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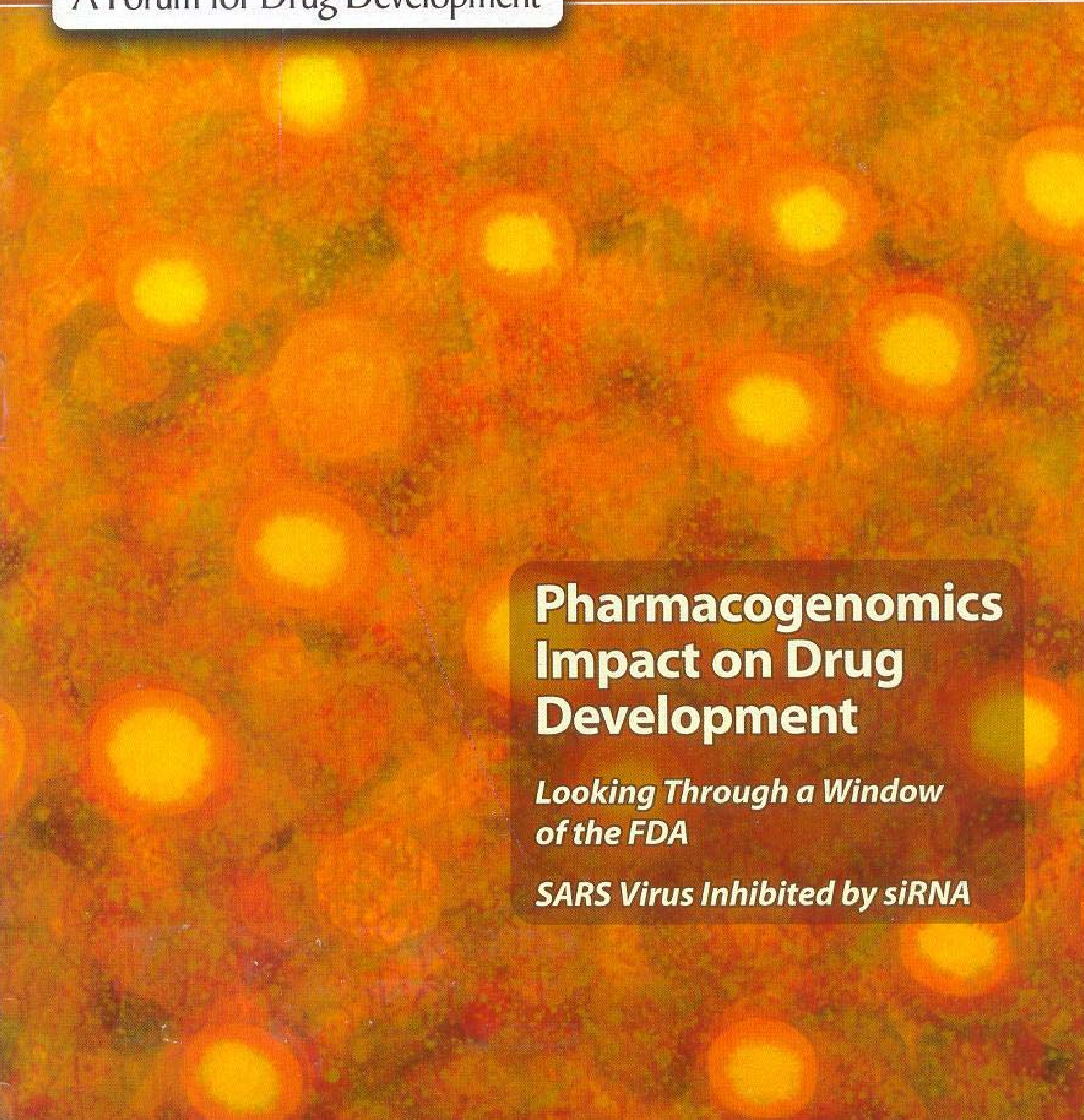

