Domanda estratta n. 1

AVVISO PUBBLICO, PER TITOLI E COLLOQUIO, PER L'ASSUNZIONE A T.D. DI N. 1 RISORSA NEL PROFILO DI RIC. SAN.,CAT. DS, NELL'AMBITO DEL PROG. PNRRMCNT2-2023-12377462: "CLINICALLY APPROVED DRUGS TARGETING PYRUVATE KINASE M2: A DRUG REPURPOSING PATHWAY TO MOVE FORWARD THE TREATMENT OF GLIOBLASTOMA", P.I. DR.SSA CLAUDIA ABBRUZZESE, CUP H83C24000370001 - APPROVAZIONE BANDO

DOMANDE ARGOMENTO SPECIFICO

1 Il candidato esponga in che cosa consiste l'autofagia cellulare e con quali tecnologie può essere studiata

2 Il candidato esponga cosa si intende per riposizionamento dei farmaci e come si eseguono i saggi di sensibilità farmacologica pre-clinici

3 Il candidato esponga le correlazioni tra cancro e metabolismo energetico con particolare riferimento all'effetto Warburg e ai meccanismi per cui risulta vantaggioso per le cellule tumorali

DOMANDE INFORMATICA

1 Cos'è Excel

2 Cos'è Word

3 Cos'è un Database

Istituti Fisioterapici Ospitalieri - ROMA

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RESEARCH

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Compensatory cross-talk between autophagy and glycolysis regulates senescence and stemness in heterogeneous glioblastoma tumor subpopulations

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Abstract

Despite tremendous research efforts, successful targeting of aberrant tumor metabolism in clinical practice has remained elusive. Tumor heterogeneity and plasticity may play a role in the clinical failure of metabolism-targeting interventions for treating cancer patients. Moreover, compensatory growth-related processes and adaptive responses exhibited by heterogeneous tumor subpopulations to metabolic inhibitors are poorly understood. Here, by using clinically-relevant patient-derived glioblastoma (GBM) cell models, we explore the cross-talk between glycolysis, autophagy, and senescence in maintaining tumor stemness. We found that stem cell-like GBM tumor subpopulations possessed higher basal levels of glycolytic activity and increased expression of several glycolysis-related enzymes including, GLUT1/SLC2A1, PFKP, ALDOA, GAPDH, ENO1, PKM2, and LDH, compared to their non-stem-like counterparts. Importantly, bioinformatics analysis also revealed that the mRNA expression of glycolytic enzymes positively correlates with stemness markers (*CD133/PROM1* and *SOX2*) in patient GBM tumors. While treatment with glycolysis inhibitors induced senescence in stem cell-like GBM tumor subpopulations, as evidenced by increased β-galactosidase staining and upregulation of the cell cycle regulators $p21^{Waf1/Cip1}$/*CDKN1A* and $p16^{INK4A}$/*CDKN2A*, these cells maintained their aggressive stemness features and failed to undergo apoptotic cell death. Using various techniques including autophagy flux and EGFP-MAP1LC3B^{+} puncta formation analysis, we determined that inhibition of glycolysis led to the induction of autophagy in stem cell-like GBM tumor subpopulations, but not in their non-stem-like counterparts. Similarly, blocking autophagy in stem cell-like GBM tumor subpopulations induced senescence-associated growth arrest without hampering stemness capacity or inducing apoptosis while reciprocally upregulating glycolytic activity. Combinatorial treatment of stem cell-like GBM tumor subpopulations with autophagy and glycolysis inhibitors blocked the induction of senescence while drastically impairing their stemness capacity which drove cells towards apoptotic cell death. These findings identify a novel and complex compensatory interplay between glycolysis, autophagy, and senescence that helps maintain stemness in heterogeneous GBM tumor subpopulations and provides a survival advantage during metabolic stress.

Keywords Glioblastoma, Tumor heterogeneity, Cancer stem cell-like cells, Metabolism, Glycolysis, Autophagy, Senescence

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Introduction

Tumors exist as heterogeneous entities that contain diverse subpopulations of cells at varying stages of differentiation and proliferative capacity and these functional differences are driven by complex genomic, epigenomic, and transcriptomic intra-tumoral heterogeneity [28, 34, 38, 41].[Glioblastoma (GBM) is the most aggressive primary malignant brain tumor in adults, characterized by a 5-year survival rate of approximately 5% due to inevitable disease relapse [23, 55, 56]. GBM tumors exhibit immense heterogeneity and contain 'stem cell-like' tumor subpopulations that are resistant to conventional therapies and have aggressive tumor seeding capacity, which contributes to the poor prognosis of this disease][2, 21, 40, 41].

