Higher Sensitivity to the Anti-Metabolic Agent Shikonin and Enhanced Bioenergetics of Ovarian Cancer Cells are Associated with Upregulation of Succinate Dehydrogenase

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Abstract

We found that the highly metabolically active phenotype is promoted by the overexpression of the mitochondrial enzyme Succinate Dehydrogenase (SDHA), which is particularly common in ovarian cancer. Prior research has examined succinate dehydrogenase insufficiency in a few uncommon diseases. There is still a need for more study because it has never been done how SDHA overexpression affects ovarian cancer metabolism. We looked into the effects of SDHA overexpression in ovarian cancer on its functional properties. We investigated the protein content of the metabolic pathways, cell proliferation, anchorage-independent growth, mitochondrial respiration, glycolytic activity, and ATP production rates in those cells using proteomics techniques and biological experiments. Last but not least, we ran a pharmacological screening to find medicines that selectively target tumor cells that overexpress SDHA. We demonstrated that cells overexpressing SDHA have improved energy metabolism and rely on both glycolysis and oxidative phosphorylation to provide the energy they require. Additionally, cells with a high SDHA phenotype were more susceptible to glucose and glutamine deprivation, which significantly decreased ATP output. We also discovered the anti-metabolic substance shikonin, which exhibits strong activity against ovarian cancer cells overexpressing SDHA. The findings highlight the underappreciated function of SDHA in the metabolic reprogramming of ovarian cancer, opening up new treatment possibilities.

Keywords: Ovarian cancer • Succinate dehydrogenase • SDHA • Shikonin

Introduction

Cancer is characterized by the reprogramming of cellular metabolism, which enables tumor cells to live and grow. The finding that tumor cells preferentially utilize glucose via the glycolysis route over Oxidative Phosphorylation (OXPHOS) in mitochondria for energy production is known as the Warburg effect, and it is a recognized marker of cancer metabolism. Previous studies have linked damaged mitochondria to cancer cells need on glycolysis. Recent research, however, has shown that many tumor forms have functional mitochondria and can obtain their energy needs not just from glycolysis but also through OXPHOS. For instance, it has been demonstrated that fractions of ovarian tumors with traits similar to cancer stem cells or with a metastatic phenotype are highly metabolically active and have enhanced mitochondrial respiration. The discovery of new treatments can be facilitated by a better understanding of the metabolic requirements of cancer cells.

We examined the metabolic characteristics of Patient-Derived Xenografts (PDXs) made from patients ovarian tumors to acquire more understanding of the ovarian cancer metabolism. We employed proteomic methods to find that increased mitochondrial metabolism in ovarian PDX models is connected to the overexpression of Succinate Dehydrogenase (SDHA) subunit A. Compared to other tumor types, ovarian carcinoma patients have a significantly higher incidence of the SDHA gene amplification or overexpression (19% of all cases), which suggests that it may be important in the reprogramming of ovarian cancer metabolism.

Succinate Dehydrogenase (SDH), sometimes referred to as mitochondrial complex II, combines the reduction of ubiquinone to ubiquinol with the oxidation of succinate to fumarate, directly linking the Tricarboxylic Acid (TCA) cycle to the mitochondrial Electron Transport Chain (ETC). The catalytic core of SDH is made up of the four subunits SDHA flavoprotein, SDHB iron-sulfur protein, SDHC and SDHD proteins, which together bind the entire protein complex to the inner mitochondrial membrane. It has been demonstrated that the succinate dehydrogenase complex's proper assembly is essential for its operation.

