

Patient-derived orthotopic xenografts: better mimic of metastasis than subcutaneous xenografts

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The majority of human solid tumours do not metastasize when grown subcutaneously in immunocompromised mice; this includes patient-derived xenograft (PDX) models. However, orthotopic implantation of intact tumour tissue can lead to metastasis that mimics that seen in patients. These patient-derived orthotopic xenograft (PDOX) models have a long history and might better recapitulate human tumours than PDX models.

The introduction of the athymic *nu/nu* mouse (nude mouse) for the growth of human tumours in 1969 changed the paradigm of basic and applied cancer research. Human tumours could now be grown for the first time in a mouse model owing to the nude mouse's lack of a thymus and T cells. Rygaard and Povlsen¹ implanted a colon cancer from a 71-year-old patient subcutaneously (s.c.) in nude mice, which grew as a well-differentiated adenocarcinoma similar to that from the donor patient. The tumours grew as local nodules and were encapsulated and did not metastasize, and they were maintained over 7 years for 76 passages. This was the first patient-derived xenograft (PDX). What is currently described as PDX does not differ substantially from what Rygaard and Povlsen described in 1969 (REF. 1).

Discrepancies have been repeatedly described between the invading and metastasizing abilities of tumours in the patient compared to the benign tumour behaviour in the s.c.-transplanted xenografts in nude mice. The vast majority of human solid tumours, growing s.c. in the nude mouse, did not metastasize. The s.c.-transplanted tumours had local expansive tumour growth with circumscribed tumour borders without apparent invasion¹. This is still the case of PDX models today².

Wang and Sordat *et al.*³ in 1982 were among the first to implant human tumours orthotopically (literally 'correct surface') in nude mice rather than 'heterotopically' (literally 'different surface', such as s.c.). Colon cancer cell suspensions were injected within the descending part of the large bowel of nude mice. Metastases as well as local tumour growth occurred. This seminal study indicated that tumour implantation at the orthotopic site, or site corresponding to the origin of the tumour in the

patient, allows the tumour to behave more similarly to the tumour in the patient and strikingly different from s.c.-transplanted tumours³.

Subsequent studies from Fidler's laboratory and others have shown that the implantation of many types of human tumours in the orthotopic sites of nude or other immunodeficient mice resulted in metastasis of human tumours⁴. However, these early models of metastasis involved orthotopic injection of either tumour cell lines or, occasionally, disaggregated patient tumours, and often had low frequencies of metastasis.

My colleagues and I pioneered the patient-derived orthotopic xenograft (PDOX) nude mouse model with the technique of surgical orthotopic implantation of intact colon cancer tissue⁵. A greater extent of metastasis was observed in orthotopic models with implanted intact tumour tissue compared with orthotopically implanted cell suspensions (for example, in stomach cancer⁶). This perhaps is due to the intact histology and cancer-cell stroma interaction of the orthotopically-implanted tumour tissue.

PDOX models from patients with colon⁵, pancreatic⁷, breast⁸, ovarian⁹, lung¹⁰ and stomach cancer¹¹, and mesothelioma¹² were established in the early 1990s, resulting in primary and metastatic tumour growth very similar to that of the patient. For example, in a clinical correlative study of 20 of 36 stomach cancers that grew orthotopically in nude mice after implantation of intact tissue, five had clinical liver metastases and all five cases resulted in liver metastases in the nude mice¹¹. Six patients had clinical peritoneal involvement of their tumour and, of these, five resulted in peritoneal metastasis in the nude mice¹¹. In another case, a patient-derived colon-cancer lung metastasis grew in the lung, but not colon or skin of nude mice¹³.

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We recently described the development of a PDOX model of HER2-positive cervical cancer. Metastasis in nude mice included peritoneal dissemination, liver metastasis, lung metastasis, as well as lymph node metastasis, reflecting the metastatic pattern in the donor patient. Primary tumours and metastases in the nude mice had histological structures similar to those in the original tumour and were stained by a HER2-specific antibody in the same pattern as was the patient's cancer¹⁴.

In the meantime, the Leder group published their famous 'OncoMouse' paper describing a transgenic mouse in which the normal mouse *Myc* gene was driven by a hormonally-inducible mouse mammary-tumour virus promoter to generate spontaneous mammary adenocarcinomas¹⁵. OncoMouse started the era of transgenic mouse cancer models, which would dominate the cancer mouse-model field for almost 25 years. The tumours in these models were spontaneous (even though they were usually driven by oncogenes with super-active viral promoters). The mice were also immunocompetent rather than deficient. More sophisticated techniques were later developed to make transgenic tumour mouse models, including homologous recombination and the use of a Cre-*loxP* system for activating oncogenes, or deactivating (knocking out) tumour suppressor genes in specific organs, sometimes resulting in tumours.

The transgenic mouse models of cancer became so dominant that xenograft models were seen as unsuitable and irrelevant to understanding human cancer. The s.c.-transplanted models seemed to retain their popularity only in the pharma companies, as it was what they were used to, data were obtained easily (only a caliper was necessary to measure the tumour size) and the data were shown to be somewhat useful for predicting clinical efficacy.

Then, in 2006, due in part to the Hidalgo group¹⁶ at the Johns Hopkins University and their associated company, a 'back to the future' event occurred — all the way back to Rygaard and Povlsen in 1969 (REF. 1). The s.c.-transplanted tumour mouse model re-emerged with great fanfare for growing patient-derived tumours. This time the mice were more immunodeficient, such as non-obese diabetic-severe combined immunodeficiency (NOD-SCID) mice, but the s.c.-transplanted tumours still did not metastasize. In order not to seem to be going back to the 1960s, the born-again s.c.-transplanted mouse models were named 'xenopatients' or 'avatars' (REF. 2), which seem to exaggerate the capability of the PDX models and their novelty. In the October 3, 2014 issue of *Science*, in the section 'On the Cover', it was stated: "To make mice better mirrors of human cancer, researchers are building 'avatars' with the cancer of a particular patient.... The work marks a sea change in cancer biology and is stirring hope that new mouse models will pave the way to more personalized care." (REF. 17) The orthotopic patient models are barely or not mentioned in the xenopatient and avatar papers^{2,18}, even though they mimic the patient much more than the s.c.-transplanted models because they metastasize.

Spending 50 years in science allows the observation of many fads that come and go and come back again. We are now back to the 1960s with the current very popular PDX fad. Orthotopic models enjoyed a modicum of popularity in the late 1980s and early 1990s, thanks in large part to the great efforts of Fidler⁴. At the present time, it seems that most scientists have either forgotten about or are unaware of orthotopic models, especially PDOX models, which are metastatic and resemble the patient's tumours. This is reminiscent of a publication in *Cell*¹⁹ in 2002 about the 'new' dimension (3rd) in cell culture that had been around for almost the entire century of the history of tissue culture but had not been learned by the vast majority of the current generation of scientists in the field. PDOX models can be of important use for individual patients as well as basic and applied research as they mimic the clinical pattern of metastasis.

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Competing interests statement

The author declares [competing interests](#): see Web version for details.