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Quantitative Classification of Tumor Cell Morphological Changes on Selectively Functionalized Biochips

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Abstract— **Proteomics information of cancer has shown that abnormalities at the levels of growth factors, receptors, intracellular mediators and transcription factors play major role in the disease progression. We report a directly quantifiable approach to measure tumor cell behavior on functionalized chips. The chips were functionalized with aptamer molecules that were selective against epidermal growth factor receptor (EGFR), a commonly overexpressed cancer biomarker. The chip-bound aptamer selectively isolated tumor cells from cell mixture samples. The isolated cells were thus bound to the chip surface. However, some normal cells also got captured on the surface. The selectivity and sensitivity of tumor isolation changed when the surface of the chip was chemically treated to create nanoscale texture. The captured cancer cells showed distinctly different behavior on the surface of the chip than that for the normal cells. The behavior quantification can serve as a novel modality to detect cancer cells from simple samples like blood, saliva or urine.**

I. Introduction

With the advent of "omics" revolution, many molecular pathways are now known that are involved in the regulation of cell behavior. Disruptions in many pathway signals and/or abnormal downregulation/overexpression of various molecules have been identified to give rise to diseased conditions. Molecular entities that can be used to indicate the state of a biological system are called biomarkers (e.g. cholestrol levels). Cancer related biomarkers have also seen huge interest and development in last decade or so. One of these biomarkers is EGFR. Given the overexpression of epidermal growth factor receptors (EGFR) in many cancerous cells (breast, lung, cervical, bladder, esophageal, ovarian cancer, etc.), it can be possibly used to detect all these cancer types [1-6].

In general, cancer diagnosis is mostly done from biopsy, cytology, in vivo scopy/imaging, and immunological markers. These approaches have shortcomings such as erroneous judgment (biopsy examination and cytology), invasive risks from anesthesia and operations (biopsy examination), high cost (in vivo scopy/imaging), low sensitivity (cytology and immunological markers), and low specificity (in vivo scopy and in vivo imaging). None of these methods meet the requirements of non-invasiveness, low-cost, high-sensitivity, high-specificity, and minimal false results. Even if the best diagnostic modality is chosen, a major problem in oncology is that treatment can be unsuccessful and/or a reservoir of tumor cells can lie dormant and eventually return, often as a metastatic lesion. In both instances, it is possible that one of the early signals of cancer cell survival will be the existence of circulating tumor cells (CTCs). CTCs are known to utilize blood circulation system to land on distant organs and form new tumors. Detection and enumeration of such cells from peripheral blood can be effective for early cancer diagnosis.

CTCs have been isolated from the blood of cancer patients [7, 8]. These cells have been seen to change with the stage of disease, i.e., the CTCs of dormant disease differ from those of metastatic cancer. The quantity of CTCs has also been correlated with disease progression and survival (especially for patients with metastatic breast cancer) [9, 10]. The work on tumor cell isolation has spanned many different approaches, materials and transduction mechanisms (e.g. immunohistochemical, flow cytometric, amperometric, mechanical, optical) [10-12].

A detection method is reported here that is based on our previous work on isolating tumor cells from a mixture of cells. The cancer and normal cells captured on the chip surface showed distinct behavior, as shown in Fig. 1.

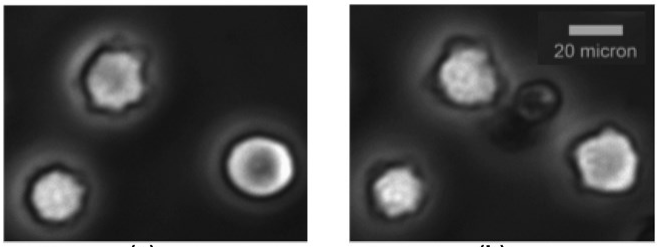

Figure 1: Mouse-derived tumor cells on the anti-EGFR aptamer grafted surface show morphological changes as seen in (a) and (b) whereas normal cells didn't change their shape, size or morphology (data not shown). The micrograph shown at (b) was taken 30 minutes after (a). Adapted from [13] with permission from American Association for Cancer Research.

The behavior of cells after capturing on the surface can be a useful cytological tool [13-15]. However, this may also face the same challenges that standard cytology and biopsy face (eroneous judgement). Towards this end, this paper presents early work done on quantifying the distinguishing features of tumor cells. Such cancer detection and quantification system can be important in a clinical laboratory setting where this can

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be deployed as part of yearly medical check-up and physical exam.

