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Review Article

Cellular Flow Cytometric Studies

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Abstract

This review focuses on flow cytometric studies at the single cell level. Currently, flow cytometry is used to analyze DNA content, cell cycle distribution, cellular viability, apoptosis, calcium flux, intracellular pH and expression of cell surface compounds in targeted cell populations. Our criteria for the selection of research papers for this review were focused on those that show current cellular applications of flow cytometry.

Keywords: Flow Cytometry; Tumor; Cell; Cell Surface Compounds; Targeting

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Introduction

Flow cytometry has been available since the 1970s and has been revolutionizing fields of medicine since its discovery. The earliest application of cytometry was to measure the physical and chemical characteristics of blood and cancer cells [1, 2]. At the present time, flow cytometry is employed as a diagnostic tool used to measure the effects of immunotherapy on tumor biology [3, 4]. It still remains a technological challenge to extract three-dimensional morphological information from cells for complete characterization and classification [5]. Recently, morphology of complicated cellular systems such as cancer can be studied on a single-cell level. Also, flow cytometry advanced the chemistry of cell labelling with fluorescent agents by applying up to 18 emission colors with high resolution detection to study cancer tumor biology. As a result, cellular composition, DNA content and lymph nodes at the single cell level have been analyzed using an integrated system of multicolor flow cytometry which provides real-time mapping of vessels [6]. The markers of metastatic cancer are circulating tumor cells (CTCs) from the primary tumor [7, 8] and flow cytometry is advancing the detection of metastatic circulating tumor cells [9]. Current flow cytometric measurements of cell morphology include changes in cell shape, loss of structure on the cell surface, cell detachment, condensation of the cytoplasm and cell shrinkage. Flow cytometry can also measure chemical changes such as the variation in cellular calcium content, pH and intracellular generation of reactive oxygen species [10]. Cells from solid tumor tissue require disaggregation before they can be analyzed by flow cytometry [11]. The cell sorting capabilities of flow cytometry have been use in the analysis of antibody conjugates and site-specific conjugated cells [12]. Other cellular processes that can be monitored by flow cytometry are: cell cycle regulation; cell survival and DNA repair; cellular differentiation; signal transduction; antioxidant and xenobiotic detoxification; stem cell biology; metabolic regulation; epigenetic mechanisms of gene regulation and tumor/stroma interactions [13-15]. DNA content varies with each phase of the cell cycle and this can be assessed using fluorescent DNA binding dyes and monoclonal antibodies to detect the expression of antigens [16]. Dead cells can now be identified at the early or late stages of necrosis using a range of viability dyes [16]. The heterogeneity of a tumor (Figure 1) and the interconnections between cellular components and tissues complicate the evaluation of the immune response. Flow cytometry as a single cell detection method can visualize and map immunological interventions as well as aid in elucidation of the underlying mechanisms of immunity.

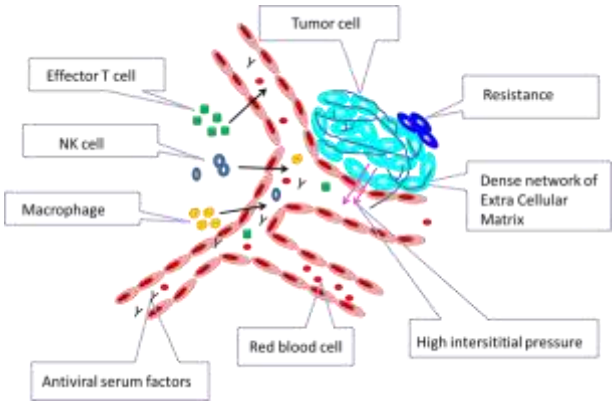

Figure 1 Heterogeneous tumor environment with immune system interaction.

