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CANCER COMMUNICATIONS Open Access

EDITORIAL

Lipid droplets: could they be involved in cancer growth and cancer–microenvironment communications?

1 | INTRODUCTION

Common characteristics of tumor cells include the reprogramming of glucose and lipid metabolism, especially the upregulation of glycolysis and lipogenesis accompanied by the accumulation of lipid droplets (LDs) predominantly in aggressive cancer cells and their microenvironment, such as the cancer cells and microenvironment in Zajdela ascites hepatoma (ZAH) [1, 2]. This work analyzed the hypothesis that LD-rich malignant cells gain an advantage for their survival, growth, and immune escape through cancer-derived LDs acting within malignant cells and their microenvironment in a synergistic manner. The effect can be amplified at hypoxia/acidosis in the tumor microenvironment, which stimulates mainly the accumulation of dispersed LDs in tumor cells [3, 4]. Our research, however, found that intracellular LDs and their orderly packed clusters appeared in dividing ZAH cells, whereas surface LD-containing vesicles in ZAH cells acted as a source of extracellular LDs. Could these LDs be involved in cancer unrestricted proliferation and cancer-induced metabolic communications with a microenvironment participating in local immunosuppressive response? Critical analysis of the literature supports a positive answer to these questions and to the proposed hypothesis.

2 | ARE LDs ESSENTIAL FOR CANCER PROLIFERATION?

In contrast to normal cells that use exogenous fatty acids (FAs) for their needs, most cancer cells, including malignant hepatocytes, are capable of synthesizing FAs themselves. The requirement of lipid for proliferating tumor cells is high for several reasons [1–3]. First, lipids represent the building blocks necessary for cell membrane production. Second, newly synthesized FAs can be stored as energy reserves. Third, lipids serve as a precursor for the synthesis of lipid second messengers and a controller for G1-checkpoint during proliferation [5]. However, induction of an impaired G1 arrest in proliferating cancer cells correlates with their ability to accumulate LDs, which are, probably, involved in bypassing this checkpoint and, thus, support further cancer growth [6]. In contrast, Currie *et al.* [7] suggest that increased LD accumulation could reflect a cell response to stress that inhibits cancer cell proliferation.

These opposite views raise important questions. When are LDs and their clusters formed: before, during, or after division of LD-rich cancer cells such as rapidly growing ZAH cells? What intravital methods are best suited for revealing LDs and their intracellular distribution within single living cells? LDs can be visualized using various lipid soluble dyes such as commonly used oil red [2, 3]. However, this is not sufficient for revealing intracellular distribution of LDs, whereas interference microscopy is capable of revealing the specifics of LDs packing more clearly as, e.g., in dividing ZAH cells (Figure 1A). Interference microscopy was popular in the 1960s as a way of color visualization of intracellular organelles, whose refractive indices differ [8]. Lipids have higher refractive indices as compared with the cytoplasm/nucleus; this property makes it possible to visualize unstained LDs in color and to study their packing into clusters. Herewith, the image of the cell should be split. As a result, a double image of the cell and its LDs is produced (Figure 1A, the arrow points to yellow LDs, their copy is green). Probably, for this reason, the method is not used nowadays. However, it was this method that made it possible to find triangle-like LD-containing structures in dividing ZAH cells. They were positioned symmetrically in daughter cells (Figure 1A); after lipid-dissolving fixation and staining with acridine orange, these structures were no longer observed (Figure 1B). These findings confirm that an increased number of LDs emerges in dividing ZAH cells and does not lead to the arrest of their proliferation. Moreover, both daugh-

Abbreviations: LD, lipid droplet; ZAH, Zajdela ascites hepatoma; FA, fatty acid; MDSC, myeloid-derived suppressor cell; TAM, tumor-associated macrophage; Treg, T regulatory lymphocyte; G-CSF, granulocyte-colony stimulating factor

