31st Annual International Conference of the IEEE EMBS
Minneapolis, Minnesota, USA, September 2-6, 2009

Detecting Alterations in Cell Ultrastructure with Optical Imaging

Vadim Backman, Hariharan Subramanian, Prabhakar Pradhan, Yang Liu, Ilker Capoglu, Jeremy D Rogers, Hemant K. Roy, Allen Taflove

Abstract— **Understanding cell functioning at the nanoscale has been hampered in part by the diffraction limited resolution of optical microscopy. We developed partial wave spectroscopic (PWS) microscopy that is capable of quantifying statistical properties of cell structure at the nanoscale. Our animal and human studies demonstrated that alterations in the nanoscale cell architecture is one of the earliest events in carcinogenesis and precedes any other known morphological changes at larger length scales (i.e. microarchitecture).**

I. Introduction

Neoplastic transformation is a protracted process of stepwise accumulation of molecular abnormalities which eventually lead to microscopic abnormalities (dysplasia). Prior to histological abnormalities, there are profound genetic, epigenetic (e.g. methylation and histone acetylation) and cellular function (decreased apoptosis and increased proliferation). Many of the gene products dysregulated early in carcinogenesis would be predicted to have structural consequences via, for example, interactions with cytoskeleton (e.g. adenomatous polyposis coli or E-cadherin in colon carcinogenesis). However, these cells appear histologically normal largely because the diffraction limited resolution renders conventional microscopy insensitive to structures less than ~200 nm. As a result, microscopy is unable to detect alterations in cell nanoarchitecture (e.g. the fundemental cellular "building blocks" with sizes <200 nm including ribosomes, nucleosomes, membranes, macromolecular complexes, etc.) that could potentially be affected by genetic/epigenetic changes in early carcinogenesis. We pose a question if in early carcinogenesis microscopically normal appearing cells do have alterations in their architecture, although these changes may occur at length scales not accessible by conventional microscopy, i.e. nanoarchitecture.

II. Results and Discussion

Recent thrust to understand biological processes at the nanoscale has been stymied by the lack of practical means of analysis of cellular nanoscale architecture. In order to assess the nanoscale we have utilized a fundamental principle of mesoscopic light transport theory that the signal in 1D arising due to the multiple interferences of light waves reflected from weak refractive index fluctuations is sensitive to any length scale of refractive index fluctuations (limited by signal-to-noise of instrumentation) [1]. While in 3D the scattering coefficient is proportional to the cube of refractive index correlation length Lc (Lc^3), in 1D it is proportional to Lc. Because optical refractive index is a linear function of the local density of intracellular solids (proteins, lipids, DNA and RNA), the spectrum of a 1D scattering signal contains information about spatial variations of density at length scales that are well below the wavelength. This is the main principle of PWS, which is capable of extracting 1D-propagating waves from different parts of a scattering particle. PWS measures the disorder strength of intracellular architecture $L_d = L_C \delta n^2$, where δn^2 is the variance of the spatial refractive index fluctuations. Our studies have shown that in realistic experimental conditions the limit of sensitivity of PWS to Lc is under 20 nm (Fig. 1). Our studies have also shown that the typical length scales in cells probed by PWS are Lc<100 nm, which corresponds to the size of the fundamental building blocks of the cell including macromolecular complexes, ribosomes, nucleosomes, etc.

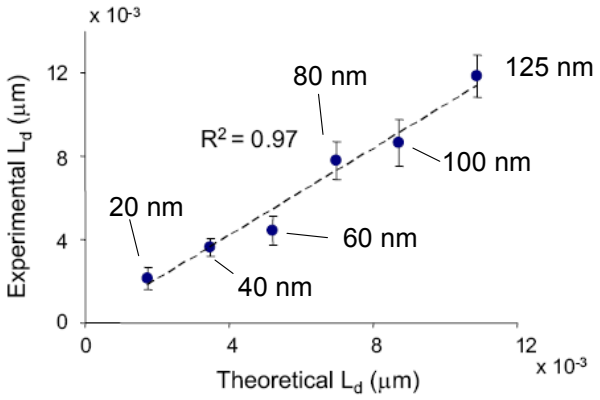

Fig. 1. Experimental validation of PWS sensitivity to nanoscale structures. Experiments were performed on nanostructured models consisting of self-assembled nanospheres of known sizes and refractive index. The linear relationship between Ld measured by PWS device and the expected Ld as well as the linear relationship between nanoparticle sizes Lc and Ld confirm the validity of PWS analysis.

Manuscript received April 24, 2009. This work was supported in part by National Institutes of Health Grants R01 EB003682, R01 CA112315, and R01 CA128641 and National Science Foundation Grant CBET-0733868.

V. Backman, H. Subramanian, P. Pradhan, I. Capoglu, J.D. Rogers, and A. Taflove are with Northwestern University, Evanston, IL 60208 USA (phone: 847-491-3536; fax: 847-491-4928; e-mail: v-backman@northwestern.edu).

