Review Article

Journal of Practical Biochemistry and Biophysics
Volume 2 Number 2, July - December 2017

Role of Flow Cytometry in Cancer Detection

Abhay Shanker Rana[1], Shikha Tyagi[2]

Author Affiliation: [1]Post Graduation M.Tech [2]Lab In-charge, Department of Biotechnology, IMS Engineering College (Dr. A.P.J, Abdul Kalam Technical University), Ghaziabad, Uttar Pradesh 201009, India.

Abstract

In this review, we will discuss the usage of flow cytometry as a diagnostic and prognostic tool for cancer. Flow cytometry analysis can help in selecting distinct therapies. Flow cytometry can be used to differentiate the typical immunophenotype of malignancies, recognition of tumor cell DNA aneuploidy and measurement of cell DNA content. Flow cytometry enables objective explanation of tumor cell heterogeneity with the help of probes that differentiate tumor and normal cells and evaluate all tumor cell properties. The cell fraction in the S phase (SPF) of cell cycle is a common parameter deduced from DNA histograms incurred by flow cytometry. Flow cytometry is a powerful tool for fast analysis of cells for marker expression, cell cycle position, apoptosis, proliferation and measurements of cell kinetics and the potential doubling time (Tpot). Tpot evaluation is helpful in speculating local control in head and neck squamous cell carcinomas. Analysis of cellular RNA content by Flow Cytometry is helpful for the objective differentiation of acute leukemia and of multiple myeloma. Various studies have been conducted to identify hematopoietic stem cells using flow cytometry. The cancer stem cell which may be responsible for the recurrence of a tumor are identified and effectively isolated by multiparametric flow cytometry. The number of Circulating tumor cells (CTCs) which have separated from the primary tumor and run into the blood or lymphatic circulation forming a secondary tumor could be a prognostic marker for cancer progression. Researchers have developed several assays to detect CTCs for cancer diagnosis.

Keywords: Cancer; Flow Cytometry; Neoplasms; DNA Content; Markers; DNA Analysis; Cancer Stem Cells; Circulating Tumor Cells.

Introduction

Cancers are among the major causes of death worldwide. There are more than 100 types of cancer, each with dissimilar causes, symptoms and treatments and can affect any part of the body. The survival of the patient is decided by the stage of the disease. With breakthroughs in research and treatment, cancer survival rates have doubled in the last four decades but still a large number of patients are either resistant to treatment or show disease reoccurrence. Many cancers can be cured if they are detected early and treated adequately. When early detection and treatment intervention is not done, patients are diagnosed at very late stages and then curative treatments have almost negligible chances of success. Flow cytometry analysis provides important diagnostic and prognostic information required to select distinct therapies. A review of studies reported over the past 6 years assessing flow cytometry, showed high sensitivity of flow cytometry compared with standard cytology for cancer detection. Numerous applications of flow cytometry

Reprint Request: Abhay Shanker Rana, Post Graduation M.Tech, Department of Biotechnology, IMS Engineering College (Dr. A.P.J, Abdul Kalam Technical University), Ghaziabad, Uttar Pradesh 201009, India.
E-mail: abhayshrana@gmail.com

Received: 13.12.2017, **Accepted:** 13.01.2018

are appearing now in the areas of pharmacology and cytoenzymology.

Overview of Flow Cytometry

Flow cytometry has evolved over the past three decades from a small lab technique to an important conventional tool for diagnosis and monitoring cancer and immune deficiencies. Flow cytometry is specifically important in situations when the investigator wishes to differentiate between multiple dissimilar cell types within a heterogeneous population and evaluate their frequencies or the expression of a particular molecule of interest. Measurement of DNA and surface antigens in hematology and immunology are the most common applications since the beginning of Flow Cytometry. However, applications of cell function measurements and measurements of cell components other than DNA are recently becoming more and more important.