SDH subunit genetic mutations cause severe assembly errors that can result in a variety of clinical diseases, such as neurodevelopmental abnormalities or some uncommon cancers. Previous research established a link between succinate buildup and the process of tumor formation in people with succinate dehydrogenase impairment. Succinate in excess inhibits HIF1's prolyl hydroxylase activity, stabilizing HIF-1 and increasing the transcription of HIF1-regulated genes that promote cancer. Additionally, a free SDHA flavoprotein accumulates often in the mitochondrial matrix as a result of faulty complex II assembly. While SDHA subunit amplification or overexpressions are relatively common in this tumor type, mutations in SDH subunits are extremely uncommon in ovarian cancer. Various in vitro and in vivo ovarian cancer models were used in this study to examine the biological effects of SDHA overexpression and its bearing on tumor phenotype. According to the research, SDHA overexpression is linked to cells enhanced capacity for colony formation and survival in anchorage-independent circumstances. This is a crucial characteristic of ovarian cancer cells that have metastatic potential and can live and proliferate in peritoneal fluid (ascites) within the abdominal cavity. Furthermore, we demonstrated that higher mitochondrial respiration and ATP generation rates were indicators of improved ovarian cancer metabolism caused by SDHA overexpression. Last but not least, we conducted a pharmacological screening and discovered the anti-metabolic substance shikonin, which is known to interfere with glycolysis and amino acid metabolism.

We demonstrated that shikonin had greater anti-tumor effectiveness and selectivity towards tumor cells that overexpressed SDHA in vitro compared to what was shown with conventional chemotherapy.

All told, the research showed that SDHA overexpression frequently happens in ovarian cancer and plays a role in the reprogramming of energy metabolism toward a phenotype that is highly metabolically active. Importantly, we demonstrated here that anti-metabolic substances like shikonin can efficiently target SDHA overexpressed tumor cells, opening up a new window for therapeutic intervention in ovarian cancer.

Discussion

One crucial mechanism of cancer is the reprogramming of cellular metabolism. Cancer cells' metabolic plasticity enables them to sustain unchecked cell proliferation and adapt to a variety of unfavorable micro environmental settings, which promotes tumor development and metastasis. In this work, a critical protein linked to an increased mitochondrial metabolism in ovarian cancers was identified as succinate dehydrogenase SDHA, a mitochondrial enzyme.

The SDHA gene amplification is quite common in ovarian cancer and has been observed in ovarian tumors at a frequency that is significantly higher than in many other malignancies (TCGA data), suggesting a potential role in the metabolic reprogramming of ovarian cancer. Succinate dehydrogenase has previously been investigated in relation to its deficit in a number of uncommon illnesses and cancers. There aren't many SDHA gain-of-function studies, though. Increased mitochondrial respiration and fumarate buildup driving pathological metabolism in some disorders have been linked to increased SDHA function. For instance, the SDHA gain-of-function phenotype causes lymphocytes B to be reprogrammed to become inflammatory, which promotes systemic inflammation and makes the disease more severe in individuals with primary antibody deficiency syndrome. Elevated SDHA contributes to a metabolic dysregulation with enhanced mitochondrial respiration in metastatic uveal melanoma, which results in resistance to therapy and a much shorter period until metastasis and patient death.

The preliminary findings in the current study suggested a potential role for SDHA overexpression in ovarian cancer and laid the groundwork for future, more thorough research. First, we looked at the expression of SDHA in a variety of healthy fallopian tubes and HGSOC PDX models, and the results revealed that the levels of SDHA protein are much lower in healthy fallopian tubes than in PDX tumors. Furthermore, we noticed that established ovarian cancer cell lines and ovarian PDXs display a variety of SDHA expressions. Importantly, the percentage (19%) of ovarian cancers in the patient population that carry SDHA amplification and/or overexpression matches the percentage (23.5%) of SDHA-high PDX tumors in the data collection.