Pharmacogenomics Impact on Drug Development

Looking Through a Window of the FDA

SARS Virus Inhibited by siRNA

Multicolor In Vivo Imaging in Mouse Models of Cancer

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FLUORESCENT PROTEINS

Cloning of the reporter gene green fluorescent proteins (GFP) from the jellyfish *Aequorea victoria* in 1992 began the era of visualization of cellular and subcellular processes in live cells in real time (1). Most importantly, it was shown that GFP could be highly expressed as a transgene in many types of organisms (2,3). Numerous cellular processes that could previously only be studied biochemically outside the cell or in fixed cells could now be visualized in real time in living cells. This powerful reporter gene has been genetically modified to increase its fluorescence intensity and to fluoresce at other distinct wavelengths, such as blue and yellow (4–7), and humanized for high expression and strong signal (8). GFP requires no other *Aequorea* proteins, substrates, or cofactors to fluoresce (9). Recently, other fluorescent proteins have been isolated from various organisms, in particular the coral *Discosoma*, which is the source of the brilliant red fluorescent protein (RFP) DsRed, that has been genetically modified to DsRed-2 (10). The availability of multicolored spectrally distinct fluorescent proteins enables simultaneous visualization of multiple processes in living cells.

IN VIVO IMAGING

Our laboratory pioneered the use of fluorescent proteins for in vivo imaging, where their very high levels of fluorescence have enabled whole-body imaging of primary and metastatic tumor growth (11), angiogenesis (12), gene expression (13), stem cell and other cell tracking (14), and infection in intact animals (15). In order to externally image and follow the natural course or inhibition of tumor progression and metastasis or other processes outlined above in vivo, high specificity, a strong signal, high resolution, and good physiological conditions are necessary. In vivo imaging with fluorescent proteins is possible without anesthetizing the animal or the injection of any type of substrate. Images can be captured from fluorescent proteins in numerous organs of the body in freely moving animals. The technology is based on the bright intrinsic fluorescence of GFP and RFP, which is partly caused by the high quantum yield of these fluorophores (7,10). The use of narrow band-pass or spectrally resolving filters can fully eliminate any autofluorescence background in the animal.

TECHNIQUES FOR IN VIVO IMAGING

External whole-body imaging of mice with primary and metastatic tumors, which are genetically labeled with the fluorescent proteins GFP and RFP, is a simple but powerful tool for investigating tumor development. For tumor cells to be visualized with this technique, they must be transduced with GFP or RFP genes, such that they become brightly fluorescent. This can be accomplished by in vitro (11,16) and in vivo (17) selection of such fluorescent tumor cells.

To produce metastasis in mice, the genetically fluorescent tumors should be transplanted orthotopically (12,16,18–26). Once the GFP- or RFP-expressing tumors have developed and metastases have formed, they can be visualized in the live mouse by use of whole-body imaging with fairly simple equipment.

A fluorescence light box, with fiber-optic lighting at about 470 nm and appropriate filters placed on top of the light box, can be used to image large tumors and can be viewed with the naked eye (11). Alternatively, the light box can be linked to a charge-coupled device (CCD) camera with appropriate filters to enable images to be captured digitally by a computer, displayed on a monitor, and digitally stored (11).

In order to visualize smaller tumors and metastases, the animal can be put on a fluorescence dissecting microscope, which incorporates a light lows unrestrained animals to be imaged without any perturbation or substrate—irradiation with nondamaging blue light is the only step needed. Images can be captured with a fairly simple apparatus, and there is no need for absolute darkness. The magnitude of bioluminescence measured in vivo varies with time after the injection and dosage of luciferin, which makes repeatable quantification very different (36).

The multicolored spectrally distinct set of fluorescent proteins is rapidly growing, especially those that emit at longer wavelengths. The multicolor fluorescent proteins form the basis of the most versatile, simple, highest-resolution, and most sensitive in vivo imaging technology. It is expected that new fluorescent proteins emitting close to 700 nm will soon be on the horizon and suitable for very deep in vivo multicolor imaging.

The portfolio of multicolor fluorescent proteins enables a second new era of biological visualization, that of in vivo imaging at the cellular level. With fluorescent proteins, multiple cellular processes can be simultaneously visualized in the intact animal that previously could only be seen in cultured cells or detected biochemically outside of the cell. The application of this imaging technology for drug discovery and development and the understanding of disease is possible by linking specific processes to expression of specific colored fluorescent proteins in any type of animal model for external noninvasive imaging.

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