icide can be used to examine the infection process and to clearly differentiate the host from the pathogen (Figure 1, I and J). Furthermore, bacteria, viruses, and diatoms can absorb and fluoresce when stained with SYBR Green I (3,5).

The SYBR Green I nucleic acid stain has superior fluorescence characteristics when compared to DAPI, acridine orange, or ethidium bromide (4). SYBR Green I is significantly less mutagenic than ethidium bromide (based on Ames testing), thus making it safer to use (10). Moreover, utilizing these culturing and staining procedures, SYBR Green I stained as well as 300 nM DAPI (Figure 1, D and E) (staining and washing of DAPI were done exactly as described above for SYBR Green I) and was superior to ethidium bromide (not shown).

The glass slide method of culturing the fungal mycelia showed no apparent differences in the number of nuclei per cell when compared to water agar-grown mycelia. In addition, the lack of water agar improved the uptake of the stains and simplified the washing of the samples. Furthermore, there was no background staining associated with the support material and growth media or water agar because only growing mycelia were stained on the glass slide. This culturing method also allowed for the staining of unfixed mycelia with DAPI and minimized the amount of washing required.

This method is fast, requires no fixation, minimizes the amount of handling and washing of the samples, produces highly reproducible results, and reduces the amount of toxic waste. Only 2.2 mL toxic liquid are produced from each assay. This method is routinely used in our laboratory to separate R. solani isolates from Ceratobasidium spp. isolates.

REFERENCES


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Transduction of Biopsy Samples: Bridging Gene Therapy between Animals and Humans

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Gene delivery systems are used for the treatment of various conditions including cancer (6). Usually, experimental systems are tested in vitro and in animals before clinical trial. However, these assessments do not always predict what will happen in human clinical samples, where transduction and expression efficiencies may vary widely between patients. Such variations may not be reflected in data obtained using a limited number of available cell lines. We have therefore developed a straightforward method for organ culture of tissue fragments such that they can be transduced ex vivo to determine whether the delivery system could be useful in a broader range of human clinical samples. Such data would enhance the prospects for approval of a clinical trial.

Our studies have utilized prostate biopsy tissues because we are currently developing a gene therapy suitable for treatment of late-stage prostate cancer. Prostate cancer shows marked biologic and genetic heterogeneity. The ability of delivery vehicles to transduce a wide range of human cancer cells is thus critical for prostate cancer gene therapy. We are investigating the use of replication-deficient human type-5 adenovirus (Ad5) or replication-abortive ovine adenovirus (OAdV) as delivery vehicles. Although Ad5 receptors are widespread, allowing gene delivery to numerous tissue types, preexisting immunity due to natural infection can prevent effective administration of the vector, and the immune response elicited by the vector results in rapid clearance (1). In contrast, OAdV is not neutralized by preexisting immunity to human Ads (2). Its receptor is unidentified but distinct from the Ad5 receptor (7). OAdV can deliver genes to several human cell types including PC-3 and LNCaP human and RM-1 murine prostate cancer cell lines (4). OAdV does not replicate productively (3), eliminating the risks associated with...
Both Ad5 and OAdV can transduce human prostate cancer cell lines, but these may not be fully representative of prostate cancers in vivo. Moreover, the relative efficiency of infection of different cell lines varies substantially. Thus, different levels of transduction are observed with OAdV and AdV5 on different cell lines. For example, OAdV can transduce RM-1 cells efficiently, but transduction of LNCaP cells is less efficient, while the reverse is true for Ad5 (unpublished data). To examine viral transduction of human prostate tissue, samples from patients undergoing radical prostatectomy (RP) or transurethral resection of the prostate (TURP) were cultured for recombinant viral transduction. Tissue fragments (about 2 × 2 mm) placed on a support of Gelfoam® (about 5 × 5 mm sections) (Pharmacia and Upjohn, Peapack, NJ, USA) and MATRIGEL® (20 µL) (BD Biosciences, San Jose, CA, USA) in 96-well tissue culture plates in T-medium (5), maintained just below the level of the specimen (about 50 µL), were cultured at 37°C and 5% CO2 overnight. They were then transduced with OAdV217A, carrying the green fluorescent protein (GFP) reporter gene under the control of the cytomegalovirus (CMV) immediate early promoter (4), and recombinant Ad5 expressing GFP under the elongation factor-1 promoter (50 µL virus at 1.5 and 3 × 1010 particles/mL culture medium for 4 h in serum-free medium). Particle number to infectious particle ratios for OAdV-217A and Ad5/GFP were less than 100:1. Four hours is a typical transduction time used in Dr. Both’s laboratory for both Ad5 and OAdV viruses on a range of tissue culture cells, including PC-3 and LNCaP prostate cancer cell lines, MCF7 breast cancer cells, RM-1 murine prostate cancer cells, and CSL503, a sheep fibroblast line. No obvious signs of cell damage have been apparent in any of these lines over the 4 h of transduction or during the subsequent culture of the cells (unpublished data). The exception to this is in the case of very high-titer transduction where virally induced cytopathic effects may be observed in some cell types. Medium was then replaced with normal T-medium with 10% fetal calf serum. Green fluorescence of the tissue under blue light excitation provided evidence for viral transduction. OAdV/GFP with the EF-1 promoter has not been constructed, but, as both EF-1 and the

