Published in final edited form as:

Free Radic Biol Med. 2015 February ; 0: 264–268. doi:10.1016/j.freeradbiomed.2014.10.732.

The Metabolic State of Cancer Stem Cells – A Valid Target for Cancer Therapy?

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Abstract

In the 1920s Otto Warburg first described high glucose uptake, aerobic glycolysis and high lactate production in tumors. Since then high glucose uptake has been utilized in the development of PET imaging for cancer. However, despite a deepened understanding of the molecular underpinnings of glucose metabolism in cancer, this fundamental difference between normal and malignant tissue has yet to be employed in targeted cancer therapy in the clinic. In this review, we highlight attempts in the recent literature to target cancer cell metabolism and elaborate on the challenges and controversies of these strategies in general, and in the context of tumor cell heterogeneity in cancer.

Keywords

Cancer stem cells; radiation; glycolysis; oxidative phosphorylation

Introduction

The successful application of any anti-cancer therapy in the clinic has its basis on the existence of a “therapeutic window” allowing for normal tissue survival while efficiently targeting cancer cells. This principal is most successfully applied in cancer surgery techniques aiming at craftily sparing normal tissues while excising cancerous tissues as efficiently as possible. Radiation therapy applies the same principle by carefully designing and planning the radiation treatment to deliver radiation doses in a manner that maximizes the dose to the tumor while minimizing the exposure of normal tissue to radiation. Additionally, radiation treatment is generally applied in fractions, taking into account the difference in repair kinetics between normal tissue and cancer, with normal tissue being more efficient at repairing sublethal damage from radiation exposure. Consequently, surgery and radiotherapy are curative treatments for a significant number of patients with localized

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disease. These same principles cannot easily be applied to systemic chemotherapy, which in contrast to surgery and radiation, when used alone cures only very few patients from cancer. It should be emphasized however, that the main reason for cancer-related fatalities is the metastatic progression of the disease, much more challenging to control by surgery or radiation. Therefore, systemic therapies are crucial for the treatment of a large number of patients with systemic disease. Targeted therapies aim to make use of specific molecular features of cancer, which are absent or present in much lower levels in normal tissue. Some targeted therapies approved for clinical use have shown great promise (Bonner et al., 2006; Chapman et al., 2011) but require careful and sometimes costly selection of patient subpopulations.

While key molecular differences between cancer and normal cells have been successfully exploited for targeted cancer therapies, one of the more common differences between cancers and normal tissues, that has yet to be exploited, are the metabolic pathways used for energy production. As it will be elaborated below, normal tissues use oxidation of glucose in the mitochondria for generating ATP, while tumor cells in general are thought to rely more on glycolysis for energy production even in the presence of sufficient levels of oxygen. The general reliance on “aerobic glycolysis”, a seemingly common characteristic of cancer cells, has been actively investigated as a potential Achilles’ heel of cancer since its discovery by Otto Warburg in 1923 (Warburg, 1923). This review will emphasize on differences in metabolic pathways between normal tissues and cancers with an emphasis on tumor heterogeneity and explore the potential of interfering with metabolic pathways as a viable anti-cancer treatment approach.

Cancer Stem Cells

The heterogeneous nature of cell populations within a tumor has been recognized for decades and can either be interpreted as the result of clonal evolution or a hierarchic organization of cancers. While the model of clonal evolution attributes the ability to form or repopulate a tumor and to seed metastases to any given viable cell in a tumor (Nowell, 1976), the cancer stem cell hypothesis limits these features to a small number of cancer stem cells (CSCs) at the apex of the hierarchy of cell populations (Reya et al., 2001). The hierarchical organization of tumors was first recognized by Rudolf Virchow in 1855, when he concluded that ‘*Omnis cellula e cellula*’ (Virchow, 1855) (“every cell originates from another existing cell like it”), and in Julius Friedrich Cohnheim’s case report of a sarcoma of the kidney in 1875 (Cohnheim, 1875). After the prospective identification of CSCs in leukemia for the first time in 1994 (Lapidot et al., 1994), the CSC hypothesis has experienced a renaissance and the search for novel drugs that target CSCs specifically, has turned into a very active area of research with some initial success (Gupta et al., 2009). It took another 10 years to prospectively identify CSCs in different solid cancers including breast cancer (Al-Hajj et al., 2003; Ginestier et al., 2007; Vlashi et al., 2013), brain tumors (Hemmati et al., 2003; Singh et al., 2003; Vlashi et al., 2009), prostate cancer (Collins et al., 2005), lung cancer (Eramo et al., 2007), colorectal cancer (Ricci-Vitiani et al., 2007), head and neck squamous cell carcinoma (Lagadec et al., 2014; Prince, 2007), melanoma (Schatton et al., 2008) and many others. The strength of the experimental evidence for the

existence of CSCs in these various tumor entities varies and is strongest in breast cancer and glioma.

