Journal of Cellular Biochemistry 17G:96–97 (1993)

OVERVIEW

Histological Surrogate Endpoints Using Quantitative Cytometry

The four presentations in Session IV emphasized the value of morphometric and cytometric features as surrogate endpoints in breast cancer. Quantitative pathologic features associated with proliferation [mitotic activity index (MAI), thymidine labeling index (TLI), % S/G_2+M, Ki-67, *neu*], cell death (apoptosis rate, % necrosis), differentiation [steroid receptors, mean nuclear area (MNA), mean nuclear volume, syntactic structure analysis (SSA) features], and to a certain degree, metastatic potential (vascularity, cathepsin D, nuclear shape, cellularity index) were discussed. DNA ploidy does not fall neatly into any of these malignant phenotype descriptions.

Jan Baak (Free University Hospital) emphasized the value of morphometrically assessed proliferation rate, especially MAI and volume weighted mitotic index. Most publications available so far indicate that DNA ploidy assessed by flow cytometry is not as strong a prognostic factor as the morphometric features MAI and MNA. He discussed how the MNA, when assessed by the interactive morphometric analysis, and to a lesser degree, DNA ploidy can add to MAI. Dr. Baak also discussed confocal laser scanning microscopy (CLSM), another powerful new tool, used for the assessment of multi-drug resistance (MDR) in tumor cells. Moreover, 3-dimensional CLSM of thick (50 μm) breast cancer sections can result in accurate measurements of nuclear volume and shape factors in individual nuclei.

Anders Zetterberg (Karolinska Hospital) described how breast cancer can be separated into two major categories with respect to ploidy level (assessed with image cytometry): (1) highly aneuploid (A-type) tumors; and (2) low grade aneuploid (diploid/near-diploid, D-type, or tetraploid/near-tetraploid, T-type) tumors. A-tumors progress rapidly and may cause death within months to a few years, while D- or T-tumors progress slowly and may only prove fatal after many years. The ploidy type/pattern (A versus D or T) is seen early in tumor development and generally remains unchanged during tumor progression. Furthermore, the development of a high degree of aneuploidy, as seen in a number of A-type premalignant lesions (including breast lesions), almost invariably seems to precede p53 mutations.

Branko Palcic (British Columbia Cancer Research Center) has been testing two (independent) hypotheses which postulate that malignant potential of ductal carcinoma *in situ* (DCIS) can be objectively assessed through measurements of nuclear features of (a) the diagnostic DCIS cells (hypothesis I) and/or (b) normal-appearing cell nuclei which can be found in the vicinity of the DCIS (hypothesis II). The cytometry system used provides a means to capture images of nuclei at the highest spatial and photometric resolution employing visible light, yielding a pixel size of 0.1 μm^2 and 1000 gray levels. Exact focus and precise segmentation of nuclear images is achieved with unique algorithms developed specifically for cytometry. Preliminary data from tissue sections indicate that the malignant potential of DCIS can be estimated with a high sensitivity and specificity (>80%) by at least one method.

Daniel Visscher (Harper Hospital) addressed functional cellular parameters of intraepithelial breast proliferation by means of commercial monoclonal antibodies suitable for use in fixed samples. Second, both neoplastic cell invasion and certain fibrocystic alterations are associated with remodeled mammary stroma. Activity of proteases, in association with other factors, may induce stromal constitutive changes which factor in neoplastic progression. Such changes include vascular proliferation (angioglycans) and altered composition of extracellular matrix glycoproteins and proteoglycans.

Independently, Drs. Palcic, Zetterberg, and Baak demonstrated the enormous improvement in image cytometers, which now can measure thousands of cells in a few minutes. This development has greatly enhanced the potential of digital image processing, which may thus have considerable advantages over flow techniques, especially if the lesions are small (as in hyperplasia, dysplasia, and carcinoma *in situ* of the breast).

Reproducibility of morphometry and cytometry has not been studied extensively enough. Yet, it is obvious that quantitative assessments can be very reproducible, depending on the care taken with a number of factors: cell and tissue processing, appropriate stains, and the measurement protocol used. Cytometric determinations are often regarded as much more reproducible than interactive morphome-

try. However, morphometric assessments can be highly reproducible, while cytometric texture features are very sensitive to small variations in cell and tissue processing. Moreover, quantitative immunohisto/cytochemical determinations with commercially available cytometry equipment may not always be as reproducible as sometimes believed and large intra- and interobserver variations in estrogen receptor, progesterone receptor, Ki-67, cathepsin D, and *neu* protein overexpression in breast cancer were reported during this session.

Jan P.A. Baak, MD, PhD, FRCPath
Professor of Pathology
Free University Hospital, Amsterdam
Pathology
Amsterdam, The Netherlands