Detection of preinvasive cancer cells

Early-warning changes in precancerous epithelial cells can now be spotted in situ.

More than 85% of all cancers originate in the epithelium that lines the internal surfaces of organs throughout the body. Although these are readily treatable provided they are diagnosed in one of the preinvasive stages, early lesions are often almost impossible to detect. Here we present a new optical-probe technique based on light-scattering spectroscopy that is able to detect precancerous and early cancerous changes in cell-rich epithelia.

Before they become invasive, at stages known as dysplasia and carcinoma in situ, early cancer cells alter the epithelial-cell architecture. In particular, the nuclei become enlarged, crowded and hyperchromatic (that is, they stain abnormally darkly with a contrast dye as a result of changes in their chromatin content). These warning signs have so far only been detectable by histological examination of biopsy specimens, but light-scattering spectroscopy now offers a biopsy-free means to measure the size distribution and chromatin content of epithelial-cell nuclei as an indicator of preinvasive neoplasia.

The diameter of non-dysplastic cell nuclei is typically 5–10 μm, whereas dysplastic nuclei can be as large as 20 μm across. Epithelial-cell nuclei can be modelled as transparent spheroids that are large in comparison to the wavelength of visible light (0.4–0.8 μm), and whose refractive index is higher than that of the surrounding cytoplasm because of their chromatin content. The spectrum of light backscattered by these particles contains a component that varies characteristically with wavelength, with this variation depending on particle size and refractive index.

For a collection of nuclei of different sizes, the light-scattering signal is a superposition of these variations, enabling the nuclear-size distribution and refractive index to be determined from the spectrum of light backscattered from the nuclei. Once the nuclear-size distribution and refractive index are known, quantitative measures of nuclear enlargement, crowding and hyperchromasia can be obtained.

However, only a small amount of the light incident on the tissue is backscattered by the epithelial-cell nuclei: the rest becomes randomized in direction by multiple scattering, producing a large background of diffusely scattered light that must be subtracted. We have used two tactics to accomplish this — mathematical modelling of the diffusive background and polarizing the incident light, which is depolarized by multiple scattering and enables the single backscattering to be observed by subtracting the depolarized component of the backscattered light.

We have tested the potential of this technique in vivo to diagnose dysplasia and carcinoma in situ in four different human organs with three different types of epithelium: columnar epithelium of the colon and Barrett’s oesophagus, transitional epithelium of the urinary bladder, and stratified squamous epithelium of the oral cavity. Our clinical investigations were all made during routine endoscopic screening or surveillance procedures, during which an optical-fibre probe delivered white light from a xenon arc lamp to the tissue surface and collected the returning light.

The tip of the optical probe was brought gently into contact with the tissue under investigation. Immediately after measuring the backscattered light, a tissue biopsy sample was taken from the same site for histological examination. We analysed the spectrum of the reflected light from each epithelium and determined the nuclear-size distribution of the epithelial cells.

We then used this nuclear-size distribution to obtain the percentage of nuclei larger than 10 μm across and the total number of nuclei per unit area (population density). As already noted, these parameters quantitatively characterize the degree of nuclear enlargement and crowding, respectively. Figure 1 shows these light-scattering spectroscopy parameters as binary plots to indicate the degree of correlation with histological diagnosis: in all four organs, there is a marked distinction between dysplastic and non-dysplastic epithelium. Both dysplasia and carcinoma in situ are associated with a higher percentage of enlarged nuclei and, on average, a higher population density, which can be used as the basis for spectroscopic diagnosis.

Our results show that light-scattering spectroscopy has the potential to detect epithelial precancerous lesions and...
pre invasive cancers throughout the body. The advantage of this technique over conventional diagnostic procedures is that it can provide objective, quantitative results in real time without the need for tissue removal. Because of its potential application in screening for endoscopically invisi-
ble lesions, this technique should significantly improve the efficiency of can-
cer screening and surveillance.

* Laser Biomedical Research Center, G. R. Harrison Spectroscopy Laboratory, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA e-mail: msfeld@mit.edu

Enzymology
Degradation of plant cell walls by a nematode

Interwoven networks of cellulose and pectin are the main components of plant cell walls, making them recalcitrant structures that can only be degraded by organisms producing a mix of synergistically acting enzymes. Animals were believed to be unable to synthesize these enzymes, depending instead on symbiotic microbes to render plants into a food source. Here we describe a metazoan pectinase gene that encodes a pectate lyase for breaking down the pectin component of plant cell walls. To our knowledge, this is the first example of non-symbiotic degradation of pectin by plant cells by an animal.

We cloned the pectate lyase gene as part of a project analysing 1,000 expressed sequence tags of preparasitic juveniles (12s) of the potato-cyst nematode Globodera rostochiensis. One expressed sequence tag (ge222) encoded a partial open reading frame with similarity to bacterial and fun-
gal pectate lyases (EC 4.2.2.2). A full-length complementary DNA sequence, which included the ge222 tag, was subsequently obtained by the rapid amplification of cDNA ends by using messenger RNA from G. rostochiensis 12s as starting material.

The full-length cDNA contained a predicted open reading frame encoding a peptide sequence (PEL-1) of relative molecular mass 28K, with a signal sequence for secre-
tion at its amino terminus. We expressed this coding region in Pichia pastoris to produce the active pectate lyase. We also found active pectate lyase in homogenates of G. rostochiensis 12s. A digoxigenin-labelled DNA probe amplified from pel-1 hybridized specifically to the subventral oesophageal gland cells, which also secrete cellulases. These oesophageal gland cells are free of any symbiotic microorganisms.

Classification of PEL-1 as a class III pectate lyase markedly reduces the number of invariant amino-acid residues in this group. Three class III pectate lyases have four conserved regions and are characterized by the presence of several cysteine residues (even in PEL-1). On the basis of comparison of the PEL-1 sequence with other class III pectate lyases, only four conserved amino-acid residues with charged side chains (two aspartate residues at posi-
tions 130 and 199, and two lysine residues at positions 137 and 160) are present in the conserved regions. It is therefore likely that one or more of these invariant residues are involved in the catalytic machinery of PEL-1 and other class III members.

Symbiont-independent degradation of plant-cell walls by animals is now recognized as being possible. An endogenous cellulase gene was first isolated from cyst nematodes; cellulases are also produced by termites and the redclaw crayfish (Chenex quadriramus). Our current finding demonstrates that, like bacteria and fungi, cyst nematodes are genetically equipped to secrete a mixture of depolymerizing cellulase and pectinase enzymes that allow the basic framework of plant cell walls to be dismantled.

Herman Popenjius*, Hein Overmars*, John Jones*, Vivian Blok†, Aska Govers*.

Figure 1 Predicted amino-acid sequence of Globodera rostochiensis PEL-1 (GenBank accession no. AF127919) aligned with bacterial (accession no. Y13340, Erwinia chrysanthemi Pel I), accession no. L32172, E. carotovora PelA and fungal (accession no. Y13340, Nectria haematococca Pel D) and accession no. U13305, N. haematococca Pel B) class III pectate lyases. Boxed shaded residues are conserved regions I to IV as described for class II pectate lyase. The alignment was made by using the program ClustalW and the BLOSUM62 sub-
stitution matrix. Asterisks, identical or conserved residues in all sequences in the alignment; colons, conserved substitutions; single dots, semiconserved substitutions.© 2000 Macmillan Magazines Ltd