Mainly a flow cytometer potentially allows for fast, sensitive and quantitative cytochemical measurements of any individual cell component that is specifically stained by distinct fluorochromes and monoclonal antibodies. Furthermore, a flow cytometer can also exploit properties of scattering, absorption and auto fluorescence, it can simultaneously acquire multiple parameters corresponding to different cell compartments of the same cells. Simultaneous measurement of nuclear DNA or RNA, and nuclear or cytoplasmic proteins, as well as membrane antigens can be achieved. The simultaneous acquisition of multiple parameters in flow cytometry is made out mainly to characterize heterogeneous cell populations. Cell sorting makes possible purification of homogenous cell, which then can be explored by other techniques.

Flow Cytometry in Cancer Detection

Flow cytometry permits high-speed analysis and sorting (1000 cells/sec) depending on morphological, biophysical, molecular, and functional cell features correlating them with each other in multi-parameter computer-aided instruments. All Flow cytometry examinations require quantitative cell staining, cell monodispersion, and high resolution analysis. For Flow cytometry to probe the heterogeneity of human cancer effectively the unequivocal discrimination of tumor from normal cells is a major requirement so that we can investigate the phenotypic diversity of tumor cells and the interaction with adjacent normal cells.

The pathways and the protein targets in cancer disease are greatly understood today, the application of biomarkers is resulting in more advanced knowledge about the disease process leading to progress in developing more effective drugs with minimum toxicity.

Measurement of DNA Content

Using probes which can distinguish tumor and normal cells and valuate differentiative as well as proliferative tumor cell properties, the objective elucidation of tumor cell heterogeneity and the tumor-host cell interaction can be achieved through quantitative cytology in the form of flow cytometry.

Abnormal nuclear DNA content is a definitive marker of malignancy. Benign and malignant human neoplasms sometimes contain clones with abnormal DNA content. Many models of DNA content aneuploidisation and evolution have been proposed but the exact mechanisms are not clearly understood. The extent of DNA aneuploidy relative to DNA diploid cells known as DNA index (DI) is a usual parameter derived from DNA histograms.

To analyze the existence of ploidy abnormality in human tumors we use DNA-specific fluorescent dyes. In Flow Cytometry cell proliferative activity does not affect the ploidy analysis unlike mitotic karyotyping but is limited only by cell preparative and staining protocols. In recent years a lot of progress has been made and the measurements of DNA content can now be achieved with high resolution and large reproducibility.

There is seen a noted variations in occurrence of DNA-defined aneuploidy. Flow cytometry studies have found that normal and reactive tissues and benign tumors show normal diploid DNA content. In some premalignant conditions like angioimmunoblastic lymphadenopathy and preleukemia and benign monoclonal gammopathy aneuploidy was detected. Diploid content is observed in chronic lymphocytic leukemia, Hodgkin's disease, and benign phase of chronic myelogeneous leukemia.

A 15 to 30% aneuploidy rate in acute leukemia and in indolent non-Hodgkin's lymphoma; a 50 to 80% aneuploidy rate in aggressive lymphoma and in multiple myeloma; and a 60 to 100% aneuploidy rate in solid tumors has been noticed (Bariogie et al., 1983).

Because of its low degree of dispersion, sensitive and selective association with neoplastia lesions and stable expression DNA content abnormality is generally used for tumor diagnosis and for detecting rare neoplastic cells in early stages of the disease.

Cellular RNA Content

Cellular or nuclear RNA content estimation gives useful information regarding transitions of cells between quiescent and proliferative states. It is known that the nucleoli are enlarged in actively growing cells and that they are distinguished in the cancer cells. Nucleolar RNA synthesis especially rRNA synthesis, is low in resting cells and increases in stimulated cells for proliferation. The role of the nucleolus in the regulation of cell proliferation and in neoplastic transformation is of particular interest in morphological investigations. Cellular RNA content is valuable for the objective differentiation of acute leukemia and of multiple myeloma. Andreeff et al introduced the method to distinguish acute lymphoblastic leukemia from acute myeloid leukemia. Following DNA digestion, propidium iodide staining confirms Frankfurt's notion that double stranded RNA content measurement in this way is a function of cellular proliferation and is very high in malignant cells than normal cells. RNA content is now being studied by using pyronine or acridine orange. The Sloan-Kettering group worked with acridine orange and has wholly published its experience.