The CSC hypothesis has been challenged by experimental evidence brought forth by laboratories questioning the hierarchical organization of cancers, especially in the context of metastatic melanoma (Quintana et al., 2008). Adding to the debate regarding the hierarchical organization of tumors is recent evidence demonstrating a remarkable plasticity of cancer cell populations (Chaffer et al., 2011; Lagadec et al., 2012; Liu et al., 2014b; Salmina et al., 2010). While such phenotypic and functional plasticity of cancer cells might allow only for snapshots of tumor cell heterogeneity in time, the CSC hypothesis has taught us about the presence of subpopulations of cancer cells in tumors that can withstand conventional chemotherapeutic agents and radiation therapy (Bao, 2006; Phillips et al., 2006; Woodward et al., 2007). Such resistance is largely attributed to the acquired ability of cancer stem cells to deal with the insult, such as pumping out or detoxifying chemotherapeutic drugs or, in the case of radiation therapy (RT) efficiently repairing DNA damage (Bao et al., 2006), or increased expression of free radical scavengers (Phillips et al., 2006). However, recent evidence from our laboratory, and others, points to an alternative reason for treatment resistance: the surviving, differentiated cells have acquired the ability to reprogram into treatment-resistant CSCs, thus contributing to tumor recurrence (Ghisolfi et al., 2012; Lagadec et al., 2012).

The Warburg effect

In the 1920s Otto Warburg published a series of scientific articles describing a specific metabolic pattern for tumor tissues consisting of a significant difference in lactate production and glucose uptake rates when compared to normal tissues (Warburg, 1923, 1925; Warburg et al., 1924; Warburg et al., 1927). The most intriguing observation made by Warburg was the persistence of lactate fermentation by cancer cells even in the presence of adequate oxygen levels, later termed the 'Warburg effect' (Racker and Spector, 1981). Warburg's seminal papers would later lead to the development of positron emission tomography in the 1970s (Ter-Pogossian et al., 1975) based on the increased glucose uptake of tumor tissue. More recently the field of tumor metabolics has merged with the field of cancer cell biology, as it becomes increasingly clear that oncogenic signaling pathways play a crucial role in tumor metabolism (Vander Heiden et al., 2009). Such advancements have sparked interest in the underlying molecular mechanisms raising hope that glucose metabolism could be a druggable target in cancer. Although today, the reliance of tumors on aerobic glycolysis is a widely accepted phenomenon, the experiments leading to this understanding have not gone without contestation. The original Warburg's observations were interpreted as indicating that the function of the mitochondria is defective, and this assumption was first challenged by Weinhouse in 1956 (Weinhouse, 1956). However, we now understand that cancer cells, although in an altered metabolic state have functional mitochondria. In a more recent study, Zu and Guppy compiled data from metabolic studies published over a period of 40 years and could not find evidence indicating a more glycolytic phenotype of cancers when compared to the corresponding normal tissue (Zu and Guppy, 2004). Instead, both normal tissues and cancers were found to retrieve only about 20% of their ATP production from glycolysis. The authors conclude that many of the studies aiming

to assess the metabolic state of cancer cells were not performed under physiological conditions matching the tumor microenvironment and that instead hypoxia, which is found in most tumors, drives aerobic glycolysis in tumor cells. However, if the Warburg effect was simply a Pasteur effect (lactate production occurring exclusively during oxygen deprivation) due to a hypoxic tumor microenvironment, then reoxygenation of hypoxic tumors should result in reversion of the Warburg effect, and should improve patient survival, something that is not observed in the clinic.

Nevertheless, dividing cells not only need energy but also building blocks for macromolecule synthesis to allow for doubling the cellular mass in support of rapid cell divisions. Fundamental work by Christofk et al. unveiled that cancers use the M2 splice isoform of pyruvate kinase to bypass mitochondrial processing of carbon sources (Christofk et al., 2008a; Christofk et al., 2008b), thus channeling carbon-3 bodies into the biosynthesis of proteins and lipids, maintaining high glucose influx, and high glutathione levels (Vander Heiden et al., 2009).

