Metformin inhibits CLIC1 chloride channel-1 conductance to impair glioblastoma stem cell proliferation

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Glioblastoma (GBM), the most common primary brain tumor in adults, is characterized by poor prognosis (overall survival of only 14.6 months), even adopting multimodal aggressive treatments such as the combination of neurosurgery, radiotherapy and chemotherapy with temozolomide). This negative outcome has been ascribed to the presence, within GBM tumor mass, of small cell populations identified as cancer stem cells (CSC), or tumor-initiating cells. CSCs display distinct phenotype and molecular characteristics from other tumor cells, high differentiation potential and unique properties of invasiveness, proliferation, self-renewing and resistance to therapy. The persistence of CSCs after standard therapy is considered responsible for tumor recurrence. Thus, the discover of new drugs selectively active on CSCs is a compelling requirement. Epidemiological and preclinical studies suggest that metformin, the first-line drug for type-2 diabetes, exerts direct antitumor activity. Clinical trials are currently ongoing in many human cancer types, although the molecular mechanisms by which metformin exerts the antiproliferative activity are still not completely identified. Several studies reported the involvement of AMP-activated kinase-dependent pathway, and the down-stream effectors (e.g. mTOR) in metformin metabolic activity. Nevertheless, contrasting evidence was obtained on the involvement of this pathway in metformin antitumor effect, and several reports showed AMPK-independent antiproliferative activity. Here we show that metformin directly inhibits chloride intracellular channel 1 (CLIC1) in human GBM CSCs, leading to tumor cell growth arrest. CLIC1 activity was reported to play a role in GBM development, progression and invasiveness, controlling the G1-S transition in the cell cycle. The inhibition of CLIC1 conductance induced by metformin reduces GBM CSC proliferation, causing arrest in G1 phase. Single point mutation in the putative CLIC1 pore region impairs metformin modulation of the channel activity, suggesting that metformin binding site is located on the extracellular portion of CLIC1. Metformin inhibition of CLIC1 mediated current and cell cycle progression is obtained through a time-dependent mechanism, in which prolonged treatments (7-10 days) allow the occurrence of antiproliferative effects for low, clinically achievable, metformin concentrations. On these premises, metformin's effects were also shown in vivo, causing a significant inhibition of GBM CSC invasiveness and metastatic diffusion. Interestingly, CLIC1 is not active in normal mesenchymal stem cells, and consequently these cells are not affected by the toxic effects of metformin, highlighting that this drug seems to be CSC-selective.

These results identify a completely new molecular target for the antiproliferative effects of metformin, which could be exploited to identify novel pharmacological approaches for GBM. Following this line we evaluated the antitumor activity, and the ability to inhibit CLIC1 ion conductance, of several known and novel biguanide derivatives. In particular, we identified novel compounds showing higher potency than metformin, but reproducing the same mechanism of action, representing promising candidates to be further developed in clinical settings.

Since the lack of drugs affecting CSC viability is the main cause of therapy failure and tumor relapse, the identification of novel and specific molecular targets and of drugs acting on this tumor cell subset represents a needed pharmacological strategy to improve GBM management.