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Cancer Cell Surface Negative Charges: A Bio-Physical Manifestation of the Warburg Effect

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The early detection of circulating tumor cells (CTCs) in blood as part of medical diagnosis will give the doctors a head start in the provision and treatment of cancer, and therefore, with the advance in Nano technology, there is an increasing expectation of some form of early detection of circulating tumor cells at a highly sensitive level, without any biomarkers, for both early cancer diagnosis and monitoring disease progression after medical intervention. This technical note reports on the recent development in detection of highly sensitive detection of cancer cells without biomarkers. This novel concept is developed based on a hallmark cancer metabolic pattern: high glycolysis rate. Secretion of high level of lactate acid by cancer cells ultimately results in negative electrical charges on their surfaces, enabling strong binding and capturing by the positively-charged nanoprobes, and subsequent magnetic separation. When nanoprobes are incubated with cancer cells in suspension, binding takes place due to charge differences, and cancer cells are then magnetically separated. The separated cells are enumerated using a flow cytometry and identified by pathological and genome sequencing methods. Preliminary results using the approach have shown exceptionally high cancer cell capture rates, therefore potentially applicable in cancer cell detection in clinical settings.

Keywords: Circulating Tumor Cells (CTCs); Warburg effect; Fe_3O_4 nanoparticles.

1. Introduction

In cancer diagnosis and therapeutics, extensive effort has been devoted to identifying cancer cells in terms of oncogenic and pathological characteristics. One of the major approaches has been the development of tumor-specific ligands for cell targeting. However, previous studies have shown that two isogenic cancer cell lines differ only by a few proteins.[1] Furthermore, any molecules present on cancer cells are not absolutely absent on normal cells.[2–4] These commonalities between cancer and normal cells at molecular level have been the fundamental problems in any biomarker-based cancer diagnosis and therapeutics. It is, therefore, critical to identify a hallmark characteristic that is shared by all cancers, regardless of the phenotype and molecular differences.

2. Warburg Effect and Lactate Secretion Generated Cancer Cell Surface Negative Charges

As is well-known, cancer cells exhibit the so-called "Warburg Effect" by which they only process

glycolysis.[5,6] High levels of glucose uptake and lactate secretion are the two most distinguishable metabolic behaviors not only widely observed in cultured cancer cells but also extensively employed in cancer clinical settings. The levels of glucose uptake and lactate secretion are observed to be thirty times greater than those of normal cells.[7,8] Most of the previous studies on the metabolic patterns of cancer cells have mainly focused on biochemistry and the cross-membrane movement of lactate. Our recent study has shown, from 22 cancer cell lines, a close correlation between the lactate acid secreted and a net of negative electrical charges that appears on cancer cell surfaces. More important, this study has shown a direct relationship between cancer cell surface electrical charges and rate of glycolysis, that is regulated by glucose uptake.

3. Cancer Cell Binding and Capturing by Surface Charged Nanoprobes

To monitor the cancer cell electrical charges, the Fe_3O_4-based nanoprobes are designed and surface-functionalized with the polyethylenimine (PEI) molecules, rendering them positively charged for cancer cell electrostatic binding, capturing and magnetic separation, as schematically shown in Fig. 1.[9–12] When mixing the positively-charged nanoprobes with cancer cells of any type, they massively bind onto the cell surfaces due to opposite electrical charges.[9,10] As the Fe_3O_4 nanoprobes are also superparamagnetic, the cancer cells, bound with a high concentration of nanoprobes on their surfaces, are easily magnetically separated. Conversely, if the nanoprobes are negatively charged with the silica functional group, few of them bind on cancer cells, resulting in insignificant cancer cell capture. In this fashion, the nanoprobes can be used to detect the cancer cell surface charges which are associated with lactate secretion, a common metabolic characteristic of all cancer cells. Note that the sizes of cancer cells such as breast cancer cells range 20–30 μm, while the typical dimension of the nanoprobe is on the order of 200–300 nm. The nanoprobe electrostatic binding can therefore take place on cancer surfaces with high concentrations. Upon strong electrostatic binding, the cancer cells can be sensitively detected, effectively captured, and magnetically removed for both diagnostic and therapeutic purposes.