The metabolic state of cancer stem cells

As highlighted above tumors are composed of heterogeneous populations of cells, a fact that is not taken into account by most studies addressing the metabolic state of tumors. With respect to the metabolic state and tumor cell heterogeneity it is perhaps irrelevant whether cancers follow the stochastic model of clonal evolution or if they are organized hierarchically. However, the different stages of cellular activity, dormant versus dividing, hypoxic versus normoxic, senescence versus quiescence, have substantially different metabolic requirements that could result in substantially different responses to metabolic targeting. In general, CSCs are considered quiescent or at least slow-cycling. In breast cancer, about 25 % of CSCs were found to be quiescent and the remaining 75 % cycled very slowly when compared to their differentiated progeny (Lagadec et al., 2009). Conversely, after exposure to ionizing radiation a large number of non-stem cells died or stopped cycling and entered a senescent state, while at the same time the quiescent CSC population was recruited into the cell cycle (Lagadec et al., 2009). Similar results were found in glioblastoma (Vlashi et al., 2009). Very few studies have investigated the metabolic state of CSCs directly. The first study investigating the metabolism of glioma CSCs and non-stem cells found that in agreement with their more quiescent/slow cycling phenotype, CSCs from low passage patient-derived glioblastoma specimen relied more on oxidative phosphorylation while their differentiated progeny generated energy mainly through aerobic glycolysis (Vlashi et al., 2011). However, when oxidative phosphorylation was blocked, glioma stem cells could readily switch to a glycolytic metabolism. In agreement with this study, Flavahan and coworkers reported that glioma stem cells could adapt to starvation by upregulating a high-affinity glucose transporter to outcompete their progeny and surrounding normal brain cells (Flavahan et al., 2013). Furthermore, a study by Craig Jordan's laboratory using patient-derived samples found quiescent leukemia stem cells to also rely primarily on oxidative phosphorylation (Lagadinou et al., 2013).

In contrary, after studying established cell lines cultured at high glucose and oxygen levels for decades, Palorini et al. reported that CSC in a osteosarcoma cell line had a glycolytic

phenotype (Palorini et al., 2014) and the same result was communicated for side population cells in the non-small cell lung cancer cell line A549 (Liu et al., 2014a). In agreement with these findings, a recent study also demonstrated that breast cancer stem cells in mouse and human tumors have a more glycolytic phenotype compared to their differentiated progeny (Feng et al., 2014). However, contradictory findings were recently reported for breast cancer stem cells exhibiting a reliance on oxidative phosphorylation when compared to the differentiated progeny (Vlashi et al., 2014).

While the number of studies investigating the metabolic requirements of different subpopulations of cells within a tumor type is rather limited, existing data suggests that CSCs from low-passage, patient-derived specimen in glioma and leukemia favor oxidative phosphorylation as their primary source of energy production, while data on breast cancer stem cells is contradictory. It is worth noting that when established cell lines, which have been adapted to high non-physiological glucose levels in growth media, are used for metabolic studies in cancer the glycolytic phenotype predominates.

The metabolic state of cancer stem cells as a therapeutic target

Metabolic targeting of cancer has become an area of increasing interest. However, as it has become clear for other targeted cancer therapies tumor heterogeneity determines the efficiency and overall success of a specific targeted drug. For this reason, patient stratification and personalized cancer medicine is becoming the *modus operandi* in the enormous effort to discover new anti-cancer drugs. The same appears to hold true in the field of cancer metabolics: a single metabolic pathway, such aerobic glycolysis is unlikely to be the next ‘Achilles heel’ of cancer. Despite the number of studies investigating the metabolic state of CSCs being small, the current data strongly suggest that the CSC population of a tumor resides in a distinct metabolic state compared to the rest of the cancer cells in the same tumor. The complete picture of the metabolic state of a tumor is likely far more complex due to heterogeneity arising not only from the hierarchical organization of tumors, but also from the heterogeneous tumor microenvironment.

One of the drugs studied extensively in the context of cancer is metformin. Its proposed mechanisms of action are incompletely understood and complex. Its major anti-diabetic effect results from down-regulation of gluconeogenesis in the liver. However, direct effects of metformin on cancer cells are thought to be exerted through inhibition of the mTOR pathway with consecutive inhibition of proliferation, thus raising the question if metformin can be effectively combined with other anti-cancer treatments that rely on proliferation of cancer cells. Nonetheless, as summarized below, some studies utilizing metabolic inhibitors to interfere with the metabolic state of CSCs have resulted in intriguing results *in vitro*. However, it is not clear whether these results would hold true when one considers the *in vivo* heterogeneity of tumors. An early study on established breast cancer cell lines used the anti-diabetic drug metformin and demonstrated partial suppression of primary mammosphere formation and *in vivo* growth delay in drug-treated samples (Huang et al., 2005). A proposed mechanism is the metformin-dependent suppression of key-regulators of the epithelial-to-mesenchymal transition (EMT) leading to loss of the CSC population (Pandya et al., 2004). This study only investigated established basal breast cancer cell lines using the expression of

