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Quantitative spectral analysis and artificial intelligence for interpretation of Pap stains

Thanks to the brilliant work of 19th Century European chemists, pathologists utilize a technology over a century old for the practice of their craft: staining of human tissues with chromophore stains and interpretation by visual inspection through a microscope. The number of chemical stains has increased steadily, although none have wider usage than the hematoxylin and eosin stain for surgical pathology and the Papanicoleau stain for cytologic preparations. The advent of immunohistochemistry in the 1970s greatly broadened our ability to analyze human tissues on a visual basis, but the data readout has remained essentially unchanged for 150 years: interpretation of color and intensity by the human eye. The result is that color is used mainly for establishing contrast, and contrast is used to provide spatial information only; color 'positivity' is a subjective determination.

The color information available in illuminated human tissue specimens is far greater than can be discerned by the human eye, even for routine stains. The human eye perceives colored light in three wide, overlapping spectral bands. In contrast, filters can be constructed for fractionation of light into much smaller, nonoverlapping spectral bands, enabling automated construction of 'stacks' of spectral images for any image field. The question can then be asked: can quantitative spectral analysis improve upon the accuracy of diagnosis over traditional human interpretation of tissue images? This question was tested by **Angelletti *et al*[1]** (p. 1555) on cytology specimens. Their methodology was to use liquid-crystal-based spectral fractionation of Papanicolaou-stained cytology preparations to obtain 'stacks' of images collected under illumination every 10 nm, from 400 to 700 nm. The resulting 'stacks' were analyzed using the Los Alamos-developed artificial intelligence GENIE (GENetic Imagery Exploitation) algorithm, which classifies images using learned spatiospectral features. The system 'learns' by automated analysis of a user-defined training set of images containing malignant and nonmalignant cells, devising its own algorithms for best fitness. The system is then 'tested' against a set of unknown images.

Proof-of-concept for the spectral analysis-GENIE system was performed on exfoliative cytology from human colonic carcinoma. In particular, this cell source enabled the authors to create known mixtures of benign *vs* malignant epithelial cells, and thus to test the accuracy of the GENIE system. Having so demonstrated such capabilities, the authors turned to the analysis of routinely processed ThinPrep™ urothelial cytology specimens. 'Training' was again performed on a user-designated set of urothelial cytology specimens. When tested on urothelial cytology specimens collected at two separate institutions over a span of 4 years, GENIE showed a combined sensitivity and specificity of 85 and 95%, respectively. Of particular note is that when 'training' was performed on cases initially diagnosed as 'equivocal' on cytology but with follow-up biopsy (surgical specimen or cytology) which was unequivocally benign or malignant, GENIE was superior to the cytopathologist interpreting the initial 'equivocal' cytology specimen.

This study demonstrates that spectral analysis linked with an artificial intelligence system can potentially be a useful adjunct to our time-honored approach to evaluation of cytology preparations. The authors do not propose that spectral analysis replace the cytopathologist. Rather, these technologies may help to expand our sensorium and hence our ability to provide useful information for patient care.

Reference

1 Angeletti C, Harvey, N, Khomitch V, *et al*. Detection of malignancy in cytology specimens using spectral-spacial analysis. Lab Invest 2005; 85:1555–1564.

Stressed out astrocytes in ataxia telangiectasia

Ataxia telangiectasia is a genetic disorder with systemic effects including immunodeficiency, neurodegeneration, radiosensitivity, scleral and cutaneous telangiectasia, and predisposition to cancer. It is the most common cause of progressive ataxia in infancy, having an incidence of at least 1 per 80 000 live births. Ataxia results from marked degeneration of the cerebellar cortex, which shows extensive loss of Purkinje cells and granule neurons with retrograde degeneration of the inferior olivary nucleus. The mutated gene on chromosome 11q, called *ataxia-telangiectasia mutated* (*Atm*), encodes a multifunctional protein belonging to a family of protein kinases that share C-terminal homology with phosphotidylinositol 3-kinases. ATM protein plays important roles in signal transduction and in the detection of DNA damage. Its truncation or destabilization in the disease state is associated with genomic instability and oxidative damage.