RNA measurements have also been found to be useful for the distinction of low- and high-grade non-Hodgkin's lymphomas (Bariogie et al., 1983).

Cell Size Related Phenotypic Markers

Many cellular properties are related to cell size, like RNA and total protein content and forward-angle light scatter. Measurement of cell size is achieved by electronic Coulter volume analysis as well as by Forward-angle light scatter analysis. Two parameter analyses of DNA content and Coulter volume for the differentiation of low and high grade lymphomas was demonstrated by Shackney et al. Lymphomas which were constituted of cells with larger Coulter volumes exhibited higher proliferative activity and matched with the higher-grade histological subtypes. Forward-angle light scatter analysis is useful in distinguishing major marrow lineages and peripheral blood cells. Multi-parameter studies by Nicola et al considering lectin binding properties demonstrated enhancement of hemopoietic stem cells through these parameters.

Cytoplasmic Immunoglobulin

Cytoplasmic immunoglobulin is a characteristic feature of plasma cells and it can readily be determined by performing direct or indirect immunofluorescence, this is very useful for multiple myeloma specifically for the classification of nonsecretory myeloma. Unlike RNA content measurements, cytoplasmic immunoglobulin determination has the potential for more effective discrimination of DNA content-derived cell cycle distribution of normal and tumor marrow cells. This happens because cytoplasmic immunoglobulin is restricted to pre-B-cells and to plasma cells.

Plasma cells characterize the last stage of B lymphocyte differentiation and recognized by the occurrence of monotypic immunoglobulin in the cytoplasm. The plasma cells of multiple myeloma patients mostly have an abnormal DNA content and higher RNA content allowing flow cytometric quantitation of plasma cells.

Flow cytometric analysis of abnormal lymphocyte populations in chronic lymphocytic leukemia (CLL) has been widely reported to show weak expression of surface immunoglobulin [5]. It is seen that almost all cases of multiple myeloma are often preceded by asymptomatic monoclonal gammopathies.

Two-parametric flow cytometry of cytoplasmic light-chain immunoglobulin and DNA can be used for prediction of survival in a newly diagnosed Multiple Myeloma treatment. It provides efficient prognostic data for AMG and also helps in many ways in research and management of myeloma. It aids us in distinguishing patients with "low producing" and "low-secretary" myeloma. It brought into focus that development of plasma cell abnormality is accompanied by a persistent decrease in the production potential of immunoglobulin.

Cytokinetic Markers

Enhanced cell proliferation, an important hallmark of cancer can be easily identified using flow cytometry. DNA analysis through Flow cytometry allows rapid and accurate measurement of large number of cells for assessing the proliferation status. Evaluation of cell cycle kinetics, proliferation and apoptosis in human cancer permits differentiation of low and high grade malignant lymphomas. The S phase cell fraction from DNA histograms deduced by flow cytometry indicates that this fraction increases with rising DNA excess in many solid tumors. By studying the uptake of tritiated thymidine the magnitude of slowly or non cycling cells is computed and the cell cycle distribution can be evaluated by flow cytometry analysis of DNA content.

Cell Surface Membrane Antigens

With the application of hybridoma generated monoclonal antibodies, surface membrane antigens are now being largely studied in lymphoid and myeloid neoplasms as a phenotypic cell marker and have recently been found important in vivo and in vitro therapeutic application. Surface marker analysis by Flow Cytometry has brought forward the therapeutic use of monoclonal antibodies for the treatment of lymphomas and leukaemias.

An interesting new application of analysis has been introduced by Ault et al. introduced a new application in surface membrane immunoglobulin analysis, they demonstrated monoclonal light chain excess among peripheral blood lymphocytes extracted from malignant lymphoma patients. The reason behind this phenomenon is not known but similar studies of myeloma raises an interesting possibility circulating tumor cells express such monoclonal excess.