Treating cancer by targeting their abnormal metabolic phenotypes has been a widely sought-after concept for many years [15, 42, 44, 66]. Yet, poor efficacy and dose-limiting toxicities demonstrated by metabolism-targeting agents in clinical trials has prevented wide-spread use of metabolic inhibitors in standard of care treatment regimens for cancer patients [11, 29, 53, 69]. GBM tumors tend to exhibit a high dependency on the 'Warburg effect', a metabolic phenotype that describes the enhanced rate of fermentative glycolysis utilized by cancer cells as compared to normal tissues [68]. Despite this, clinical trials utilizing glycolysis inhibitors such as dichloroacetate (DCA) to treat GBM tumors have yet to demonstrate any clear survival benefit for patients [9, 35].[The problems with treating tumors with anti-metabolic drugs arise when we consider the complex heterogeneity and plasticity of cancer cells. Heterogeneous tumor subpopulations may possess different energy and metabolic needs, therefore, targeting a single metabolic phenotype may not affect all subpopulations equally. Moreover, metabolic networks are highly interconnected and can be rewired to compensate for deficiencies in certain pathways][1, 20, 24]. In order to design better metabolism-based therapeutic strategies for the treatment of GBM tumors, metabolic preferences of distinct tumor subpopulations must be taken into consideration. Moreover, the effect of metabolism-targeting interventions on additional metabolic and growth-related compensatory processes must be assessed for the rational development of novel combinatorial therapies.

Cancer cells exist in a state of uncontrolled proliferation [16]. Induction of genotoxic or energetic stress can overwhelm cancer cells and lead to cell death. This is the guiding principle behind cancer-targeting therapies [16, 48]. If cancer-targeting agents cause irreparable damage, this can stimulate tumor suppressor pathways that will either activate a programmed cell death pathway, or can drive cells to exit the cell cycle and enter into a dormant, non-proliferative state known as senescence [10, 48]. The majority of cancer-targeting agents used in clinics are highly toxic and induce programmed cell death to stop tumor growth [48]. On the other side, senescence has also been deemed a desirable therapeutic outcome for cancer treatment as it has the potential to limit tumor cell proliferation without causing extensive damage to surrounding tissues from cell death and inflammatory signals [10]. However, the role of senescence in the response of heterogeneous tumor subpopulations to metabolism-targeting interventions is unclear. Autophagy is an intracellular catabolic process that can breakdown and recycle cellular constituents in response to nutrient deprivation [22, 70]. The role of autophagy in cancer is controversial, as it has been demonstrated that autophagy may act as a pro-survival or pro-death signal depending on the stimuli and stage of tumor development [67]. Recently, upregulation of autophagy has been shown to act as an adaptive resistance mechanism in response to chemotherapeutic stress in cancer cells [5, 25, 33, 57]. Hence, combination therapies targeting autophagy-mediated resistance mechanisms may represent an attractive therapeutic strategy to augment chemotherapy. Despite these insights, the role of autophagy in the response of heterogeneous tumor subpopulations to metabolic inhibitors remains unclear.

[This study aims to investigate metabolic phenotypes of heterogeneous GBM tumor subpopulations and explore the role of cell-growth related processes, such as autophagy and senescence, in response to metabolism-targeting interventions. Using a clinically-relevant and patient-derived model of GBM, comprehensive metabolic profiling was performed on stem cell-like and non-stem-like tumor subpopulations.] It was found that stem cell-like CD133/PROM1^{HIGH} patient-derived GBM cells possess enhanced levels of glycolytic enzymes and glycolytic activity as compared to their non-stem-like counterparts. Moreover, bioinformatic analysis demonstrated that glycolytic enzyme expression positively correlates with stemness markers in GBM patient tumor specimens. While treatment with glycolytic inhibitors induced senescence and inhibited cell growth in stem cell-like GBM subpopulations as compared to non-stem-like cells, blocking glycolysis alone did not hamper their stemness capacity. Further mechanistic analysis concluded that this was due to compensatory upregulation of autophagy. Reciprocally, inhibition of autophagy in stem cell-like tumor subpopulations corresponded with an increase in glycolytic activity, while also promoting senescence-related growth arrest without affecting stemness capacity. Combinatorial inhibition of glycolysis and autophagy led to a cumulative decrease in cell growth, driving cells towards apoptotic programmed cell death instead of