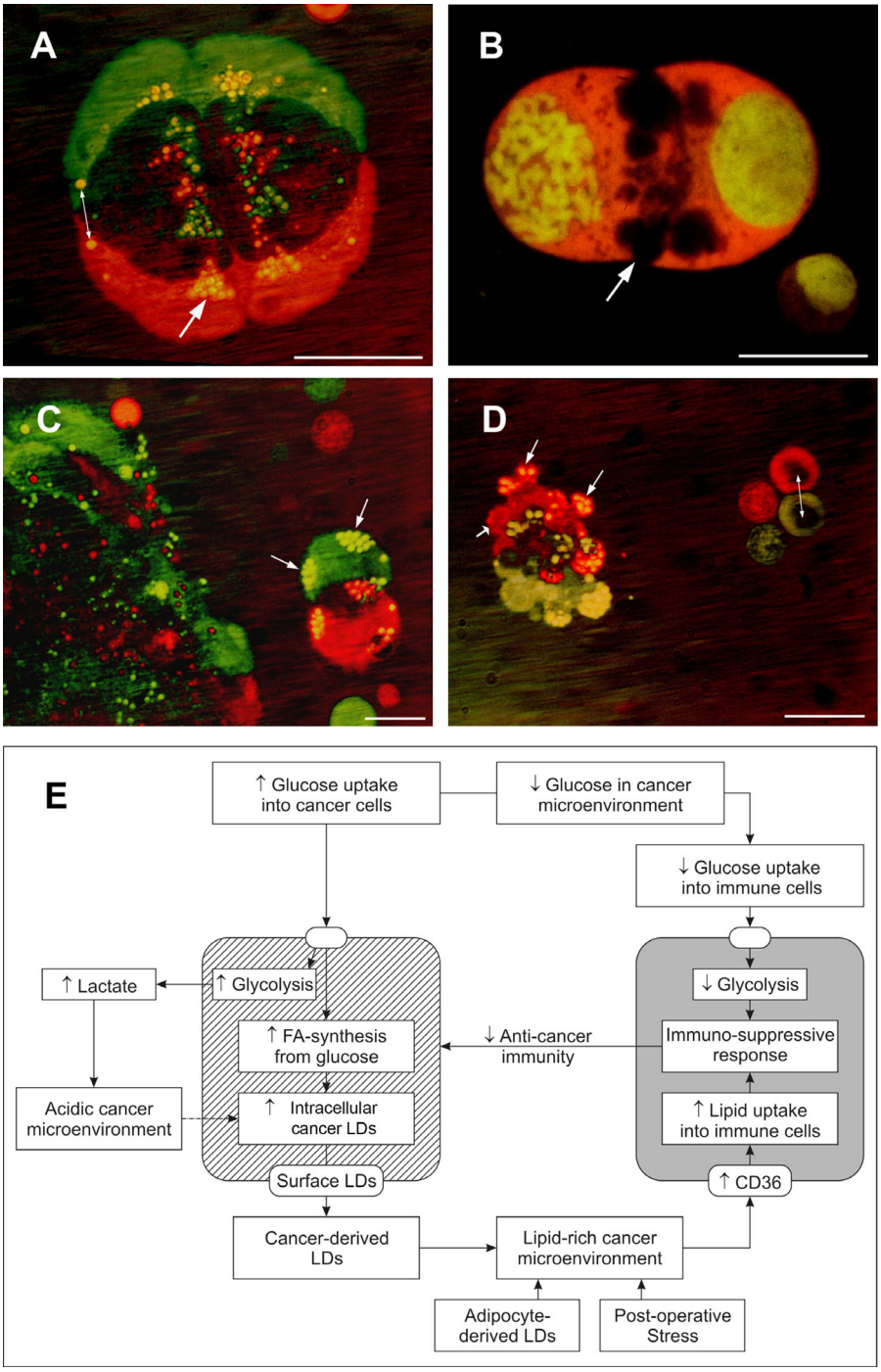

FIGURE 1 An illustration (micrographs A–D) of how cancer-derived LDs and their clusters participate in proliferation of ZAH cells (A–B), in communications between cancer cells and their microenvironment (C–D), and of how these effects can be coordinated (scheme E). (A–B) Comparison of interference (A) and fluorescence (B) microscopic images of LD-containing clusters in a dividing ZAH cell. The location of LD-containing clusters before (A) and after (B) lipid-dissolving standard fixation and acridine orange staining are shown. LD-containing clusters are of unexpected triangle-like form (A, arrow). Groups of clusters were disposed on both sides of the cell division line; after fixation, however, they were removed as a whole interconnected structure (B, arrow to black regions). Scale bar: 10 μm. (C–D) The appearance of LD groups in the form of separate cap-like clusters on a ZAH cell surface (C, arrows). Scale bar: 10 μm. This type of LD clustering, probably, acts as the basis for further formation of different cell surface vesicles containing unstained LDs (D, long arrows) or without LDs (D, short arrow). Then these LD-containing vesicles can be released to ascitic fluid to support immunosuppressive conditions. Double-headed arrows (A and D) indicate the magnitude of cell splitting by interference microscopy. (E) Metabolic model of glucoselipid feedback interactions between cancer cells, their microenvironment and immune cells. Cancer-derived LDs can be involved in the creation of a lipid-enriched microenvironment by which the anticancer immune response is reduced. Abbreviations: LD, lipid droplet; ZAH, Zajdela ascites hepatoma

ter cells before their separation into two new ZAH cells (Figure 1B) acquire LDs that are assembled into LD clusters of an unusual form (Figure 1A). An earlier work has described polarization of LDs during NIH 3T3 cell division in vitro [6]. However, LD-containing clusters have not been found, probably because ZAH cells grow in more acidic conditions. It has been noted that extracellular acidosis can increase the size and number of LDs, but only in transformed (not normal) cells; therefore, prevention of acidosis-stimulating formation of LDs is a promising target of limiting the growth and invasion of tumor cells [3].

