Functional Flow Cytometry Testing: An Emerging Approach for the Evaluation of Genetic Disease

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In this issue of Clinical Chemistry, Nahas and coworkers describe a flow cytometry–based method to evaluate the functional capacity of the protein ataxia telangiectasia kinase and apply this method as a rapid functional test to detect mutations associated with ataxia telangiectasia (AT) (1). The rationale for this approach was to identify a reliable screening method for AT, not only because of the extremely time-consuming conventional assays of radiation sensitivity, which are based on establishing a fibroblast line from each patient, but also because of the currently costly—as well as time-consuming—mutation analysis of the 15 000 nucleotides that constitute the ATM gene (ataxia telangiectasia mutated). A flow-based method focused on detecting phosphorylation of the DNA-repair protein H2AX has recently been described as an approach to screening for AT and, potentially, AT carriers (2). This method, however, focuses on co-staining of cells in the G0/G1 phase with propidium iodide to define cell cycle changes, a rather cumbersome approach as a potential clinical test. The alternative method described by Naha et al. in this issue involves evaluating intracellular phosphorylation of another key DNA-repair protein, SMC1 (structural maintenance of chromosome 1) after γ-irradiation–or bleomycin treatment–induced cell damage. The initial evaluation of this flow cytometry–based method established that it could unambiguously identify AT patients. It also appears to distinguish heterozygote AT carriers from controls. Furthermore, The investigators evaluated other disorders involving DNA-repair defects that share some clinical features with AT and found that the flow-based SMC1-phosphorylation assay appears to distinguish AT patients. Therefore, this assay may prove to be a very useful screening test for AT, and it may be useful in studies evaluating the potential risk posed by a heterozygous ATM mutation. The observation that bleomycin can be substituted for γ irradiation is important because such a substitution would eliminate the need for an irradiator to perform this assay. The data presented for bleomycin have thus far been limited, however, so consideration of incorporating this modification into a clinical assay will require more extensive evaluation of bleomycin-induced SMC1 phosphorylation. In addition, the authors’ preliminary data demonstrating that cells can be evaluated within 48–72 h of obtaining the sample without compromising the results extends the potential of this method as a clinical-screening test.

The flow cytometric approach used by the authors is in line with the general appreciation that this technology affords the capacity to evaluate specific aspects of cell function in addition to the more conventional assays based on characterizing proteins on cell surfaces or intracellularly. An early report established that flow cytometry could detect phosphorylation of STAT1 (signal transducer and activator of transcription 1) following ex vivo exposure of mononuclear cells to interferon γ (3). Furthermore, this approach confirmed that monocytes obtained from patients with a genetic defect in the interferon γ receptor failed to phosphorylate STAT1 following interferon γ exposure (3). Importantly, the sensitivity of the flow method compared very favorably with immunoblotting. Since this initial description, the applications of flow cytometric methods for detecting intracellular protein phosphorylation in response to specific ligand–receptor interactions have expanded dramatically (4–6). This methodology has been complemented by the expanding commercial availability of reagents for the specific detection of a large number of phosphorylated intracellular proteins (7). In all cases, the advantages of this approach include the ready availability of analytical flow cytometers, the relative ease of the method, the very rapid turnaround time of the assay, and the capacity to evaluate and characterize a large number of cells as discrete events. There are undoubtedly additional targets that could be linked to a functional flow cytometry assay, depending on the availability of specific reagents with...
the capacity to clearly distinguish between the native protein and the post–cell signaling form (e.g., a phosphorylated intracellular protein).

The method described by Nahas and colleagues expands this approach into the area of specific cellular-repair mechanisms associated with DNA damage. The clinical utility of this assay will depend on the verification of these initial data and will require rigorous additional evaluation before it can be considered as a clinical test. This process includes the development of a well-defined reference interval, which will likely determine the actual utility of the assay for reliably detecting heterozygous disease carriers. As is the case with any clinical laboratory test, appropriate controls are critical for establishing the reliability of specific results. In the case of functional flow cytometry, it is necessary to ensure that the specific stimulation (i.e., bleomycin exposure in this assay) and fixation/permeabilization steps, as well as the detection reagents, are all performing appropriately. This requirement necessitates that a control sample be evaluated in parallel with patient samples.

The application of reliable and technically manageable functional testing will serve an important role in laboratory evaluation of genetic defects, particularly in settings with varied clinical phenotypes associated with specific genetic defects. Functional testing is also emerging as a necessary step in the evaluation of genetic diseases, owing to the increasing complexity involved in interpreting mutation data (8). It is clear that methodologic approaches, turnaround times, and costs for specific genetic analyses will change in the future, but this fact will not obviate functional testing as part of a complete laboratory evaluation of genetic disorders. Furthermore, functional testing that identifies heterozygosity could enhance the utility of specific assays such as the SMC1-phosphorylation assay described in this issue. Overall, this report underscores the expanding utility of functional flow cytometry for the evaluation and/or monitoring of specific disorders.

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References