surface molecules CD24^{low}/CD44^{high} for identification of CSCs. However, this marker combination has been shown to be inadequate in isolating breast cancer stem cells for the cell lines used in these studies (Fillmore and Kuperwasser, 2008). This marker combination is expressed in 80 – 95 % of the cells in these lines while the estimated frequency of CSCs is magnitudes lower. In contrary, a study by Oliveras-Ferraro et al. (Oliveras-Ferraro et al., 2010) used the luminal breast cancer line, MCF-7 and demonstrated adaptation of the cells to chronic exposure to metformin and acquisition of a gene expression profile that indicated the acquisition of a CSC state by large portions of the cell population. However, this study did not confirm the CSCs phenotype by operational means (Oliveras-Ferraro et al., 2010). In a different study by Jung et al. the number of MCF-7 mammospheres and their size was decreased after metformin treatment, accompanied by a down-regulation of Oct 4 expression (Jung et al., 2011). This study made the assumption that all cells in primary 3D mammospheres are breast cancer stem cells even though it is known that mammosphere cultures consist of a mixture of cells in different stages of differentiation (Ponti et al., 2005). Therefore the observed decreases in mammospheres and Oct 4 gene expression in the studies by Jung et al. are unlikely to only reflect changes in the CSC population.

It is worth to also note that the concentrations of metformin used to observe an anticancer effect in the above-described studies ranged from 1 to 10mM. Plasma concentrations that can be achieved in humans are in the low micromolar range (Scheen, 1996). Importantly, attempts to increase plasma concentrations of metformin to achieve concentrations effective against tumors is unlikely to be clinically tolerable due to lactic acidosis (Graham et al., 2011).

Still, as we are writing this review more than 200 clinical trials on metformin and cancer have been registered with the NIH. The rationale for the use of metformin comes from the observation that type 2 diabetes is associated with an increased risk for breast, colon, prostate, kidney and pancreatic cancer (Giovannucci et al., 2010) and an initial cohort study on 12,000 diabetic patients showing a reduced cancer risk in patients treated with metformin (Evans et al., 2005). However, a more recent larger cohort study on 95,820 patients with type 2 diabetes studying the effect of metformin on cancer risk did not show a decreased risk in the metformin group (Tsilidis et al., 2014). So far, no large prospective phase III trial has reported outcomes for non-diabetic cancer patients treated with metformin. Some early phase I studies claim a beneficial effect of metformin based on differences in the apoptotic index of pretreatment biopsies and surgical specimen obtained after metformin treatment. However, the average increase in the number of apoptotic cells is in the single digit range, at best (Cazzaniga et al., 2013; Kalinsky et al., 2014) and thus, marginal when compared to the effect of a single 2 Gy fraction of radiation. In essence, the current body of literature does not support the idea that addition of metformin will lead to a major break through in cancer therapy.

Concluding remarks

Cancer metabolism is experiencing a renaissance in an attempt to develop novel targeted cancer therapies. Since its initial description by Warburg, we have developed a sophisticated understanding of the genetic, epigenetic, and metabolic alterations in cancers and their inter

dependence with the tumor microenvironment. The tumor cell heterogeneity of most solid cancers and the dynamic equilibrium between different cell populations further complicates an already complex interplay of pathways that underlie the malignant phenotype of cells. Targeting a single pathway in bulk tumor populations, whether it is driven by a receptor tyrosine kinase or a metabolic enzyme is unlikely to be the next ‘magic bullet’ against cancer. However, careful patient selection (Davis et al., 2014; Iglesias et al., 2013) and integration of metabolic inhibitors into biology-driven novel treatment concepts against cancer that do not ignore tumor cell heterogeneity could hold the key to widen therapeutic windows of existing therapies.

Acknowledgments

Grant Support

FP was supported by a generous gift from Steve and Cathy Fink and by grants from the *National Cancer Institute* (1R01CA137110, 1R01CA161294) and the Army Medical Research & Materiel Command’s Breast Cancer Research Program (W81XWH-11-1-0531).

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Highlights

Bulk tumor cell populations rely on aerobic glycolysis

Cancer stem cells are in a specific metabolic state

Cancer stem cells in breast cancer, glioblastoma and leukemia rely on oxidative phosphorylation of glucose