INSIDE LAB INVEST

Laboratory Investigation (2004) **84**, 4–5. doi:10.1038/labinvest.3700019

Microdissection and quantitative PCR as a prognostic tool

Analyzing the molecular expression of oncogenic proteins is becoming increasingly valuable as a prognostic indicator for the management of malignant tumors. Towards this end, immunohistochemistry of tumor tissue sections is a first-line tool for pathologists. However, this method is inherently subjective and does not provide true quantitative information. Extraction of tumor tissues for quantitative molecular analysis is an alternative, but is reduced in value when tumor cells represent a variable fraction of a solid tumor mass. Laser-capture microdissection of tumor cells followed by a quantitative PCR is an excellent method for evaluating the expression of oncogenes in solid tumors, and is now under intense study as a prognostic tool. A case in point is HER2/Neu (erbB-2), a proto-oncogene on human chromosome 17, which is overexpressed in breast carcinoma and other cancers including lung cancer, ovarian cancer, synovial sarcoma and osteosarcoma. The degree of overexpression is correlated with poor prognosis in patients with breast cancer.

In this issue, Fellenberg *et al* (p 113) analyze paraffin-embedded pre-operative biopsies of osteosarcoma for HER2/Neu expression by microdissection and quantitative PCR. Tumor response to pre-operative chemotherapy was assessed in the subsequently resected osteosarcoma specimens. These authors demonstrate that the quantitative expression of HER2/Neu in the initial biopsy tissue, when compared with the housekeeping gene porphobilinogen deaminase, correlated well with the tumor response to pre-operative chemotherapy. As all tumor samples expressed HER2/Neu, immunohistochemistry was not of predictive value. This study lends credence to the use of microdissection and quantitative molecular analysis in the prognostic work-up of solid tumors, with the pathologists and pathology laboratory playing the central role.

TIMP-2–MMP-2 Interactions mediate glioma invasion

Glioblastoma multiforme is the most common and most lethal primary brain tumor in adults. Extensive infiltration of brain tissue by tumor cells makes complete surgical resection impossible and current radiation and chemotherapy is minimally effective. Understanding the mechanisms of glioma invasion will accelerate the search for therapeutic targets to

inhibit or block this process. In this issue, Lu *et al* (p 8) report the isolation of a highly invasive subpopulation of tumor cells that were derived from a well-characterized human glioblastoma cell line U87MG. The investigators provide evidence that increased invasiveness of the derived cell line (called 'U87CI') was related to upregulation of TIMP-2, which promoted the activation of MMP-2. The latter finding suggests that under some conditions, metalloproteinase inhibitors may activate metalloproteinases and promote tumor cell invasion. This finding provides new insights into mechanisms of glioma invasion and may facilitate the rational design of novel therapies that are desperately needed for this malignancy.

Rapid mutation analysis using PCR and fluorescent resonance energy transfer

DNA mutation analysis by RFLP can be a useful diagnostic tool, but often involves time-consuming, cumbersome procedures that cannot be automated. Götting *et al* (p 122) have developed a rapid-cycle PCR method that yields reliable genotyping results in less than 45 min. Their method detects mutations by melting curve analysis of amplification products that are produced with fluorescently labeled oligonucleotide probes. The authors used the method to detect a mutation in the ABCC6 gene (c.3421C>T) that is associated with pseudoxanthoma elasticum (PXE). PXE is a heritable disorder of connective tissue that affects the skin, eyes, and the gastrointestinal and cardiovascular systems. A rapid and accurate diagnosis has traditionally been difficult because PXE is a rare disease with no specific biochemical markers and a highly variable phenotype. However, the new genotyping assay can now be employed for the rapid and reliable identification of this pathogenic mutation.

Molecular detection of smallpox virus in archival pathology specimens

In one of the greatest public health victories of our time, smallpox was eradicated from the human population in 1979. Today, the only repositories of the causative agent (*Variola* virus) that are sanctioned by the World Health Organization are the Centers for Disease Control and Prevention (Atlanta, USA) and the State Research Center of Virology and Biotechnology (Novosibirsk, Russia). However, the potential use of *Variola* as an agent of bioterrorism has caused increased concern that unregulated sources of the virus could exist making improved methods for its detection a necessity. In this issue, Schoepp *et al* (p 41) describe the detection and

identification of *Variola* virus in a human arm and hand specimen that was preserved for over 50 years in unknown fixatives. The paper features spectacular pathology and state-of-the-art molecular diagnostics. The authors use an integrated diagnostic approach employing routine histology, electron

microscopy, and ultimately DNA extraction and real-time PCR using fluorogenic 5' nuclease (TaqMan[®]) assays for definitive identification of a subtype of *Variola major*. This approach will be useful in the surveillance and detection of the virus in archival pathology specimens.