Although it is well established that cancer proliferation is followed by metabolic deregulation of cellular gene expression, little is known about which lipid-related factors contribute to metabolic deregulation and communication by cancer proliferation. Recently, it has been found that cancer cell proliferation is co-supported with oncogene (squalene epoxidase, a key enzyme in cholesterol synthesis) and LDs [9]. This observation relates well with the finding that increased LDs and their clusters (Figure 1A) form during cancer proliferation.

3 | CAN CANCER CONTROL MICROENVIRONMENTAL ALTERATIONS AND IMMUNITY BY EXTRACELLULAR LIPIDS?

In most cases, aggressive cancer cells containing an increased number of LDs [2] were also found in a cancer microenvironment frequently characterized by low pH and oxygen [3]. These metabolic changes are typical of ascitic fluid in which ZAH cells grow in the rat peritoneal cavity. What is the source of these LDs in a cancer microenvironment? Can cancer LD-containing surface vesicles be responsible for accumulation of extracellular LDs in acidosis-driven conditions? To answer these questions, a specific distribution of LD clusters in different ZAH cells was investigated. Considerable attention has been given to the study of the assumption that acidosis-adapted cancer cells can alter the intracellular movement of cytoplasmic LDs to the cell surface [10]. Our investigation observed the emergence of ZAH cells that had surface LD clusters (Figure 1C), as well as surface vesicles containing LDs (Figure 1D, two long arrows) or without LDs (Figure 1D, short arrow). This suggests that some aggressive cancer cells and their surface LD-containing vesicles are a key contributor of LDs in hepatoma ascitic fluid. As a result, a cancer microenvironment becomes rich with lipid/LDs that may reprogram the immunometabolism and give rise to an immunosuppressive response in different immune cells, such as dendritic cells, myeloid-derived suppressor cells (MDSCs), tumor-associated macrophages (TAMs), and T regulatory lymphocytes (Tregs). Certain examples can be given to support this assumption.

