Pictures in Molecular Medicine
Watching real-time metastasis *in vivo*

Fig. 1. Real-time whole-body imaging of green fluorescent protein (GFP)-expressing human PC-3 prostate cancer growth and metastasis implanted by surgical orthotopic implantation (SOI) in nude mice. White arrows, primary tumour; arrowheads, mesenteric lymph nodes; pink arrows, inguinal lymph nodes; green arrow, axillary lymph node.

Visualizing cancer cells that stably express green fluorescent protein (GFP) is far more sensitive and rapid than the traditional cumbersome procedures of histopathological examination, immunohistochemistry or other imaging modalities. In particular, GFP labeling markedly improved the ability to visualize metastases in fresh soft organs and bone with the resolution of a single cell [1]. A major advantage of GFP-expressing tumour cells is that visualization requires no preparative procedures, substrates or contrast agents [1].

The GFP-based fluorescent optical tumour imaging system therefore presents many powerful features for non-invasive whole-body imaging [2]. Only the tumours and metastases contain the heritable GFP-encoding gene and are therefore selectively imaged with very high intrinsic contrast to other tissues, which is why no contrast agents are needed. GFP expression in the tumour cells results in genetically stable autofluorescence over indefinite time periods, allowing the quantitative real-time imaging of tumour growth and metastasis formation, as well as their inhibition by agents of all types. Therefore, no probes, dyes or substrates need be administered to the subjects being imaged. The very bright GFP fluorescence enables internal tumours and metastases to be observed externally in critical organs such as colon, liver, bone, brain, pancreas, breast, lymph nodes, prostate and other organs [2–4]. Powerful examples of GFP imaging are described below.

Imaging growth and metastasis of prostate cancer *in vivo*

Fig. 1 shows whole-body imaging performed weekly after orthotopic tumour implantation of the GFP-expressing human prostate cancer PC-3 in nude mice. The images demonstrated real-time development of primary tumour size and evolving metastases. Image measurement of these changes indicated that primary tumour growth increased in size between day 14 and day 28. Abdominal lymph node metastases increased qualitatively and quantitatively from day 14 to day 28. Inguinal lymph node metastases only appeared by day 21. Axillary lymph node metastases appeared only by day 28.

External imaging of micrometastasis

The sensitivity of external imaging is limited by light scattering in intervening tissue, especially in skin. Opening a reversible skin-flap in the light path markedly reduces signal attenuation, increasing detection sensitivity many-fold [5]. The observable depth of tissue is thereby greatly increased and many tumour cells that were previously hidden are now clearly observable. For example, Fig. 2 shows GFP-expressing Lewis lung tumour micrometastasis consisting of a few cells viewed through a skin-flap over the chest wall on the lung of a nude mouse that was implanted with

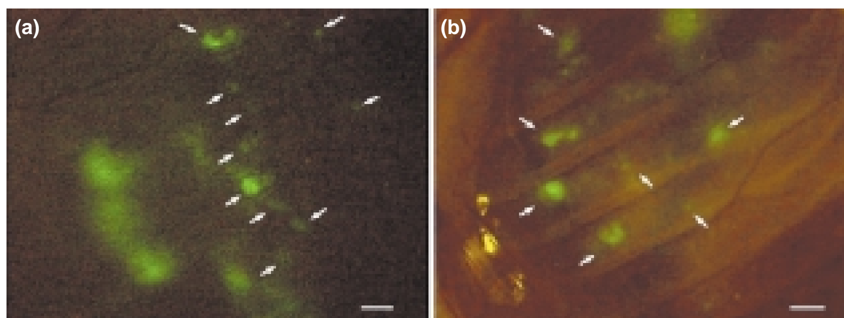

Fig. 2. External direct view of green fluorescent protein (GFP)-expressing Lewis lung cancer micrometastasis on primary and metastatic lung in a nude mouse. (a) Two–three cell micro-foci in the ipsilateral (primary) lung at day five after surgical orthotopic implantation (SOI). Scale bar = 50 µm. (b) Micrometastases (arrows) in the contralateral (opposite) lung at day seven after SOI. Scale bar = 1 mm.

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the primary tumour. Contralateral micrometastases were imaged through a corresponding skin-flap.

Prospectives

Whole-body imaging of tumour growth and metastasis down to the microscopic level will enable real-time *in vivo* study of many previously unsolved problems. An important and currently little understood phenomenon is that of tumour-cell dormancy and the phenomenon of interference between the primary tumour and metastatic deposits. Apparently, the presence of a primary tumour can suppress the growth, angiogenesis and colonizing growth of a second distinct tumour [5]. Research into tumour interference has largely used ectopic tumours such as subcutaneous xenografts. The externally viewed GFP orthotopic tumour model affords several new and powerful approaches to this question. Tumors can be placed into their natural environments and allowed to grow without surgical interference. Furthermore, and most importantly, tumours can be labeled in two colours such as GFP and red fluorescent protein (RFP) which allows great flexibility in placement and timing of the tumours, which can then be followed for interference and other phenomena [5].

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Blood-bank testing for infectious diseases: how safe is blood transfusion?

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Remarkable progress has been made in transfusion safety from infection over the past three decades. Donor deferrals for at-risk behaviors, the introduction of more-sensitive viral-screening assays and the recent introduction of nucleic-acid amplification technology have nearly eliminated transmission of HIV and hepatitis C virus (HCV) by blood transfusion in North America. Nevertheless, risks of other infectious agents for which such robust screening tools have not been developed, such as bacteria and parasites, still remain. As a result of these successes, the non-infectious risks such as misidentification of patients and inadequate and inappropriate transfusion have become the primary sources of transfusion risk.

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Safety from transfusion-transmitted infectious disease has improved remarkably over the past three decades with the implementation of more-stringent donor-eligibility criteria and the development of increasingly sensitive serological and, most recently, nucleic-acid assays for viral detection [1]. Testing is routine for: HIV-1 and -2; hepatitis B virus (HBV) and HCV; human T-cell leukemia virus (HTLV)-I and -II; syphilis; and, for selected blood recipients, cytomegalovirus. The major remaining source of post-transfusion viral infection is if blood is collected from the donor during the earliest stages of infection (the so-called 'seronegative window period'), at a time when the donor is asymptomatic but viremic, and is still nonreactive in tests for viral antibodies or antigens. Therefore, nucleic-acid amplification testing (NAT) has been implemented to increase the sensitivity of detection in donations during the seronegative window period. Future material improvements in transfusion safety will probably require a more intense focus on other real and theoretical infection risks and on serious noninfectious hazards.

Immunoassays and NAT

Enzyme-linked immunoassay (ELISA) is the primary method used to screen blood donors. The use of monoclonal antibodies and synthetic peptides have made these tests more sensitive and specific, and have facilitated the automation needed for daily screening of hundreds or thousands of samples under the constant pressure for rapid release of blood for transfusion. New technologies to improve sensitivity and specificity, including chemiluminescent immunoassays (ChLIA), are awaiting approval by the Food and Drug Administration (FDA).

Until recently, exquisitely sensitive and specific NAT was not feasible for donor screening owing to the lack of automation, time pressures, space restrictions in blood centers and cost. However, in 1999, two systems were applied to US donors under an investigational new drug (IND) exemption from the FDA: the Roche Molecular Systems COBAS Ampliscreen™ tests for HCV and HIV and the Gen-Probe/Chiron Procleix™ Pooled Plasma HIV-1/HCV Amplified Assay. The latter received FDA licensure in February 2002. Testing is carried out on pooled samples from 24 and 16 donors, respectively, reducing the number of tests required, the time to perform testing and the cost. HIV and

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