Next, we functionally validated our ovarian cancer models and demonstrated that, in contrast to SDHA knockdown, conditional overexpression of SDHA greatly boosted succinate dehydrogenase enzyme activity in ovarian cancer cell lines. Additionally, fumarate buildup was connected to the cells' elevated succinate dehydrogenase activity. There have also been reports of fumarate buildup in cells with increased SDHA in other illnesses. For instance, it is found that patients with primary antibody deficiency syndrome have elevated fumarate levels in lymphocytes B with SDHA gain-of-function phenotype. We examined the impact of SDHA overexpression on cell proliferation and in vivo tumor formation in order to assess the biological roles of SDHA in ovarian cancer. The in vitro investigations showed that in some cell lines, SDHA overexpression is linked to a reduction in cell proliferation, whilst in other cell lines, the proliferation of cells is only slightly influenced. In vivo studies indicated that mice bearing the OVCAR3 tumor model had a propensity for slower tumor growth. In vitro cell proliferation was decreased by the overexpression of SDHA in renal cancer cells, while in vivo tumor growth was suppressed in a nude mice model. According to many studies, multiple myeloma cell lines in vitro cell growth and invasion were suppressed by elevated SDHA expression.

Additionally, in anchorage-independent settings, SDHA overexpression is linked to the production of noticeably more and larger cancer cell colonies. It has been demonstrated that less tumorigenic or normal cells go through growth inhibition and/or apoptosis, but aggressive and metastatic tumor cells can survive and spread quickly in the lack of anchoring to the extracellular matrix. These findings show that, despite reduced cell proliferation caused by SDHA overexpression, these cells are better able to survive and form colonies in suspension within a semi-solid matrix. This is extremely important to the biology of ovarian cancer because this particular tumor type produces huge volumes of ascitic fluid in the peritoneal cavity, which contains floating cancer cells that must develop the capacity to survive, multiply, and spread in order to advance the disease.

Similar to this, it is observed that multiple myeloma cells overexpressing SDHA are less invasive than control cells. Based on this body of research, it was hypothesized that SDHA overexpression may enhance cell survival and proliferation under anchorage-independent circumstances, in contrast to tumor cells with low SDHA levels, which more effectively divide, migrate, and invade adherent cell cultures.

The goal of the current study was to compare the bioenergetics profiles of ovarian cancer cell lines overexpressing SDHA to those of their corresponding SDHA-low counterparts.

The bioenergetic requirements of the cell lines varied significantly, which was related to the SDHA overexpression status. The mitochondrial respiration and maximal and reserve glycolytic capacity were both markedly increased in the cell lines overexpressing SDHA. The SDHA overexpression cells had a phenotype that was extremely metabolically active, as evidenced by a markedly higher total ATP output. Others have previously described metabolic flexibility and variation in bioenergetic profiles in ovarian cancer and other tumor types. Chemotherapy causes metabolic alterations in ovarian cancer cells, giving them the metabolic flexibility they need to withstand a chemotherapy assault. Other investigations have discovered a subset of ovarian cancer cells that are extremely metabolically active, characterized by elevated OXPHOS, high glucose absorption, and high glycolysis. Similar to our recent findings, the high bioenergetic signature of these cells enhanced their ability to form spheroids and survive in anchorage-independent settings.

According to higher rates of ATP synthesis from glycolysis than from OXPHOS in our investigation, which is also consistent with published results, all cell lines, independent of their SDHA status, preferred to employ the glycolysis pathway to meet their energy requirements. Glycolysis is the most significant mechanism for energy production and cell survival and glucose have a major role in the proliferation of ovarian cancer cells. In a different study, it was noted that as ovarian cancer cells moved from a benign to a highly aggressive cell phenotype, their metabolic profile shifted to a more glycolytic one.