While it is well known that neurons (especially Purkinje cells) are particularly sensitive to oxidative stress and would be vulnerable in ataxia telangiectasia, the possible role of impaired astrocytic function has not been extensively studied. The Bergmann glia are specialized astrocytes of the cerebellar cortex, which are aligned next to the Purkinje cell layer and function during development to guide the migration of external granule cells during the formation of the internal granular layer. They persist into adulthood where they are believed to play a 'supportive' role in health and become 'reactive' in disease. A second type of astrocytes is diffusely distributed throughout the internal granular layer. There is emerging interest in the role of astrocytes in providing antioxidant support for neurons and the possibility that loss of this function may contribute to neuronal death.

In this issue, **Liu *et al*[1]** (p. 1471) provide evidence that oxidative stress of astrocytes may contribute to neuronal degeneration in ataxia telangiectasia. The investigators show that cultured astrocytes from an *Atm* knockout mouse (*Atm*−/−) exhibit abnormalities of growth, increased spontaneous DNA synthesis and increased expression of molecules related to oxidative and endoplasmic reticulum stress. There was activation of redox-sensitive extracellular signaling protein kinase 1 and 2 (ERK1/2) in primary cultures of *Atm*−/− astrocytes and Bergmann glia from *Atm*−/− animals. ERK1/2 activation was also detected in cerebral cortical neurons and astrocytes. This study suggests that the absence of ATM may lead to intrinsic astrocytic defects, resulting in impaired oxidative support for nearby neurons *in vivo* and that antioxidants could have a therapeutic role in ataxia telangiectasia.

Reference

1 Liu N, Stoica G, Yan M, *et al*. ATM deficiency induces oxidative stress and endoplasmic reticulum stress in astrocytes. Lab Invest 2005;85: 1471–1480.

Two small-molecule tyrosine kinase inhibitors are harmful to ALK-expressing lymphoma cells

Chromosomal translocations are one of the key mechanisms resulting in aberrant expression and constitutive activation of tyrosine kinases, in particular in hematopoietic cells. Inactivation of the catalytic activity of such oncogenic tyrosine kinases by small molecule inhibitors represents an effective therapy for certain cancers, as best exemplified for the BCR/ABL kinase in chronic myelogenous leukemia.

In this issue, **Marzec *et al*[1]** (p. 1544) report that two structurally related quinazoline derivatives directly inhibit the activity of anaplastic lymphoma kinase (ALK) that is ectopically expressed and persistently activated in a distinct subtype of T-cell lymphoma and other malignancies, typically due to the t(2;5)(p23;q35) translocation. This translocation fuses a distal portion of the ALK gene that encodes the entire cytoplasmic region of the ALK protein with the proximal portion of the nucleophosmin (NPM) gene. Inhibition of the catalytic activity of NPM/ALK suppressed growth and induced apoptotic cell death of T-cell lymphoma cells. In addition, it completely inhibited tyrosine phosphorylation of the STAT3 transcription factor that is the key effector of the NPM/ALK-mediated oncogenicity. The authors also demonstrate that NPM/ALK phosphorylates STAT3 independently of Jak3 and other members of the Jak tyrosine kinase family.

These results represent 'proof-of-principle' evidence that oncogenic ALK can be effectively targeted by a small molecule inhibitor and that inhibition of ALK activity is detrimental for the malignant cells carrying the kinase. In addition, the study provides further insight into the mechanisms of the ALK-mediated cell transformation.

Reference

1 Marzec M, Kasprzycka M, Ptasznik A, *et al*. Inhibition of ALK in T-cell lymphoma cells induces apoptosis and suppresses proliferation and STAT3 phosphorylation independently of Jak3. Lab Invest 2005;85:1544–1554.