Early Studies

The beginnings of flow cytometry started more than 50 years ago. In the 1940s, Papanicolaou and Traut demonstrated that they could identify cells from cervical cancer by staining tissues with specifically designed stains [17]. In 1953, Coulter patented the first cytometric instrument which was used for accurate determination of the number of white or red blood cells, based on electronic impedance measurements (US patent number 2,656,508) [18]. Based on these advances, single-cell analyzers were constructed in the 1960s. In 1965, Fulwyler developed the first microfluorometer (flow cytometer) called the *Los Alamos Flow Microfluorometer* [19]. The flow microfluorometer was a cell separator. This instrument separated cells based on cell volume. In 1968, Göhde developed the first fluorescence-based apparatus which worked as a pulse cytophotometer [18]. However, at this time, fluorescence techniques were not as popular in research as absorption spectroscopies. Thus, the first commercial flow cytometer was built around a Zeiss fluorescent microscope. The commercial name of this cytometer was The *Cytograph* which had an onboard He-Ne laser system at 633 nm for scatter measurements. This instrument could segregate and sort live and dead cells depending on their uptake of Trypan blue. These advances were followed by a fluorescence apparatus called the *Cytofluorograph* with an air-cooled argon laser operating at 488 nm. In 1976, eight years after the first fluorescence based flow cytometer was introduced, it was agreed at the Conference of the American Engineering Foundation in Pensacola, Florida, that the name "*flow cytometry*" would be used [19]. Many commercially available cytometers are now equipped with several lasers for multiple wavelength generation. Laser diodes emitting at approximately 640 nm were introduced into flow cytometers in the early 1990s as replacements for the He-Ne laser [20]. Small He-Ne lasers continued to be used in flow cytometers for some time, but laser diodes are now used predominantly. Violet laser diodes were the next laser type to see wide usage in flow cytometry. The development of polychromatic flow cytometry and multicolor technology has enabled the detection of five or more markers simultaneously. Flow cytometry provides a high throughput, multi-dimensional analysis of cells flowing in suspension. Today, modern instruments have several lasers and detectors which enables multiple and accurate identification of cells from animals, plants, bacteria, yeast or algae and particles such as chromosomes or nuclei [21, 22].

Flow cytometry analyses particles in a fluid as they pass through lasers [23]. Any cell or particle that is 0.2 to 150 μm in size can be analyzed using a flow cytometer. The purpose of the fluidics system is to transport particles in a stream of fluid to the laser beam where they are illuminated. The fluorescence can then be measured to determine the amount and type of cells present in a sample [24-29]. Choosing the correct lasers and filter configurations for fluorescent protein analysis is similar to designing the detection optics for fluorescent probe or probes. The excitation wavelength should be as close to the excitation maxima as possible, although the broad excitation curves for fluorescent proteins allow some flexibility in laser choice [30-32].

Various Analyses

Flow cytometry is the preferred assay to use for cells sorting, cell counting, drug screening and phenotypic analysis [33]. The preclinical and clinical usefulness of flow cytometry as a diagnostic tool has increased due to advances in cancer research with the development of new cancer drugs and treatments. Traditionally, flow cytometry has been used for the analysis of tumor biology [34].

Flow cytometry allows multiparameter analysis of single cells. Figure 2 illustrates several major facets of cellular studies that can be performed by using flow cytometry. Besides cell sorting and counting, cytometric measurements enable monitoring of cellular interactions, expression of surface molecules, cellular proliferation, secretion and cell mobility. Cell proliferation cytometric analyses can characterize cell growth and differentiation. Measurement of cell proliferation is used to evaluate drug toxicity and inhibition of tumor cell growth. Proliferation measurements using flow cytometry is an assay of DNA content [35, 36]. Detection of secreted proteins is difficult as proteins released from the cell before detection or may degrade rapidly. Intracellular staining methods can then be used for detection of target proteins using flow cytometry. Flow cytometry is used to establish secretion profiles of granules and cytokines and cytokines associated with immune cells can influence cell mobility [37]. Monitoring of cell-cell interactions using flow cytometry is achieved by labelling cells with different dyes. Expression of common cell surface molecules such as glycolipids, glycoproteins and clusters of differentiations (often abbreviated as CD) are also measured by flow cytometry. CD markers are surface molecules providing targets for immunophenotyping of cells such as $CD34^+$, $CD31^-$, CD117 for stem cells, $CD45^+$ for all leukocyte groups, $CD45^+$, CD11b, $CD15^+$, $CD24^+$, $CD114^+$, $CD182^+$ for granulocyte, CD4, $CD45^+$, $CD14^+$, $CD114^+$, CD11a, CD11b, $CD91^+$,CD16 for Monocyte, $CD45^+$, $CD3^+$ for T lymphocyte, $CD45^+$, $CD3^+$, $CD4^+$ for T helper cell, CD4, CD25 for T regulatory cell, $CD45^+$, $CD3^+$, $CD8^+$ for cytotoxic T cell, $CD45^+$, $CD19^+$, $CD20^+$, $CD24^+$, CD38, CD22 for B lymphocyte, $CD45^+$, $CD61^+$ for thrombocyte and $CD16^+$, $CD56^+$, $CD3^-$, CD31, CD30, CD38 for natural killer cells [38, 39]. Cell adhesion molecules (CAMs) are proteins located on the cell surface. Cell adhesion molecules belong to four protein families: the immunoglobulin superfamily (IgSF CAMs), the integrins, the cadherins, and the selectins [40]. Calcium-independent cell adhesion molecules are IgSF CAMs and lymphocyte homing receptors; calcium-dependent CAMs are integrins, cadherins, and selectins [41].