In a report by Han *et al.*, the Fe_3O_4 nanoparticles were rendered fluorescent and electrically charged either positively (MNCs⊕) or negatively (MNCs Θ). They carried out systematic *in-vitro* experiments on cell binding of HeLa cells via electrostatic interactions with charged nanoparticles.[10] As shown in Fig. 2, the green fluorescence from the positively-charged nanoparticles (MNCs⊕) is visible around

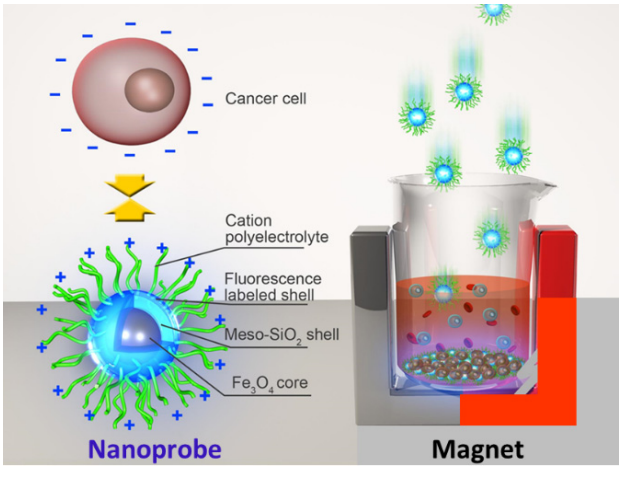

Fig. 1. Schematic diagram showing the nanoprobe design and cancer cell capturing.[9] Mechanism of Cancer Cell Surface Negative Electrical Charges.

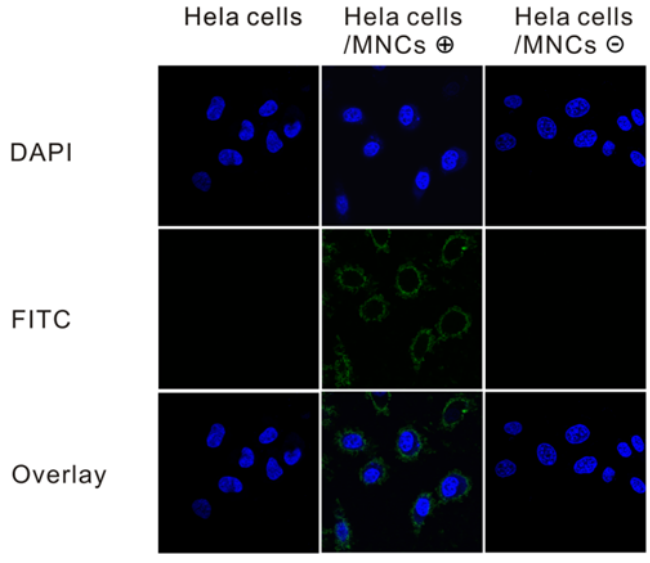

Fig. 2. Confocal fluorescent images showing treated cancer cells (HeLa cells) with positively- (MNCs⊕) and negatively- (MNCs Θ) charged magnetic nanoparticles. Note the cancer cells are shown in blue fluorescence while the green circles surrounding them are the positively-charged nanoparticles (middle column). No green fluorescent is seen on the right column for the negatively-charged nanoparticles since they do not bind on the cancer cells.[10]

the cancer cells (the blue colored cell nucleus is stained by DAPI) due to strong electrostatic force (middle column in Fig. 2).[10] In sharp contrast, no green fluorescence can be observed on the membrane of cancer cells treated by the negatively-charged nanoparticles (MNCs Θ). These are strong experimental evidences that show the negatively-charged cancer cell surfaces, consistent with the hypothesis based on the Warburg Effect.