II. Materials and Methods

Nanotextured and plain glass slides were used for cancer cell isolation. In either case, the surfaces were functionalized with the anti-EGFR aptamer through multi-step layer by layer assembly of molecules (Fig 2). First of all, the slides were cleaned with Piranha solution (H_2O_2:H_2SO_4, 1:1) followed by silanization. A single-stranded DNA (ssDNA) sequence was then attached to the surfaces through a homo-bifunctional linker. The linker would bind to the silane on one end and the amine group of ssDNA on the other end. After ssDNA attachment and blocking of unreactive linker functional groups, anti-EGFR RNA was hybridized to the ssDNA on the chips. After incubating the chips with tumor cell mixtures, time lapsed images were taken.

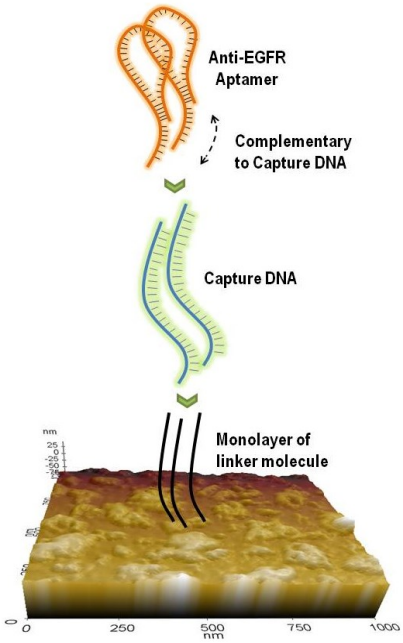

Figure 2: Surface modification with EGFR specific aptamer (not to scale)

These recorded images were then processed though several steps towards final contour detection. After initial image enhancement, cell boundaries were detected and images were converted to binary format for processing. The temporal changes in contours of cells from frame to frame gave information like size, the changes in cell boundary, the growth, the formation of pseudopods, etc. Based on these features several feature vectors were defined that would differentiate one cell type from the other.

The image enhancement operations showed dependence of the compoutation speed on a number of factors, e.g. the density of cells in a frame, the frequency of frame capture by the camera, the depth of field of the imaging plane, the resolution of the captured image, the ability to reimage the same cell (or set of cells) at exactly same position.

The processes consisted of tone detection (dark versus bright areas), identification of bright region (cell tagged), segmentation (conversion to binary image), angular sectoring (to breakdown circular cell into parts), boundary tracing (cell growth and motility) and detection of protrusions (pseuodpods).

III. Results

EGFR-specific aptamers selectively isolated EGFR overexpressing human glioblastoma (hGBM) cells with high specificity. Tumor cells, when bound to such functionalized surfaces, showed distinct morphological patterns and enhanced activity as compared to healthy cells, which remained calm. These cells showed clear changes in cell shapes from spherical to semi-elliptical with very flat orientation, formed pseudopods (possibly to cover much more surface area) and showed rapid growth. Cell behavior on both EGFR-specific surface and control surface was quantified and different feature vectors like non-uniformity (Figure 3), Hausdorff distance, frame-to-frame differential pseudopod formations were calculated. The non-uniformity feature was based on the formation of finger-like projections protruding out of the cell walls. This also made the focal point move and required manual focusing of the microscope. A frequency of 4 images per minute was found to be optimal to essentially capture the slow movements of the cells.

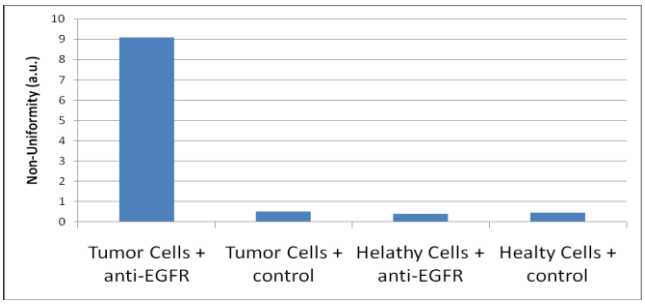

Figure 3 : Nonuniformity (au) in cell shape calculated from time-lapsed images of tumor cells and healthy cells on anti-EGFR aptamer and mutant aptamer substrates.

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