summed up as 'implant or insult'. Implantation uses an orthotopic, xenograft inoculation of a previously defined cell line or tissue fragment. The alternative—an insult—such as treatment with a carcinogen, can be used to induce tumour formation. Recombinant or specific knockout genetically modified animals are fascinating and useful for some studies but unfortunately are outside the scope of this discussion.

Inoculation allows for a shorter time frame to treatable disease in general. It usually implies a cell line that is or could be characterized in terms of mutations, protein under- or over-expression, cell signaling, and drug sensitivity or resistance. An advantage is that the tumour is also often localised. What is sometimes referred to as metastatic disease in such models may simply reflect vascular emboli or spillage associated with the disruption of anatomic barriers during the inoculation rather than a phenotypic characteristic of a tumour.

In contrast to the implant model is the insult model. This generally takes significantly longer to develop treatable disease. The tumour burden is less predictable with respect to timing and size, and by nature it is more diffuse. It is generally more reliable, though, and it is simpler to employ.

Regular dosing of an N,N-diethyl nitrosamine (DENA) is an example that falls into this category. Such tumours are often well characterized with respect to their toxicology on both a whole-organism and cellular basis. A major drawback is a very unnatural etiology for the underlying disease.

Knowing some of the more general aspects of these methods, we now will briefly examine rat, rabbit, woodchuck, pig and dog models. In rodents, there are numerous tumour models from both the implant and insult categories, and also a third method that bridges the two strategies. The bridging method is to implant a tumour orthotopically using a cell line originally derived from an insult, typically drug exposure with the same species and strain of rat. The Novikoff N1S1 rat cell line derived from DENA exposure is an example. More recently, with the goal of in vivo visualization using bioluminescence, ultrasound and PET, the McA-RH7777 line was transfected with firefly luciferase [14]. This is a derivative of the Morris hepatoma, originally derived from exposure to N-2 fluorenylphthalamic acid. Using a syngeneic model in this manner preserves the immune system and allows for a tumour derived from the organ of interest, but again it is drug-induced and the animals are smaller.

Moving up in size, the next animal is the rabbit. By far the most popular tumour is an implantable tumour of epithelial origin known as VX2. It grows very quickly after inoculation and perhaps in fact too quickly. It has been used variously as a model for corneal, tongue, breast, auricular, bladder, liver, kidney, colorectal, lung and brain cancers to name but a few, and even has been invoked in a benign condition, uterine fibroids [15]. The famous virologist and Nobel laureate Peyton Rous [16] was given a lead early in his career by his mentor Dr. Richard Shope [17]. Rous took a papilloma project and built an entire career on this and similar viral tumours. He was certainly the most prominent physician and researcher to do this, but was by no means the last to do so. VX2 has its origins in the 1930s from wild cottontail rabbits in Iowa and Kansas bearing viral papillomas [18] treated over time with tars, methylcholanthrene or a benzanthracene to induce mutations and rapid malignant transformation [19]. Rous and his colleagues published a plethora of papers over the subsequent 30 or more years on the subject.

As a model for HCC, VX2 has significant limitations. It suffers from a high rate of spontaneous necrosis and does not grow in vitro. Inoculation must therefore be performed using fresh or frozen material, and studies have shown that the most consistent results are obtained using fresh pieces rather than processed and frozen cells. A recent report in which 142 animals were used documented these issues well. Success rates nearly doubled using fresh rather than frozen tissues for tumours grown in both hind limb and liver. Lower rates of tumour outside of the inoculation region (what some may call metastasis) were noted when fragments were used compared to cell suspensions [20]. The tumour kinetics are far out of the normal range and the survival time for untreated animals, while clearly a function of the inoculation size and method, is on the order of weeks.

In terms of understanding the VX2 tumour, there is a small body of work that has been published on expression of cytokines induced in the surrounding tissues and matrix metalloproteinases expressed by the tumour but little else. Of perhaps greater significance, we are aware of no published reports describing any characterization of the molecular biology of the tumour itself. Wall and Shani in their critique [1] summarised the importance of this omission quite well: ‘Recent discoveries of unexpected variation in genome organization and regulation may reveal a heretofore unknown lack of homology between model animals and target animals that could account for a significant proportion of the weakness in predictive ability.’ Unappreciated alternative and redundant pathways abound and make the task of modeling more difficult than it seems at the outset. This despite the fact that mammalian biochemical pathways are generally conserved.

Woodchuck hepatitis virus [21] is one of the closest true mimics to human HCC known. It is fairly closely related to human hepatitis B, but it does not incite cirrhosis and requires 1–4 years to develop after neonatal inoculation. Reports of its use in the literature are mainly focused on understanding the molecular biology and immunology of the disease [22].

A pig model for HCC that was recently reported [23] might at first glance appear more suited due to the size of the animals and the greater similarity of the liver anatomy to that of humans. Weekly intraperitoneal dosing of carcinogen for 3 months was followed by 10–12 months for development of innumerable small tumours. It is a drug-induced model and therefore several issues arise. The timing of tumour development is a problem. The multiplicity of the tumours becomes an issue in addition to the unnatural etiology. It is worth noting that any model involving carcinogens also has a small but real risk of exposure to the staff conducting the experiment. As the authors note, the time frame could potentially be shortened by the use of phenobarbital or a similar promoter. They also point out that the technique itself represents a significant improvement over previous methods such as dietary dosing for 5 years. Mini-pigs would seem most suitable under these circumstances as outbred swine would grow to an intractable size in the interval required to develop tumours.

The final animal model we will mention is canine venereal sarcoma. This model requires mild immune suppression in mongrel dogs, and tumours grow to a treatable size within 8–12 weeks after inoculation [24, 25]. Continuous immune suppression is required to prevent spontaneous regression. As with VX2, the cell of origin is unrelated to HCC and there is very little in the way of characterization that has been done on molecular biology of this model.

This brings us to our third and final question: what would make an improved animal model? This of course depends heavily on what we want to accomplish with it. For purposes of this discussion we need a large animal model that is relevant and practical. We need animal models that are much closer to the target disease in humans both in scale and biology than what we currently have. We need to use them judiciously. Costs must be viewed in the context of the price of doing the wrong thing and drawing incorrect or misleading conclusions. A large animal model derived from a relevant cell of origin that can be studied in vitro with well-characterized molecular biology is needed for investigation of existing percutaneous methods and emerging technologies.

Tumour growth rates should be closer to those seen in the human to achieve a better balance between costs and fidelity. The necrosis we observe should be the necrosis we cause, not the rapid, spontaneous, uncontrolled debris so characteristic of some models. Angiogenic tumours that are truly hypervascular will be invaluable particularly for studies of new embolic agents.

Our goal should include mimicry of the setting as well: cirrhosis and a normal immune system. It has been said over and over again that the host makes the disease, and that is nowhere more true than in the world of neoplasia. Minimal or no immune suppression clouding the data would be ideal. This will become all the more important as immune therapies continue to advance.

Cancer is a complex process and the solutions required will likewise be complex. Based on the foregoing, the target is challenging. Despite all of the research investment of the past, it could be argued that we haven't accomplished much. The nature and strength of the conclusions reported in the literature with existing models are suspect at best given the limitations of these models. We stand therefore at a fork in the road. We can continue down the same path, using these same models. We can draw more unfounded, weak conclusions. We can congratulate ourselves on our learned ways and complex technology. Down the other path we can acknowledge the weaknesses and vow to do better. The impressive plasticity and genetic instability of tumours has been noted [26], but there is no amount of adaptation to a scalpel. We should strive for no less.

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