The lactate-secretion-generated cancer cell surface negative changes can be explained by the cross-membrane movement of mobile ions. The charge neutrality of most human cells is maintained by the ion pumps through the plasma membrane. In cancer cells, the cross-membrane movement of lactate is the pathway of glycolysis in hypoxia. The charges of immobile ions can easily encounter massive amount of Na^+ present in the interstitial space and extracellular environment. These immobile ions are fully accessible by Na^+ for charge neutralization. It is the highly elevated level of glycolysis that utilizes sufficient glucose available and secretes a large amount of lactate as a continuous source of mobile anions moving from cell interior to exterior, consequently creating a net of negative charges. Therefore, the perpetuating ion movement is solely responsible for the signature pattern of cancer cell surface negative charges. Our findings mechanistically link one of the most fundamental metabolic pattern, glycolysis, with a hallmark characteristic of cancer cell surface charges. A direct relationship is established between the cancer surface electric charge behavior and the lactate secretion based on a large group of cancer cell lines (22 cell lines). The surface negative charge is found to be a universal property of cancer cells regardless of their molecular and genetic differences. The operating mechanism identified in this study has laid an important foundation for the fundamental understanding of cancer cells. Furthermore, the cancer cell surface charges can be used as an analyte for studying the metabolism of cells since it directly correlates to glycolysis. In conclusion, the cancer cell surface negative change is a bio-physical manifestation of the Warburg effect.

4. Potential in Nano Detection of Tumor Cells in Clinical Blood

The nanoprobes described in Fig. 1 provide a new strategy in highly sensitive detection of cancer cells without any biomarkers. In sharp contrast to the current approaches, cell targeting, binding and capturing can be effectively achieved based on the electrical charge differences between the negative cancer cells and the positively-charged magnetic nanoprobes. This novel concept is developed based on a hallmark cancer metabolic pattern: high glycolysis rate. Secretion of high level of lactate acid by cancel cells ultimately results in negative electrical charges on their surfaces, enabling strong binding and capturing by the positively-charged nanoprobes, and subsequent magnetic separation. As shown in Fig. 1, when nanoprobes are incubated with cancer cells in suspension, binding takes place due to charge differences, and cancer cells are then magnetically separated. The separated cells are enumerated using a flow cytometry and identified by pathological and genome sequencing methods. Our preliminary results using the above approach have shown exceptionally high cancer cell capture rates, therefore potentially applicable in cancer cell detection in clinical settings.

One of the clinical implications, as described in Ref. 9, is the detection of circulating tumor cells (CTCs) in blood. CTCs are defined as those that detach from the primary tumor and enter blood therefore responsible for metastasis as they develop secondary lesions.[13,14] As such, there is an increasing need for early detection of circulating tumor cells at a highly sensitive level for both early cancer diagnosis and monitoring disease progression after medical intervention. The current CTC detection technologies rely on immunomagnetically labeled cells or morphological differences between cancer and normal cells, therefore with limitations due to biomarker non-specificity and morphological non-exclusivity. More important, both methods can only achieve capturing of a few CTCs per 7.5. ml of blood sample. This sensitivity level is still too low, and new detection mechanisms need to be identified for much higher detection sensitivity. The charge-based nanoprobe will serve as a highly promising technology for CTC detection.

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Prof. Donglu Shi is currently the Chair and Graduate Director of the Materials Science and Engineering program at University of Cincinnati. His most recent works on nano-biomedicine pioneer several novel approaches in developing multifunctional nano systems that have enabled successful tumor diagnosis and therapeutics. These include drug/gene delivery and release by intelligent triggering mechanisms; magnetic hyperthermia and photothermal ablation of cancer cells for tumor suppression. His current research deals with sensitive detection of cancer cells by surface electrically-charged, fluorescent, and superparamagnetic nanoparticles. Some of his major research has been published in *Advanced Materials, ACS Nano*, and *Biomaterials*. Donglu Shi has been invited to write review articles on cancer diagnosis and therapeutics by *Advanced Materials*, *Small* and *Advanced Functional Materials*. Donglu Shi has published 280 refereed SCI journal publications including *Nature* and *Physical Review letters*, with an *h*-index of 47. He is currently the Editor-in-Chief of *Nano LIFE*, and Associate Editor of *Materials Science & Engineering: C*, and *Journal of Nanomaterials*.