One such example is that dendritic cells lose their capability of antigen cross-presentation after high-LD accumulation and in this way reduce the recruitment of interferon-secreting T cells to tumor cells, which increases their survival [11]. Another example is that MDSCs promote tumor growth by blocking the antitumor T cell response. It has been found that tumor-derived cytokines (such as granulocyte-colony stimulating factor [G-CSF] and granulocyte-macrophage colony-stimulating factor) are responsible for an increased expression of lipid transport receptors in MDSCs. As a result, an increased lipid uptake from the tumor microenvironment into MDSCs is observed. Intracellular accumulation of lipids increases the oxidative metabolism and activates the immunosuppressive pathways in MDSCs [12]. A similar effect is demonstrated by TAMs that are enriched with lipids in a tumor environment and acquire immunosuppressive properties [13], because TAMs express elevated levels of lipid transport receptors supporting LD formation [14]. In other words, lipid-dependent fatty acid metabolism controls the immunosuppressive phenotype of TAMs. Therefore, inhibition of lipid uptake and LD formation by a specific inhibitor of fatty acid synthase C75 [14] and/or degradation of LDs enables inhibiting the suppressive function of TAMs [15]. Moreover, systemic immune changes were prevented by G-CSF blockade, revealing a remarkable plasticity in the systemic immune state [16].

$CD8^+$ T cells are a master effector of antitumor immunity, and their presence at tumor sites has to do with favourable outcomes, whereas Treg cells are described as a potent suppressor of effector cells. Under a tumor microenvironment containing increased lipid/LDs, an anticancer dysfunction of $CD8^+$ T cells [17] and activation of Treg cells [18] have been observed.

Recent reports confirm the important role of LDs as a hub for different immune suppressive cytokines, and enzymes like cyclooxygenase-2 [19] preferentially give rise to a tolerogenic response in a cancer microenvironment. Altogether, these data suggest that similar lipid-reprogramming pathways are activated in different immunocytes, which may act synergistically to suppress anticancer immunity in a lipid-rich microenvironment. Figure 1E illustrates this effect.

4 | CONCLUSIONS AND PERSPECTIVES

The metabolism of cancer cells is reprogrammed to support their proliferation and to restrict a host ability to generate an effective anti-immune response. These metabolic alterations include lipogenesis that gives rise to LD accu-

mulation, especially in aggressive cancer cells such as ZAH cells. This work revealed an increased formation of LDs and their clusters (Figure 1A) during the proliferation of ZAH cells. It is not known how LDs act under proliferation. Probably they serve as a lipid hub, supporting the survival of cancer cells under stress conditions. One of the most puzzling features of LDs is their ability to form unexpected ordered clusters in dividing ZAH cells, as discovered in this work (Figure 1A). Further studies are required to reveal the mechanism responsible for the formation of dispersed/clustered LDs and to use them subsequently in developing anticancer therapies.

An important remaining question is how the cancer metabolism can modify its microenvironment in which cancer cells gain an ability to avoid detection and destruction from anticancer immune responses. The results of this work support the hypothesis that not only adipocytes or surgery-induced stress [20] but also cancer-derived LDs can be a source of extracellular LDs, whose FAs are often used as a fuel or signaling molecules in different immune cells. The main consequences of such metabolic changes could give rise to activated oxidation of FAs, reduced glycolysis, and increased lipid uptake, which can serve as the basis for a suggestion that inhibition of FA uptake by immune cells and/or accumulation of FAs in the form of LDs can decrease the immunosuppressive response in a tumor microenvironment.

This suggestion is supported by the selective pharmacological inhibition of upregulated fatty acid transport protein 2 that abrogates the activity of MDSCs and substantially delays the progression of cancer in mice [21]. Although other possibilities to decrease the lipid-mediated immunosuppression have been analyzed in a recent publication, the important questions remain open. Do LDs play a causal or communication role or are a consequence of tumorigenesis? Could cancer-derived LDs be a target(s) for anticancer clinical therapies? What are the effective pathways for its correction? Answering these questions will provide a critical insight into the importance of LDs and their clusters for metabolic communications between cancer cells, their environment and immune cells. Further studies are needed to confirm this intriguing possibility in other cancers and to search for potential targets for anticancer treatment.

DECLARATIONS

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

All data generated or analyzed during this study are included in this published article and its supplementary information file.

COMPETING INTERESTS

The author declares that she has no competing interests.

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AUTHOR'S CONTRIBUTIONS

P.S. was the sole contributor to this article. The author designed the concept, wrote the paper, and prepared the manuscript and the figure.

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