Circulating Tumor Cells

Circulating tumor cells (CTCs) were first spotted in 1869 by Thomas Ashworth and have since been of great significance in defining the metastatic spread of carcinomas. CTCs are cells which have disunited from the primary tumor and flow into the blood or lymphatic circulation producing a secondary tumor.

Current advancements in technology now reproducible detection of CTCs by a simple blood test. The number of CTCs can be used as a predictive marker for cancer progression. Researchers have created several assays to detect CTCs for cancer diagnosis. High CTC counts depict aggressive cancer, higher metastasis, and decreased relapse time. As blood collection is very easy, CTCs prove to be a useful marker for cancer progression and survival. CTCs can also help in therapeutic management, therapy effectiveness and give knowledge about drug resistance mechanisms.

The molecular characterization of CTCs offers a unique ability to assess genotypic and phenotypic features of a cancer without the need for invasive biopsy [16]. CTC isolation techniques rely on antibodies against epithelial cell-adhesion molecule (EpCAM), EpCAM is a protein that sticks out of the surface of CTCs not in healthy cells. Methods derived from immunocytochemistry and reverse transcriptase–polymerase chain reaction have also been useful in the detection and characterization of CTCs but neither technique leads to direct isolation of CTCs. These methods are generally deficient for any kind of functional characterization because they will need a cell-fixation step, which makes it unfeasible to preserve CTC viability.

The Cell Search system enables the reliable detection of CTCs in blood and is suitable for the routine assessment of metastatic breast cancer patients in the clinical laboratory. Blood samples should be shipped at room temperature and CTC counts are stable for at least 72 h [17]. Cell Search platform (Veridex LLC, Huntingdon Valley, PA, USA) and is currently the most widely practiced cytometric technology and the only one that is FDA approved for the counting of CTCs.

Cancer Stem Cell

Recently, a form of cancer cell called the cancer stem cell (CSC) has been observed and noted for tumors. CSCs might be accountable for the reoccurrence of a tumor after a successful therapy and are believed to stand a rich metastatic potential. For the progress of competent treatment strategies, formation of dependable methods for the recognition and proficient isolation of CSCs is really important. Equivalent to their stem cell counterparts in bone marrow or small intestine, several differentiation surface antigens have been defined, thus letting researchers to recognize them in the tumor mass and to find out their degree of differentiation. Moreover, functional properties and characteristic of CSCs can be examined.

Side population analysis is based on the stem cell-specific activity of certain ATP-binding cassette transporter proteins, which are able to transport fluorescent dyes out of the cells. Furthermore, the stem cell-specific presence of aldehyde dehydrogenase isoform 1 can be used for CSC labeling (Greve, Kelsch, Spaniol, Eich & Gotte, 2012). The method of choice for the examination of Cancer Stem Cells is Multiparametric flow cytometry. It enables the simultaneous analysis of varying cellular features with high performance and reliability. More than that, it enables the separation of living cells on the basis of marker expression or functional properties by fluorescence-activated cell sorting. The main plus point of this technique is its capability to isolate rare cells, which is a requirement for identifying small cell populations within the tumor bulk. Quantification is possible too and can be attained either by the addition of count check beads to the sample or by volume-based flow cytometry.

Sorting of CSCs

The mechanical sorting systems are completely isolated, thus cutting out the risk of aerosol

generation. FACS-Calibur, Becton Dickinson, NJ, operates with a catcher tube located in the upper part of the flow cuvette. If the analyzing unit finds out a cell as a sorting target, the mechanical unit enters the flow stream, collects the cell, and brings it into another separate tube.

Future Prospects of Flow Cytometry

Enumeration of circulating prostate micro particles (PMPs), a type of Extracellular vesicles discharged by prostate cancer in urine, blood and seminal fluid, may constitute a non-invasive method to prioritize and distinguish patients of prostate cancer with intermediary risk and high risk. Conventional flow cytometry isn't designed to analyze submicron events as the optics can detect light scatter from greater than 3 microns. New generation tools like nanoscale flow cytometry are able to examine cases 100-1000nm in diameter. Nanoscale flow cytometry of EVs in plasma, serum, or urine provides high-content information in a high-throughput manner.