To test whether SDHA overexpression shifts cellular metabolism in response to food deprivation, ovarian cancer cells of glucose or glutamine in various sets of tests were starved. In order to adjust to shifting nutritional conditions, the cells displayed significant metabolic plasticity, alternating between glycolysis and oxidative phosphorylation. We found that glutamine deprivation boosted glycolytic ATP yield, suppressing ATP synthesis via mitochondrial respiration, but glucose limitation dramatically reduced ATP production from glycolysis, which was offset with a higher ATP yield from OXPHOS. Cells can become more tolerant in the changing tumor microenvironment thanks to this metabolic flexibility. In a study, it was discovered that mouse ovarian tumor cells that were enriched in the stem cell population had a highly flexible metabolic profile. In contrast to parental tumor cells without stem cell characteristics, which exhibited the glycolytic Warburg effect and were unable to effectively control cellular metabolism under stress, the cells were able to boost OXPHOS and glycolysis under the right conditions. However, in our investigation, all ovarian cancer cell lines shown some degree of metabolic flexibility in response to metabolic stress (limitation of glucose or glutamine). It suggests that established human ovarian cancer cell lines, like mice ovarian tumor cells with stem cell characteristics, have already developed enough metabolic plasticity to adapt to a limited supply of nutrients. However, our research revealed that cell lines with and without SDHA overexpression have varied capacities to make up for the loss of ATP synthesis following food deprivation.

The matching control cells, on the other hand, showed an enhanced metabolic capability that let them maintain a consistent ATP generation rate under nutrient-deficient conditions. These findings imply that cells with high energy metabolism, which are characterized by SDHA overexpression, may be more vulnerable to cellular stress or a lack of vital nutrients. It has been demonstrated that ovarian cancer cells with high levels of glycolytic and mitochondrial activity have considerable sensitivity to suppression of either glycolysis or OXPHOS, which results in a bioenergetic malfunction and cell death. A different study found that glutamine was essential for the high-invasive ovarian cancer cell lines' increased metabolism, and that their deprivation caused them more harm than their low-invasive counterparts.

Others, however, asserted that cancer cells' extremely adaptive metabolic phenotype enables them to endure cellular stress or substrate shortage. However, the findings and those of earlier studies suggest that some cancer cells with a high metabolic profile and high energy requirements may be less able to deal with food shortage.Therefore reasoned that cells overexpressing SDHA would be particularly susceptible to medications that alter the metabolism of glucose and/or glutamine. We conducted a screening of anti-metabolic drugs and discovered the powerful medication

shikonin, which kills SDHA overexpressing ovarian cancer cells more efficiently than SDHA-low counterparts. Additionally, SDHA overexpression significantly increased the sensitivity of cancer cells to shikonin, which had a potent anti-tumor activity that was superior to that of conventional chemotherapy.

According to our theory, cells that overexpress SDHA have a high energy metabolism that depends on glucose for glycolysis and glutamine for the maintenance of TCA cycle flux and OXPHOS in the mitochondria. As a result, these cells may not survive if the metabolism of either glucose or glutamine is disrupted. Given that ATP and other biosynthetic precursors are produced as a result of the combination of glycolysis and glutaminolysis, which has been previously found to encourage the fast proliferation of cancer cells, simultaneous blocking of both pathways may be a promising treatment approach. For instance, it was showed that blocking both glutaminolysis and glycolysis had a potent synergistic effect on the viability of ovarian cancer cells.

Conclusion

In conclusion, the research offered fresh perspectives on the part that SDHA overexpression plays in the reprogramming of energy metabolism in ovarian cancer. We demonstrated that SDHA overexpressing cells have an extremely active metabolism and rely on both glycolysis and oxidative phosphorylation to meet their energy requirements. The ability of cells to survive anchorage-independent settings is improved, even if this phenotype might not promote a higher rate of cell proliferation. Importantly, ovarian cancer cells with increased SDHA are more susceptible to glucose and glutamine deprivation, which is connected to a marked decrease in ATP output in those cells were discovered. Finally, using a pharmacological screening technique, we discovered the anti-metabolic chemical shikonin, which showed a precise and powerful activity against ovarian cancer cells overexpressing SDHA. Shikonin pre-clinical testing as a possible therapeutic strategy for SDHA-amplified malignancies will be the main focus of the future research. The development of novel strategies to specifically target the metabolism that is unique to ovarian cancer is the long-term aim, and this work represents a significant step in that direction.

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