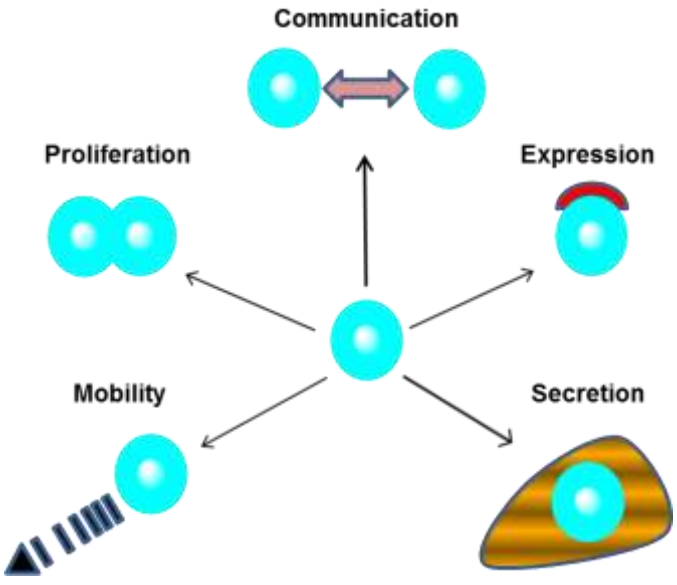

Figure 2 Cell Activity in Heterogeneous Tumor

A review by Brown and coworkers presents applications and principles of flow cytometry in clinical hematology [42] for the study of hematologic malignancies and disorders of myeloid and

lymphoid such as myeloid leukemia, chronic myeloproliferative neoplasms and chronic myelodysplastic neoplasms. Lymphoid disorders studied by flow cytometry include acute lymphoblastic leukemia/lymphomas, B-cells, T-cell, and NK-cell lymphomas, large granulocytic leukemia with Kir antibodies, plasma cell myelomas and Sezary syndrome. Flow cytometry has been shown to be effective for evaluating changes in cell number and proliferation during breast cancer chemotherapy [43, 44]. Flow cytometry identifies and segregates (or isolates) particular types of cells based on expression of marker molecules on their surface [45-47]. Begg and coworkers applied this technique to the study of fibrosarcoma and adenocarcinoma tissues [48]. Also, the biology of cancer tumors before and after irradiation has been studied by flow cytometry [49-51]. The intracellular distribution of important chemotherapeutic antibiotics belonging to the anthracycline group (e.g. adriamycin) was detected by flow cytometry [52, 53]. Flow cytometric investigation showed that GNRs conjugated with doxorubicin and cyclo(Arg-Gly-Asp-D-Phe-Cys) peptide demonstrated greater cellular uptake and cytotoxicity compared to non-targeted GNRs conjugated with doxorubicin [54]. Recently, synthetic glycocluster tumor antigen conjugates which are promising for the development of tumor vaccines, have been evaluated by flow cytometry [55, 56]. Other studies include serotonin signaling [57], the administration of thymidine analogues [58], and targeted delivery of therapeutics such as cytokines and antibodies [59]. All targeting agents can be monitored at several stages of activity by using flow cytometry. Recent advances in flow cytometry have made it possible to screen biopharmaceuticals and provide their distribution, targeting efficiency and cell vitality conditions.

Conclusion

The most recent studies demonstrate that flow cytometry can be used for disease detection and treatment monitoring. Flow cytometry is likely the preferred method of diagnosing and immunophenotyping various types of cancers. The advances in immunotherapy are likely to benefit from the availability of flow cytometry in preclinical and clinical research. Flow cytometry provides fast multi-parameter quantification of the biological properties of individual cells at subcellular and molecular levels.

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