Bead-based flow cytometric assays with high sensitivities could ease the detection of low abundant proteins, making better our knowledge of biological pathways and helps in disease diagnosis at early stages. Micro-bead based multiplexed protein immunoassays have rapidly grown in the past 10 years.

New creative concepts in radiation-induced signaling processes and the integration of radiation with new targeted agents are considerable areas for future flow cytometry-based research. Additionally high-throughput assays for radiation-associated population studies are very likely to be formed sooner.

Conclusion

The advancement and application of flow cytometry-based technologies is causing high impacts on the diagnosis, monitoring, and prognostication of patients with cancer and also classification of the disease. Flow Cytometry has had significant effects on patients receiving solid organ allografts and allogeneic hematopoietic stem cell transplants.

The capability of flow cytometry to bring forth huge amounts of multi dimensional, high-complexity data has certainly placed this technology as a major platform for use in clinical pharmacology for years to come. The instruments are getting smaller as well as less expensive and number of clinically useful antibodies is growing day by day, this is creating more chances for clinical laboratories to adapt flow cytometry in the diagnosis and prognosis of the disease.

The rising field of "cytomics" represents a structured, whole-cell–based elucidation of cellular physiology that encompasses aspects of genomics and proteomics and it attempts to design a clear comprehensive picture of individual cells. The flow cytometer can be seen as a rational platform for building the foundation of this emerging field.

Key Messages

"Flow cytometry analysis provides important diagnostic and prognostic information required to select distinct therapies. A review of studies reported over the past 6 years assessing flow cytometry, showed high sensitivity of flow cytometry compared with standard cytology for cancer detection"

"Flow cytometry is specifically important in situations when the investigator wishes to differentiate between multiple dissimilar cell types within a heterogeneous population and evaluate their frequencies or the expression of a particular molecule of interest."

"The pathways and the protein targets in cancer disease are greatly understood today, the application of biomarkers is resulting in more advanced knowledge about the disease process leading to progress in developing more effective drugs with minimum toxicity."

References

1. Barthel B, Martin N, Schumann J, Johnson T, Drewinko B, Swartzendruber D, Gohde W, Andreeff M, and Freireich E . Flow Cytometry in Clinical Cancer Research. Cancer Research 1983 Sep;43:3982-3997.
2. Verschoor C, Lelic A, Bramson J and Bowdish E. An Introduction to Automated Flow Cytometry Gating Tools and Their Implementation. Frontiers in Immunology 2015;6:380. doi:10.3389/fimmu.2015.00380.
3. Jaye D , Bray R, Gebel H, Wayne, Harris A and Waller E. Translational Applications of Flow Cytometry in Clinical Practice. J Immunol 2012; 188:4715-4719; doi: 10.4049/jimmunol.1290017.
4. Giaretti W. Origins of ... Flow cytometry and applications in oncology. J Clin Pathol 1997;50:275-277. doi : 10.1136/jcp.50.4.275.
5. Lewis R, Cruse J, Pierce S, Lam J, Tadros Y. Surface and cytoplasmic immunoglobulin expression in B-

cell chronic lymphocytic leukemia. Experimental and Molecular Pathology. http://dx.doi.org/10.1016/j.yexmp.2005.04.009