Y. Liu is with University of Pittsburgh, Pittsburgh, PA, USA.

H.K. Roy is with NorthShore University Health Systems, Evanston, IL 60208 USA.

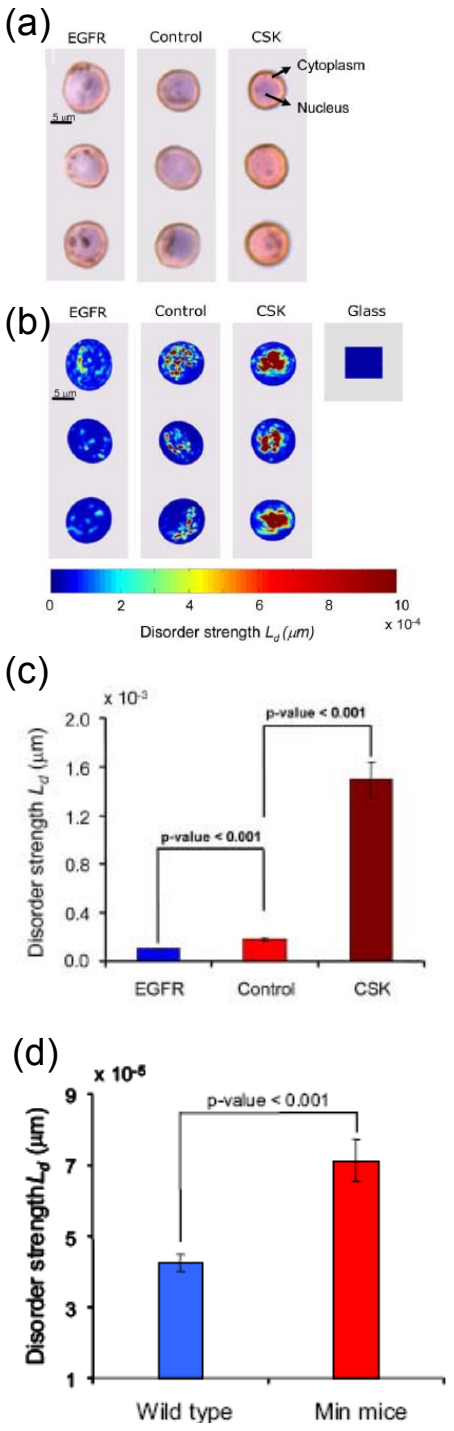

Fig. 2. (a-c) Disorder strength of cell nanoarchitecture correlates with the neoplastic potential in histologically indistinguishable HT-29 cell lines (EGFR-knockdown, empty vector control, and CSK-knockdown). (d): Disorder strength differences among histologically normal-appearing intestinal cells in the MIN-mouse model of colon cancer.

We performed a number of studies demonstrating that PWS can detect subtle genetic/epigenetic changes associated with carcinogenesis.

(1) Cell cultures: We used Sh-RNA approach against a tumor suppressor gene, c-terminal src kinase (CSK) and the proto-oncogene, epidermal growth factor receptor (EGFR) in the human colon cancer cell line HT-29. The knockdown was modest (<50%). Thus, microscopically the three cell lines were indistinguishable (Fig. 2(a)). However, the PWS parameter, Ld, was markedly altered ($p<0.01$, Fig. 2(b,c)) with a clear increase from the least aggressive cells (EGFR knockdowns), intermediate (empty vector controls) and the most aggressive (CSK knockdowns).

(2) Animal studies: Ld increase appears to be a common theme in cells undergoing neoplastic transformation. Ld was markedly increased in the normal-appearing intestinal cells in the MIN-mouse model of intestinal carcinogenesis compared to those from the wild-type mice before the development of neoplastic lesions (6 weeks old mice, $p<0.01$, Fig. 2(d)). The same trend was observed in the colonic cells from the AOM-treated rats (model of sporadic colon carcinogenesis) well before the appearance of neoplastic lesions, in the pre-ACF and pre-adenoma stage (2 weeks after AOM injection, $p<0.01$).

Across all cell types we studied, Ld is remarkably well conserved—the ratio of the standard deviation to the mean for control cells is <15%. This is in sharp contrast to neoplastic cells in which the variability can approach 100%. This highly conserved nature of the disorder may indicate its significance in cell housekeeping.

III. Conclusion

Our data indicate that PWS has the potential to detect cell changes that would otherwise be missed by conventional histopathology. PWS may potentially provide another dimension to histopathology and complement and expand its use. If the results are confirmed in humans, we may have to redefine our understanding of the significance of cell architecture and what we consider to be a ‘‘histologically normal’’ cell.

References

[1] H. Subramanian, P. Pradhan, Y. Liu, I. Capoglu, X. Li, J. Rogers, A. Heiftez, D. Kunte, H. K. Roy, A. Taflove, V. Backman, “Optical Methodology for Detecting Histologically Unapparent Nanoscale Consequences of Genetic Alterations in Biological Cells”, *PNAS*, vol. 105, no. 51, 20124–20129, 2008.