<table>
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<th>Biopsy</th>
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<tr>
<td>RP 1</td>
<td>CaP–Gleason 6-7</td>
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<td>RP 2</td>
<td>Benign Hyperplasia of Prostate</td>
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<td>TURP 2</td>
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<td>TURP 3</td>
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<td>OAdVGFP/Ad5GFP Transduction</td>
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<td>RP 3</td>
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Table 1. Summary of Human Prostate Tissue Sample Transductions

Figure 1. Transduction of prostate biopsy tissue with recombinant viruses expressing GFP. Green fluorescence provides evidence for viral transduction and transgene expression. Exclusion of propidium iodide indicates tissue viability. Panels A and B represent photomicrographs of a whole piece of TURP3 tissue cultured ex vivo (advanced hormone refractory prostate cancer) observed under fluorescent microscopy using a long focal-length objective. They are both pictures of the same piece of tissue observed under panel A (FITC fluorescence showing GFP distribution) and panel B (Texas Red® fluorescence filters showing distribution of propidium iodide uptake). Similarly, panels C and D represent explant tissue sections viewed under FITC illumination. They are separate pieces of tissue from the same biopsy [i.e., TURP4 (benign prostate) transfected with Ad5GFP (panel C) or OAdVGFP (panel D)].
This approach may thus provide a tissue from a diverse range of patients, enabling the use not only of human biopsy specimens but also of explant cultures could also be examined (Table 1). Thus, RP and TURP tissue samples have been cultured short term ex vivo. Their successful transduction by OAdV and Ad5 suggests that these vectors may be suitable gene delivery vehicles for a range of human prostate cancers. The potential to effect and evaluate tissue-specific gene expression for enzyme pro-drug therapy is now evident. With a sensitive assay, gene delivery may be quantitatively assessed. While it is possible that individual biopsy samples could be tested by organ culture before treatment for a given patient, we envision that the technique described above would have more potential for showing that gene delivery, and hence therapy, can be applied to a wider range of specimens than that defined by known prostate cancer cases; hence, it is more likely to be effective in a wider range of patients. These experiments were designed to test host range, rather than to provide ex vivo vaccines, although it is possible that transduction of explant cultures could also be extended to such an application. While we have focused on prostate cancer, this is a potentially generic system, enabling the use not only of human biopsy material but also of relevant diseased tissue from a diverse range of patients. This approach may thus provide a bridge between animal experiments and clinical studies.

REFERENCES


Portable Plant-Tissue Macerator

With the recent expansion in the use of beneficial transgenic traits in crop plants, a need has arisen for the rapid detection of individual plants expressing proteinaceous gene products. This detection facilitates the selection of the desired plants for breeding, phenotypic evaluation, etc. ELISA has become the primary analytical method for detecting and quantifying transgenic protein levels in plants (2). The need for rapid and reliable qualitative analysis has led to the development of ELISA lateral-flow strip tests (Strategic Diagnostics, Newark, DE, USA and others). These strip tests can be placed in a plant-tissue extract and will display a diagnostic band only when the protein of interest is present. The ability to test plants under field conditions has also required a rapid method for extracting leaf tissue using portable equipment. The most widely used method is the sampling of leaf tissue followed by maceration with a motorized pestle (Kontes, Vineland, NJ, USA). We have developed a portable plant-tissue macerator that we observe to be faster and that produces a more consistent extract when compared to the mortar and pestle technology.

Several manufacturers (Retsch, Haan Germany, and SPEX CertiPrep, Metuchen, NJ, USA) and laboratories (Dow AgroSciences LLC, Indianapolis, IN, USA) have developed laboratory leaf macerators that make use of a hard metal sphere in a small vessel such as is found in 96-well microplates. Leaf material and extraction buffer are added to the vessel, and the vessel is then sealed and placed in a shaker. Rapid shaking causes the hard sphere to macerate the leaf tissue.

We have developed a portable, single-vessel unit that makes use of a battery-powered reciprocating saw (Makita Cordless Recip Saw, model 4390D; Makita Corporation of America, Buford, GA, USA) and a custom-made attachment that holds a 2-mL plastic tube (Eppendorf® Safe-lock 2.0 mL microcentrifuge tubes; Brinkman Instruments, Westbury, NY, USA)