6. Papasotiriou, I., Chatziioannou, M., Pessiou, K., Retsas, I,. Dafouli, G., Kyriazopoulou. Detection of Circulating Tumor Cells in Patients with Breast, Prostate, Pancreatic, Colon and Melanoma Cancer: A Blinded Comparative Study Using Healthy Donors. Journal of cancer therapy 2015 doi: 10.4236/jct.2015.67059.
7. Gupta V, Zhang Q, and Liu Y. Evaluation of Anticancer Agents Using Flow Cytometry Analysis of Cancer Stem Cells. Methods Mol Biol. 2011;716: 179–191. doi:10.1007/978-1-61779-012-6_11.
8. Lu Y, Liang H, Yu T, Xie J, Chen S, Dong H, Sinko P, Lian S, Xu J, Wang J, Yu S, Shao J, Yuan B, Wang L, and Jia L . Isolation and Characterization of Living Circulating Tumor Cells in Patients by Immunomagnetic Negative Enrichment Coupled With Flow Cytometry. Cancer 2015, doi: 10.1002/cncr.29444
9. Greve B, Kelsch R, Spaniol K, Theodor H, Gotte M. Flow Cytometry in Cancer Stem Cell Analysis and Separation. Published online 6 February 2012 in Wiley Online Library (wileyonlinelibrary.com) doi: 10.1002/cyto.a.22022.
10. Siddiqui K, Pardhan S. Prostate extracellular vesicles in patient plasma as a liquid biopsy platform for prostate cancer using nanoscale flow cytometry. Oncotarget Jan 2016. doi: 10.18632/oncotarget.6983.
11. Mannello F. Understanding breast cancer stem cell heterogeneity: time to move on to a new research paradigm. BMC Medicine 2013;11:169. http://www.biomedcentral.com/1741-7015/11/169.
12. Ahluwalia, M., Wallace, P., & Peereboom, D. Flow Cytometry as a diagnostic tool in Lymphomatous or Leukemic Meningitis. Cancer, 2012;118(7):1747-1753. doi: 10.1002/cncr.26335.
13. Li, J., Ji, L., Chen J., Zhang, W., & Ye, Z. Wnt/5ØýÞ-Catenin Signaling pathway in Skin Carcinogenesis and Therapy. BioMed Research International Volume 2015, Article ID 964842, http://dx.doi.org/10.1155/2015/964842.
14. Zhang, B, Yang, J, Zou, Y, Chen, M, Hong, G, Dai, H. Plasmonic micro-beads for fluorescence enhanced, multiplexed protein detection with flow cytometry. Chemical science 2014 doi: 10.1039/C4SC01206B.
15. Miller, M.C., Doyle, G.V. and Terstappen, L.W. Significance of circulating tumor cells detected by the Cell Search system in patients with metastatic breast colorectal and prostate cancer. JJ Oncol. 2010;2010:617421. doi: 10.1155/2010/617421.
16. Krebs M, Hou J, Ward T, Blackhall F, Dive C. Circulating tumour cells: their utility in cancer management and predicting outcomes. Ther Adv Med Oncol 2010;2(6):351-365 doi: 10.1177/1758834010378414.
17. Riethdorf S, Fritsche H, Muller V, Rau T, Schindlbeck C, Rack B, Janni W, Coith C, Beck K, Janicke F, Jackson S, Gornet T, Cristofanilli M and Pantel K. Detectionof Circulating Tumor Cells in Peripheral Blood of Patients with Metastatic Breast Cancer: A Validation Study of the Cell Search System. Clin Cancer Res 2007, doi:10.1158/1078-0432.CCR-06-1695.
18. Barlogie B, Alexanian R, Pershouse M, Smallwood L, and Smith L. Cytoplasmic Immunoglobulin Content in Multiple Myeloma. J. Clin. Invest (1985). 002 1-9738/85/08/0765/05.
19. Bhatt A N, Mathur R, Farooque A, Verma A and Dwarakanath B S. Cancer biomarkers - Current perspectives. Indian J Med Res 2010 Aug;132; 129-149.
20. Plaks V, Koopman C D and Werb Z. Circulating Tumor Cells. Science. 2013 Sept;13:341(6151): doi:10.1126/science.1235226.
21. Williams SCP, Circulating tumor cells. www.pnas.org/cgi/doi/10.1073/pnas.1304186110.
22. Papanikolaou X, Rosenthal A, Dhodapkar M, EpsteinJ, Khan R, Rhee1 F, Jethava Y, WaheedS, Zangari M, Hoering A, Crowley J, Alapat D, Davies F, Morgan G and Barlogie B. Blood Cancer Journal 2016;6:e410; doi:10.1038/bcj.2016.19.